Ocean Optics Protocols For Satellite Ocean Color Sensor Validation, Revision 4, Volume V:

Biogeochemical and Bio-Optical Measurements and Data Analysis Protocols

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Preface

This document stipulates protocols for measuring bio-optical and radiometric data for the Sensor Intercomparison and Merger for Biological and Interdisciplinary Oceanic Studies (SIMBIOS) Project activities and algorithm development. The document is organized into 7 separate volumes as:

**Ocean Optics Protocols for Satellite Ocean Color Sensor Validation, Revision 4**

**Volume I: Introduction, Background and Conventions**

**Volume II: Instrument Specifications, Characterization and Calibration**

**Volume III: Radiometric Measurements and Data Analysis Methods**

**Volume IV: Inherent Optical Properties: Instruments, Characterization, Field Measurements and Data Analysis Protocols**

**Volume V: Biogeochemical and Bio-Optical Measurements and Data Analysis Methods**

**Volume VI: Special Topics in Ocean Optics Protocols**

**Volume VII: Appendices**

The earlier version of *Ocean Optics Protocols for Satellite Ocean Color Sensor Validation, Revision 3* (Mueller and Fargion 2002, Volumes 1 and 2) is entirely superseded by the seven Volumes of Revision 4 listed above.

The new multi-volume format for publishing the ocean optics protocols is intended to allow timely future revisions to be made reflecting important evolution of instruments and methods in some areas, without reissuing the entire document. Over the years, as existing protocols were revised, or expanded for clarification, and new protocol topics were added, the ocean optics protocol document has grown from 45pp (Mueller and Austin 1992) to 308pp in Revision 3 (Mueller and Fargion 2002). This rate of growth continues in Revision 4. The writing and editorial tasks needed to publish each revised version of the protocol manual as a single document has become progressively more difficult as its size increases. Chapters that change but little, must nevertheless be rewritten for each revision to reflect relatively minor changes in, e.g., cross-referencing and to maintain self-contained consistency in the protocol manual. More critically, as it grows bigger, the book becomes more difficult to use by its intended audience. A massive new protocol manual is difficult for a reader to peruse thoroughly enough to stay current with and apply important new material and revisions it may contain. Many people simply find it too time consuming to keep up with changing protocols presented in this format - which may explain why some relatively recent technical reports and journal articles cite Mueller and Austin (1995), rather than the then current, more correct protocol document. It is hoped that the new format will improve community access to current protocols by stabilizing those volumes and chapters that do not change significantly over periods of several years, and introducing most new major revisions as new chapters to be added to an existing volume without revision of its previous contents.

The relationships between the Revision 4 chapters of each protocol volume and those of Revision 3 (Mueller and Fargion 2002), and the topics new chapters, are briefly summarized below:

**Volume I:** This volume covers perspectives on ocean color research and validation (Chapter 1), fundamental definitions, terminology, relationships and conventions used throughout the protocol document (Chapter 2), requirements for specific *in situ* observations (Chapter 3), and general protocols for field measurements, metadata, logbooks, sampling strategies, and data archival (Chapter 4). Chapters 1, 2 and 3 of Volume I correspond directly to Chapters 1, 2 and 3 of Revision 3 with no substantive changes. Two new variables, Particulate Organic Carbon (POC) and Particle Size Distribution (PSD) have been added to Tables 3.1 and 3.2 and the related discussion in Section 3.4; protocols covering these measurements will be added in a subsequent revision to Volume V (see below). Chapter 4 of Volume I combines material from Chapter 9 of Revision 3 with a brief summary of SeaBASS policy and archival requirements (detailed SeaBASS information in Chapter 18 and Appendix B of Revision 3 has been separated from the optics protocols).

**Volume II:** The chapters of this volume review instrument performance characteristics required for *in situ* observations to support validation (Chapter 1), detailed instrument specifications and underlying rationale (Chapter 2) and protocols for instrument calibration and characterization standards and methods (Chapters 3 through 5). Chapters 1 through 5 of Volume II correspond directly to Revision 3 chapters 4 through 8, respectively, with only minor modifications.

**Volume III:** The chapters of this volume briefly review methods used in the field to make the *in situ* radiometric measurements for ocean color validation, together with methods of analyzing the data (Chapter 1),
detailed measurement and data analysis protocols for in-water radiometric profiles (Chapter 2), above water measurements of remote sensing reflectance (Chapter III-3), determinations of exact normalized water-leaving radiance (Chapter 4), and atmospheric radiometric measurements to determine aerosol optical thickness and sky radiance distributions (Chapter 5). Chapter 1 is adapted from relevant portions of Chapter 9 in Revision 3. Chapter 2 of Volume III corresponds to Chapter 10 of Revision 3, and Chapters 3 through 5 to Revision 3 Chapters 12 through 14, respectively. Aside from reorganization, there are no changes in the protocols presented in this volume.

**Volume IV**: This volume includes a chapter reviewing the scope of inherent optical properties (IOP) measurements (Chapter 1), followed by 4 chapters giving detailed calibration, measurement and analysis protocols for the beam attenuation coefficient (Chapter 2), the volume absorption coefficient measured in situ (Chapter 3), laboratory measurements of the volume absorption coefficients from discrete filtered seawater samples (Chapter 4), and in situ measurements of the volume scattering function, including determinations of the backscattering coefficient (Chapter 5). Chapter 4 of Volume IV is a slightly revised version of Chapter 15 in Revision 3, while the remaining chapters of this volume are entirely new contributions to the ocean optics protocols. These new chapters may be significantly revised in the future, given the rapidly developing state-of-the-art in IOP measurement instruments and methods.

**Volume V**: The overview chapter (Chapter 1) briefly reviews biogeochemical and bio-optical measurements, and points to literature covering methods for measuring these variables; some of the material in this overview is drawn from Chapter 9 of Revision 3. Detailed protocols for HPLC measurement of phytoplankton pigment concentrations are given in Chapter 2, which differs from Chapter 16 of Revision 3 only by its specification of a new solvent program. Chapter 3 gives protocols for Fluorometric measurement of chlorophyll concentrations, and is not significantly changed from Chapter 17 of Revision 3. New chapters covering protocols for measuring, Phycoerythrin concentrations, Particle Size Distribution (PSD) and Particulate Organic Carbon (POC) concentrations are likely future additions to this volume.

**Volume VI**: This volume gathers chapters covering more specialized topics in the ocean optics protocols. Chapter 1 introduces these special topics in the context of the overall protocols. Chapter 2 is a reformatted, but otherwise unchanged, version of Chapter 11 in Revision 3 describing specialized protocols used for radiometric measurements associated with the Marine Optical Buoy (MOBY) ocean color vicarious calibration observatory. The remaining chapters are new in Revision 4 and cover protocols for radiometric and bio-optical measurements from moored and drifting buoys (Chapter 3), ocean color measurements from aircraft (Chapter 4), and methods and results using LASER sources for stray-light characterization and correction of the MOBY spectrographs (Chapter 5). In the next few years, it is likely that most new additions to the protocols will appear as chapters added to this volume.

**Volume VII**: This volume collects appendices of useful information. Appendix A is an updated version of Appendix A in Revision 3 summarizing characteristics of past, present and future satellite ocean color missions. Appendix B is the List of Acronyms used in the report and is an updated version of Appendix C in Revision 3. Similarly, Appendix C, the list of Frequently Used Symbols, is an updated version of Appendix D from Rev. 3. The SeaBASS file format information given in Appendix B of Revision 3 has been removed from the protocols and is promulgated separately by the SIMBIOS Project.

In the Revision 4 multi-volume format of the ocean optics protocols, Volumes I, II and III are unlikely to require significant changes for several years. The chapters of Volume IV may require near term revisions to reflect the rapidly evolving state-of-the-art in measurements of inherent optical properties, particularly concerning instruments and methods for measuring the Volume Scattering Function of seawater. It is anticipated that new chapters will be also be added to Volumes V and VI in Revision 5 (2003).

This technical report is not meant as a substitute for scientific literature. Instead, it will provide a ready and responsive vehicle for the multitude of technical reports issued by an operational Project. The contributions are published as submitted, after only minor editing to correct obvious grammatical or clerical errors.
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Chapter 1

Overview of Biogeochemical Measurements and Data Analysis in Ocean Color Research

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1.1 INTRODUCTION

A total of 9 biogeochemical and bio-optical observations are listed in Tables 3.1 and 3.2. Phytoplankton pigment concentrations determined by the HPLC method, and fluorometric chlorophyll $a$ and pheopigments are required measurements for which detailed protocols are described in Chapters 2 and 3, respectively. Observation of chlorophyll $a$ fluorescence intensity in situ is listed as highly desired, and protocols for its measurement and data analysis are also included in Chapter 3. Six additional biogeochemical observations are listed as specialized measurements. These include concentrations of Phycobiliprotein (Phycoerythrin), and suspended particulate measurements including Coccolith concentrations, total Suspended Particulate Matter (SPM), Particulate Organic Carbon (POC), Particulate Organic Nitrogen (PON, and Particle Size Distribution (PSD). Methods of measurement and data analysis for these specialized observations, most of which are related to applications of ocean color image data to ocean process studies, are reviewed briefly in the present chapter.

1.2 PHYTOPLANKTON PIGMENT CONCENTRATIONS

High Performance Liquid Chromatography (HPLC) Measurements and Analysis (Chapter 2)

Mueller and Austin (1995) simply adopted the JGOFS HPLC protocols for measuring phytoplankton pigment concentrations by reference (UNESCO 1994), and supplemented them with some brief instructions on sampling and sample handling procedures. Although this approach embraced protocol documentation describing a complete methodology, and represented a community consensus, the lack of a comprehensive end-to-end protocol statement has proved to be a source of confusion and debate within the ocean color community. Furthermore, the JGOFS protocols (UNESCO 1994) specified that pigment concentrations should be reported in units of pigment mass per mass of seawater (ng Kg$^{-1}$), rather than in units of pigment mass per volume of seawater (either $\mu$g L$^{-1}$, or mg m$^{-3}$). The use of volumetric concentrations is critical because radiative transfer in the ocean, and absorption by pigments, are volumetric processes. One could use the mass concentration values preferred by JGOFS, but it would be essential to supplement them with densities computed from CTD data, and make the conversion to volumetric concentrations. Therefore, a complete set of protocols for HPLC measurement of phytoplankton pigment concentrations was added as Chapter 13 of Revision 2.0 to the Ocean Optics Protocols (Fargion and Mueller 2000), updated as Chapter 16 of Revision 3 (Mueller and Fargion 2002), and updated here again as Chapter 2 of the present volume. Chapter 2 provides complete protocols for obtaining water samples, filtering them, freezing the filtered samples in liquid nitrogen, sample handling and storage, extraction, HPLC calibrations and measurements, data analysis and quality control. A new HPLC solvent program in Chapter 2 replaces that specified in the previous version of the protocols (Bidegare et al. 2002).

Fluorometric Measurement of Chlorophyll $a$ Concentration (Chapter 3)

For reasons similar to those described above for HPLC pigment measurements, it was decided that the protocols for fluorometric measurement of the concentrations of chlorophyll $a$ and pheopigments were too briefly abstracted in Mueller and Austin (1995). Therefore, new detailed protocols for this measurement were added as Chapter 14 to Revision 2 (Fargion and Mueller 2000), updated as Chapter 17 of Revision 3 (Mueller and Fargion 2002), and reproduced here without significant change as Chapter 3. Chapter 3 provides complete protocols for obtaining water samples, filtering them, freezing the filtered samples in liquid nitrogen, sample handling and storage, extraction, fluorometer calibrations and measurements, data analysis and quality control.
In addition, Chapter 3 discusses geographic and temporal variability in the relationship between fluorimetric chlorophyll concentrations and combined concentrations of total chlorophyll pigments determined by the HPLC methods (Chapter 2). It is both easier and less expensive to measure chlorophyll \( a \) and pheopigment concentrations using the fluorimetric method, which has the added advantage of allowing shipboard analyses at sea during lengthy cruises. When these data are used for remote sensing algorithm development or validation, however, regional and temporal (\textit{i.e.} cruise-to-cruise) dispersions and/or biases may be introduced unless the fluorimetric data are first statistically adjusted (on a local basis) to agree with HPLC determinations of the concentration of total chlorophylls. A cost-effective strategy is to acquire, on each cruise, a majority of filtered pigment samples for fluorimetric chlorophyll \( a \) and pheopigment analysis, supplemented by a smaller number of replicate samples for HPLC pigment analysis. The HPLC replicates should provide a representative distribution over geographic location, depth and time during a cruise, and will be used to determine a local regression relationship between the two measurements. This approach is now required for pigment data submitted for SeaBASS archival and SIMBIOS validation analysis.

\textit{Phycoerythrin and other Phycobiliproteins}

\( R_{RS}(\lambda) \) may be enhanced by fluorescence by phycoerythrin (PE) in a band near 565 nm (\textit{e.g.} Hoge \textit{et al.} 1998; Wood \textit{et al.} 1999). The detection from aircraft of laser-induced phycoerythrin fluorescence is already well established (Hoge \textit{et al.} 1998). It is more difficult to detect and quantify solar induced phycoerythrin fluorescence, but some work has been done in that area as well (Morel \textit{et al.} 1993; Morel 1997; Hoge \textit{et al.} 1999; Subramaniam \textit{et al.} 1999).

Various phycoerythrins differ from one another in chromophore composition. All phycoerythrins contain phycoerythrobilin chromophores [PEB; maximum \( a(\lambda) \) near \( \lambda \sim 550 \text{ nm} \)]; many others also contain phycourobilin chromophores [PUB; maximum \( a(\lambda) \) near \( \lambda \sim 500 \text{ nm} \)], which extends the range of wavelengths absorbed by the pigment molecule into the blue regions of the spectrum. The ratio of PUB:PEB chromophores in the PE pigments synthesized by different \textit{Synechococcus} strains greatly affects the absorption spectrum of the whole cells (Wood \textit{et al.} 1985). Clearly, the dependence of \( a(\lambda) \) on the PUB:PEB ratio of phycoerythrin will affect also \( R_{RS}(\lambda) \) in water masses dominated by cyanobacteria. The PUB:PEB ratio for the PE in a given water mass may be characterized using scanning fluorescence spectroscopy (Wood \textit{et al.}, 1999; Wyman, 1992).

The measurement of phycoerythrin is not yet as routine, nor as accurate, as the measurements of chlorophylls and carotenoids. The techniques introduced by Stewart and Farmer (1984) work well for measuring biliproteins in freshwater and estuarine species, but are less successful for natural populations of marine species. Wyman (1992) reported a linear relationship between the \textit{in vivo} fluorescence emission intensity of PE measured in the presence of glycerol and the PE content of \textit{Synechococcus} strain WH7803. Scanning spectral fluorescence measurements have been used to estimate PE concentration of extracted bulk samples (Vernet \textit{et al.}, 1990). Nevertheless, there are few direct measurements of separated PE proteins from natural samples. High Performance Capillary Electrophoresis (HPCE) is a powerful analytical tool currently used in clinical, biochemical, pharmaceutical, forensic, and environmental research. In HPCE, high voltages (typically 10-30 KV) are used to separate molecules rapidly in narrow-bore (25-100 \( \mu \text{m} \)), fused-silica capillaries based on differences in the charge-to-mass ratio of the analytes. HPCE is an automated analytical separation system with reduced analysis times and on-line quantification of compounds, ideally suited to the separation and quantification of water-soluble proteins (like phycobilins) from seawater. HPCE methods for separation analyses of phycoerythrin from cyanobacterial cultures and natural samples are currently under development and may be included in a future revision to the ocean optics protocols (C. Kinkade, Pers. Comm.).

1.3 \textit{In Situ} Chlorophyll \( a \) Fluorescence

Protocols for measuring and analyzing profiles of \textit{in situ} fluorescence by chlorophyll \( a \), \( F(z) \) (Table 3.1 in Chapter 3, Volume I) are described in Chapter 3. When measured together with \( c(z,660) \) profiles (Chapter 2, Volume IV), the structure of \( F(z) \) provides valuable guidance for selecting depths of water samples, analyses of structure in \( K(z,\lambda) \) derived from radiometric profiles, and various aspects of quality control analysis. It is often useful to digitally record one-minute averages of \( F(z, \text{lat}, \text{lon}) \) in water pumped from a near-surface depth (\( z \sim 3 \text{ m} \)) to measure horizontal variability while underway steaming between stations, especially in water masses where mesoscale and sub-mesoscale variability is strong (Section 4.2, Chapter 4, Vol. I). If supplemented by frequent
fluorometric chlorophyll $a$ samples filtered from the flow-through system, the alongtrack profile of $F(z, \text{lat}, \text{lon})$ can be “calibrated” in units of chlorophyll $a$ concentration (mg m$^{-3}$).

### 1.4 SUSPENDED PARTICLES

#### Suspended Particulate Matter

All total suspended particulate material (SPM) dry weight (mg L$^{-1}$) will be determined gravimetrically as outlined in Strickland and Parsons (1972)$^1$. In general, samples are filtered through preweighed 0.4 µm polycarbonate filters. The filters are washed with three 2.5 mL - 5.0 mL aliquots of DIW and immediately dried, either in an oven at 75$^\circ$ C, or in a dessicator. The filters are then reweighed in a laboratory, back on shore, using an electrobalance with at least seven digits of precision.

#### Particulate Organic Carbon and Particulate Organic Nitrogen

Protocols for measuring concentrations in seawater of Particulate Organic Carbon (POC) and Particulate Organic Nitrogen (PON), as specified for JGOFS (UNESCO 1994, Chapter 15), are also adopted here. The units of POC and PON are µg C Kg$^{-1}$ and µg N Kg$^{-1}$, respectively. Therefore, it is mandatory that each of these measurements be accompanied by Conductivity, Temperature and Pressure measurements so that the density of seawater $[\text{Kg m}^{-3}]$ may be calculated.

#### Particle Size Distributions

Particle size distributions can potentially provide important information about the shape of the volume scattering function, which strongly influences the bidirectional aspects of remote-sensing reflectance (Chapter 4 of Volume III and, e.g., Morel and Gentili 1996). Particle size distributions have been measured for many years using Coulter Counters and related to IOP, including $c(\lambda)$ (e.g. Kitchen et al. 1982). More recently, several investigators have used the Spectrix Particle Size Analyzer to measure particle size distributions (see, e.g., Chapter 2, Vol. VI). Protocols for measurements and analyses of particle size distributions are not included in this version of the ocean optics protocols, but should be written and added to a future revision of this protocol volume.

#### Coccolith Concentrations

Concentrations of coccoliths, calcium carbonate (CaCO$_3$) platelets detached from coccolithophorids (sp.), are measured as cell counts [number density per unit volume] using a microscope with polarization optics (Balch et al. 1991). An epifluorescence microscope is used to count plated and naked intact cells, before and after the coccoliths are dissolved by acidification. Also measured, before and after acidification, are the Volume Scattering Function (VSF) values at three angles, from which the volume specific backscattering coefficient for coccoliths is determined by subtraction (Voss et al. 1998).

### 1.5 FUTURE DIRECTIONS

Future additions to this volume include chapters providing detailed protocols for Phycoerythrin measurement and data analysis, measurements and analyses of coccolith concentrations, and methods for measurement and analyses of SPM, PSD, and the organic suspended particulate fractions POC and PON.

### REFERENCES


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$^1$ In some previous versions of the Ocean Optics Protocols (Mueller and Austin 1992, 1995; Fargion and Mueller 2000), it was incorrectly stated that suitable protocols were part of the JGOFS core measurements protocols (UNESCO 1994). The JGOFS protocols do not include SPM measurements of the type specified here.


UNESCO, 1994:  Protocols for the Joint Global Ocean Flux Study (JGOFS) Core Measurements, Manuals and Guides 29: 170pp


Chapter 2

HPLC Phytoplankton Pigments: Sampling, Laboratory Methods, and Quality Assurance Procedures

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2.1 INTRODUCTION

Marine phytoplankton utilize chlorophyll \( a \) as their major light harvesting pigment for photosynthesis. Other accessory pigment compounds, such as chlorophylls \( b \) and \( c \), carotenoids and phycobiliproteins, also play a significant role in photosynthesis by extending the organism's optical collection window, thereby improving absorption efficiencies and adaptation capabilities. The important chlorophyll degradation products found in the aquatic environment are the chlorophyllides, phaeophorbides, and phaeophytns. The presence, or absence, of the various photosynthetic pigments is used to separate the major algal groups, and to map the chemotaxonomic composition of phytoplankton in the oceans.

The unique optical properties of chlorophyll \( a \) have been used to develop spectrophotometric (Jeffrey and Humphrey 1975) and fluorometric (Holm-Hansen et al. 1965) measurement techniques. With the commercial availability of fluorometers for routine measurements of chlorophyll \( a \), this pigment became a universal parameter in biological oceanography for estimating phytoplankton biomass and productivity. These optical methods can significantly under- or overestimate chlorophyll \( a \) concentrations, because of the overlap of the absorption and fluorescence bands of co-occurring chlorophylls \( b \) and \( c \), chlorophyll degradation products, and accessory pigments (Trees et al. 1985; Smith et al. 1987; Hoepffner and Sathyendranath 1992; Bianchi et al. 1995; Tester et al. 1995).

The application of HPLC to phytoplankton pigment analysis has lowered the uncertainty for measuring chlorophylls \( a \), \( b \), and \( c \), chlorophyll degradation products, and carotenoid pigments, HPLC techniques are recommended. For low uncertainty determinations of chlorophylls \( a \), \( b \), and \( c \), chlorophyll degradation products, and carotenoid pigments, HPLC techniques are recommended. It should be noted, however, that the reverse-phase C\(_{18}\) HPLC method recommended by the Scientific Committee on Oceanographic Research (SCOR) (Wright et al. 1991) is not capable of separating monovinyl chlorophyll \( a \) from divinyl chlorophyll \( a \), nor monovinyl chlorophyll \( b \) from divinyl chlorophyll \( b \). This method, therefore, only provides concentration estimates for these co-eluting pigment pairs; methods for optically resolving monovinyl chlorophyll \( a \) and divinyl chlorophyll \( a \) are given below.

Divinyl chlorophyll \( a \), the major photosynthetic pigment found in Prochlorococcus, accounts for 10 % to 60 % of the total chlorophyll \( a \) in subtropical and tropical oceanic waters (Goericke and Repeta 1993; Letelier et al. 1993; Andersen et al. 1996; Bidigare and Ondrusek 1996; Gibb et al. 2000). Divinyl chlorophyll \( a \) is spectrally different from normal (monovinyl) chlorophyll \( a \) and its presence results in a significant overestimation of total chlorophyll \( a \) concentration as determined by the conventional HPLC methods (Goericke and Repeta 1993; Letelier et al. 1993; Latasa et al. 1996). To avoid these errors, it is recommended that monovinyl and divinyl chlorophyll \( a \) be spectrally resolved, or chromatographically separated, to obtain an unbiased determination of total chlorophyll \( a \) for ground-truthing satellite ocean color algorithms and imagery. Total chlorophyll \( a \), TChl \( a \), is the sum of divinyl chlorophyll \( a \), monovinyl chlorophyll \( a \), chlorophyllide \( a \), and chlorophyll \( a \) epimers and allomers. These co-eluting chlorophyll species can be resolved spectrally following C\(_{18}\) HPLC chromatography (Wright et al. 1991) and quantified using dichromatic equations at 436 nm and 450 nm (Latasa et al. 1996). Alternatively, these two chlorophyll species can be separated chromatographically and individually quantified using C\(_{8}\) HPLC techniques (see below).
The protocols specified below for HPLC pigment analyses follow closely those prescribed in the *JGOFS Core Measurement Protocols* (UNESCO 1994). Both sets of protocols include:

1. Use of Whatman GF/F glass fiber filters, approximately 0.7 µm pore size;
2. Extraction in aqueous acetone; and
3. Calibration with standards.

The present protocols differ from the JGOFS protocols in one critical respect. Absorption of light in seawater, or any other medium, is a volumetric process, even though the volume absorption coefficient may vary with the density of the medium. For ocean color and optical analyses, therefore, the concentrations in seawater of all phytoplankton pigments shall be expressed in units of mass per unit volume of seawater (µg L⁻¹ or mg m⁻³). This differs from the JGOFS protocols, which specify that concentrations in seawater of all phytoplankton pigments should be expressed in ng Kg⁻¹.

In addition to HPLC analyses, it is recommended that the standard fluorometric methodology used for measuring chlorophylls and pheopigments (Holm-Hansen *et al.* 1965, Strickland and Parson 1972) also be applied to the same extracted pigment samples used for HPLC analysis. Protocols for fluorometric measurements of chlorophyll *a* and pheopigments are given here in Chapter 3 of the present volume. For a more in depth review of guidelines for measuring phytoplankton pigments in oceanography see Jeffrey *et al.* (1997)

### 2.2 SAMPLING PROTOCOLS FOR PHYTOPLANKTON PIGMENTS

**Water Samples**

Water samples should be taken using, *e.g.*, Niskin bottles at the site of, and simultaneously with, the surface in-water upwelled radiance and reflectance measurements, and at depth increments sufficient to resolve variability within at least the top optical depth. The \(K(z,\lambda)\), profiles over this layer will be used to compute optically weighted, near-surface pigment concentration for bio-optical algorithm development (Gordon and Clark 1980).

When possible, samples should be acquired at several depths distributed throughout the upper 200 m of the water column [or in turbid water, up to seven diffuse attenuation depths, *i.e.* \(\ln(E(z,\lambda)/E(z,\lambda))=7\), to provide a basis for relating fluorescence signals to pigment mass concentrations.

Samples should be filtered as soon as possible after collection. If processing must be delayed for more than an hour, hold the samples on ice, or in a freezer at 4°C, and protect them from exposure to light. For delays longer than several hours, the samples should be stored in liquid nitrogen. Use opaque sample bottles, because even brief exposure to light during sampling and/or storage might alter pigment values.

**Filtration**

Whatman GF/F glass fiber filters, with approximately 0.7 µm pore size, are preferred for removing phytoplankton from water. The glass fibers assist in breaking the cells during grinding, accommodate larger sample volumes, and do not form precipitates after acidification. Twenty-five mm diameter GF/F glass fiber filters should be used with vacuum (7-8 inches of mercury) or positive pressure (1-2 psi). Positive pressure filtration is recommended, because it filters larger volumes of water at reduced filtration times. The only problem with vacuum filtration is that unobservable air leaks may occur around the filtration holder, and as a result the pressure gradient across the filter is much less than what is indicated on the vacuum gauge. When positive filtration is used, any leakage around the filter holder results in observable dripping water.

Inert membrane filters, such as polyester filters, may be used when size fraction filtration is required. When this is done, it is recommended to also filter a replicate sample through a GF/F to determine the total concentration. Summing the various size-fractionated concentrations may not produce an accurate estimate of the total, because of the potential for cell disruption during filtration.

There has been an ongoing discussion of filter types and retention efficiencies for natural samples. Phinney and Yentsch (1985) showed the inadequacy of GF/F filters for retaining chlorophyll *a* in oligotrophic waters, as did Dickson and Wheeler (1993) for samples from the North Pacific. In response to Dickson and Wheeler (1993),
Chavez et al. (1995) compared samples collected in the Pacific Ocean using GF/F and 0.2 µm membrane filters with small filtered volumes (100 mL to 540 mL). Their results showed a very close agreement between the two filter types, with GF/F filters having only a slightly positive 5% bias.

Filtration volume can directly affect the retention efficiency for GF/F filters. Particles can be retained by filters through a variety of ways, such as filter sieving, filter adsorption, electrostatic and van der Waals attractions (Brock, 1983). When water flows through the pores of a Nuclepore filter, streamlines are formed that can align small particles longitudinally, with the result that cell diameter becomes important with these filters. It is known, on the other hand, that Whatman GF/F filters can retain particles much smaller than their rated pore size. Generally, at small volumes (100 mL to 300 mL) filter adsorption, and electrostatic and van der Waals attractions are important, whereas at larger volumes (>2,000 mL) sieving dominates. This has been tested in oligotrophic waters off Hawaii in which small (<500 mL) and large volumes (> 2 L to 4 L) retained similar amounts of chlorophyll a on the two types of filters, whereas for intermediate sample volumes the GF/F filters showed lower concentrations. During several cruises off the Hawaiian Islands, differences in retention efficiencies were found for GF/F filters to be a function of sample volume; large sample volumes (2 L and 4 L) retained about 18% more chlorophyll a than replicate 1 L samples.

Filtration volumes are usually limited by the concentration of particles present in each sample. For HPLC analysis it is important to filter as large a volume as possible, so as to accurately measure most of the major pigments. A qualitative check to determine whether a large enough volume has been filtered is to count the number of accessory pigments (chlorophylls b, c1, c2, c3, and carotenoids) quantified, excluding chlorophyll degradation products (Trees et al. 2000). Most algal groups (excluding phycobiliprotein-containing groups) contain at least four HPLC-measurable accessory pigments (see Jeffrey et al. 1997). Therefore, pigment samples that do not meet this minimum accessory pigment criterion may have detection limit problems related to low signal-to-noise ratios for the HPLC detectors and/or inadequate concentration techniques (e.g. low filtration volumes). It is generally recommended that the following volumes be filtered for HPLC pigment analyses: 3 L to 4 L for oligotrophic waters, 1 L to 2 L for mesotrophic waters, and 0.5 L to 1 L for eutrophic waters.

It is recommended to not pre-filter seawater samples to remove large zooplankton and particles, because this practice may exclude pigment-containing colonial and chain-forming phytoplankton, such as diatoms and *Trichodesmium* sp. Forceps may be used to remove large zooplankton from the GF/Fs following filtration.

**Sample Handling and Storage**

Samples should be filtered as quickly as possible after collection and stored immediately in liquid nitrogen. Liquid nitrogen is the best method for storing samples with minimum degradation for short, as well as, longer storage times (e.g. 1 year). Placing samples in liquid nitrogen also assists in pigment extraction by weakening the cell wall and membrane during this rapid temperature change. Ultra-cold freezers (-90 °C) can be used for storage, although they have not been tested for longer than 60 days (Jeffrey et al. 1997). Conventional deep freezers should not be used for storing samples more than 20 hours before transferring them to an ultra-cold freezer, or liquid nitrogen. Again, storage of samples in liquid nitrogen immediately after filtration is the preferred method.

Samples should be folded in half with the filtered halves facing in. This eliminates problems of rubbing particles off the filter during placement in sample containers and storage.

It is strongly recommended to use aluminum foil wrappings for sample containers. This simple, but effective, container is both inexpensive and easy to use. Cut small pieces of heavy-duty aluminum foil into approximately 4 cm squares. Fold each piece in half, and using a fine-point permanent marker, write a short sample identifier (e.g. first letter of the cruise and a sequential sample number) on the foil. Writing on the folded foil, prior to placement of the filter, both avoids puncturing the foil with the marking pen, and improves the legibility of the sample identifier. Place the folded filter in the aluminum foil. Fold the three open sides to form an envelope that is only slightly larger than the folded filter (~3 cm x 1.5 cm).

The use of foil containers minimizes the size requirement of the storage container. It is also acceptable to use either cryogenic tubes, or HistoPrep tissue capsules, but they occupy more storage volume per sample, and they are more expensive than aluminum foil. If fluorometric analysis is to be done soon after collection, it is still recommended to place the samples in liquid nitrogen to assist in pigment extraction, and on removal from the liquid nitrogen to place them immediately in chilled 90% acetone.

7
Recordkeeping

Information regarding sample identification should be logged in a laboratory notebook with the analyst’s initials. For each filter sample record the sample identifier (as written on the sample container), station number for the cruise, water volume filtered (VFILT) in mL, and depth of the water sample, together with the date, time, latitude, and longitude of the bottle cast during which the sample was acquired.

2.3 LABORATORY METHODS FOR HPLC PHYTOPLANKTON PIGMENT ANALYSIS

Internal Standard and Solvent Preparation

In addition to daily calibration of the HPLC system with external standards, an internal standard (e.g. canthaxanthin) should be used to determine the extraction volume. It is important to verify that the internal standard employed is not a naturally occurring analyte in the field samples to be analyzed by HPLC. Canthaxanthin is recommended as an internal standard because it has a restricted distribution in ocean waters, and it is readily available in high purity from commercial sources. For additional background on the use of internal standards see Snyder and Kirkland (1979). The internal standard should be added to the sample prior to extraction and used to correct for the addition of GF/F filter-retained seawater and sample volume changes during extraction. When new external and internal standards are prepared they should be verified against previous standards and a standard reference solution if available. An internal standard with an HPLC peak removed from those of all the pigments, canthaxanthin, is added at a fixed concentration to the HPLC-grade acetone solvent used to extract the pigments from the filtered samples. A sample of canthaxanthin spiked acetone solvent is injected into the HPLC system and its peak area $A_{\text{STD}}$ is recorded to provide a baseline internal standard for monitoring the solvent concentration in each extracted sample.

Extraction

Filters are removed from the liquid nitrogen, briefly thawed (~1 min), and placed in glass centrifuge tubes for extraction in acetone. Three mL HPLC-grade acetone is added to each tube, followed by the addition of a fixed volume of internal standard (typically 50 µL canthaxanthin in acetone). Alternatively, canthaxanthin spiked HPLC-grade acetone solvent may be prepared in advance, in a batch large enough for all samples, and 3 mL is added to each tube in a single step. Since GF/F filters retain a significant amount of seawater following filtration (ca. 0.2 mL per 25 mm filter), the final acetone concentration in the pigment extracts is ~ 94 % (acetone:water, by volume); by measuring the canthaxanthin peak area $A_{\text{STD}}$ for each sample, the ratio $A_{\text{STD}} / A_{\text{Sample}}$ may be used to adjust for sample to sample variations in the extraction volume.

Samples are disrupted by sonication, placed in a freezer, and allowed to extract at 0°C for 24 h. Alternatively, the cells can be mechanically disrupted using a glass/Teflon tissue grinder and allowed to extract at 0°C for 24 h. If after disrupting the cells, it is necessary to rinse the tissue grinder, or mortar and pestle, then a known volume of 90 % acetone, measured using a Class A volumetric pipette, should be used. The ease with which the pigments are removed from the cells varies considerably with different phytoplankton. In all cases, freezing the sample filters in liquid nitrogen improves extraction efficiency.

Prior to analysis, pigment extracts are vortexed and centrifuged to minimize cellular debris. To remove fine glass fiber and cellular debris from the extract, as well as enhance the life expectancy of the HPLC column, filter the extract through 13 mm PTFE (polytetrafluoroethylene) membrane syringe filters (0.2 µm pore size). The use of Nylon filters is not recommended as they may bind certain hydrophobic pigments.

Apparatus

The HPLC system consists of solvent pumps, sample injector, guard and analytical columns, absorption (and fluorescence) detector, and a computer. A temperature-controlled autosampler is optional, but highly recommended, to chill the samples chilled prior to injection and to reduce uncertainties during sample preparation and injection. A variety of companies manufacture HPLC systems (e.g. Agilent Technologies, Beckman, ThermoQuest, Waters
Associates). For a review of hardware and software requirements for measuring chlorophylls and their degradation products, as well as carotenoids, see Jeffrey et al. (1997).

**HPLC Eluants and Gradient Programs**

There are several currently recognized HPLC methods for separating chlorophylls, chlorophyll derivatives and taxonomically important carotenoids. The C$_{18}$ method of Wright et al. (1991) is recommended by SCOR and separates more than 50 chlorophylls, carotenoids, and their derivatives using a ternary gradient system. This HPLC method is described in detail below. The separation of the various pigments requires about 30 minutes. Prior to injection, 1000 µL of the aqueous acetone pigment extract is diluted with 300 µL HPLC-grade water to increase the affinity of pigments for the column during the loading step. This procedure results in sharper peaks, allowing greater loading than can be obtained with undiluted samples.

This method does not separate monovinyl and divinyl chlorophylls $a$ and $b$. The presence of divinyl chlorophylls $a$ and $b$, can cause errors if they are not separated either physically on the column, or by a channels ratio method from the monovinyl forms. Latasa et al. (1996) showed that the use of a single response factor (only for monovinyl chlorophyll $a$) could result in a 15 % to 25 % overestimation of total chlorophyll $a$ concentration if divinyl chlorophyll $a$ was present in significant concentrations. Although monovinyl and divinyl chlorophyll $a$ co-elute, each compound absorbs differently at 436 nm and 450 nm and it is therefore possible to deconvolve the absorption signals due to these pigments (Latasa et al. 1996).

Alternatively, these two chlorophyll species can be separated chromatographically and individually quantified using the C$_{8}$ HPLC techniques described by Goericke and Repeta (1993) and Van Heukelem and Thomas (2001). The latter technique uses a two solvent system and elevated column temperature to achieve desired separations.

Regardless of the method or column-packing material used (C$_{18}$ or C$_{8}$), it is important that HPLC performance be validated before and during use. This would include validation that resolution between peaks is acceptable, or when peaks are not chromatographically resolved, that equations based on spectral deconvolution are possible in order to quantify relative proportions of each pigment in a co-eluting pair.

**Determination of Algal Chlorophyll and Carotenoid Pigments by HPLC (Wright et al. 1991):**

**a. Equipment and reagents:**

1. **Reagents:** HPLC grade acetone (for pigment extraction); HPLC-grade water, methanol, acetonitrile and ethyl acetate; 0.5 M ammonium acetate aq. (pH = 7.2); and BHT (2,6-di-tert-butyl-p-cresol, Sigma Chemical Co.).

2. **High-pressure injector valve** equipped with a 200µL sample loop.

3. **Guard-column** (50 mm x 4.6 mm, ODS-2 Spherisorb C$_{18}$ packing material, 5 µm particle size) for extending the life of the primary column.

4. **Reverse-phase HPLC column** with end capping (250 mm x 4.6 mm, 5 µm particle size, ODS-2 Spherisorb C$_{18}$ column).

5. **Variable wavelength or filter absorbance detector** with low volume flow through cell. Detection wavelengths are 436 nm and 450 nm.

6. **Data recording device:** a strip chart recorder, or preferably, an electronic integrator and computer equipped with hardware and software for chromatographic data analysis.

7. **Glass syringe** (500 µL) or HPLC autosampler.

8. **HPLC Solvents:** solvent A (80:20, by volume; methanol:0.5 M ammonium acetate aq., pH=7.2; 0.01 % BHT, w:v), solvent B (87.5:12.5, by volume; acetonitrile:water; 0.01 % BHT, w:v) and solvent C (ethyl acetate). Solvents A and B contain BHT to prevent the formation of chlorophyll $a$ allomers. Use HPLC-grade solvents. Measure volumes before mixing. Filter solvents through a solvent resistant 0.4 µm filter before use, and degas with helium, or an in-line vacuum degassing system, during analysis.
9. Calibration standards: Chlorophylls \( a \) and \( b \) and \( \beta \)-carotene can be purchased from Sigma Chemical Co. (St. Louis, MO 63178, USA). Other pigment standards can be purchased from the International Agency for ¹⁴C Determination, VKI Water Quality Institute, Agern Allé 11, DK-2970 Hørsholm, Denmark. The concentrations of all standards in the appropriate solvents should be determined, using a monochromator-based spectrophotometer, prior to calibration of the HPLC system (Latasa et al. 1999). Spectrophotometric readings should be made at a bandwidth \( \leq 2 \) nm and the optical density (\( OD \)) of the pigment standards should range between 0.2 to 0.8 \( OD \) units at \( \lambda_{\text{max}} \) (Marker et al. 1980). The recommended extinction coefficients for the various phytoplankton pigments can be found in Appendix E of Jeffrey et al. (1997). Absorbance is measured in a 1 cm cuvette at the peak wavelength \( \lambda_{\text{max}} \), and at 750 nm to correct for light scattering.

10. Concentrations of the standards are calculated as

\[
C_{\text{STD}}^i = \frac{10^6 [A^i(\lambda_{\text{max}}) - A^i(750)]}{bE_{\text{tm}}^i},
\]

where \( C_{\text{STD}}^i \) is the concentration (\( \mu g \ L^{-1} \)) of the standard for pigment \( i \), \( A^i(\lambda_{\text{max}}) \) and \( A^i(750) \) are absorbances at \( \lambda_{\text{max}}^i \) and 750 nm, respectively, \( b \) is the pathlength of the cuvette (cm), and \( E_{\text{tm}}^i \) is the weight-specific absorption coefficient (L g⁻¹ cm⁻¹) of pigment \( i \). Values for \( \lambda_{\text{max}}^i \) and \( E_{\text{tm}}^i \) are given in Appendix E of Jeffrey et al. (1997). Standards stored under nitrogen in the dark at -20°C do not change appreciably over a one-month period, provided that they are stored in containers proven to prevent evaporation (e.g. glass or Teflon bottles/vials).

b. Procedure:

1. Set up and equilibrate the HPLC system with eluant A at a flow rate of 1 mL min⁻¹.
2. Calibrate the HPLC system using working standards prepared, on the day of use, by diluting the primary standard with the appropriate solvent (Jeffrey et al. 1997, Appendix E). When preparing calibration standards, one should only use dilution devices for which the precision and uncertainty have been validated with the solvent to be measured. Prepare at least 5 concentrations (\( \mu g \ L^{-1} \)) of working standards for each pigment spanning the concentration range appropriate for the samples to be analyzed.
3. For each working standard, mix 1000 \( \mu L \) with 300 \( \mu L \) of distilled water, shake, and equilibrate for 5 min prior to injection (diluting the standards and sample extracts with water increases the affinity of pigments for the column in the loading step, resulting in an improved separation of the more polar pigments). Rinse the sample syringe twice with 300 \( \mu L \) of the diluted working standard and draw 500 \( \mu L \) of the working standard into the syringe for injection. Place the syringe in the injector valve, overfilling the 200 \( \mu L \) sample loop 2.5-fold. To check for possible interferences in the extraction solvent and/or filter, prepare a blank by extracting a glass fiber filter in 90 % acetone, mixing 1000 \( \mu L \) of the 90 % acetone filter extract and 300 \( \mu L \) distilled water, and injecting the mixture onto the HPLC system. For each pigment \( i \), plot absorbance peak areas (arbitrary system units) against working standard pigment masses (concentrations multiplied by injection volume). The HPLC system response factor \( F^i \) (area \( \mu g^{-1} \)) for pigment \( i \) is calculated as the slope of the regression of the peak areas of the parent pigment (plus areas of peaks for structurally-related isomers if present) against the pigment masses of the injected working standards (\( \mu g \)). Structurally related isomers (e.g. chlorophyll \( a \) allomer) contribute to the absorption signal of the standards and disregarding them will result in the over-estimation of analytes in sample extracts (Bidigare 1991).
4. Prepare pigment samples for injection by mixing a 1000 \( \mu L \) portion of the aqueous acetone pigment extract and 300 \( \mu L \) distilled water, shake, and equilibrate for 5 min prior to injection. Inject the sample onto the HPLC column. Samples that are pre-mixed with distilled water (or other injection buffer) should not be allowed to reside in autosampler compartments for extended durations, because hydrophobic pigments will precipitate out of solution (Mantoura et al. 1997).
For additional information regarding HPLC method implementation and injection conditions see Wright and Mantoura (1997).

5. Following injection of the sample onto the HPLC system, use the following solvent system program to separate the chlorophyll and carotenoid pigments: 0.0’ (90%A, 10%B), 1.0’ (100%B), 11.0’ (78%B, 22%C), 27.5’ (100%B, 90%C), 29.0’ (100%B), and 30.0’ (100%B). Degas solvents with helium or an in-line vacuum degassing system during analysis. It should be noted that method performance varies significantly between HPLC systems because of differences in dwell volume, equilibration time, and injection conditions. It is, therefore, recommended that analysts validate that desired peak separations are attained for pigment pairs of interest by calculating the peak resolution indices Rs as

$$R_s = \frac{2(t_{R2} - t_{R1})}{w_{B1} + w_{B2}},$$

(2.2)

where \(t_{R1}\) and \(t_{R2}\) are the retention times (min) of peaks 1 and 2, and \(w_{B1}\) and \(w_{B2}\) are the widths (min) of peaks 1 and 2 at their respective bases (Wright 1997). Peak separation values \(R_s < 1.0\) are insufficient for accurate quantification of peak areas (Wright 1997).

6. Peak identities are routinely determined by comparing the retention times of sample peaks with those of pure standards. Peak identities can be confirmed spectrophotometrically by collecting eluting peaks from the column outlet (or directly with an on-line diode array spectrophotometer). Absorption maxima for the various phytoplankton pigments can be found in Part IV of Jeffrey et al. (1997).

7. Calculate individual pigment concentrations as

$$C_{Sample}^{i} = \frac{A_{Sample}^{i}V_{Extracted}}{F_{i}^{}V_{Injected}^{i}V_{Sample}^{i}A_{Cantha}^{i}}$$

(2.3)

where \(C_{Sample}^{i}\) is the individual pigment concentration (µg L⁻¹), \(A_{Sample}^{i}\) is the area of individual pigment peak for a sample injection, \(V_{Extracted}\) is the volume extracted (mL, to nearest 0.1 mL), \(V_{Injected}\) is the volume injected (mL, measured to the nearest 0.001 mL), \(V_{Sample}\) is the sample volume filtered (L, measured to the nearest 0.001 L), and the other coefficients are defined above.

8. This method is designed for the separation of chlorophyll and carotenoid pigments, but it is also capable of separating the major chlorophyll breakdown products.

9. The uncertainty of the HPLC method was assessed by performing triplicate injections of a mixture of phytoplankton and plant extracts; coefficients of variation (standard deviation/mean x 100 %) ranged from 0.6 % to 6.0 %. The use of an appropriate internal standard, such as canthaxanthin, will decrease the uncertainty.

2.4 QUALITY ASSURANCE PROCEDURES

Quality assurance procedures outlined here should be routinely employed to assure accurate, precise and representative results.

As a means of monitoring an instrument's performance, individual pigment response factors \(F^i\) should be charted as functions of time (Clesceri et al. 1998). These quality control graphs should be retained with the data analysis logbooks to document the quality of each data set.

A selected number of samples should be analyzed in duplicate (or triplicate) to assess representativeness and uncertainty in the method and instrumentation. In multi-ship/investigator studies, replicate samples should be collected and archived for future intercalibration checks.

Fortified samples should be analyzed as part of the quality assurance effort. Fortified samples are prepared in duplicate by spiking a sample with known quantities of the analytes of interest at concentrations within the range.
expected in the samples. Fortified samples are used to assess the method's uncertainty in the presence of a typical sample matrix.

The method detection limit (MDL) for the analytes of interest can be determined by measuring seven replicate standard injections (Glaser et al 1981). The standard deviation $S_c$ of the seven replicate measurements is calculated, and the MDL is computed as

$$\text{MDL} = t(6,0.99)S_c,$$

where $t(6,0.99)$ is the Student's $t$ value for a one-tailed test at the 99% confidence level, with (N-1)=6 degrees of freedom. For this particular sample size (N=7) and the 99% confidence level, $t(6,0.99) = 3.707$ (Abramowitz and Segun 1968, Table 26.10).

System and spiked blanks should be routinely analyzed. A system blank consists of a filter, reagents, and the glassware and hardware utilized in the analytical scheme. The system blank is quantified under identical instrumental conditions as the samples and is analyzed by appropriate quantitative methods. The system blank may not contain any of the analytes of interest above the MDL or corrective action must be taken. A spiked blank is defined as a system blank plus an authentic external standard containing the analytes of interest. Each set of samples should be accompanied by a spiked blank and is quantified under the same instrumental conditions as the samples.

2.5 PROTOCOL STATUS AND FUTURE DIRECTIONS FOR RESEARCH

Recent studies have identified the presence of novel bacterial phototrophs in coastal and oceanic waters. These include proteorhodopsin-containing *Bacteria* (Béjà et al. 2000, 2001) and aerobic anoxygenic phototrophic *Bacteria* (Kolber et al. 2000, 2001). Sequence analysis of BAC clone libraries prepared from Monterey Bay, Station ALOHA and the Southern Ocean revealed that numerous uncultivated members of the $\gamma$-Proteobacteria contain genes that code for proteorhodopsin. This membrane-bound pigment contains trans-retinal, absorbs at blue-green to green wavelengths, and functions as a light-driven proton pump. In an unrelated study, Kolber et al. (2000) used an infrared fast repetition rate (IRFRR) fluorometer to document the widespread occurrence of aerobic anoxygenic phototrophs (AAPs) in the world oceans. These microbes possess low amounts of bacteriochlorophyll a ($\lambda_{\text{max}} = 358$ nm, 581 nm and 771 nm) and unusually high levels of bacteriocarotenoids ($\lambda_{\text{max}} = 454$ nm, 465 nm, 482 nm and 514 nm). They require molecular oxygen for growth. One of us (RRB) has initiated HPLC pigment analysis of these latter clones and retinal-related compounds to determine if the Wright et al. (1991) method can be used for their separation and quantification.

REFERENCES


Chapter 3

Fluorometric Chlorophyll \( a \): Sampling, Laboratory Methods, and Data Analysis Protocols

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3.1 INTRODUCTION

In addition to HPLC analyses, it is recommended that the standard fluorometric methodology used for measuring chlorophylls and pheopigments also be applied to (i) the same extracted pigment samples used for HPLC analysis, and (ii) additional independent samples. Analysis of fluorometric chlorophyll \( a \) concentration is a far simpler procedure than HPLC analysis, especially at sea. On a given research cruise, therefore, it is economically feasible to acquire and process many more fluorometric than HPLC samples and to statistically relate fluorometric and HPLC chlorophyll \( a \) concentrations using linear regression analysis. This additional analysis will also enable a direct link to the historical bio-optical algorithms and database development during the CZCS validation experiments.

Protocols for fluorometric determination of the concentrations of chlorophyll and pheopigments were developed initially by Yentsch and Menzel (1963) and Holm-Hansen \textit{et al.} (1965), and are described in detail by Strickland and Parsons (1972). Holm-Hansen \textit{et al.} (1965) and Strickland and Parsons (1972) used first principles of fluorescence spectroscopy to derive these fluorometric equations. The equation proposed by Yentsch and Menzel (1963) is only indirectly linked to first principles, through debatable assumptions, and its use is not recommended. Although these measurements have been shown to contain errors as compared to HPLC determinations (Trees \textit{et al.} 1985; Smith \textit{et al.} 1987; Hoepffner and Sathyendranath 1992; Bianchi \textit{et al.} 1995; Tester \textit{et al.} 1995), the CZCS phytoplankton pigment concentration algorithms were based on them entirely. The SeaWiFS protocols for this analysis will be those given in Strickland and Parsons (1972) as updated by this chapter.

Pigment databases generally show a log-normal distribution, which is consistent with that proposed by Campbell (1995) for bio-optical properties. Therefore, it is appropriate to perform log-linear regressions on HPLC determined total chlorophyll \( a \) (chlorophyllide \( a \), chlorophyll \( a \) epimer, chlorophyll \( a \) allomer, monovinyl chlorophyll \( a \) and divinyl chlorophyll \( a \)) and fluorometrically determined chlorophyll \( a \), using model I regressions. Standard Model I regressions were selected because HPLC determined total chlorophyll \( a \) concentrations are to be predicted from fluorometrically determined chlorophyll \( a \) [Model I regressions are appropriate for both predictions and determining functional relationships, whereas Model II regressions should not be used to predict values of \( y \) given \( x \) (page 543, Sokal and Rohlf 1995)].

Examples of regression models predicting log HPLC total chlorophyll \( a \) (following Chapter 2 HPLC protocols) from log fluorometric chlorophyll \( a \) are shown in Figures 3.1, 3.2, and 3.3 for three cruises in different geographic areas. In each example, the regression slopes are significantly different from a one-to-one relationship, although for the Gulf of California (GoCAL November 1996, Figure 3.3) the slope is close to unity. One-to-one ratios have also been found for other geographic areas, but not necessarily during all seasons. Therefore, the relationship (slope and offset) between HPLC total chlorophyll \( a \) and fluorometric chlorophyll \( a \) must be determined for a selected number of samples for each cruise, so that a cruise-specific scaling factor can be applied to other fluorometric samples.

The protocols specified below for fluorometric chlorophyll \( a \) analyses follow closely those prescribed in the JGOFS Core Measurement Protocols (UNESCO 1994), but they differ in one important respect. Absorption of light in seawater, or any other medium, is a volumetric process, even though the volume absorption coefficient may
vary with the density of the medium. For ocean color and optical analyses, therefore, the concentration of chlorophyll \(a\) shall be expressed in units of mass per unit volume of seawater, either in \(\mu g\) L\(^{-1}\), or mg m\(^{-3}\). This differs from the JGOFS protocols, which specify that concentrations in seawater of chlorophyll \(a\) and pheopigments should be expressed in \(\mu g\) kg\(^{-1}\).

### 3.2 SAMPLE ACQUISITION AND STORAGE

Water samples should be taken using, \textit{e.g.}, Niskin bottles at the site of, and simultaneously with, the surface in-water upwelled radiance and reflectance measurements, and at depth increments sufficient to resolve variability within at least the top optical depth.

The \(K(z)\), profiles over this layer will be used to compute optically weighted, near-surface pigment concentration for bio-optical algorithm development (Gordon and Clark 1980). When possible, samples should also be acquired at several depths distributed throughout the upper 200 m of the water column (or in turbid water, up to seven diffuse attenuation depths, \textit{i.e.} \(\ln(E(0)/E(z))=7\), to provide a basis for relating fluorescence signals to pigment mass concentration.

Samples should be filtered as soon as possible after collection. If processing must be delayed for more than an hour, hold the samples on ice, or in a freezer at 4\(^\circ\)C, and protect them from exposure to light. For delays longer than several hours, the samples should be stored in liquid nitrogen. Use opaque sample bottles, because even brief exposure to light during sampling and/or storage might alter pigment values.

**Filtration**

Whatman GF/F glass fiber filters, with approximately 0.7 \(\mu m\) pore size, are preferred for removing phytoplankton from water. The glass fibers assist in breaking the cells during grinding and no precipitate forms after acidification. Twenty-five mm diameter GF/F glass fiber filters should be used with a vacuum or positive pressure with a pressure differential equivalent to 180-200 mm of mercury. Large filtration volumes are not required, because of the increased sensitivity of the fluorescence measurement.

Inert membrane filters, such as polyester filters, may be used when size fraction filtration is required. When this is done, it is recommended to also filter a replicate sample through a GF/F to determine the total concentration. Summing the various size-fractionated concentrations may not produce an accurate estimate of the total, because of the potential for cell disruption during filtration.

There has been an ongoing discussion on filter types and retention efficiencies for natural samples. Phinney & Yentsch (1985) showed the inadequacy of GF/F filters for retaining chlorophyll \(a\) in oligotrophic waters, as did Dickson and Wheeler (1993) for samples from the North Pacific. In response to Dickson and Wheeler (1993), Chavez \textit{et al.} (1995) compared samples collected in the Pacific Ocean using GF/F and 0.2 \(\mu m\) membrane filters with small filtered volumes (100-540 mL). Their results for small volumes showed a very close agreement between the two filter types with GF/F filters having only a slightly positive 5% bias.

Filtration volume can directly affect the retention efficiency for GF/F filters. Particles can be retained by filters through a variety of ways, such as filter sieving, filter adsorption, electrostatic and van der Waals attractions (Brock, 1983). When water flows through the pores of a Nuclepore filter, streamlines are formed that can align small particles longitudinally, with the result that cell diameter becomes important with these filters. It is known, on the other hand, that Whatman GF/F filters can retain particles much smaller than their rated pore size. Generally, at small volumes (100-300 mL) filter adsorption, and electrostatic and van der Waals attractions are important, whereas at larger volumes (> 2,000 mL) sieving dominates. This has been tested in oligotrophic waters off Hawaii in which small (< 500 mL) and large volumes (> 2-4 liters) retained similar amounts of chlorophyll \(a\) on the two types of filters, whereas for intermediate sample volumes the GF/F filters showed lower concentrations. As a general rule, it is recommended that the following volumes be filtered for these water types: 0.5-1.0 liter for oligotrophic, 0.2-0.5 liter for mesotrophic, and 0.1 liter and less for eutrophic water.

It is recommended to not pre-filter seawater samples to remove large zooplankton and particles, because this practice may exclude pigment-containing colonial and chain-forming phytoplankton, such as diatoms and \textit{Trichodesmium} sp. Forceps should be used to remove large zooplankton from the GF/Fs following filtration.
Sample Handling, and Storage

Samples should be filtered as quickly as possible after collection, and the filters stored immediately in liquid nitrogen. Liquid nitrogen is the best method for storing filter samples with minimum degradation for short, as well as, longer storage times (e.g. 1 year). Placing samples in liquid nitrogen also assists in pigment extraction by weakening the cell wall and membrane during this rapid temperature change. Ultra-cold freezers (-90°C) can be used for storage, although they have not been tested for longer than 60 days (Jeffrey et al. 1997). Conventional deep freezers should not be used for storing samples more than 20 hours before transferring them to an ultra-cold freezer, or liquid nitrogen.

Again, storage of samples in liquid nitrogen immediately after filtration is the preferred method. The addition of MgCO₃ at the end of the filtration process to stabilize chlorophyll has not been used for many years as a routine oceanographic method, because of the uncertainty in pigment absorption by MgCO₃.

If samples are to be stored for any length of time prior to fluorometric analysis, they should be folded in half with the filtered halves facing in. This eliminates problems of rubbing particles off the filter during placement in sample containers and storage.

It is strongly recommended to use aluminum foil wrappings for sample containers. This simple, but effective, container is both inexpensive and easy to use. Cut small pieces of heavy-duty aluminum foil into approximately 4 cm squares. Fold each piece in half, and using a fine-point permanent marker, write a short sample identifier (e.g. first letter of the cruise and a sequential sample number) on the foil. Writing on the folded foil, prior to placement of the filter, both avoids puncturing the foil with the marking pen, and improves the legibility of the sample identifier. Place the folded filter in the aluminum foil. Fold the three open sides to form an envelope that is only slightly larger than the folded filter (~3cm x 1.5cm).

The use of foil containers minimizes the size requirement of the storage container. It is also acceptable to use either cryogenic tubes, or HistoPrep tissue capsules, but they occupy more storage volume per sample, and they are more expensive than aluminum foil. If fluorometric analysis is to be done soon after collection, it is still recommended to place the samples in liquid nitrogen to assist in pigment extraction, and on removal from the liquid nitrogen to place them immediately in chilled 90% acetone.

Recordkeeping

Information regarding sample identification should be logged in a laboratory notebook with the analyst’s initials. For each filter sample record the sample identifier (as written on the sample container), station number for the cruise, water volume filtered (V_{FILT}) in mL, and depth of the water sample, together with the date, time, latitude, and longitude of the bottle cast during which the sample was acquired.

3.3 LABORATORY METHODS FOR FLUOROMETRIC DETERMINATION OF CHL. a AND PHEOPIGMENT CONCENTRATIONS

Chlorophyll and pheopigments can be determined using either a Turner Designs (or Sequoia) fluorometers equipped with the standard light sources and Corning excitation and emission filters, following the manufacture's recommendation for measuring extracted chlorophyll. The fluorometric instrument should be warmed-up for at least 30 to 45 minutes prior to making measurements.

Because of the acidification requirement for the standard fluorometric method (Holm-Hansen et al. 1965), differences in excitation and emission wavelength bands between fluorometers can produce uncertainties (Trees et al. 1985). The sensitivity with which a particular instrument is able to differentiate between chlorophyll and pheopigment is a function of the excitation wavelength. This effect is measured during calibration of the fluorometer and is called the tau factor (τ). Saijo and Nishizawa (1969) have shown that τ can vary from 1 to 11.5, depending upon the excitation wavelength (in the range between 410 nm and 440 nm). For example, a comparison between a Turner Designs (Model 10-005R) analog fluorometer and a Turner Designs (Model 10-AU-005) digital fluorometer showed statistically significant differences for 42 oceanic samples (slope = 1.06), even though both were calibrated with exactly the same standards (Figure 3.4). The departure from a unit slope is attributable to differences in the excitation bands for the two fluorometers.
**Fluorometer Calibrations**

Bench fluorometers used to measure concentrations of extracted chlorophyll and pheopigments should be calibrated using authentic chlorophyll \(a\) standards, as prescribed also in the HPLC Protocols (Chapter 2). Chlorophyll \(a\) standards can be purchased from Sigma Chemical Co. (St. Louis, MO 63178, USA).

If a fluorometer has been shipped for a cruise, or if it has been unused for several weeks, it is strongly recommended that it be recalibrated with an authentic chlorophyll \(a\) standard. The use of solid standards, like those provided by Turner Designs and other manufacturers, can only provide a check for instrumental drift. They cannot be used as primary pigment standards. However, the solid standard should be used at frequent intervals during each day's analyses to monitor instrument drift.

The concentration of the chlorophyll \(a\) standard, in the appropriate solvent, must be determined using a monochromator-based spectrophotometer prior to calibrating the fluorometer. The recommended extinction coefficients for chlorophyll \(a\) in several solvents can be found in Appendix E of Jeffrey et al. (1997). Absorbance is measured in a 1 cm cuvette at the peak wavelength \(\lambda_{\text{max}}\), and at 750 nm to correct for light scattering. The bandwidth of the spectrophotometer should be between 0.5 and 2 \(\mu\)m, with the standard concentration being such that the absorbance falls between 0.1 and 1.0 optical density units (Clesceri et al., 1998a). The concentration of the standard is calculated as

\[
C_{\text{STD}} = \frac{10^6 [A(\lambda_{\text{max}}) - A(750)]}{bE_{\text{1cm}}},
\]

where \(C_{\text{STD}}\) is the concentration (\(\mu\)g L\(^{-1}\)) of the chlorophyll \(a\) standard, \(A(\lambda_{\text{max}})\) and \(A(750)\) are absorbances at \(\lambda_{\text{max}}\) and 750 nm, \(b\) is the pathlength of cuvette (cm), and \(E_{\text{1cm}}\) is the specific absorption coefficient (L g\(^{-1}\) cm\(^{-1}\)) of chlorophyll \(a\) in 90\% acetone. For 90\% acetone \(E_{\text{1cm}}\) = 87.67 L g\(^{-1}\) cm\(^{-1}\), and for 100\% acetone \(E_{\text{1cm}}\) = 88.15 L g\(^{-1}\) cm\(^{-1}\), when applied to the absorption measured at the peak wavelength \(\lambda_{\text{max}}\) (Jeffrey et al. 1997, Appendix E). The peak wavelength \(\lambda_{\text{max}}\) must be determined by inspection of the measured spectrum, because its location may shift due to interactions between the particular solvent and mixture of pigment compounds in each sample. Standards stored under nitrogen in the dark at -20\(^\circ\)C do not change appreciably over a one-month period, provided that they are stored in containers proven to prevent evaporation (e.g. glass or Teflon bottles/vials).

The stock chlorophyll \(a\) standard, with its concentration measured on a spectrophotometer as described above, should be diluted using calibrated gas-tight syringes, and Class A volumetric pipettes and flasks. The minimum number of dilutions of the stock standard for calibrating a fluorometer depends on whether it is a digital model (Turner Designs 10-AU-005), or it is an analog model with a mechanical mode for changing sensitivity (e.g. Turner Designs 10-005). A minimum of 5 dilutions is required for calibrating a digital fluorometer. Analog fluorometers with a variety of door settings, such as the Turner Designs Model 10-005, must be calibrated for each door setting using at least three standard concentrations per door. The diluted standard pigment concentrations used in calibrating the fluorometer must bracket the range of concentrations found in the samples being analyzed.

Each diluted chlorophyll \(a\) standard is placed in the fluorometer and the signal (\(F_a\)) is recorded, after waiting a short period of time (60 seconds) for it to stabilize. The standard is removed and diluted HCL acid (2 drops of 5 \%, or 1 drop of 10 \%, both concentrations by volume) is added and mixed within the test tube. The tube is then placed back into the fluorometer, and after stabilization, the acidified fluorescence signal (\(F_a\)) is recorded. Following acidification of the chlorophyll \(a\) standard, the fluorescence signal stabilizes relatively quickly. This is not the case for natural samples that contain a mixture of pigment compounds, however, and stabilization time may vary from sample to sample. Stabilization time has to be the same for both pigment standards and for natural samples. To minimize this source of uncertainty, and to standardize this measurement technique, it is recommended that both acidified natural sample and acidified pigment standards be allowed to react with the acid for one minute prior to recording the acidified fluorescence signal (\(F_a\)). Two drops of 5 \% (by volume) hydrochloric acid is added to each of the pigment standards and natural samples. Once the acid is added, the sample in the test tube should be mixed by inverting the tube several times, using parafilm as a stopper. All fluorometric measurements for both pigment standards and natural samples should be carried out at room temperature. A 90 \% (by volume) acetone blank (\(Blk_a\)) and an acidified acetone blank (\(Blk_b\)) should also be measured, even though the acidified blank (\(Blk_a\)) is frequently found to be equal to the non-acidified blank (\(Blk_b\)). The fluorometer’s sensitivity to pheopigments, \(\tau\), is calculated as
\[ \tau = \frac{F_b - Blk_b}{F_a - Blk_a}, \quad (3.2) \]

and is averaged over all concentrations of the chlorophyll standard. For the mechanical door model fluorometers, data from the higher gain door settings will often become noisy and computed \( \tau \) values will begin to decrease. These data should be excluded from the average. The fluorometer’s response factor, \( F_R \) (\( \mu g \) L\(^{-1}\) per fluorescence signal), is determined as the slope of the simple linear regression equation

\[ C_{\text{STD}} = F_R (F_b - Blk_b), \quad (3.3) \]

calculated for the sample of diluted concentrations of the pigment standard, and forcing a zero intercept. With a digital fluorometer, the regression analysis is applied to the data from the entire 5, or more, concentrations and a single \( F_R \) factor is determined for the instrument. With a mechanical fluorometer, the regression is applied to the data from the 3, or more, concentrations of the standard, and a separate \( F_R \) factor is determined, for each door setting. As a means of monitoring an instrument’s performance, \( F_R \) factors from successive calibrations should be charted as functions of time (Clesceri et al., 1998b). These quality control graphs should be retained with the data analysis logbooks to document the quality of each data set for which that fluorometer is used.

**Solvent Preparation.**

It is recommended that 90% acetone (by volume) be used to extract pigments for the fluorometric analysis. Richard and Thompson (1952) were the first to propose 90% acetone as a solvent to extract pigments from marine phytoplankton. Their results indicated improved extraction efficiencies, and also showed that the procedure minimized the activity of the naturally occurring chlorophyllase enzyme, which degrades the pigment. With a graduated cylinder, make up 90% acetone by first pouring in distilled water, followed by 100% acetone. Using volumetric pipettes, or auto-pipettes, accurately measure 8 mL to 10 mL of 90% acetone and place it in a centrifuge tube. Record this volume as \( V_{\text{EXT}} \). A number of such tubes containing acetone are then stored in a freezer and individually removed as filter samples are collected. Pre-chilling the solvent in this way reduces the possibility of temperature induced pigment degradation.

**Extraction**

Filters are removed from liquid nitrogen and placed in the chilled centrifuge tubes for extraction in \( V_{\text{EXT}} \) mL of 90% acetone. Samples are disrupted by sonication, placed in a freezer, and allowed to extract at 0°C for 24 h. Alternatively, the cells can be mechanically disrupted using a glass/Teflon tissue grinder and allowed to extract at 0°C for 24 h. If after disrupting the cells, it is necessary to rinse the tissue grinder, or mortar and pestle, then a known volume of 90% acetone, measured using a Class A volumetric pipette, should be used. The ease at which the pigments are removed from the cells varies considerably with different phytoplankton. In all cases, freezing the sample filters in liquid nitrogen improves extraction efficiency. Prior to analysis, pigment extracts are swirled into a vortex to remove particles from the sides of the tube, and then centrifuged to minimize cellular debris.

**Measurement**

Following the same measurement procedure described above under Fluorometer Calibration, each extracted sample is placed in the fluorometer and its non-acidified and acidified responses, \( F_b \) and \( F_a \), are measured and recorded. The concentration of chlorophyll [Chl] (\( \mu g \) L\(^{-1}\)) in the sample is calculated as

\[ [\text{Chl}] = \left\{ \left( F_b - F_a - Blk_b + Blk_a \right) \frac{\tau}{\tau - 1} \right\} F_R \frac{V_{\text{EXT}}}{V_{\text{FILT}}}, \quad (3.4) \]

and pheopigments concentration [Pheo] (\( \mu g \) L\(^{-1}\)) as

\[ [\text{Pheo}] = \left\{ \left( F_a - Blk_a \right) \frac{\tau}{\tau - 1} \right\} F_R \frac{V_{\text{EXT}}}{V_{\text{FILT}}}, \quad (3.5) \]

where volumes extracted \( V_{\text{EXT}} \) and filtered \( V_{\text{FILT}} \) are in mL. Pheopigment concentrations determined using the standard fluorometric method of Holm-Hansen et al. (1965) have not been reported in published articles for many years. This is based on the fact that (i) there is always a residual amount of pheopigments in all natural samples (Smith and Baker, 1978; 25% of the summed chlorophyll plus pheopigment), (ii) pheopigment concentrations are
overestimated in the presence of chlorophyll $b$ (Lorenzen and Jeffrey, 1980; Vernet and Lorenzen, 1987), and (iii) HPLC measured pheopigments, generally contribute very little to the chlorophyll $a$ pigment pool (e.g., Hallegraeff, 1981; Everitt et al., 1990; and Bricaud et al., 1995). Trees et al. (2000a) assembled an extensive HPLC pigment database (5,617 samples) extending over a decade of sampling and analysis, and including a variety of environments ranging from freshwater to marine, oligotrophic to eutrophic, and tropical to polar, and found that the average pheopigment to chlorophyll $a$ ratio was only 0.037. This global scale result emphasizes the problems associated with estimating pheopigments using the standard fluorometric method.

3.4 In Situ CHLOROPHYLL $a$ FLUORESCENCE PROFILES

An in situ fluorometer should be employed to measure a continuous profile of chlorophyll fluorescence. The fluorometer should be mounted on the same underwater package as the water sampler, ideally together with a CTD, transmissometer and other inherent optical properties (IOP) sensors. In some cases it may be desirable to also include a radiometer on this package, if shading effects associated with the package and/or ship are not significant.

In situ fluorometers produce nearly continuous profiles of artificially stimulated fluorescence. Fluorometer data (in volts) should be corrected by subtracting an offset, determined by shading the instrument on deck. These unscaled fluorescence responses are adequate to provide guidance in K-profile analysis and interpretation.

To produce vertical continuous profiles of pigment concentration, HPLC-derived pigment concentrations from water samples taken at discrete depths may be interpolated, with the aid of in situ fluorescence profiles. These fluorescence interpolated profiles should then be used with $K_d(z,\lambda)$ profiles to compute the optically weighted average pigment concentration over the top attenuation length (Gordon and Clark 1980).

The A/D channel used to acquire and record signal voltages from the in situ fluorometer must be calibrated, and its temperature-dependent response to known voltage inputs characterized. The range dependent A/D bias coefficients should be determined at approximately $5^\circ$C intervals over the range from 0-25$^\circ$C to characterize the temperature sensitivity of the data acquisition system.

Zero fluorescence offsets should be measured on deck before and after each cast; the optical windows should be shaded to avoid contamination of the zero offset value by ambient light. Before each cast, the fluorometer windows should be cleaned following the manufacturer's instructions.

3.5 PROTOCOL STATUS AND FUTURE DIRECTIONS FOR RESEARCH

In order to minimize interferences caused by the overlapping excitation and emission wavebands of chlorophylls $a$, $b$, $c$ and pheopigments, Turner Designs (Sunnyvale, CA) manufactures the multi-spectral fluorometer TD-700. This instrument was recently tested using samples collected at the US JGOFS Hawaii Ocean Time-series Station ALOHA (22.75$^\circ$N, 158$^\circ$W). A set of replicate monthly (May - Dec 2000) pigment samples collected between the surface and 175 m were analyzed by HPLC using the protocols described in Chapter 2. Duplicate samples were subsequently analyzed in 100% acetone with the TD-700 using the manufacturer's calibration. The results of these comparisons are illustrated in Figures 3.5, 3.6 and 3.7 for chlorophylls $a$, $b$, and $c$, respectively. The Model I regression equations predicting each HPLC pigment (in mg m$^{-3}$) from the equivalent TD700 estimate are:

- HPLC Chl $a = 0.729[TD-700 Chl a] + 0.0144; (r^2 = 0.894)$.
- HPLC Chl $b = 0.607[TD-700 Chl b] – 0.0163; (r^2 = 0.816)$.
- HPLC Chl $c = 1.083[TD-700 Chl c] – 0.00249; (r^2 = 0.906)$.

These equations differ significantly from a one-to-one relationship. The present comparisons differ also from those published in Trees et al. (2000a), although care must be used in this comparison since the concentrations were expressed there in ng L$^{-1}$ (which accounts for the factor of 10$^{-3}$ differences in the respective offset coefficients). These results call into question the stability of the fluorometer. It is also evident that the equations provided by the manufacturer must be verified with HPLC data, and that these calibration relationships should be reviewed frequently.

It is interesting and noteworthy that the TD-700 fluorometer did not detect pheopigments in any of the samples analyzed.
Figure 3.1: Comparisons between fluorometrically determined chlorophyll and HPLC determined total chlorophyll a (chlorophyllide a, chlorophyll a epimer, chlorophyll a allomer, monovinyl chlorophyll a, and divinyl chlorophyll a) from samples collected during Atlantic Meridional Transect 3 cruise (30°N to 30°S, October 1996).

$\log_{10}\text{HPLC} = 0.916 \log_{10}\text{Fluor} - 0.365$

$r^2 = 0.888, n = 179$

Figure 3.2: Same as Figure 3.1 for data collected during the Marine Optical Characterization Experiment (MOCE) 4 cruise.

$y = 0.432x^{0.856}$

$log_{10}\text{HPLC} = 0.856 \log_{10}\text{Fluor} - 0.364$

$r^2 = 0.733, n = 285$

Figure 3.2: Same as Figure 3.1 for data collected during the Marine Optical Characterization Experiment (MOCE) 4 cruise.
Figure 3.3: Same as Figure 3.1 for data collected during the Gulf of California cruise (Gulf of California, November 1996).

Fluorometric Chlorophyll $a$ (mg m$^{-3}$)

$y = 0.665x^{1.065}$  \( r^2 = 0.937 \)

$log_{HPLC}=1.065log_{Fluor}-0.178$

$s=0.937, n=300$

Figure 3.4: Comparison of fluorometrically determined chlorophyll $a$ using the VisLab Turner Fluorometer (10-005R) and the Moss Landing Marine Laboratory Turner Fluorometer (10-AU-005). Samples were analyzed from a MOBY Nov 96 cruise and a Gulf of California cruise (Mueller, Nov 96).
Figure 3.5. Comparison between chlorophyll a determined by the TD700 equation supplied by the manufacturer and that measured by HPLC methods.

Figure 3.6: Same as Figure 3.5 for chlorophyll b.
REFERENCES


