AN ABSTRACT OF THE THESIS OF

Alice M. Murphy for the degree of Master of Science in Oceanography presented on June 14, 1996. Title: Phytoplankton Fluorescence and Survival Below the Euphotic Zone in the California Current System.

Abstract approved:

Timothy J. Chow

High-resolution surveys of the California Current in the summer of 1993 with an instrumented Seasoar vehicle revealed high levels of photosynthetic pigment fluorescence well below the base of the euphotic zone (~ 200 m). The survey region, located about 200 km offshore of northern California, enclosed a portion of a meandering jet and an adjacent cyclonic eddy. This deep biomass was physically separated from biomass in the euphotic zone, and may have resulted from the subduction of recently upwelled, near-shore biomass. These observations raise questions about the fluorescence activity and survival capability of photoautotrophs during prolonged periods of darkness.

A laboratory experiment was set up to simulate conditions experienced by subducted phytoplankton populations. In vivo multi-excitation chlorophyll a fluorescence and metabolic activity were monitored in cultures of the diatom T. weissflogii subjected to two months of complete darkness. At the end of this two month dark incubation period, in vivo chl a fluorescence in the diatom cultures had levelled off to 45% of initial values, and the contribution of accessory pigments to chl a fluorescence showed no significant change. Cells were metabolically active during dark incubation, and exponential growth was established upon re-exposure to a light:dark photoperiod. If natural populations also possess this ability to survive and retain fluorescence capacity in the dark, the chlorophyll a fluorescence signal should persist below the euphotic zone over time scales relevant to subducted phytoplankton assemblages.

Observations of phytoplankton below the euphotic zone have a wide scope of implications, ranging from the cellular level to meso and global scales. On the cellular level, deep phytoplankton assemblages raise questions about photoadaptation and dark survival of phytoplankton. These deep features also lend evidence for water mass
subduction, a component of mesoscale circulation which is not well understood. Finally, these deep features may represent a substantial flux of carbon relative to the normal rain of biogenic material out of the euphotic zone. The biomass observed at ~200 m during the EBC program contained an estimated $2.2 \times 10^4$ metric tons carbon, and thus represents a substantial source of food to mesopelagic consumers. If these phytoplankton originated from a coastal upwelling source, the transport of this biomass off the continental shelf and to depth potentially represents an advection term in the carbon budget of the northeast Pacific which has been previously overlooked.
Phytoplankton Fluorescence and Survival Below the Euphotic Zone
in the California Current System

by

Alice M. Murphy

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed June 14, 1996
Commencement June 1997
Master of Science thesis of Alice M. Murphy presented on June 14, 1996

APPROVED:

Redacted for privacy

Major Professor, representing Oceanography

Redacted for privacy

Dean of the College of Oceanic and Atmospheric Sciences

Redacted for privacy

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for privacy

Alice M. Murphy, Author
ACKNOWLEDGEMENTS

This work would not have been possible without the generous support and even-tempered encouragement of my major professor, Tim Cowles. Thanks also to my committee members, Ricardo Letelier, Dan Shafer, and Jerry Heidel. Ricardo Letelier’s enthusiasm and insightful comments are especially appreciated. Co-workers Lynne Fessenden and Nathan Potter played critical roles during my graduate career. Lynne Fessenden, a great facilitator, smoothed my path through graduate school from the day I arrived until the completion of my thesis. Her efforts, ranging from lab training to thesis editing, were considerable. Nathan Potter’s programming skills and Unix expertise were instrumental in processing the data presented here. From his extensive bag of computer tricks, Nathan always came up with an effective way to accomplish the task at hand.

This thesis reflects the efforts of many. All participants in the multi-investigator Eastern Boundary Currents project are acknowledged for their contributions. In particular, Mike Kosro provided the satellite and ADCP figures presented here. I benefitted from discussions with physical oceanographer Jack Barth, and I commend him for his interest in biological aspects of the study. HPLC analyses were performed as a courtesy of Robert Bidigare, Mikel Latasa, and Kristi Hanson. Sandy Moore analyzed the nutrient samples, and Margaret Sparrow and Bruce Eversmeyer assisted in the POC analyses. This work was supported by a grant from the Office of Naval Research (ONR # N00014-92J-1535) as part of the Eastern Boundary Currents/Accelerated Research Initiative.
# TABLE OF CONTENTS

1. INTRODUCTION

- Oceanographic setting: the California Current System ........................................ 2
  - Circulation ........................................................................................................... 2
    - The jet as a boundary between the coastal and open ocean ....................... 3
    - Biological response to CCS circulation ....................................................... 4
  - Subduction of phytoplankton in the California Current System ...................... 5

2. OBSERVATIONS OF DEEP, POSSIBLY SUBDUCTED PHYTOPLANKTON IN THE CALIFORNIA CURRENT SYSTEM ......................................................... 8

- Introduction ........................................................................................................... 9

- Eastern Boundary Currents project: field surveys .............................................. 9

- Field observations from the EBC project .......................................................... 11
  - Large scale physical and biological fields ....................................................... 11
    - Circulation ....................................................................................................... 11
    - In situ chlorophyll fluorescence ..................................................................... 12
    - Circulation ....................................................................................................... 15
    - Deep fluorescence feature ............................................................................. 15

- Conclusions ............................................................................................................ 19

3. EFFECTS OF DARKNESS ON MULTI-EXCITATION IN VIVO FLUORESCENCE AND SURVIVAL IN A MARINE DIATOM ........................................ 21

- Introduction ........................................................................................................... 22

- Methods ................................................................................................................ 24
  - Phytoplankton culture and experimental design ............................................. 24
  - Variables measured ............................................................................................ 25
    - In vivo multi-excitation fluorescence .............................................................. 25
  - Cell counts .......................................................................................................... 26
  - Pigment, carbon and nutrient measurements .................................................. 27
  - Statistical methods .............................................................................................. 27

- Results .................................................................................................................... 28
TABLE OF CONTENTS (continued)

Cell counts ................................................................. 28
\textit{In vivo} fluorescence and \textit{in vitro} chlorophyll \textit{a} .................. 28
Multi-excitation fluorescence ratios ........................................ 33
Particulate organic carbon .................................................. 35

Discussion ........................................................................ 36

Conclusions ...................................................................... 41

4. SYNTHESIS AND CONCLUSIONS ................................. 43

Possible origin and transport pathways of the fluorescence feature .... 43

Ecological Implications ...................................................... 47

Carbon transport .............................................................. 47
\hspace{1em} Cross-shelf carbon transport ...................................... 48
\hspace{1em} Contribution to the 'biological pump' ............................ 49

Deep phytoplankton features as a food source ....................... 50

Summary .......................................................................... 51

BIBLIOGRAPHY .............................................................. 53

APPENDICES ................................................................. 61

Appendix A Results summary ............................................... 62
Appendix B Safire calibration .................................................. 65
Appendix C Variability in FDA stained cell counts ...................... 66
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.</td>
<td>The Eastern Boundary Currents project study area (approximated by dashed lines), including the shiptrack for Small Scale Survey 1</td>
<td>10</td>
</tr>
<tr>
<td>2.2.</td>
<td>The Seasoar towed instrumentation package</td>
<td>11</td>
</tr>
<tr>
<td>2.3.</td>
<td>ADCP currents at 25 m from LSS 1 (June 7-28 1993) overlaid on sea surface temperature from June 12 1993</td>
<td>12</td>
</tr>
<tr>
<td>2.4.</td>
<td>Contours of Chl a fluorescence (a) and meridional (north-south) ADCP currents (b) along line 12 of LSS 1</td>
<td>13</td>
</tr>
<tr>
<td>2.5.</td>
<td>Chl a fluorescence and sigma-t profiles typical of (a) inshore and (b) offshore locations</td>
<td>14</td>
</tr>
<tr>
<td>2.6.</td>
<td>SSS1 survey area (boxed region in (a)) and ADCP vectors at 25 m (b) overlaid on SST</td>
<td>16</td>
</tr>
<tr>
<td>2.7.</td>
<td>Chl a fluorescence and sigma-t profiles through deep fluorescence feature at 38ºN, 126.2ºN (line 2 of SSS1, June 1993)</td>
<td>17</td>
</tr>
<tr>
<td>2.8.</td>
<td>Contours of chl a fluorescence along zonal transects of Small Scale Survey 1 (June 29-July 2 1993)</td>
<td>18</td>
</tr>
<tr>
<td>2.9.</td>
<td>Chl a fluorescence on sigma-t for a single Seasoar uptrace along SSS1 line 2 at 38ºN, 126.3ºW</td>
<td>19</td>
</tr>
<tr>
<td>3.1.</td>
<td>Contours of chlorophyll a fluorescence along an east-west transect at 38ºN in the California Current System (June 1993)</td>
<td>23</td>
</tr>
<tr>
<td>3.2.</td>
<td>Total cells (living plus dead) during original dark incubation trial. Solid circles represent mean of replicates</td>
<td>29</td>
</tr>
<tr>
<td>3.3.</td>
<td>Growth of T. weissflogii upon re-exposure of darkened cultures to a light-dark photoperiod</td>
<td>29</td>
</tr>
<tr>
<td>3.4.</td>
<td>In vivo chl a fluorescence (a) and in vivo Chl a fluorescence per cell (b) during original dark incubation and 4 day repeat trial</td>
<td>31</td>
</tr>
<tr>
<td>3.5.</td>
<td>In vitro chl a concentration during dark incubation, determined by extraction in acetone and HPLC analysis</td>
<td>32</td>
</tr>
<tr>
<td>3.6.</td>
<td>In vitro (extracted) chl a per cell during dark incubation</td>
<td>32</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>3.7.</td>
<td>Predicted (----) and measured (solid circles) <em>in vivo</em> Chl <em>a</em> fluorescence per <em>in vitro</em> (extracted) chl <em>a</em> concentration during first 6 weeks of darkness</td>
<td>33</td>
</tr>
<tr>
<td>3.8.</td>
<td>Multi-excitation fluorescence ratios, B-ratio (a) and G-ratio (b), during dark incubation</td>
<td>34</td>
</tr>
<tr>
<td>3.9.</td>
<td>Particulate organic carbon per cell over time in the dark during original experiment (a) and repeat trial (b)</td>
<td>35</td>
</tr>
<tr>
<td>3.10.</td>
<td>Particulate organic carbon per <em>in vivo</em> chlorophyll <em>a</em> fluorescence over time in the dark (original experiment)</td>
<td>36</td>
</tr>
<tr>
<td>4.1.</td>
<td>Spring 1993 alongshore wind (solid line) at 39°N, 123.7°W, and weekly Bakun upwelling indices at 39°N, 125°W (dashed line)</td>
<td>44</td>
</tr>
<tr>
<td>4.2.</td>
<td>Conceptual model of possible origin and transport of the deep fluorescence feature observed during the EBC project</td>
<td>48</td>
</tr>
</tbody>
</table>
Chapter 1

INTRODUCTION

Photoautotrophic phytoplankton growth in the ocean is restricted to the euphotic zone, where there is adequate light for photosynthesis. However, during a recent study of the California Current System, fluorescence attributed to photosynthetic pigments was observed at depths well below the euphotic zone. These unexpected observations have a range of implications, from the cellular level to meso and global scales. On the cellular level, deep phytoplankton assemblages raise questions about the ability of these cells to survive and retain photosynthetic capacity during prolonged periods of darkness. These deep features also provide evidence of water mass subduction, a component of mesoscale circulation which is not well understood. In this sense, deep phytoplankton assemblages have potential utility as water mass tracers. Finally, this transport pathway for phytoplankton biomass of coastal origin may have considerable impact on offshore food web dynamics as well as on the flux of carbon to deeper waters.

There are several ways in which physical processes affect phytoplankton distributions within the complex mesoscale fields of the California Current System. One such pathway, which forms the focus of this thesis, is subduction of phytoplankton from the ocean’s surface layer to depth. In introducing this study, I will summarize recent findings on the physical and biological structure of the California Current System. In Chapter Two I describe field studies conducted during the Eastern Boundary Currents project in 1993, when the deep fluorescence feature was observed. I report the results of a laboratory experiment on the effects of darkness on in vivo fluorescence in Chapter Three, and in Chapter Four I discuss the ecological implications of deep phytoplankton assemblages, based on a synthesis of laboratory and field observations.
Oceanographic setting: the California Current System

Circulation

The California Current flows southward along the west coast of North America from the Strait of Juan de Fuca to the tip of Baja California. Large-scale California Current flow is related to basin scale and regional winds, especially an anticyclonic wind system which circulates around a subtropical high pressure region over the Northeast Pacific. The traditional view of the California Current, derived largely from the California Cooperative Fisheries Investigations program (CalCOFI), is that of a broad, slow surface current that carries cold, low-salinity water southward from the subarctic Pacific. This current represents the eastern boundary of the North Pacific gyre, and spans a region from the coast to about 1,000 km offshore (Lynn and Simpson, 1987).

Superimposed on the anticyclonic gyre circulation, which occurs year-round, are seasonal currents which result from wind-driven upwelling and downwelling. Coastal upwelling occurs in the spring and summer off North America where divergence of surface waters results from wind-driven Ekman transport in the presence of a coastal boundary. An upwelling front, characterized by isopycnals that slope upwards toward the coast, develops over the continental shelf. A geostrophic current develops along the front, flowing southward and roughly parallel to the coast. The geostrophic flow is caused by the horizontal density gradient across the front. Light, warm waters offshore have greater values of geopotential height than the cold, dense waters inshore, and a horizontal pressure gradient results. This pressure gradient, when balanced by the Coriolis force, drives southward flow in the northern hemisphere. During the winter, downwelling favorable winds lead to a poleward countercurrent sometimes referred to as the Davidson Current; this poleward current develops within 100 km of the coast, with highest velocities over the continental shelf (Lynn and Simpson, 1987).

A more complex picture of the California Current System (CCS) has emerged in the past two decades as a result of several intensive sampling programs (e.g., Coastal Upwelling Ecosystems Analysis program (CUEA), Coastal Ocean Dynamics Experiment (CODE), and the Coastal Transition Zone (CTZ) program). From these investigations a model of a meandering frontal jet amidst a mesoscale eddy field was formed, and superimposed on the traditional view of large-scale California Current flow. The complexity of the circulation in the CCS was first revealed by satellite images of sea surface temperature and ocean color, which show horizontal gradients in temperature and chlorophyll generated by the upwelling. These images reveal a convoluted boundary
between upwelled and oceanic water, with filaments of cold water with high chlorophyll concentrations extending from the shelf to more than 200 km offshore (Bernstein et al., 1977; Abbott & Zion 1985). Also visible in these images are closed circulations, or eddies, typically about 100-200 km in diameter, located on either side of the filaments.

Mesoscale surveys during the CTZ program off of northern California showed that the filaments and eddies visible in satellite images are associated with a strong, surface intensified equatorward jet. The filaments represent the offshore limbs of a jet that is thought to originate at the upwelling front and meander seaward of the continental shelf as the upwelling season advances (Strub et al., et al., 1991). In regions where the wind stress is very strong and persistent (e.g., off northern California and southern Oregon), the upwelling front and the associated jet move offshore soon after the onset of upwelling. In these regions the frontal jet is observed over the shelf only at the beginning of the upwelling season (Huyer, 1990), which typically occurs in mid March off northern California (Kosro et al., 1991). Instabilities in the jet are thought to cause the meanders and lead to separated eddies on either side of the jet (e.g., Brink, 1992). The jet ranges from about 50-75 km in width, penetrates to depths of 100 m or more, reaches velocities of 0.5-1.0 m s\(^{-1}\), and meanders up to a few hundred km offshore (Huyer et al., 1991; Kosro et al., 1991; Strub et al., 1991).

The relationship between the currents and water masses over the shelf and the larger scale California Current System has been the focus of recent and ongoing research. The jet velocities (> .5 m s\(^{-1}\)) measured during the CTZ program are significantly higher than the seasonal mean velocities of about 5-10 cm s\(^{-1}\) determined from the CalCOFI data set (e.g., Hickey, 1979; Hickey, in press). The alongshore station spacing in the CalCOFI survey grid (~ 70 km) probably resulted in underestimates of current velocities by missing a high velocity narrow core (Huyer et al., 1991). Estimates of volume transport in the jet off Pt. Arena (Huyer et al., 1991; Kosro et al., 1991), and across CalCOFI line 60 near Pt. Reyes (Lynn et al., 1982) were similar (ranging from 2-6 Sv), which suggests that the frontal jet transports a substantial portion of California Current flow.

**The jet as a boundary between the coastal and open ocean**

The frontal jet may represent the core of the larger scale California Current (Huyer et al., 1991; Strub et al., 1991; Kosro et al., 1991) and act as both a barrier and a vehicle of exchange between the coastal and open ocean. The distribution of properties and materials across the jet provides insight into its role in separating coastal and oceanic waters. Waters inshore of the jet are characterized by lower temperatures and higher density, salinity, nutrient and chlorophyll levels than those offshore (Chavez et al., 1991).
Transects across a jet off Pt. Arena during the CTZ program showed gradients of increasing temperature and decreasing density from the inshore to the offshore edge of the jet. A salinity minimum was located in the jet core: these waters were lower in salinity than surface waters offshore and onshore at the same latitude, suggesting a northern origin (Huyer et al., 1991). Low levels of nitrate and chlorophyll in surface waters of the jet reported by Chavez et al. (1991) indicate that the jet core itself was not transporting significant amounts of upwelled water to the open ocean. However, waters with relatively high levels of nutrients and phytoplankton and zooplankton biomass were found up to a few hundreds of km offshore, on the inshore side of jets (Chavez et al., 1991; Hood et al., 1991; Mackus et al., 1991; Smith and Lane, 1991). These patterns most likely arise from a combination of transport along the inshore edge of the jet and from introduction of nutrients into the euphotic zone through local upwelling within the jet and associated eddies. The cross jet structure described here implies that while the meandering jet delineates a boundary between coastal and oceanic waters, it also serves to extend the upwelling signal seaward of its coastal origin.

Two distinct phytoplankton communities were identified in the coastal transition zone off northern California: a coastal, diatom-dominated (e.g., Chaetoceros spp., Thalassiosira spp.) community found to the south and inshore of the jet; and a community of small solitary phytoplankton characteristic of oceanic waters to the north and offshore (Chavez et al., 1991; Hood et al., 1991). Zooplankton distribution patterns in the coastal transition zone showed a banding parallel to the axis of the CTZ jet, and zooplankton biomass was highest along the inshore/southern edge of the jet and declined offshore; additionally, zooplankton species were found in the jet that weren't found locally on either side, suggesting a northern origin (Mackus et al., 1991). These patterns indicate that the jet forms an ecological boundary between coastal and offshore plankton communities, and advects northern species equatorward.

**Biological response to CCS circulation**

Upwelling regions are dynamic environments where physical processes have a pronounced influence on biomass distributions (reviewed by Branch et al., 1987; Small and Menzies, 1981). Circulation in these areas affects primary production and phytoplankton distributions by advecting water parcels from one location to another, altering nutrient distributions, and by mixing cells through a range of light regimes. Upwelling blooms are typically dominated by diatoms; these pioneer species are capable of exploiting pulses of nutrients which are periodically introduced into the surface layer (Smetacek, 1985). Their success lies both in their fast growth rates in nutrient-rich,
turbulent environments and in effective seeding of the new environment by resting stages from previous populations (Smetacek, 1985). Off northern California, centric diatoms (e.g., Chaetocerus and Thalassiosira spp.), have been observed during upwelling conditions (Abbott et al., 1990; Hood et al., 1990; Hood et al., 1991).

The response of phytoplankton to wind-driven upwelling proceeds through a series of steps from bloom formation to decay. Upwelling circulation off the northwest coast of North America responds rapidly (within less than 1 day; Small and Menzies, 1981) to episodic wind events which occur throughout the spring and summer and persist for 3-10 days (Huyer 1976; Small and Menzies, 1981). At the onset of upwelling, utilization of upwelled nutrients is low due to low levels of phytoplankton biomass and the short residence time of autotrophic cells in the euphotic zone. As the water column is stabilized due to wind relaxation and surface heating, phytoplankton biomass accumulates in the euphotic zone. MacIsaac et al. (1985) showed (in the upwelling system off Peru) that this period is characterized by a light induced 'shift up' to increased nutrient uptake, photosynthesis, and synthesis of macromolecules. The high abundance of phytoplankton cells reduces light penetration and draws down nutrients in the euphotic zone (Brown and Field, 1986). Wind relaxation and cessation of upwelling ultimately results in nutrient depletion of the surface layer and decay of the phytoplankton bloom. This entire sequence from the onset of upwelling to nutrient depletion was completed in 8 to 10 days during a study of the Peruvian upwelling system by MacIsaac et al. (1985).

It is well established that wind-driven upwelling acts to enrich the biological components of coastal waters. Recently, it has been recognized that additional physical forces act to enhance primary production in systems such as the California Current System. For example, during a study of an eddy/jet feature off of Cape Mendocino (northern California) Hayward and Mantyla (1990) observed nutrient input to the euphotic zone resulting from isopycnal doming within the cyclonic eddy and upward tilting of isopycnals associated with high-velocity flow in the coastal jet. The mesoscale eddy/jet feature was uncoupled from the local winds, and represented an an important nutrient source -- distinct from wind-driven upwelling -- which strongly affected chemical and biological structure.

Subduction of phytoplankton in the California Current System

In the meandering jet model, the jet defines a convoluted boundary between the coastal and open ocean, while extending the upwelling signal seaward of its coastal origin.
By drawing upwelled waters hundreds of kilometers offshore, the mesoscale jet and eddy field may serve to enrich offshore waters with coastal flora and fauna. Further enrichment may occur if organisms exit the jet and are deposited in offshore waters during seaward excursions. Water mass subduction potentially represents one such mechanism for deposition of coastal biomass in waters seaward of the continental shelf, through the removal of plankton from high-flow regions in a surface-intensified jet.

Water mass subduction refers to the vertical displacement of a water mass from the surface to depth, or from one depth to a deeper depth. The process is not well understood, and is described synonymously in the literature by the terms subduction, subsidence and downwelling. Several physical mechanisms have been proposed as causes of subduction. Downwelling due to changes in relative vorticity has been invoked as a subduction mechanism (Pollard and Regier, 1992; Swenson et al., 1992); conservation of potential vorticity requires that downwelling occur to compensate for positive gains in relative vorticity. Other physical mechanisms include convergence at fronts (Flament et al., 1985), flow along sloping isopycnals (Washburn et al., 1991) and interaction of the jet with a large scale deformation field (Onken et al., 1990).

Evidence of subduction in the California Current System has been reported using a variety of physical, biological, and chemical indicators (Flament 1985; Hood et al., 1991; Kadko et al., 1991; Washburn et al., 1991). Subduction of water with biologically derived characteristics in the CCS was suggested as early as 1964, when Stefansson and Richards observed oxygen maxima at depths that increased with distance offshore out to 400-500 km off the coasts of Washington and Oregon. The oxygen layers were well below the productivity maxima, and the authors proposed that water containing photosynthetically produced oxygen was advected offshore and downward. Small and Menzies (1981) suggested that subsidence of phytoplankton from surface waters to depth may occur in the Oregon upwelling system when a two-cell circulation pattern develops. In the two-cell circulation model (e.g., Wroblewski, 1977; Peterson et al., 1979), the density gradient at the upwelling front separates two circulation cells, one inshore and one offshore of the front. In the nearshore cell, water upwelled adjacent to the coast is transported offshore, then sinks along isopycnals at the density front, providing a mechanism for transport of phytoplankton from surface waters to depth. While studying a filament off Pt. Arena during CODE, Flament et al. (1985) inferred, from temperature and salinity properties, that heavy surface waters were subducted beneath lighter northern waters where the filament converged with water to the south. Downwelling velocity was estimated to be 9 m per day.
Subducted water masses were identified throughout the study region of the Coastal Transition Zone program. They were located both within and outside of the jet, and vertical velocity estimates were on the order of 10 m per day. Evidence for water mass subduction during the CTZ program was based largely on observations of surface-derived characteristics at depth. These surface-derived characteristics include $^{222}$Rn:$^{226}$Ra deficiencies, oxygen saturation, and high levels of phytoplankton biomass. At the sea surface, deficiencies in the $^{222}$Rn:$^{226}$Ra ratio occur due to gas exchange with the atmosphere, while these two species are in equilibrium in the ocean’s interior. Thus deficiencies at depth represent water that has recently (within a week) been at the surface. Kadko et al. (1991) detected $^{222}$Rn:$^{226}$Ra deficiencies accompanied by high levels of oxygen saturation and chlorophyll at depths below the euphotic zone. These waters were also lower in nutrients and warmer and fresher than surrounding water at depth. Their conclusion that subduction was occurring in this region was buttressed by further observations of phytoplankton biomass below the euphotic zone reported by Washburn et al. (1991) and Hood et al. (1991).

Subduction of phytoplankton biomass from surface waters to depth is one of several ways in which physical processes affect phytoplankton distributions within the complex mesoscale fields of the California Current System. In a recent study of the California Current System (the Eastern Boundary Currents program), fluorescence attributed to photosynthetic pigments was observed at depths well below the euphotic zone, providing evidence that water mass subduction is occurring in this system as the coastal jet meanders offshore. In the following chapter I present these field observations in the context of the biological and hydrographic regime of the study area off northern California.
Chapter 2

OBSERVATIONS OF DEEP, POSSIBLY SUBDUCTED PHYTOPLANKTON IN THE CALIFORNIA CURRENT SYSTEM

Alice M. Murphy and T. J. Cowles

College of Oceanic and Atmospheric Sciences
Oregon State University
Corvallis, Oregon
Introduction

The Eastern Boundary Currents (EBC) project was an interdisciplinary study of the mesoscale jet and eddy field off of northern California. High resolution surveys of physical and biological fields were conducted in this mid-latitude region of the California Current System (CCS) during the summer of 1993. This part of the CCS is the location of the strongest southward spring-summer winds (up to 2 dynes cm$^{-2}$; Hickey, in press), and a complex flow field results. The objective of the EBC project was to better understand mesoscale physical dynamics in this region and the related biological response. This chapter provides a brief, general description of phytoplankton distribution patterns within the mesoscale fields of the California Current System, and focuses on an observed deep, possibly subducted fluorescence feature.

Eastern Boundary Currents project: field surveys

The Eastern Boundary Currents project study area was centered about 200 km off the coast of northern California, from the continental slope to about 128°W (Figure 2.1). The results from two large-scale surveys (in June and August) were used to guide four small-scale surveys, which focused on specific features on scales of 100 km or less. While this chapter includes a description of patterns which were predominant throughout the surveys, the focus is Small Scale Survey 1 (SSS1), which sampled a cyclonic eddy and a portion of an adjacent jet from June 30 to July 2. The survey grid consisted of zonal sections with approximately 10 km spacing over a 70 x 120 km area centered at about 38.8°N, 126.2°W. This 'quasi synoptic' survey covered the entire grid in 3 days.

A horizontally towed, undulating (0-300m), instrumented vehicle (Seasoar; Figure 2.2) obtained high resolution measurements of temperature, salinity, pressure, density, and multi-excitation fluorescence along survey lines (1-2 km horizontal and 2-3 m vertical resolution). The suite of instruments mounted on the Seasoar resolve physical and biological structure on the same temporal and spatial scales. The Seasoar was equipped with: dual SeaBird Electronics (SBE) temperature and conductivity sensors, an SBE pressure sensor and a 9/11-plus CTD for hydrographic measurements; a Towed Optical Fluorescence Unit (TOFU) to measure fluorescence of photosynthetic pigments; and an Optical Plankton Counter (OPC) for zooplankton abundance measurements. Satellite AVHRR imagery of sea-surface temperature, TOPEX altimetry, and Acoustic Doppler
Current Profiler (ADCP) velocity measurements were also obtained during the EBC program. A summary of CTD observations from the SeaSoar tows including data acquisition and processing protocols is provided in Kosro et al. (1995).

The Towed Optical Fluorescence Unit (TOFU), described in Desiderio et al. (in press), is an in situ multi-excitation fluorometer designed to measure fluorescence of photosynthetic pigments. The instrument, mounted inside Seasoar, recorded fluorescence of phytoplankton in water pumped through the instrument’s flow cell. Unlike conventional fluorometers, multi-excitation fluorometers measure fluorescence as a function of separate excitation wavebands. The TOFU used three visible excitation wavebands (violet, blue and green) to excite fluorescence which was recorded at 20 10 nm emission wavebands (from 546 to 733 nm), using a monochromator and photodiode array system. The fluorescence data was merged with CTD data collected simultaneously during Seasoar deployments.
Figure 2.2. The Seasoar towed instrumentation package. The Seasoar undulates between the surface and 300 m depth, obtaining high resolution physical and biological measurements. The intake for TOFU is on the nose.

Field observations from the EBC project

Large scale physical and biological fields

Circulation

The flow field in all EBC surveys was dominated by a surface-intensified (velocities $>0.5 \text{ ms}^{-1}$ at times) baroclinic jet, continuous through the study area (Huyer et al., 1994; Kosro et al., 1994). The large scale circulation pattern is illustrated in Figure 2.3. This map of ADCP currents at 25 m overlain on satellite sea surface temperature (SST) shows a southwesterly flowing jet entering the survey grid from the north at about 124.5°W, making a cyclonic bend at about 38°N, then exiting to the south, again at about 124.5°W. Both cyclonic and anticyclonic eddies were found both inshore and offshore of the jet.
throughout the EBC surveys, and one was selected for further study in the ensuing Small Scale Survey 1.

Figure 2.3. ADCP currents at 25 m from LSS 1 (June 7-28 1993) overlaid on sea surface temperature from June 12 1993. Light colors denote cool water, and scale arrow (upper right) represents .5 m s\(^{-1}\). Provided by P.M. Kosro.

**In situ chlorophyll fluorescence**

The spatial distribution of *in situ* chlorophyll *a* (chl *a*) fluorescence relative to current velocities shows that the jet delineates the boundary between inshore regions rich in chlorophyll and offshore regions with low chlorophyll levels. Sections of chlorophyll fluorescence contours and meridional ADCP velocities along line 12 of Large Scale Survey 1 provide an illustration of this pattern (Figure 2.4). The jet, located at ~ 124.5°W, was oriented equatorward at this location, and the zonal component of the velocity is not shown. Visual inspection shows that the transition from high to low levels of chlorophyll fluorescence occurs on the inshore side of the highest equatorward flow.
Figure 2.4. Contours of chl $a$ fluorescence (a) and meridional (north-south) ADCP currents (b) along line 12 of LSS 1. Vertical lines in (a) denote locations of profiles shown in fig. 2.5. ADCP currents provided by P.M. Kosro.
The regions inshore and offshore of the jet are distinguished not only by differences in fluorescence intensity, but also by the vertical distribution of this fluorescence. Two predominant vertical patterns of chlorophyll fluorescence, representing both coastal and oceanic waters, were observed in the survey area: regions inshore of the jet where chl \( a \) fluorescence was concentrated in surface waters, and offshore regions where chl \( a \) fluorescence was concentrated in a deeper layer. These patterns are demonstrated in vertical profiles from locations inshore and offshore of the jet along line 12 of LSS1 (Figure 2.5. The locations of these profiles are denoted by the vertical lines in Figure 2.4a. The inshore profile (Figure 2.5a) exhibits high surface chlorophyll levels which decline abruptly at the base of the mixed layer (about 50 m). In the offshore profile (Figure 2.5b)), maximum chlorophyll levels are lower than those inshore (by a factor of about 5) and are concentrated deeper. Peak fluorescence levels occur in the pycnocline at about 85 m, just

Figure 2.5. Chl \( a \) fluorescence and sigma-t profiles (binned on 2 db) from locations (a) inshore and (b) offshore of the jet. Profiles are from positions marked with vertical lines in Figure 2.4, along line 12 of LSS 1. Note the difference in fluorescence scales between (a) and (b). 300 fluorescence counts \( \sim 1 \mu g \text{ l}^{-1} \).
below the mixed layer. This pattern is representative of the well-known deep chlorophyll maxima (e.g., Steele and Yentsch, 1960; Cullen and Eppley, 1981) which are located at the base of the euphotic zone (1% light level) in oceanic waters (Chavez et al., 1991).

**Small Scale Survey 1**

*Circulation*

A cyclonic eddy identified during the June large scale survey was selected for further study in Small Scale Survey 1 (SSS1). This eddy originated over the shelf break off Pt. Arena in April and translated WSW at ∼ .05 m s⁻¹ on average during the survey period (Kosro, 1994). During SSS1, the 60 km eddy was located on the inshore edge of a strong (.5 ms⁻¹) baroclinic jet, more than 200 km offshore. Figure 2.6a shows the SSS1 survey region embedded in the large scale SST field, and a closer view of the survey region is provided in Figure 2.6b; the eddy/jet feature is visible in the ADCP vectors, and strong, cyclonic circulation is evident. The southwestward flowing jet makes a sharp cyclonic bend along the southern edge of the eddy and exits the survey grid on the eastern edge.

**Deep fluorescence feature**

Vertical profiles of *in situ* chlorophyll fluorescence in Small Scale Survey 1 differ from the predominant patterns described for the EBC large scale survey area. In addition to a deep chlorophyll maximum evident in the pycnocline just below the mixed layer, there was a region of comparable fluorescence levels at approximately 200 m depth (Figure 2.7). The deep feature was physically separated from the chlorophyll biomass at the base of the euphotic zone (∼ 50m). This feature was sampled by a CTD cast in which extracted chl a was measured. Based on data from this cast, 300 fluorescence counts represent approximately 1 μg l⁻¹ chl a in the deep (200 m) region of biomass.

Zonal sections of chlorophyll fluorescence contours from SSS1 (Figure 2.8) illustrate the areal extent of this feature, which is approximately 50 km x 60 km wide and 100 m deep, and spans a volume of ∼ 350 km³. For this volume estimation, the border of the feature was defined as the region where chl a values lie between .25 and .5 μg l⁻¹, and depths were restricted to those below the deep chlorophyll maximum. The total chl a content of the feature was ∼ 4.4 x 10⁵ kg, which was 2.5 times that in surface waters directly above the deep feature. Based on a carbon:chlorophyll value of 50 (e.g., Landry and Lorenzen 1989), this biomass contains 2.2 x 10⁴ metric tons (2.2x 10¹⁰ g) of carbon, and represents a substantial flux of carbon from surface waters to these depths.
Figure 2.6. SSS1 survey area (boxed region in (a)) and ADCP vectors at 25 m (b) overlaid on SST. Scale arrow (lower right in (b)) = .5 m s\(^{-1}\). Provided by P.M. Kosro.

The majority of biomass in the deep feature is concentrated in the offshore (west) portion of the survey area, where the equatorward jet bounds the eddy’s offshore side. Fluorescence was lowest in the return flow in the southeastern portion of the survey grid. Zooplankton biomass distributions in SSS1, measured with the Optical Plankton Counter, were inversely correlated with phytoplankton within the jet and were directly correlated with zooplankton within the eddy (Huntley et al., 1994; Gonzalez et al., 1996).
The eddy sampled in SSS1 was tracked with satellite imagery of SST and sampled again in late summer during Small Scale Survey 3 (September 1-5). At this time, the eddy had evolved into a dipole pair and the deep fluorescence feature was not observed.

These field observations of deep phytoplankton assemblages pose several physical and biological questions. For example, how long has this deep biomass been below the euphotic zone? How does *in situ* chlorophyll fluorescence change with time in the dark? Does the contribution of accessory pigments to chlorophyll *a* fluorescence change with time in the dark? Are the phytoplankton assemblages observed below the euphotic zone alive or dead, and what is their fate? Some of these biological questions are addressed in the laboratory study presented in Chapter Three, which examines changes in *in vivo* fluorescence and metabolic activity of a marine diatom during 2 months of darkness.

The primary physical question is: How did this assemblage of photosynthetic organisms reach waters well below the euphotic zone? Several physical mechanisms have been invoked as causes of subduction and the question arises as to whether phytoplankton

![Figure 2.7. Chl *a* fluorescence and sigma-t profiles (binned on 2 db) through the deep fluorescence feature at 38°N, 126.2°W (line 2 of SSS1, June 1993).](image)
are being subducted along isopycnals or sinking across them. One approach to this question is to look at the distribution of chlorophyll fluorescence on density. If subduction is occurring due to flow along isopycnals, fluorescence distributions should describe well-defined, Gaussian shaped layers parallel to isopycnal surfaces, whereas a uniform distribution of fluorescence on sigma-\(t\) would point towards sinking across isopycnals (Washburn et al., 1991). Washburn et al. (1991) found that the vertical distribution of phytoplankton was related to the position of isopycnal surfaces in subducted water masses of the coastal transition zone off northern California. Similarly, fluorescence in Small Scale Survey 1 of the EBC project appears to be roughly banded on 2 isopycnal surfaces. This is exemplified by the plot of chlorophyll fluorescence on sigma-\(t\) from line 2 of SSS1 shown in Figure 2.9. The deep chlorophyll maxima located at about 50 m is associated with the 25.0 sigma-\(t\) surface, and the deeper biomass peak is associated with sigma-\(t\) values ranging from 26.0 to 26.5. Although the association of fluorescence with these two

Figure 2.8. Contours of chl \(a\) fluorescence along zonal transects of Small Scale Survey 1 (June 29-July 2, 1993). 300 fluorescence counts \(-1 \mu g l^{-1}\).
isopycnal bands is prevalent throughout SSS1, there is some indication that fluorescence crosses isopycnals along at least one of the survey lines (line 4, not shown).

![Graph](image)

**Figure 2.9.** Chl *a* fluorescence on sigma-t for a single Seasr uptrace along SSS1 line 2 at 38°N, 126.3°W. Fluorescence was binned on 0.1 sigma-t units.

**Conclusions**

High resolution, simultaneous observations of both physical and biological fields provide a description of the mesoscale structure of the California Current System. The predominant patterns of *in situ* chlorophyll fluorescence observed during the Eastern Boundary Currents program are consistent with results from previous studies of the California Current System. A meandering, equatorward jet delineates a boundary between biologically rich coastal waters and oligotrophic waters offshore. The deep phytoplankton assemblage described here supports conclusions by previous investigators that phytoplankton are subducted from surface waters to depth in the mesoscale jet and eddy field of the California Current System. Washburn et al. (1991) estimated a total subducted volume for the CTZ survey region of 100-1000 km³, and a vertical chl *a* flux of
(5-9) x 10^3 kg d^-1. The CTZ estimates were based on discrete samples along CTD transects; the high-resolution SeaSoar surveys of the EBC project were able to map an entire deep fluorescence feature, and the volume of this feature alone (350 km^3) falls within the range of subducted volume estimated for the entire CTZ survey region.

These high concentrations of phytoplankton biomass in waters seaward of the continental shelf suggest that organic carbon of coastal origin is transported across the shelf. Additionally, subduction of this biomass represents a mechanism for quickly removing large concentrations of phytoplankton from the euphotic zone. This results in a high vertical flux of organic carbon relative to the normal rain of biogenic material out of the euphotic zone, providing a food supply for mesopelagic consumers and possibly increasing the amount of organic carbon ultimately sequestered in the deep ocean. These ecological implications of deep fluorescence features are discussed in chapter 4.

The subduction evidence reported here and in the literature is based partly on the assumption that phytoplankton could not have grown below the euphotic zone at the depths at which they were observed. However, the extent to which advective processes can be inferred from observations of deep fluorescence features is limited by our understanding of the behavior in vivo fluorescence in the dark. Fluorescence is a non-conservative property, and is affected in situ by processes such as photoadaptation, phytoplankton growth and senescence, and losses due to sinking and grazing. These factors complicate the application of deep fluorescent features as subduction indicators. To assess the utility of phytoplankton as indicators of water mass subduction, it is necessary to identify the time scales over which changes occur in non-conservative properties such as in vivo fluorescence. This issue is addressed in the following chapter.
Chapter 3

EFFECTS OF DARKNESS ON MULTIPLEX CITATION IN VIVO FLUORESCENCE AND SURVIVAL IN A MARINE DIATOM

Alice M. Murphy and Timothy J. Cowles

College of Oceanic and Atmospheric Sciences
Oregon State University
Corvallis, Oregon
Introduction

Photoautotrophic phytoplankton growth in the ocean is restricted to the euphotic zone, where there is adequate light for photosynthesis. The presence of phytoplankton below the euphotic zone implies physical displacement of surface waters to depth, and observations of deep phytoplankton assemblages have been presented as evidence for water mass subduction in the California Current System (Hood et al., 1991; Kadko et al., 1991; and Washburn et al., 1991). In this sense, phytoplankton have potential utility as water mass tracers.

During a high-resolution survey of the California Current System in the summer of 1993, using a towed in situ fluorometer, we observed photosynthetic pigment biomass not only in the euphotic zone but also at depths well below the euphotic zone. A deep region of chlorophyll $a$ (chl $a$) fluorescence was centered at 150-200 m (Figure 3.1); it was physically separated from a "deep chlorophyll maximum" (Steele and Yentsch, 1960; Cullen and Eppley, 1981) centered at 50-60 m, representing the base of the euphotic zone. The survey region, located roughly 200 km offshore of northern California, enclosed a portion of a meandering jet and an adjacent cyclonic eddy.

These observations suggest that water mass subduction occurs in this system as the coastal jet meanders offshore. However, the extent to which advective processes can be inferred from observations of deep fluorescence features is limited by our understanding of the behavior of in vivo fluorescence in the dark. Fluorescence is a non-conservative property, and is affected in situ by processes such as photoadaptation, phytoplankton growth and senescence, and losses due to sinking and grazing. These factors complicate the application of deep fluorescent features as tracers of water mass subduction.

To assess the applicability of phytoplankton as indicators of subduction, it is necessary to identify the time scales over which changes occur in non-conservative properties such as fluorescence. If in vivo chl $a$ fluorescence is detectable in the dark for a finite and predictable period of time, a maximum persistence time for the fluorescence signal below the euphotic zone can be inferred; and from this, an estimate of minimum subduction velocity can be obtained. Multi-excitation chlorophyll fluorescence can also provide insight into physical regimes by providing light history information. The pigment composition of photoautotrophs includes accessory pigments which absorb light at wavelengths outside the chlorophyll absorption band and transfer a portion of this energy to chlorophyll $a$. The physiological response of phytoplankton to low light includes an increase in the fluorescence excitation of chl $a$ by accessory pigments relative to the excitation by chl $a$ itself. (Neori et al., 1984; Soohoo et al., 1985). By recording
fluorescence as a function of separate excitation wavebands, multi-excitation fluorescence techniques provide a means of quantifying the contribution of accessory pigments to chlorophyll \( a \) fluorescence. Neori et al. (1984) applied multi-excitation fluorescence techniques to natural phytoplankton assemblages, and observed a shift in excitation of \( a \) fluorescence toward accessory pigment wavebands with depth. This shift was intensified in vertically stratified waters, suggesting that when the water column is unstable, the residence time of the cells at any depth is insufficient for the induction of significant photoadaptation.

Although the photoadaptive response of phytoplankton to low light has been studied intensively, little is known about the behavior of \textit{in vivo} \( a \) fluorescence during prolonged periods of darkness. The primary objective of the present study was to identify time scales over which \textit{in vivo} \( a \) fluorescence, and the potential contribution of accessory pigments to this fluorescence, changes under dark conditions. A secondary
objective was to assess the viability and metabolic activity of phytoplankton cells during prolonged periods of darkness.

In order to address these objectives, a laboratory experiment was set up to simulate conditions experienced by subducted phytoplankton populations. *In vivo* multi-excitation chlorophyll *a* fluorescence and metabolic activity were monitored in cultures of the diatom *T. weissflogii* subjected to two months of complete darkness. At the end of this two month dark incubation period, *in vivo* chl *a* fluorescence in the diatom cultures had levelled off to 45% of initial values, and the contribution of accessory pigments to chl *a* fluorescence showed no significant change. *In vivo* chl *a* fluorescence decreased relative to chl *a* concentration determined *in vitro* during the first 6 weeks of darkness, indicating that the amount of chl *a* biomass represented by a given *in vivo* fluorescence signal in the field depends on how long the biomass has been below the euphotic zone. Cells were metabolically active during dark incubation, and exponential growth was established upon re-exposure to a light:dark photoperiod. If natural populations also possess this ability to survive and retain fluorescence capacity in the dark, it is not possible to obtain estimates of advective time scales based on a maximum persistence time of chlorophyll *a* fluorescence below the euphotic zone. Nevertheless, deep phytoplankton assemblages lend evidence for water mass subduction since they are indicative of water that has been at or near the surface at some time. Additionally, these features may represent a substantial flux of carbon from the euphotic zone, relative to the normal rain of biogenic material.

**Methods**

**Phytoplankton culture and experimental design**

The diatom genus *Thalassiosira* was selected for the experiment because it is has been observed during upwelling conditions in the California Current system (Abbott et al., 1990; Chavez et al., 1991; Hood et al., 1990; Hood et al., 1991), and the deep biomass observed in the field is likely to have originated from an upwelling source. A unialgal culture of *T. weissflogii* (strain 636; Northeast Pacific Culture Collection, University of British Columbia) was grown in IMR seawater medium (Eppley et al., 1967) in a controlled-environmental chamber at 16° C under a 14:10 hr light:dark photoperiod. The culture was transferred to darkness during the logarithmic phase of growth and divided among fifty four 125 ml Erlenmeyer flasks wrapped in foil to exclude light. The flasks were placed in a dark controlled-environmental chamber where they were held at 12° C
for 62 days. This incubation temperature was chosen because it is within the range of temperatures that a phytoplankton assemblage would be exposed to during a vertical excursion from warm waters of the euphotic zone during bloom conditions, to cooler waters at a depth of 200 m.

During the dark incubation period, triplicate flasks were periodically removed for sampling and the following measurements were made: multi-excitation fluorescence; cell numbers (living and dead); and concentrations of pigments (chlorophyll $a$ and accessory pigments), nitrate, and particulate organic carbon (POC). Sampling frequency ranged from daily during the first week to weekly at the end of the dark incubation period. *In vivo* fluorescence and cell numbers were measured at each sampling; other measurements were made less frequently (Appendix A). Flasks were swirled once per day, to avoid formation of local layers of anoxia, and again before sampling. In addition to the original experimental trial, two repeat trials were performed. In one trial, *in vivo* fluorescence and cell counts were monitored for the first three days of dark incubation. In the second trial, cell counts (living and dead) and POC were monitored during 10 weeks of darkness.

At the end of the 2 month dark incubation period, viability was assessed upon re-exposure of darkened cultures to light. The flasks remaining on day 62 were returned to a 14:10 h light:dark photoperiod at 16°C. Cell numbers were monitored over the following three weeks.

**Variables measured**

*In vivo multi-excitation fluorescence*

A Spectral Absorption and Fluorescence Instrument (SAFIRE; Desiderio et al., 1996) was used to measure chlorophyll fluorescence as a function of separate excitation wavebands. The SAFIRE uses six excitation wavebands (4 in the visible and 2 ultraviolet) to excite fluorescence at 16 emission wavebands (from ultra-violet to far red). It is equipped with a xenon flash lamp and rotating filter wheel, both operating at 30 Hz. In the present study, chlorophyll $a$ fluorescence emission (685 nm) was examined as a function of 3 visible excitation wavebands (violet, blue and green). The SAFIRE was calibrated with a *Thalassiosira weissflogii* culture in log growth phase (Appendix B).

During sample processing, a 50 ml aliquot of culture from each replicate flask was diluted 20-fold then passed through the instrument's flow cell with gravity flow. A flow rate of 50 ml s$^{-1}$ resulted in a travel time of .1 s from the excitation source to the chlorophyll $a$ fluorescence emission detector. Precautions were taken to avoid light exposure during the fluorescence sampling process.
The contribution of accessory pigments to chlorophyll a fluorescence is quantified by calculating ratios of chlorophyll fluorescence emission as a function of excitation wavelength. The ratios are collectively referred to here as multi-excitation fluorescence (MEF) ratios, and individually as the B-ratio and the G-ratio. The B-ratio (CHLB:CHLV) is obtained by dividing chlorophyll emission (685 nm) from blue excitation (485 nm) by chlorophyll emission resulting from violet excitation (435 nm). Similarly, the G-ratio (CHLG:CHLV) is obtained by dividing chlorophyll emission from green excitation (520) by chlorophyll emission resulting from violet excitation (435 nm). Quantum corrections were applied to correct for the spectral dependencies of the lamp output. The excitation power output for each wavelength was obtained through direct power measurement using a calibrated pyro-electric head and a Newport Model # 8825-C Power Meter. The excitation power measurements corresponded to a quantum ratio of (1: .7: .4) for (violet : blue : green) excitation wavebands.

**Cell counts**

Counts of living and dead cells were made on each replicate flask throughout the two month experimental period. Additionally, live/dead cell counts were made during a 2 month repeat trial of the dark incubation.

Cell metabolic activity was assessed by staining cultures with the vital stain fluorescein diacetate (FDA) using the protocol of Dorsey et al. (1989) and obtaining cell counts under an epifluorescence microscope. The stain fluorescein diacetate provides an index of metabolic activity, and hence cell viability, in marine phytoplankton (Bentley-Mowat 1982; Dorsey et al., 1989; Selvin et al., 1988). FDA readily penetrates intact membranes of living cells. It is not fluorescent, but once inside a living cell it is hydrolysed by esterases to give fluorescein, which fluoresces green.

A 10 ml stock solution of fluorescein diacetate (Sigma Chemical Company, F-7378, Lot No. 104H5010) was made up in reagent grade dimethylsulfoxide (DMSO) at a concentration of 5 mg ml\(^{-1}\) and stored frozen. A 30 μM working stock was prepared immediately prior to each sampling session by diluting the stock solution 400-fold in filtered (.2 μm) seawater. The working stock was kept in the dark at 12° C during the FDA assay, which rarely took more than a half hour.

Aliquots of phytoplankton culture were stained using a final FDA concentration of 10 μM. These samples were kept in the dark at 12° C for 6 to 7 minutes before filtering onto .8 μm black Poretics membrane filters. The filters were then mounted on slides for epifluorescence microscopic examination.
Epifluorescence observations were made on an Olympus BHTU microscope equipped with a reflected light fluorescence attachment (BH2-RFL) and illuminated by a mercury lamp source (Osram HBO 100W/2). The optical system incorporated a 490 nm excitation filter, a 500 nm dichroic mirror, and a long pass 515 nm barrier filter for epifluorescence detection.

Concentrations of living (FDA positive) and dead (FDA negative) cells were determined by enumeration of cells within an ocular grid. The green fluorescence of fluorescein is readily distinguished from the red autofluorescence of chlorophyll. Cells that fluoresced green were classified as living, or FDA positive, and those that fluoresced red were classified as dead, or FDA negative. A minimum of 100 cells were counted from each sample. Assuming a random distribution of cells across the filter, these counts give a 95% confidence interval of the estimate within ± 20% of the true mean (Lund et al., 1958).

**Pigment, carbon and nutrient measurements**

Aliquots (10 ml) of culture for pigment and particulate organic carbon (POC) measurement were filtered onto 25 mm glass fiber filters (Whatman GF/F) and then frozen. Chlorophyll a and phaeopigment concentrations were determined *in vitro* with a Turner Designs fluorometer calibrated with commercial chlorophyll a (Sigma Chemical Company) after extraction in 90% acetone (Strickland & Parsons, 1972). Six frozen filters were shipped to the Department of Oceanography at the University of Hawaii for determination of photosynthetic pigments by High Performance Liquid Chromatography (HPLC). For POC determination, filters were dried at 60°C for 24 hours and analyzed on a Carlo Erba NA1500 C/N analyzer. For nutrient determination, 10 ml aliquots of culture were stored frozen in acid washed, 20 ml polycarbonate scintillation vials. Nitrate plus nitrite concentration was determined using a Technicon II Auto-Analyzer and the protocol of Whitledge et al., (1986).

**Statistical methods**

Simple linear regression (SLR) was used to assess changes in measured variables as a function of time in the dark. To determine whether measured variables changed significantly with time in the dark, 2 sided p-values were calculated for tests of the hypothesis that the coefficient of time ($\beta_1$) in the SLR model is equal to zero.

Standard errors for the mean of replicate measurements at each time point were calculated and are represented by error bars in the figures. Standard errors for ratios of
two variables (e.g., carbon per cell in figure 3.9) were calculated using rules for propagation of error as outlined in Taylor (1982).

**Results**

The means and standard errors (SE) of the variables measured during dark incubation are provided in Appendix A, in addition to the sampling schedule and the number of replicates for each day of sampling.

**Cell counts**

Concentration of total cells (living plus dead) decreased over the dark incubation period from $3.4 \times 10^4$ cells ml\(^{-1}\) to $2.2 \times 10^4$ cells ml\(^{-1}\), or 65% of the initial value (Figure 3.2). The percentage of FDA positive cells showed no significant change from a mean value of 82% during the first three weeks of incubation (p-value=.60). During the remaining 6 weeks the stained cell counts were highly variable, and a 2 month repeat trial was performed in order to clarify the cell counts.

During the 2 month repeat study period, the mean total cell concentration did not change significantly from a mean value of $3.8 \times 10^4$ cells ml\(^{-1}\) (p-value=.75). Nor did the number of living cells change significantly with time (p-value=.94). The mean concentration of living cells was $3.2 \times 10^4$ cells ml\(^{-1}\) during this time, which represents 84% of the total cells.

When the cultures that had been in the dark for two months were returned to a light:dark photoperiod, exponential growth was re-established, with no apparent time lag (Figure 3.3). The relative growth rate (u) was .43, which lies in the same range as growth rates of *T. weissflogii* measured from cultures grown under a light:dark photoperiod without prolonged exposure to darkness. The mean concentration of [nitrate + nitrite] in the culture for the 2 month period was 367 μM and did not change significantly with time (p-value=.27).

**In vivo fluorescence and in vitro chlorophyll a**

*In vivo* chlorophyll a fluorescence, measured by SAFIRE, increased approximately 10% during the first 24 hours, dropped rapidly during the next week, then gradually levelled off to roughly 45% initial values (Figure 3.4a). Chlorophyll a fluorescence per cell showed a similar pattern (fig. 3.4b), levelling off at about 65% initial values by the
Figure 3.2. Total cells (living plus dead) during original dark incubation trial. Solid circles represent mean of replicates. Error bars = ± 1 SE of replicate means. The apparently low values between days 26 and 41 may be due to a systematic offset during this interval, while a different person was enumerating cells.

Figure 3.3. Growth of *T. weissflogii* upon re-exposure of darkened cultures to a light:dark photoperiod. The line through the data points is an exponential fit: $N_t = N_0 e^{\mu t}$, where $N_t$ is the number of cells at time $t$, $N_0$ is the number of cells at time 0, $\mu$=growth rate (t$^{-1}$) and $t$ is time, in days. $N_0=900$, $\mu=.43$. 
end of the incubation period. To verify the initial increase in these parameters, the first 3 days of dark incubation were repeated. The data from this repeat trial (trial 2) are overlaid on the original data in Figure 4 but were not included in statistical analyses.

After the initial increase in fluorescence during the first 24 hours, the decay in *in vivo* chlorophyll *a* fluorescence as a function of time in the dark can be described by the following simple linear regression model:

\[
\text{in vivo fluorescence counts} = 130.6 - 17.94 \times \ln \text{day} \quad \text{(eq. 1)}
\]

\[\text{SE}=3.25 \quad \text{(SE}=1.14)\]

\[r^2 = .86; n = 43\]

where fluorescence counts are measured by SAFIRE at 685 nm emission and 435 nm excitation. A lack-of-fit F-test revealed no evidence that the simple linear regression model is inadequate for these data (p>.05).

*In vitro* (extracted) chlorophyll *a* concentration did not change significantly from a mean value of 61 µg l\(^{-1}\) during the first 6 weeks of dark incubation (p=.23); after week six, the mean extracted chl *a* concentration decreased to 37 µg l\(^{-1}\) (Figure 3.5). *In vitro* chlorophyll *a* concentration determined by HPLC showed a similar trend of relatively constant values followed by a decline sometime between weeks 4 and 7. Chlorophyll *a* per cell was highly variable and did not change significantly with time in the dark, although a plot of the data (Figure 3.6) gives some indication of an increase during the first week and a half of darkness. The concentration of phaeopigments was less than 3% of chlorophyll *a* concentration in all samples, as determined by HPLC and acetone extraction.

*In vivo* chlorophyll *a* fluorescence decreased relative to *in vitro* (extracted) chlorophyll *a* concentration during the first 6 weeks of darkness (p=.008). This relationship is depicted in Figure 3.7, which shows both predicted and observed values of the ratio of *in vivo* fluorescence to extracted chlorophyll *a* concentration during the first 6 weeks of darkness. The predicted curve was generated as follows. Predicted values of *in vivo* chlorophyll *a* fluorescence were calculated from the regression model for the decay in *in vivo* fluorescence (eq. 1) over time in the dark. These values were divided by the mean extracted chl *a* concentration (61 µg l\(^{-1}\)) observed during the first 6 weeks of dark incubation. It should be noted that the concentration of Chl *c* relative to chl *a*, determined by HPLC, did not change significantly during the experiment (p=.24). This ensures that the relationship between *in vivo* and *in vitro* chl *a* measurements was not confounded by the contribution of Chl *c* to the extracted chl *a* measurement.
Figure 3.4. *In vivo* chl a fluorescence (a) and *in vivo* chl a fluorescence per cell (b) during original dark incubation and 4 day repeat trial. In (a), values from trial 2 are normalized to the mean on day 0 of trial 1, and dashed line is fitted from the regression model described in text (eq. 1). Error bars = ± 1 SE of replicate means.
Figure 3.5. *In vitro* chl *a* concentration during dark incubation, determined by extraction in acetone and HPLC analysis. Error bars = ± 1 SE of mean of replicates.

Figure 3.6. *In vitro* (extracted) chl *a* per cell during dark incubation.
Multi-excitation fluorescence ratios

The multi-excitation fluorescence ratios (B-ratio and G-ratio), for the 2 month period, are shown in Figure 3.8. Although the data suggest an initial increase in both ratios during the first several days, neither the B-ratio nor the G-ratio changed significantly with time in the dark (p-value = .21 and .65, respectively). However, during this period the molar ratio of fucoxanthin to chlorophyll a increased chl significantly from .56 to .62 (p-value = .03).
Figure 3.8. Multi-excitation fluorescence ratios, B-ratio (a) and G-ratio (b), during dark incubation. Error bars = ± 1 SE of replicate means.
**Particulate organic carbon**

Changes in cellular carbon content during dark incubation are shown in Figure 3.9. Carbon is expressed on a cellular basis by dividing the carbon concentration (pg C ml$^{-1}$) measured in a given flask by the total cell concentration (cells ml$^{-1}$) measured from the same flask. Carbon per cell increased 2.5 and 1.7-fold during the first 5-6 weeks of the original experiment and a 10 week repeat trial, respectively. A subsequent decline in carbon per cell occurred during the final few weeks. This trend is evident in the repeat trial and is suggested by the data from the original experiment. Particulate organic carbon per *in vivo* chl *a* fluorescence also peaked at week 6 (Figure 3.10) and the data suggest a subsequent decline.

![Graph showing particulate organic carbon per cell over time](image)

**Figure 3.9.** Particulate organic carbon per cell over time in the dark during original experiment (a) and repeat trial (b). Error bars represent ± 1 SE. Solid circles represent means of duplicate samples where error bars are present, and single data points where no error bars are present.
Figure 3.10. Particulate organic carbon per *in vivo* chlorophyll *a* fluorescence over time in the dark (original experiment). Units for carbon per fluorescence are $((\text{pg C m}^{-1}) \text{fluorescence counts}^{-1}) \times 10^{-3}$. Error bars represent ± 1 SE. Solid circles represent means of duplicate samples where error bars are present, and single data points where no error bars are present.

**Discussion**

Under conditions of prolonged (2 months) darkness, a metabolically active population of *T. weissflogii* was maintained that was capable of exponential growth when returned to light favorable conditions. The persistence of the fluorescence signal in the dark and the growth upon re-exposure to light indicate that both the photochemical apparatus and biochemical carbon fixation pathways remained functional. There is some uncertainty associated with our estimates of metabolic activity due to variability in the the FDA assay results; this is addressed in Appendix C. Nonetheless, the percentage of metabolically active cells was at least 50% and most likely between 72% and 84% during dark incubation.

The ability of diatoms to survive months of complete darkness is not a surprising result. Diatoms, particularly polar species which are exposed to months of winter
darkness, are known for their dark survival capabilities (e.g., Bunt and Lee, 1972; Palmisano and Sullivan, 1982; Smayda and Mitchell-Innes, 1974). Dark survival mechanisms include: 1) reduction in cellular metabolism; 2) resting spore formation and resting stages without morphological change; and 3) heterotrophy (Smetacek, 1985).

The particulate organic carbon data suggest that *T. weissflogii* may be capable of heterotrophy. The increase in cellular carbon during dark incubation suggests that cells were taking up dissolved organic carbon present in the culture flasks. Such a shift from autotrophy to heterotrophy under conditions of light stress was suggested for the pennate diatom *Fragilaria sublinearis* isolated from antarctic sea ice (Bunt & Lee, 1972). During three months of incubation in complete darkness, the cellular carbon content of this diatom increased while cell numbers decreased; they suggested that surviving cells may be reutilizing organic compounds derived from senescent members of the population.

It has been established that some diatoms are capable of heterotrophic nutrition under conditions of light stress (Hellebust and Lewin, 1977 and authors therein; Lewin and Hellebust, 1978; Palmisano and Sullivan, 1982). Although facultative heterotrophy is fairly widespread in pennate diatoms which are frequently found in nearshore, shallow-water, benthic environments where organic compounds are abundant, there is little evidence for heterotrophic nutrition among open-water centric diatoms which typically exist in waters low in dissolved organic carbon (Hellebust and Lewin, 1977; Sloan and Strickland, 1966). However, our data suggest that the centric diatom *T. weissflogii* may be capable of incorporating dissolved organic carbon (DOC) into cellular material. The decline in cellular carbon following the peak at weeks 5-6, however, indicates that heterotrophic activity may be limited. To adequately assess this organism’s capacity for heterotrophy, measurements of radiolabelled organic carbon uptake are needed.

A potential experimental artifact in the POC data is the contribution of bacteria to the carbon signal measured on the glass fiber filters. The cultures used in this experiment were not axenic, and rod shaped bacteria 1.6 μm in length were present in the culture flasks. These bacteria are large enough to be retained on GF/F filters, which have a particle retention size of .7 μm. Bacteria were not counted during dark incubation, and it is not possible to directly quantify the contribution of bacteria carbon to POC. However, a rough estimate was made, based on a comparison of microscopic cell counts of bacteria in a log-phase *T. weissflogii* culture grown under a L:D photoperiod with a culture which had been in the dark for 12 weeks. These counts indicate that bacteria carbon comprised approximately 4% of total POC at day 0, and probably comprised less than 10% of total POC at weeks 5-6, when the measured POC was at a maximum. Based on these estimates, the effect of bacteria carbon on the increase in calculated POC per cell is
negligable. Another possible artifact which cannot be assessed is the flocculation of DOC into POC.

The ability of photoautotrophs to exist heterotrophically under conditions of light stress represents a potential survival mechanism in environments where cells are periodically removed from the euphotic zone. In such environments, uptake of DOC could provide a cellular carbon supply adequate for maintenance metabolism until cells are returned to favorable light conditions, provided that sufficient organic substrates are available. One characteristic of diatoms which are facultative heterotrophs is their ability to retain both their pigmentation and photosynthetic capacity when grown in the dark on organic substrates for considerable periods of time (Hellebust and Lewin, 1977 and authors therein), which is consistent with the results presented here. Facultative heterotrophy is a potentially effective survival mechanism for phytoplankton existing in a physically dynamic system such as the California Current.

The initial increase of \textit{in vivo} chl \textit{a} fluorescence (bulk and cellular measurements) during the first 48 hours of darkness is an expected physiological response to light stress. During photoadaptation, the light harvesting capacity of phytoplankton cells is enhanced by an increase in cellular content of photosynthetic pigments (Kiefer et al., 1976). This is accompanied by an increase in accessory pigments relative to chl \textit{a}, which is manifest in an enhancement of excitation of chl \textit{a} fluorescence by accessory pigments relative to excitation by chl \textit{a} itself. Thus the rise in MEF ratios observed during the first week of dark incubation (Figure 3.8), although not statistically significant, is consistent with established photoadaptive responses of phytoplankton to light stress.

Photoadaptive enhancement of MEF ratios have been attributed to both an increase in absorption by accessory pigments relative to chl \textit{a} and an increase in energy transfer efficiency between light harvesting pigments and reaction center chl \textit{a} molecules (Neori et al., 1984; Soohoo et al., 1985). Our results do not support these concepts. The principal accessory pigments of diatoms are chl \textit{c} and fucoxanthin (Jorgensen, 1977), and an enhancement of excitation of chl \textit{a} fluorescence by these pigments should be reflected in the MEF ratios. However, while the molar ratio of fucoxanthin to chl \textit{a} increased by 10\% (from .56 to .62) over several weeks of dark incubation, the MEF ratios remained unchanged, which indicates that fucoxanthin was not efficiently absorbing and/or transferring excitation energy to chl \textit{a}. This result implies that the MEF ratios are not sensitive to changes in molar pigment ratios that are less than or equal to 10\%.

The ratio of \textit{in vivo} fluorescence intensity per chl \textit{a} varies with environmental conditions (Prezelin, 1981 and authors therein), and the decline in this ratio observed during the first 6 weeks of the present experiment affects our interpretation of field data.
The concentration of chlorophyll represented by a given amount of fluorescence measured \textit{in vivo} depends on how long the observed biomass has been below the euphotic zone. After 6 weeks of darkness, the model presented in Figure 3.7 predicts that a given \textit{in vivo} fluorescence count represents 1.7 times the amount of chlorophyll represented by the same fluorescence value at day 0.

What is the cause for the decline of \textit{in vivo} fluorescence relative to chl \(a\) concentration over time in the dark? Possible causes include a reduction in the chlorophyll-specific absorption cross section and a reduction in the quantum yield of fluorescence (i.e., quanta emitted/quanta absorbed) via photochemical or non-photochemical fluorescence quenching. The former is more likely to have occurred during the present study and the reasoning is as follows.

The probability that a pigment molecule will absorb an impinging photon can be quantified as an absorption cross section, with dimensions of area per unit compound (Dubinsky, 1992). The chlorophyll-specific absorption cross section of intact cells is affected by biochemical and structural factors, such as nutrient status and shading among cells and between constituents packaged within the cells. In the present experiment, nutrient replete conditions persisted during dark incubation, so the effect of nutrient depletion on absorption cross section is not relevant here. Shading between cells in SAFIRE’s sample volume is unlikely as well, since cell numbers did not increase during dark incubation, and flocculation of cells was not observed during microscopic cell counts. Since chl \(a\) per cell did not change significantly during the several weeks of dark incubation considered here, shading due to photoadaptive increase in cellular pigment concentration is also improbable.

The chlorophyll-specific absorption cross section may have been reduced due to structural changes in cellular components during dark incubation. Berner et al. (1989) observed a decrease in absorption cross section due to an increase in the number of thylakoids per stack in the grana during dark adaptation of the chlorophyte \textit{Dunaliella tertiolecta}. Lipids, glycerol, and other cell constituents are likely to influence light absorption as well, although these factors have not been thoroughly investigated (Dubinsky, 1992). The carbon data presented here illustrate that changes in cell composition were occurring during the dark incubation period; such changes may have affected the absorption of chlorophyll molecules within the cells.

Changes in the quantum yield of fluorescence (a.k.a. fluorescence yield) are considered to be a major source of variation in \textit{in vivo} fluorescence per unit chlorophyll \(a\) in the field (Falkowski and Kiefer, 1985). The quantum yield of fluorescence is controlled by photochemical and non-photochemical quenching, which reflect pathways of excitation.
energy within the photosynthetic apparatus of algal cells. The absorption of visible light by a chlorophyll molecule causes a transition of an electron from the ground state to an excited state. After a photon is absorbed, several reaction pathways compete for the deactivation of excited chlorophyll. The major pathway is photosynthetic electron transport, and a competing process of deactivation is the emission of chlorophyll a fluorescence. The effect of photochemical utilization of energy (i.e., photosynthesis) is to quench fluorescence, and thus the degree of photochemical quenching reflects the state of photosynthetic electron transport: photochemical quenching is maximal when all reaction centers are open. In the present study, algal growth commenced immediately upon re-exposure of darkened cultures to light, indicating that the photosynthetic pathway remained intact during the incubation period; thus photochemical quenching of fluorescence is expected. However, an increase in photosynthetic electron transport capacity during several weeks of complete darkness is improbable, and thus a reduction in fluorescence yield via photochemical quenching is an unlikely cause for the decline in in vivo fluorescence per unit chlorophyll a.

Non-photochemical fluorescence quenching results in de-excitation of chlorophyll a via nonradiative decay (i.e., heat loss). It is a form of photoprotection, induced in vivo under conditions in which absorbed light intensity is greater than what can be used by photosynthetic electron transport (Horton et al., 1994). Since it is induced under high light conditions, and not expected to occur during dark incubation, a reduction in fluorescence yield due to non-photochemical quenching of fluorescence is unlikely in the present study. It is also unlikely that such quenching occurred in the sample volume of the fluorometer due to excitation from the lamp. Upon re-exposure of a darkened culture to light, nonphotochemical quenching occurs within 1-2 minutes of excitation (Govindjee, 1986). The travel time in SAFIRE’s flow cell from the excitation source to the fluorescence detector was approximately 0.1 seconds, which ensures that algal cells passed through the sample volume before nonphotochemical quenching could have occurred.

To summarize, potential causes for the observed reduction in in vivo fluorescence per unit chl a include a reduction in fluorescence yield via fluorescence quenching and a reduction in the chlorophyll-specific absorption cross section. Neither photochemical nor nonphotochemical fluorescence quenching pathways are consistent with our experimental conditions. A decrease in chlorophyll absorption, due to structural changes in cell components during dark incubation, appears to be the most probable cause for the observed reduction in in vivo fluorescence per unit chlorophyll a.
Conclusions

Cultures of *T. weissflogii* were able to survive two months in the absence of light, then establish exponential growth upon re-exposure to light. During dark incubation, the *in vivo* chl *a* fluorescence signal persisted, although its intensity relative to chl *a* determined *in vitro* declined. This latter result implies that the amount of pigment biomass represented by deep fluorescence features depends on the length of time the phytoplankton assemblage has been below the euphotic zone. If our results for *T. weissflogii* extend to natural populations, then light limitation of photosynthesis does not preclude the survival of subducted phytoplankton assemblages and the consequent accumulation of chlorophyll *a* at depths below the euphotic zone. Thus it is not possible to obtain estimates of advective time scales based on a maximum persistence time of the chlorophyll *a* fluorescence signal below the euphotic zone. Nevertheless, deep phytoplankton assemblages lend evidence for water mass subduction since they are indicative of water that has been at or near the surface at some time.

Multi-excitation fluorescence characteristics have been demonstrated to be sensitive to both taxonomic composition and photoadaptive state of the phytoplankton (Hilton et al., 1989; Neori et al., 1984; Soohoo et al., 1985; Yentsch and Phinney, 1985), which complicates the characterization of natural assemblages based on MEF ratios. We have demonstrated that the MEF ratios in a single diatom species are unaffected by prolonged periods of darkness. These observations serve to narrow the range of possible interpretations of MEF characteristics slightly. The deep fluorescence feature observed in the California Current System possessed multi-excitation fluorescence characteristics which can be distinguished from assemblages near the surface and in adjacent flow regimes (T.J. Cowles, unpublished). Since MEF ratios did not change significantly during the dark incubation reported here, the observed differences between deep and surface assemblages in the field are more likely to reflect differences in taxonomic composition than photoadaptive changes within a single species. However, the slight (although not statistically significant) increase in MEF ratios during the first few days of darkness implies that the differences in multi-excitation fluorescence observed in natural populations may reflect short term (on the order of days) exposure to dark conditions.

Given the ability of diatom populations to retain fluorescence activity during prolonged periods of darkness, why aren’t regions of deep chlorophyll biomass more prevalent in coastal upwelling systems? A possible explanation is that the complete set of physical and biological conditions necessary for subduction of phytoplankton occur infrequently. Alternatively, subducted phytoplankton cells may be cropped by midwater
grazers before biomass accumulates at depth. Finally, inadequate sampling (both vertically and the horizontally) may be responsible for the apparent paucity of subducted phytoplankton assemblages. Surface waters tend to be the focus of biological sampling efforts, since phytoplankton assemblages are not expected to be found in the dark. Additionally, features may be missed due to inadequate horizontal resolution. Limitations in resolution of biological features may be imposed by the episodic nature of coastal upwelling blooms, even in high resolution surveys using *in situ* instrumented vehicles. A pulse of coastally generated phytoplankton biomass carried offshore in a jet and subducted to depth could potentially be missed in a non-synoptic, large scale survey.

These deep phytoplankton features may represent a substantial flux of carbon relative to the normal rain of biogenic material out of the euphotic zone, thereby providing a food source to mesopelagic organisms. If these phytoplankton originate from a coastal upwelling source, the transport of this biomass offshore and to depth potentially represents an advection term in the carbon budget that has been previously overlooked. These ecological implications are discussed in the following chapter.
Chapter 4

SYNTHESIS AND CONCLUSIONS

The purpose of this chapter is to synthesize the laboratory results with the field observations of deep, possibly subducted phytoplankton assemblages. I will discuss potential pathways for the accumulation of phytoplankton below the euphotic zone seaward of the continental shelf, based on observations from the present study and those reported in the literature. Specifically, I will discuss possible origin and transport mechanisms of the deep fluorescence feature observed during the Eastern Boundary Currents project, the role of the feature as a food source to mesopelagic consumers, and its possible role in coastal and oceanic carbon budgets.

Possible origin and transport pathways of the fluorescence feature

Chlorophyll concentrations in the deep assemblage relative to surrounding waters suggest that the feature did not originate locally. The chl a concentration of the feature, located approximately 200 km offshore, ranged from about 1.5 to 2 \( \mu g L^{-1} \). Chl a concentrations in this offshore region were typically < 1 \( \mu g L^{-1} \) during the EBC surveys, even within cyclonic eddies where isopycnals were domed towards the surface. Additionally, chl a in these waters was typically concentrated in 20-40 m thick bands (usually at the deep chlorophyll maxima), whereas the deep feature spanned ~ 40 to 125 m of depth and occupied a volume of ~ 350 km\(^3\). The chlorophyll biomass in the deep feature was an estimated 2.5 times larger than the biomass in the surface waters directly above it. These observations suggest that the feature originated near the coast, where high levels of phytoplankton biomass occur periodically due to upwelling events.

If the fluorescence feature was transported offshore and to depth along sloping isopycnals, as suggested by the distribution of chl a on sigma-t (see fig. 2.9), then distributions of isopycnal surfaces will provide insight into potential source waters. Isopycnal analyses of the EBC large scale surveys reveal that all isopycnal surfaces slope steeply towards the coast, with typical depth differences of > 100 m between the shallowest point and the deepest point offshore (Huyer et al., 1996). The highest fluorescence values in the feature are centered between the 26.2 and 26.4 isopycnals.
These surfaces were never shallower than 100 m and 140 m, respectively, during the June large scale survey (J. Barth, pers. comm.), which sampled sampled waters seaward of the continental shelf. However, these isopycnals do rise to depths within the euphotic zone very close to the coast, and were observed in the upper 50 m of the water column within 5 km of the California coast during CODE Huyer, 1984). Thus if the phytoplankton biomass was constrained to this isopycnal range, the origin of this feature must have been very near the coast.

Specific upwelling events can be used to identify the timing of potential phytoplankton blooms near the coast. Alongshore winds and the Bakun upwelling index at 39°N during spring of 1993 are shown in Figure 4.1. The winds were measured at a NOAA meteorological station off Pt. Arena and the Bakun upwelling index data was made available by the NOAA/NMFS Pacific Fisheries Environmental Group in Monterey, CA. The Bakun upwelling index provides a broader scale indication of the rate of upwelling than the local winds measured at a single meteorological station. The upwelling index is

![Figure 4.1. Spring 1993 alongshore wind (solid line) at 39°N, 123.7°W, and weekly Bakun upwelling indices at 39°N, 125°W (dashed line). Negative wind speeds indicate equatorward winds; 1 m s⁻¹ = 2.2 miles per hour. Upwelling indices > 50 reflect strong upwelling; negative values reflect downwelling.](image-url)
derived from atmospheric pressure data, and the units may be thought of as indicative of the average amount of water (cubic meters) upwelled through the bottom of the Ekman layer each second along 100 m of coastline on a scale of about 200 miles (Bakun, 1973). Upwelling events off the Oregon coast with indices > 50 were identified as "strong" events by Small and Menzies (1981). Three periods of upwelling favorable winds stand out in the wind data: one beginning ~ April 10 (julian day 101); one in late April-early May (beginning ~ julian day 116); and one beginning in early June (~ julian day 156) and persisting through the late June survey period. These events are evident in the Bakun index as well, although the early April event is weak.

The deep phytoplankton biomass observed in Small Scale Survey 1 was associated with an eddy/jet feature, and was situated where the equatorward jet bounded the offshore edge of the cyclonic eddy. The question arises as to whether the biomass was entrained and transported in the eddy, or was transported in the meandering jet, and passed the eddy at the time of the survey by chance. The eddy originated over the shelf break in April and translated WSW at about .05 m s\(^{-1}\) on average during the survey period (Kosro et al., 1994). The local wind records show an upwelling favorable wind event in early April, which registers as a weak event in the Bakun upwelling index. It is possible that the biomass observed in SSS1 originated from the early April wind event off Pt. Arena, and was subsequently entrained and transported in the eddy. Alternatively, if either of the larger and more persistent upwelling events in May and June wind were the biomass sources, then the biomass was generated after the eddy separated from the coastal region. By late May, the eddy was well offshore (~ 38.5N, 125W); if the biomass originated during the May-June coastal upwelling events, it was most likely transported offshore in the jet prior to its association with the eddy.

Potential source regions for the deep feature can be identified from the timing of upwelling favorable wind events and estimated horizontal velocities. Velocities in the EBC jet ranged from ~ .1 m s\(^{-1}\) to > .5 m s\(^{-1}\), with higher velocities near the surface. Thus the horizontal velocity that a phytoplankton assemblage is exposed to depends on its vertical position in the jet, as well as where it is positioned horizontally relative to the high velocity core. If I assume that the biomass originated from an early June bloom (e.g., June 10) and was transported along the edge of the jet at a mean velocity of .20 m s\(^{-1}\) up until the time of the survey, the source region would be 350 km upstream, or just north of the Large Scale Survey 1 (LSS1) grid (see Figure 2.3). Since the jet was closest to the coast in the northeastern portion of the survey grid during the June large scale survey, and did not return to the coast during the remainder of the study period, it is plausible that the feature originated in or upstream of this region.
Alternatively, horizontal velocities can be estimated based on the timing of upwelling favorable wind events and a speculated source region. For simplicity I will assume that the biomass originated north of Pt Arena at ~ 39.5N, 124W, approximately 300 km upstream of the SSS1 survey region. If a June bloom is the source, and biomass was transported along the jet between the assumed date of June 10 and the time of the survey, the mean horizontal velocity was .17 m s\(^{-1}\). If the source was a May bloom (e.g., May 10), the horizontal velocity is reduced to .07 m s\(^{-1}\). An earlier source date requires that the the biomass spent more time in low flow regions, i.e. at depth or at the outer edge of the jet.

Although observations of phytoplankton at depth (~200 m) and seaward of the coastal upwelling center suggest that water mass subduction is occurring in this system as the coastal jet meanders offshore, the possibility that phytoplankton are sinking through the water, rather than subducted with it, warrents consideration. The primary argument against sinking and in favor of subduction is that reported phytoplankton sinking rates of ~1 m d\(^{-1}\) (Bienfang, 1981; Bienfang et al., 1983) are not rapid enough to transport cells to the depths at which they were observed. For example, if the biomass observed at 200 m in SSS1 arrived at that depth by sinking at a rate of 1 m d\(^{-1}\), it would have been near the surface (25 m) 175 days prior to the late June survey. This requires a phytoplankton bloom in January, which is inconsistent with coastal upwelling dynamics along this coast (e.g., Small and Menzies, 1981). It is more plausible that the deep biomass originated from a surface bloom following the onset of upwelling favorable winds in May or June. Transport of this biomass offshore and to depth would require a net vertical velocity of 3-9 m day\(^{-1}\).

It is possible for diatoms to sink much faster than 1 m d\(^{-1}\) under certain conditions, and it has been argued that rapid mass sinking of diatom blooms is a widespread and recurring survival mechanism (Smetacek, 1985). Sinking is of value to survival in species able to withstand long periods of darkness (as was demonstrated for the diatom *T. weissflogii* in our lab experiment), while sinking out of nutrient depleted surface water. Sinking velocities on the order of 100 m d\(^{-1}\) have been estimated for diatom assemblages observed in the field (Platt et al., 1983; Billett et al., 1983; v. Bodundgen et al., 1981 in Smetacek, 1985). These high rates are effected by a combination of particle aggregation (facilitated by mucous formation in senescent cells), and physiologically controlled buoyancy reduction. In most diatoms, sinking rates are inversely correlated with growth rates; actively growing cells are nuetrally buoyant, whereas sinking rates increase upon nutrient depletion (Smetacek, 1985 and authors therein). Sinking rates can subsequently decrease when high nutrient conditions (Bienfang, 1981) or low light levels (Bienfang et al., 1983)
are encountered. Sinking rates of nutrient-depleted individual cells and chains of diatoms measured in vitro are less than 10 m d\(^{-1}\) (Smayda, 1970).

The above considerations suggest that vertical transport of the phytoplankton biomass observed in the EBC surveys could have resulted from a combination of both sinking and subduction processes. Given these two mechanisms, there are a variety of scenarios which could explain the origin, timing and transport pathways of the deep fluorescence feature reported here. A simplified conceptual model of the possible origin and transport of the deep feature observed during the EBC project is shown in Figure 4.2. This figure depicts a coastal upwelling phytoplankton bloom which is advected offshore and equatorward in a meandering jet. During this time, the phytoplankton assemblage is subject to a net horizontal velocity of 0.20 m s\(^{-1}\) and a net vertical velocity of 10 m d\(^{-1}\). This vertical velocity may result from physiologically controlled sinking from surface waters to a deeper density layer (~26.4) followed by flow along sloping isopycnals; the water mass in which the biomass is located is downwelled further due to a gain in relative vorticity as the jet approaches the cyclonic meander in which the feature is observed. Given these velocities, the biomass is transported 350 km downstream from its origin north of Pt. Arena in a period of 20 days, arriving at the area of the SSS1 survey grid at a depth of 200 meters. If the laboratory results for *T. weissflogii* fluorescence under dark conditions (presented in Chapter 3) hold true for natural populations, the fluorescence signal should persist below the euphotic zone over this time scale. Forthcoming papers by EBC investigators are likely to include analyses of potential source waters and of the physical mechanisms governing vertical velocities in the EBC jet.

**Ecological Implications**

**Carbon transport**

The net exchange of carbon dioxide between the atmosphere and the ocean, and thus the ocean’s role in the global carbon cycle, is dominated by carbon transport into and out of the ocean’s upper layer (Michaels et al., 1994). Our observations of phytoplankton biomass at 200 m depth in waters seaward of the continental shelf have potential implications for two components of the oceanic carbon budget: cross-shelf exchange and vertical flux from surface waters to depth. These field observations illustrate the potential importance of advective processes in controlling carbon distributions. Michaels et al. (1994) suggested that advection is the most difficult component of the carbon budget to
interpret and measure, and that imbalances in the carbon budget off Bermuda may be due
to failure to account for horizontal advection of biomass.

Cross-shelf carbon transport

It has been suggested that that carbon budgets for continental shelf ecosystems are not
balanced, and that large fractions of organic matter produced on continental shelves are
exported (e.g., Malone et al., 1983; Walsh et al., 1981). Malone et al. estimated that $4.8 \times 10^6$
metric tons C, or 90% of primary production produced during the spring diatom
bloom, may be exported from shelf waters of the New York Bight to the continental slope.
On a unit area basis, this is equivalent to the annual shelf export of $3 \times 10^9$ tons C yr$^{-1}$
calculated by Walsh et al. (1981) for shelf ecosystems in general. These carbon budgets,
based on autotrophic production of organic carbon and heterotrophic consumption, are
subject to debate since there are inaccuracies associated with measurements of each
component. For example, Rowe et al. (1986) tested the export hypothesis for the NW
Atlantic shelf by searching for an imbalance between primary production and heterotrophic use of carbon, and found no evidence to support the hypothesis that a significant amount of carbon is exported from the continental shelf.

The EBC observations of high concentrations of phytoplankton biomass in waters seaward of the continental shelf suggest that organic carbon of coastal origin is exported off the shelf in the California Current System. To put the magnitude of this possible export pathway into perspective, the carbon content of the feature can be expressed relative to the amount of carbon fixed in the coastal upwelling region over the continental shelf. Based on an estimated annual primary productivity of 420 g C m\(^{-2}\) yr\(^{-1}\) for upwelling ecosystems (Martin et al., 1987), the estimated carbon content of the deep feature (2.2 x 10\(^4\) metric tons C) is equivalent to the amount of carbon fixed annually under a 5 km x 10 km area in an upwelling region. Or, expressed on the scale of an upwelling event (~ 1 week), the feature represents the amount of carbon fixed under a 5 km x 220 km area in one week during the upwelling season. This latter estimate is based on the assumption that 75% of the 420 g C m\(^{-2}\) yr\(^{-1}\) is fixed during a four month upwelling season.

Processes that are likely to play a dominant role in the export of coastal phytoplankton biomass from the shelf include horizontal transport of phytoplankton along the edge of meandering jets, and eddy shedding, or ‘cyclogenesis’. Subduction of surface waters to depth may play a secondary role in offshore deposition by removing biomass from high flow regions of surface-intensified jets. However, it is likely that although the jets meander both seaward and shoreward along a general southerly path, they do not re-enter shelf waters once they have separated from the shelf (J. Barth, pers. comm.). Thus, once phytoplankton biomass is transported off the shelf in a meandering jet, it not likely to return.

**Contribution to the ‘biological pump’**

Subduction of phytoplankton may provide a mechanism for enhancing the ‘biological pump’ by contributing to the flux of organic matter from surface waters to the deep ocean. The continual downward flux of biogenic carbon in the ocean, and eventual sequestration in the sediments, is considered a central component of global carbon cycles (Karl & Winn, 1991). This component of the ‘biological pump’ (e.g., Moore & Bolin, 1987) plays an important role in determining the nature of the oceanic carbon sink. It has been estimated that ~ 7.4 gigatons (1 gigaton = 10\(^{15}\) g.) of carbon can be removed annually from surface waters of the world’s oceans via the biological pump (Martin et al., 1987). This value
represents 10-20% of global primary production and approximates the annual input of CO₂ to the atmosphere by fossil fuel burning (Karl & Winn, 1991).

Only a portion of the carbon removed from surface waters is ultimately sequestered in ocean sediments. An estimated 50% of the organic matter exported from open ocean surface waters is regenerated to CO₂ via respiration by mesopelagic organisms at depths less than 300 m; 90% is regenerated by 1500 m and less than 5% reaches sediments on the ocean floor (Martin et al., 1987). These numbers were derived from sediment trap fluxes measured during the Vertical Transport and Exchange (VERTEX) program in the Northeast Pacific, with station locations ranging from the western edge of the California Current, offshore to the central gyre, and extending southward to the North Pacific Equatorial Current. All stations were characterized by nutrient depletion in the mixed layer and deep chlorophyll maxima located in or above the nitracline.

The potential contribution of the observed deep biomass feature to the biological pump via vertical transport from surface waters to deep ocean sediments can be assessed using the carbon flux data reported by Martin et al. (1987). To evaluate this, I assumed the simplest possible vertical transport pathway, in which the observed biomass (containing 2.2 x 10⁴ metric tons C) was deposited on the seafloor directly below it. Based on this transport pathway and the open ocean regeneration rates reported by Martin et al. (1987), the deep feature could contribute .29 - .58 g C m⁻² to ocean sediments seaward of the continental shelf. This contribution is substantial relative to reported deposition rates of 1 to a few grams C m⁻² yr⁻¹ (e.g., Martin et al., 1987; Mann & Lazier, 1991 (chapter 10)) for deep ocean sediments. The estimated carbon deposition reported here represents a single event. If a few of these events were to occur in a single year, carbon deposition could surpass conventional estimates for open ocean sedimentary flux. This implies that sediment trap data may underestimate vertical carbon flux by missing episodic advection events, a possibility proposed by Michaels et al. (1994).

**Deep phytoplankton features as a food source**

Phytoplankton at 200 m represent a food source for mesopelagic organisms. The main hypotheses proposed to explain vertical migration of zooplankton share the assumption that food is more abundant and of better quality in the surface layer than at depth (Williamson et al., 1996). The observations reported here suggest that midwater food concentrations may occasionally exceed surface concentrations: the carbon content below the euphotic zone in this survey was ~ 2.5 times the quantity in surface layer above it. If 50% of the carbon flux from the surface layer is regenerated in the upper 300 m (Martin et al., 1987), then the deep feature reported here contributed 1.1 x 10⁴ to 2.2 x 10⁴
metric tons carbon as food to consumers in these waters, which corresponds to 23 - 47 ug l⁻¹. This range depends on the portion of initial biomass which had already been respired at the time of observation: the high end assumes that 50% of initial biomass has already been respired, and the low end assumes that 50% of the biomass observed at the time of the survey will be respired. The vertical transport rate will also affect the amount of carbon respired in the water column.

The magnitude of the biomass feature as a zooplankton food source is illustrated by a consideration of abundances and respiratory requirements of zooplankton in the California Current System. Fessenden and Cowles (1995) estimated the respiratory requirements of calanoid copepods in Oregon coastal waters to range from .6 to 6.5 ug C copepod⁻¹ d⁻¹. Copepods dominate the nearshore zooplankton community during the upwelling season, with reported abundances ranging from 500 to 2,000 per m³ over the Oregon shelf (Peterson and Miller, 1976). If these abundances and respiratory requirements apply to grazer populations in the EBC study area, the deep feature contained enough carbon to fulfill the respiratory requirements of the zooplankton community for 6 to 20 days. This estimate is conservative, since zooplankton abundances are likely to be lower offshore where the deep phytoplankton feature was observed than over the shelf in the upwelling region.

Summary

There are several ways in which physical processes affect phytoplankton distributions within the complex mesoscale fields of the California Current System. Observations of phytoplankton at depths below the euphotic zone have been presented here as evidence that water mass subduction is occurring in this system as the coastal jet meanders offshore. The subduction evidence reported here, and in the literature, is based partly on the assumption that phytoplankton could not have grown below the euphotic zone at the depths at which they were observed. However, the extent to which advective processes can be inferred from observations of deep fluorescence features is limited by our understanding of dark survival and the behavior of in vivo fluorescence at depth.

In the laboratory study presented in chapter 3, in vivo fluorescence of the diatom T. weissflogii levelled off to 45% of its initial value during two months of complete darkness, and a metabolically active population was maintained that was capable of exponential growth when returned to favorable conditions (light). This indicates that the in vivo fluorescence signal can persist in the dark over time scales relevent to subducted
phytoplankton assemblages. If our lab results for *T. weissflogii* extend to natural populations, then light limitation of photosynthesis does not preclude the survival of subducted phytoplankton assemblages and the consequent accumulation of chlorophyll *a* at depths below the euphotic zone. Thus it may not be possible to obtain estimates of advective time scales based on a maximum persistence time of chlorophyll *a* fluorescence below the euphotic zone.

Given the ability of diatom populations to survive and fluoresce during prolonged periods of darkness, why haven’t regions of deep chlorophyll biomass been observed more often in systems such as the California Current? One explanation is that the complete set of physical processes resulting in subduction of phytoplankton occur infrequently. Alternatively, subducted phytoplankton cells may be cropped by midwater grazers before biomass accumulates at depth. The final and most probable explanation is that inadequate sampling (both vertically and the horizontally) is responsible for the apparent paucity of subducted phytoplankton assemblages. Surface waters tend to be the focus of biological sampling efforts, since phytoplankton assemblages are not expected to be found in the dark. Additionally, inadequate horizontal resolution may account for some features remaining unobserved. Even in high resolution field surveys using *in situ* vehicles such as the Seasoar, limitations in resolution of biological features may be imposed by the episodic nature of coastal upwelling blooms. A pulse of coastally generated phytoplankton biomass carried offshore in a jet and subducted to depth could easily be missed in a non-synoptic, large scale survey.

Observations of phytoplankton below the euphotic zone have a wide scope of implications, ranging from the cellular level to meso and global scales. On the cellular level, deep phytoplankton assemblages raise questions about photoadaptation and dark survival of phytoplankton. These deep features also lend evidence for water mass subduction, a component of mesoscale circulation which is not well understood. Finally, these deep features may represent a substantial flux of carbon relative to the normal rain of biogenic material out of the euphotic zone. The biomass observed at ~ 200 m during the EBC program contained an estimated 2.2 x 10^4 metric tons of carbon, and thus represents a substantial source of food to mesopelagic consumers. If these phytoplankton originated from a coastal upwelling source, transport of this biomass off the continental shelf and to depth potentially represents an advection term in the carbon budget of the northeast Pacific which has been previously overlooked.
BIBLIOGRAPHY


APPENDICES
## Results summary

Sampling schedule and replicate means ± 1 SE of variables measured during dark incubation. Accessory pigment concentrations determined by HPLC, and multi-excitation fluorescence ratios, calculated at each *in vivo* fluorescence sampling, are not shown. 

( ) = number of replicates sampled on given day. Dash indicates no sampling.

<table>
<thead>
<tr>
<th>Day Number</th>
<th><em>In vivo</em> Chl a Fluorescence (intensity)</th>
<th>Cell Counts (total cells ml(^{-1}))</th>
<th>Extracted Chlorophyll a (µg l(^{-1}))</th>
<th>HPLC Chl a (µg l(^{-1}))</th>
<th>[Nitrate + Nitrite] (µM)</th>
<th>Particulate Organic Carbon (µg ml(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>137 ± 1 (3)</td>
<td>3.7E4 ± 2.1E3 (3)</td>
<td>62.4 (1)</td>
<td>37.7 (1)</td>
<td>354 ± 3 (3)</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>150 ± 3 (2)</td>
<td>5.0 E4 ± 1.9E3 (3)</td>
<td>58.5 (1)</td>
<td>39.3 (1)</td>
<td>338 ± 19 (3)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>119 ± &lt;1 (2)</td>
<td>4.2E4 ± 5.4E3 (3)</td>
<td>66.1 ± 7.3 (2)</td>
<td>-</td>
<td>405 ± 45 (3)</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>109 ± 1 (3)</td>
<td>2.2E4 ± 2.8E3 (3)</td>
<td>63.0 (1)</td>
<td>38.2 (1)</td>
<td>375 ± 36 (3)</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>110 ± 2 (3)</td>
<td>2.8E4 ± 1.8E2 (3)</td>
<td>-</td>
<td>-</td>
<td>360 ± 17 (3)</td>
<td>1.1 ± .2 (2)</td>
</tr>
<tr>
<td>5</td>
<td>103 ± 1 (3)</td>
<td>2.5E4 ± 1.1E3 (3)</td>
<td>57.7 (1)</td>
<td>-</td>
<td>463 ± 36 (2)</td>
<td>-</td>
</tr>
</tbody>
</table>
### Appendix A (continued)

<table>
<thead>
<tr>
<th>Day Number</th>
<th><em>In vivo</em> Chl <em>a</em> Fluorescence (intensity)</th>
<th>Cell Counts (total cells ml⁻¹)</th>
<th>Extracted Chlorophyll <em>a</em> (μg l⁻¹)</th>
<th>HPLC Chl <em>a</em> (μg l⁻¹)</th>
<th>[Nitrate + Nitrite] (μM)</th>
<th>Particulate Organic Carbon (μg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>82 ± 7 (3)</td>
<td>2.8E4 ± 8.8E2 (3)</td>
<td>64.7 ± 4.9 (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>97 ± 4 (3)</td>
<td>3.0E4 ± 4.4E2 (3)</td>
<td>-</td>
<td>-</td>
<td>356 ± 1 (3)</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>91 ± 4 (3)</td>
<td>2.7E4 ± 3.1E3 (3)</td>
<td>69.5 (1)</td>
<td>38.4 (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>79 ± 7 (3)</td>
<td>2.4E4 ± 6.7E3 (3)</td>
<td>57.7 ± 5.2 (2)</td>
<td>-</td>
<td>354 ± 2 (3)</td>
<td>1.3 (1)</td>
</tr>
<tr>
<td>20</td>
<td>84 ± 7 (3)</td>
<td>2.6E4 ± 2.7E3 (3)</td>
<td>57.7 ± 11.4 (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>73 ± 1 (3)</td>
<td>1.7E4 ± 3.1E3 (3)</td>
<td>-</td>
<td>39.6 (1)</td>
<td>363 ± 3 (3)</td>
<td>1.6 (1)</td>
</tr>
<tr>
<td>34</td>
<td>68 ± 4 (3)</td>
<td>2.4E4 ± 2.5E3 (3)</td>
<td>56.4 ± 1.3 (2)</td>
<td>-</td>
<td>363 ± 2 (3)</td>
<td>2.1 (1)</td>
</tr>
<tr>
<td>41</td>
<td>60 ± 2 (3)</td>
<td>1.8E4 ± 1.3E3 (3)</td>
<td>57.7 ± 0 (2)</td>
<td>-</td>
<td>363 ± 5 (3)</td>
<td>2.1 (1)</td>
</tr>
</tbody>
</table>
### Appendix A (continued)

<table>
<thead>
<tr>
<th>Day Number</th>
<th>$In \text{vivo} \text{ Chi a}$ Fluorescence (intensity)</th>
<th>Cell Counts (total cells ml$^{-1}$)</th>
<th>Extracted Chlorophyll a (μg l$^{-1}$)</th>
<th>HPLC Chi a (μg l$^{-1}$)</th>
<th>[Nitrate + Nitrite] (μM)</th>
<th>Particulate Organic Carbon (μg ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>61 ± 2 (3)</td>
<td>2.0E4 ± 2.5E3 (3)</td>
<td>31.0 (1)</td>
<td>31.0 (1)</td>
<td>337 ± 18 (3)</td>
<td>-</td>
</tr>
<tr>
<td>55</td>
<td>59 ± 7 (3)</td>
<td>1.9E4 ± 1.0E3 (3)</td>
<td>34.0 (1)</td>
<td>-</td>
<td>367 ± &lt;1 (2)</td>
<td>1.4 ± .3 (2)</td>
</tr>
<tr>
<td>62</td>
<td>54 ± 4 (2)</td>
<td>1.7E4 ± 3.4E3 (2)</td>
<td>39.1 ± 5.0 (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Appendix B

Safire calibration

A calibration was performed to determine whether the relationship between in vitro (extracted) chlorophyll concentration and in vivo fluorescence - measured by SAFIRE - was linear within the range of experimental concentrations. A Thalassiosira weissflogii culture in log growth phase was diluted into a series of 8 flasks of varying cell concentrations. Both extracted and in vivo chlorophyll fluorescence were determined on aliquots from each flask. Chlorophyll a concentrations in the culture flasks ranged from .16 to 25.73 μg l⁻¹. The extracted chlorophyll concentration per unit in vivo chlorophyll a fluorescence was calculated using a simple linear regression model. The instrument response was linear within the experimental range of chlorophyll a concentrations (Figure below), and can be described by the following model:

\[
\text{Extracted chl } a = 0.021 \times \text{in vivo fluorescence counts} \\
(\text{SE}=0.001)
\]

\[r^2=0.99; \ n=8\]

where in vivo fluorescence is measured by SAFIRE at 685 nm emission and 435 nm excitation and extracted chl a is measured in μg l⁻¹.

![Graph showing the relationship between in vivo chlorophyll a fluorescence and extracted chlorophyll a concentration.](image)
Variability in FDA stained cell counts

The high variability in numbers of FDA stained cells during the original run of the laboratory experiment presented in chapter 3 is puzzling, and we suspect that the values observed during the mid and latter part of the experiment are erroneous. During the repeat trial, stained cells comprised 84% of total cell numbers for the duration of the 10 week incubation. This percentage is comparable to the percentage of living cells observed during the first 3 weeks of the original run (82%), suggesting that the low counts of stained cells observed during the mid and latter part of the original experiment were erroneous. The FDA measurements made during the repeat trial were verified by an independent measure of metabolic activity. On two sampling dates during the repeat trial, sample aliquots were stained with the vital stain Celltracker\textsuperscript{TM} (Molecular Probes, Inc.) at a final concentration of 5 \textmu M, in addition to aliquots stained with FDA. Cell counts from these trials indicated that 85% (S.E.=7%; n=3) of the total cells were alive, confirming the FDA results.

The stained cell counts during the mid and latter part of the first trial may be erroneous due to a problem in methodology. There were two differences in the FDA staining procedures between the original and repeat trials. During the original trial, the FDA stock solution was stored frozen in a 20 ml scintillation vial and was thawed on each sampling date. Thus, the stock solution was repeatedly thawed and refrozen during the experimental period. A fresh batch of working solution was made on each sampling date from the thawed stock solution, and was kept in the dark at 12\textdegree C during sampling. During the repeat trial, the FDA stock solution was stored frozen in individual 10 \textmu l aliquots. On each sampling day, a 10 \textmu l aliquot was thawed and used to make working solution, and the working solution was kept in the dark and on ice during sampling. The variability in counts of stained cells in the original trial may be attributed to the fact that the working solution was not kept on ice during sampling and/or to repeated thawing and refreezing of the stock solution. However, fresh stock solution was made up once during the original experiment, and the percentage of living cells was low (30%) on the following day.

It is likely that there is another cause of variability in counts of FDA positive cells in addition to (or rather than) the temperature of the working solution and the repeated thawing and refreezing of the stock solution. We offer no conclusive explanation, but certain observations made during the repeat trial are worth noting. In eight cell counts out of 30, distinct differences in the percentage of stained cells were observed across the filter.
On these occasions, cells were stained bright green in the center of the filter and cells on the periphery of the filter were stained very faintly or not at all. The relative area of faint/unstained cells to stained cells was variable and difficult to quantify. Based on rough visual estimates, the area of faint/unstained cells ranged from 1% to 40% of the filter, and the percentage of stained cells in these regions ranged from 0% to 20%. When these anomalies were observed, cell counts were made in the region of the filter where the stain was most apparent, which could result in an overestimation of the percentage of living cells. In the most extreme case, if all cells were FDA negative across 40% of the filter, and cell counts were made in the remaining 60% of the filter in which 85% of the cells were FDA positive, the mean percentage of living cells across the entire filter would be 51%. A more realistic scenario is that in which 20% of the cells are FDA positive across 20% of the filter, and 85% of the cells are FDA positive across the remaining 80% of the filter. In the latter case, the mean percentage of living cells across the entire filter would be 72%.

In light of the variability associated with the stained cell counts, how confident are we in these numbers? Results from the repeat trial demonstrate that metabolic activity can be sustained in a large portion of a population of *T. weissflogii* cells held in the dark for 10 weeks. The estimate of 84% living cells for the repeat trial may be high; nonetheless, the percentage of living cells was at least 50% and most likely between 72% and 84%. The across-filter variability in stained cells observed in the repeat trial was also observed on one date during the original run and may have occurred on other dates as well. Selvin et al. (1988) reported an anomalously low value of FDA positive cell counts during a dark incubation of *Prorocentrum lima*. This dinoflagellate maintained cell division when held in the dark for three weeks, and metabolic activity, as assessed by FDA, was as high as 98% to 100% in all samples but one. On one sampling date, staining was irregular and only 30% of the cells were identified as FDA positive; no explanation was offered. There is clearly uncertainty associated with our estimates of cell viability based on the FDA assay. However, we can confidently say that under conditions of prolonged (2 months) darkness, a metabolically active population of *T. weissflogii* is maintained that is capable of exponential growth when returned to light favorable conditions.