2001

Pigment labeling determination of carbon to chlorophyll

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PIGMENT LABELING DETERMINATION OF CARBON TO CHLOROPHYLL

A Thesis

Presented to

Moss Landing Marine Laboratories

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

James G. Smith

May 2001

UMI Number: 1405518

UMI Microform 1405518

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ABSTRACT

PIGMENT LABELING DETERMINATION OF CARBON TO CHLOROPHYLL

by James G. Smith

Nutrient stress is thought to result in measurable increases in the C:Chl ratio of natural phytoplankton communities. It is hypothesized that increases in C:Chl should be evident from nutrient rich coastal waters to stable oligotrophic gyres. Determination of the specific activity of chlorophyll carbon from pigment labeling experiments allows estimates of algal carbon unique to photosynthetic cells. This technique was performed along a transect from Monterey Bay, CA to Station Aloha, HI in the fall of 1998. C:Chl ranged from 15 to 163. Highest values were encountered in the mesotrophic transition zone between eutrophic coastal conditions dominated by eukaryotic phytoplankton and the oligotrophic open ocean characterized by a large percentage of prokaryotes. Nitrate levels did not explain the observed trend in C:Chl. The transition zone may indicate an area of increased stress as comparatively stable coastal and oceanic phytoplankton communities react to the hydrographic/biogeochemical interface that separates the end-member communities.

ACKNOWLEDGEMENTS OF FINANCIAL SUPPORT

National Science Foundation

San Jose State Faculty Wives

Ethel and Earl Myers Oceanographic Trust

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The ratio of phytoplankton organic carbon to phytoplankton chlorophyll (C:Chl) is a key ecological parameter which 1) provides estimates of algal carbon biomass from routine measurements of Chl *a* and 2) provides information regarding the physiological state of phytoplankton expressed through the relative degree of cellular pigmentation (Cloern, et al., 1995). Due to the difficulties of isolating phytoplankton biomass from other trophic forms of carbon in the ocean, methods to estimate in-situ C:Chl ratios have been of broad interest (Eppley, et al., 1977; Redalje and Laws, 1981; Welschmeyer and Lorenzen 1984; Gallegos and Vant 1996). Numerous lab studies have demonstrated C:Chl range widely within and among species and also serve to provide realistic working limits for in-situ C:Chl. The recent review by Cloern, et al. (1995) cites a range of C:Chl varying from 30-330 (wt/wt) in over 250 laboratory determinations. Fortunately, these studies show C:Chl to vary predictably with several environmental variables, particularly irradiance and nutrient supply. For instance, under conditions of low irradiance and replete nutrients, C:Chl has been shown to fall to its lower limit; as cells acclimate to high irradiance and low nutrients, the C:Chl ratio increases predictably. For any given alga, it is reasonable to assume that the lower and upper limits of C:Chl may vary over a 5-fold range as a result of acclimation to irradiance and nutrient stress (Cloern, et al., 1995).

On the basis of this laboratory evidence, it has often been assumed that C:Chl ratios should vary predictably in natural phytoplankton assemblages. One

such prediction is that C:Chl should decrease with depth in the water column and this has been supported by a number of studies (Welschmeyer and Lorenzen 1984; Goericke and Welschmeyer 1998; Cullen, 1982). Laboratory results also lead to a hypothesis that C:Chl is lowest in near-shore, eutrophic, turbid environments and is higher in the clear, oligotrophic, open ocean. Unfortunately, data to support this hypothesis are not readily available. The purpose of the work described here was to apply a consistent set of methodological routines to samples collected along a transect from coastal to oceanic environments to test the hypothesis that C:Chl increases in the offshore direction, as predicted by laboratory experiments.

Estimating the C:Chl ratio of native phytoplankton populations is confounded by the difficulty in isolating phytoplankton biomass from all other sources of carbon. Most often, chlorophyll a is measured to serve as a proxy for phytoplankton biomass. However, it is the carbon content of phytoplankton that is needed to calculate growth rate (μ) and for understanding the role of phytoplankton in the oceanic carbon cycle. To achieve estimates of phytoplankton carbon Redalje and Laws (1981) introduced the technique of pigment labeling. This method directly estimates μ by measuring the amount of ^{14}C that is incorporated into chlorophyll. Combining μ with estimates of primary production, allows the calculation of biomass. These three fundamental ecological parameters are related by the equation:

$dP / dt = \mu * Cp$ $\quad$ $dP / dt =$ Primary Production

μ = Growth Rate

Cp = Phytoplankton Carbon Biomass

In the study described here, μ was estimated by the pigment labeling technique and primary production was estimated by the ^{14}C uptake technique (Steeman Nielsen (1952). Once Cp was calculated, measures of chlorophyll were incorporated to estimate C:Chl.

Total particulate organic carbon (POC) provides an upper limit of phytoplankton carbon (Cp) due to the potential presence of detrital and heterotrophic biomass. This non-phytoplankton component can be large, especially in oligotrophic regions of the oceans (Banse, 1977). Measures of algal biomass are many, each with its advantages and drawbacks. Microscope and flow cytometric cell counts can be converted to biomass, but care must be taken to only count phytoplankton cells. The high abundance of very small prokaryotic phytoplankton and of *Prochlorococcus* sp. in the oligotrophic ocean makes phytoplankton cell counts in the oceanic region difficult to quantify with microscopes. Flow cytometers work well for counting small cells (Campbell and Vaulot, 1993), but are not as accurate in communities of larger phytoplankton. These cell counts require a conversion factor to achieve a biomass estimate and this ratio of cell volume to cell carbon is known to vary (Strathman, 1967) (Caperon and Meyer, 1972) and thus may impose uncertainties in microscopic estimates of algal carbon. Adenosine triphosphate (ATP) can also be used as an indicator of biomass, but is not specific to phytoplankton (Hunter and Laws,

1981). Chlorophyll a (chl a) is routinely measured and is the most widely accepted proxy for phytoplankton biomass. The advent and refinement of satellite remote sensing of chlorophyll makes this proxy of biomass of particular interest. Yet, to correctly convert remotely sensed or extracted chlorophyll to phytoplankton biomass, accurate knowledge of the phytoplankton carbon to chlorophyll a ratio is needed. This study presents estimates of algal carbon to chlorophyll ratios of in-situ phytoplankton populations from Monterey Bay, California to Station ALOHA, Hawaii.

Phytoplankton growth rate is another basic ecological parameter that is difficult to quantify in the ocean. The most widely accepted assessment of phytoplankton primary production is the uptake of ^{14}C as introduced by Steeman Nielsen (1952). This provides an estimate of carbon uptake per unit volume. Advances in ultra clean incubation techniques (Fitzwater, et. al, 1982) have improved these estimates of primary production and now are widely accepted. As a result, estimates of global primary production have slightly increased. The measure of primary production is not the same as growth rate. Growth rates can be measured in the lab through the use of axenic cultures. In-situ estimates are much more difficult as phytoplankton are continually adapting to fluctuating levels of available light and nutrient stress may be limiting growth rates. Grazer and bacterial activity will further complicate estimates of growth rate.

Despite these difficulties, in-situ growth estimates have been made. Changes in cell counts and the frequency of cell division (Swift and Durbin, 1972)

have been used to estimate growth rates. Most often, the equation:

$$(\mu) = 1/Cp \times dCp/dt$$

is used to estimate growth rate (μ=growth rate per time, Cp=phytoplankton biomass, t=time). The change in Cp over time is easily measured by carbon uptake experiments, although the interpretation of results is confounded by food web interactions. Unfortunately, the measurement of total phytoplankton biomass at any given time is subject to the errors discussed above.

The present study will estimate Cp by combining the technique of pigment labeling and estimates of carbon uptake. In doing so, in-situ phytoplankton growth rates will be measured directly. Combing estimates of Cp and chlorophyll will generate the ratio of C:Chl An accurate knowledge of the ratio of phytoplankton carbon to chlorophyll a (C:Chl a) would allow the routine and frequent measure of ocean chlorophyll a to be converted to phytoplankton biomass. Culture studies show this ratio to vary from 10 to >330 (Cloern, et al., 1995) depending on species (Chan, 1980), temperature (Verity, 1981, Thompson et al., 1992), nutrient availability (Verity et al., 1988), light (Geider and Osborne 1986, Nielsen and Sakshaug, 1993) and simulated season (Curl and Small, 1965, Eppley et al., 1977, Anderson and Rudehael, 1993). The effects of temperature, nutrients and light are highly variable (Figure 1), making accurate, in-situ measurements difficult. Laboratory results can serve as a guide to what might be expected as the measurements progress from nutrient rich, cold waters with a shallow euphotic zone to warmer, nutrient depleted waters with a deeper

euphotic zone. Laboratory trends predict increased C:Chl as available nutrients decrease and light increases. In contrast, increases in temperature have the opposite effect.

The technique of isotopic labeling of chlorophyll a carbon (Redalje and Laws, 1981) offers an alternative method of estimating both μ and Cp of natural phytoplankton communities, free from the interferences associated with non-algal carbon. For this technique, phytoplankton are incubated with a known activity of $H^{14}CO_3$ and given time to achieve isotopic equilibrium among all algal carbon compounds. This means that all algal carbon compounds, including chlorophyll, will have the same specific activity (dpm/gC). The specific activity of carbon contained in chlorophyll a is determined after chromatographic purification and isolation of the pigment. The amount of activity found in chlorophyll is directly related to the growth rate and allows its calculation. Combining growth rate with ^{14}C uptake allows the calculation of phytoplankton biomass.

Previous experimenters had used long-term ^{14}C incubations to determine plant biomass (Laws and Bannister, 1980). These studies were based on the concept of uniform labeling; that after sufficient cell doublings, the specific activity (dpm/μgC) of the plant material was equal to the specific activity of the dissolved inorganic carbon (DI^{14}C). Measures of phytoplankton cell activity (dpm/cell) could then be converted to biomass (μgC/cell) (Laws and Wong, 1978, Hunter and Laws, 1981). If labeled to anything less than uniform labeling, the absolute specific activity of the algal carbon must be determined to allow the calculation of

biomass. This is not easy in systems other than axenic algal cultures due to the necessity of isolating phytoplankton carbon from animal, bacterial and detrital carbon.

To overcome this problem, Redalje and Laws (1981) assumed that chl a turn over (synthesis and degradation) was quick enough to allow the level of labeling of chl a to equal the level of labeling of the total phytoplankton cellular carbon, i.e., that all carbon compounds reached isotopic equilibrium in a relatively short time. Redalje (1983) found this condition to be met is as little as two hours. Welschmeyer and Lorenzen (1984) further tested the assumption that the specific activity (SA) of chlorophyll a and of total algal carbon was not significantly different at the end of a labeling experiment and found the two to be equal. Further, they showed that total plant carbon and chlorophyll carbon become labeled at the same rates. This is a critical assumption of the labeling technique. Using this assumption, chlorophyll a carbon activity represents the carbon activity of the entire phytoplankton cell. Isolation and purification of chlorophyll a, unique to photosynthetic organisms, allows phytoplankton carbon activity to be separated from all other sources of carbon.

Carbon specific growth rates of in-situ phytoplankton populations could also be calculated by combining the specific activity of the chlorophyll carbon and of the DIC. Growth rate is calculated by determining the amount of ^{14}C found in chl a at the end of the incubation period. Isolating pure chlorophyll minimizes errors associated with food web interactions. Conventional carbon uptake

experiments measure all activity typically found on a GF/F filter after a period of incubation. This activity most likely is not solely due to phytoplankton uptake, but contains contributions from ^{14}C incorporation in all particulate forms, including grazers and bacteria that have consumed particulate and dissolved photosynthetic products repeatedly. While current carbon fixation experiments may be underestimated by up to a factor of two (Welschmeyer, 1999, Fitzwater et al., 1982), the determination of growth rate through pigment labeling avoids this problem. The calculation of growth rate is affected only by the amount of ^{14}C that is fixed into chlorophyll. Purifying chlorophyll minimizes the effects of other trophic levels on the estimate of growth rate.

Goericke and Welschmeyer (1993) demonstrated that the pigment labeling technique most closely estimates the synthesis rate of chlorophyll a, not necessarily the algal cell growth rate. They showed that the rate of chlorophyll a synthesis is equal to the rate of carbon synthesis only when growth is balanced. The rate of ^{14}C incorporation into chl a scales with the rate of chl synthesis and not with the rate of ^{14}C uptake. Chlorophyll is not turned over in response to changing light conditions (Goericke and Welschmeyer, 1993). It is well known that photo adaptation can significantly alter C:Chl as cells increase chlorophyll production in response to low light, or divide without new chlorophyll production in times of high light. Photoadaptation will likewise disconnect chl synthesis rates estimated from chlorophyll labeling and true cellular growth rates. Therefore, every effort must be made to keep phytoplankton populations at light levels near

their in-situ condition in order to use the chlorophyll labeling technique to estimate algal growth rates.

Technological advances have lead to the pigment labeling technique being applied in several studies of natural phytoplankton (Table 1). High-pressure liquid chromatography (HPLC) has replaced the original thin-layer chromatography (Redalje and Laws, 1981; Welschmeyer and Lorenzen, 1984) and allows quick and reliable isolation and quantification of chlorophyll a. The technique has also been extended to carotenoid labeling in an effort to obtain taxon specific growth rates (Gieskes and Kraay, 1989).

These published results for C:Chl of natural phytoplankton populations from chlorophyll labeling experiments (7 to 270) vary widely and are specific to regional bodies of water. While culture studies under various conditions of light, nutrients and temperature may be used to predict the behavior of C:Chl across nutrient-rich coastal waters to nutrient-poor open ocean waters, no comprehensive study has been published that test those predictions. The goal of the present study was to conduct labeling experiments along a transect and measure C:Chl and in-situ growth rates for phytoplankton carbon. The transect extends from the eutrophic coastal Monterey Bay, CA, through the California Current and ends at low nutrient, blue water Station ALOHA, HI (Figure 2). Based upon expected trends in the physical environment, changes in species composition and previously published results, the working hypothesis will be that

C:Chl and growth rate will vary as a function of oceanographic province and C:Chl will increase steadily from the near shore to the oceanic regions.

MATERIALS AND METHODS

Sampling and Hydrography

Thirteen stations were sampled along a transect from Monterey Bay, California to the Hawaii Ocean Time Series (HOTS) station at Station ALOHA during September 1998 on board the R/V Moana Wave. The first eight stations were to be separated by 110km. The next ten stations were to be separated, nominally, by 375km. Actual separation was determined by steaming distance between stations occupied once per day.

Conductivity, temperature and depth (CTD) were sampled from a Seabird 9E during rosette down casts. Analysis of temperature changes determined the mixed layer depth using the technique of Lentz (1992) (Δ= 0.10 °C/m).

Light profiles of photosynthetically active radiation (PAR) were taken daily to 200m. Natural log of mixed layer PAR was plotted against depth and the negative slope of the regression line was used as the light attenuation value (k). The light attenuation coefficient was used to calculate the average PAR of the mixed layer and the percent of surface PAR at the bottom of the mixed layer. These values were used to determine the number of neutral density nickel screens, which mimicked average mixed-layer irradiance conditions, in deck board incubations. Based upon the average amount of incident irradiation found in the mixed layer in Monterey Bay, two screens were used for each incubation.

Screen treatments did not change along the transect to allow inter-comparison between stations.

Most water samples for labeling experiments and discrete hydrographic analysis were taken with 10L Niskin bottles on a 24-bottle rosette off a standard hydrowire. Station 7 was sampled from two-bucket casts off the ships leeward side. Sampling at the HOTS station occurred with 30L Go-Flo bottles. Sampling depth occurred approximately in the middle of the mixed layer. Water samples were transferred through silicon tubing directly into incubation or sample vessels. All sampling occurred approximately 4 hours before dawn to ensure all incubation experiments were initiated before sunrise.

Nitrate

For nitrate analysis, 20mL plastic scintillation vials were rinsed three times, filled two-thirds with sample water and frozen. Caps were re-tightened after freezing. Nitrate analysis was performed in the Trace Metals Laboratory of Moss Landing Marine Laboratories (MLML) using the flow-through, ultraviolet spectrophotometric absorption technique (Guenther, 1999). Standards (Milli Q water, 0.2μm filtered low nitrate surface seawater and 25μM nitrate) and sample absorbance were measured (HP 8452A diode array spectrophotometer with 2nm resolution) from 200 to 400nm in a 1cm flow-through cuvette (Hellma with Suprasil I windows). Four solenoid pumps propelled the 3 standards and the sample at 1.2mLs per minute. Standards were re-measured every fifteen samples. Absorbance spectra were saved and nitrate values were calculated in

a Quickbasic (v. 4.5) program written by Dr. Ken Johnson (Monterey Bay Aquarium Research Institute). Nitrate standards of 2.0, 4.0 and 20.0μM, made in low nitrate seawater, were treated as samples and run through the system to check for instrument drift.

Particulate Organic Matter

Seawater [5.44L (1.044L at station 1)] was passed through a Nitex® screen (pore size 303μm), collected in acid-washed (10% HCl) bottles, filtered through 25mm GF/F filters, dried for 24 hours at 65°C and stored under vacuum until particulate carbon (POC) and nitrogen (PON), analysis could be preformed. At each station, blank filters were collected, dried and stored in the same manner as the sample filters to account for any effects due to storage. In the lab, samples were acid fumed (over a thin layer of concentrated HCl) for 12 hours and then dried again for an additional 24 hours at 60°C. GF/F filters were not pre-combusted to avoid any perturbation to pore shape and diameter. Un-combusted clean filters were run as blanks and their values subtracted from sample values. Carbon and nitrogen were measured on a 440 Elemental Analyzer with reduction at 700°C and combustion at 850°C in nickel sleeves that had been combusted at 700°C for at least 4 hours. Cystein standards were weighed on a Perkin Elmer A4-4 analytical balance in aluminum boats that had been combusted at 525°C for at least 4 hours and analyzed with each run of 10 samples.

Total Chlorophyll a

Total chlorophyll a was sampled at the beginning of each incubation experiment directly from the sampling bottles. Quantitative aliquots, nominally 200mLs, were filtered (10mm Hg vacuum) through 25mm GF/F filters and extracted in 5mLs of 90% acetone for at least 24 hours at -5°C. Samples were vortexed for at least 30 seconds, spun down to remove filter debris for 5 minutes at 3000 rpm and analyzed at sea on a Kratos 950 fluorometer. The Kratos 950 fluorometer was configured for single-step determination of chl a (Welschmeyer, 1994); excitation interference filter of 436nm, emission interference filter of 680nm, blue lamp (F4T4.5B2). Calibration coefficients were calculated daily with pure chlorophyll a in 90% acetone, stored at –0.5°C.

HPLC and Pigment Labeling

Seawater (2.2L) was filtered through 25mm GF/F filters for HPLC analysis. Samples were covered in aluminum foil, placed in cryo-vials and stored in liquid nitrogen. These samples were analyzed by HPLC (see below for procedure) and served as initial pigment concentrations.

Approximately 4L of water was sampled into acid-cleaned (10% HCl) polycarbonate bottles for pigment labeling experiments. Two hundred to 600µCi of $H^{14}CO_3$ was added, bottles were topped off and gently mixed (lower activities were used at near shore stations; higher activities at offshore stations). Bottles were placed in one-layer thick neutral density screen bags and into a blue acrylic deck-board incubator, with one layer of neutral density screen built in, yielding a

final irradiance exposure of approximately 22% surface I_o. Percent irradiance was approximated and was not measured directly. Surface seawater was continually flushed through the incubators and incubator water temperature was monitored through the daylight hours to ensure temperatures remained near ambient. At the HOTS station, incubation bottles were placed in open-mesh bags, fastened to a polypropylene in-situ line and returned to the sampling depth. The in-situ line was held taught with a 34Kg termination weight; surface floats and navigational aids allowed tracking while the array drifted. All incubations lasted for 24 hours, from pre-dawn to pre-dawn.

At the end of each incubation, two 140mL samples were removed for total chlorophyll analysis as described above. Two 50µL aliquots were added directly to buffered scintillation flour (4mLs fluor + 0.45mLs of 0.1N NaOH: Aquasol II – New England Nuclear) for analysis of total labeled dissolved organic carbon ($DI^{14}C$). Two 140mLs samples were filtered through 25mm GF/F filters, rinsed with 0.2µm seawater to remove DIC, acid fumed (concentrated HCl) for at least 2 minutes and placed into 5mLs scintillation fluor (Scintisafe) in plastic scintillation vials for analysis of total labeled particulate organic carbon ($PO^{14}C$). Scintillation counting was preformed at sea to check on adequate addition of label; final counts were made six months later in the lab on a Packard 2000 CA/LL Liquid Scintillation counter at 1 and 30-minute count times using an internal standard for quench correction. The remainder of the sample bottle (ca 4L) was filtered quantitatively through a 25mm GF/F filter to be used for HPLC analysis of

pigment-labeling. Filters were folded, placed in aluminum foil, put into a cryo-vial and stored in liquid nitrogen until pigment purification was preformed later in the lab.

Initial and labeled final pigment samples were extracted for 24 hours in 1.2mLs nitrogen bubbled 90% acetone and stored at -20°C. Samples were vortexed for 30 seconds and spun down at 3000 rpm for 5 minutes. Pigment quantification and isolation was preformed on a Varian Model 5060 high-pressure liquid chromatography system (HPLC) with in-line double detectors: Kratos FS 450 fluorometer (blue excitation filter = Corning 5-60, red emission filter = Corning 2-64) and a Linear UVIS 200 spectrophotometer (absorbance at 440nm). A small quantitative aliquot (100 to 200μL) from each sample was injected on the HPLC system to determine 'initial' and 'final' pigment concentrations using HPLC analysis. The sample was quantitatively diluted 1.5-fold with nanopure water to reduce solvent strength and manually injected with a syringe into a 1mL injection loop. A three solvent system was used on a C-8 column (Dynamax, 25cm length 4.6mm ID, 5μm particle size, 100A pore) with a C-8 guard column, column filter and a flow rate of 1.5mLs per minute. Solvents used were: A) 20% aqueous ammonium acetate, 80% methanol, B) acetonitrile, C) acetone. The gradient was as follows (time, % solvent A, % solvent B, % solvent C): (0, 100, 0, 0), (8, 0, 100, 0), (13, 0, 70, 30), (17, 0, 20, 80), (19, 0, 20, 80), (20, 100, 0, 0), (26, 100, 0, 0). Peak areas were measured and multiplied by

the appropriate response factor to calculate the concentration of mono-vinyl and di-vinyl chlorophyll a.

The pigment-labeling sample was processed for chromatographic analysis as follows, similar to the method described in Welschmeyer et al. (1993). The remainder of the radiolabeled sample extract was diluted 50% with nanopure water and loaded onto a C-18 solid phase extraction cartridge (Baker SPE 1mg), washed with 50% acetone (ca. 1mL), washed with nanopure water (ca. 1mL) and eluted with 100% acetone yielding a final elution volume of approximately 1.0mLs. Samples were diluted (2 parts sample to 1 part water) to aid in the separation of carotenoid peaks. Up to 1mL of diluted sample was manually injected onto the same HPLC system as above but with a different column and elution gradient. The large injection volume is necessary to ensure enough chlorophyll a is collected to give a detectable scintillation count. The change to a C-18 column (Dynamax, 25cm length 4.6mm ID, 5μm particle size, 100A pore), C-18 guard column and new elution gradient was done to allow separation of carotenoids and chlorophylls C_1 and C_2. The solvents were the same, but the gradient was different: (time, % solvent A, % solvent B, % solvent C): (0, 100, 0, 0), (8, 0, 100, 0), (13, 0, 70, 30), (17, 0, 20, 80), (25, 0, 20, 80), (26, 100, 0, 0), (33, 100, 0, 0). Individual pigment peaks were collected and saved for other analysis. Divinyl and monovinyl chlorophyll a were collected as one fraction, since C-18 columns with this solvent system do not separate mono- and di-vinyl

chl a. Samples were nitrogen bubbled and stored at -10°C until all samples were initially purified.

The mono- and di-vinyl chlorophyll a fraction was acidified (15μL 10% HCL) and re-injected on to the same HPLC system with a C-18 column, but without pre-injection sample dilution to maximize final radioassay signals. Acidification of chl a and subsequent HPLC isolation of pheophytin is necessary to obtain a radio-chemically pure pigment as chl a is known to co-elute with colorless compounds that contain radioactivity (Goericke, 1992). Solvent B was changed to 100% methanol and the gradient was as follows: (time, % solvent A, % solvent B, % solvent C): (0, 100, 0, 0), (10, 0, 100, 0), (12, 0, 70, 30), (15, 0, 60, 40), (28, 0, 60, 40), (29, 100, 0, 0), (35, 100, 0, 0). These changes were necessary to ensure baseline separation of mono- and di-vinyl pheophytin a. This is illustrated in Figure 3. Absorbance was set at 665 nm. Divinyl pheophytin and monovinyl pheophytin were collected separately into clean 5mL scintillation vials (plastic) and 3 mLs of scintillation fluor (Scintisafe) was added. Samples were nitrogen bubbled for 2 hours to remove acetone from the HPLC eluent that would quench the liquid scintillation counts.

Scintillation Counting

Radioactivity, as counts per minute (cpm), was measured on a Packard 2000 CA/LL scintillation counter (6hr count time per sample). Background counts were measured by adding varying amounts of 60% solvent B and 40% solvent C eluent, collected from the HPLC system, to scintillation fluor so that the total

volume was the same in 13 vials. These were counted for 6hrs. Activity remained constant while quench indicating parameters varied, therefore, it was concluded that background could not be quenched and the average of 11.798 cpm was subtracted from all samples (DI^{14}C, PO^{14}C and labeled pigments) cpms. A quench curve was created with radioactive tolulene and varying amounts of 60/40 eluent. Sample tSIE (the external standard quench parameter of this counter) values were compared with the curve to estimate counting efficiency. CPMS were divided by the efficiency to calculate disintegrations per minute (dpm).

Response Factors

Pure mono-vinyl chlorophyll a (Sigma Scientific) was dissolved in 90% acetone and its absorbance measured from 350 to 800nm on a Hewlett Packard 8452A spectrophotometer. Absorbance at 750nm was subtracted from absorbance at 664nm and the extinction coefficient of 87.67 (Jeffrey and Humphrey, 1975) was used to calculate chl a concentration. Pheophytin a was created by acidifying 1mL of chlorophyll a extract with 20μL of 10% HCl. The same scan was preformed and absorbance at 750nm was subtracted from absorbance at 667nm and the extinction coefficient of 51.2 (Jeffrey and Humphrey, 1975) was used to calculate pheophytin concentrations. These pigments were quantitatively injected on the HPLC system described above using a C-18 column. Each pure pigment was injected twice to obtain absorbance values at 440 and 665nm. Peak area per weight of pure pigment

injected was calculated to create the response factor. It is assumed that di-vinyl chlorophyll and pheophytin a will have the same response factor as their mono-vinyl counterparts at 665nm.

Calculations

The original formulas of Redalje and Laws (1981), expanded by Welschmeyer and Lorenzen (1984), allow for the calculation of an initial carbon (C_o), and a final carbon (C_p). The necessary parameters and relevant equations are:

1. I = specific activity of inorganic carbon (dpm / μg)
2. A = activity of particulate organic carbon (dpm / L)
3. R = specific activity of isolated chlorophyll carbon (dpm / μg)
4. L = relative specific activity (range: 0 – 1.0)
   = (1.05 * R) / I (1.05 to account for isotopic discrimination)
5. μ = growth rate
6. ΔC = Change in phytoplankton carbon concentration = (1.05 * A) / I
7. C_o = Initial phytoplankton carbon concentration = $\Delta C / (e^{\mu t} - 1)$
8. C_p = Final phytoplankton carbon concentration = $\Delta C / (1 - e^{-\mu t})$
9. t = incubation time in days

Growth rates (μ) were calculated through the P_1, F_2-P_2 Model (Goericke and Welschmeyer, 1993). The equation is:

10. $L = 1 - [1.0424 * e^{(-\mu * t)}] + [0.0424 * e^{(-16 * \mu * t)}]$

Iterative computing is used to solve for μ.

The three components of the calculation of C:Chl are chlorophyll, carbon uptake and the labeling coefficient (L). The first two scale linearly with C:Chl, while L does not. All three are vulnerable to errors. Chlorophyll is the most routine of the measurements, but estimates of chlorophyll at the end of an incubation can be influenced by grazing and photoadaptaion during the incubation. Production estimates will usually show some carbon uptake. Both equations 7 and 8 rely upon the one estimate of carbon uptake that occurs at the end of the experiment. If growth and grazing are balanced, the two will be equal. This is rarely the case. Also, grazing will destroy chlorophyll, but retain the isotopic signature in a particulate form and influence the final estimate of phytoplankton carbon. Therefore, estimates of carbon uptake contain some measure of ^{14}C in forms other than algae, such as grazers and bacteria. These food-web interactions make the final estimate of algal carbon an overestimate. Therefore, only initial estimates of phytoplankton carbon will be considered. Equation 7 is the working equation for the calculation of phytoplankton carbon. Equation 4 is unaffected by grazing as no preference has been observed by grazers in their consumption of either labeled or unlabeled phytoplankton (Laws, 1984). Photoadaptation is the biggest concern in the measurement of L.

Production values are adjusted to account for losses of carbon uptake due to grazer-based $^{14}CO_2$ respiration and grazer based $DO^{14}C$ release. It is the amount lost as excreted $DO^{14}C$ and respired $^{14}CO_2$ that is not retained upon filters. These losses are highest in oceanic areas where smaller grazers

dominate the zooplankton community and have higher weight-specific metabolic rates. The correction values were obtained from ^{14}C- based dilution experiments which determined the production rates at ambient levels of grazing and at levels which were 'diluted' to mimic the absence of grazing pressure (Welschmeyer, 1999). The derived correction factors increase production values by 1.14 at station one, 1.66 at stations two and three, and by 1.95 for the remainder of the stations. Correction factors provided by Welschmeyer (pers. comm.).

RESULTS

Thirteen experiments were conducted to assess carbon to chlorophyll ratios in natural phytoplankton assemblages. Filter fluorescence of bulk chlorophyll a, nitrate, particulate organic matter (POM) and hydrographic conditions were also measured. Stations 1 through 5 were separated by approximately 110 kilometers, while the remaining stations were separated from 330 to 550 kilometers (Figure 2). Station separation depended upon distance traveled between water collection times, nominally at 0300 each day. Station 9's incubation may have become contaminated as growth rate was very low, chlorophyll levels were reduced by half from beginning to end of incubation and carbon fixation was low. It was excluded from results and discussion.

Hydrography

Mixed layer depth was similar at Stations 1 and 2, averaging 10m (SD=0.71) (Table 2). Mixed layers at stations 3 through 13 ranged from 22 to 54m, with an average of 37m (SD=11.5). Station 6 had the deepest mixed layer

depth of 58m. Changes in sigma theta confirmed these results. PAR through the upper depths of the water column is presented in Table 2 as a percent of the surface value averaged through the mixed layer. The depth of the 1% light level generally increased as the cruise progressed toward Hawaii (Figure 4). The average percent of surface irradiance (I_o) measured in the mixed layer ranged from 25 to 61 percent, with the lowest values at stations 2 through 5 (25 – 38.8%). Deck-board incubations were exposed to 25% of ambient irradiance. Mean incubator temperature and surface CTD temperature mirrored each other as both steadily increased along the transect (Figure 5). Incubator temperatures represent daylight averages for each incubation period. Daylight warming and cruise progression into warmer waters caused incubator values to be slightly higher than the CTD. Despite this, the two temperatures were always within 1.6 °C of the other (Table 2). Incubator temperatures showed a decrease from station 2 to 3, while CTD values showed a decrease from stations 3 to 4.

Nitrate

Nitrate (NO_3), as an average of the mixed layer, was low through out the transect (Table 2). Negative values can be attributed to limits of resolution inherent to the technique. The UV Method is not ideal for oligotrophic systems as values much below 0.5μM cannot be resolved with certainty. Station 1 had the highest nitrate at 1.5μM. The remaining stations up to HOTS were all below 0.5μM nitrate, except for station 10 at 1.3μM (Figure 6). Fall average surface nitrate concentrations in Monterey Bay from 1989 to 1996 were always below

5μM, with several occurrences of surface nitrate values below 1μM (Pennington and Chavez, 2000).

Particulate Organic Matter

One sample per transect station was taken to represent the mixed layer and analyzed for particulate organic carbon (POC) and particulate organic nitrogen (PON) (Table 2). POC ranged from a high of 328 μg/L at Station 1 to a low of 40μg/L at station 11. After Station 1, POC values were below 85μg/L, with the exception of Station 7 that had 110μg/L (Figure 7). PON values followed a similar trend. Highest near shore, and then falling off quickly as the cruise progressed toward Hawaii. Station 1 had a PON value of 29μg/L. Particulate carbon to particulate nitrogen ratios were highest at Station 1 (11.34) and then ranged from 4.99 to 7.17 at the remaining stations (Table 2).

Bulk Chlorophyll a

Station 1 had the highest value of near 1.50μg/L. This agrees with published data of an average surface chlorophyll concentration of approximately 1.75μg/L from 1989 to 1996 in Monterey Bay (Pennington and Chavez, 2000). The next two stations dropped to about 0.27μg/L, and the remaining stations were all approximately 0.10μg/L (Figure 8). Assimilation values (μgC/μgChl*d) showed no distinct trend, with values ranging from 25 to 122 for grazing adjusted production and from 13 to 62 for non grazing adjusted production (Table 4).

Primary Production

Carbon fixation (μgC/L*day) was highest at station 1 with a value of 22.99 (Table 4). Stations 2 and 3 were approximately 10.0 and the remaining stations were between 1.18 and 5.40 (Figure 8). The mean of the last ten stations was 2.8 (SD=1.4). Grazing-adjusted carbon fixation was higher by the values presented earlier.

HPLC Pigment Analysis

All samples were assessed for initial and final concentrations of mono-vinyl chlorophyll a, di-vinyl chlorophyll a, zeaxanthin, peridinin and fucoxanthin through pigment analysis via HPLC. Initial values are seen in Table 3. Station 1 had 0.60μg/L of mono-vinyl chl a, Stations 2 and 3 had about 0.175μg/L and the remaining mixed layer samples held about 0.020μg/L. All calculations of chlorophyll did not include any contribution of area due to the chlorophyllide peak just before the chlorophyll peak. Di-vinyl chl a was not detected until Station 3 (Figure 9). Its highest concentration, 0.077μg/l, was measured at station 4, all other stations that contained di-vinyl chl a had concentrations of approximately 0.03μg/L. Summed mono- and di-vinyl chlorophyll a concentrations were slightly lower than those estimated from bulk filter fluorescence techniques. Trends along the transect were similar between HPLC and bulk filter fluorescence estimates of total chlorophyll.

Zeaxanthin concentrations ranged from 0.029 to 0.060μg/L and showed no distinct trend along the transect. However, zeaxanthin to total chlorophyll a did show a pattern (Figures 10). This ratio was low at the first three stations

(0.048 to 0.142). From Station 3 to 4 the ratio increased and thereafter ranged from 0.352 to 0.596 through the rest of the stations, with a peak at Station 11. Fucoxanthin concentrations decreased from a high at station 1 of 0.097μg/L to 0.015 at Station 2 (Figure 11). There was another decrease from 0.019 to 0.005μg/L at the transition from Station 3 to 4. Fucoxanthin concentration held steady at this low value for the remainder of the stations. Fucoxanthin to total chl a was highest (0.102) at Station 1 and then ranged from 0.039 to 0.702 at Stations 2 to 13 with no distinct trend. Peridinin concentration was also highest at Station 1 (0.039 μg/L) (Figure 12). The remainder of the stations were constant in the range of 0.001 to 0.005μg/L. Peridinin to total chl a ranged between 0.01 and 0.06. The highest value (0.059) was encountered at Station 6.

Pigment Labeling

Thirteen pigment labeling experiments were conducted along the transect, each producing an estimate of carbon to chlorophyll and of phytoplankton growth rate (Table 3). Grazing adjusted carbon to chlorophyll values ranged from 15.06 to 163.26. Lowest values were measured at either end of the transect, with the highest values at Stations 2 through 5 (Figure 13). Ratios gradually declined from a high at station 2 until the end of the transect. Carbon to chlorophyll ratios never exceeded POC to chlorophyll ratios (Figure 14). Estimates of total algal carbon from pigment labeling (Table 3) were highest at stations 1 through 3 for both grazing adjusted and non-grazing adjusted estimates of phytoplankton carbon. The remaining stations had a mean algal carbon concentration of 6.13

μg/L (std dev = 4.20) and of 11.94 ug/L (std dev = 8.19) for the grazing adjusted samples. Total carbon, adjusted for grazing, at stations 2 and 3 consisted of 80 and 77 percent algal carbon respectively (Figure 7). No other station had more than 39 percent algal carbon while the mean of the others was 18.5% (SD=9.77). Growth rates ranged from 0.15 to 0.99 per day. Growth rate was 0.43 per day at Station 1, dipped to the lowest values at Stations 2 through 5, and generally increased throughout the remainder of the transect (Figure 15).

During this same cruise, Culley (2000) performed estimates of bacteria-like particles (blp) and virus-like particles (vlp) at all stations using epifluorescent techniques. Concentrations of bacteria and viruses are highest in Monterey Bay, drop to lower values at Stations 2 and 3, and then to a lowest, steady value from Station 4 to Hawaii. Bulk chlorophyll to BLP ratios show a similar trend, with a slight increase from Station 2 to 3. These data are presented to elucidate part of the heterotrophic community (Figure 16).

The empirically derived results of this experiment can be fit to the model of Cloern et. al (1995). The model was used to calculate a value of nutrient limited growth rate from the experimental values of C:Chl and the measured values of temperature and irradiance. These results are presented in Figure 17.

DISCUSSION

The observed ratios of carbon to chlorophyll (60 to 145) fall within the range seen in the literature for chemostat and batch culture experiments under various nutrient, light and temperature regimes for a variety of phytoplankton taxa

(Cloern et. al, 1995, Laws et. al, 1983). Production values, chlorophyll a concentrations and the degree of labeling (L) are all within accepted ranges and indicate the technique is valid. When primary production rates were corrected for grazing, C:Chl ratios increased (Table 3), reflecting the direct effects of carbon uptake upon the calculation of C:Chl. A C:Chl ratio of 58.64 at Station 1 falls within previous values for coastal environments as observed by Welschmeyer and Lorenzen (1984) in Dabob Bay, Washington (7 – 77) and by Redalje (1983) off the Southern California Bight and the Coast of Baja California (12 – 34) in twenty-four hour chlorophyll labeling experiments. Carbon to chlorophyll values were lowest at the most near shore station, increased to highest values at Stations 2 through 5 and gradually decreased to values similar to the first station.

Station Aloha, of the HOTS program, has a long-term data set of prochlorococcus cell counts via flow cytometery (Campbell and Vaulot, 1993), and of both monovinyl- and divinyl-chlorophyll a (HOTs Website, 2000). Taking this data, along with carbon per cell ratios of Calliau et al. (1996) of 49fgC/cell and of Campbell et al. (1994) of 53fgC/cell, allows calculation of C:Chl for prochlorococcus at the HOTS location. These values ranged from 15 to 381 in the upper 100m during the fall of 1998 (Figure 18) and show the wide variety in open-ocean C:Chl and how quickly the ratio can change. The present results fall within this range.

The previously cited studies suggested that elevated C:Chl represents a stressed phytoplankton population largely due to low nutrient availability and/or

elevated light levels. Therefore, Stations 2 through 5 and 7 may have contained stressed populations or high light-adapted algae. The latter condition seems inconsistent with the estimates of exponentially weighted mean percent I_o in the mixed layer at these same stations. Reductions in available nutrients and increases in light penetration from the near shore to the off shore were expected to raise C:Chl (Figure 1). In contrast, increasing temperature farther off shore was expected to decrease C:Chl (Figure 1). This trend due to temperature is observed in the offshore stations. The model of Cloern et. al (1995) predicts a drop in C:Chl of 1.2 times over the observed increase in temperature. The actual decrease in C:Chl is approximately 2 fold, indicating that temperature is not a major influencing factor. Despite these predictions from laboratory cultures, no single measured parameter could fully explain the observed trend in the field. The data might indicate that it is an interaction of physical, biological and chemical influences that govern phytoplankton biochemical composition. Different forcing factors, working on different community compositions, may change the dominant parameter influencing carbon to chlorophyll from one body of water to another. The most influential factor or combination of factors, could not be determined in the present study. Despite this, the observed trend in carbon to chlorophyll can be viewed in the context of species composition, nutrient state and hydrographic control of mixed layer depth, light penetration and temperature.

Species composition has an effect on C:Chl in laboratory cultures and field studies. Chan (1980) showed that diatoms had lower C:Chl ratios (32-35) than dinoflagellates (92-120) in nutrient replete cultures. Diatoms are thought to be the dominant plankter in temperate and high latitude waters (Lalli and Parson, 1997). Fucoxanthin, while also present in prymnesiophytes and some dinoflagellates, is the major diagnostic pigment for diatoms (Jeffrey, et al. 1997). The fucoxanthin/chl a was highest at Station 1 (Figure 11). This indicates that diatoms, with a low predicted C:Chl, are at their largest abundance at Station 1. Peridinin, the major diagnostic pigment of dinoflagellates, was also highest at Station 1, buts its ratio against total chl a was rather steady throughout the transect (Figure 12). As this diagnostic pigment decreased in concentration, zeaxanthin to total chl a increased after Station 3 (Figure 10). Zeaxanthin is indicative of prokaryotes, specifically prochlorococcus and cyanoabacteria in the phytoplankton. Di-vinyl chlorophyll a, present only in prochlorococcus, did not become detectable until Station 3. From Station 4 westward, di-vinyl chl a constituted approximately half of the total chl a concentration (Figure 9). Stations 2 through 4 are an area of changing phytoplankton community composition and also represented some of the highest C:Chl values encountered. Diatom dominance decreased as smaller prochlorophytes become an important part of the ecosystem. Perhaps this transition zone of fluctuating water masses of different physical and chemical properties made it difficult for either the coastal or oceanic phytoplankton end-member populations to establish dominance such

that they experience a continual state of stress. This may contribute to elevated C:Chl levels.

After Station 5, the relative abundances of di-vinyl and mono-vinyl chl a, fucoxanthin and zeaxanthin became stable. C:Chl ratios range from 15 to 91. This area is most likely dominated by the smaller prochlorococcus that have been observed with C:Chl levels of 25 to 38 in laboratory cultures using the pigment labeling technique (Calliau et al., 1996). Throughout the HOTs ten-year data set, it was often observed that C:Chl ratios could change by a factor of two in one month (Figure 18). Carbon to chlorophyll values are highly variable and can rapidly change. Campbell et al. (1994) estimate that prochlorococcus make up about 41% of the phytoplankton carbon at HOTS. She also estimates a phytoplankton C:Chl mean of 128 (SE=9.9) for the surface mixed layer. Clearly, the results presented here fall within the range observed by the HOTs group.

Total particulate carbon to bulk filter fluorescence estimates of chlorophyll a can be viewed as the upper limit of C:Chl (Figure 14). This limit will rarely be reached as only during times of extreme blooms could algal carbon ever reach high enough levels as to make all other carbon sources insignificant. Estimates of algal carbon were predictably high near shore and decreased after Station 3. Of more interest was the percent of total POC that is algal (Figure 7). The high algal carbon percentages at Stations 2 and 3 indicate an ecosystem dominated by phytoplankton. Conversely, heterotrophic biomass must be a small portion of total carbon at these two stations. Counts of virus-like particles and bacteria-like

particles were highest near shore, lower at Stations 2-3, and decreased to a consistently lower value at the remaining stations (Figure 16) (Culley, 2000). Bacteria counts dip below the trend at station two, while viral counts did not. Total chl a to BLP (Figure 16) did show higher values at Stations 2, 3 and 4 than is seen at the following open-ocean stations. Despite this variability in the trends, the viral and bacterial components of the heterotrophic biomass did not decline sufficiently to account for the high percentage of algal carbon. Estimates of microzooplankton abundance were not available. While algal carbon dominated the POC, this does not necessarily indicate a flourishing phytoplankton community at Stations 2 – 5. Chlorophyll a concentrations were approximately 1.5 times higher than the open ocean stations, while growth rates (μ=0.19 – 0.33) were some of the lowest encountered and production values were on par with the open ocean stations. This may be indicative of a body of water full of dying phytoplankton at the end of a bloom.

Light intensity can influence C:Chl; generally, as light intensity increases, C:Chl also increases (Calliau et al, 1996, Goericke and Welschmeyer, 1993, Geider and Osborne, 1986). Phytoplankton adjust to higher light intensities by cessation of chlorophyll production while continuing to divide (Falkowski, 1984). At all stations the depth of the 1% light level was well below the bottom of the mixed layer. Therefore, phytoplankton were never mixed below the euphotic zone. But light limitation was occurring at any light level below saturation and there were differences in the average intensity of light seen in the mixed layer.

These differences may provide some insight into the observed C:Chl. The highest C:Chl values were seen at Stations 2 through 5. It is these same stations that had lower levels of average light intensity (avg=32.54%) in the mixed layer (Figure 4) than the open ocean stations 6 through 13 (avg=55.63%). While lab cultures indicate light availability and C:Chl parallel each other, this field study indicates the contrary. Lab cultures are generally given the time to photoacclimate and adjust their chlorophyll levels. In-situ conditions are most likely much more dynamic. The phytoplankton sampled at stations 2 through 5 may have been acclimated to a previously higher light level in the mixed layer. Phytoplankton may not have had enough time to adjust to the current lower level of light to achieve optimal photosynthesis. This light stress may contribute to higher C:Chl. After Station 6, average light intensity in the mixed layer increased and remained steady. This higher light and steady state condition of the open ocean may allow phytoplankton populations not only sufficient light for photosynthesis, but enough time to acclimate to these conditions.

Culture work indicates that nutrient stress should cause a rise in C:Chl. This may be the case in the present study as well. After the first station, carbon uptake and total chlorophyll both rapidly declined, community composition begins to shift to smaller prokaryotic algal and nitrate became close enough to zero to make detection dubious. Station 1 may represent the only coastal, eutrophic environment. Its available nitrate may be just enough to keep its phytoplankton community growing at a steady state. Low levels of nitrate in the next few

stations may not have been sufficient to support the phytoplankton community that is similar to Station 1 (Figure 6). Trace metals, such as Fe, are known to be limiting to phytoplankton. If iron limitation was occurring, most likely it was happening at these stations farther off shore and more removed from terrigenous sources. These plankton may have been nutrient stressed, which may explain the elevated C:Chl. Once into the oceanic stations, low levels of nitrate may have been sufficient to allow the resident phytoplankton population to thrive. Liu et al. (1997) found prochlorococcus growth rates of 0.64 to 0.78 per day in the equatorial and subtropical North Pacific Ocean despite the fact that surface nitrate concentrations were often at or near zero.

Trace nutrients were not measured, but could be playing a key role in the elevated C:Chl ratios seen in the transition stations. Iron, because of its role in the molecular structure of chlorophyll, deserves special mention. Diatoms still constitute a significant part or the phytoplankton community at Stations 2 and 3 as indicated by the ratio of fucoxanthin to chlorophyll a. Iron limitation increases with distance from shore and may be limiting to the larger diatoms. Insufficient iron could limit chlorophyll production and contribute to elevated C:Chl levels in the transition stations. Open-ocean end-member community phytoplankton may be acclimated to low levels of iron and other trace nutrients.

Fitting the measured values of C:Chl, temperature and irradiance to the model of Cloern, et al. (1995), produces an estimate of nutrient limited growth rate (Figure 17). This estimate is similar to growth rates estimated from pigment

labeling technique (Figure 15). Lowest μ are observed at Stations 2-5 and are coincident with the highest values of C:Chl. In the model, low μ can be attributed to a condition of stress caused by nutrient limitation. Therefore, measured values of C:Chl may be indicative of nutrient stress in the transition zone. While measures of nitrate do not explain the observed trend, perhaps some other nutrient may be the cause of the elevated C:Chl in the transition zone.

CONCLUSIONS

The observed trend of C:Chl found in this study show highest values coincident with the major transition in phytoplankton community structure as evidenced by chemotaxonomic pigment analysis. Community composition, available light, temperature and available nutrients are all intertwined to compose the environment that the phytoplankton community experiences. Each alone, or several acting together, ultimately govern the observed carbon to chlorophyll ratios. Replete nutrients, high light and a diatom dominated community may control Station 1 C:Chl. A prochlorococcus dominated community at a steady light and nutrient state, may leave temperature to influence oceanic C:Chl. It is the transition zone from coastal to open ocean that has the highest C:Chl and this may be due to several stressors. Lower average light in the mixed layer, depressed nitrate levels and a mixing of relatively stable coastal and oceanic populations of phytoplankton may cause an overall stressed population and lead to elevated C:Chl. Clearly, despite careful efforts at constraining C:Chl estimates as tightly as possible, much noise exists within the data and within the

interpretation. The ratios fall within published values and are below the ratio of total particulate organic carbon to total chlorophyll a estimates. Therefore, in-situ phytoplankton carbon to chlorophyll ratios may be highest in regions of highly stressed populations and not simply highest in open ocean, oligotrophic regions.

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C:Chl	Reference:	Location / Sample
240 - 270	Redalje and Laws, 1981	Kaneohe Bay, Hawaii
7 - 77	Welschmeyer and Lorenzen, 1981	Dabob Bay, Washington
10 - 91	Bidigare et. al, 1990	Sargasso Sea
87 - 195	Ditullio and Smith, 1996	Ross Sea, Antarctica
65 - 240	Goericke and Welschmeyer, 1998	Sargasso Sea
12 - 33	Cailliau et. al, 1996	*Prochlorococcus sp.* Cultures

Table 1 - Carbon to Chlorophyll Ratios from Pigment Labeling

Station (#)	Sampling Depth (m)	Mixed Layer (m)	PAR Mixed Layer Avg (%)	1% Light Depth (m)	CTD Temp (°C)	Incubator Temp (°C)	NO_3 (µM)	POC (µg/L)	PN (µg/L)	POC/PN
CTD 1	5,8	10	49.21	28.14	16.80	17.77	1.50	328.17	28.93	11.34
CTD 2	10	9	25.77	49.79	17.70	18.80	0.18	77.57	12.98	5.98
CTD 3	15	41	36.69	66.26	17.50	18.90	NA	83.37	15.13	5.51
CTD 4	30	31	38.84	71.40	18.10	18.76	0.20	71.83	10.02	7.17
CTD 5	26	36	28.87	79.95	19.30	20.88	0.29	73.36	10.93	6.71
CTD 6	26	58	60.98	93.22	21.20	22.05	0.48	53.01	8.30	6.39
CTD 7	0	22	61.66	108.61	22.10	22.40	0.51	109.83	16.98	6.47
CTD 8	1,5,10,15	25	60.21	111.24	22.40	24.00	0.34	57.37	11.35	5.05
CTD 10	17	33	60.95	126.17	25.00	25.48	1.32	52.03	7.01	7.42
CTD 11	19	37	50.02	127.21	25.60	26.13	-0.15	40.40	7.82	5.16
CTD 12	17	44	49.61	104.90	26.50	26.72	0.10	49.86	6.71	7.43
CTD 13	10	54	46.00	106.60	26.40	27.05	0.66	50.08	9.99	5.01

Table 2 - Hydrographic and Chemical Data Summary
CTD 7 sampled from a bucket cast. Mixed layer represents the bottom of the mixed layer. PAR is the exponentially weighted mean of incident irradiance in the mixed layer. 1% light depth is the depth at which incident irradiance is attenuated to 1% of the surface value. CTD temperature was taken from the depth of sampling. If more than one depth was sampled, temperature was averaged. Incubator temperature is an average over the 24 hour incubation period.

Station (#)	Kratos Bulk Chl a (μg/L)	Mono-vinly Chl a (μg/L)	Di-vinyl Chl a (μg/L)	Zeaxanthin (μg/L)	Fucoxanthin (μg/L)	Peridinin (μg/L)
CTD 1	1.500	0.605	0.000	0.046	0.097	0.039
CTD 2	0.247	0.156	0.000	0.033	0.015	0.004
CTD 3	0.450	0.206	0.029	0.037	0.019	0.003
CTD 4	0.107	0.047	0.077	0.059	0.005	0.004
CTD 5	0.083	0.036	0.034	0.029	0.005	0.002
CTD 6	0.080	0.025	0.027	0.034	0.004	0.002
CTD 7	0.086	0.029	0.039	0.041	0.004	0.001
CTD 8	0.096	0.028	0.034	0.044	0.005	0.005
CTD 10	0.074	0.020	0.035	0.037	0.005	0.001
CTD 11	0.078	0.023	0.033	0.052	0.004	0.001
CTD 12	0.088	0.017	0.029	0.043	0.005	0.001
CTD 13	0.063	0.015	0.023	0.037	0.004	0.001

Table 3 - Pigment Data Summary

All pigment concentrations are initial values from the beginning of each incubation. Kratos chlorophyll a measurements achieved from bulk filter fluorescence. Other pigment concentrations from HPLC peak analysis.

Station (#)	Carbon Fixation (μgC/L*d)		Assimilation Value (μgC/μgChl*d)		Carbon to Chlorophyll a (wt/wt)		Phytoplankton Carbon (μg/L)		Growth Rate	Labeling Coefficient
	Grazing Adjusted	Non Grazing Adjusted	Grazing Adjusted	Non Grazing Adjusted	Grazing Adjusted	Non Grazing Adjusted	Grazing Adjusted	Non Grazing Adjusted	(u)	(L)
CTD 1	26.21	22.99	27.56	24.18	66.85	58.64	63.57	55.76	0.43	0.32
CTD 2	18.40	11.08	80.31	48.38	271.01	163.26	62.08	37.40	0.30	0.23
CTD 3	16.29	9.81	50.86	30.64	201.63	121.46	64.57	38.90	0.28	0.21
CTD 4	4.63	2.38	34.80	17.85	212.45	108.95	28.29	14.51	0.19	0.14
CTD 5	6.40	3.28	74.34	38.12	223.91	114.83	19.27	9.88	0.33	0.25
CTD 6	6.25	3.20	75.44	38.69	106.09	54.40	8.79	4.51	0.59	0.42
CTD 7	10.68	5.48	113.05	57.97	178.84	91.71	16.89	8.66	0.54	0.39
CTD 8	4.04	2.07	42.76	21.93	119.18	61.12	11.27	5.78	0.37	0.28
CTD 10	6.86	3.52	86.05	44.13	55.12	28.26	4.39	2.25	1.01	0.62
CTD 11	2.24	1.15	25.52	13.09	112.02	57.45	9.81	5.03	0.26	0.19
CTD 12	4.31	2.21	47.42	24.32	29.37	15.06	2.67	1.37	1.03	0.63
CTD 13	8.94	4.58	122.41	62.77	83.88	43.02	6.12	3.14	0.83	0.55

Table 4 – Production and Pigment Labeling Data Summary
Grazing adjusted values represent an upward adjustment to carbon uptake measurements to account for grazer interactions. The labeling coefficient and growth rate are unaffected by grazers.

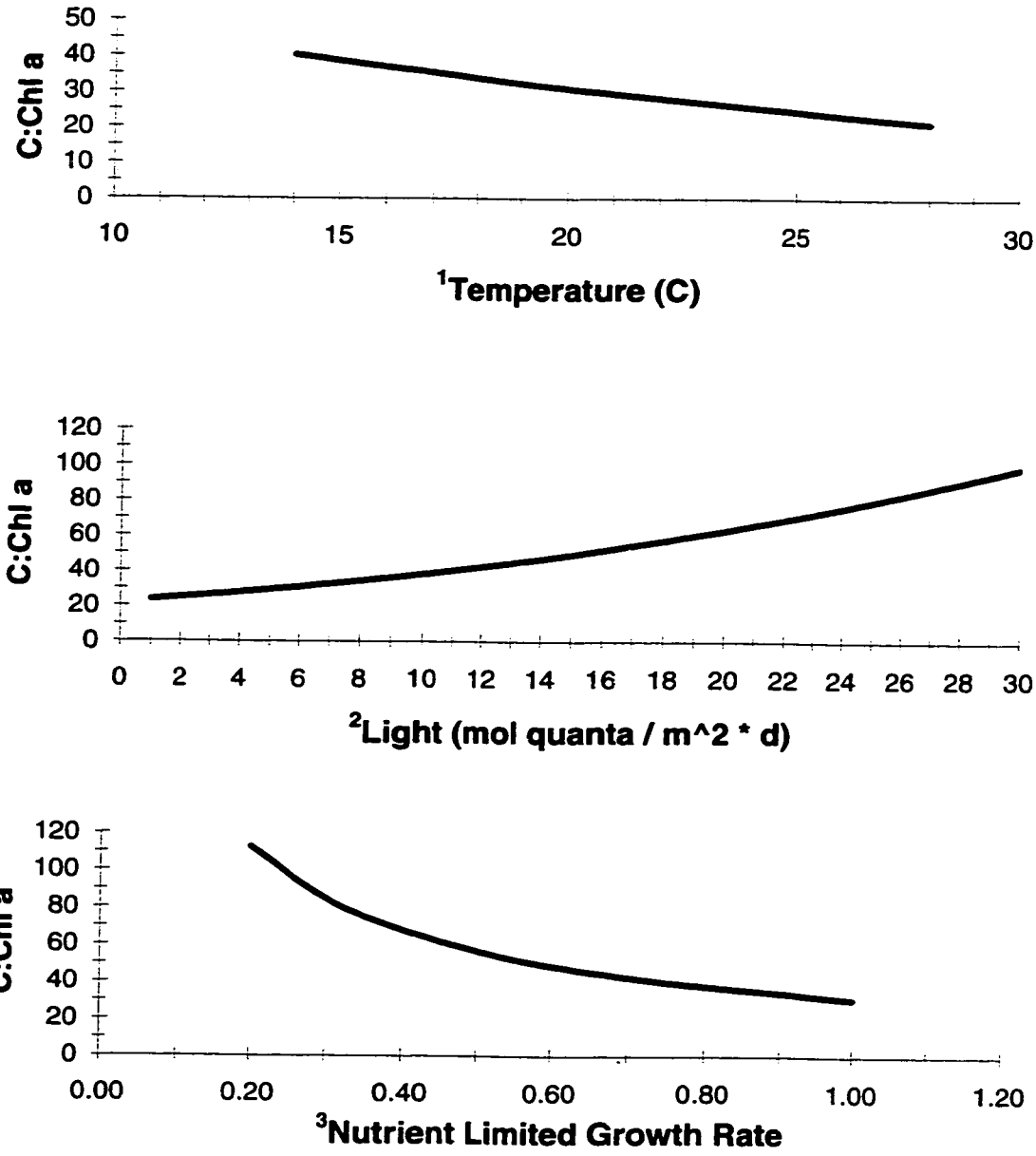


Figure 1 - Predicted Values of C:Chl vs Temperature, Light and Relative Growth Rate

From the model of Cloern, et al., 1995

Chl:C = 0.003 + 0.0154 exp(0.050 * T) exp(-0.059 * I) *μ'

[1]Temperature: I = 6 mol quanta / m^2*day, *μ' = 1/day

[2]Light: Temp = 20°C, *μ' = 1/day

[3]Relative Growth Rate: Temp = 20°C, I = 6 mol quanta / m^2*day

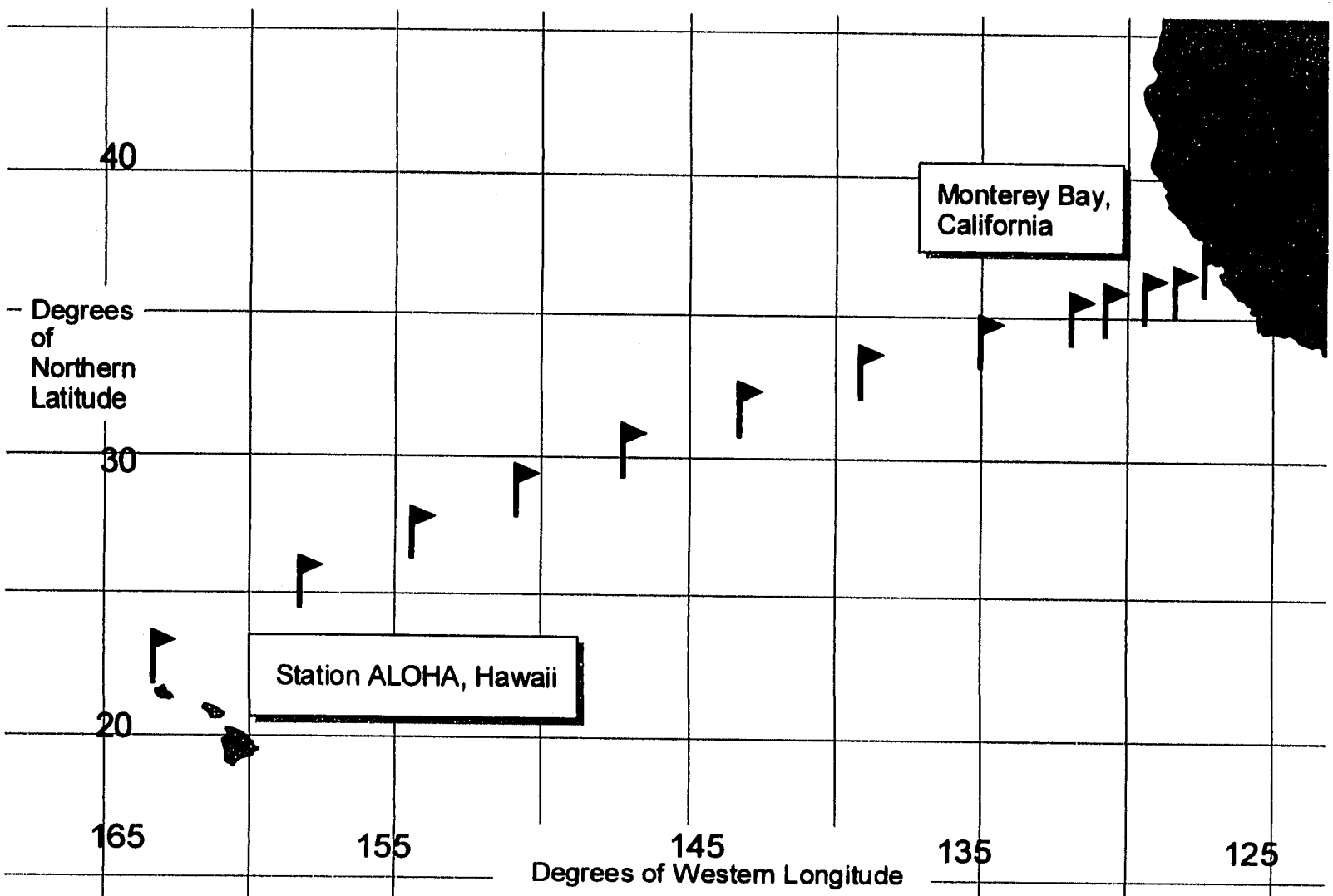


Figure 2 – GRZ 98 Cruise Track

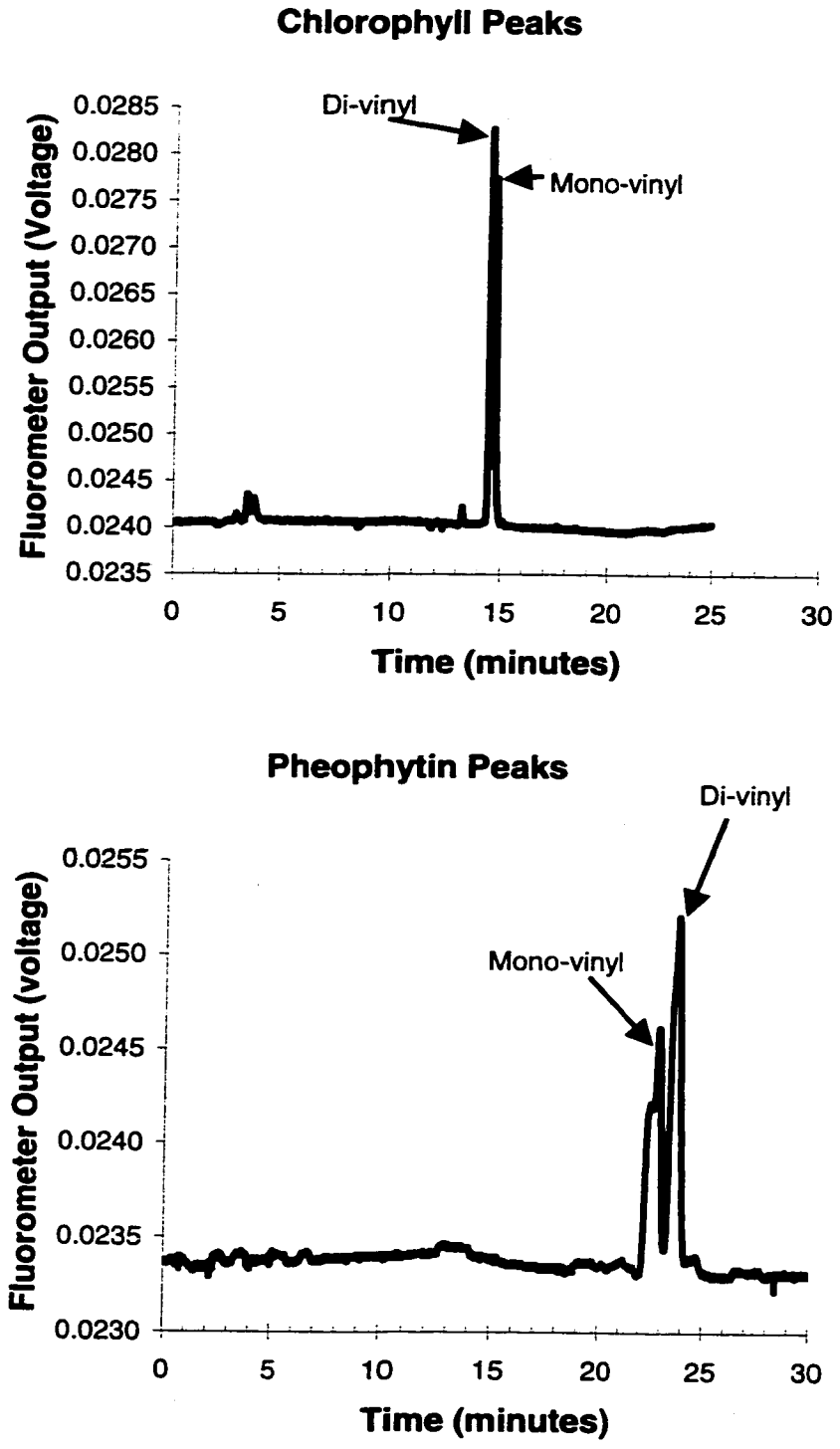


Figure 3 – HPLC Chromatograms of Chlorophyll and Pheophytin
Acidification to pheophytin increases retention time and resolution

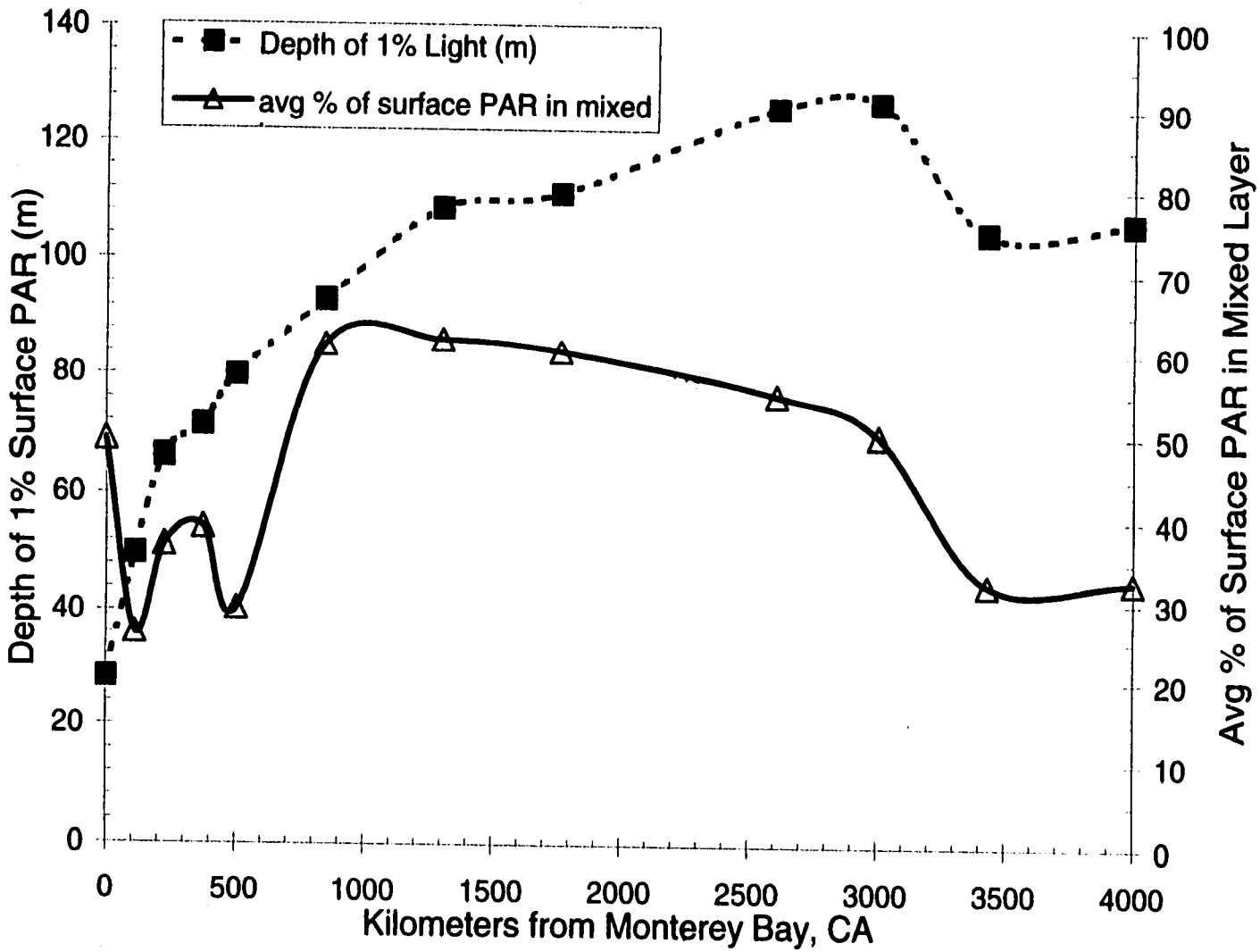


Figure 4 – Light Attenuation and Depth of Euphotic Zone
Depth of 1% surface PAR is depth at which surface irradiance is attenuated to 1%. Average percent of surface PAR is the exponentially weighted mean percent I_0 in the mixed layer.

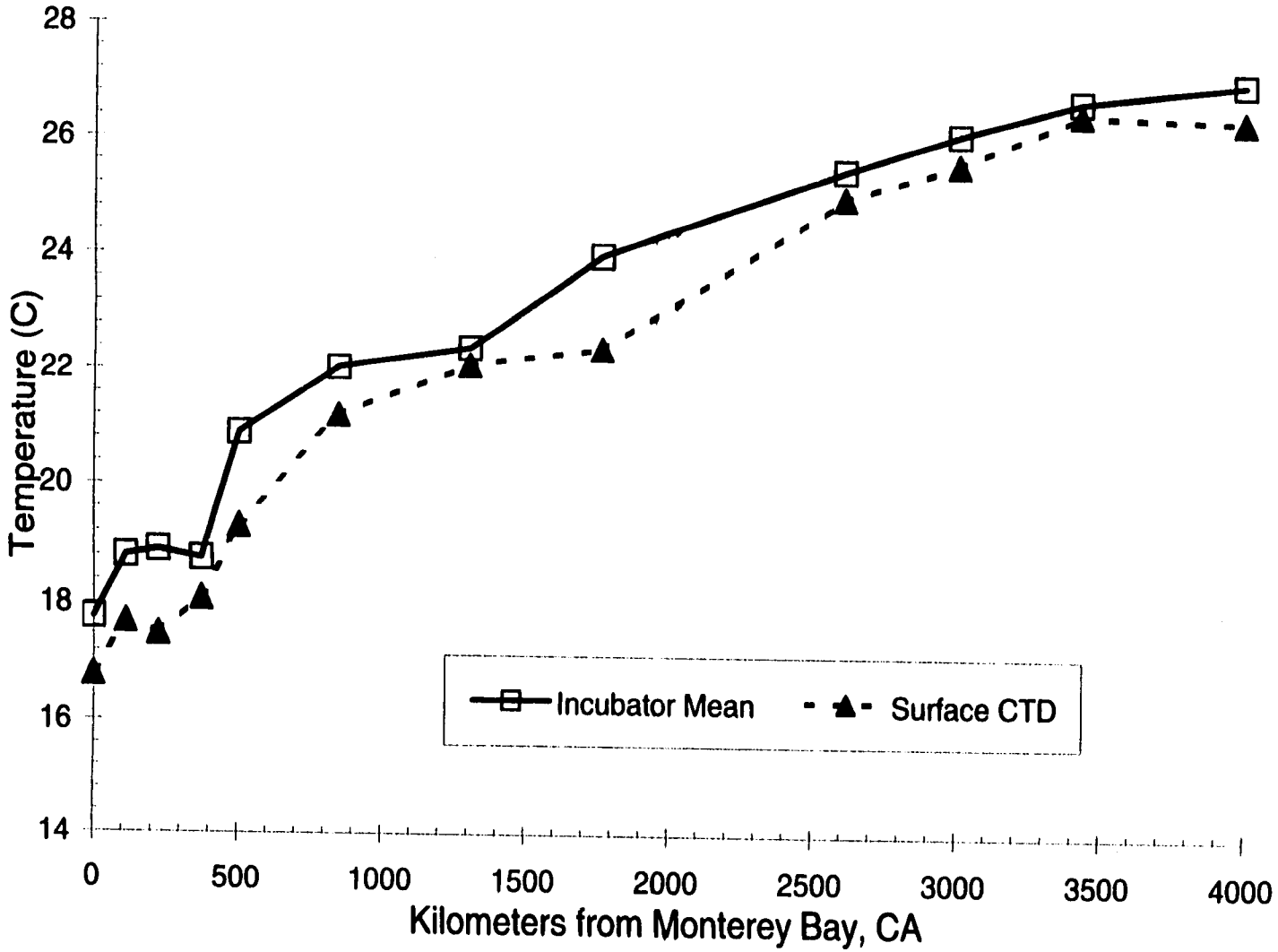
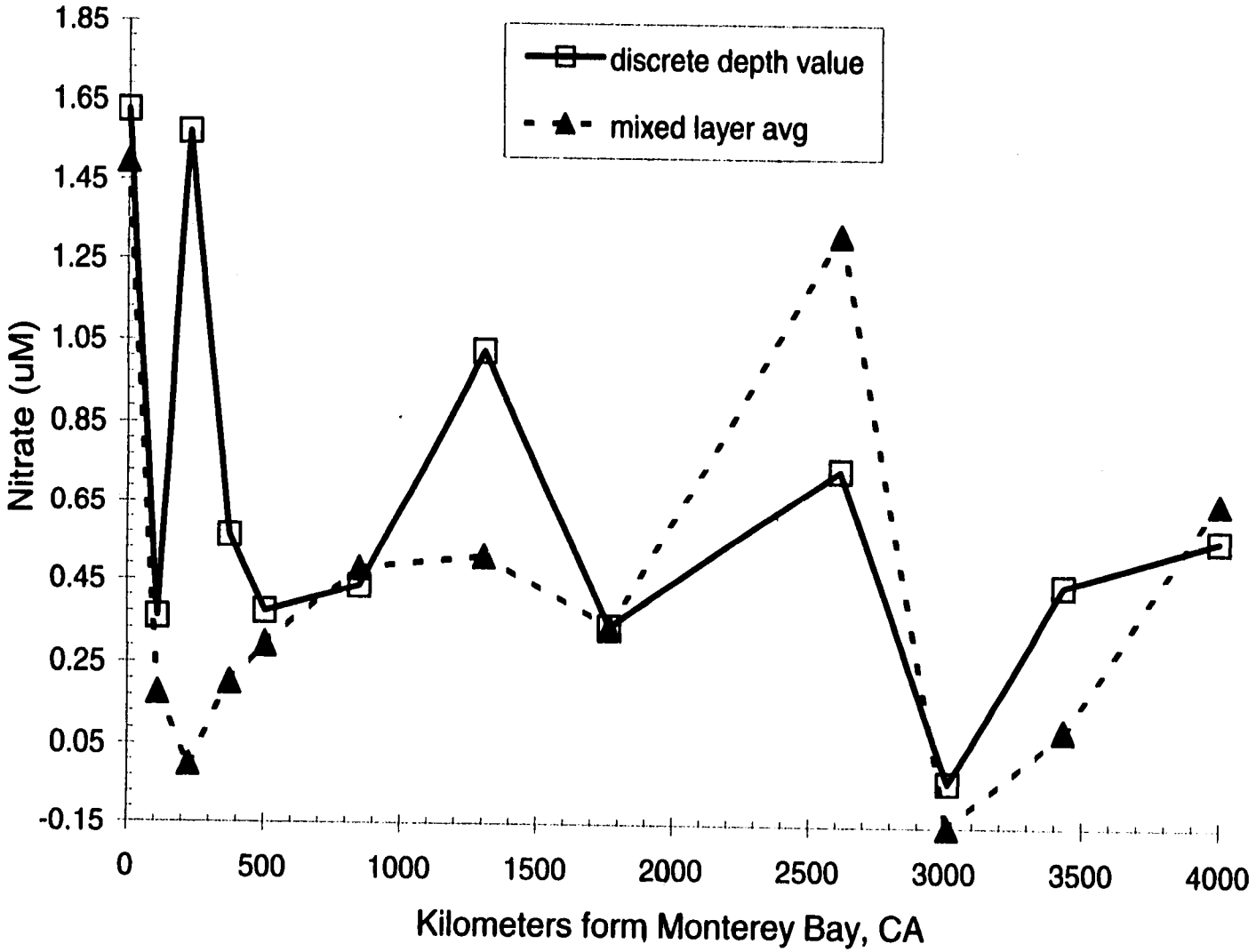


Figure 5 – Incubator and Surface Temperatures
Incubator temperature is the daily average.



.Figure 6 – Nitrate Concentrations Along Transect
Discrete depth value taken at depth of pigment labeling sample

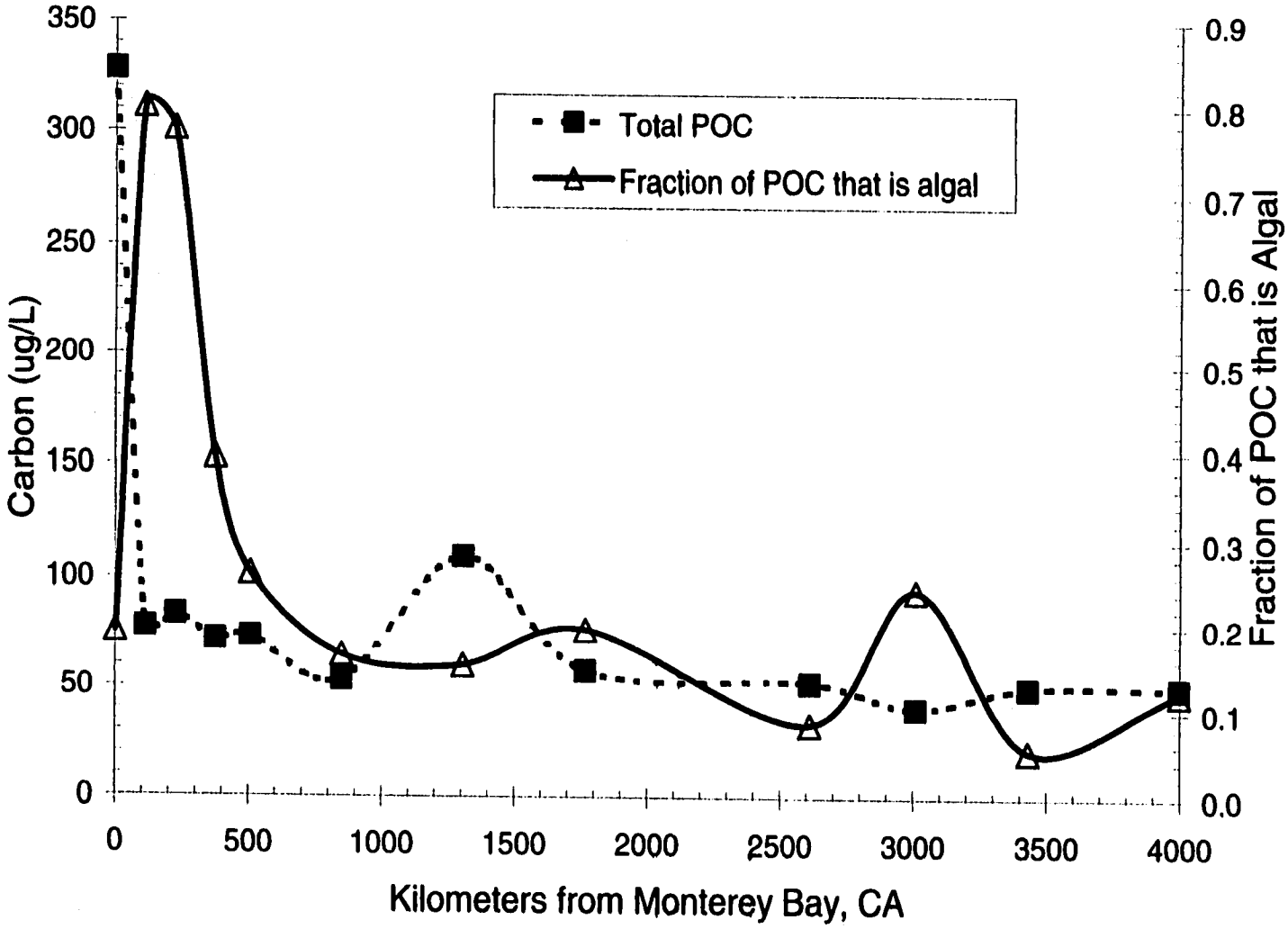


Figure 7 – Total Particulate Organic Carbon and Fraction that is Algal
Algal fraction computed as total POC divided by algal carbon

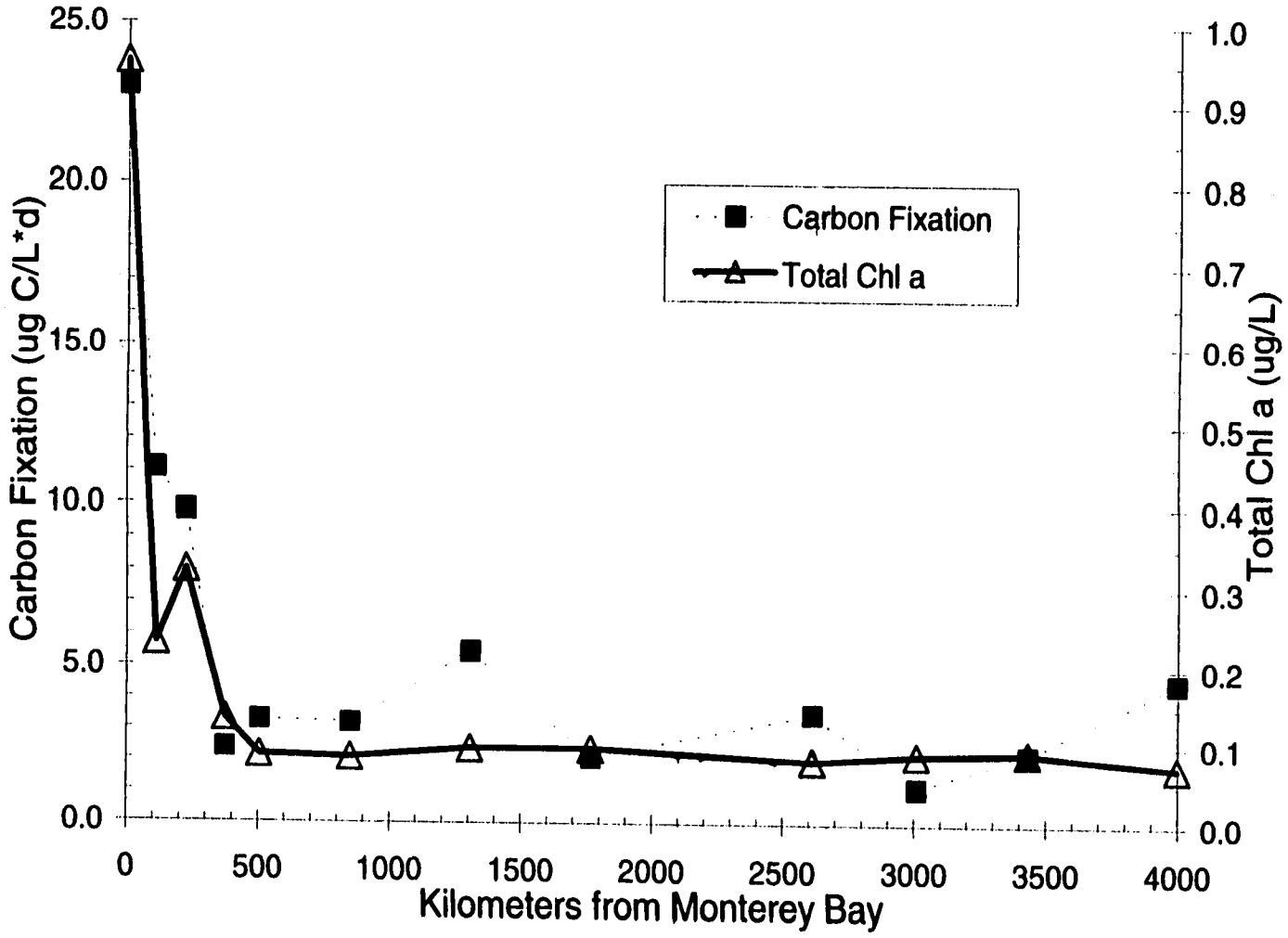


Figure 8 – Carbon Fixation Rates and Total Chlorophyll a Concentrations
Total Chl a from bulk filter fluorescence

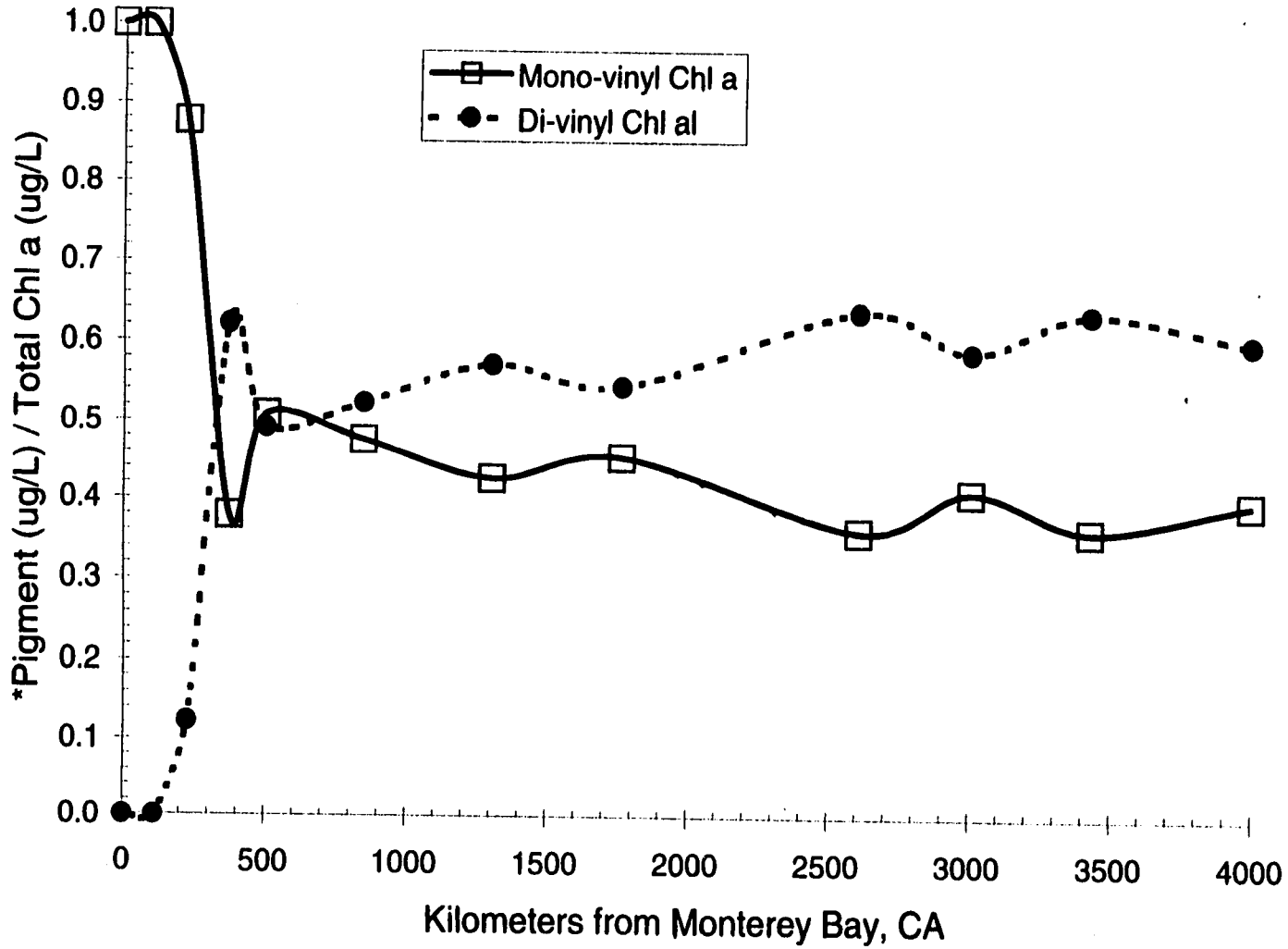


Figure 9 – Pigment Concentrations Relative to Total Chlorophyll a Concentrations
Ratio calculated as concentration to concentration

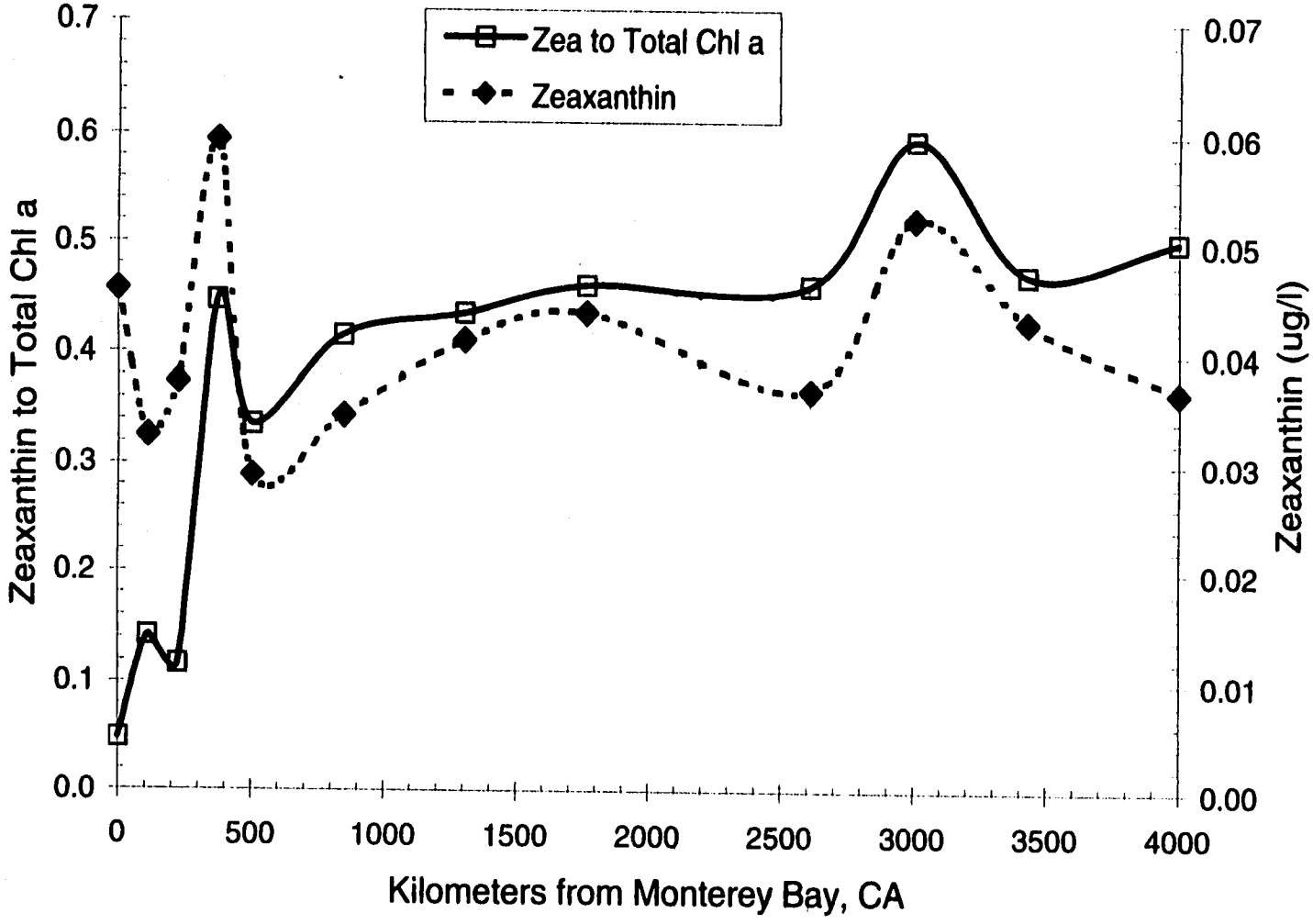


Figure 10 – Mixed Layer Zeaxanthin Concentrations Along Transect

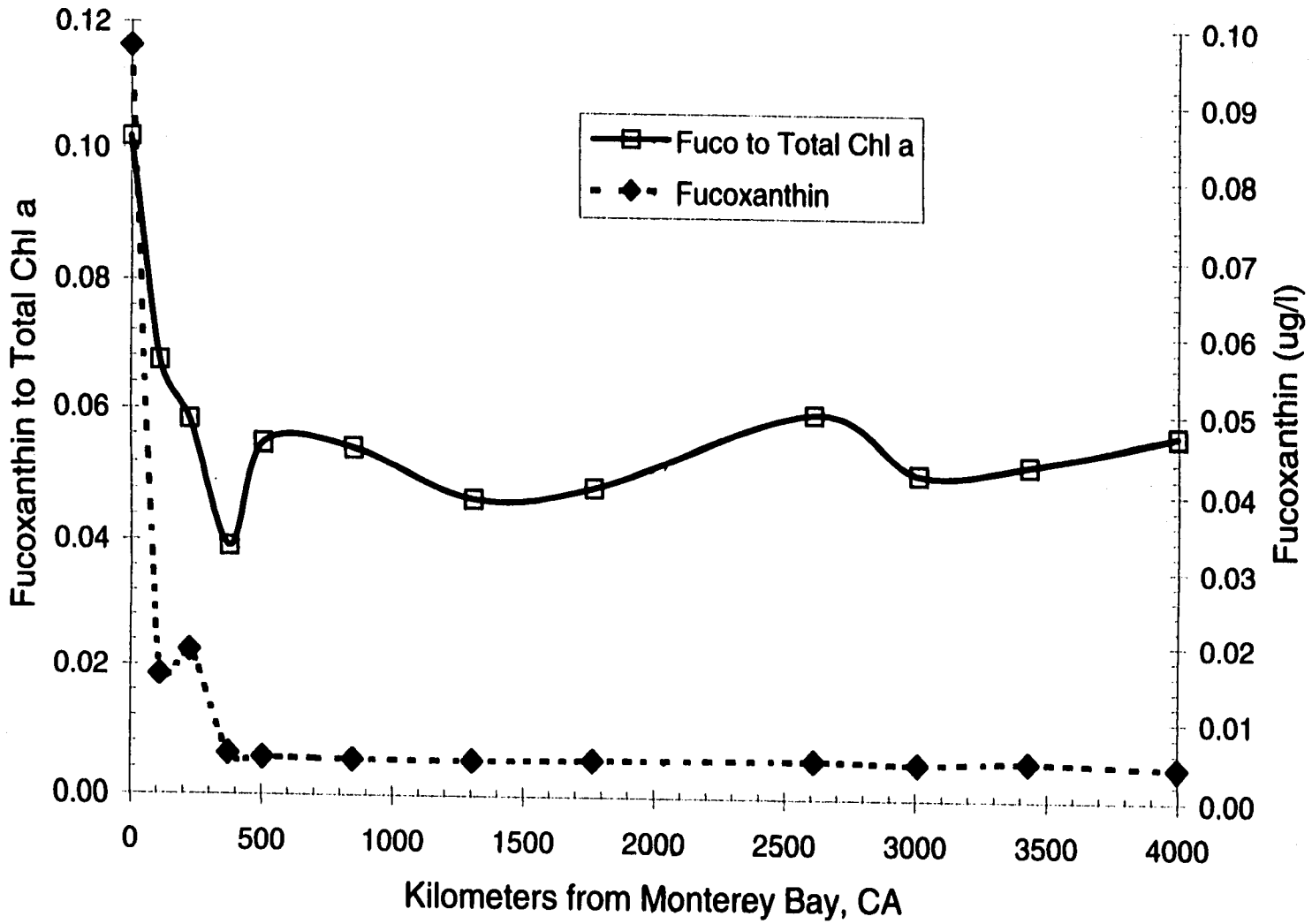


Figure 11 – Mixed Layer Fucoxanthin Concentrations Along Transect

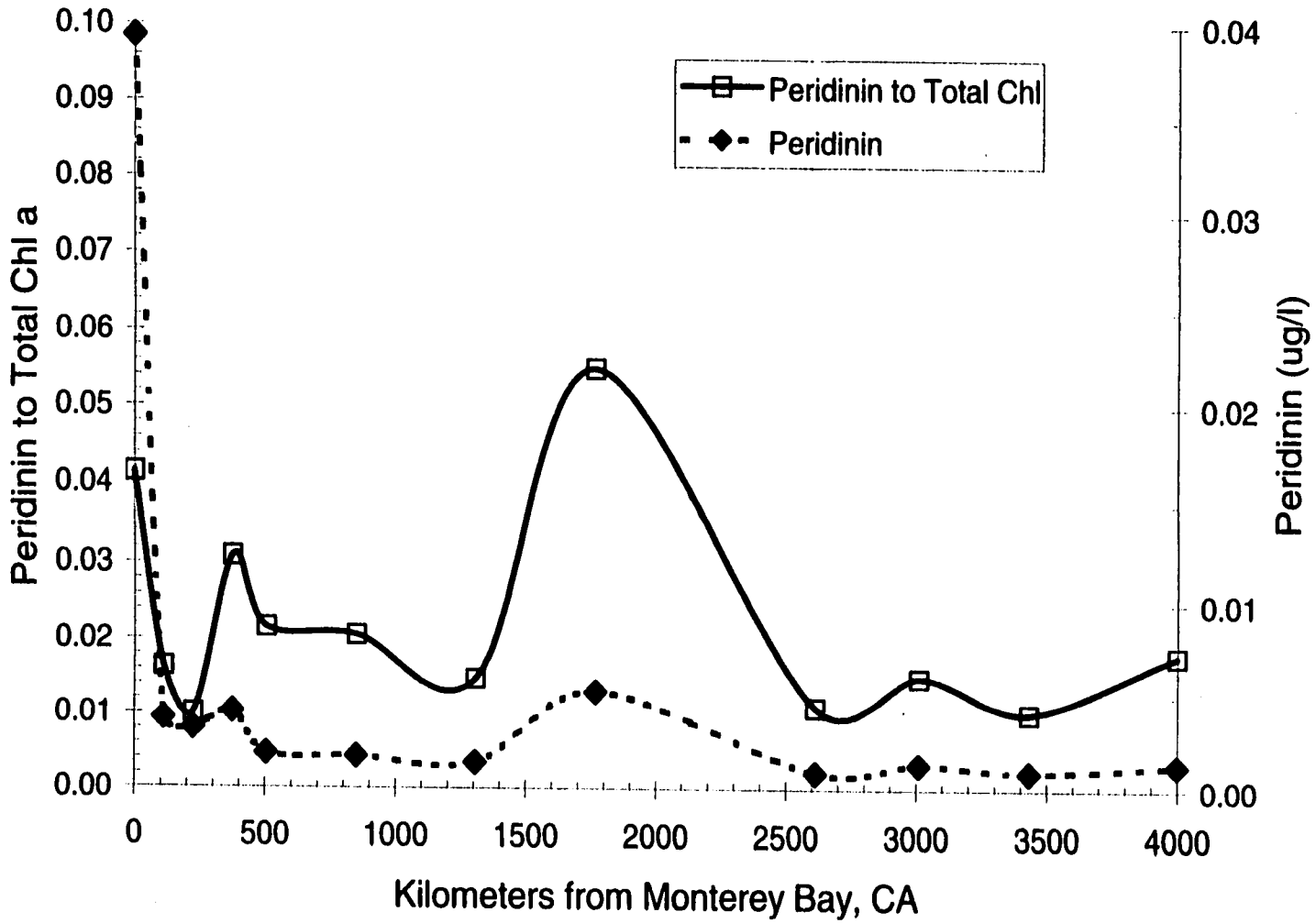


Figure 12 – Mixed Layer Peridinin Concentrations Along Transect

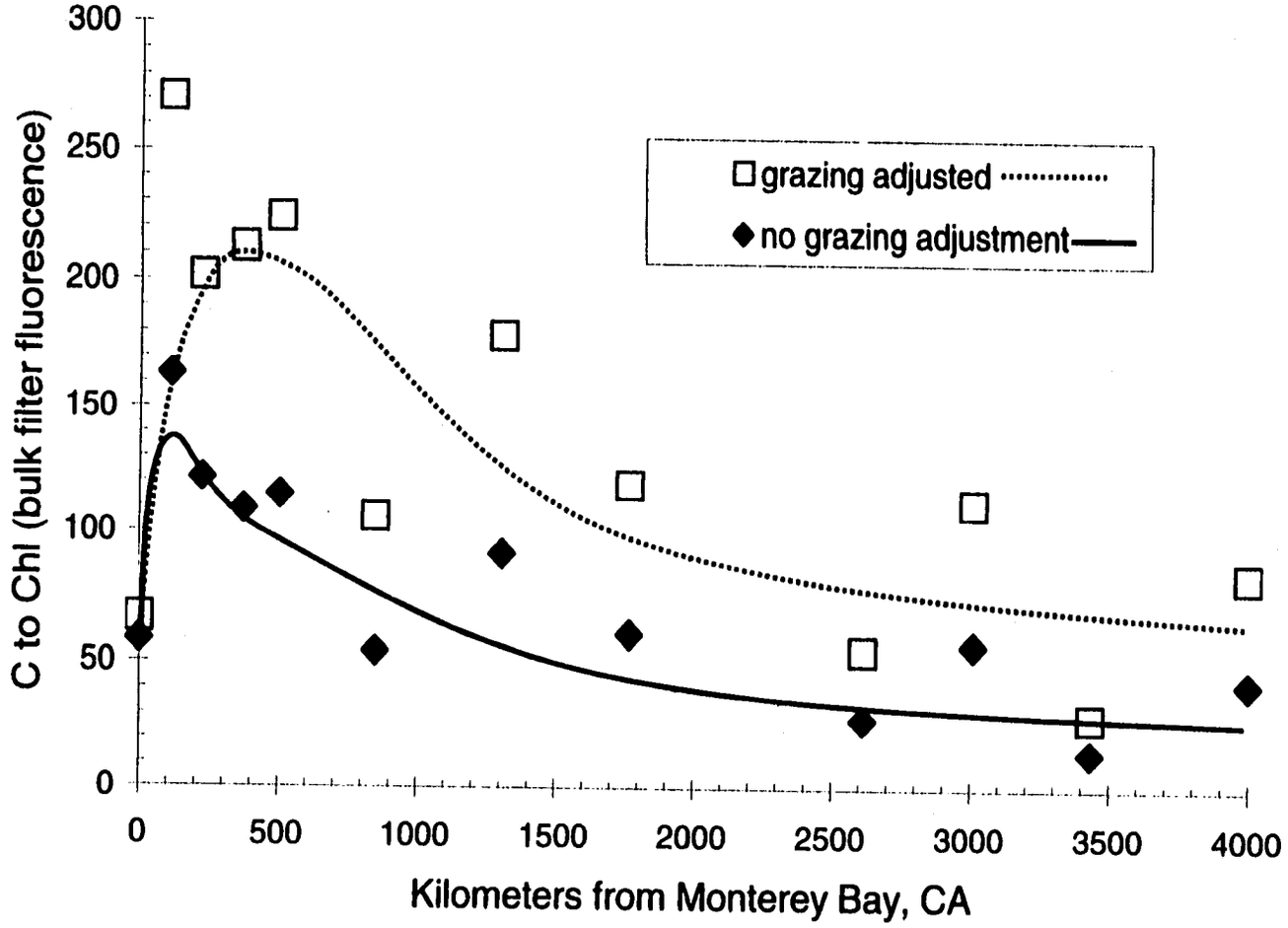


Figure 13 – In-situ Carbon to Chlorophyll a Ratios from Chlorophyll Labeling
Grazing adjusted values had carbon uptake values corrected up to account for grazer activity

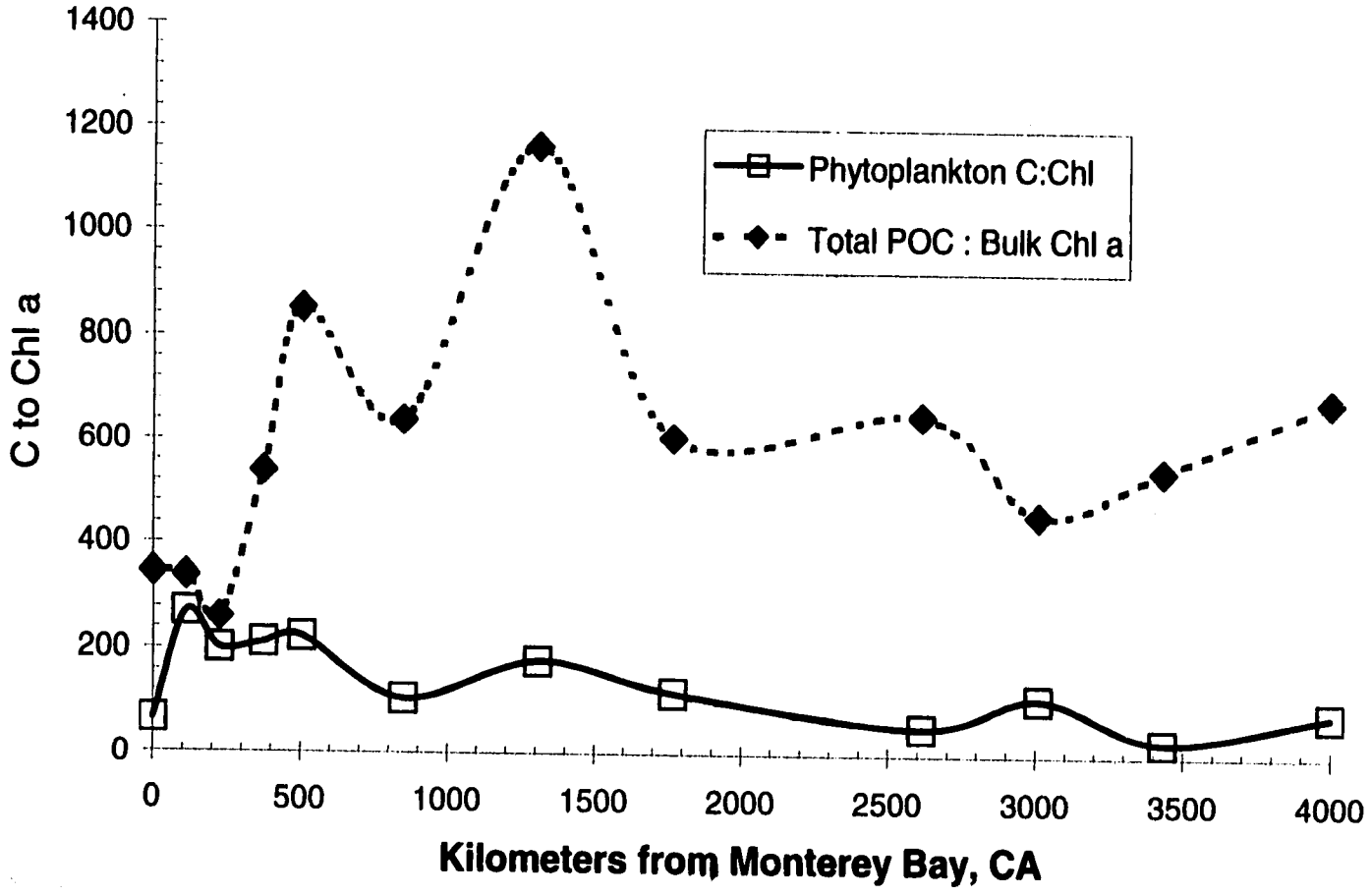


Figure 14 – Total POC:Chl and C:Chl from Pigment Labeling
Phytoplankton C:Chl values are adjusted for grazing (carbon uptake adjusted up). Chlorophyll values are from bulk filter fluorescence.

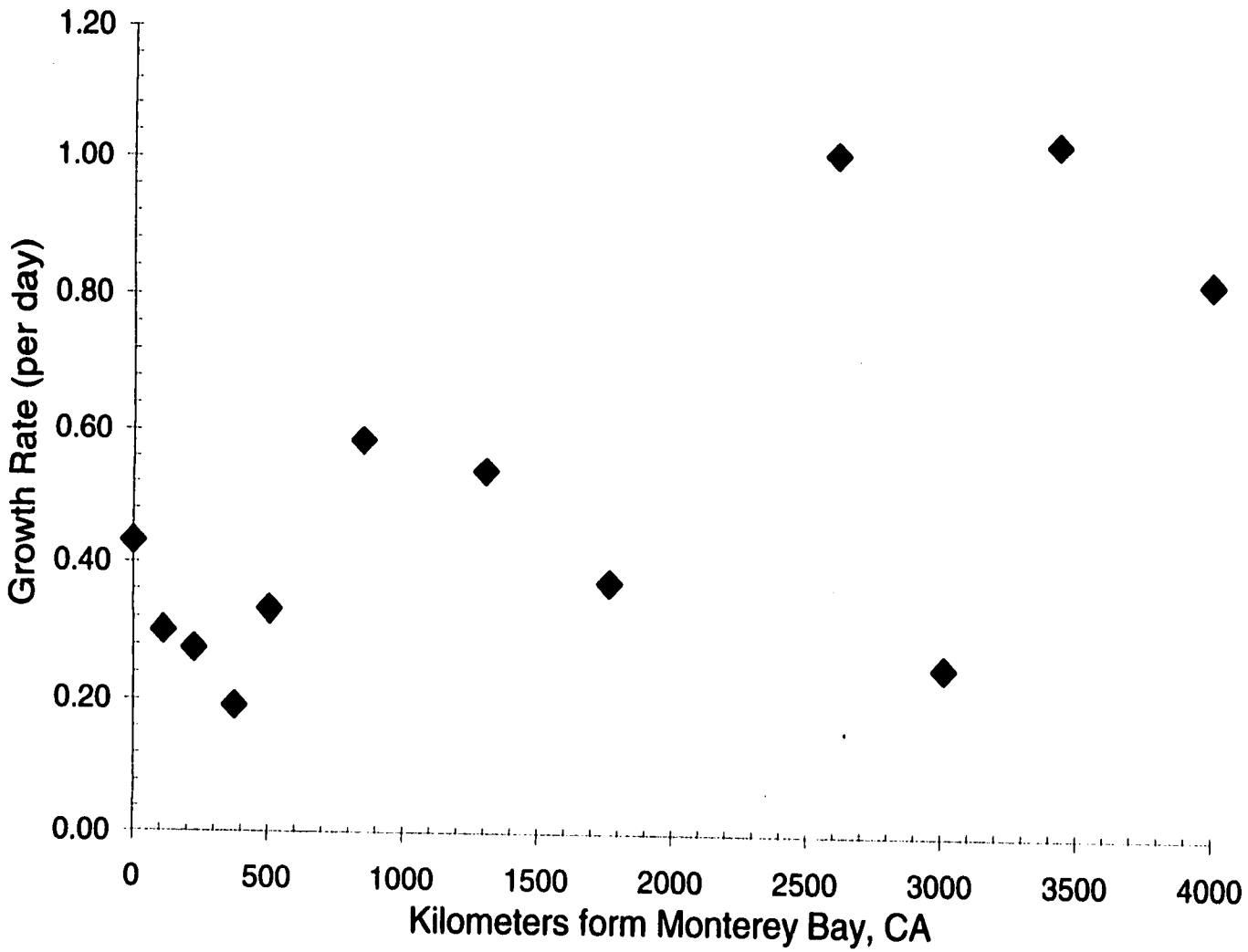


Figure 15 – In-situ Phytoplankton Growth Rates
Calculated from pigment labeling technique

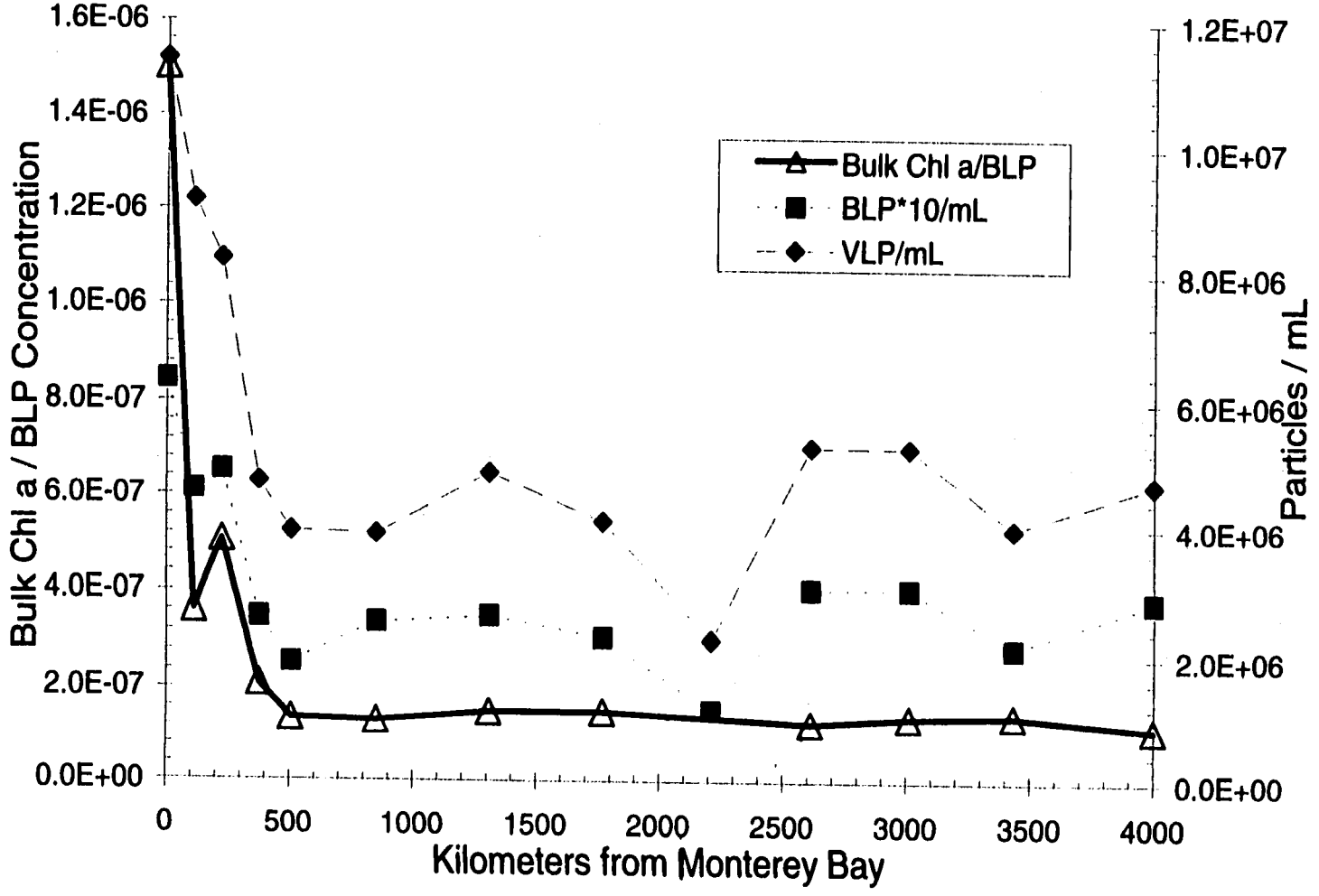


Figure 16 – Bacteria-like Particle (BLP) and Virus-like Particle (VLP) Concentrations and Ratio of Total Chlorophyll a to BLP

BLP and VLP counts from Culley, 1999

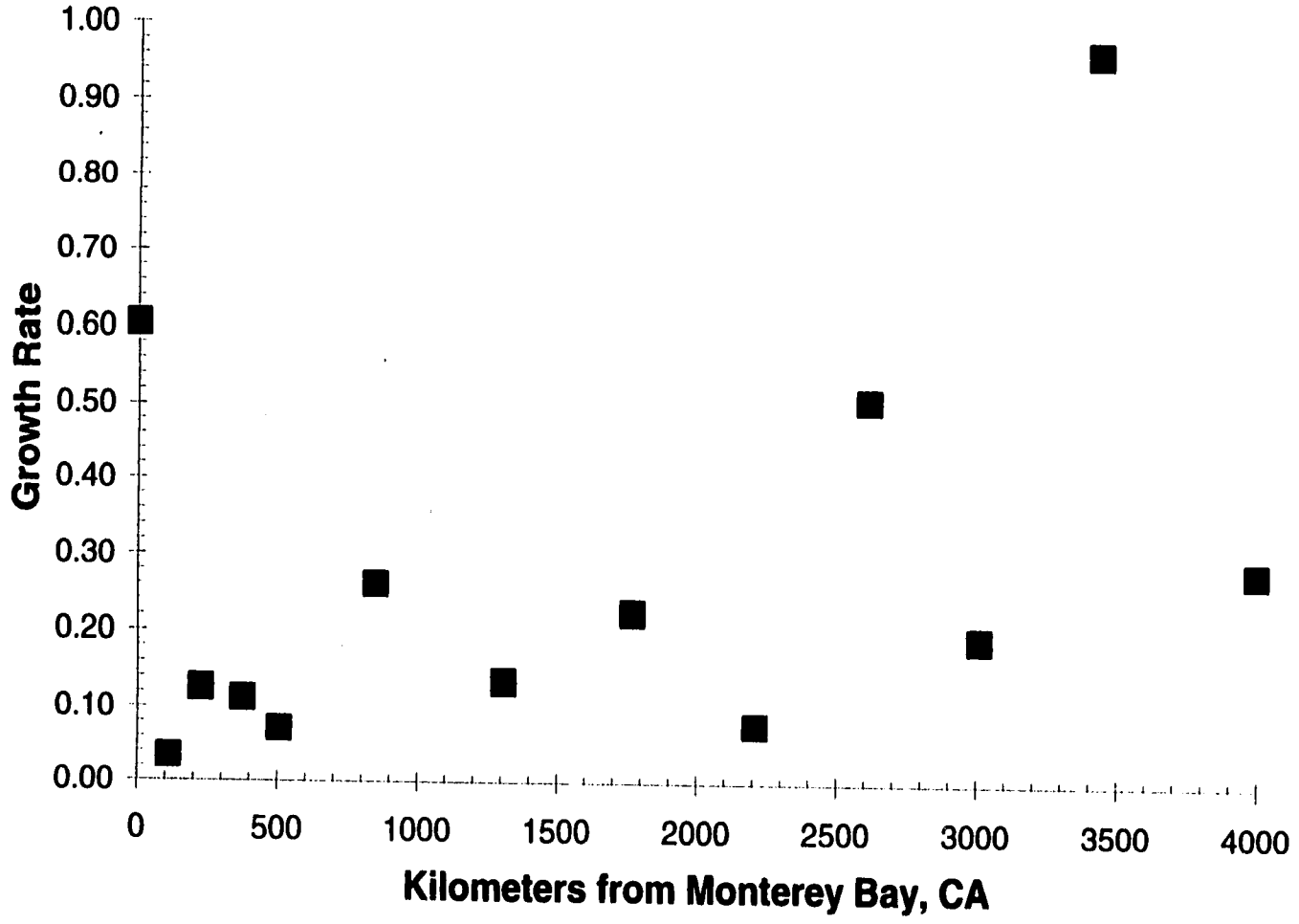


Figure 17 – Predicted Growth Rates Modeled from Measured Values of C:Chl, Temperature and Light
Model from Cloern, et al. (1995)

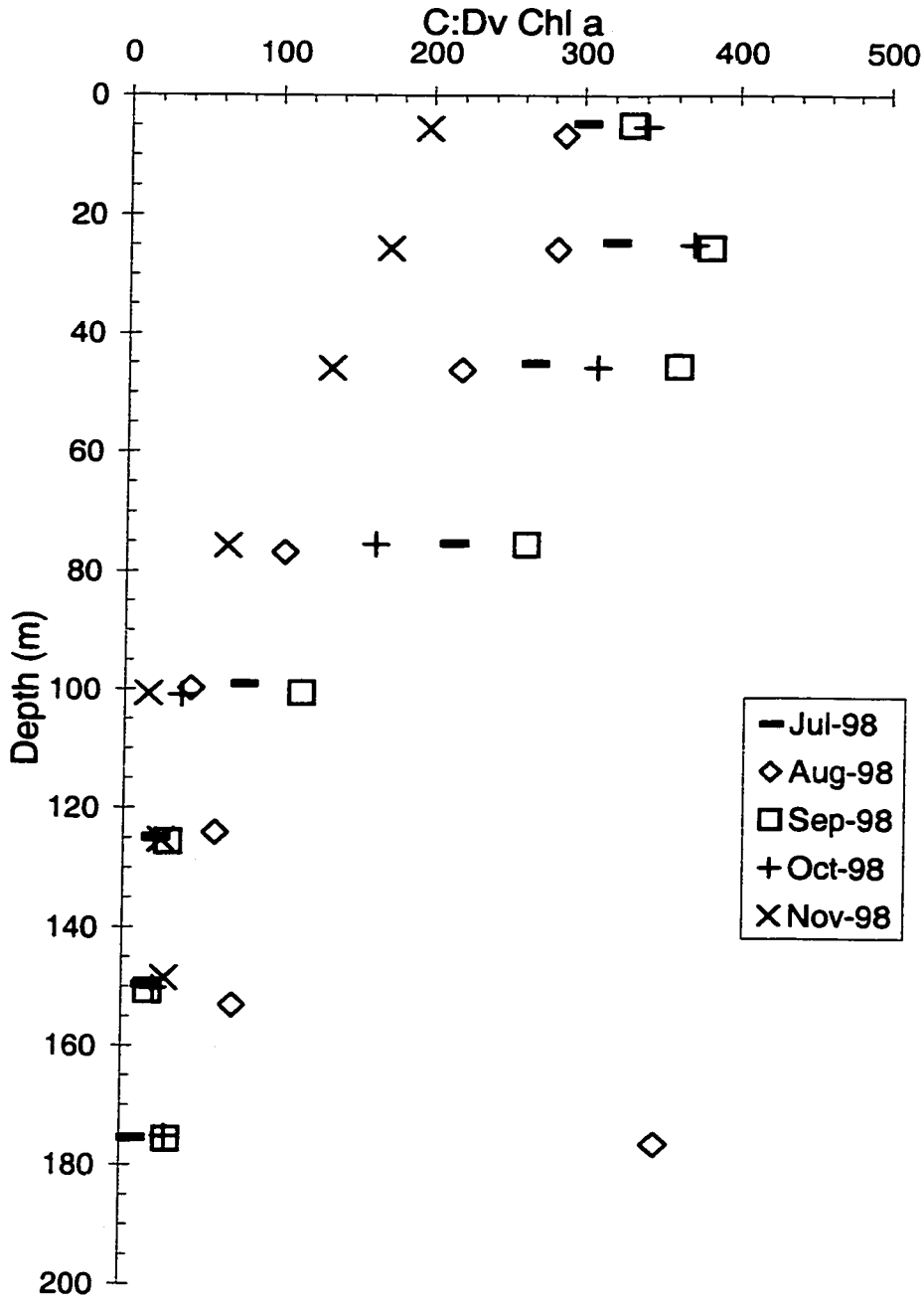


Figure 18 – HOTs Prochlorococcus Carbon to Di-vinyl Chlorophyll a
Hawaii Ocean Time Series (Station ALOHA) Data
Prochlorococcus cell counts from flow cytometry (Campbell and Vaulot, 1993)
Di-vinyl Chlorophyll a concentrations from HOTs Website, 2000
Carbon to Prochlorococcus Ratios: Calliau et al. (1996) & Campbell et al. (1994)