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DETERMINATION OF PLANKTONIC PRIMARY PRODUCTION PARAMETERS IN THE ATLANTIC OCEAN USING IN SITU INHERENT OPTICAL PROPERTIES

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DETERMINATION OF PLANKTONIC PRIMARY PRODUCTION PARAMETERS IN THE ATLANTIC OCEAN USING IN SITU INHERENT OPTICAL PROPERTIES

CHRISTOPHER DAVID LOWE

A thesis submitted to the University of Plymouth
in partial fulfilment for the degree of

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Faculty of Science

In Collaboration with
Plymouth Marine Laboratory

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"We apologise for the inconvenience."

God's Last Message to his Creation, Mostly Harmless, Douglas Adams.

"it is...it is...it is green"


"If you gaze long into an abyss, the abyss will gaze back into you."

Friedrich Nietzsche

Southern Pacific 2-8-8-4 ac9 class locomotive, picture credit R. H. Kindig.
Christopher David Lowe

Determination of planktonic primary production parameters in the Atlantic Ocean using in situ inherent optical properties

Bio-optical measurements from three of the Atlantic Meridional Transect programme cruises in 2003-2004 were examined to determine spatial variation and correlations between phytoplankton variables. These cruises each crossed approximately 106° of latitude between the UK and the Falkland Islands, covering a range of environments in the Atlantic Ocean.

Measurements of primary production were made using a Fast Repetition Rate Fluorometer (FRRF), concentration of phytoplankton pigments using High Performance Liquid Chromatography (HPLC) and in situ particle absorption using a novel double cast technique with an ac9+ nine wavelength absorption/attenuation meter. Ancillary data in the form of salinity, temperature and chlorophyll concentration profiles were used to determine the spatial distribution of communities and to provide data for calibration. Cluster analysis of pigment data, using multivariate Brae-Curtis statistical analysis, produced effective partitioning of the cruises into functional regions for further work.

The study showed that reference blanking was important in oceanic measurements with the FRRF and that separate blanks were required for each chamber. Blanks of unfiltered water from below the euphotic zone coincided best with blanks taken under in situ irradiance in the water column. Positive log linear relationships were shown between FRRF photosynthetic quantum efficiency (Fq) and, contrary to expectations, between photosystem II cross sectional area (εPSII) and measurements of chlorophyll a concentration.

Comparisons between FRRF and automated flow cytometry data suggested that the photochemistries of the prokaryotes Synechococcus and Prochlorococcus are significantly different and that the photochemistry of Prochlorococcus is similar to that of eukaryotes.

The concentration ratio of chlorophyll a:total pigment was found to follow a positive log linear relationship with chlorophyll a concentration, similar to that of the FRRF variables. A possible causative link between the relative loads of chlorophyll a and total pigment with FRRF variables was suggested.

The novel twin filtered and unfiltered casts of the ac9+ produced acceptable particulate absorption spectra. Spectra representing chlorophyll a and total pigment concentrations demonstrated the same positive log linear relationship of the pigment measurements suggesting that this method could be used to determine pigment concentrations and therefore primary production parameters.

Direct correlations were found between FRRF primary production parameters and both pigment ratios and absorption ratios, suggesting that absorption measurements could be used to a proxy for primary production parameters.
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Chapter 1

Introduction

1.1 The AMT cruises

The Atlantic Meridional Transect (AMT) [Aiken et al., 2000] utilises the biannual passage of the RRS James Clarke Ross between the UK and the Falkland Islands to undertake scientific measurements. Since 1995, 13 cruises have been made along this track with a further three cruises between the UK and South Africa. This has given a unique time series of measurements on an ocean basin spatial scale, encompassing the major regions of the North and South Atlantic Ocean.

1.2 Aims

This study was based upon in situ optical measurements taken on three cruises (AMTs 12, 13 and 14) that latitudinally transected the Atlantic Ocean. These cruises have the overall aim of describing large scale and long term patterns of physical, chemical and biological variables in the Atlantic, the optical characteristics of phytoplankton being the focus of this thesis. The primary aim was to clarify the nature of relationships in phytoplankton pigment ratios so as to develop a methodology for the calculation of magnitude of photosynthetic parameters based on inherent optical properties. The methodologies for examining primary production are shown diagrammatically in figure 1.1, with four main steps:

1. acquisition of optical and ancillary data on three AMT cruises,
2. analysis of the relationship of pigment ratios to chlorophyll $a$,
3. developing an optically derived method for determining the rate of photosynthesis,
4. linking the pigment and optical relationships to inherent optical properties.

![Diagram of Plankton, Pigment load, Photophysiology, Optical properties, Pigment ratio, Quantum efficiency, Absorption spectra, Primary production]

Figure 1.1: The three methods of measuring primary production explored in this thesis. Three phytoplankton measurements (pigment load, photo-physiology and optical properties) can be made through three methods (HPLC, FRRF and ac9+) to determine measures of primary production (pigment ratios, quantum efficiency and absorption ratios).

1.3 Thesis structure

This thesis consists of two introductory chapters followed by two methodology chapters, four data chapters, a discussion chapter and a concluding chapter. The introductory chapters (chapters 1 and 2) discuss the Atlantic Ocean and measurements taken onboard the AMT cruises to give a background to the study area and a general background to optics and bio-optics. The first methodology chapter (chapter 3) describes the methods used to develop suitable blanks for optical instruments in oceanic waters. The second methodology chapter (chapter 4) is the general methods chapter covering the protocols.
used in the thesis. The first data chapter (chapter 5) examines phytoplankton primary production rates determined optically on AMTs 12-14, and examines relationships between parameters measured using this technique. The following chapter (chapter 6) puts these relationships into a biological frame by discussing how pigment loads and pigment concentration ratios could explain patterns in primary production parameters. The third data chapter (chapter 7) examines how pigment relationships can be measured optically through *in situ* absorption spectra taken concurrently with primary production and pigment data. The three primary data chapters are then discussed in a final data chapter (chapter 8) in the context of each other to demonstrate interlinking between primary production parameters, pigment loads, pigment ratios and absorption spectra. The following chapter (chapter 9) discusses the results obtained in the context of the hypotheses of the thesis and suggests future work. The final chapter (chapter 10) states the conclusions of this thesis.

### 1.3.1 The Atlantic Ocean - Physical Structure

As in terrestrial systems, breaking down the ocean into smaller systems to derive relationships within each of these systems, where a universal relationship is not apparent, is highly advantageous [Begon et al., 1986, Aiken et al., 1992]. The Atlantic Ocean, as crossed by the AMT, can be summarized into five major regions based on the physical and biological characteristics of the water column [Longhurst, 1998, Zubkov et al., 2000]. In some parts of the ocean, under stable weather conditions, the water column stratifies into a surface mixed layer and a deeper, generally colder layer, between which is a transition region characterised by a rapid change in temperature, salinity and water density [Hooker et al., 2000]. This transition region is hereafter referred to as the thermocline. The region between the thermocline and ocean surface is the surface mixed layer and the depth of the thermocline below the surface is termed the mixed layer depth, MLD.

The northern and southern extents of the Atlantic, referred to as the Northern and Southern Temperate Regions, are generally well mixed by a combination of wind stress and convective cooling [Hooker et al., 2000]. There is little or no thermocline present during the winter, although a seasonal thermocline may occur during the summer [Aiken et al., 2004].

Closer to the equator, Coriolis forces and weather systems cause two anticyclonic vortices termed the North Atlantic Subtropical Gyre and the South Atlantic Gyre. Here, surface waters move towards their centres [Brown et al., 1991] and this, along with
stable weather conditions in the surface waters, causes a permanent thermocline to be present [Moran et al., 2004]. The movement of water towards the centres of the gyres results in upwelling where the gyres meet (at the equator) giving rise to the fifth major region, the Equatorial Upwelling [Longhurst, 1998]. Here a permanent thermocline may also be present, although the advection of surface waters into the gyre means that this thermocline is much shallower than in the gyres [Brown et al., 1991].

The temperate water bodies are generally characterised by high concentrations of chlorophyll (representing phytoplankton) throughout the water column [Aiken et al., 2000]. The stability of the gyres leads to extreme oligotrophy (low nutrient concentration) as the organisms present use up nutrients, which are then exported as organic matter that sinks to deep water [Lalli and Parsons, 1997]. This leads to very low chlorophyll concentrations in the surface waters with a distinct peak in concentration at the thermocline, where both light and nutrients are available [Letelier et al., 2004]. The Equatorial Upwelling is generally stratified, but the high rate of upwelling leads to higher nutrient levels, and hence chlorophyll concentrations, in the surface waters. In areas of high upwelling, the thermocline may be forced to the surface so that no stratification is present [Brown et al., 1991].

Previous work on defining these systems within the Atlantic Ocean was reviewed by Longhurst [1998], developing on a methodology based on AMT data [Longhurst, 1995]. This methodology utilises physical variables to determine the boundaries of provinces, largely based upon modelled datasets, and identifies seven regions through which the AMT cruise track runs as described in table 1.1 and shown in figure 1.2. Table 1.1 also shows how these provinces correspond to the regions described by Zubkov et al. [2000].

Table 1.1: The biogeochchemical provinces identified by Longhurst [1998] related to regions described by Zubkov et al. [2000].

<table>
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<tr>
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<tbody>
<tr>
<td>NADR</td>
<td>North Atlantic Drift Region</td>
<td>Northern Temperate</td>
<td>NT</td>
</tr>
<tr>
<td>NAST(E)</td>
<td>North Atlantic Sub-Tropical Gyre (East)</td>
<td>Northern Gyre</td>
<td>NASG</td>
</tr>
<tr>
<td>NATR</td>
<td>North Atlantic Tropical Gyre</td>
<td>Northern Gyre</td>
<td>NASG</td>
</tr>
<tr>
<td>WTRA</td>
<td>Western Tropical Atlantic</td>
<td>Equatorial Upwelling</td>
<td>EU</td>
</tr>
<tr>
<td>SATL</td>
<td>South Atlantic Tropical Gyre</td>
<td>Southern Gyre</td>
<td>SAG</td>
</tr>
<tr>
<td>SSTC</td>
<td>South Sub-Tropical Convergence</td>
<td>Southern Temperate</td>
<td>ST</td>
</tr>
<tr>
<td>FKLD</td>
<td>Falklands Current</td>
<td>Southern Temperate</td>
<td>ST</td>
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</table>

From the point of view of this study, it was desirable to place samples into a specific region. Such a region should be clearly definable but need not be part of a complex
Figure 1.2: Biogeochemical Provinces as described by Longhurst [1998]. The AMT cruises pass through the NADR (North Atlantic Drift Province), NAST(E) (North Atlantic Subtropical Gyre, East), NATR (North Atlantic Tropical Gyre), WTRA (Western Tropical Atlantic), SATL (South Atlantic Tropical Gyre), SSTC (South Subtropical Convergence) and FKLD (Falklands Current).

When qualitatively describing variations of parameters over large areas, the regions of Zubkov et al. [2000] have therefore been used in this study. However, in order to maintain a high number of samples within a region, a broader classification was desirable, so throughout this investigation stations were simply denoted as belonging to one of two groups: 'gyre' and 'non-gyre'. These were defined following Poulton [2002] and Omachi [2003] who both used phytoplankton diversity marker pigments in a multivariate statistical analysis package (PRIMER, Clarke and Gorley, 2001) to cluster empirically samples into provinces for comparative analyses.
1.3.2 Mixed Layer Depth Calculation

In this study vertical partitioning of the water column was required to allow distinctions to be made between physical layers containing functionally and taxonomically distinct phytoplankton communities. For optical measurements, this was undertaken using sea temperature and salinity and their variation with depth in the profile. From these data, density was calculated and used to measure the Brunt-Väisälä frequency, a measurement of the stability of the water column that shows the amount of energy required to mix the water column at that depth [Pond and Pickard, 1983]:

\[ N^2 = \frac{g}{\rho} \Delta \sigma_t \]

\( N^2 \) = Brunt-Väisälä frequency (s\(^{-2}\))
\( g \) = Acceleration due to gravity at sea level (9.81 ms\(^{-2}\))
\( \Delta \) = 'Change of per metre' (m\(^{-1}\))
\( \rho \) = Density (kg m\(^{-3}\))
\( \sigma_t \) = Density change due to salinity = \( \rho - 1000 \) (kg m\(^{-3}\))

The position of the mixed layer depth (MLD) is shown by a high value of the Brunt-Väisälä frequency, and since this consistently produced measurements of mixing depths that correlated well with subjective partitioning of the water column it was therefore used as the method for deriving MLD.

1.4 Phytoplankton Ecology and Taxonomy

This section examines the ecology, physiology and taxonomy of phytoplankton and discusses factors that may affect the community distribution and variation in functionality.

1.4.1 Physical and chemical requirements of phytoplankton

The chemical and biological characteristics of the Atlantic are interlinked with each other, since both are driven by the physical characters of the ambient water body [Hooker et al., 2000]. The nutrient composition of a section of the water column dictates the organisms that are able to survive there, and the use of nutrients by these organisms alters the nutrient concentration. This feedback drives the majority of the chemical and biological processes in the ocean.

Phytoplankton, like plants, require water, light and nutrients to live. Water is ubiquitous in the marine environment, so the availability of light and nutrients dictate the
spatial distribution of phytoplankton.

In mixed regions there is an even distribution of nutrients in the water. Under these conditions, the distribution of phytoplankton within the water column is also constant; the phytoplankton are unable to move against the currents that cause mixing [Clark et al., 1999]. In stratified water, like those found throughout much of the AMT cruise track, a surface layer forms that is mixed in the same way as a water column in a temperate climate [Hooker et al., 2000]. Within this surface mixed layer the community is homogeneous, however the nutrient supply is used up over time by the phytoplankton, so the nutrients are lost from the mixed layer [Letelier et al., 2004]. Nutrients from the more nutrient replete, deeper waters diffuse upwards, but a community of phytoplankton adapted to low light conditions is present at the thermocline and utilises much of the nutrient load diffusing across this boundary [Letelier et al., 2004]. The high concentration of pigments used to sustain this community results in a strong signal in fluorescence profiles; as a result the region where this community dominates is termed the fluorescence maximum (fmax).

1.4.2 Phytoplankton Taxonomy

Phytoplankton are a diverse group of single celled autotrophic organisms which, although they photosynthesise, are not plants. Like macroalgae, to which many are closely related, they are derived from disparate evolutionary lineages resulting in considerably more variation in their photosynthetic pathways than is found in plants [Cavalier-Smith, 1993]. This variation in photochemistry and morphology means that phytoplankton are able to survive in conditions under which plants would not and under a wider range of conditions [Moore and Chisholm, 1999]. Taxonomically, the smallest plankton are dominated by kingdom Monera, while the larger phytoplankton are represented by kingdom Protista [Mauseth, 1998].

In the sea, the phytoplankton are composed of three functional groups based on size. These are the micro (20μm-200μm), nano (2μm-20μm) and picoplankton (0.2μm-2μm) [Lalli and Parsons, 1997, Nybakken, 1997, Zubkov et al., 2000].

1.4.3 Microplankton

The microplankton are the largest celled phytoplankton in the ocean and account for the majority of phytoplankton biomass in regions where light and resources are not limiting [Sze, 1993]. They are composed almost exclusively of the diatom and dinoflagellate
families:

- Diatoms are large pill-box or pinnate shaped cells encased in a silica shell. They tend, along with dinoflagellates, to dominate in high nutrient waters, and are sometimes limited by the availability of silicate [Lalli and Parsons, 1997, Sze, 1993].

- Dinoflagellates, when in high concentrations, can produce toxins that prevent other algal groups competing with them in a bloom situation [Sze, 1993]. Dinoflagellates are often responsible for red tide harmful algal blooms encountered in coastal regions [Clark et al., 1999].

1.4.4 Nanoplankton

The nanoplankton are a disparate group of families including nanoflagellates and coccolithophores and generally dominate in the deep chlorophyll maximum community [Gibb et al., 2000]:

- Nanoflagellates is a catchall term that comprises a number of small celled phytoplankton taxa that generally dominate in much of the oligotrophic gyres [Gibb et al., 2000].

- Coccolithophores are instantly recognisable due to their covering of calcareous plates (coccoliths) [Hasle et al., 1997]. Coccolithophores are generally included within the nanoplankton due to their size and life history [Vidussi et al., 2001]. They will often bloom in huge numbers at continental shelf edges, turning the sea a milky blue colour that is easily visible on satellite images [Weeks et al., 2004]. The coccolithophore Emiliania huxleyi is generally considered to be the most cosmopolitan species in the oceans having an almost worldwide distribution [Hasle et al., 1997].

1.4.5 Picoplankton

The picoplankton are the smallest class of photosynthesising organisms found in the sea, their lower limit being approximately 0.2μm in diameter. Most picoplankton are prokaryotes, which are physiological and photochemically very distinct from eukaryotes in that they carry their nuclear material naked within the cytoplasm of the cell [Albets et al., 1994]. Instead of having chloroplasts (as in eukaryotic autotrophs), the photosynthetic apparatus is maintained on thylakoid membranes, vesicles which are held free within the cytoplasm of the cell and not enclosed in organelles.
Picoeukaryotes also fall into this group, but these are generally present in considerably smaller numbers than Prochlorococcus and Synechococcus on the AMT transect [Heywood, J and Zubkov, M., unpublished data]. Unlike other autotrophs, cyanobacteria do not use chlorophyll $a$ as a pigment in the antenna structure of PSII (see section 2.7), but instead use a class of photochemicals called phycobilins [Glover et al., 1987, Ting et al., 2002]. These absorb light in a different set of wavelengths to chlorophyll $a$ (resulting in their cyan colour by which they are named, [Mauseth, 1998]) allowing cyanobacteria to fill a niche missed by other species. Cyanobacteria still produce chlorophyll $a$, but in concentrations that can be up to three times lower than in other phytoplankton [Glover et al., 1987].

Prochlorophytes (genus Prochlorococcus) were recently discovered by Chisholm et al. [1988], and have been found to dominate the majority of the oceanic phytoplankton assemblages [Zubkov et al., 2000] between approximately 40° South and 40° North, although they are usually absent outside this area [Partensky et al., 1999]. Like the cyanobacteria, these organisms are prokaryotic, but unlike cyanobacteria they do use chlorophyll $a$ as a photosynthetic pigment in the antenna structure of PSII [Ting et al., 2002]. This chlorophyll $a$ is in its di-vinyl form, as opposed to the mono-vinyl form used by all other phytoplankton, and so is diagnostic of this genus. Currently there are understood to be two strains of Prochlorococcus with very different life history strategies with respect to their functional position in the phytoplankton. One strain, which is adapted to photosynthesise in extremely low light conditions, lives below the thermocline in high nutrient waters. The other strain is adapted to high light conditions and can be found living within the surface mixed layer [Moore and Chisholm, 1999].

1.5 The Atlantic Meridional Transect (AMT)

The cruise tracks of AMT 12-14, the cruises on which this thesis is based, are shown in figure 1.3. AMT cruises pass through approximately 100° of latitude from the UK (50°N) to the Falkland Islands (50°S). This large scale track provides the opportunity to measure physical, chemical and biological variables over a very long transect and investigate spatial variation within them. The repetition of the AMTs, twice a year for 10 years, has given a temporal element to the dataset such that annual and inter-annual variation can be investigated [Aiken et al., 2000, Poulton, 2002].
Figure 1.3: The cruise tracks of (top left) AMT 12 and (top right) 14 between the Falkland Islands and the UK (cruises going deep into the Northern Gyre) and (bottom) AMT 13 between the UK and the Falkland Islands (a cruise concentrating on the African Equatorial Upwelling Region). The colour bar represents chlorophyll a concentration from the SeaWiFS satellite [Images Lavender, S., unpublished data].
In September, the AMT cruises sample in the boreal (Northern hemisphere) autumn and austral (Southern hemisphere) spring, returning to the UK in the following April thus sampling the austral autumn and boreal spring.

1.5.1 AMT Structure and Phytoplankton Community Distribution

As described in section 1.3.1, the Atlantic can be divided into distinct regions, of which the AMT passes through several. These were sampled by physical measurements taken with a CTD (Conductivity, Temperature, Depth) instrument that profiled the water. As ancillary data, a chlorophyll fluorometer was used to give a measure of phytoplankton concentration. Figure 1.4 shows temperature and chlorophyll plots along the transect of AMT 14 demonstrating the five regions described by Zubkov et al. [2000].

From knowledge of the general requirements (water temperature, water clarity and nutrient availability) of the various phytoplankton types, an expected overview can be made of the biological structure of the Atlantic as shown diagrammatically in figure 1.5. Within temperate waters, where the water is well mixed, domination by large-celled dinoflagellates and diatoms can be expected [Aiken et al., 2004, Gibb et al., 2000] (figure 1.5, community A). Within the oligotrophic gyres, Prochlorococcus is likely to dominate within the surface mixed layer along with some Synechococcus (figure 1.5, community B). Below the surface mixed layer in the nutrient picocline (the region of nutrient change between the oligotrophic surface mixed layer and nutrient replete deeper water) a community dominated by nanoflagellates can be expected [Gibb et al., 2000, Zubkov et al., 2000] (figure 1.5, community C). At the equator, some patches may be dominated by larger cells if upwelling currents reach the surface, but if not then the structure from the gyres will be maintained, but compressed at shallower depths [Brown et al., 1991]. At depths when photosynthesis cannot produce sufficient energy for phytoplankton to survive, no viable phytoplankton populations will be present (figure 1.5, community D).
Figure 1.4: Latitudinal sections of temperature (top) and calibrated chlorophyll fluorescence (bottom) taken from the AMT 14 CTD rosette. Black points represent samples and profiles.
Figure 1.5: Expected community structure on the AMT cruises as discussed in section 1.5.1. Community A, diatoms and dinoflagellates, B, light adapted *Prochlorococcus* and cyanobacteria, C, nanoflagellates and dark adapted *Prochlorococcus* and D, no viable phytoplankton community.
Chapter 2

Optics and bio-optical interactions

2.1 The Electromagnetic Spectrum

The electromagnetic spectrum is made up of a continuum of wavelengths resulting from photons with differing energies. Although theoretically infinite, the limits of this spectrum are in practice considered to be gamma radiation (wavelengths of less than $10^{-11}$ m) to radio waves (wavelengths of the order of 1 m). In between, from short to long wavelengths there are X-rays, ultraviolet light, visible light, infrared light and microwaves (Figure 2.1) [Hecht, 2001]. All of these radiation types are emitted by the sun, but the Earth's atmosphere shields its surface from many of these wavelengths, filtering out harmful radiation and thus allowing life to exist on the planet [Jensen, 2000]. The atmosphere is particularly transparent to wavelengths between approximately 450 nm (blue) and 750 nm (red), and so the eyes of most organisms have evolved to make use these available wavelengths [Falkowski and Raven, 1997]. This range is therefore the spectrum visible to humans.

2.2 Interactions of light with water

Since over 70% of the Earth is covered in water, much of the light entering the atmosphere will come into contact with water bodies when it reaches the Earth's surface. When light interacts with water it can either be absorbed or scattered. These interactions are represented and quantified in a number of different ways [Kirk, 1994].

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Figure 2.1: The electromagnetic spectrum. The visible spectrum makes up a small fraction of this continuum, but is vital for photosynthesis.

2.2.1 Coastal and Oceanic waters

According to Morel and Prieur [1977] the world’s oceans can broadly be partitioned into two groups from an optics point of view, called case 1 and case 2 waters. Case 1 waters are those where all light interactions are caused by either the water itself or biogenic particles; these waters are commonly found in the open ocean. Here although almost all particulates are marine and biological in origin, there are some atmospheric inputs even in the middle of the oceanic basins. However, although these are biologically important [Baker et al., 2003], they are optically insignificant. Case 2 waters are ‘contaminated’ with terrestrial material in the form of riverine runoff and atmospheric inputs [Baker et al., 2003, Clark et al., 1999, Miller, 1994]. The cruise tracks of the AMTs are almost exclusively off the continental shelf and outside the influence of terrestrial inputs, therefore this investigation is centred on case 1 waters.
2.3 Inherent Optical Properties

The Inherent Optical Properties (IOPs) are those interactions that are identical in quantity regardless of the ambient light [Preisendorfer, 1976]. These interactions are absorption, scattering and attenuation. The simplest IOP to measure is beam attenuation \( c \), which is the sum of absorption \( a \) and scattering \( b \). As such it quantifies the total loss of light as it passes through a medium.

\[
c = a + b
\]  
(2.1)

If we consider a beam of light passing through a medium, in this case water, a part of the incident flux is absorbed by the water and particles within the water column. This can be quantified by dividing the fraction of the radiant flux absorbed by the path distance of the medium through which it is lost, known as the absorption coefficient. However, in order to define absorption the term absorbance, \( A \), is used. If we consider a beam of parallel light, \( \Phi_0 \), hitting a system then part of the radiant flux, \( \Phi_a \), is absorbed by the system. Therefore:

\[
A = \frac{\Phi_a}{\Phi_0}
\]  
(2.2)

If we consider an infinitely thin layer of thickness \( \Delta r \) and the incident flux lost to absorption is \( \Delta A \) then:

\[
a = \frac{\Delta A}{\Delta r}
\]  
(2.3)

Similarly for scattering:

\[
b = \frac{\Delta B}{\Delta r}
\]  
(2.4)

And since \( c = a + b \):

\[
c = \frac{\Delta C}{\Delta r}
\]  
(2.5)

In reality, infinitely thin layers cannot exist so we must derive an equation for a medium with finite thickness. Therefore, if we consider an infinitely thin layer within a medium of finite thickness, the infinitely thin layer is at a distance \( r \) into the medium. This medium is then illuminated with an incident flux \( (\Phi_0) \) that diminishes to \( \Phi \) over the distance \( r \) and the change of radiance between the two being denoted as \( \Phi_\Delta \) (negative
since it is a loss of radiance), then:

$$\Delta C = -\Phi_A/\Phi_r$$  \hspace{1cm} (2.6)

If we consider the depth of the medium and therefore integrate between widths 0 and r:

$$\ln(\Phi/\Phi_0) = -cr$$  \hspace{1cm} (2.7)

This demonstrates an exponential drop in flux with an increase in medium penetration (Beer Lambert’s Law) and may be rewritten as:

$$c = -1/r \ln(\Phi_0/\Phi)$$  \hspace{1cm} (2.8)

$$c = -1/r \ln(1 - C)$$  \hspace{1cm} (2.9)

We can therefore determine c if we know the proportion of radiant flux lost as it passes through a medium and the thickness of the medium through which the light has travelled. In a system where scattering is negligible it follows that:

$$a = -1/r \ln(1 - A)$$  \hspace{1cm} (2.10)

And in a system where absorption is negligible that:

$$b = -1/r \ln(1 - B)$$  \hspace{1cm} (2.11)

### 2.3.1 Absorption characteristics of pure water

Atoms and molecules interact with light, absorbing and emitting photons. The energy level at which a particular molecular species can absorb light is highly specific and is dependent on the energy required either to excite an electron within the shell of that atom or molecule to a higher state, or to cause vibration of bonds between atoms in a molecule [Hecht, 2001]. The absorption coefficient quantifies this interaction in terms of flux lost per metre of medium (m\(^{-1}\)). In the same way as air in the atmosphere attenuates a large part of the spectrum, water has a similar effect in the ocean [Lalli and Parsons, 1997]. Figure 2.2 shows an absorption spectrum of pure, bi-distilled, deionised water in the visible spectrum. Note that the longer wavelengths show high absorption compared to the shorter wavelengths. As a result, the shorter (blue) wavelengths penetrate further into the ocean from the sun than do the longer (red) wavelengths [Kirk, 1994].
Figure 2.2: The absorption spectrum of pure water in the visible spectrum measured by spectrophotometer at Plymouth Marine Laboratory.
2.3.2 Scattering

Total scatter is the combination of reflection, refraction and diffraction and, like absorption, is quantified by the scattering coefficient (b). Scatter cannot easily be directly measured; it is often calculated, as described in section 2.8.1, using absorption and beam attenuation, which can be directly measured.

Scattering is a result of light interacting with particles in the water column [Kirk, 1994]. If light is scattered from its direct downwelling path, it will travel through the water at an angle away from the vertical. Simple geometry tells us that it has to travel through a greater distance within the medium to reach a certain depth and therefore the likelihood of it being absorbed before it reaches that depth is increased. The primary net result of this is to increase the attenuation of light in the water column.

In case 1 waters, the effect of scattering compared to absorption is comparatively small due to the very low concentration of particles in the water column. Scattering that does occur is due to biogenic particles, which interact in the same way as inorganic or terrestrial particulate matter [Kirk, 1994].

2.4 Bio-optical interactions

Bio-optical interactions are defined as interactions between the ambient light field and the biota or products of the biota. Considered here are the use of light in photosynthesis by planktonic autotrophs.

2.4.1 Photosynthesis

A fundamental process of life is the use of light energy to fix carbon and so provide energy for biological systems. Living systems cannot directly utilise light energy, but can through a series of reactions, convert it into C-C bond energy that can be released by glycolysis and other metabolic processes. As the eyes of many animals have evolved to use the most available radiation, plants have evolved to use pigments that capture these wavelengths for photosynthesis [Falkowski and Raven, 1997]. As photosynthesis is a series of chemical reactions driven by energy from light that fixes carbon into carbohydrates and evolves oxygen, the basic chemical equation is:

$$6CO_2 + 6H_2O + h\nu \rightarrow C_6H_{12}O_6 + 6O_2$$

(2.12)
The band of wavelengths collected by photosynthetic pigments is known as Photosynthetically Available Radiation (PAR) and is defined as being between 400 and 700nm, which corresponds closely to visible light [Tyler, 1966]. PAR is a major limiting factor for carbon fixation as photosynthesis can only occur where PAR is present in sufficient quantities for the energy collected by absorption to outweigh the energy cost of synthesising and maintaining the photosynthetic apparatus. The region where this is the case is termed the euphotic zone [Banse, 2004].

2.5 Phytoplankton pigments

2.5.1 Chlorophyll a

A number of pigments and pigment groups are used by phytoplankton in photosynthesis. The most important pigment is chlorophyll a, which absorbs light strongly in two bands (see figure 2.3, top), one centred at a wavelength of 676nm and a more dispersed, but more significant, band between 400nm and 490nm [Aiken, 2001].

Chlorophyll a is used by all photosynthetic organisms as the photosynthetic reaction centres [Govindjee, 1975], and so are the only pigments capable of transferring absorbed light energy into the photosystems of the photosynthetic pathway. Photosystems are arrangements of chlorophyll a and other pigments located within the chloroplasts in eukaryotes, the organelles of the cell where photosynthesis takes place. Some prokaryotes have only one photosystem, photosystem II, so numbered because, while it was probably the first to evolve, it was the second one discovered [Mauseth, 1998]. Eukaryotes have photosystem II (PSII) and photosystem I (PSI). PSI uses chlorophyll a in the form referred to as P700, while photosystem II uses a form of chlorophyll a known as P680 [Mauseth, 1998]. Both of these forms of chlorophyll a function in photosynthesis, however only PSII is given detailed consideration in this study as the reactions of PSI are generally not rate determining in photosynthesis [Mauseth, 1998].

Chlorophyll a is present in a number of forms dependent on the species of phytoplankton in which it is found. Mono-vinyl chlorophyll a, generally simply referred to as chlorophyll a and abbreviated to Chla, is present in all eukaryotic autotrophs. The di-vinyl form of chlorophyll a is found only in Prochlorococcus and is referred to as DvChla. The sum of all chlorophyll a species will be referred to as total chlorophyll a (TChla).
2.5.2 Accessory pigments

Although only chlorophyll \( a \) reaction centres can transfer energy to the photosystems (see section 2.7) other molecules are capable of absorbing light energy and transferring that energy to the chlorophyll \( a \) molecules. These chemicals are referred to as the accessory pigments; they absorb light over a greater range of wavelengths than chlorophyll \( a \) and so increase the efficiency of light absorption. In summary, the pathways by which light energy can enter the photosynthetic systems are:

\[
\text{Sun} \rightarrow \text{Chlorophyll } a \rightarrow \text{Photosystem II} \\
\text{Sun} \rightarrow \text{Accessory Pigments} \rightarrow \text{Chlorophyll } a \rightarrow \text{Photosystem II}
\]

Accessory pigments are highly variable between taxa and are often used as diagnostic markers for the presence of a taxa as discussed in section 2.6. These pigments fall into three categories, the chlorophylls (\( a, b \) and \( c \)), the carotenoids and the phycobilins. Note that chlorophyll \( a \) can also act as an accessory pigment and is not only found as a reaction centre [Ting et al., 2002]. Figure 2.3 shows the absorption spectra of these groups of pigments. Chlorophylls \( b \) and \( c \) have a similar absorption spectrum to that of chlorophyll \( a \), however the spectrum for chlorophyll \( b \) is slightly compressed (figure 2.3, top) such that the red absorption peak is at 650nm, allowing absorption in a wider band at the red end of the spectrum [Govindjee, 1975, Jensen, 2000, Jeffrey et al., 1997a]. Chlorophyll \( c \) does not have an absorption band in the red end of the spectrum, but has a very wide range of absorption in the blue end of the spectrum [Jensen, 2000, Jeffrey et al., 1997a, Wozniak et al., 1998].

The carotenoids absorb light at wavelengths where chlorophyll \( a \) does not; that is to say in a wide band between 400nm and 550nm, with a maximum absorption at approximately 440nm (figure 2.3, bottom) [Jensen, 2000, Sokoletsky et al., 2003]. Phycobilins absorb light in the region between 500nm and 650nm (figure 2.3, bottom) [Jensen, 2000, Mauseth, 1998, Ting et al., 2002].

The carotenoids fall into two functional groups: those that absorb light and transfer it to the reaction centres, termed photosynthetic carotenoids (PSCs); and those that absorb light, but do not transfer the energy and so radiate it as heat and light (photoprotectant carotenoids, PPCs). PPCs, as discussed in section 2.7.1, fulfill the function of protecting the photosynthetic pathways from damage when the cell is exposed to very high light levels as, for example, found in the surface waters of the gyres. Under these conditions, if only photosynthetic pigments were present, the energy input would result
Figure 2.3: Positions of oceanic phytoplankton pigment absorption peaks from Jensen [2000]. Top shows the absorption spectra of chlorophyll $a$ and the more compressed absorption spectrum of chlorophyll $b$, with the red absorption peak shifted towards the blue. The bottom plot shows the absorption spectrum of $\beta$-carotene, a carotenoid, which absorbs in a similar region to the blue absorption of the chlorophylls, and the absorption peaks of two phycobilins (phycoerythrin and phycocyanin), that are between the two absorption peaks of the chlorophylls.
in the production of large numbers of highly reactive free radicals that would damage the photosynthetic pathways. Absorption by PPCs reduces the energy flow into the system to levels where damage is minimised [Muller et al., 2001], thus allowing the phytoplankton to survive in a niche that would otherwise not be available [Hulburt, 1977].

The accessory pigment load of a community is often considered as a single entity to allow ease of comparison with other measures. More specifically, the total accessory carotenoid load (TAC) will often be discussed, that is the sum of the PSC and PPC concentrations. Similarly, the total pigment load (Tpig) of the sample will also be discussed; this is the sum of all pigment concentrations.

2.6 Diagnostic Pigments

The identification of community structure by pigment composition has frequently been used to fractionate the community by taxonomic group [Aiken et al., 2004, Barlow et al., 2001, 2004, Claustre, 1994, Uitz, 2002, Vidussi et al., 2001]; indeed, computer software is commercially available that can be used to make further inferences about community structure based on these data [Puraya et al., 2003, Garibotti et al., 2003, Havskum et al., 2004, Hutchinson, 1961, Lutz et al., 2003, Mackey et al., 1996, Schluter and Mohlenburg, 2003]. In this study, these methodologies were adapted to split the community into separate functional groups and examine the pigment relationships within these groups.

The quantities of phytoplankton pigments present were examined in large datasets by Trees et al. [2000], who were able to demonstrate a strong linear correlation between TAC and TChla. Although this relationship was striking, Trees et al. [2000] found considerable variation in the regression lines between cruise datasets, suggesting that other factors were affecting the relationship.

Two ratios, TChla:Tpig and TChla:TAC, have been used to investigate the nature of this relationship by determining correlations between them and other variables. Aiken et al. [2004] demonstrated, in a seasonal study at a sampling station in the Western English Channel, that there was a log linear relationship between the ratio of TChla:TAC and TChla. It was suggested that this could be seen as an indication of the state of health of the phytoplankton community. The reasoning was that phytoplankton cells would preferentially synthesise the relatively energy-expensive and unstable chlorophyll a under optimal growth conditions, thus maximising their ability to photosynthesise. Communities undergoing rapid growth or turnover would therefore have a relatively high ratio of chlorophyll a to other pigments, as well as a high chlorophyll a concentration.
Since the hypothesis of Aiken et al. [2004] suggests a pigment ratio varying with chlorophyll a concentration, it is in direct conflict with that of Trees et al. [2000], which suggests a constant pigment ratio. An explanation of this might be that the relatively constant physical and chemical variables, and corresponding relatively stable phytoplankton community, at a single sampling station enabled the relationship described by Aiken et al. [2004] to be seen. However, on larger scales (as in oceanographic cruises) these variations might be masked by geographic and taxonomic variations.

2.7 Absorbing light - the reaction centres and photosystems

To enter the photosystems light must first be absorbed, and this is achieved in phytoplankton by units called antenna complexes (figure 2.4). In each antenna complex there is a single chlorophyll a molecule, termed the reaction centre, that passes energy into the photosystems [Mauseth, 1998]. To increase the efficiency of this reaction centre a large number of pigment molecules are associated with it. Chlorophyll a, b, and c molecules as well as accessory carotenoids are clustered around this reaction centre molecule and are held in position by their hydrophobic tails, which are held in a structure called the thylakoid membrane. The close proximity of these pigments enables them to donate energised electrons between pigment molecules until they reach the reaction centre molecule in a process called resonance energy transfer [Falkowski and Raven, 1997]. This means that the entire antenna structure acts much like a single large molecule as seen in figure 2.4.

Carotenoids are relatively less efficient than chlorophylls in this method of energy transfer, thus reducing the light harvesting abilities of cyanobacteria which do not use chlorophylls in their antenna structures. Instead they use pigment structures called phycobiliproteins, which carry phycobilins as their light harvesting pigment. However, cyanobacteria still use the single chlorophyll a molecule as the reaction centre within the antenna complex [Govindjee, 1975].

The reactions of photosynthesis are divided into two distinct pathways, the Light Dependent Reactions and the Light Independent Reactions. As is shown in figure 2.5, light energy is absorbed by the PSII antenna structure and passed to the reaction centre where it raises an electron to a higher energy level. This electron is expelled and passes through a series of intermediates to the cytochrome b6 complex and from there to PSI. To
Figure 2.4: The antenna pigment complex of PSII embedded through the component pigment’s hydrophobic tails into the thylakoid membrane (yellow). Quanta of light are absorbed by the accessory pigments (light green), which transfer the absorbed energy to the chlorophyll reaction centre (dark green) to enter PSII, from Mauseth [1998].
replace this electron in PSII, water is split to produce O₂ and 4H⁺ per 2 H₂O molecules. An electron is donated in this reaction, O₂ is expelled and the osmotic potential from the H⁺ is used in the synthesis of ATP. This is the reaction route used for energy fixation in prokaryotes. In eukaryotes the electron donated from PSII is energised by light absorption and used to reduce NADPH and a proton from NADH₂. Again this H⁺ is used in the synthesis of ATP. The ATP created in these reactions is stored in C–C bonds by carbon fixation of CO₂ [Falkowski and Raven, 1997]. In the Light Independent Reactions, carbon dioxide (from the atmosphere or, in the case of marine organisms dissolved in water) is captured and modified by the addition of hydrogen to form carbohydrates, which have the empirical formula of [CH₂O]. The incorporation of CO₂ into organic compounds is known as carbon fixation [Mauseth, 1998].
2.7.1 Quenching

Under high light conditions the amount of energy being absorbed by the phytoplankton can saturate the photosystems such that they cannot process all of the energy entering the system. Under these conditions the chlorophyll $a$ molecules of the reaction centres can, instead of handing energy to the photosystems, produce a highly oxidising, and therefore damaging, free radicals [Muller et al., 2001]. To reduce the occurrence of this, phytoplankton have developed protective strategies, called quenching, to remove this energy from the system. Energy can be lost at the point of absorption by the photoprotectant carotenoids, which absorb light energy and, instead of transferring this energy to the reaction centres, radiate it as either heat or light in the form of fluorescence [Muller et al., 2001]. However, above a certain level of irradiance these mechanisms will become swamped and therefore ineffective, and damage of the photosystems will result [Demmig-Adams and Adams III, 1992]. This damage results in a decrease in the variable fluorescence of the sample due to an increase in the initial fluorescence of a sample under subsaturation radiance ($F_o$) (see section 2.10.1) and is termed photoinhibition. This can also be observed in standard fluorometer measurements as a decrease of fluorescence for a given chlorophyll $a$ concentration in surface waters [Behrenfeld and Boss, 2003]. This process allows phytoplankton to survive and photosynthesise in regions of the water column where no viable population could otherwise exist [Ficek et al., 2000].

2.7.2 Primary photosynthetic pigment absorption and water column community structure

The absorption of light and the use of that energy, along with the availability of nutrients, determine the community structure within the water column [Gibb et al., 2000]. In the surface waters light levels are high, to the extent that the energy being absorbed cannot be used and the photosystems are quenched. Under these conditions Prochlorococcus, which can tolerate very low nutrient conditions and has a large load of PPCs, dominates [Gibb et al., 2000]. At fluorescence maximum ($f_{max}$) the nanoflagellates dominate, since these are less tolerant of low nutrient conditions but are able to exploit the lower irradiance levels in this layer [Gibb et al., 2000]. Their pigment load contains more PSCs and, unlike the prokaryotes, they also synthesise large quantities of chlorophyll $b$ as an accessory pigment [Ting et al., 2002]. Since most of the red spectrum at the depth of the $f_{max}$ has been filtered out by the absorption of the water itself, the phytoplankton using chlorophyll $b$ have a competitive advantage, and under these conditions the community structures...
discussed in chapter 1 and shown in figure 1.5 result.

### 2.7.3 Non living biological light interactions

Not all interactions of light with biogenic material involve living organisms; excreta, shed sections of cell walls, dissolved material, cell contents resulting from messy eating by predators and dead organisms are all present in the water column [Morel and Maritorena, 2001]. Many of these materials interact with light, at some wavelengths significantly more than the living biological material. A major component of these materials is Coloured Dissolved Organic Matter (CDOM), defined as any biogenic material in the water of less than 0.2 μm in diameter, and is therefore considered dissolved. At 450 nm CDOM may absorb up to eight times more light than the phytoplankton in oligotrophic waters [Krause-Jensen et al., 1998]. A typical absorption spectrum of CDOM is shown in figure 2.6. Absorption of CDOM decreases exponentially with wavelength and so measurements of dissolved matter are normally made at wavelengths of less than 450nm [Tilstone et al., 2003].
2.7.4 Particulate vs. pigment absorptions: The package effect

The absorption characteristics of pigments extracted into a solvent as in HPLC (a methodology used to identify and quantify pigments, see section 4.3.1) differ from those in vivo due to the package effect [Kirk, 1994]. The pigments are encapsulated in the thylakoid membranes of the chloroplasts in the eukaryotes and on various photosynthetic membranes in the prokaryotes [Mauseth, 1998], and are also complexed with the photosystem molecules. Consequently, the wavelengths at which they absorb light, as well as the magnitude of absorption, are slightly altered [Bissett et al., 1997]. However, in the open ocean small cells dominate the phytoplankton community, and since these small cells are relatively unaffected by the package effect, problems arising from this mechanism are likely to be minimised [Nelson et al., 1993].

2.8 Absorption measures

Margalef [1967] reported that in areas of high productivity the ratio of the light absorption by phytoplankton at 670nm and 490nm (a_670:a_490) increased compared to less productive waters. The instruments available at the time meant that it was not possible then to measure this effect quantitatively. With the development of particulate absorption spectra [Ferrari and Mingazzini, 1995, Tassan and Ferrari, 1995], the profiling spectrophotometer [Wetlabs inc, 2004a, Green et al., 2003] and the phytoplankton absorption methods described in this study it is now possible to quantitatively measure phytoplankton community absorption both discretely and as depth profiles [Green et al., 2003].

The maximum diagnostic absorption concentration of chlorophyll a in vivo is at 676nm, with the bulk of the rest of the pigments showing a maximum absorption at approximately 490nm [Kirk, 1994] (see figure 2.7). These wavelengths fit with the observations of Margalef [1967] and are consistent to the pigment ratio theory of Aiken et al. [2004]. If we therefore substitute the absorption of the phytoplankton at 676nm for the concentration of chlorophyll a, and the absorption at 490nm for the total pigment concentration, then an optically derived variation of the pigment ratios can be produced.

The findings of Margalef [1967] were investigated in a number of laboratory culture studies. Watson and Osborne [1979] and Holmboe et al. [1999] showed variations in a_480:a_665 ratios in extracted pigments. This ratio equates to the pigment ratio of total pigment absorption:chlorophyll a absorption (a_Tpig:a_chla) and they showed high ratios
Figure 2.7: Absorption spectra of a typical phytoplankton sample showing the major pigment groups and total absorption [Wozniak et al., 1998].

at extreme nitrogen deprivation. Schlueter et al. [1997] showed these ratios were also high in nutrient replete cultures suggesting that other factors were affecting absorption ratios. Heath et al. [1990] showed that these ratios also varied with taxonomic makeup of cultures under similar conditions. So, while it has been demonstrated that a number of factors affect absorption ratios, no robust correlations related to these relationships have been described.

2.8.1 Determination of absorption and scattering

Attenuation, absorption and scattering measurements are all passive measurements of water parameters, in that their measurement does not affect the sample being measured. This is in contrast to an active measurement such as fluorometry that relies upon a biological response (fluorescence) from a stimulus to make the measurement. These passive measurements have the advantage that they can be determined remotely and therefore relationships based on these parameters have the potential to be measured using techniques such as satellite observations.

Traditionally, attenuation has been measured using beam transmissometers, which simply measure the attenuation of monochromatic light over a known distance [Chelsea
Instruments Ltd, 1996]. To do this, a light source of known intensity is directed towards a photoreceptor while passing through a chamber open to the environment. Interactions between the light and water (scattering and absorption) take place, and the amount of light that remains after these effects is measured by the photoreceptor. In recent years, a new methodology has been developed using modified beam transmissometers to measure attenuation and absorption. If we know absorption and attenuation then scattering can be calculated by equation 2.1.

The ac9+ (figure 2.8) produced by Wetlabs Inc. measures absorption and attenuation at nine wavelengths: 412nm, 440nm, 488nm, 510nm, 532nm, 555nm, 610nm, 676nm, 715nm. The instrument consists of two separate beam transmissometers, which are not open to the environment; instead water is pumped through tubes between the light source and the detector. The first tube has matt black sides such that any light scattered by particles in the water column is absorbed by the wall of the tube; any light absorbed by components of the water is also lost to the photodetector. This is therefore similar to a standard beam transmissometer in which attenuation is measured as the combined effects of scattering and absorption are accounted for. The second tube has highly polished walls, which means that light is scattered rather than being lost, and is measured by the photoreceptor. The effect of scattering is therefore negated and only light absorption is measured [Wetlabs inc, 2004a].

In this investigation, the absorption characteristics are used. These wavelengths are relevant for in situ measurements of phytoplankton absorption characteristics as described in table 2.1. The main wavelengths used are 488nm (total accessory pigment and carotenoid absorption) and 676nm (chlorophyll a red peak maximum absorption), as shown in figure 2.7.

2.9 Determination of Biotic Absorption and Scattering

The absorption and scattering of particles (mainly phytoplankton), as opposed to dissolved constituents of the water, can also be determined in a novel method using the ac9+, which was investigated during this study. A double cast technique was employed to do this, measuring CDOM absorption in the first casts and total absorption in the second. This was done by connecting a 0.2μm cartridge water filter to the inflow of each tube of the ac9+ on the first cast, such that only dissolved material or small non photosynthetically active particles pass through [Zubkov et al., 2000] as shown in figure 2.9. The measurements of the ac9+ will therefore only show the absorption of the Coloured
Figure 2.8: Diagrammatic layout of the ac9+ instrument from Wetlabs inc [2004a]. Note the parallel tubes used to measure absorption and attenuation.
Table 2.1: Absorption wavelengths measured by the ac9+ and their significance [Staehr et al., 2004].

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Attribute</th>
</tr>
</thead>
<tbody>
<tr>
<td>412</td>
<td>UV photo protective pigments and CDOM maximum absorption</td>
</tr>
<tr>
<td>440</td>
<td>Maximum absorption in the blue peak of chlorophyll a</td>
</tr>
<tr>
<td>488</td>
<td>Maximum carotenoid absorption</td>
</tr>
<tr>
<td>510</td>
<td>Phycobilin absorption</td>
</tr>
<tr>
<td>532</td>
<td>Phycobilin absorption</td>
</tr>
<tr>
<td>555</td>
<td>Minimum pigment absorption</td>
</tr>
<tr>
<td>650</td>
<td>Maximum chlorophyll b absorption</td>
</tr>
<tr>
<td>676</td>
<td>Maximum absorption in the red peak of chlorophyll a</td>
</tr>
<tr>
<td>715</td>
<td>Baseline zero particulate absorption</td>
</tr>
</tbody>
</table>

Dissolved Organic Matter (CDOM) and scattering of very small particles, which have come through the mesh [Wetlabs inc, 2004b, Green et al., 2003]. The second cast, without the filters, took measurements of both the dissolved and particulate components of the water column. The casts were taken in this order so that particulate material from the unfiltered cast did not contaminate the measurements tubes of the ac9+, thus removing the necessity to clean the instrument between casts.

The double cast system allows the derivation of the absorption and scattering effects of the phytoplankton; the optical properties of the phytoplankton are equal to those of the total minus those of the CDOM:

\[ a_{\text{particulate}} = a_{\text{total}} - a_{\text{CDOM}} \]  \hspace{1cm} (2.13)

Attenuation, absorption and scattering spectra for the total water column, the dissolved constituents of the water column and the plankton within the water column can therefore be constructed [Kirk, 1994]. These properties are affected by the physical state of the water and as such corrections must be applied for water temperature and salinity [Pegau et al., 1992, Wetlabs inc, 2004b, Zaneveld et al., 1992].

2.10 Fluorescence

When light is absorbed by chlorophyll a, an electron in the molecule is excited to a higher energy level. But the limited availability of open reaction centres means that much of this energy is not used for photosynthesis, since the energy leaving the system must always equal that absorbed. If the possible routes of energy loss are examined, then three sinks of energy can be determined [Falkowski and Kiefer, 1985].
Figure 2.9: ac9+ setup to measure CDOM. Water passes through 0.2μm filters before entering the measurement tubes, thus removing any particles including photoactive organisms.
1. The energy can enter the photosynthetic pathway, allowing the chlorophyll molecule to return to its ground state.

2. The molecule can return to its ground state by passing energy to another molecule, in this case oxygen, creating an oxygen radical. This radical is highly reactive and as such harmful to the photosynthetic system, but as the photosynthetic system is highly efficient this pathway is minimised.

3. Energy can also be removed from the system as heat loss to the surrounding medium and as a fluorescence to the environment [Falkowski and Kiefer, 1985].

Since the photosynthetic apparatus of autotrophs interacts with the light field in a dynamic way it does not have the same optical properties that would be observed in non photoactive matter. In inert materials, the energy lost by an electron falling back to its original energy state after being absorbed will have the energy transmitted as a photon at the frequency equating to the energy of that drop [Kirk, 1994] by:

\[ \epsilon = h\nu \]  

\( \epsilon \) = Energy drop of electron returning to ground state, photon energy, (J)  
\( h \) = Planck's constant \((6.63 \times 10^{-34} \text{Js})\)  
\( \nu \) = Frequency of resulting light, \((s^{-1})\)

However, in chlorophyll \(a\), instead of energy being fluoresced directly back into the environment, it is absorbed by the antenna complex of PSII. When this energy is in the photosystem it can either be processed through the photosynthetic pathways or, if the photosystem is non functional, fluoresced back into the environment. The transfer of energy through the antenna structure to the photosystem means that this energy loss is termed delayed fluorescence, and may take approximately \(10^{-3}\) seconds relative to the \(10^{-9}\) seconds of direct fluorescence. This delayed fluorescence can be used to measure the concentration of chlorophyll \(a\) in a sample - a traditional measurement of phytoplankton presence in oceanography [Aiken, 2001, Chelsea Instruments Ltd, 2002b, Kirk, 1994, Margalef, 1967]. The phytoplankton are subjected to monochromatic light at a wavelength of 470nm and an intensity such that the PSII reaction centres become saturated and are no longer able to process the energy. A photo-detector is then used to measure the amount of light that is fluoresced at 683nm by the chlorophyll \(a\) [Chelsea
Instruments Ltd, 2002b]. Since the light is at saturation intensity, all or almost all, of the chlorophyll a molecules will fluoresce and so the fluorescence is directly proportional to the concentration of chlorophyll a [Laney, 2002]. The wavelength of fluorescence is different to that of the excitation energy, and therefore there is no contamination of the measurement by the light source [Aiken, 2001, Chelsea Instruments Ltd, 2002b].

2.10.1 Active Fluorescence

The physiological state of phytoplankton and their rate of photosynthesis can be measured by the interactions of light at sub saturation intensities within PSII reaction centres [Kolber et al., 1990].

If the energy being absorbed by the system is considered to be unity then the sum of energy loss though photochemistry ($K_p$), heat ($K_h$) and fluorescence ($K_f$) must also be unity:

$$K_p + K_h + K_f = 1 \quad (2.15)$$

When a photosynthetic system saturated by light is considered, this can be simplified. All photosynthetic reaction centres can be considered inoperable as they are saturated, and therefore the energy loss to the system must be as a result of heat and fluorescence loss. As this represents the maximum fluorescence and heat loss from the system the suffix $m$ is used:

$$K_{hm} + K_{fm} = 1 \quad (2.16)$$

An assumption is made that the proportion of energy lost from the system by heat and fluorescence are constant at any irradiance level [Kolber et al., 1990]:

$$\frac{K_{fm}}{K_{hm}} = \frac{K_f}{K_h} \quad (2.17)$$
These assumptions can be used to derive the relative fluorescence emission rate, $F$, of the system. If the energy absorbed by the system is $E_a$, and the reactions competing for the returning energy of the excited chlorophyll $a$ to ground state is summed as $\sum K_i$, then the proportion of energy being returned as fluorescence ($k_f$) can be used to determine $F$:

$$F = \frac{E_a K_f}{\sum K_i} \quad (2.18)$$

From this, the fluorescence yield ($\Phi F$) of the system can be calculated:

$$\Phi F = \frac{F}{E_a} = \frac{K_f}{K_p + K_h + K_f} \quad (2.19)$$

As previously discussed, the efficiency of the photosynthetic system means that in a relaxed state, with all reaction centres being open, the energy flow into the photosynthetic system is much higher than the energy loss through heat and fluorescence.

$$K_p \gg K_h + K_f \quad (2.20)$$

However, when all the reaction centres are closed $K_p$ can be considered 0 thus fluorescence yield is maximum and denoted as $\Phi F_m$. The same methodology can be used to determine the energy that is taken in by the potential photochemical yield of PSII ($\Phi P_0$).

$$\Phi P_0 = \frac{K_p}{K_p + K_h + K_f} = \frac{\Phi F_m - \Phi F_0}{\Phi F_m} = \frac{F_v}{F_m} \quad (2.21)$$

Subtracting the minimum fluorescence yield from the maximum ($\Phi F_m - \Phi F_0$) gives the variable rate of fluorescence of the system ($F_v$). The photochemical efficiency of PSII has been shown to be directly linked to the PSII photochemical yield, $\frac{F_v}{F_m}$, and is therefore an integral part of determining of rates of primary production using optical methodologies [Kolber et al., 1998].
The Fast Repetition Rate Fluorometer (FRRF)

The methodology that has been developed to investigate sub-saturation fluorescence is known as Fast Repetition Rate (FRR) fluorometry [Kolber et al., 1998], and at present the only commercially available instrument that utilises this methodology in situ is Chelsea Instruments's FASTtrackTM FRR fluorometer (hereafter FRRF). The layout of the optics of the FRRF can be seen in figure 2.10. Mechanically, this is similar to traditional fluorometers in that a sample is subjected to light and the resulting fluorescence is measured. However, it does this by subjecting the sample to a series of rapid, sub-saturation, flashlets at a rate at which PSII is unable to process the resulting energy so some of it is retained within the system. The fluorescence from the initial sub saturation flashlet (divided by the excitation energy from the FRRF to give a fluorescence ratio not affected by variations in the LED light emissions) indicates the phytoplankton's ability to absorb light. When the next flashlet is applied to the sample it is analogous to having a flashlet of a slightly higher intensity and thus the resulting fluorescence signal is slightly higher. This continues through the flash sequence, typically 100 flashes with an interval of approximately 5 μs seconds between flashlets [Chelsea instruments Ltd, 1998], until the saturation level of the sample is reached (figure 2.11). At this point, the fluorescence measurement is equivalent to that of a standard fluorometer.

As described in section 2.7.1, under high light conditions (particularly in the clear waters of the gyres), the plankton adapt their physiology to prevent damage to their photosynthetic systems. This can take the form of production of photo protectant pigments or changes in the structure of the cell contents. Under these conditions the ability of the phytoplankton to use light is reduced. The FRRF therefore has two chambers, the light chamber and the dark chamber (figure 2.10). The light chamber is open to the ambient light conditions, so that under high light conditions, these measurements have undergone photochemical quenching. The dark chamber is enclosed preventing ambient light from reaching the sample and thus ameliorating quenching. Therefore, the photosynthetic parameters are measured when the phytoplankton are closer to its maximum photosynthetic capacity [Chelsea Instruments Ltd, 1998].

Using the Kolber et al. [1998] model, six parameters are measured using active fluorescence during the excitation and relaxation phases for each chamber (see figure 2.11).

1. The initial fluorescence (\(F_0\), arbitrary units) is the initial fluorescence when the sample is subjected to an excitation flashlet.
Figure 2.10: Diagrammatic representation of the FRRF measurement heads. LEDs produce blue light (blue arrows) exciting chlorophyll α molecules in the sample. Delayed fluorescence from PSII (red arrows) is measured perpendicular to the excitation pathway. Energy collected is increased through a Photo Multiplier Tube (PMT) to an extent determined by the gain setting of the instrument prior to reading the fluorescence. A small detector embedded in the LED array measures the light output from the LEDs to allow normalisation. The left diagram shows these measures in the 'Light Chamber' that is open to the ambient light conditions of the sample. The right diagram shows the ‘Dark Chamber’ where the measured sample has been allowed to recover from photochemical quenching (see section 2.7.1) [Chelsea Instruments Ltd, 1998].
2. The maximum fluorescence \( (F_m, \text{arbitrary units}) \) is the maximum fluorescence yield from a light saturated sample.

3. The photosynthetic cross sectional area of PSII \( (\sigma_{PSII}, \text{Å}^2 \text{(quanta)}^{-1}) \) is a measurement of the size of PSII antenna complexes in the sample.

4. Variable fluorescence \( (F_v, \text{arbitrary units}) \) is the ability of the phytoplankton to retain energy absorbed while that light is being processed, which is calculated as \( F_m - F_o \).

5. Photosynthetic Quantum Efficiency (PQE); this value is defined as \( \frac{F_v}{F_m} \) (dimensionless) and measures the proportion of open, functioning reaction centres and therefore the amount of energy that the system is capable of processing at any one time.

6. The turnover rate of PSII, \( \tau \) (seconds).

2.11.1 Primary production parameters measured by the FRRF

Two primary production parameters as determined by the FRRF were used in this investigation. These are Photosynthetic Quantum Efficiency \( \left( \frac{F_v}{F_m} \right) \) and photosynthetic cross sectional area of PSII \( (\sigma_{PSII}) \). These two parameters were used to compare to proxies of primary production from pigment and absorption measurements.

The measurement of \( \frac{F_v}{F_m} \), as described in section 2.10.1 is a measure of the functioning PSII reaction centres and as such is a measure of the maximum capacity of energy throughput of PSII [Aiken, 2001]. It can be used as an indication of the physiological status of a phytoplankton sample; the higher the \( \frac{F_v}{F_m} \), the higher the proportion of functioning PSII centres, and so the ‘healthier’ the population. \( \frac{F_v}{F_m} \) is affected by photochemical quenching; the dark-adapted measurements are relatively constant in the water column, whereas the light-adapted measurements decrease near the surface where light levels are highest and potentially damaging [Long et al., 1994].

When the number of flashes is plotted against the fluorescence, the initial gradient of the phytoplankton absorption ability can be calculated. This is termed the photosynthetic cross sectional area of PSII, \( \sigma_{PSII} \) and is a measure of the likelihood of a photon striking the antenna complex of PSII and the probability that the strike energy will enter the photosystem rather than be fluoresced or lost as heat [Mauzerall and Greenbaum, 1989]. It is dependent on the number and types of pigment associated with the antenna.
complex, and as such can be considered to be analogous to the ‘size’ of the antenna complex of the reaction centre. This means that the magnitude of $\sigma_{PSII}$ is dependent on the pigment load [Moore et al., 2005], which in turn can be affected by physiological state and taxonomy.

2.12 Determining primary production rates using the FRRF

Traditional measurements of the rate of photosynthesis have relied upon the uptake of radioactively tagged carbon isotopes by photosynthesising organisms in incubators over 6 to 12 hours followed by a measurement of radioactivity within a filtered sample of the culture [Chavez and Barber, 1987, Peterson, 1980, Vaillancourt et al., 2003b]. FRRF fluorimetry has several advantages over traditional discrete incubation measures of primary production:

- it is an almost instantaneous, automatic methodology and thus changes in the physiology are minimised,
- it is a self contained instrument that can be attached to profiling optical rigs and therefore produce depth profiles.
- it measures gross electron transfer rate and therefore gross primary production as opposed to net primary production measured by isotope carbon uptake.
- no approximation of the rate of respiration is required to determine the rate of production.

The primary production parameters, $\frac{F_e}{F_m}$ and $\sigma_{PSII}$ can be placed into formulae to determine primary production in terms of oxygen evolution or carbon fixation. However other parameters in these equations are currently not well constrained [Laney, 2003, Suggett et al., 2001, Vaillancourt et al., 2003a] and therefore these equations are not used in this research. Direct outputs of the FRRF, namely $F_m$, $\sigma_{PSII}$ and $\frac{F_e}{F_m}$ are not affected by such problems (beyond those assumptions stated in section 2.10.1), and have therefore been considered quantitative measures.

With these caveats in mind, FRRF data were used to examine relationships between variations of the production measures:

- $\sigma_{PSII}$ and $\frac{F_e}{F_m}$ relative to $F_m$;
- $\sigma_{PSII}$ and $\frac{F_e}{F_m}$ relationships with chlorophyll a concentration determined through fluorescence and HPLC.
Figure 2.11: Raw data output from the FRRF with the parameters of $F_0$ (first point), $F_m$ (maximum fluorescence), $\sigma_{PSII}$ (rate of energy uptake) and $\tau$ (relaxation coefficient).
2.12.1 Previously reported relationships of $\frac{F_a}{F_m}$ and $\sigma_{PSII}$ with other parameters

$\frac{F_a}{F_m}$ was reported by Aiken et al. [2004] to vary in a log linear fashion with chlorophyll $a$ concentration, which indicates that the chlorophyll $a$ concentration and its relative concentration to accessory pigments may be a causative factor limiting $\frac{F_a}{F_m}$. The suggested reason for this correlation was that under optimal conditions, phytoplankton would synthesise more chlorophyll $a$ which, though energetically expensive, is a limiting factor in the rate of photosynthesis. Under the conditions where photosynthetic rate and therefore resource acquisition (and Darwinian fitness) can be maximised by this action it is reasonable for the cell to increase its rate of synthesis of chlorophyll $a$.

$\sigma_{PSII}$ relationships have been less well described. Aiken et al. [2004] did not report on the relationships between pigments or pigment ratios with $\sigma_{PSII}$, but recent research by Moore et al. [2005] and Suggett et al. [2004] suggest that the taxonomic makeup of a community has the greatest effect on $\sigma_{PSII}$. Suggett et al. [2004] showed that *Synechococcus* has a low $\sigma_{PSII}$ relative to the eukaryotes but that *Prochlorococcus* has a relatively high $\sigma_{PSII}$. Studies of mono cultures found that $\sigma_{PSII}$ was not positively correlated with chlorophyll $a$ concentration in a linear fashion [Moore et al., 2005, Suggett et al., 2004] but in fact increases with a decrease in chlorophyll $a$ concentration [Aiken et al., in press]. This may be because $\sigma_{PSII}$ is a measurement of the relative size of the antenna pigment structure of PSII. Under low chlorophyll $a$ concentrations, the relative concentration of accessory pigments will be high, and the number of accessory pigment molecules relative to chlorophyll $a$ within the antenna pigment structure will also be high, so the antenna complex will be relatively large.

2.13 Hypotheses

Arising from the work of Aiken et al. [2004] and the observations of Margalef [1967], the following hypotheses were framed:

1. The FRRF is a viable instrument for measuring primary production parameters in oceanic waters.

2. Primary production parameters from the FRRF vary with measures of pigment concentration.

3. Pigment ratios are the causative factor of this variation.
4. Absorption ratios can be used as a proxy for pigment ratios and therefore for FRRF primary production parameters (assuming hypothesis 3 is correct).
Chapter 3

Method Development - Blanks in Optical instruments

Measurements of photosynthetic rates through optical methods have been routinely carried out for over half a decade [Aiken et al., 2000, 2004, DiTullio et al., 2003, Suggett et al., 2001, Vaillancourt et al., 2003b] using FRRFs. However, the methodologies for the use of FRRFs are not well standardised and different researchers are using different approaches. The low levels of phytoplankton concentrations in the oligotrophic gyres mean that in this study, blanks and calibration of optical instruments were of particular importance. This chapter discusses the blanking procedures and the software configuration in the deployment of the optical instruments used on AMT.

3.1 FRRF settings and data processing

The settings used in configuring the FRRF (gain setting and interflash delay) are specified in this chapter. These settings relate to the control electronics of the FRRF and as such, are not calibrated in terms of physical units.

3.1.1 FRRF Gain

The FRRF is supplied with a facility that automatically alters the gain setting of the FRRF to maximise sensitivity in low chlorophyll concentration environments without saturating the instrument in highly productive areas [Chelsea Instruments Ltd, 1998]. There are two problems with this. Firstly, the gain settings not only multiply the signal, but also any noise within the system. Secondly, the auto gain feature causes data loss and production of spurious data as the gain changes [Aiken, J. Pers. Comm.]. The instrument
will tend to change gain as it goes through an area of change in the water column, often a region of particular interest. To nullify these issues, which were encountered on AMT 12, the FRRF was set to a single gain on AMTs 13 and 14. The gain was selected so that the instrument could detect the low levels of chlorophyll present in the surface waters of the gyres, and at a level where the instrument was unlikely to saturate at $f_{\text{max}}$. For these reasons, a gain setting of 16 was generally selected. However, on AMT 13 the FRRF was accidentally deployed with a gain setting of 1 for part of the transect. This adversely affected data quality as measurements were often taken as zero and any non-zero data were lost in noise.

### 3.1.2 Saturation interflash delay

The FRRF uses a ratio between the fluorescence from the sample and a reference measurement taken within the LED array used to create the flashlets. For determination of this ratio, stability of the reference signal is desirable. When an interflash delay of 0 was used, a significant reduction in the intensity of the reference signal was apparent as the flash sequence progressed. This was due to the inability of the capacitors, used to power the LEDs, to charge before a discharge was required again [Chelsea Instruments Group Pers. Comm.]. When the interflash delay was increased to 1 the reference signal oscillated significantly, coupled with a reduction in the decrease rate of the reference measure. An interflash delay of 4 generated a sufficiently flat reference profile to be considered stable, but the increase in interflash delay meant that light energy was not supplied to the photosynthetic apparatus of the sample at a sufficient rate to saturate the system [Laney, 2003, Moore et al., 2005]. As a result of this, although potentially adding error to processed data, an interflash delay of 0 had to be used on the AMT cruises. On the basis of these findings relating to gain and interflash delay the FRRFs used on AMTs 13 and 14 were set up as shown in table 3.1.

### 3.1.3 Data processing

Examination of the data output from the proprietary analysis software (Chelsea Instruments FRS v1.8) showed errors. In particular, the first point would often be an outlier, thus affecting the values of $F_o$ and $\sigma_{P_{SII}}$ (see figure 3.1). This error was corrected by taking a five point average of the fluorescence profile gradient from points 2 to 6, which was then extrapolated back to reposition point 1 (see figure 3.1). This procedure was repeated for the excitation signal in the raw data. The data were then processed using the
Table 3.1: The setup parameters used on the FRRFs on AMTs 13 and 14

<table>
<thead>
<tr>
<th>Run Menu</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td>65535 Acquisitions</td>
</tr>
<tr>
<td>7.</td>
<td>16 Flash sequences per acquisition</td>
</tr>
<tr>
<td>8.</td>
<td>100 Saturation flashes per sequence</td>
</tr>
<tr>
<td>9.</td>
<td>4 Saturation flash duration (in instrument units)</td>
</tr>
<tr>
<td>A.</td>
<td>0 Saturation interflash delay (in instrument units)</td>
</tr>
<tr>
<td>B.</td>
<td>ENABLED Relaxation flashes</td>
</tr>
<tr>
<td>C.</td>
<td>20 Relaxation flashes per sequence</td>
</tr>
<tr>
<td>D.</td>
<td>4 Relaxation flash duration (in instrument units)</td>
</tr>
<tr>
<td>E.</td>
<td>120 Relaxation interflash delay (in instrument units)</td>
</tr>
<tr>
<td>F.</td>
<td>1000 ms Sleeptime between acquisition pairs</td>
</tr>
<tr>
<td>G.</td>
<td>16 PMT Gain in Normal Mode</td>
</tr>
<tr>
<td>H.</td>
<td>DISABLED Analog Output</td>
</tr>
<tr>
<td>I.</td>
<td>DISABLED Desktop (verbose) Mode</td>
</tr>
<tr>
<td>J.</td>
<td>ACTIVE Light Chamber (A)</td>
</tr>
<tr>
<td>K.</td>
<td>ACTIVE Dark Chamber (B)</td>
</tr>
<tr>
<td>L.</td>
<td>ENABLED Logging mode to internal flashcard</td>
</tr>
<tr>
<td>M.</td>
<td>90 Upper Limit Autoranging Threshold value</td>
</tr>
<tr>
<td>N.</td>
<td>15 Lower Limit Autoranging Threshold value</td>
</tr>
</tbody>
</table>

proprietary software with the profile divided into 1m depth bins (samples being averaged for each bin).

3.1.4 FRRF blanks

Blanks in optical instruments are used to obtain a baseline reference value. *Cullen and Davis* [2003] reported on the significance of blanks in oceanographic measurements, specifically noting the impact of the inappropriate selection of blanks on FRRF measurements. They suggested that the common use of deionised water as a blank could result in artificial patterns in $F_m$ and therefore $F_m^*$. *Cullen and Davis* [2003] suggested that an 'ideal' blank would be an in situ measurement of the blank at ambient irradiance with all phytoplankton removed. However, for practical purposes they suggested that one of the following should be used:

1. deionised water,
2. filtered sea water with all biological material removed,
3. water taken from below the euphotic depth where no viable phytoplankton should be present.
Figure 3.1: Raw data correction of the first fluorescence point. The line shows the regression line used to calculate the corrected $F_0$. 
In accordance with the findings of Cullen and Davis [2003] blanks were taken on AMTs 12-14 using three different sources:

1. water from the ship's milliQ deionised water system,
2. surface water taken from the ship's non toxic sea water supply and filtered through a 0.2μm capsule filter to remove any photosynthetically active organisms [Zubkov et al., 2000],
3. unfiltered water taken from 1000m on the deep CTD casts.

The FRRF was suspended in a bucket made of black polypropylene (a material which is non-fluorescent in the wavelength used by the FRRF [Rongrong et al., 2000]) and half filled with the blank to be used. Fifty acquisitions were taken, 25 from the FRRF's dark chamber and 25 from the light chamber. These blank files were then averaged after the first point had been corrected (see section 3.1.3).

Blanks were applied to the raw profile data by the following method:

- 25 acquisitions were taken for both chambers, each consisting of the 120 flashlets which make up an FRRF saturation measurement (see figure 2.11),
- for each flashlet a mean of the 25 acquisitions was taken,
- each of these 120 means were subtracted from the corresponding flashlet of each acquisition in the profile,
- blanks were not applied to the reference channel of the data since these values are not determined by the ambient water,
- the first point correction was applied to the profile data (figure 3.1),
- the profile was processed using proprietary software (FRS v1.8).

To determine which blank source should be used, blanks were applied to profiles taken at the same sampling station as those blanks. To determine the quality of a blank, an assumption was used that below the depth where no photochemical quenching occurs (where the $F_{mL}/F_{m}$ profile become linear) and above the thermocline, the $F_{mL}/F_{m}$ of the light and dark chambers should be equal [Chelsea Instruments Ltd, 2002a]. In this depth range, there should be sufficient signal to be measured but no quenching from surface irradiance. For the purposes of this study, this is referred to as the equality assumption.
Figure 3.2: Application of blanks to FRRF data. Raw excitation data (top), excitation measured as a blank (middle) and blank corrected data (bottom). For each flashlet, corrected = raw - blank.
Figure 3.3: FRRF blanks taken on AMT 14. Shown is the variation from the assumption that \( \frac{F_{\text{m}}}{F_{\text{m}}} \) for the light and dark chambers should be equal at depths where no quenching occurs. U, M, F and K represent unblanked, blanked with MilliQ deionised water, 0.2\( \mu \text{m} \) filtered surface water and 1000m unfiltered water respectively. The central line shows the mean value of the blank, the boxed area the standard deviation and the bars the range of the data.
The $f_m$ data from the four profiles that fulfilled these prerequisites were collated and plotted (figure 3.3). The original unblanked data showed approximately twice the deviation (0.08) from the equality assumption, than those of the blanked data (0.04). Since the deviation in the unblanked data was greater than 10% of the maximum $f_m$ of 0.65, this strongly suggested that that blanks should be used. There was very little difference between the three blanking methods, but blanks taken using milliQ water showed the smallest deviation from the equality assumption (0.04) and the smallest variance.

As described in section 3.1.4, Cullen and Davis [2003] suggested that the only viable method of taking blanks is to run profiles with in situ water at the ambient light conditions with photosynthetic organisms removed. Although no examples of this method exist in the literature, the twin cast deployment of optics instruments on AMT 14 allowed this to be attempted. Two profiles were already being taken for the ac9+ data (see section 2.9), where one of the profiles had a 0.2μm capsule filter. This was utilised for the FRRF blank by attaching the outflow from the ac9+ to the dark chamber of the FRRF (figure 3.4). The water pumped through the ac9+ entered the FRRF throughout the profile and gave a blank profile that could be applied to the dark chamber of the standard profile.

The blank profile produced blanks at a large number of depths. Ideally, these should have been applied to the concurrent unfiltered raw data prior to processing. Practically however, the manipulation of the raw data in this way was unwieldy and therefore blank correction was applied after processing, where data and blanks had been placed into one metre bins and could easily be manipulated.

The profiles were processed in the same way as the standard profile and then the $F_o$ and $F_m$ values were subtracted from the unblanked profile. The results of this method showed that for much of the water column the blanked profile was very similar to the values with the blank taken from the 1000m water. Corrected $F_o$ and $F_m$ were higher than those taken from either filtered sea water or milliQ deionised water (figure 3.5, right).

There are two possible implications of this. Firstly, it may be that the 0.2μm filter allowed small numbers of photosynthetically active organisms through to the FRRF. Secondly, small particles present in the water column and passing through the filter may have had a scattering effect on the light. The stability of the blank, where large variations in the un-blanked profiles were evident (figure 3.5, left), implied that the
Figure 3.4: Setup of the FRRF with the ac9+ pump flow-through system for filtered blanks.

latter mechanism caused the variation. Notably, as can be seen in figure 3.5, the blanks run on discrete filtered sea water from concurrent profiles showed values very similar to the milliQ and filtered surface sea water values. These blanks were filtered through 0.1μm filters under more controlled laboratory conditions aboard the ship [Kim, Y., unpublished data] so all particulates were removed, not just photosynthetically active organisms [Lalli and Parsons, 1997, Zubkov et al., 2000]. Since this error was caused by non photosynthetically active particles, it was logical to apply the discrete deep water blank (with its higher values) as it removed the effect of these particulates while retaining the effect of autotrophic organisms.

From these experiments, two conclusions could be drawn. When considering discrete blanks, milliQ and filtered surface sea water blanks marginally adhered best to the presumption that the light and dark chambers should give equal values of $E/F'_{in}$ where no photo quenching is present. However, when considering profiles of in situ blanks, the deep water blanks seemed to coincide well with the profile. Since this corresponded to the 'ideal' blank proposed by Cullen and Davis [2003], unfiltered deep water blanks were
Figure 3.5: FRRF blank profiles. Left: $F_m$ profile of CTD station 62, a northern subtropical gyre on AMT 14. Right: The concurrent $F_m$ blank values: irregularly dashed line for milliQ de-ionised water; regularly dashed line for filtered surface sea water; solid line for unfiltered deep water; open circles for filtered FRRF downcast; and the filled circles for filtered FRRF upcast. Red crosses show discrete blanks from the main CTD water sampling rosette passed through a 0.1μm filter.
applied to all profiles.

3.1.5 FRRF Blanks - Discussion

The different methodologies of blanking used on AMT showed several issues that should be addressed if FRRF data is to be considered viable. Firstly, blanking is a necessity in the oligotrophic ocean and separate blanks must be used for the light and dark chambers. Secondly, correction of the $F_0$ value by extrapolation of raw fluorescence data also affected $F/F_0$ values. Once these two issues had been successfully addressed, parameters generated by the FRRF fell within levels that would be expected in these regions [Kolber et al., 1998, Kolber and Falkowski, 1993].

3.2 Blanks of the WetLabs ac9+

The use of the ac9+ in oligotrophic waters meant that it was operating near its detection limits [Wetlabs inc, 2004a]. Under these conditions the calibration and blanking of the instrument became highly important.

Wetlabs inc [2004b] suggest that tracking of instrument drift is undertaken using dry air measurements (with the instrument running in dry nitrogen gas). This gas was not available during the AMTs and so this was not possible, and attempts to run similar tracking methods on deck with deionised milliQ water proved problematic; it proved impossible to remove air bubbles from the measurement tubes of the ac9+ making blank measurements meaningless. The use of the ship’s deionised water supply as a blank was also ineffective because gyre water is optically more pure than that produced by the milliQ system [Wetlabs inc, 2004b]. Therefore, the ac9+ used was placed in the PML clean water calibration facility on return to shore after AMT 14. Unfortunately, this blank was also optically less clear than gyre water at some wavelengths.

From the perspective of particulate absorption, this was not an issue as the double casts using the 0.2μm filters provided an ideal in situ blank for the instrument at ambient conditions in the same way as the ‘ideal’ blank for the FRRF. To calculate the particulate absorption spectrum the CDOM profile was removed from the total profile as shown in figure 3.6, therefore any systematic error that could have been removed by blanking was already accounted for. If the total water or CDOM absorption and attenuation data were to be used then these issues would have to be considered, and extreme care would be needed in blanking this instrument.
Figure 3.6: ac9+ absorption spectra as determined by double casts. Top is an absorption profile from an unfiltered ac9+, middle is with 0.2µm filters (CDOM cast) and bottom is the unfiltered minus the filtered profile (particulate spectrum).
Chapter 4

Methodology

The methods within this study fall into five distinct groups and as such the methodologies and results are discussed in five sections.

4.1 Deployment of field instrumentation

Data were collected on three AMT cruises: AMTs 12 to 14. AMTs 12 and 14, in May-June 2003 and 2004 respectively, went deep into the North Atlantic so as to sample the oligotrophic North Atlantic Subtropical Gyre. AMT 13, in September-October 2003, sampled within the high nutrient upwelling off Mauritania in West Africa. All of these cruises followed the same cruise track in the Southern hemisphere, so as to create a time series of data for the oligotrophic South Atlantic Gyre.

Several physical dataset profiles were available as part of the standard measurements taken with the main CTD (Conductivity, Temperature, Depth) rig used for sampling. This rig was deployed concurrently with optical instruments and therefore could be used in conjunction with optical measurements. The suite of measurements taken included:

1. Seabird 9/11 plus CTD profiles taken from the main water sampling rosette to characterise the structure of the water column.

2. Wetlabs ac9+ measurements of absorption and attenuation at 9 wavelengths (see section 2.8.1). Data from the main CTD rosette was used to correct the ac9+ for the effects of temperature and salinity. It was used to measure chlorophyll absorption at 676nm as a biomass indicator [Drain, 2003]. Additionally, on AMTs 13 and 14, the instrument was used with a 0.2μm capsule filter for the determination of CDOM and particulate absorption spectra [Green et al., 2003].
3. FRRF measurements of the cross-section of PSII, the quantum yield and rate of photosynthetic electron transport [Chelsea Instruments Ltd, 1998]. During the cruises blank measurements were taken with the instrument every three days with 1000m water from the CTD, deionised clean water from the ship's MilliQ system and water filtered through a 0.2μm capsule filter off the ship's underway non toxic seawater supply (see chapter 3).

4. High Performance Liquid Chromatography (HPLC) samples taken from three depths for each 11am cast. Depths were dependent on the structure of the data returned by the main CTD, but generally covered a surface sample, one at 6m, one at 20m (or in the gyres 50m) and one at the fmax.

The optics deployments used an optics rig, which carried the FRRF and the ac9+, as shown in figure 4.1. This was deployed at the mid-day 11am cast on each day and often additionally at the 2am pre-dawn cast. The main CTD rosette also carried an FRRF, deployed on all 300m casts. Water samples were taken from a water sampling rosette attached to this CTD from which discrete samples for HPLC analysis were taken.

4.2 Descriptive partitioning of the Atlantic into functional areas

4.2.1 Community structure

The determination of biogeochemical provinces (see section 1.3.1) was simplified for the purposes of this study into gyre and non-gyre regions. In this investigation, pigments were used as the biological factor to establish whether a sample station was in a gyre or non-gyre region. HPLC surface pigment data (see section 4.3.1) were analysed using the PRIMER v5 (Plymouth Routines In Marine Ecological Research) [Clarke and Gorley, 2001] software package. HPLC concentrations were transformed to the fourth root so as to increase the effect of the presence or absence of a pigment, and decrease the effect of pigment concentration. Therefore, this transformation decreased the influence of highly dominant pigments, and increased the effect of the lowest concentration pigment. Without this transformation any relationships would be almost entirely driven by the concentration of chlorophyll a, and community structure changes represented by the presence of other pigments would be obscured.

These data were used to construct a Braé-Curtis similarity matrix. This matrix
Figure 4.1: The optics deployment rig used on AMT's 12-14 showing the positions of the ac9+, FRRF and CTD instruments.
showed the relative similarity between each sample, signifying the similarity as a percentage. These similarities were then ranked and clusters plotted as dendrograms. When plotted in this manner it was simple to divide the data into two distinct categories with the greatest degree of similarity between the two categories. On examination of the sample stations in each category it was possible to label one group as ‘non-gyre’ with stations from the two ends of the transect and around the equator. The other category with the stations lying between the ‘non-gyre’ stations was labelled the ‘gyre’ category.

4.3 Quantification of relationships between factors based on discrete samples

4.3.1 HPLC Pigment ratios

Pigment analysis by High Pressure Liquid Chromatography (HPLC) was undertaken at the National Oceanographic Centre (NOC) [Root, S., Poulton, A., Kim, Y. N. and Holeton, C., unpublished data]. HPLC uses a carrier solvent, in this case acetone, to dissolve pigments and then passes them through a diffraction column into a spectrophotometer. This allows for the splitting and identification of pigments within a sample, and calculation of concentrations through absorption spectra [Barlow et al., 1997, Mantoura and Llewellyn, 1983, Wright et al., 1991]. Using this technique, chlorophylls a, b and c, di-vinyl chlorophyll a and the carotenoids were detected and quantified [Mantoura and Llewellyn, 1983], but since phycobilins are not soluble in acetone, they were not represented in this dataset [Jeffrey et al., 1997a]. The pigments quantified on all cruises are listed in table 4.1. Reported relationships between pigment concentrations implied linearity between the chlorophyll a concentration and both total pigment and total accessory carotenoid loads [Trees et al., 2000]. In order to test this linearity, the AMT datasets were analysed by plotting the ratio of TChla:Tpig against TChla. The contrasting hypothesis of Aiken et al. [2004], that the TChla:Tpig pigment ratio varies with chlorophyll a concentration was also tested.

The large spatial scale AMT datasets were split upon two premises:

1. that there would be variations in photochemistry geographically between the gyres and upwelling regions;

2. that there would be differences between the photochemistry of different phytoplank-
Table 4.1: The pigments measured on AMTs 12-14 by HPLC

<table>
<thead>
<tr>
<th>Chlorophyll</th>
<th>Carotenoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll a</td>
<td>19-Butanoyloxyfucoxanthin</td>
</tr>
<tr>
<td>Di-vinyl chlorophyll a</td>
<td>Fucoxanthin</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>19-Hexanoyloxyfucoxanthin</td>
</tr>
<tr>
<td>Chlorophyll c₂</td>
<td>Alloxanthin</td>
</tr>
<tr>
<td>Chlorophyll c₃</td>
<td>Peridinin</td>
</tr>
<tr>
<td></td>
<td>Violaxanthin</td>
</tr>
<tr>
<td></td>
<td>Zeaxanthanine</td>
</tr>
<tr>
<td></td>
<td>Diadinoxanthin</td>
</tr>
</tbody>
</table>

ton taxa.

The first of these was relatively simple to establish using province analysis as described in section 4.2. The two sub tropical provinces were separated from the dataset and analysed separately from the rest of the dataset. The second premise was more complex, and required taxon based ratios.

4.3.2 Taxon based ratios

The phytoplankton community was split taxonomically into two to reflect the greatest changes in physiology, and potentially photochemistry. As such, the division of prokaryotes from eukaryotes was selected [Trees et al., 2000, Mauseth, 1998]. To divide the phytoplankton population, differences in the accessory pigment composition of the two groups were used; the methodology of Vidussi et al. [2001] was adapted for this purpose.

Many of the accessory pigments are considered to be diagnostic of a taxon (see Table 4.2) and can therefore be considered as markers for the presence or absence of that taxon. Vidussi et al. [2001] showed that the concentration of these pigments within a sample representing a taxon is proportional to the chlorophyll a produced by that taxon.

Diagnostic pigments have been generally used to give primarily a descriptive indication of the community structure over spatial or temporal scales [Gibb et al., 2000, Vidussi et al., 2001]. In this study, the proportion of the community made up of a functional group was determined by dividing the sum of the concentration (mg m⁻³) of the diagnostic pigments for a group by the total Diagnostic Pigment (DP) concentration (mg m⁻³),
Table 4.2: The diagnostic pigments of various taxa within the phytoplankton, after Vidussi et al. [2001]. Pico = picoplankton, Nano = nanoplanckton, Micro = microplankton.

<table>
<thead>
<tr>
<th>Diagnostic Pigment name</th>
<th>Abbreviation</th>
<th>Taxon</th>
<th>Size fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeaxanthanine</td>
<td>Zea</td>
<td>Prokaryotes</td>
<td>Pico</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>Chlb</td>
<td>Picoeukaryotes</td>
<td>Pico</td>
</tr>
<tr>
<td>Di-vinyl Chlorophyll b</td>
<td>DvChlb</td>
<td>Prochlorophytes</td>
<td>Pico</td>
</tr>
<tr>
<td>Alloxanthin</td>
<td>Allo</td>
<td>Cryptophyta</td>
<td>Nano</td>
</tr>
<tr>
<td>19-Hexanoyloxyfucoxanthine</td>
<td>Hex</td>
<td>Nanoflagellates</td>
<td>Nano</td>
</tr>
<tr>
<td>19-Butanoyloxyfucoxanthin</td>
<td>But</td>
<td>Nanoflagellates</td>
<td>Nano</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>Fuc</td>
<td>Diatoms</td>
<td>Micro</td>
</tr>
<tr>
<td>Peridinin</td>
<td>Peri</td>
<td>Dinoflagellates</td>
<td>Micro</td>
</tr>
</tbody>
</table>

Therefore:

\[
Proportion_{pico} = \frac{DP_{pico}}{DP} \quad (4.1)
\]

\[
Proportion_{nano} = \frac{DP_{nano}}{DP} \quad (4.2)
\]

\[
Proportion_{micro} = \frac{DP_{micro}}{DP} \quad (4.3)
\]

This methodology uses ratios of pigments present in the sample to fractionate the community into micro, nano and picoplankton (see chapter 1 for definitions). For the purposes of this study, some alterations were made to this methodology. The functional groups classified by Vidussi et al. [2001] have been modified to form two groups; the prokaryotes and eukaryotes. Here, the prokaryotes are defined as cyanobacteria and prochlorophytes, which differs from the Vidussi et al. [2001] definition of picoplankton in that it does not include picoeukaryotes. This group was removed by excluding from the calculation Chlb, which is diagnostic of these organisms. Flow cytometry measurements made at NOC [Zubkov, M. and Heywood, J., unpublished data] also showed this group to be present in very low numbers relative to prochlorophytes and cyanobacteria. Therefore, the prokaryotes are defined by the relative concentration of zeaxanthanine compared to the total concentration of these diagnostic pigments. Equations 4.4 to 4.8 were used to determine the relative concentrations of Tchl a and Tpig in the prokaryotes and eukaryotes.
The phytoplankton community was taken to be represented by the pigments listed in table 4.2 and split based on this assumption using a Diagnostic Pigment (DP).

\[ DP = \text{Zea} + \text{Chlb} + \text{Allo} + \text{Fuc} + \text{But} + \text{Fuco} + \text{Peri} \]  
(4.4)

According to Vidussi et al. [2001], the proportion of the concentration of one of these relative to the DP equates to the proportion of chlorophyll a being produced by the taxon to which it is specific. Based upon this assumption, the prokaryote chlorophyll a content was calculated by:

\[ T\text{chl}_{a\text{prok}} = (\text{Zea}/DP).T\text{chl}_a \]  
(4.5)

And the total pigment of the prokaryotes was calculated as:

\[ T\text{pig}_{\text{prok}} = T\text{chl}_{a\text{prok}} + \text{Zea} \]  
(4.6)

The concentration of chlorophyll a attributable to the eukaryotes is therefore:

\[ T\text{chl}_{a\text{euk}} = T\text{chl}_a - T\text{chl}_{a\text{prok}} \]  
(4.7)

and the total pigment:

\[ T\text{pig}_{\text{euk}} = T\text{chl}_{a\text{euk}} + T\text{AC}_{\text{euk}} = T\text{chl}_{a\text{euk}} + DP - \text{Zea} \]  
(4.8)

### 4.3.3 Community Structure Dominance

Initially, the pigments in the community were split by assigning them to either prokaryotes or eukaryotes. For the AMT data, the eukaryotes consisted almost entirely of nanoflagellates due to a lack of larger cells in open ocean populations [Gibb et al., 2000]. Initially, a simple split was made such that if the accessory pigment of zeaxanthanine was greater than 50% of the total load (Zea>Allo+Hex+But+Fuc+Peri) the community was considered to be dominated by prokaryotes, otherwise it was dominated by eukaryotes.

Whilst the classification of a sample as either prokaryote or eukaryote groups was easy to apply, its usefulness was limited to those cases where a sample was clearly dominated by one group. Where this was not the case, a method using fuzzy logic was developed to apportion the sample partly to one group and partly to another. Where neither prokaryotes or eukaryotes completely dominated a sample this allowed the sample to be
split and the pigments within that sample could be deviled into those produced by the prokaryotes and those produced by the eukaryotes.

Several different methods were used to determine the chlorophyll a concentration in the different taxonomic fractions and total pigment concentration. Where the Diagnostic Pigment had been determined, the proportion of this within each group could be used to apportion the chlorophyll a concentration to the group. However, Glover et al. [1987] suggested that the proportion of chlorophyll a produced by cyanobacteria is different to that of the eukaryotes. Cyanobacteria such as *Syneccoccus* use phycobilins as their primary photopigment with chlorophyll a as an accessory [Trees et al., 2000], which results in them having approximately one third of the chlorophyll a relative to other photoautotrophs. Likewise, *Prochlorococcus* (the other prokaryotic group found on the AMT transects) do not produce the mono-vinyl chlorophyll used by other photoautotrophs, instead using di-vinyl chlorophyll a as their primary photosynthetic pigment. Accordingly, a different determination of chlorophyll a was used for the prokaryotes. It was assumed that di-vinyl chlorophyll a is produced by *Prochlorococcus* in the same ratio as mono-vinyl chlorophyll a in the eukaryotes, and that the cyanobacterium *Syneccoccus* produces one third the amount of the mono-vinyl chlorophyll a as the eukaryotes. Given the presumption of a 1:1 ratio of the generation of Chla in prokaryotes and eukaryotes, the concentration of Chla from the prokaryotes should be:

\[
T\text{Chla}_{\text{prok}:1} = \text{Zea}/DP\cdot T\text{Chla} \quad (4.9)
\]

Since the concentration of Chla provided by *Prochlorococcus*, DvChla is known, the contribution of *Syneccoccus* and can be calculated using the 1:1 assumption:

\[
T\text{Chla}_{\text{syn}} = T\text{Chla}_{\text{prok}:1} - Dv\text{Chla} \quad (4.10)
\]

The input from *Syneccoccus* can then be determined with a 1:3 ratio of chlorophyll from *Syneccoccus* relative to eukaryotes:

\[
T\text{Chla}_{\text{syn}} = (T\text{Chla}_{\text{prok}:1} - Dv\text{Chla})/3 \quad (4.11)
\]

Therefore, total chlorophyll a from the prokaryotes is:

\[
T\text{Chla}_{\text{prok}} = Dv\text{Chla} + ((\text{Zea}/DP\cdot T\text{Chla}) - Dv\text{Chla})/3 \quad (4.12)
\]
And the total chlorophyll $a$ from eukaryotes is:

$$TChla_{euk} = TChla - TChla_{prok} \quad (4.13)$$

Chlorophyll $a$ has several accessory pigments [Barlow et al., 2001, 2004, Cowles et al., 1993, Vidussi et al., 2001] and so choosing the right combination of these for pigment ratios within groups was complex. For example, either the total pigment complement ($T_{pig}$), including all accessory pigments or the total accessory carotenoids ($TAC$) could be considered. Within the accessory pigments the whole group could be considered or only the carotenoids. The carotenoids could also be subdivided into the photosynthetic and photoprotectant carotenoids (PSCs and PPCs). In this study, the $T_{pig}$ and $TAC$ were used. This accounted for all taxonomic diagnostic pigments and allowed for differentiation of these groups using \textit{in situ} optics (see section 2.8.1).

After taxonomic splitting, further methods of assigning pigments to different groups presented themselves. For the purposes of simplicity, the example used here is for the prokaryotes. From the DP calculation, the percentage of the population ($\%_{prok}$) contributed by the prokaryotes was known. The simplest method was to apply this percentage to the $TAC$ and then add the prokaryotic chlorophyll $a$.

$$TAC_{prok} = TAC.(\%_{prok}/100) \quad (4.14)$$

$$T_{pig}_{prok} = TAC_{prok} + TChla_{prok} \quad (4.15)$$

However, this is an over simplification since the diagnostic pigments of other groups are included and so these pigments must be excluded.

$$TAC_{prok} = (TAC - DP) + (DP.(\%_{prok}/100)) \quad (4.16)$$

Again, however, some of the accessory pigments are not ubiquitous within groups. For example, the prokaryotes do not contain chlorophylls, instead the only accessory pigments they produce are zeaxanthanine and $\beta$-carotene ($\beta$-car), therefore:

$$TAC_{prok} = Zea + (\beta - car.(\%_{prok}/100)) \quad (4.17)$$
If we are simply splitting the population into prokaryotes and eukaryotes, the determination of $TAC_{euk}$ and $Tpig_{euk}$ from any of these methods is simple since:

$$TAC_{euk} = TAC - TAC_{prok}$$  \hspace{1cm} (4.18)

$$Tpig_{euk} = Tpig - Tpig_{prok}$$  \hspace{1cm} (4.19)

As it was desirable for the same measure of $TAC$ and $Tpig$ to be used on each cruise the pigments in these groups had to be standardised. $\beta$-carotene was not measured on AMT 14, and so the taxonomically split pigment groupings were defined as:

$$TAC_{prok} = Zea$$  \hspace{1cm} (4.20)

$$Tpig_{prok} = Zea + TChla_{prok}$$  \hspace{1cm} (4.21)

and

$$TAC_{euk} = TAC - TAC_{prok} = TAC - Zea$$  \hspace{1cm} (4.22)

$$Tpig_{euk} = TAC_{euk} + TChla_{euk}$$  \hspace{1cm} (4.23)

4.4 Processing of optical data

4.4.1 FRRF Processing

As a result of the findings described in chapter 3, 1000m water from the main CTD rosette was used as blanks for the FRRF, using the nearest geographic blank for each profile. During preprocessing, the first point in the data array was replaced with an extrapolated value. FRRF raw data were processed using the factory supplied definition file for the instrument as used by FRS v 1.8 Chelsea Instruments propriety software. The output of this process was then reduced to two values for each profile as described in section 4.4.3.

4.4.2 ac9+ Processing

As with the FRRF, the raw data output from the ac9+ required a number of processing stages. The first step was to apply the ac9+ calibration file to the raw data using the
WaP 5.3 [Wetlabs inc, 2002] proprietary software. This subtracted the absorption of pure water and returned an absorption value for each wavelength at each depth. However, these data must be corrected for temperature and salinity, so both of these sets of ancillary data were extracted from concurrent CTD profiles and transformed using the equation [Wetlabs inc, 2004b]:

\[ a_{mts} = a_m - \left[ \Psi_t(t - t_r) \right] + \Psi_s(S - S_r) \]  

(4.24)

\( a_{mts} \) = Temperature and Salinity corrected absorption  
\( a_m \) = Raw measured value  
\( \Psi_t \) = Linear dependence of pure water absorption on temperature (from Wetlabs inc [2004b])  
\( t \) = Measured temperature  
\( t_r \) = Standardised temperature  
\( \Psi_s \) = Linear dependence of pure water absorption on salinity (from Wetlabs inc [2004b])  
\( S \) = Salinity  
\( S_r \) = Standardised salinity

Finally, a scatter correction for the water itself must be applied. Three methods are suggested by Wetlabs [Wetlabs inc, 2004a]. The simplest method assumes that the absorption of particles and CDOM at 715nm is zero [Varela et al., 1998] (equation 4.25).

\[ a_t(\lambda) - a_w(\lambda) = a_{mts}(\lambda) - a_{mts}(\lambda_{ref}) \]  

(4.25)

The second method (equation 4.26) corrects for changes in the temperature of the water and the third (equation 4.27) corrects for both temperature and salinity changes.

\[ a_t(\lambda) - a_w(\lambda) = a_{mts}(\lambda) - \varepsilon [a_{mts}(\lambda) - a_{mts}(\lambda_{ref})] \]  

(4.26)

\( \lambda \) = Wavelength [nm]  
\( a_t(\lambda) \) = Total corrected absorption  
\( a_w(\lambda) \) = Absorption of pure water  
\( a_{mts}(\lambda) \) = Absorption measured corrected for temperature and salinity  
\( a_{mts}(\lambda_{ref}) \) = Absorption measured at the reference wavelength (here 715nm) corrected for temperature and salinity
The third methodology (equation 4.27) recognises that water scattering properties are both temperature and salinity dependent. Therefore, it was possible to apply scattering corrections to the absorption values using the methodology of Zaneveld et al. [1992]:

\[ a_t(\lambda) - a_w(\lambda) = a_{mts}(\lambda) - \frac{a_{mts}(\lambda_{ref})}{[c_{mts}(\lambda) - a_{mts}(\lambda_{ref})]} [c_{mts}(\lambda) - a_{mts}(\lambda)] \]  

(4.27)

When these corrections were applied it was found that the correction of absorption at 715nm using a value of zero was the only method that produced acceptable results. Correction using the other two methodologies resulted in sizable negative values at several wavelengths (see examples in figure 4.2), possibly as a result of these methodologies being developed within case 2 waters where there is often a high proportion of terrestrial particles [Varela et al., 1998].

Particulate absorption was calculated from the ac9+ by subtracting the CDOM absorption at each wavelength from the total absorption as described in more detail in section 2.9. As well as producing the particulate spectrum this provided a high quality blank for the removal of systematic errors from the data. Any systematic error would be in both datasets and thus subtracting the CDOM absorption from the total absorption nullified any errors.

4.4.3 Cast Processing to Provide Summary Values

In order to perform direct comparisons between datasets collected during casts, each instrument’s data were consolidated into two single measurements for the water column: the surface mixed layer and the fmax. For surface mixed layer values, data were averaged over the depth of the profile above the thermocline where there was no photo-quenching. In practice, this was taken as the region above the thermocline where data were constant as shown in figure 4.3. The double cast system used with the ac9+ meant that the fmax could not be definitely identified as it could move up or down in the period between casts. For this reason, and because it reduced the influence of noise within the dataset, data
Figure 4.2: Total absorption spectra with water scatter correction using three methodologies. Top left, taking \( a_{715} \) as 0 (equation 4.25), top right, correcting using water temperature (equation 4.26), and bottom, temperature and salinity correction (equation 4.27).
Figure 4.3: An example profile demonstrating how a single value in a profile was calculated. The surface water where quenching of the $F_m$ (green line) takes place was rejected as was data below the mixed layer. The mean of the $\frac{F_v}{F_m}$ (red line) and $F_m$ values in the surface mixed layer and fmax regions were then taken.

around the fmax were placed into a 5m bin; an average was taken of the 5m around the fmax suggested by the profile.

4.5 Quantification of relationships between factors based on optics profiles

4.5.1 ac9+ band ratio and area ratio methodologies

Absorption bands returned by the ac9+ were used as proxies for specific pigments and the specific wavelength used for each group followed the spectral absorption discussed in chapter 2 and shown in figure 2.7. The 440nm absorption was used as a proxy for the total pigment load, 488nm absorption for the absorption of the total accessory carotenoid load and 676nm absorption for the chlorophyll a absorption.

To account for all pigments that are present in the phytoplankton, a more integrated approach was also adopted. Instead of using only the maximum absorption wavelengths,
the area under the plot of all wavelengths was used. The area of the trapezoids between each band were calculated and total pigment absorption was calculated by determining the area between neighbouring bands and taking the sum of these for all wavelengths (see figure 4.4).

A modification of this method was used for chlorophyll $a$, which has two absorption peaks. Since the largest at 440nm is within the region in which the carotenoids absorb, it is therefore of no use in determining specific chlorophyll $a$ concentration. The second absorption band covers the region between 650nm and 715nm, peaking at 676nm, an absorption wavelength that is diagnostic of chlorophyll $a$ and therefore this band can be used. However, chlorophyll $b$ absorbs in the region around 650nm, which means the whole of the area of the peak cannot be used to determine a spectral area of chlorophyll $a$. To retain a dimensionless ratio of $aT_{Chla}:aT_{pig}$ an area must be determined. Since the absorption at 676nm is diagnostic of chlorophyll $a$ and the spectral half width of chlorophyll $a$ is known to be 9nm [Fishwick, J. unpublished data], the absorption at 676nm was multiplied by 9 to give an area that equated only to chlorophyll $a$ and therefore could be used in a spectral area ratio.

Figure 4.4: Spectral area as calculated from ac9+ data. The sum of the area of trapezoids a-h gives the total pigment absorption. As the absorption of chlorophyll $b$ prevents the use of the region between 650nm and 715nm as an indicator of chlorophyll $a$, the diagnostic absorption at 676nm is multiplied by the spectral half width of the peak (±9nm) to give diagnostic chlorophyll $a$ area.
4.6 Statistics

In this investigation the $r^2$ value was used as the primary statistic to describe the fit of a correlation to the data. The $r^2$ value is a measure of how well the fit of a regression line describes the variability of the data [Shaw and Wheller, 2000]. An $r^2$ value of 1 means that all variability is accounted for by the fit, whereas an $r^2$ value of 0 means that none of it is.

Because the instruments were being used at their detection limits, there was a large amount of noise in the data. The random errors inherent in these observations means that $r^2$ values for the fits are typically low. This does not invalidate the fits, as they are typically made using sufficient data that random errors can be accounted for. The low $r^2$ values do, however, mean that the fit is very sensitive to non-Gaussian systematic errors. It is for this reason that care has been taken to keep such errors to a minimum in the blanking process.

The homogenous nature of the gyre (where most data was collected) resulted in a very low dynamic range in datasets. If the data were of greater dynamic range a log linear $\chi^2$ test could have been used to demonstrate statistical significance between variables [Wrigley, 1985]. Type II regressions were used since both the dependent and independent variables were subject to measurement error [McArdle, 2003]. In the case of HPLC data, although error could not be calculated due to the single point nature of the data, it was still considered to be present.

As an indication of the variance of optically acquired data from the FRF and ac9+, plots of data from these instruments are shown with error bars. These bars represent the standard deviation of the measurements, in the unquenched surface mixed layer in the case of surface samples and the 5m around the fmax in the case of fmax data (see, for example, figures 5.12 and 5.13). HPLC samples are discrete data points and therefore error bars are not applied to these data (for example, figure 6.6).

4.7 Summary

As stated in chapter 1, three methods of measuring primary production parameters were explored in this study, and therefore three methodologies have been described to determine these parameters. The FRF gave a direct measure of primary production by measuring the response of PSII to light, HPLC provided insights into the taxonomy and the physiology of the phytoplankton community and the ac9+ provided a passive
measure of the pigment signatures. The results of the use of these methodologies are discussed in chapters 5 to 8.
Chapter 5

Active fluorescence

This chapter examines AMT measurements taken with the FRRF instrument and considers correlations between the parameters measured. These values provide a benchmark of primary production parameters for comparison with observation from other instruments in the following chapters.

5.1 AMT - sections

The FRRF dark chamber maximum fluorescence ($F_{mD}$) and photosynthetic quantum efficiency ($\frac{F_{mD}}{F_{mD}}$) data measured were plotted against depth and latitude for AMTs 12-14 (top plots in figures 5.1 to 5.3) along with temperature data taken from CTD measurements (bottom plots in figures 5.1 to 5.3). These plots show the biological and physical structure of the AMT through the North Atlantic Subtropical Gyre (NASG) and South Atlantic Gyre (SAG) with deep mixed layers bordered and split by more mixed water masses (described in section 1.3.1).

Figures 5.1 and 5.3 show the sections from AMT 12 and 14 respectively: the cruises which concentrated on entering the gyres. The highly mixed Northern and Southern upwelling regions, at latitudes of greater than 40°N and S respectively, were evident in the $F_{m}$ plots as areas of high chlorophyll (high $F_{m}$) concentrations reaching to the surface. The two oligotrophic gyres were also evident, with the thick low chlorophyll concentration mixed layer overlying a fluorescence maximum that reached down as far as 200m. At the equator, a region of upwelling indicating a shallower, but more intense, fluorescence maximum than within the gyres. The Northern gyre’s MLD was slightly deeper than that of the Southern gyre as these cruises were undertaken during the boreal autumn when the summer stability of weather conditions allowed a larger degree of stratification.
Figure 5.1: Summarised AMT 12 data (May-June 2003) of FRRF chlorophyll fluorescence. Top panel as $F_{mD}$ (shades) and $\frac{F_{mD}}{F_{mD}}$ (contours), bottom panel, CTD temperature. Black points represent samples and profiles.
Figure 5.2: Summarised AMT 13 data (September-October 2003) of FRRF chlorophyll fluorescence. Top panel as $F_mD$ (shades) and $F_{mD}$ (contours), bottom panel, CTD temperature. Black points represent samples and profiles.
Figure 5.3: Summarised AMT 14 data (May-June 2004) of FRRF chlorophyll fluorescence. Top panel as $F_{mD}$ (shades) and $\frac{F_{mD}}{F_{mD}^0}$ (contours), bottom panel, CTD temperature. Black points represent samples and profiles.
The $\frac{F_m}{F_m^L}$ sections showed that this parameter followed $F_m$ in general form, but that the maximum values of $\frac{F_m}{F_m^L}$ were slightly deeper than those of $F_m$.

Figure 5.2 shows the same sections for AMT 13, which, instead of going deep into the NASG, sampled the upwelling region off Mauritania. The Northern and Southern temperate regions were evident as homogenous, high fluorescence profiles at the two ends of the transect. Both gyres were also evident, although since the cruise track of AMT 13 (figure 5.2) did not go further than the edge of the Northern gyre, this region was not well characterised. Within the upwelling zone, between 20°N and 25°N, there was an intense increase in fluorescence in the surface waters, consistent with nutrients being injected into the surface through upwelling of a nutrient rich deep water. This would have provided the phytoplankton population with sufficient nutrients and light to bloom, resulting in a high fluorescence [Aiken et al., 2004, Falkowski and Raven, 1997, Kirk, 1994].

The AMT 13 data showed considerable data drop out rates and invalid data points resulting in inconsistencies within the section (figure 5.2), particularly within the oligotrophic gyres. These are shown in figure 5.2 as patchy inconsistent contours in the surface waters of the gyres between 20°S and 10°S and between 20°N and 30°S. These aberrations are discussed further in section 5.1.1 and were removed in later analysis, but are presented here as an indication of the extent of this problem on AMT 13.

### 5.1.1 AMT depth profiles

Figures 5.4 to 5.8 show typical depth profiles of FRRF data from each cruise and each oceanographic region. These figures are ordered so as to show regions in the order which they were encountered along a North to South cruise track but are discussed as region types. Production parameters are shown from the light chamber of the FRRF ($\frac{F_m}{F_m^L}$) as these showed the effect of the ambient light field on the photosynthetic rates of the phytoplankton.

Figures 5.5 and 5.7 show FRRF profiles from the NASG and SAG respectively, which had low concentrations of chlorophyll ($F_m$) and low light chamber $\frac{F_m}{F_m^L}$ ($\frac{F_m}{F_m^L}$) in the surface waters. The low $\frac{F_m}{F_m^L}$ was a result of photochemical quenching, the physiological protection of the photosystems by the phytoplankton under light intensities that would otherwise damage the photosystem. As light intensity decreased with increasing depth, so quenching decreased and therefore $\frac{F_m}{F_m^L}$ increased.

At the thermocline, a distinct chlorophyll maximum was present, presumably because
Figure 5.4: FRRF data representative of the Northern Temperate region. Top left, $F_m$, top right, $\frac{E_{\text{dil}}}{E_{\text{dil}}}$, bottom left, Temperature and bottom right, $\sigma_{\text{PSII}}$. Solid black lines show AMT 12 data and dotted black lines AMT 13.
Figure 5.5: FRRF data representative of the Northern Gyre region. Top left, $F_{m}$, top right, $\frac{F_{nL}}{F_{mL}}$, bottom left, Temperature and bottom right, $\sigma_{PSII}$. Solid black lines show AMT 12 data, dotted black lines AMT 13 and solid grey lines AMT 14.
Figure 5.6: FRRF data representative of the Equatorial region. Top left, $F_m$, top right, $F_{mL}$, bottom left, Temperature and bottom right, $\sigma_{PSII}$. Solid black lines show AMT 12 data, dotted black lines AMT 13 and solid grey lines AMT 14.
Figure 5.7: FRRF data representative of the Southern Gyre region. Top left, $F_m$, top right, $F_{mL}$, bottom left, Temperature and bottom right, $\sigma_{PSII}$. Solid black lines show AMT 12 data, dotted black lines AMT 13 and solid grey lines AMT 14.
Figure 5.8: FRRF data representative of the Southern Temperate region. Top left, $F_m$, top right, $\frac{F_L}{F_{op}}$, bottom left, Temperature and bottom right, $\sigma_{PSH}$. Solid black lines show AMT 12 data, dotted black lines AMT 13 and solid grey lines AMT 14.
light and nutrients would be available to sustain a sizable population of autotrophs. There was a concurrent slight increase in $\frac{E_{ph}}{F_{mD}}$ due to taxonomic photochemical and physiological adaptation which maximised the use of the limiting amount of light reaching that depth. The oligotrophic gyres are highly stable, and therefore little nutrient turnover occurs from waters below [Marum et al., 2003]. Consequently, the water in these regions is nutritionally very poor. At the interface of the surface mixed layer and the thermocline, nutrients are available, but there is little light. In this region the phytoplankton produce large quantities of chlorophyll $a$, resulting in a high $\frac{E_{ph}}{F_{mD}}$, and so will collect light efficiently.

In the majority of gyre measurements from AMT 13 (figure 5.5, dotted lines) substantial errors occurred. $F_m$ was zero at many points, probably in part because in the NASG the gain setting was accidentally set to 1 (see chapter 3 for discussion of the effects of gain settings), although there may also have been blanking or instrument errors. As a result of these factors it was not possible to determine $\frac{E_{ph}}{F_{mD}}$ and $\sigma_{PSII}$ from several AMT 13 profiles.

The profiles obtained from the SAG (figure 5.7) were similar in most respects to the NASG (figure 5.5). However, at the fluorescence maximum in some profiles there was a distinct dip in $\frac{E_{ph}}{F_{mD}}$ (shown clearly in figure 5.9). It has been suggested [Omachi, 2003] that this was due to depletion of a limiting trace element, probably iron in this area.
of relatively high stability and high biomass. Saharan dust atmospheric inputs mean that this was less likely to be an issue in the NASG [Baker et al., 2003], whereas less atmospheric input enters the SAG and therefore this effect was more obvious.

The equatorial upwelling region (figure 5.6) shared many of the features of gyre profiles, but the structure of the water column was compressed by the physical forcing of the underlying water upwelling into the surface mixed layer. For the profiles encountered during AMT, these upwelling regions did not break the surface and therefore these were not true upwelling measurements as defined by Longhurst [1998]. They did however show considerable differences to the gyre profiles and so are considered separately.

Due to instrument failure, no measurements were taken of FRRF parameters in the Northern Temperate region on AMT 14. Figures 5.4 (AMTs 12 and 13) and 5.8 (AMTs 12-14) are profiles in Northern and Southern temperate waters, showing that they differed from gyre data in several respects. Physically, these regions would be subject to a large amount of wind mixing as well as mixing through conductive cooling [Hooker et al., 2000]. The degree of mixing would therefore have been much higher than in the gyres, such that nutrient rich water was being brought up from beneath the euphotic zone providing resources that were in short supply in the gyres. This resulted in a concentrated population of phytoplankton throughout the euphotic zone as was evident from the $F_m$ profiles in figures 5.4 and 5.8.

5.2 Relationships within the FRRF data

Initial plots of $\frac{F_{\infty}}{F_m}$ and $\sigma_{PSII}$ with $F_m$ showed large amounts of scatter, with several points that appeared anomalous as they fell outside theoretical limits. The reasons for these apparent errors were therefore investigated.

5.2.1 Nutrient limitation

As was discussed in section 5.1.1, gyre profiles often showed localised minima in $\frac{F_{\infty}}{F_m}$ at the $F_{\text{max}}$ possibly due to iron deprivation in these areas of high biological activity [Omachi, 2003]. To account for this effect, which could alter the normal relationship of $\frac{F_{\infty}}{F_m}$ with $F_m$, several profiles were examined. At those stations where a localised $\frac{F_{\infty}}{F_m}$ minimum was apparent the $\frac{F_{\infty}}{F_m}$ in the profile was taken instead. In the region where $\frac{F_{\infty}}{F_m}$ max was present it was likely that the nutrient limitation stress would be ameliorated and therefore data should fit the rest of the dataset better.
Figure 5.10: FRRF measurements from the fmax from all three AMTs accounting for the localised $\frac{F_{psx}}{F_{psy}}$ minimum: top, $\frac{F_{psx}}{F_{psy}}$ and bottom, $\sigma_{PSH}$ with $F_{m}$. Dots represent samples with no localised $\frac{F_{psx}}{F_{psy}}$-min, crosses are data at the $\frac{F_{psx}}{F_{psy}}$-min and open circles are where $\frac{F_{psx}}{F_{psy}}$-min data have been replaced with $\frac{F_{psx}}{F_{psy}}$-max data.
Figure 5.10 shows the output of this analysis for $f_{\text{max}}$ data on all cruises. $F_{\text{m}}$ data taken from the $F_{\text{mmax}}$ (figure 5.10, top) fell below the $F_{\text{m}}$ range of the unaffected dataset. By comparison, the corrected dataset taken from the local $F_{\text{mmax}}$ conformed much more closely to the unaffected dataset. The $\sigma_{\text{PSII}}$ measurements (figure 5.10, bottom) were less informative, although at the points where there was an $F_{\text{m}}$ local minimum a localised $\sigma_{\text{PSII}}$ maximum was often recorded and indeed brought these data into alignment with the unaffected dataset. This study is concerned with general patterns in the photochemistry of the phytoplankton and not changes brought about by micronutrient deprivation. Data taken at localised $F_{\text{m}}^\text{min}$ were therefore replaced with $F_{\text{mmax}}$ data for further analysis.

5.2.2 Taxonomic variation

Automated Flow Cytometry (AFC) data describing the relative abundance of three groups of the picoplankton ($\text{Prochlorococcus}$, $\text{Synechococcus}$ and picoeukaryotes) were taken on the AMT cruises [Zubkov, M. and Heywood, J., unpublished data]. Almost all stations showed a dominance of $\text{Prochlorococcus}$, no stations showed a dominance of picoeukaryotes and only 10 showed a dominance by $\text{Synechococcus}$; these 10 stations being in the more productive temperate regions of the transect.

Figure 5.11 (top) shows FRRF data for both the surface and $f_{\text{max}}$, split according to the picoplankton groups. The stations dominated by $\text{Prochlorococcus}$ all followed a log linear increase of $F_{\text{m}}$ with $F_{\text{m}}$. However, $\text{Synechococcus}$-dominated samples corresponded well with outlying data points suggesting that the $F_{\text{m}}$ of this organism (at the wavelength associated with eukaryotic photosystems) was comparatively low [Moore et al., 2005]. Again, $\sigma_{\text{PSII}}$ (figure 5.11, bottom) was relatively unaffected by this association. On the basis of these findings only those samples dominated by $\text{Prochlorococcus}$ were used.

5.2.3 Quality checked relationships

Figures 5.12 to 5.14 show the relationship between $F_{\text{m}}$, $F_{\text{m}}$ and $\sigma_{\text{PSII}}$. $F_{\text{m}}$ was constrained between 0.2 and 0.65 as demonstrated in culture experiments by Kolber and Falkowski [1993], and generally were in the lower part of that range due to the oligotrophic nature of the dataset. $\sigma_{\text{PSII}}$ was in the range between 400 - 1000 $\text{Å}^2$(quanta$)^{-1}$ and generally in the upper region of this range, also due to the oligotrophic nature of the samples [Moore et al., 2005]. Since the instrument was taking measurements at the limit of its sensitivity, there was a large amount of noise in the dataset. As a consequence the
Figure 5.11: Splitting AMT data at both surface and fmax according to dominant picoplankton taxon for top, $\frac{F_R}{F_m}$ and bottom, $\sigma_{PSII}$ with $F_m$. Filled circles are *Prochlorococcus* dominated, while open circles are *Synechococcus* dominated.


\[ r^2 \] values were relatively low.

Figures 5.12 and 5.13 show the relationship between \( \frac{F_m}{F_m} \) with \( F_m \) in the surface waters and \( F_{m\max} \) respectively. \( F_m \) in the \( F_{m\max} \) was consistently higher than that in the surface waters, with all the values being greater than 1.5. They showed little variation in \( \frac{F_m}{F_m} \) or \( \sigma_{PSII} \) with \( F_m \). Almost all the surface waters had an \( F_m \) value of under 2, and most below 1. The noise inherent in the FRRF data meant that statistics associated with patterns in the data would generally not show significance. However, as shown in table 5.1, some patterns were apparent with the equations for these plots.

When the surface mixed layer and \( F_{m\max} \) data were considered together some general patterns were apparent (figure 5.14). \( \frac{F_m}{F_m} \) values of between 0.2 and 0.65 fell within the theoretical ranges for this parameter [Kolber and Falkowski, 1993, Smyth et al., 2004] and appeared to follow the published pattern of a log linear relationship with \( F_m \) [Aiken et al., 2004]. However, \( \sigma_{PSII} \) did not follow the expected inverse log relationship suggested by Moore et al. [2005], Suggett et al. [2004] and Aiken et al. [in press]. Instead, these data showed a log linear relationship in the same positive form as the relationship of \( \frac{F_m}{F_m} \) with \( F_m \). \( \frac{F_m}{F_m} \) appeared to be low at low \( F_m \) values increasing to a constant high value as \( F_m \) increased (between 0.5 and 0.6 when \( F_m \) reached 1). This was best described in the surface data of AMT 14 (figure 5.12), where 57% of the variance of the dataset was described by a log linear relationship between \( \frac{F_m}{F_m} \) and \( F_m \).

When considering \( \sigma_{PSII} \) relative to \( F_m \) any relationship was less clear. Generally, \( \sigma_{PSII} \) should have shown a negative correlation with \( F_m \) since (under more optimal conditions) a number of PSII reaction centres should have been present which could use absorbed light efficiently. Large cross sectional areas of each PSII reaction centre were not therefore needed. However, AMTs 12 and 14 data appeared to contradict this, instead following a similar relationship to the positive log linear relationship of \( \frac{F_m}{F_m} \). Data from AMT 13 appeared to follow a negative log linear relationship of \( \sigma_{PSII} \) with \( F_m \).

The equations derived for these relationships were plotted on the same axes for an easy comparison between them (figure 5.15). The top graph shows plots of \( F_m \) against \( \frac{F_m}{F_m} \) with four of the six plots, including all of the \( F_{m\max} \) equations, demonstrating a very similar gradient. Two of the surface equations (AMTs 12 and 14) showed strong negative correlations instead of the small positive gradients demonstrated by the other four plots. AMT 13 equations had a very different intercept from those of AMTs 12 and 14, suggesting a possible blanking issue with this dataset. Application of blanks to FRRF data systematically affected \( F_m \) and \( F_o \), therefore if blanks were incorrect then the
Figure 5.12: Relationships in surface waters between $F_m$ and (left) ($\frac{F_v}{F_m}$) and (right) $\sigma_{\psi_{II}}$ in (top) AMT 12 ($y = -0.114\ln(x)+0.62$, $r^2 = 0.72$ and $y = 20.5\ln(x)+485$, $r^2 = 0.08$), (middle) AMT 13 ($y = 0.0338\ln(x)+0.435$, $r^2 = 0.25$ and $y = -50.5\ln(x)+695$, $r^2 = 0.17$) and (bottom) AMT 14 ($y = -0.211\ln(x)+0.4858$, $r^2 = 0.05$ and $y = 107\ln(x)+446$, $r^2 = 0.57$).
Figure 5.13: Relationships in Fmax waters between $F_m$ and (left) ($\frac{E_m}{F_m}$) and (right) $\sigma_{PSII}$ in (top) AMT 12 ($y = -0.072\ln(x)+0.58$, $r^2 = 0.47$ and $y = 97.4\ln(x)+514$, $r^2 = 0.42$), (middle) AMT 13 ($y = 51.3\ln(x)+501$, $r^2 = 0.48$ and $y = 0.01\ln(x)+0.42$, $r^2 = 0.02$) and (bottom) AMT 14 ($y = -69.3\ln(x)+630$, $r^2 = 0.21$ and $y = -0.0106\ln(x)+0.52$, $r^2 = 0.04$).
Figure 5.14: Relationships in all data between \( F_m \) and (left) \( \frac{F_v}{F_m} \) and (right) \( \sigma_{FSH} \) in (top) AMT 12 \( (y = 0.048 \ln(x)+0.45, r^2 = 0.48 \) and \( y = 35.9 \ln(x)+504, r^2 = 0.06) \), (middle) AMT 13 \( (y = 0.016 \ln(x)+0.42, r^2 = 0.08 \) and \( y = 108.3 \ln(x)+436, r^2 = 0.53) \) and (bottom) AMT 14 \( (y = 0.039 \ln(x)+0.45, r^2 = 0.47 \) and \( y = 85 \ln(x)+465, r^2 = 0.71) \).
Figure 5.15: Plots of equations describing data of $F_m$ vs $\frac{F_m}{F_{m}}$ (top) and $\sigma_{PSII}$ (bottom). Dashed black lines show surface equations and grey lines equations from $\text{fmax}$ data.
Table 5.1: Equations of the Log linear correlations of the form $y = \gamma \ln(x) + \alpha$ between FRRF $\frac{F_e}{F_m}$ and $\sigma_{PSII}$ with $F_m$.

<table>
<thead>
<tr>
<th>Cruise</th>
<th>Layer</th>
<th>$y$ axis</th>
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<th>$\gamma$</th>
<th>$r^2$</th>
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<td>12</td>
<td>Surface $\frac{F_e}{F_m}$</td>
<td>-0.114</td>
<td>0.62</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{PSII}$</td>
<td>20.5</td>
<td>485</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fmax $\frac{F_e}{F_m}$</td>
<td>-0.072</td>
<td>0.58</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{PSII}$</td>
<td>97.4</td>
<td>514</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All $\frac{F_e}{F_m}$</td>
<td>0.048</td>
<td>0.45</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{PSII}$</td>
<td>51.3</td>
<td>501</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Surface $\frac{F_e}{F_m}$</td>
<td>0.0338</td>
<td>0.435</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{PSII}$</td>
<td>-50.5</td>
<td>695</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fmax $\frac{F_e}{F_m}$</td>
<td>0.01</td>
<td>0.42</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{PSII}$</td>
<td>35.9</td>
<td>504</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All $\frac{F_e}{F_m}$</td>
<td>0.016</td>
<td>0.42</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{PSII}$</td>
<td>-69.3</td>
<td>630</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Surface $\frac{F_e}{F_m}$</td>
<td>-0.211</td>
<td>0.4858</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{PSII}$</td>
<td>107</td>
<td>446</td>
<td>0.57</td>
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</tr>
<tr>
<td></td>
<td>Fmax $\frac{F_e}{F_m}$</td>
<td>-0.0106</td>
<td>0.52</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{PSII}$</td>
<td>108.3</td>
<td>436</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All $\frac{F_e}{F_m}$</td>
<td>0.039</td>
<td>0.45</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{PSII}$</td>
<td>85</td>
<td>465</td>
<td>0.71</td>
<td></td>
</tr>
</tbody>
</table>

Intercept on these regressions would have shifted. When $F_m$ was plotted against $\sigma_{PSII}$, in the same manner, the resulting spread of equations was greater. AMT 13 showed equations with gradients opposite to those of AMTs 12 and 14.

5.2.4 Relationships between FRRF data and HPLC pigments

As the CTD rosette was deployed at the same time as the FRRF, the concurrent water samples allowed replacement of the $F_m$ values with calibrated HPLC chlorophyll $a$ concentrations [Aiken et al., 2004, Suggett et al., 2001]. Figures 5.16 to 5.18 show the linearity between these two measurements on AMT 12 to 14, while figures 5.20 to 5.22 show the relationship between chlorophyll $a$ concentration and $\frac{F_e}{F_m}$ and $\sigma_{PSII}$ when fluorometric chlorophyll replaces $F_m$.

Data were taken from the FRRF in the region where light intensity was low enough for there to be no measurable photochemical quenching [Chamberlin et al., 1990]. The correlation between FRRF $F_m$ and HPLC gave an indication of the viability of the FRRF dataset [Suggett et al., 2001]. Figures 5.16 to 5.18 and table 5.2 show the correlation between these values. Figure 5.16 shows the AMT 12 correlation of average unquenched $F_m$ values with HPLC total chlorophyll $a$ and total pigment in the surface mixed layer.
(top) and at the fmax (bottom). There was no clear pattern in the surface mixed layer data, but the fmax data showed a linear relationship although the $r^2$ values were low ($r^2=0.22$ and 0.32 for TChla and Tpig vs. $F_m$ respectively).

Figure 5.17 shows the same correlations for AMT 13. In these data, a clear positive linear relationship was evident in both surface mixed layer and fmax data. A large number of bad data points were removed from the AMT 13 dataset (see section 5.1 and 5.1.1) suggesting much of these data may have been of lower quality than those for AMTs 12 and 14. However, the data showed a similar proportion of the variance of the data explained as AMT 12, with $r^2$ values of 0.28 and 0.56 for Chla and Tpig respectively.

AMT 14 data (shown in figure 5.18) again showed good correlations between chlorophyll $a$, total pigment concentrations and $F_m$ in surface waters with $r^2$ values of 0.62 and 0.64 respectively. The fmax data also had a clear linear relationship with $r^2$ values of 0.55 for both correlations. The form of these data (with two different gradients in the regressions between the surface mixed layer and fmax data, see table 5.2) suggested the presence of two distinct populations, and possible reasons for this are discussed in section 5.3.

For comparison between regressions, the relationships were plotted together in figure 5.19. In these plots it was clear that the fmax and surface mixed layer data produced two distinct relationships. The plots of surface data were similar for both AMT 13 and 14 (AMT 12 was not plotted due to a lack of correlations) and the fmax equations for all three cruises were very similar. This suggested either an artefact produced by the instrument at low chlorophyll $a$ concentrations or other factors affecting the response of the instrument. This relationship was duplicated in the plot of $F_m$ against HPLC derived total pigment concentration.

Figures 5.20 to 5.22 show the result of plotting $\frac{F_m}{F^*}$ and $\sigma_{PSII}$ against HPLC chlorophyll $a$ values, with equations and $r^2$ values shown in table 5.3. This substitution resulted in an increase in the dynamic range of surfaced mixed layer data such that surface and fmax data overlapped more than with $F_m$ values.

Figure 5.20 shows data from AMT 12 where the surface mixed layer and fmax data appeared to show negative log linear relationships, but with low $r^2$ values of 0.06 and 0.03. This lack of a relationship, which was also in evidence when correlated with $F_m$ instead of HPLC chlorophyll $a$, was presumably a result of the very low dynamic range seen in these data relative to AMTs 13 and 14. As with the $F_m$ correlation, no clear relationship was present in $\sigma_{PSII}$ data, although a slight positive correlation was apparent. Figure 5.21
Table 5.2: Equations of the Log correlations between HPLC TChla and Tpig with FRRF $F_m$.

<table>
<thead>
<tr>
<th>Cruise</th>
<th>Layer</th>
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<th>$\gamma$</th>
<th>$r^2$</th>
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<tbody>
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<td>12</td>
<td>Surface</td>
<td>Chla</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tpig</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fmax</td>
<td>Chla</td>
<td>0.14</td>
<td>0.03</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tpig</td>
<td>0.25</td>
<td>0.06</td>
<td>0.32</td>
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<tr>
<td>13</td>
<td>Surface</td>
<td>Chla</td>
<td>0.24</td>
<td>0.31</td>
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<td></td>
<td></td>
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<td>0.17</td>
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<tr>
<td></td>
<td>Fmax</td>
<td>Chla</td>
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<td>0.08</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tpig</td>
<td>0.11</td>
<td>0.14</td>
<td>0.56</td>
</tr>
<tr>
<td>14</td>
<td>Surface</td>
<td>Chla</td>
<td>0.04</td>
<td>0.19</td>
<td>0.62</td>
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<td></td>
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<td>Tpig</td>
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<td>0.10</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Fmax</td>
<td>Chla</td>
<td>-0.27</td>
<td>0.14</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tpig</td>
<td>-0.49</td>
<td>0.37</td>
<td>0.55</td>
</tr>
</tbody>
</table>

shows data from AMT 13, where a positive log linear relationship was apparent in both surface and $F_m$ plots. However, in this case a negative log linear relationship was evident in the $\sigma_{PSII}$ plots. Figure 5.22 shows data from AMT 14, with a positive log linear relationship of $\frac{F_m}{F_m}$ with chlorophyll $a$ in the surface waters ($r^2$ value of 0.33), however the zero gradient shown in the regression of the $F_m$ data resulted in an $r^2$ of 0. Once more any pattern in $\sigma_{PSII}$ was unclear, although fitting a log linear relationship resulted in a weak positive correlation.

The regression equations were plotted on the same axes for examination of correlation between cruises (Figure 5.23). $\frac{F_m}{F_m}$ equations showed AMT 12 and 14 plots being of similarly small gradients, with AMT 13 again showing a much larger positive gradient in the surface waters and negative gradient in the $F_m$ data. For the $\sigma_{PSII}$ data, a similar relationship was shown, where AMTs 12 and 14 showed low positive gradients with AMT 13 showing a large positive gradient in the surface waters and negative gradient in the $F_m$ data.

5.3 Discussion

As expected, the datasets for the two subtropical gyres showed low chlorophyll ($F_m$) concentrations in overlying waters and a higher concentration, deeper, chlorophyll maximum at the thermocline [Aiken et al., 2000]. At the equatorial upwelling, the depth of the thermocline decreased but the general form was the same. At each end of the transects, this structure broke down as no stratification was present and instead parameters were
Figure 5.16: Linearity in AMT 12 data between HPLC chlorophyll a (left) and total pigment load (right) with FRRF $F_m$ in surface waters (top, no correlation in surface), and at the fmax (bottom. $y = 0.027x + 0.137, r^2=0.22$ and $y = 0.062x + 0.248, r^2=0.32$)

Table 5.3: Equations of the Log linear correlations between FRRF $\frac{F}{F_m}$ and $\sigma_{PSII}$ with HPLC TChla.

<table>
<thead>
<tr>
<th>Cruise</th>
<th>Layer</th>
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<th>$\gamma$</th>
<th>$r^2$</th>
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<td>0.47</td>
<td>0.022</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fmax</td>
<td>0.48</td>
<td>-0.012</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Surface $\sigma_{PSII}$</td>
<td>521</td>
<td>15</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fmax</td>
<td>660</td>
<td>56.5</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Surface $\frac{F}{F_m}$</td>
<td>0.76</td>
<td>0.18</td>
<td>0.78</td>
<td></td>
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<tr>
<td></td>
<td>Fmax</td>
<td>0.42</td>
<td>0.004</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Surface $\sigma_{PSII}$</td>
<td>147</td>
<td>93</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fmax</td>
<td>595</td>
<td>35.7</td>
<td>0.11</td>
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<tr>
<td>14</td>
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<td>0.061</td>
<td>0.33</td>
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<tr>
<td></td>
<td>Fmax</td>
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<td>0.002</td>
<td>0.00</td>
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<tr>
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<td>Surface $\sigma_{PSII}$</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Fmax</td>
<td>679</td>
<td>40.9</td>
<td>0.15</td>
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Figure 5.17: Linearity in AMT 13 data between HPLC chlorophyll a (left) and total pigment load (right) with FRRF $F_m$ in surface waters (top. $y = 0.3074x + 0.238, r^2 = 0.41$ and $y = 0.1726x + 0.106, r^2 = 0.46$), and at the fmax (bottom. $y = 0.0755x + 0.0518, r^2 = 0.28$ and $y = 0.139x + 0.107, r^2 = 0.56$)
Figure 5.18: Linearity in AMT 14 data between HPLC chlorophyll a (left) and total pigment load (right) with FRRF $F_{m}$ in surface waters (top. $y = 0.1854x + 0.0433$, $r^2=0.62$ and $y = 0.0954x + 0.0139$, $r^2=0.64$), and at the fmax (bottom. $y = 0.14x - 0.27$, $r^2=0.55$ and $y = 0.37x - 0.49$, $r^2=0.55$)
Figure 5.19: Plots of equations describing data of $F_m$ vs HPLC chlorophyll a concentration (top) and total pigment (bottom). Dashed black lines show surface equations and grey lines equations from fmax data.
Figure 5.20: Relationships on AMT 12 in surface (left) and fmax (right) waters between HPLC chlorophyll $a$ with top, ($\frac{F}{F_m}$) ($y = 0.022x + 0.47$, $r^2=0.06$ and $y = -0.012x + 0.48$, $r^2=0.03$) and bottom, $\sigma_{PSII}$ ($y = 15x + 521$, $r^2=0.05$ and $y = 56.5x + 660$, $r^2=0.12$).
Figure 5.21: Relationships on AMT 13 in surface (left) and fmax (right) waters between HPLC chlorophyll \( a \) with top, \( \frac{F_\text{F}_\text{m}}{F_\text{m}} \) \( (y = 0.18x + 0.76, r^2 = 0.78 \text{ and } y = 0.004x + 0.42, r^2 = 0.01) \) and bottom, \( \sigma_{PSII} \) \( (y = 93x + 147, r^2 = 0.03 \text{ and } y = 35.7x + 595, r^2 = 0.11) \).
Figure 5.22: Relationships on AMT 14 in surface (left) and fmax (right) waters between HPLC chlorophyll a with top, $(\frac{F_v}{F_m})$ ($y = 0.061x + 0.59$, $r^2 = 0.33$ and $y = 0.002x + 0.5$, $r^2 = 0.00$) and bottom, $\sigma_{PSII}$ ($y = 30x + 657$, $r^2 = 0.09$ and $y = 40.9x + 679$, $r^2 = 0.15$).
Figure 5.23: Plots of equations describing data of HPLC chlorophyll $a$ concentration vs $F_{m}/F_{n}$ (top) and $\sigma_{PSII}$ (bottom). Dashed black lines show surface equations and grey lines equations from $f_{max}$ data.
relatively constant throughout the profile.

On examination of AMT 13, it appeared that the FRRF was not well characterised and not operating normally as the data from this cruise appeared more noisy. The data was clearly unusable in many casts, particularly in the NASG where the gain function of the instrument had been set to 1 resulting in zero detection levels in many profiles (figure 5.5 and appendix E). Although used for describing variation through the transects; while discussing FRRF correlations, no distinction was made between the dark and light chambers of the FRRF as data were being taken from regions where the phytoplankton community were not photochemically quenched. Therefore, given that blanks were applied, both chambers should give equal values [Chelsea Instruments Ltd, 1998].

The degree of noise in the FRRF data was countered, as far as possible, by taking the mean of the unquenched readings from the surface layer. The more discrete nature of the fmax dictated that for this region a mean of 5, 1 metre binned measurements centred around the fmax was taken. The result of these errors, and the fact that the instrument was working at the limits of its ability to detect fluorescence, meant that relationships derived from these data were often not able to account for a large amount of the variance. Nevertheless, relationships were qualitatively evident and in some cases the degree of variance explained by them was relatively high.

Within the FRRF data a log linear relationship was apparent between $\frac{F_\infty}{F_m}$ and $F_m$. Further examination of variations of these relationships between cruises seemed to imply two sets of relationships, with the surface water showing a greater gradient than the measurements taken at the fmax. This implied either that in the low chlorophyll concentrations of the surface water the FRRF was operating below its detection limit ($0.1 \text{mg m}^{-1}$) [Chelsea Instruments Ltd, 1998] and producing spurious relationships or that some aspect of these regions was affecting measurements.

The value of $\sigma_{PSII}$ would be expected to be related to $F_m$ in a negative log linear fashion as suggested by Moore et al. [2005], Suggett et al. [2004] and Aiken et al. [in press], but in fact the calculated relationships where this occurred were very weak, describing as little as 5% of the variance of the dataset. However, data from AMTs 12 and 14 showed strong positive correlations of $\sigma_{PSII}$ with chlorophyll $a$ measures. When the whole dataset (all three cruises) was considered together a negative log linear relationship appeared to be present, although the degree of variance explained was again too small to consider the relationship quantitatively. It is suggested there may be a taxonomic artefact within the AMT 13 data, a cruise in the upwelling region that showed a larger
proportion of eukaryotes and a negative correlation. AMTs 12 and 14, being gyre based, were prokaryote dominated cruises and showed a positive correlation.

In order to allow correlation of the photosynthetic parameters with more universally used measurements, the relationship of $F_m$ to chlorophyll $a$ was studied. This would be expected to show a linear relationship [Aiken, 2001, Drain, 2003] and indeed this was found to be the case. AMT 12 surface mixed layer data fell within a small range of $F_m$ and so no relationship was apparent; however surface waters for AMTs 13 and 14 and all the fmax data showed linearity, but again with different gradients for surface and fmax data.

The substitution of $F_m$ with HPLC derived chlorophyll $a$ into plots of $\frac{E_p}{F_m}$ and $\sigma_{PSII}$ resulted in a distinct spreading in the lower chlorophyll $a$ value data relative to when it was plotted against $F_m$. The effect of this was to reduce the gradient of the log linear regression. Again, measurements from AMT 13 were distinctly different to those from AMTs 12 and 14. $\frac{E_p}{F_m}$ from AMTs 12 and 14 both showed the expected [Aiken et al., 2004] positive log linear relationship with a small positive gradient in the surface waters and a small negative gradient in fmax water. Relationships with $\sigma_{PSII}$ were weak, but appeared to show a slight positive gradient in contradiction to the expected results as suggested by Moore et al. [2005], Suggett et al. [2004] and Aiken et al. [in press].

All of these datasets showed distinctly different relationships in the surface mixed layer compared to those data taken at the fmax. Assuming this was not an instrument artefact, the most likely cause was a change in the fluorescence response of the phytoplankton community to the excitation of the FRRF. As will be shown in chapter 6, the surface mixed layer phytoplankton community was dominated by prokaryotes [Zubkov et al., 2000] that have different photo-physiology to the eukaryotes found in the fmax community and shelf seas [Ting et al., 2002].
Chapter 6

Pigments

Chapter 6 examines relationships in the composition of phytoplankton pigments along the AMT track. The general community structure is first investigated, followed by relationships between the concentrations of different pigments and groups of pigments.

6.1 Community structure

Figure 6.1 shows the community pigment structure of the AMT 12 section. AMTs 13 and 14 are not shown, but follow a similar structure. Chlorophyll a concentration (top) represents phytoplankton abundance, the total prokaryotic accessory pigment (middle) represents prokaryotic phytoplankton abundance and total eukaryotic pigment (bottom) represents eukaryotic phytoplankton abundance (nanoflagellates).

In the gyres, between 30°S and 10°S and 10°N and 30°N, a low pigment concentration could be seen in the surface mixed layer. This was less distinct in the prokaryote pigments, which indicates the dominance of this group. Below the surface mixed layer, a high concentration chlorophyll maximum with concurrent high prokaryote and eukaryote pigments could be seen; the eukaryotic pigments being of higher concentration than the prokaryotes. In the equatorial region, the pattern of pigments took much the same form as that in the gyres, although the dominance of prokaryotic pigments in the surface mixed layer was found to be much more evident than in the gyres (figure 6.1). At the Northern and Southern extent of the transect this structure was found to break down, due to the large amount of mixing that occurs in these areas. Here, all pigments were found to be present in high concentrations as would be expected where neither nutrients nor light were limiting growth [Lalli and Parsons, 1997]. The concentration of prokaryote specific pigments in samples where they dominated was generally found to be lower than those
specific to nanoflagellates in the areas where nanoflagellates dominated. Prokaryote pigment concentrations were at a maximum of 0.1mgm\(^{-3}\) whereas nanoflagellate pigments in the majority of the transect reached 0.25mgm\(^{-3}\) and in temperate regions peaked at 0.7mgm\(^{-3}\).

6.2 Biogeochemical Provinces

Pigments in the surface mixed layer were used to identify biogeochemical provinces [Longhurst, 1998, Omachi, 2003] based upon multivariate statistical analysis, producing similarity clusters of pigment composition [Clarke and Gorley, 2001]. Data from the fmax are not shown because the community in this region was much less variable than in the surface waters, such that the regions were not clearly defined. Placing of a station into a 'gyre' or 'non-gyre' group was therefore based only on the surface mixed layer pigments.

The results of these analyses are shown in the dendrograms in figures 6.2 to 6.4. The dashed lines indicate the degree of similarity (where two distinct populations became apparent); the lower the degree of similarity the greater was the difference between the groups. All of these plots showed a clear distinction between two major groups.

Clusters were designated as being 'gyre' or 'non-gyre' by examination of the stations within those clusters. The general position of gyres was known in that they lay between the temperate stations at the two ends of the transect and were split by an upwelling region. On this basis the gyre cluster was easily identified. The stations identified as belonging to either a 'gyre' or a 'non-gyre' region generally clustered well, and regions that, based on their position (see appendix C), would be expected to be gyre were clearly separated from those that would be expected to be in a temperate region. One group contained the highest and lowest station numbers as would be expected from the temperate mixed waters (at each end of the transect). A small number of the station numbers in the middle of the cruise were also identified with this group, suggesting the presence of the equatorial upwelling region. The equatorial region was less well-identified as this technique did not account for the vertical structure of the profile, but depended on the pigment structure in surface waters. This technique may not have clearly identified the equatorial region since the community structure of this region was similar to the gyre communities (see figure 6.1). The 'gyre' or 'non-gyre' status was then plotted over the track of each cruise, as shown in figure 6.5.
Figure 6.1: Pigment structure of the AMT 12 section, total chlorophyll $a$ (top), total prokaryotic accessory carotenoids (middle) and total eukaryote accessory carotenoids (bottom).
Figure 6.2: Cluster analysis of surface mixed layer pigments from AMT 12. Splitting into 2 main groups is achieved at 78% similarity as shown by the dashed line. Station positions are shown in appendix C.
Figure 6.3: Cluster analysis of surface mixed layer pigments from AMT 13. Splitting into 2 main groups is achieved at 70% similarity as shown by the dashed line. Station positions are shown in appendix C.
Figure 6.4: Cluster analysis of surface mixed layer pigments from AMT 14. Splitting into 2 main groups is achieved at 77% similarity as shown by the dashed line. Station positions are shown in appendix C.
Figure 6.5: The ‘gyre’ and ‘non-gyre’ status of CTD stations overlayed on cruise tracks of (top left) AMT 12 and (top right) 14 between the Falkland Islands and the UK (cruises going deep into the Northern Gyre) and (bottom) AMT 13 between the UK and the Falkland Islands. Yellow points represent ‘gyre’ stations, red points ‘non-gyre’ stations.
6.3 Pigment correlations

Figure 6.6 shows linear correlations between chlorophyll $a$ and pigment groups. Figure 6.6 (left) shows the linear relationship between TChla and Tpig as originally proposed by Trees et al. [2000]. Data from AMTs 12, 13 and 14 showed $r^2$ values of 0.96, 0.95 and 0.94 respectively, and when all cruises were considered together the resulting $r^2$ value was 0.95. These data strongly suggest that the total pigment concentration increased linearly with the TChla concentration. When Tpig was replaced with the concentrations of the TAC pigment load, as shown in figure 6.6 (right), the $r^2$ values of linear correlation in data from AMT 12, 13 and 14 dropped to 0.84, 0.72 and 0.80 respectively and 0.76 when considered together. This implied that the removal of chlorophylls from Tpig to give TAC moved the correlation of these data away from linearity of that TChla is a large component of Tpig, in which case the $r^2$ is bound to decrease with removal of TChla.

Figure 6.7 shows plots of the variation of the ratio of TChla:Tpig with TChla to explain the variance not accounted for in the linear relationship between TChla and Tpig, suggested by Aiken et al. [2004], in the linearity of Tpig and TChla concentrations proposed by Trees et al. [2000]. A linear relationship between Tpig and TChla concentration would imply a zero gradient on a graph of TChla:Tpig vs. Tpig (the rate at which TChla is synthesised compared to total pigment). Data from AMTs 12 and 14 appeared to show a zero gradient (slopes of 0.02 and 0.01 respectively), but as a result of this the proportion of variance in the dataset explained was very low. However, AMT 13 data appeared to show a decrease in this ratio at low TChla concentrations suggesting a possible log linear relationship. When a log linear curve was fitted to this an $r^2$ value of 0.23 resulted. The noise apparent at low TChla concentration suggested a possible variation in the pigment ratio. A possible cause for this lack of clarity was the presence of distinct populations with different pigment characteristics [Aiken et al., 2004, Barlow et al., 2001, 2004, Havskum et al., 2004, Mackey et al., 1996, Vidussi et al., 2001, Uitz, 2002].

In order to resolve these relationships more clearly, the data were split according to physical separation and taxonomic boundaries. The initial split was based on depth, between the surface mixed layer and the fmax. The average surface mixed layer data are shown in figure 6.8 (left) and those taken from the fmax in figure 6.8 (right). This splitting showed clearer patterns than those arising from the whole dataset. The fmax data were clustered at higher chlorophyll $a$ concentrations and showed a slight positive gradient on a log linear graph with $r^2$ values of 0.04, 0.19 and 0.03 for AMTs 12 to 14.
Figure 6.6: The linear relationship between chlorophyll concentration and concentration of Total Pigments (Tpig) (left) and Total Accessory Carotenoid (TAC) (right) in HPLC data from AMT 12 (top, Tpig = 2.04x + 0.01, r^2=0.96, TAC = 0.82x - 0.01, r^2=0.82), 13 (middle, Tpig = 1.83x + 0.38, r^2=0.95, TAC = 0.82x + 0.04, r^2=0.8) and 14 (bottom, Tpig = 2.01x + 0.03, r^2=0.94, TAC = 0.94x + 0.02, r^2=0.8).
Figure 6.7: Pigment ratios of TChla:Tpig varying with TChla in AMTs 12 (top), 13 (middle) and 14 (bottom). Log linear correlation for AMT 12, $y = 0.02 \ln(x) + 0.51$, $r^2=0.1$, AMT 13, $y = 0.06 \ln(x) + 0.6$, $r^2=0.44$ and AMT 14, $y = 0.01 \ln(x) + 0.48$, $r^2=0.06$. 
Table 6.1: Linear correlations between TChla and pigment groups: \( y = \gamma(x) + \alpha \).

<table>
<thead>
<tr>
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<th>y axis</th>
<th>Cruise</th>
<th>( \gamma )</th>
<th>( \alpha )</th>
<th>( r^2 )</th>
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</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>2.01</td>
<td>0.03</td>
<td>0.94</td>
</tr>
<tr>
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<td>-0.01</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
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<td>13</td>
<td>0.82</td>
<td>0.04</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>0.94</td>
<td>0.02</td>
<td>0.80</td>
</tr>
<tr>
<td>TAC prokaryotes</td>
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<td>0.02</td>
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</tr>
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</tr>
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<td></td>
<td></td>
<td>14</td>
<td>0.69</td>
<td>0.24</td>
<td>0.82</td>
</tr>
<tr>
<td>TAC eukaryotes</td>
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<td>0.88</td>
<td>-0.03</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>0.87</td>
<td>0.04</td>
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<tr>
<td></td>
<td></td>
<td>14</td>
<td>0.77</td>
<td>0.01</td>
<td>0.88</td>
</tr>
</tbody>
</table>

respectively. Because these data lay on a near zero gradient the \( r^2 \) values did not describe variability in the dataset. The surface data were spread over a larger range and showed a steeper gradient for AMTs 12 and 13 (consequently the \( r^2 \) values are higher, 0.24, 0.57 and 0.13 for AMTs 12, 13 and 14 respectively).

When the data were split according to provinces (derived from the multivariate statistical analyses shown in figures 6.2 to 6.4), the resulting log linear plots (figure 6.9) had a higher variance explained, with \( r^2 \) values ranging from 0.17 to 0.64. The non-gyre regions were less well described with \( r^2 \) values ranging from 0.02 to 0.45. The data were then split taxonomically rather than geographically; see section 4.3.1 for an explanation of the method. Plotting these data on the same axes (figure 6.10) showed that prokaryote dominated samples were present in low chlorophyll regions and eukaryotes dominated in high chlorophyll regions.

Linear plots of the data describe the variation in the linearity of the bulk dataset (figure 6.10). Samples with high proportions of eukaryote DP concentrations relative to prokaryotic DP and with high TChla concentrations showed high conformity to linearity (\( r^2 \) values of between 0.82 and 0.88), as did those with a high proportion of prokaryotic DP and low TChla concentration samples (\( r^2 \) values of between 0.82 and 0.89). However, as can be seen in table 6.1, there were distinct differences in the fitted gradients for these two taxa. These two groups explained the loss of linearity in the total dataset when comparing variations of TAC with TChla instead of Tpig with TChla in figure 6.6.

As would be expected from the linearity of this group (figure 6.10), eukaryote dominated samples showed a log linear pigment ratio relationship with a near zero gradient,
Figure 6.8: Pigment ratios of TChla:Tpig varying with TChla in AMTs 12 (top) 13 (middle) and 14 (bottom) split between surface mixed layer data (left) and the fmax (right). AMT 12 surface \( y = 0.03 \ln(x) + 0.54, r^2 = 0.24, \) fmax \( y = 0.02 \ln(x) + 0.52, r^2 = 0.04, \) AMT 13 surface \( y = 0.05 \ln(x) + 0.57, r^2 = 0.57, \) fmax \( y = 0.04 \ln(x) + 0.54, r^2 = 0.19, \) and AMT 14 surface \( y = 0.35 \ln(x) + 0.54, r^2 = 0.13, \) fmax \( y = 0.02 \ln(x) + 0.48, r^2 = 0.03. \)
Figure 6.9: Pigment ratios of TChla:Tpig varying with TChla in AMTs 12 (top), 13 (middle) and 14 (bottom) split between surface mixed layer data (left, S) and the fmax (right, F) and between gyre stations (open circles, G) and non-gyre stations (filled circles, U). AMT 12: SG, $y = 0.06\ln(x)+0.61$, $r^2=0.33$, SU, $y = 0.08\ln(x)+0.57$, $r^2=0.22$, FG, $y = 0.04\ln(x)+0.56$, $r^2=0.14$, FU, $y = 0.02\ln(x)+0.52$, $r^2=0.02$. AMT 13: SG, $y = 0.08\ln(x)+0.66$, $r^2=0.52$, SU, $y = 0.05\ln(x)+0.57$, $r^2=0.17$, FG, $y = 0.14\ln(x)+0.73$, $r^2=0.52$, FU, $y = 0.02\ln(x)+0.53$, $r^2=0.05$. AMT 14: SG, $y = 0.06\ln(x)+0.62$, $r^2=0.28$, SU, $y = 0.09\ln(x)+0.56$, $r^2=0.61$, FG, $y = 0.02\ln(x)+0.49$, $r^2=0.02$, FU, $y = 0.07\ln(x)+0.53$, $r^2=0.69$. 
suggesting constant proportions of the pigment contributed by TChla as shown in table 6.2 and figure 6.11. However, the prokaryote samples showed a distinct log linear relationship of pigment ratio with TChla concentration and this explained the deviation from linearity of this group between TChla and Tpig in figure 6.10. The flat regression in the eukaryotes resulted in a very low proportion of variance explained, reaching as low as 0.02 in AMT 12. The larger gradient of the prokaryote however returned high $r^2$ values of between 0.32 and 0.62.

The taxonomic relationships suggested that splitting the pigment data by species composition was effective in describing the different populations. The fact that taxonomic splitting produced very similar groupings to the geographic splitting supports this; although the variation can be described by changes in position in the water column
and regions it should be the community that dictates the pigment composition and not the physics of the environment. The communities representing the gyre surface waters were generally dominated by prokaryotes with low pigment concentrations. In the temperate regions, surface water nutrients and light were both available to the eukaryotes, so these taxa were able to dominate and the pigment concentration was higher. In the fmax, the community was dominated throughout by the eukaryotes with high pigment concentration.

The methodologies used so far have split the data into one of two categories relating to geographical position, water depth or the dominant taxonomy of the sample. These methods did not show the community variability and so the data were analysed further by dividing the data according to the DPs as described in section 4.3.3. As discussed in section 4.3.3, the prokaryote pigment load was derived from two taxa with differing photochemistry, Prochlorococcus and Synechococcus. Prochlorococcus uses DvChla as its primary photo pigment, which was identified and quantified separately from MvChla by HPLC. Synechococcus uses MvChla but not as its primary photopigment as in the eukaryotes and so the quantity of MvChla relative to Tpig in this organism may not be the same as in the eukaryotes.

For the purposes of this study, an initial assumption was made that the pigment load of MvChla was the same in both Synechococcus and eukaryotes. Pigment ratios based on this assumption (figure 6.12, left) show that the gradients and intercepts of both prokaryotes and eukaryotes were similar for data from all cruises, but the intercept was lower in the eukaryotes relative to the prokaryotes (see table 6.3). The r² values of the prokaryote data were generally lower than when samples were apportioned to a class (figure 6.11), however the eukaryotes showed a better fit with this methodology.

When the MvChla load of Synechococcus was set to be 1/2 that of the eukaryotes [Glover et al., 1987], the form of the plots was very different (figure 6.12, right). The plot relating to the prokaryotes showed a considerably higher gradient than that relating to the eukaryotes. Also, the r² value of the prokaryote regression was much higher than when taken as a single class, varying from 0.47 to 0.63. However, the r² values for the eukaryotes of between 0.01 and 0.12 was lower than the classified grouping (shown in figure 6.11). These followed the difference in slope shown in figures 6.8 and 6.9, suggesting that this method may more accurately represent the pigment load of the phytoplankton groups. Again, the flat eukaryotic graph suggested a stable pigment ratio in this group whereas the prokaryotes showed a distinct increase in the pigment load of
Figure 6.10: Correlation between TChla and TAC split between prokaryotes and eukaryotes. AMT 12 prokaryotes (top left, $y = 0.69x + 0.02$, $r^2=0.82$) and eukaryotes (top right, $y = 0.88x - 0.03$, $r^2=0.82$). AMT 13 prokaryotes (middle left, $y = 0.72x + 0.04$, $r^2=0.89$) and eukaryotes (middle right, $y = 0.87x + 0.04$, $r^2=0.88$) and AMT 14 prokaryotes (bottom left, $y = 0.69x + 0.24$, $r^2=0.82$) and eukaryotes (bottom right, $y = 0.77x + 0.01$, $r^2=0.88$).
Figure 6.11: Pigment ratios of two populations of phytoplankton based on Diagnostic Pigment composition of samples. Prokaryotes (Zea dominated) closed circles, nanoplankton (Fuc, Hex, Diad, Peri and Allo dominated) open circles. AMT 12 prokaryotes (top, closed circles, $y = 0.03\ln(x)+0.39$, $r^2=0.32$) and eukaryotes (top, open circles, $y = 0.01\ln(x)+0.33$, $r^2=0.02$), AMT 13 prokaryotes (middle, closed circles, $y = 0.05\ln(x)+0.43$) and eukaryotes (middle, open circles, $y = 0.02\ln(x)+0.35$, $r^2=0.38$) and AMT 14 prokaryotes (bottom, closed circles, $y = 0.05\ln(x)+0.59$, $r^2=0.27$) and eukaryotes (bottom, open circles, $y = 0.03\ln(x)+0.5$, $r^2=0.26$).
Table 6.3: Log linear correlations between TChla and taxonomically split pigment ratios in the form y = \gamma \ln(x) + \alpha.

<table>
<thead>
<tr>
<th>x axis</th>
<th>y axis</th>
<th>Cruise</th>
<th>\alpha</th>
<th>\gamma</th>
<th>\mathbf{r}^2</th>
</tr>
</thead>
<tbody>
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<td>TChla:Tpig Pro 1:1 MvChla</td>
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<td>0.07</td>
<td>0.04</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>0.80</td>
<td>0.07</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>0.63</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>TChla:Tpig Euk 1:1 MvChla</td>
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<td>0.55</td>
<td>0.04</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>0.58</td>
<td>0.06</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>0.48</td>
<td>0.03</td>
<td>0.18</td>
</tr>
<tr>
<td>TChla:Tpig Pro 1:3 MvChla</td>
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<td>0.91</td>
<td>0.12</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>0.97</td>
<td>0.13</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>0.93</td>
<td>0.12</td>
<td>0.47</td>
</tr>
<tr>
<td>TChla:Tpig Euk 1:3 MvChla</td>
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<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td>0.55</td>
<td>0.03</td>
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</tr>
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<td></td>
<td></td>
<td>14</td>
<td>0.43</td>
<td>0.01</td>
<td>0.00</td>
</tr>
</tbody>
</table>

TChla relative to pigment load as the TChla concentration increased. The higher values for the prokaryotes (than the eukaryotes) suggested that the TChla load was higher for a given Tpig concentration.

6.4 Discussion

The community structure derived from HPLC pigment analysis clearly showed the segregation of data into distinct regions. As predicted by the findings of Gibb et al. [2000] and Zubkov et al. [2000], the gyres were clearly delineated by a deepening of the nanoflagellate dominated sub surface chlorophyll maximum and thickening of the overlying low pigment concentration, prokaryote dominated surface mixed layer. The equatorial upwelling region was in evidence by the thinning of this structure, and the Northern and Southern Temperate regions were shown by a breakdown of the structure to a homogenous eukaryote dominated community [Gibb et al., 2000].

The plotting of total pigment against chlorophyll a showed strong linearity as suggested by Trees et al. [2000]. However, when total pigment was replaced by total accessory carotenoid, this correlation became much weaker, corresponding to the hypothesis of Aiken et al. [2004] that the ratio of chlorophyll a to accessory pigment load is variable. This implied that the inclusion of TChla concentration in Tpig increased linearity, as it would by definition increase the tendency towards a linear 1:1 relationship. The influence of chlorophyll b and chlorophyll c must also be considered, since neither are contained within TAC and chlorophyll c is not present in prokaryotic pigment loads [Jeffrey et al.,
Figure 6.12: Splitting of pigment ratios by fuzzy logic based on relative diagnostic pigment concentration. Left assuming prokaryotes have the same relative chlorophyll a concentration as eukaryotes (1:1), right assuming the concentration in Syneccoccus is 1/3 that of eukaryotes (1:3). Closed circles, prokaryote component (P), open circles eukaryote component (E). AMT12, 1:1P, \( y = 0.04\ln(x)+0.07, r^2=0.13 \); 1:1E, \( y = 0.04\ln(x)+0.55, r^2=0.32 \); 1:3P, \( y = 0.12\ln(x)+0.91, r^2=0.63 \); 1:3E, \( y = 0.01\ln(x)+0.50, r^2=0.00 \). AMT13, 1:1P, \( y = 0.07\ln(x)+0.80, r^2=0.23 \); 1:1E, \( y = 0.06\ln(x)+0.58, r^2=0.53 \); 1:3P, \( y = 0.13\ln(x)+0.97, r^2=0.53 \); 1:3E, \( y = 0.03\ln(x)+0.55, r^2=0.12 \). AMT14, 1:1P, \( y = 0.02\ln(x)+0.63, r^2=0.05 \); 1:1E, \( y = 0.03\ln(x)+0.48, r^2=0.18 \); 1:3P, \( y = 0.12\ln(x)+0.93, r^2=0.47 \); 1:3E, \( y = 0.01\ln(x)+0.43, r^2=0.00 \).
1997b]. On examination of the data it was apparent that a major deviation from the linear relationship was present at the lower TChla and TAC concentrations and that this deviation took the form of a decrease in the ratio of TChla to TAC. Deviation from linearity at a single point over large temporal scales was explained by Aiken et al. [2004] through log linear relationships between TChla and pigment ratios. However these relationships have been shown not to hold on large oceanic datasets, such as those produced by the AMTs [Aiken, J., pers. comm.]. Noise in the data at low concentrations meant that no pattern was obvious.

The presence of a relationship between the pigment ratios and chlorophyll a concentration in the English Channel [Aiken et al., 2004] and its lack in large scale datasets suggest either:

1. that the English Channel is a special case or part thereof with factors acting on the phytoplankton unusual to the region, or

2. that a similar relationship is present within the general phytoplankton community, but has not been resolved.

When pigment ratios of TChla:TAC were plotted against TChla it appeared that two populations were present. The first showed a zero gradient demonstrating a linear relationship of TAC to TChla, whereas the second showed a distinct decrease of the ratio at low concentrations. These communities were split using two main methodologies: physical separation and taxonomic variation. Each of these splits produced two distinct populations, both of which produced log linear relationships in the ratio of TChla:TAC varying with TChla. Splitting of upwelling from gyre regions and the surface mixed layer from the fmax produced data groups very similar to those produced by partitioning distinct taxonomic communities.

6.4.1 Conclusions

Splitting phytoplankton communities based on physical variation gave results that corresponded strongly with splitting them taxonomically. The degree of linearity of TAC relative to TChla rather than with Tpig was increased by the taxonomic and geographic splitting. Variation from linearity appeared to be greater in the prokaryotes, and there was considerable variation between cruises in this fraction of the community. It is therefore possible that the ratio of TChla:Tpig compared to TChla may in fact be constant in eukaryotes and only vary in the prokaryotes.
It was clear from these analyses that several communities were present, each of which had varying degrees of TChla:Tpig and TChla concentration. It was also apparent that splitting these communities into two main groups (be it using geographic or taxonomic criteria) resolved the main structure of these relationships. The hypothesis of a log linearity of pigment ratios relative to chlorophyll a concentration was therefore supported.
Chapter 7

Absorption

Chapter 7 examines the pigment relationships discussed in chapter 6 using *in situ* optical measurements. Absorption measurements related to specific pigments are examined, as are the absorption signatures of pigment ratios. All data in this chapter are from AMTs 13 and 14; the ac9+ was not used to measure particulate absorption profiles on AMT 12.

7.1 Spectral absorption

Figure 7.1 shows calibrated ac9+ data corrected for the effects of temperature and salinity as described in section 4.4.2. The absorption of water (see figure 2.2 in chapter 2) has also been removed. Further examples of these data can be seen in appendix G and appendix H. Figure 7.1 (top) shows the absorption of all dissolved and particulate components in the water column, with high absorption in the blue (0.15 m\(^{-1}\) at 412 nm) decreasing to near zero at 650 nm before increasing slightly at 676 nm and 715 nm.

Figure 7.1 (middle) shows the result of adding a 0.2 \(\mu\)m capsule filter to the ac9+, therefore reading only the dissolved components of the water column [Nybakk, 1997; Zubkov et al., 2000]. This plot took much the same form as the total absorption plot, figure 7.1 (top), with the peak in the blue (absorption at 412 nm of 0.14 m\(^{-1}\)) again decreasing to nearly zero at 650 nm before increasing slightly. It took much of the expected form of CDOM absorption (high absorption in the blue dropping to very low in the red) but the increase at 715 nm was not expected. A possible reason for this increase was that very small particles (<0.2 \(\mu\)m) could pass through the filter. These particles would not be photoactive organisms, but could result in an increase in attenuation through both absorption and scattering at this wavelength. Another possible reason for this is that absorption measures are highly affected in the near infra red and so this increase may be
Figure 7.1: Reconstructed absorption spectrum from the fmax of CTD 66 (a South Atlantic Gyre Station) AMT 13 ac9+ data. Circles show position of measured wavelength for (top) total absorption, (middle) CDOM absorption and (bottom) particulate absorption.
as a result of temperature affects not being fully constrained.

Figure 7.1 (bottom) shows the result of subtracting the CDOM absorption spectrum from the total absorption. It shows the absorption spectrum of the particulate fraction of the water column, mainly from phytoplankton pigments in case I waters [Nelson et al., 1993]. There were two distinct peaks, one with a maximum at 440nm representing the total pigment load of the sample and a smaller second peak at 676nm representing the chlorophyll $a$ in the sample [Staehr et al., 2004]. These values were nearly an order of magnitude lower than the dissolved fraction, with the peak at 440nm being $0.017m^{-1}$ and at 676nm being $0.004m^{-1}$, and took on the expected form for a particulate spectrum [Kirk, 1994].

7.1.1 Depth Profiles

Figure 7.2 shows depth profiles of two ac9+ absorption wavelengths together with the ancillary data of PAR (light level) and FRRF $F_m$ (equates to chlorophyll concentration). Further examples of these profiles are shown in appendix I and appendix J. The CDOM absorption was particularly strong at short wavelengths and so a plot of the absorption at 412nm ($a_{412}$) was used to represent this (top left, figure 7.2). This showed a exponential increase with depth from near zero at the surface to $0.13 \text{ m}^{-1}$ at 100m. This pattern can be explained together with the PAR measurement (figure 7.2, top right) because, as CDOM absorbs light, it is photo-oxidised to a non-absorbent species. Consequently the low level of absorption at the surface increased with depth as light decreased [Miller, 1994].

Figure 7.2 (bottom left) shows the result of subtracting the $0.2\mu m$ capsule filtered absorption at 676nm from the unfiltered value. This gives the particulate absorption at 676nm, an absorption wavelength diagnostic of chlorophyll $a$ in vivo. The figure 7.2 (bottom) plots demonstrate the clear link between particulate $a_{676}$ and $F_m$. The $F_m$ peaked at 120m while $a_{676}$ rose from $0.002m^{-1}$ to $0.008m^{-1}$ at the same depth indicating that $a_{676}$ can be used as a measure of $F_m$ and chlorophyll $a$.

Figure 7.3 shows a full ac9+ particulate spectrum cast (all 9 wavelengths and all depths), and therefore the absorption of all particulates and pigments in the water column. Figure 7.3 (top) clearly shows the overlying mixed layer with low absorption at the 440nm peak and very low absorption at 676nm. Absorption at all wavelengths decreased at the 70m fluorescence maximum and dropped to near zero below the fmax indicating a lack of pigments.
Figure 7.2: ac9+ derived absorption and ancillary profiles from CTD 62 of AMT 14 (a North Atlantic Subtropical Gyre station). Top left shows CDOM (filtered) absorption at 412nm compared to the Photosynthetically Active Radiation (top right). Bottom left shows the particulate (total - filtered) absorption at 676nm compared to FRRF $F_m$ that equates to chlorophyll (bottom right).
Figure 7.3: Full section of ac9+ derived particulate absorption from AMT 13 CTD 66 (a South Atlantic Gyre station) in 2D (top) and 3D (bottom) showing high absorbance at all wavelengths at the fluorescence maximum with an overlying region of low absorbance in the surface mixed layer.
Figure 7.3 lower shows the same data in three dimensions. The surface data shows a pronounced peak at 440nm and a much less clear peak at 676nm where pigment concentration would have a lower influence [Lalli and Parsons, 1997]. This shape remained the same throughout the water column and became much more distinct at the 70m fmax.

7.1.2 Absorption Linearity

Figure 7.4 shows direct comparisons between wavelengths associated with different pigments or groups of pigments for AMT 13 (top) and AMT 14 (bottom). As discussed in section 2.8.1, a676 is diagnostic of chlorophyll a while a440 and a488 are diagnostic of Tpig and TAC load respectively [Staehr et al., 2004]. Also, a676 is generally lower than a488 which in turn is always lower than a440. The measurements shown in chapter 6 (from HPLC) data suggested that when a440 and a488 are plotted against a676 a linear correlation would be expected. Figure 7.4 (top), together with the equation parameters in table 7.1, clearly showed this linearity for AMT 13 data with a high r² value of 0.88 for both a440 and a488. On AMT 14 (figure 7.4 bottom) linearity was again evident with r² values of 0.57 and 0.64 for a440 and a488 respectively. A much larger number of data points were collected on this cruise than on AMT 13, with the data concentrated at lower absorptions. Some of the higher absorption AMT 14 data showed much greater standard deviations than that from AMT 13. In general, the linearity of these plots mirrored closely the HPLC data (see section 6.3), implying a good correlation between the absorption at specific wavelengths and the pigments they were used to represent.

7.1.3 Band Ratios

Figure 7.5 shows relationships between the absorption wavelengths representing the pigments and pigment ratios discussed in chapter 6 with a676:a440 representing TChla:Tpig, a676:a488 representing TChla:TAC and a676 representing TChla.

In figure 7.5 a676:a488 was greater than a676:a440, since a440 was greater than a488. For both AMT 13 (figure 7.5, top) and AMT 14 (figure 7.5, bottom) a positive log linear relationship is shown. For AMT 13 the r² values (0.84 and 0.78 for a676:a440 and a676:a488 respectively) were higher than for AMT 14 (0.60 and 0.72 for a676:a440 and a676:a488 respectively) due to the higher error levels in the AMT14 dataset described in section 7.1.2. Again the results supported the hypothesis [Staehr et al., 2004] that these data represent specific pigments and pigment groups.

Figure 7.5 shows a676 increasing in the proportion of a676:a440 and a676:a488 as a676
Figure 7.4: Absorption correlations between $a_{676}$ (chlorophyll $a$) and, left, $a_{440}$ (total pigment) and right, $a_{488}$ (total carotenoids) on top, AMT 13 ($a_{440}$ $y = 2.11x + 0.01$, $r^2=0.88$, $a_{488}$ $y = 1.75x + 0.01$, $r^2=0.88$) and, bottom, AMT 14 ($a_{440}$ $y = 2.04x + 0.01$, $r^2=0.57$, $a_{488}$ $y = 1.62x + 0.01$, $r^2=0.64$).
increases and therefore takes the same form as that of the pigment ratio data described in section 6.3. This parallels the increase in TChla:Tpig as TChla increases.

### 7.1.4 Area Linearity

Since the absorption spectra of carotenoids vary depending on their role [Kirk, 1994], a second methodology was used in which the absorption spectrum was constructed in a series of trapezia between ac9+ wavelengths. The total area of the spectrum was used as a proxy for Tpig and the area resulting from the absorption at 676nm multiplied by the spectral half width of the red peak of chlorophyll a (9nm) was used as a proxy for chlorophyll a concentration, creating a comprehensive determination of total pigment in a single measure. The error associated with these measurements was higher, since the individual band error from direct measurement was compounded by the multiplication of several bands together. Overall, the absorption of all wavelengths gave a more holistic...
Table 7.1: Linear correlations between ac9+ absorption variables in the form $y = \gamma(x) + \alpha$.

<table>
<thead>
<tr>
<th>$x$ axis</th>
<th>$y$ axis</th>
<th>Cruise</th>
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<th>$\alpha$</th>
<th>$r^2$</th>
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<tbody>
<tr>
<td>a676</td>
<td>a440</td>
<td>13</td>
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<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>2.04</td>
<td>0.01</td>
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<tr>
<td>a488</td>
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<td>0.01</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>1.62</td>
<td>0.01</td>
<td>0.64</td>
</tr>
<tr>
<td>Chla</td>
<td>Both</td>
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<td>0.02</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Tpig</td>
<td>Both</td>
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<td>0.03</td>
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<tr>
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<td>0.02</td>
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</tr>
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<td>Both</td>
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<td>0.02</td>
<td>0.12</td>
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<tr>
<td>- $F_{max}$</td>
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<td>0.72</td>
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<td></td>
</tr>
<tr>
<td>a440</td>
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<td>0.03</td>
<td>0.78</td>
</tr>
<tr>
<td>- Surface</td>
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<td>0.02</td>
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</tr>
<tr>
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</tr>
<tr>
<td>$\beta_{\text{Chla}}$</td>
<td>$\beta_{\text{Tpig}}$</td>
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<td>8.15</td>
<td>1.25</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>5.59</td>
<td>1.05</td>
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</tr>
<tr>
<td>$F_m$</td>
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<td>1.29</td>
<td>0.44</td>
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<tr>
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<td>1.49</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>- $F_{max}$</td>
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<td>0.32</td>
<td>0.2</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>$\beta_{\text{Tpig}}$</td>
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<td>Both</td>
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<td>7.87</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both</td>
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</tr>
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<td>7.1</td>
<td>0.28</td>
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<td>1.93</td>
<td>1.1</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>

measurement of total pigment absorption, so although these errors may have been higher, the accuracy of the measurement was also higher. However, since chlorophyll $a$ absorbs not only at 676nm but also in the main Soret band at 440nm (see figure 2.7) [Aiken, 2001, Falkowski and Kiefer, 1985], this absorption measure is not a total chlorophyll $a$ absorption, but rather a constant proportion of that total.

Figure 7.6 shows the linearity of $a_{\text{Chla}}$ compared to $a_{\text{Tpig}}$ as calculated by spectral area. The data showed a linear relationship for both cruises with $r^2$ values of 0.93 and 0.96 for AMTs 13 and 14 respectively. As with the band absorption measure, this methodology showed strong linear correlation between $a_{\text{Chla}}$ and $a_{\text{Tpig}}$. Also, the equation parameters in table 7.1 showed that, between cruises, this measure was more consistent than was the band ratio methodology.

7.1.5 Spectral area ratios

Figure 7.7 shows the pigment ratio proxy from spectral area of ac9+ data versus the $a_{\text{Chla}}$. Again, the calculations of spectral area compounded with the ratio calculation meant that the error associated with some of these measurements was high, particularly
Figure 7.6: Spectral area of ac9+ total pigment absorption vs chlorophyll a absorption for AMT 13 (top, $y = 8.15x + 1.25$, $r^2=0.93$) and AMT 14 (bottom, $y = 5.59x + 1.05$, $r^2=0.96$).
Table 7.2: Log linear correlations between chlorophyll $a$ absorption measures and absorption ratios in the form $y = \gamma \ln(x) + \alpha$.

<table>
<thead>
<tr>
<th>x axis</th>
<th>y axis</th>
<th>Cruise</th>
<th>$\gamma$</th>
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<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_{676}$</td>
<td>$a_{676}:a_{440}$</td>
<td>13</td>
<td>0.81</td>
<td>0.11</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
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<td>0.72</td>
</tr>
<tr>
<td>$a_{676}:a_{488}$</td>
<td>13</td>
<td>1.02</td>
<td>0.14</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>1.11</td>
<td>0.14</td>
<td>0.60</td>
</tr>
<tr>
<td>$a_{Chla}$</td>
<td>$a_{Chla}:A_{pig}$</td>
<td>13</td>
<td>0.11</td>
<td>0.03</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>0.13</td>
<td>0.27</td>
<td>0.78</td>
</tr>
</tbody>
</table>

in some AMT 14 data points. The equivalent HPLC data for absorption ratios suggested that these plots should form positive log linear relationships, and in fact both AMT13 and AMT14 (figure 7.7) did indeed show this correlation with $r^2$ values of 0.78 and 0.83 respectively. Again, the high $r^2$ values associated with these data and their similarity to the concurrent HPLC data suggested that spectral area calculated in this way could be used as a proxy for pigment data. There was also a similarity in several of the regression parameters (table 7.2).

### 7.2 Discussion

The collection of in situ particulate absorption data required the use of novel methodologies to remove the effect of dissolved components from the total absorption. The general form of absorption plots fitted well with the expected form of CDOM and in vivo particulate spectra. CDOM absorption profiles showed increasing absorption with depth at all wavelengths, and concurrent light intensity data suggested that these data could potentially be used as a quantitative measure of CDOM. Depth profiles of particulate absorption also paralleled measurements of pigments taken using the FRRF, giving a preliminary indication that these parameters may correlate. Taken as a whole the particulate absorption varied as would be expected through the water column while retaining the distinctive pigment spectral form. At all wavelengths distinct increases in absorption were shown at the fmax and dropped to near zero below this region.

In oligotrophic waters the measurements were taken at the detection limits of the ac9+ instrument and as such blanking methodologies were important [Wetlabs inc, 2004b, Cullen and Davis, 2003] requiring corrections during processing due to changes in salinity and temperature [Wetlabs inc, 2004b]. The more complex methodologies (using temperature and salinity data from the CTD) used to correct for scattering in absorption data
Figure 7.7: Spectral area of ac9+ pigment ratios of total pigment:chlorophyll $a$ absorption vs chlorophyll $a$ absorption for AMT 13 (top, $y = 0.11\ln(x)+0.03$, $r^2=0.83$) and AMT 14 (bottom, $y = 0.13\ln(x)+0.27$, $r^2=0.78$).
resulted in large negative absorptions and therefore proved to be ineffective. However, the simpler method (using 715nm as a zero absorption band [Varela et al., 1998]) resulted in consistently acceptable absorption spectra for both the dissolved and particulate fractions. Problems also arose in these waters with calibration drift, but these were overcome to some extent by the use of the double cast system.

Investigation of wavelength absorption specific to pigment and pigment group was used to test the hypothesis that these data could be used as a proxy for HPLC pigment concentrations. When bands specific to total pigment and total carotenoid concentrations were plotted against the band for chlorophyll \( a \), linearity was evident for both cruises (AMTs 13 and 14) and the relationships were also similar to each other. Further, when spectral area was used instead of band ratios, this linearity described even more of the variance in the data, and the results obtained by plotting these bands and spectral areas directly against the concentration of these pigments supported the hypothesis that these data could be used as proxies for pigment concentrations. In the case of chlorophyll concentration, the area and band measurements showed the same \( r^2 \) values (these two datasets were directly comparable).

The plotting of absorption ratios against the absorption band of chlorophyll \( a \) showed clear conformity to a positive log linear relationship as did the relationship based on spectral area. The data of AMT 14 showed considerable noise in this dataset but even so the variance described by the regression was still high. Again the relationships between AMTs 13 and 14 were similar, and the ratios based on spectral area showed a better fit to the relationship than data taken from band ratios.

7.2.1 ac9+ Sources of Error

The measurement of \textit{in situ} particulate absorption was subject to a number of errors, some of which remained unconstrained in this investigation. As the particulate absorption spectra were dependent on two casts there was a possibility that the water column may have changed in between the two casts. This could be particularly true of measurements at the \( f_{\text{max}} \), a region that can be compressed into a relatively thin layer. To reduce the impact of this, the data around the \( f_{\text{max}} \) were averaged over a 5 metre bin.

When initially examined, prior to determining the particulate spectrum, the total absorption of the sample did not result in a log linear relationship. This suggested that no systematic error was in fact present, since a constant added to the denominator of a fraction of a linear relationship produces a log linear relationship rather than a linear
relationship. In addition, subtraction of the dissolved absorption spectrum from the total absorption spectrum should remove systematic errors as they would be present in both datasets and so cancel each other out.

The ac9+ was not calibrated between cruises and therefore the calibration may have shifted. Any systematic error should be cancelled out again by the subtraction, but scaling errors might not be. The similarity in data between cruises suggested this was not the case, however this remains an unknown error.

7.2.2 Conclusions

In this chapter it has been demonstrated that:

- absorption measurements specific to pigments showed linear relationships and ratios of these pigments followed a log linear relationship with the absorption of chlorophyll a.

- pigment specific absorptions and their ratios were directly correlated and as such these absorption measures could be used as measures of pigments.
Chapter 8

Cross-correlation of primary production measures

Chapter 8 aims to develop correlations between the three methodologies that in chapters 5 to 7 were used to examine primary production parameters, demonstrating log linear relationships between 'production' measures or 'ratios' and 'pigment' parameters. It is hypothesised that $F_m$ [Laney, 2002], chlorophyll $a$ concentration [Trees et al., 2000] and $a_{676}$ [Sokoletsky et al., 2003] could all be representative of 'pigment' in the sample and that $F_{m}', \sigma_{PSII}$ [Kolber and Falkowski, 1993], TChla:Tpig [Aiken et al., 2004], $a_{676}:a_{440}$, $a_{676}:a_{444}$ and $a_{chla}:a_{Tpig}$ [Margalef, 1967] could be representative of a measure of 'production'. In order to test the hypothesis that these methods are measurements of the same parameters, correlations between these parameters were examined.

8.1 HPLC and FRRF parameters

The relationship between FRRF $F_m$ as a measure of chlorophyll $a$ was explored in chapter 5. Subsequently in chapter 6 log linear relationships between pigment ratios determined by HPLC and TChla were shown similar to those that had been demonstrated in chapter 5 between chlorophyll $a$ and $F_{m}'. The form of the relationships shown in chapters 5 and 6 would suggest a positive linear correlation between pigment ratios and $F_{m}'. Although it had been suggested that a linear negative relationship would be expected with $\sigma_{PSII}$ [Aiken et al., in press], the correlations in chapter 5 suggest a possible positive linear correlation between pigment ratios and $\sigma_{PSII}$. Figures 8.1 to 8.3 show the correlations for cruises AMTs 12, 13 and 14 respectively with correlation parameters shown in table 8.1.
Table 8.1: Linear relationships between TChla:Tpig HPLC data and FRRF production variables in the form $y = \gamma(x) + \alpha$.

<table>
<thead>
<tr>
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<th>y axis</th>
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<th>$\alpha$</th>
<th>$\gamma$</th>
<th>$r^2$</th>
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</thead>
<tbody>
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<td>TChla:Tpig</td>
<td>$\frac{F_{m}}{F_{m}^{c}}$ Surface</td>
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<td>0.191</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1.88</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>0.36</td>
<td>0.41</td>
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</tr>
<tr>
<td></td>
<td>$\frac{F_{m}}{F_{m}^{c}}$ fmax</td>
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<td>0.13</td>
<td>0.74</td>
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</tr>
<tr>
<td></td>
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<td>0</td>
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<td>0.066</td>
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<tr>
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<td>294</td>
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</tr>
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</table>

The correlations obtained on AMTs 12 and 14 were relatively similar for the surface water as shown in table 8.1. Data from the fmax showed positive relationships between pigment ratios (TChla:Tpig) and $\frac{F_{m}}{F_{m}^{c}}$. $\sigma_{PSII}$ also showed a weak positive relationship in both of these layers. However, in AMT 13 data the pattern was different. Although both layers showed positive correlations between pigment ratios and $\frac{F_{m}}{F_{m}^{c}}$, these had a much higher gradient than in AMTs 12 and 14. Interestingly, $\sigma_{PSII}$ showed a weak positive correlation in the fmax but a high gradient negative correlation in the surface waters. As was noted in chapter 5, the FRRF data from all cruises and particularly AMT 13 were very noisy and as a result some of the $r^2$ values for these relationships were low (dropping to below 0.1). In many cases it was therefore difficult to draw definite conclusions from the FRRF correlations. However, it did appear that there was a general positive correlation between $\frac{F_{m}}{F_{m}^{c}}$ and TChla:Tpig and between $\sigma_{PSII}$ and TChla:Tpig.

8.2 ac9+ data with HPLC and FRRF parameters

The low number of ac9+ particulate spectrum profiles taken on AMT 13 meant that only 3 data points were concurrent with viable FRRF profiles. The AMT 13 and 14 datasets were therefore amalgamated for the following analysis.

8.2.1 ac9+ correlations with HPLC

In chapter 7 it was demonstrated that, in accordance with the findings of Sokolentsky et al. [2003], absorption relationships derived from ac9+ data closely followed those de-
Figure 8.1: Correlations in AMT 12 data between FRRF $\frac{F_{FD}}{F_{MD}}$ (top) and $\sigma_{PSII}$ (bottom) with HPLC pigment ratios (TChla:Tpig) in left, surface waters ($y = 0.1912x + 0.3309$, $r^2=0.0328$ and $y = 0.74x + 0.13$, $r^2=0.27$) and right, fmax ($y = 451.79x + 284$, $r^2=0.34$ and $y = -664x + 899$, $r^2=0.10$).
Figure 8.2: Correlations in AMT 13 data between FRRF $\frac{F_{\text{F}} F_{\text{P}}}{F_{\text{mP}}}$ (top) and $\sigma_{\text{PSII}}$ (bottom) with HPLC pigment ratios (TChla:Tpig) in left, surface waters ($y = 1.8831x - 0.4759$, $r^2 = 0.69$ and $y = -0.004x + 0.42$, $r^2 = 0$) and right, fmax ($y = -2400x + 1872$, $r^2 = 0.5$ and $y = 92.03x + 494$, $r^2 = 0.03$).
Figure 8.3: Correlations in AMT 14 data between FRFF $\frac{F_{gD}}{F_{\text{mD}}}$ (top) and $\sigma_{PSII}$ (bottom) with HPLC pigment ratios (TChla:Tpig) in left, surface waters ($y = 0.41x + 0.36$, $r^2=0.5$ and $y = 0.066x + 0.47$, $r^2=0.16$) and right, fmax ($y = -198.4x + 481$, $r^2=0.05$ and $y = 294x + 450$, $r^2=0.23$).
rived from HPLC pigment data. However, in order to show that they are proxies for each other, direct comparisons were made. Figure 8.4 shows plots of ac9+ band absorption measurements against concurrent HPLC pigment measurements. The closed circles in figure 8.4, top plot, show the correlation of a\(_{676}\) with HPLC TChla. A positive linear correlation between these datasets would be expected [Sokoletsy et al., 2003]. Although most of these data were clustered at low TChla concentrations, the majority of the data points did indeed conform to linearity and the regression accounted for 81% of the variance as can be seen in table 8.2. The open circles in figure 8.4 show the same relationship between a\(_{440}\) and the Tpig. Here again, although there were a small number of outliers, a clear positive linear relationship was present, the r\(^2\) value of this relationship being 0.77.

A plot of a\(_{r\text{Chla}}\) versus TChla is not shown as the absorption data would take on the same form as the a\(_{676}\) versus TChla regression because it is the same data multiplied by a factor. Figure 8.4, (bottom), shows the spectral area of Tpig versus HPLC Tpig, again demonstrating linearity between these two factors (87% of the variance is accounted for).

These plots supported the use of absorption (at these specific wavelengths) to measure pigments and indicated that further examination of pigment ratios relative to absorption ratios was worthwhile. As was shown in figure 8.4 and table 8.2, the use of spectral areas, where available, resulted in a more robust relationship than absorption bands. Spectral area relationships accounted for a larger proportion of the variance in the dataset and therefore were a better proxy for pigment measurements.

### 8.2.2 Absorption ratios and HPLC pigment ratios

Figure 8.5 and table 8.3 show the correlation between band absorption ratios and HPLC derived pigment ratios. Since the data described in section 8.2.1 supported the correlation of pigments to absorption at diagnostic wavelengths, the ratios would be expected to show a positive linear relationship. Although this was the case, the increase in error resulting from the production of ratios meant that regressions of a\(_{676}:a_{440}\) and a\(_{676}:a_{488}\) versus TChla:Tpig were weak (r\(^2\) values of 0.31 and 0.30 respectively).

Figure 8.6 shows the spectrally reconstructed absorption ratio from the ac9+ data plotted against HPLC pigment ratios. Unlike previous relationships, in which area derived measures of pigments showed significantly improved regressions to band methodologies, the r\(^2\) value of this relationship (at 0.27) was slightly less than that for the band ratio methods. Although this indicated that pigment ratios could be measured using band absorption ratios, the variance explained by this relationship is comparatively low.
Figure 8.4: Correlations of $a_{676}$ with chlorophyll $a$ (top, closed circles, $y = 0.05x + 0.02$, $r^2=0.81$) and $a_{440}$ with chlorophyll $a$ (top, open circles, $y = 0.04x + 0.03$, $r^2=0.77$) on the combined AMT 13 and 14 dataset and of $a_{Tpig}$ with HPLC Tpig (bottom, $y = 8.1x+7.87$, $r^2=0.87$).
Table 8.2: Linear relationships between absorption measures and FRRF $F_m$ in the form $y = \gamma(x) + \alpha$.

<table>
<thead>
<tr>
<th>x axis</th>
<th>y axis</th>
<th>Cruise</th>
<th>$\alpha$</th>
<th>$\gamma$</th>
<th>$\gamma^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_{676}$</td>
<td>Chla</td>
<td>Both</td>
<td>0.02</td>
<td>0.05</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Tpig</td>
<td>Both</td>
<td>0.03</td>
<td>0.04</td>
<td>0.77</td>
</tr>
<tr>
<td>$F_m$</td>
<td>Both</td>
<td>0.02</td>
<td>0.0035</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>- Surface</td>
<td>Both</td>
<td>0.02</td>
<td>0.0049</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>- $F_{max}$</td>
<td>Both</td>
<td>0.00</td>
<td>0.006</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>$a_{440}$</td>
<td>$F_m$</td>
<td>Both</td>
<td>0.03</td>
<td>0.007</td>
<td>0.78</td>
</tr>
<tr>
<td>- Surface</td>
<td>Both</td>
<td>0.02</td>
<td>0.018</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>- $F_{max}$</td>
<td>Both</td>
<td>0.01</td>
<td>0.010</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>$a_{Chla}$</td>
<td>$a_{Tpig}$</td>
<td>13</td>
<td>1.25</td>
<td>8.15</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>1.05</td>
<td>5.59</td>
<td>0.96</td>
</tr>
<tr>
<td>$F_m$</td>
<td>Both</td>
<td>1.29</td>
<td>0.18</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>- Surface</td>
<td>Both</td>
<td>1.49</td>
<td>0.27</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>- $F_{max}$</td>
<td>Both</td>
<td>0.20</td>
<td>0.32</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>$a_{Tpig}$</td>
<td>Tpig</td>
<td>Both</td>
<td>7.87</td>
<td>8.10</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.68</td>
<td>1.18</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.10</td>
<td>2.42</td>
<td>0.28</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>1.10</td>
<td>1.93</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>

Again, the main reason for this was that errors were compounded by the creation of spectral areas and by the process of producing ratios.

### 8.2.3 Absorption ratios and FRRF

The mechanism resulting in the correlation of FRRF data to HPLC pigments was suggested by Aiken et al. [2004] as a causative factor in determining primary production parameters as the pigment complement of the antenna structure of PSII impacts the absorption of light by the photosystems and the transfer of energy within the photosystem. However, in order for this to be used as a direct measure, correlations between absorption and primary production parameters are necessary. Direct correlations between FRRF parameters with ac9+ absorption, band ratios, and area ratios were therefore made. Figure 8.7 shows direct correlations between ac9+ $a_{440}$, $a_{676}$, $a_{TPig}$, and $a_{Chla}$ with FRRF $F_m$. These are all measures of pigment concentration and therefore would be expected to show positive linear correlations [Sokoltsky et al., 2003, Laney, 2002]. In fact, all of these measures showed a similar pattern to those reported in chapter 5 (when comparing $F_m$ with HPLC pigments).

There appeared to be two distinct populations in the dataset (both with linear relationships), separated according to their position in the water column: whether surface
Table 8.3: Linear relationships between $\text{a}_{\text{Chla}}:\text{a}_{\text{Tpig}}$ ac9+ data and FRRF production variables in the form $y = \gamma(x) + \alpha$.

<table>
<thead>
<tr>
<th>x axis</th>
<th>y axis</th>
<th>Cruise</th>
<th>$\alpha$</th>
<th>$\gamma$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_{676:440}$</td>
<td>TChla:Tpig</td>
<td>Both</td>
<td>0.48</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>$\frac{F_{\text{m}}}{F_{\text{m}}}$</td>
<td>Both</td>
<td>1.6</td>
<td>-0.1</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>- Surface</td>
<td>Both</td>
<td>-0.54</td>
<td>0.95</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>- Fmax</td>
<td>Both</td>
<td>0.85</td>
<td>0.35</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>$\sigma_{\text{PSII}}$</td>
<td>Both</td>
<td>0.97</td>
<td>-0.005</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>- Surface</td>
<td>Both</td>
<td>0.94</td>
<td>-0.0004</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>- Fmax</td>
<td>Both</td>
<td>0.07</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$a_{676:488}$</td>
<td>TChla:Tpig</td>
<td>Both</td>
<td>0.59</td>
<td>0.4</td>
<td>0.31</td>
</tr>
<tr>
<td>$\frac{F_{\text{m}}}{F_{\text{m}}}$</td>
<td>Both</td>
<td>0.65</td>
<td>0.23</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>- Surface</td>
<td>Both</td>
<td>-0.25</td>
<td>0.89</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>- Fmax</td>
<td>Both</td>
<td>0.8</td>
<td>0.35</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>$\sigma_{\text{PSII}}$</td>
<td>Both</td>
<td>0.1</td>
<td>-0.0004</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>- Surface</td>
<td>Both</td>
<td>0.88</td>
<td>-0.0002</td>
<td>0.09</td>
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</tr>
<tr>
<td>- Fmax</td>
<td>Both</td>
<td>0.74</td>
<td>-0.00006</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$a_{\text{Chla}:\text{Tpig}}$</td>
<td>TChla:Tpig</td>
<td>Both</td>
<td>0.16</td>
<td>0.058</td>
<td>0.27</td>
</tr>
<tr>
<td>$\frac{F_{\text{m}}}{F_{\text{m}}}$</td>
<td>Both</td>
<td>0.18</td>
<td>0.09</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>- Surface</td>
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<td>-0.11</td>
<td>0.23</td>
<td>0.5</td>
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</tr>
<tr>
<td>- Fmax</td>
<td>Both</td>
<td>0.1</td>
<td>0.13</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>$\sigma_{\text{PSII}}$</td>
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<td>0.2</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
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<td>-2.5x10^{-5}</td>
<td>0.2</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>- Fmax</td>
<td>Both</td>
<td>-1.5x10^{-5}</td>
<td>0.18</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>
Figure 8.5: Correlations of $a_{676}:a_{440}$ (top, $y = 0.5x + 0.48$, $r^2 = 0.3$) and $a_{676}:a_{488}$ (bottom, $y = 0.4x + 0.59$, $r^2 = 0.31$) with HPLC TChla:Tpig on the combined AMT 13 and 14 dataset.
Figure 8.6: Correlation of $a_{TChla}:a_{Tpig}$ and with HPLC TChla:Tpig on the combined AMT 13 and 14 dataset, $y = 0.058x + 0.16$, $r^2=0.27$. 
mixed layer or fmax. This suggested that the dataset populations may have arisen from
taxonomic variation (described in chapter 5); they were separated at low and high $F_m$,
suggesting different responses to FRRF excitation. When these data were considered as
a single relationship, the $r^2$ values varied between 0.44 and 0.78 (see table 8.2). However,
when separated into low $F_m$ populations (surface mixed layer data with $F_m$ values of
less than 2) and high $F_m$ populations ($F_m$ values of greater than 2 originating from the
fluorescence maximum) then two distinct patterns emerged. The $r^2$ values relating to
the low $F_m$ population varied between 0.12 and 0.52, whereas the $r^2$ values relating to
the high $F_m$ population were 0.72 and 0.89. These were distinctly different regressions
as can be seen from the parameters in table 8.2.
Figure 8.8: Correlations in the surface mixed layer of FRRF $F_m$ with absorption measures, top $a_{676}$ (left, $y = 0.0049x + 0.02$, $r^2=0.12$) and $a_{440}$ (right, $y = 0.018x + 0.02$, $r^2=0.52$), bottom chlorophyll a absorption (left, $y = 0.27x + 1.49$, $r^2=0.14$) and total pigment absorption (right, $y = 2.42x + 7.1$, $r^2=0.28$) on the combined AMT 13 and 14 dataset.
Figure 8.9: Correlations in the fmax of FRRF $F_m$ with absorption measures, top $a_{676}$ (left, $y = 0.006x + 0$, $r^2=0.72$) and $a_{440}$ (right, $y = 0.01x + 0.01$, $r^2=0.89$), bottom chlorophyll $a$ absorption (left, $y = 0.32x + 0.2$, $r^2=0.72$) and total pigment absorption (right, $y = 1.93x + 1.1$, $r^2=0.79$) on the combined AMT 13 and 14 dataset.
8.2.4 Absorption ratio correlation with $\frac{F_{m}}{F_{m}}$ and $\sigma_{PSII}$

Chapter 5 demonstrated the positive log linear relationship between $\frac{F_{m}}{F_{m}}$ with $F_{m}$ and the pigment concentration. The positive correlations between $a_{676}$, pigment load and $F_{m}$ (shown in section 8.2.3), along with the positive log linear relationship with absorption (shown in sections 7.1.3 and 7.1.5), therefore suggested that $\frac{F_{m}}{F_{m}}$ should co-vary linearly with absorption ratios. Data presented in figure 8.10 showed that a clear positive linearity was in fact present ($r^2$ values of 0.37 and 0.20). Figure 8.13 (top) also showed a linear relationship for the spectral area $\alpha_{F_{m}}:\alpha_{Trig}$ ($r^2$ of 0.23). These relationships again showed low $r^2$ values since both the $x$ and $y$ terms of the equations showed large random error. This, combined with the low dynamic variability of the dataset (which is based only on gyre data), resulted in weak correlations (see table 8.3).

As a result of splitting these data between surface and $f_{max}$ (in the same way as for the measurements of $F_{m}$), a clearer picture developed. For $a_{676}:a_{440}$, $a_{676}:a_{488}$ (figure 8.12) and $a_{Trig}:a_{Trig}$ (figure 8.14, top) in the surface waters the $r^2$ value remained similar at 0.14, 0.04 and 0.50 respectively. All of these data showed a negative gradient (see table 8.3). However, in the $f_{max}$ the same relationships showed positive correlations ($r^2$ values of 0.28, 0.36 and 0.28 respectively), and a considerable increase for the $r^2$ value of the dataset taken as a whole as shown in figures 8.12 and 8.14.

As discussed in chapter 5, FRRF measurements of $\sigma_{PSII}$ did not give the expected results and patterns were not well defined. In these data a negative log linear relationship was expected with $F_{m}$ and $T_{Chla}$ [Aiken et al., in press], and although this appeared to be the case the relationship was not clear. It is therefore surprising that a clear relationship was found between $\sigma_{PSII}$ and absorption ratios. Figure 8.11 shows this for $a_{676}:a_{488}$ and $a_{676}:a_{488}$ (top and bottom plots respectively) with $r^2$ values of 0.34 and 0.26 respectively. The plot using $a_{Chla}:a_{Trig}$ (figure 8.13) again shows the relationship, although less clearly, returning an $r^2$ value of 0.21 for a negative linear relationship. The splitting of these data (as undertaken for $\frac{F_{m}}{F_{m}}$) resulted in a significant breakdown of these relationships (figure 8.14, bottom), since the decrease in dynamic range made any relationship less apparent.

8.2.5 Discussion

The correlations shown in this chapter were, as expected, generally weak as a result of the inherent noise resulting from instruments operating near their detection limits. FRRF data were particularly affected by the noise and therefore any correlations shown must
Figure 8.10: Correlations of $a_{476}:a_{440}$ (top, $y = 0.16x - 0.1$, $r^2=0.37$) and $a_{676}:a_{488}$ (bottom, $y = 1.59x - 0.01$, $r^2=0.44$) with $F_v/F_m$ on the combined AMT 13 and 14 dataset.
Figure 8.11: Correlations of $a_{676}:a_{440}$ (top, $y = -0.46x + 0.95$, $r^2=0.07$) and $a_{676}:a_{488}$ (bottom, $y = -0.23x + 0.91$, $r^2=0.04$) with $\sigma_{PSII}$ on the combined AMT 13 and 14 dataset.
Figure 8.12: Correlations of $a_{676:a_{440}}$ (left) and $a_{676:a_{488}}$ (right) with $F_v/F_m$ split between the surface mixed layer (top, $y = -0.54x + 0.94$, $r^2=0.14$ and $y = -0.25x + 0.89$, $r^2=0.04$) and $f_{max}$ (bottom, $y = 0.85x + 0.23$, $r^2=0.28$ and $y = 0.8x + 0.35$, $r^2=0.36$) on the combined AMT 13 and 14 dataset.
Figure 8.13: Correlations of $a_{Chla}$ vs. $a_{Tpig}$ with $F_v/F_m$ (top, $y = 0.18x + 0.09$, $r^2=0.23$) and $\sigma_{PSII}$ (bottom, $y = -5.8 \times 10^{-5}x + 0.20$, $r^2=0.21$) on the combined AMT 13 and 14 dataset.
Figure 8.14: Correlations of $a_{\text{Chla}}$ with $F_j$ (left) and $\sigma_{\text{PSII}}$ (right) split between the surface mixed layer (top, $y = -0.11x + 0.23$, $r^2=0.50$ and $y = -2.5\times10^{-5}x + 0.2$, $r^2=0.14$) and $f_{\text{max}}$ (bottom, $y = 0.09x + 0.13$, $r^2=0.20$ and $y = -1.4\times10^{-5}x + 0.18$, $r^2=0.01$) on the combined AMT 13 and 14 dataset.
be considered qualitative. The absorption data were taken exclusively from the gyres (so as to prevent clogging of the filters used on the ac9+ in more productive waters), which were the regions where measurements were most problematic for the FRRF [Chelsea Instruments Ltd, 1998]. The low dynamic range that resulted from measurements being taken from the homogenous oligotrophic gyres also meant that regressions were weaker than would have been the case if a greater range of data had been available. Also, as discussed in section 4.6, a low degree of explained variance would be expected since these ratios are measurements of deviation from linearity between the numerator and the denominator. On the basis that the linearity in these relationships accounted for around 97% of variance, as was the case for pigment linearity (see section 6.3 in chapter 6), ratios were accounting for a small deviation from this linearity. Therefore, the noise as well as the small variation partially accounted for the relatively weak relationships between these ‘production’ parameters.

Direct comparison of absorption and HPLC data with FRRF data suggested two distinct populations in the FRRF data. Both $F_m$ and $\frac{E_\alpha}{F_m}$ showed distinct populations for low and high chlorophyll a and $\alpha_{TChla}$ values which, because of their varying phytoplankton communities, split into the surface mixed layer and fmax datasets. $F_m$ showed clear linearity in both of these populations, but different gradients. However, while $\frac{E_\alpha}{F_m}$ had an expected positive correlation in the fmax, it showed a distinct negative correlation with absorption band and spectral area ratios in the surface mixed layer.

Given the lack of relationship apparent in $\sigma_{PSII}$ data with HPLC pigments (chapter 5) and the positive correlation shown between HPLC TChla:Tpig shown in this chapter, it was surprising that both band and area absorption ratios in fact showed a clear negative linear correlation with $\sigma_{PSII}$. As opposed to the situation with $F_m$ and $\frac{E_\alpha}{F_m}$, splitting these data between the surface mixed layer and fmax degraded the relationships. This indicated differences in measurements of $F_m$, and possibly $F_v$, between layers although these did not in turn affect $\sigma_{PSII}$. 

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8.2.6 Summary

1. All three methods investigated showed clear log linear relationships between 'pigment' and 'primary production' measures.

2. Correlation between methodologies resulted in a degradation of these relationships, but the log linearity was generally still present.

3. These relationships implied an increase in the proportion of chlorophyll a in the total pigment load when the total pigment load, both increased and under higher rates of photosynthesis.
Chapter 9

Discussion

The core of this thesis was an investigation of optical and ancillary measurements made on three large scale cruises (AMTs) between the United Kingdom and the Falkland Islands. Measurements using three main techniques were examined to quantify and explain relationships of phytoplanktonic primary production parameters. The FRRF was used to measure directly the photosynthetic quantum efficiency (\( \frac{F_{m}}{F_{n}} \)) and the effective cross sectional area of PSII (\( \sigma_{PSII} \)). Ratios of pigment concentration, measured using HPLC, were examined as a causative factor to explain the variations of FRRF parameters encountered. Absorption measurements, using an ac9+, were then used as an alternative measure of these pigment ratios and by inference used to determine primary production through a passive measurement. The relationships derived in this investigation were though qualitatively apparent not well characterised by \( r^2 \) values; there were three main explanations for this:

1. All of the instruments were operating at the limits of their sensitivity in the clear oligotrophic waters of the open ocean gyres.

2. The data were all taken from the gyres, which are homogenous areas. The dynamic range of measurements were therefore very low and regressions unclear.

3. Ratio measurements show small variations in the strong linear relationships between the numerator and denominator of the ratio varying with chlorophyll a measurements. Therefore changes in the dependent variable are small in comparison to changes in the independent variable.

Two methodological developments were made. Firstly, FRRF blanking protocols were investigated and a suitable methodology for blanking FRRFs in oceanic conditions
suggested. Secondly, the ac9+ was used in a novel way employing twin casts and filters to measure particulate absorption by subtracting dissolved constituent absorption from total absorption.

Blanks of FRRF were compared between deionised water, filtered surface sea water and unfiltered water from 1000m, which showed that the water from 1000m best described an in situ blank with all phytoplankton removed. After blanks had been applied to FRRF data, both $\frac{\varepsilon_k}{F_m} \text{ and } \sigma_{PSII}$ showed positive log linear relationships with measures of chlorophyll $a$ concentration. This was in line with predictions for $\frac{\varepsilon_k}{F_m}$, but against predictions for $\sigma_{PSII}$. However, $\sigma_{PSII}$ did show the suggested negative log linear relationship with the chlorophyll $a$ diagnostic absorption, $a_{676}$.

The ratios of pigment groups (determined by HPLC) were examined to determine whether the relative concentration of TChla to other pigment groups varied with increasing TChla. Samples dominated by either prokaryotes or eukaryotes were considered separately, and in each case the pigment ratio of TChla:Tpig followed a log linear relationship with TChla. A mechanism was suggested to explain the hypothesis that variation of $\frac{\varepsilon_k}{F_m}$ and $\sigma_{PSII}$ with chlorophyll $a$ was a mechanism for causing this change in ratio. Absorption spectra (using the novel ac9+ double cast) were investigated to determine whether pigment ratios and therefore FRRF production values could be estimated. Absorption ratios, diagnostic of chlorophyll $a$ and total pigment were found to correlate well with equivalent HPLC pigment data. Absorption ratios were also found to describe the variation in $\frac{\varepsilon_k}{F_m}$ as a positive linear relationship and these ratios described a negative correlation with $\sigma_{PSII}$, in line with suggestions but contrary to ratios derived from HPLC data. A reason for this disparity is suggested in section 9.1.4.

In order to be confident in the relationships shown in this study statistical significance must be demonstrated. The noise in the data as a result of the instruments used being at their detection limits and the low dynamic range of the data precluded this in this study. The relationships shown in this thesis were generally log linear in nature. As the log linear factor was the dependent variable the method often used to reduce variance by taking the log of the independent variable [Raitos et al., 2005, Zar, 1984] was not possible.

9.1 Discussion of hypotheses

In order to establish whether links could in fact be made between absorption ratios and pigment ratios, and therefore FRRF $\frac{\varepsilon_k}{F_m}$ and $\sigma_{PSII}$, four hypotheses were made and
tested. These will be discussed in turn so that conclusions can be drawn in chapter 10.

9.1.1 Hypothesis 1 - The FRRF is a viable instrument for measuring primary production parameters in oceanic waters.

The FRRF had been used in many previous oceanographic cruises as a method of measuring primary production parameters [Aiken, 2001, Aiken et al., 2000, 2004, Moore et al., 2005, Vaillancourt et al., 2003a,b]. However, issues of running blanks, together with operation setup parameters, has never been adequately explored. Consequently, many of these issues may have introduced systematic error into the datasets resulting in the production of false patterns [Laney, 2003].

As was discussed in chapter 3, the FRRF was working at its detection limits in the subtropical gyres [Chelsea Instruments Ltd, 1998] and so blanks were of great significance. The elevated FRRF first point reading was particularly an issue here since it caused an elevated $F_0$ and therefore lower $F_m$ and $F_n$. Because measurement of $\sigma_{PSII}$ was calculated, using the propriety FRS software from the gradient of the points between $F_0$ and $F_m$ [Chelsea Instruments Ltd, 1998], elevation of the first point resulted in a lower $\sigma_{PSII}$ (see figures 2.11 and 3.1). Blanks for the FRRF were examined in detail and, since inequality was found between light and dark chamber data in regions where they should be equal, it was clear the FRRF required blanking procedures to be applied. Therefore, it was equally clear that separate blanks should be applied to each chamber of the FRRF. An investigation of blanks was undertaken to determine the most appropriate type of blank. Examination of variance of the difference between the two chambers showed significant improvements of all blanked data compared to the un-blanked data. Although there was little difference between the blanks used, water from the ship's MilliQ deionised water system was marginally the best blank based on the assumption of equality between the light and dark chambers in unquenched phytoplankton. However, further analysis showed that water taken from the CTD at 1000m was the most applicable blank as it was most similar to the 'ideal blank' value measured throughout the water column as specified by Cullen and Davis [2003]. Given the small variation in these blanks, the 1000m blank was used throughout.

The blanking methods were gain specific and, if more than one gain was used, a blank must be taken and calculated for each gain [Laney, 2003]. Data loss, which occurred during the gain switching, was also prevented. An attempt was made to utilise the auto-gain facility during AMT 12 which might have given more reliable measurements in extremely
oligotrophic waters. Apart from some data dropouts at the fmax, the data were similar to the data from AMT 14, which followed a similar cruise track but with the auto-gain facility switched off. This suggested that gain switching would not have greatly improved the quality of data collected and that the loss of data from using the autogain facility was not justifiable in oceanic waters.

Overall, the complexity of the blanking methodologies suggested that in oceanic waters (with very low chlorophyll concentrations), the use of data from this instrument should be treated with suspicion if it is run 'out of the box', that is simply using the proprietary software and calibration files without attempting to apply blanks to, or quality check, data.

One major change in methodology not considered in this study that should be considered in future is the use of software other than proprietary FRS software supplied by Chelsea Instruments. An example would be the v series of data reduction programs [Laney, 2003]. The current version, v5, is considered to be superior to the FRS v1.8 software as it has more robust curve fitting algorithms and statistical outputs [Laney, 2002]. Also, the calibration files (used to take into account instrument characterisation) are more effective than FRS and account for the first point issue described above. However, the blanking methodologies described in chapter 3 would still need to be applied. The characterisation files required to run the v5 data reduction programs were not taken during the data acquisition phase and therefore the v5 program could not be used.

9.1.2 Hypothesis 2 - FRRF Primary production parameters vary with measures of pigment concentration.

In general, on all cruises the FRRF data were noisy and correlations between parameters \( \frac{F_c}{F_m} \) and \( \sigma_{PSII} \) with both \( F_m \) and TChla were weak (chapter 5). The \( \frac{F_c}{F_m} \) showed a positive log linear relationship with these parameters as previously shown by Aiken et al. [2004]. However, contrary to the suggestions of Suggett et al. [2004], Moore et al. [2005] and Aiken et al. [in press], \( \sigma_{PSII} \) showed little correlation. Major outliers in FRRF data were often attributed to taxonomic variation, mainly the dominance of *Synechococcus* in outlying samples (figure 5.11). In all cases where FRRF data were examined with respect to these factors it became clear that two populations were present, one at high and one at low \( F_m \), corresponding to measurements taken from the surface mixed layer and the fmax. Two possible reasons were suggested. Firstly, in the surface mixed layer the FRRF was operating below its minimum detection limit [Chelsea Instruments Ltd,
and therefore presumed to be producing spurious data. The FRRF manual [Chelsea Instruments Ltd, 1998] quotes the minimum instrument detection limit as 0.1 mg m\(^{-3}\) of chlorophyll \(a\), and as can be seen in figure 6.1, chlorophyll \(a\) concentrations between the surface and \(f_{\text{max}}\) regularly fell below this range (particularly in the gyres). Secondly, the phytoplankton population in the surface mixed layer could have been reacting to the FRRF in a different manner to that at the \(f_{\text{max}}\). It is known that prokaryotes react to the FRRF in a different manner to eukaryotes [Chelsea Instruments Ltd, 2005, Moore et al., 2005, Suggett et al., 2001, 2004]. Most notably, samples dominated by \textit{Synechococcus} were found in this study to be different to those dominated by eukaryotes and \textit{Prochlorococcus} (figure 5.11). A commercial laboratory instrument is now available that can take measurements at the absorption wavelengths used by prokaryotes [Gorbunov and Falkowski, 2004] and another is in development for taking these measurements \textit{in situ} [Chelsea Instruments Ltd, 2005].

Although the noise within the FRRF data (see discussion of Hypothesis 1) precluded statistical detection of the relationship, it appeared that, as expected, \(\frac{F_{\text{m}}}{F_{\text{m}}^{c}}\) varied with chlorophyll \(a\) in a positive log linear fashion [Aiken et al., 2004]. A negative relationship was expected between \(\sigma_{\text{PSII}}\) and \(F_{\text{m}}\) (as discussed in section 2.12.1). Although, as in the case of \(\frac{F_{\text{m}}}{F_{\text{m}}^{c}}\), \(\sigma_{\text{PSII}}\) was not shown to be clearly correlated with \(F_{\text{m}}\) (shown in section 5.2.3), the data appeared to show a positive log linear relationship. Also, \(\sigma_{\text{PSII}}\) followed a positive log linear relationship with chlorophyll \(a\) in contrast to the negative relationship suggested.

\textbf{9.1.3 Hypothesis 3 - Pigment ratios are the causative factor of variation in primary production parameters.}

This section discusses the potential link between the FRRF data relationships and HPLC measured pigment loads (chapters 5, 6 and 8). The sections of taxon specific pigment concentrations (section 6.1) clearly showed the different populations of phytoplankton species present along the AMT cruise track, following closely the structure shown by Gibb et al. [2000]. The use of multivariate cluster analysis (to determine gyre or non-gyre status) and the splitting of profiles into surface mixed layer and \(f_{\text{max}}\) populations acted as effective ways to group data. Further attempts to split the data based upon the dominant taxon also resulted in a greater resolution of the patterns (chapter 6).

Published data suggest that the total pigment load of a phytoplankton sample should be directly proportional to the chlorophyll \(a\) concentration [Trees et al., 2000]. However,
this linearity has not been shown to be consistent between oceanographic cruises [Trees et al., 2000]. The linearity of these variables was strong within AMT data, but when the TAC instead of total pigment load were considered, deviations from linearity were apparent (section 6.3). The large difference in linearity and distinct trend in the decrease of TAC relative to TChla suggested that chlorophylls were forcing the linearity within total pigment. When the data were split on the basis of geographic regions or taxonomy, clear log linear relationships were apparent between TChla:Tpig and TChla. This relationship was clearer in the prokaryotes than eukaryotes, which showed a gradient tending towards zero (section 6.3), suggesting a near constant ratio.

The fuzzy logic method, developed to subdivide each sample based on the proportion of the pigment load, produced useful results showing conformity to regressions similar to those resulting from the dominance methodology (section 6.3). However, the uncertainty of the relative chlorophyll pigment loads meant that this could not be used as a quantitative method. Nevertheless, when comparisons were made with the dominance plots as suggested by Glover et al. [1987] and Trees et al. [2000], the data in section 6.3 suggested that the chlorophyll a load of Synecococcus was indeed lower than that of the eukaryotes. As a result of these findings along with the findings of chapter 5 it is suggested that the photochemistry of Synecococcus varies considerably with that of the eukaryotes affecting this ratio. It is also suggested that Prochlorococcus has a similar photochemistry to the eukaryotes in that it does not use phycobilins as its accessory pigments and can use chlorophyll a (even though in its Di-vinyl form) as an accessory pigment. The antenna structure and photo-physiology of Prochlorococcus would therefore be expected to be more similar to the eukaryotes than that of Synecococcus.

As shown in section 6.3 the ratio of TChla:Tpig showed a log linear relationship with TChla as suggested by Aiken et al. [2004]. The increase in the proportion of TChla to Tpig, with an increase in TChla, meant that as chlorophyll a increased, there was an increase in the relative load of chlorophyll a. Consequently total pigment load was not proportional to chlorophyll a as had been suggested by Aiken et al. [2004]. The log linear relationship was similar in form to that shown by the FRRF $\frac{F_{m}}{F_{m}}$ with $F_{m}$ and TChla (section 8.1). The linearity between $\frac{F_{m}}{F_{m}}$ and TChla:Tpig (shown in chapter 8) was therefore in line with expectations.

In order to explain this relationship, it is suggested that chlorophyll a is high in areas where $\frac{F_{m}}{F_{m}}$ is high, therefore the areas with high chlorophyll a concentrations are areas where photosynthesis is most efficient. As chlorophyll a is the reaction centre of the
antenna complexes of PSII [Falkowski and Raven, 1997], its concentration is a limiting factor in the rate of photosynthesis. The fact that chlorophyll $a$ is both energetically expensive to synthesise and unstable means that maintaining a high concentration requires a large energy outlay [Govindjee, 1975]. It is therefore logical that a higher concentration of chlorophyll $a$ can only be maintained when higher rates of photosynthesis are possible. On the basis of this assumption, it is also logical that if the relative concentration of chlorophyll $a$ to accessory pigments increases then the rate of photosynthesis will increase accordingly.

When the relationship of pigment with pigment ratios (shown in section 6.3) was considered, as TChla changed, the relationship was driven by the change of the numerator (TChla) relative to the denominator (Tpig). Equally, when $\frac{F_{m}}{F_{m}}$ was considered, it was the $F_{o}$ change that drove the relationship as $F_{m}$ increased.

As was discussed in section 2.10.1, $F_{o} = F_{m} - F_{o}$, and therefore any change in $F_{o}$, along with $F_{m}$, determines the capacity of the phytoplankton to process light energy; a low $F_{o}$ suggests that there are a small number of non functional, or closed, reaction centres, and a large $F_{m}$ would suggest a large number of reaction centres in total. A low $F_{o}$ relative to $F_{m}$ suggests a high $F_{o}$ [Chelsea Instruments Ltd, 1998]. Therefore the large amount of energy that these reaction centres could process would mean that the reaction centres could be maintained and kept open (thus maximising the energy throughput of PSII).

It is suggested that since $F_{m}$ relates to chlorophyll $a$ concentrations, and pigment ratios follow the same relationship as $\frac{F_{m}}{F_{m}}$ with TChla, a large TChla concentration denotes a population with an energy replete, well maintained photosynthetic mechanism. Consequently $F_{o}$ is low and as TChla is high, $F_{m}$ is high. Therefore $F_{o}$ and $\frac{F_{m}}{F_{m}}$ are also high.

In oceanic conditions, it is suggested that, under light and nutrient stress, the optimal increase in $\sigma_{PSII}$ that would make use of light under low photosynthesis conditions may not be energetically viable. Instead, the relative cross sectional area of PSII antenna complexes appeared to be smaller than when measured under high chlorophyll $a$ concentrations. Under the low nutrient and high light conditions of the upper ocean, where chlorophyll $a$ concentrations are low, light is not the factor limiting photosynthesis, instead photosynthesis is nutrient limited [Babin et al., 1996]. The low energy flow through photosynthesis means that chlorophylls within the antenna complex of PSII, which are energetically expensive to synthesise and maintain, cannot be maintained. In-
stead, the less efficient but more stable and easily synthesised carotenoids \cite{Govindjee, 1975} are used to absorb and transfer energy; this results in a lower $\sigma_{PSII}$ \cite{Mauzerall and Greenbaum, 1989}. Under lower light regimes, but higher nutrient conditions, acquisition of light becomes more limiting and therefore the energy absorption and transfer to PSII is ecologically more important. Under these conditions, there is the potential to photosynthesise when $\sigma_{PSII}$ is greater and so more energy can be captured and used.

Therefore, it is suggested that the measurement of $\sigma_{PSII}$ is defined by three major factors.

1. The taxonomic makeup of the community results in different accessory pigments and relative concentrations of pigment groups with the pigments absorbing at different efficiencies, which in turn affects $\sigma_{PSII}$.

2. The ambient light affects the relative concentrations of photosynthetic and photoprotectant carotenoids in the antenna structure, such that the presence of photoprotectant pigments particularly affects the transfer of energy to the reaction centres and thus the magnitude of $\sigma_{PSII}$.

3. The availability of resources, in the form of light and nutrients, affects the potential of the phytoplankton to fix carbon and store energy. Above a certain energy potential, it becomes energetically viable to synthesise and maintain chlorophyll molecules in higher concentrations thus making the antenna complexes much more efficient at absorbing light and transferring energy to the reaction centres.

\subsection{9.1.4 Hypothesis 4 - Absorption ratios can be used as a proxy for pigment ratios and therefore for FRRF primary production parameters}

This section examines the correlation between absorption at different wavelengths (chapter 7) and pigment concentrations (chapter 6) and confirms absorption measures can be used as proxy measures of pigment loads and therefore photosynthetic parameters (chapter 5). Absorption profiles are not a measurement of the fluorescence, like the FRRF, or a measurement of a processed extracted sample like HPLC; instead, they take a passive measure of an \textit{in situ} parameter linked to pigment concentrations. This section therefore discusses whether this method can be used to measure the pigments in oceanic waters as suggested by \textit{Behrenfeld and Falkowski} \cite{1997}. As pigments have been shown to link
to FRRF production parameters (chapter 8), the capacity of this method to determine primary production parameters is also discussed.

The novel double cast methodology (described in section 2.8.1) produced viable particulate absorption spectra. The first step in examining these data was to investigate the linearity of chlorophyll \( a \) absorption and total pigment absorption. Correlations between absorption proxies for TChla and Tpig (in section 7.1.2), returned strong linear relationships suggesting that these methodologies should be applicable. Also, direct comparison of absorption data with HPLC measured data (section 8.2.1) showed strong relationships.

On the basis of this, \( ac9+ \) derived ‘production’ measurements of \( a_{TChla}:a_{Tpig} \) were compared to ‘production’ measurements from the FRRF and HPLC (section 8.2.2). Using absorption data on its own, clear log linear relationships were apparent between absorption ratios as proxies for TChla:Tpig against the absorption proxy for TChla. Absorption ratios were shown to be correlated with pigment ratios (section 8.2.2) suggesting that the use of passive measurements of pigments as a proxy for primary production parameters is in fact feasible.

When \( ac9+ \) data were compared to the FRRF data, some differences were apparent from what would be expected given the relationship of FRRF data with HPLC data. The positive log linear relationship of chlorophyll \( a \) absorption to \( \frac{F_p}{F_m} \) was observed (section 5.2.4) as was the linearity between the absorption ratios relating to \( a_{TChla}:a_{Tpig} \) with \( \frac{F_p}{F_m} \) in section 8.2.2. However, unlike the HPLC data, there was a clear negative log linear relationship of \( \sigma_{PSII} \) with chlorophyll \( a \) absorption and a negative linear relationship with \( a_{TChla}:a_{Tpig} \). The relationship with \( \frac{F_p}{F_m} \) (section 8.2.2) resolved well when split between surface and fmax data, however the \( \sigma_{PSII} \) data were much more coherent as a single group. This supported the argument shown by the HPLC data (section 6.3) that there were two populations showing different \( \frac{F_p}{F_m} \) relationships with \( a_{676} \). However, in this case the populations showed consistent trends in \( \sigma_{PSII} \).

If the surface mixed layer is considered, it was shown in chapter 6 that this region was dominated by prokaryotes. The suggestion that the chlorophyll \( a \) load of these taxa varied relative to the eukaryotes was also supported in chapter 6 [Glover et al., 1987, Ting et al., 2002].

On the assumption that in reality the absorption of light by Prochlorococcus (which dominated the prokaryotes population) is the same as eukaryotes, as supported by the consistency of \( \sigma_{PSII} \) with chlorophyll \( a \) absorption, it is reasonable to assume that the resulting issue of \( \frac{F_p}{F_m} \) not correlating as expected was a result of different functional types.
of pigments in the antenna complex of PSII. The pigments used by cyanobacteria are known to absorb at those wavelengths that the FRRF uses to excite chlorophyll a and this can therefore result in a change in the fluorescence measurements \cite{Moore2005}. Instruments in development, which use different wavelengths to characterise cyanobacteria primary production, should help resolve this issue \cite{Gorbunov2004, Chelsea2005}. However, the linear tendencies demonstrated between absorption data and FRRF production parameters suggest that measurement of primary production parameters using passive IOPs is a viable methodology.

As has been discussed a number of times, a negative correlation between FRRF $\sigma_{PSII}$ and pigment measures would be expected, as suggested by Suggett et al. \cite{Suggett2004}, Moore et al. \cite{Moore2005} and Aiken et al. \cite{Aiken2016}. However, positive correlations emerged between $\sigma_{PSII}$ and FRRF $F_m$ (section 5.2.3) and HPLC Tchla (section 5.2.4). Chla absorption at 676nm contradicted these findings (section 8.2.2), resulting in a decrease in $\sigma_{PSII}$ as initially suggested by Suggett et al. \cite{Suggett2004}, Moore et al. \cite{Moore2005} and Aiken et al. \cite{Aiken2016}. The optics data taken in this study were rigourously quality checked and careful measures were taken to reduce errors in the data. Errors in HPLC data however cannot be well described as these data were produced externally. A potential explanation for this disparity must therefore be that the pigment data have large amounts of errors associated with them. The weakness of HPLC data correlations with ac9+ and FRRF data relative to the correlations between these instruments support this suggestion.

If however the HPLC data are correct then there is a difference between the correlations of primary production parameters measured by the FRRF with HPLC data and ac9+, which must be explained. This variation could be a result of the package effect altering the wavelength and magnitude of pigment absorption through its physiological coupling to the photosynthetic system and the positioning of reaction centres in the cell \cite{Nelson1993}. The empirical link being investigated in this study applies to the functional absorption of these samples and so logically applies to the light absorption of the sample \textit{in vivo}. This is therefore indicative of the ecological significance of this absorption. Extraction of pigments into acetone, to measure actual concentration as in HPLC readings, may not give this measure.

The difference between \textit{in vivo} and \textit{in vitro} absorption measurements could explain the seemingly contradictory FRRF data. In the data it was found that $\frac{E}{F_m}$ followed a clear relationship with pigment ratios, but $\sigma_{PSII}$ did not. Also $\sigma_{PSII}$ followed a clear relationship with absorption ratios, but $\frac{E}{F_m}$ was less clear. When these are considered
in the context of absorption and pigment load, a possible reason emerges. \( \sigma_{PSII} \) is a measure of the rate of the phytoplankton's ability to absorb light and saturate PSII [Moore et al., 2005]. In this case, it is logical that since the functional absorption of light is a factor in the measurement rather than an actual measure of the concentration of a pigment it will therefore give a better relationship. However, when \( F_{m}^{/} \) is considered the absorption is no longer an issue as it relates to the capacity of the PSII reaction centres present rather than to their ability to absorb [Moore et al., 2005].

It is suggested that discrepancies between correlations with HPLC data and absorption measures are a result of absorption being a passive measure of phytoplankton absorption \textit{in vivo}. It is therefore a functional representation of the antenna complex absorption rather than a concentration measurement made in the laboratory or based on fluorescence. Hence, it may reflect the organisation of the cell's organelles to maximise the absorption capability of the complexes under low light conditions and in turn to maximise the possible rate of photosynthesis.

In future investigations of these relationships, two issues will have to be resolved in order to give a clear measure of pigment concentrations. Firstly, the package effect as discussed above should be quantified. Secondly, the absorption of the non-pigment particulate components (such as cell walls and dead material) will have to be accounted for in some way [Kirk, 1994]. In the laboratory, this is undertaken by bleaching the pigments from the sample so that only this material remains. The absorption of this material can then be quantified and removed from the total particulate absorption [Ferrari and Mingazzini, 1995, Tassan and Ferrari, 1995]. To bleach samples in this way would be impractical \textit{in situ}, but could perhaps be achieved during profiles with the introduction of a bleaching agent such as \( \text{H}_2\text{O}_2 \), or application of a high intensity ultra violet light.

9.2 Future Work

The suggestions for future work are split between the three techniques used:

9.2.1 FRRF

- The presence of large quantities of cyanobacteria in some areas produced considerable variation in the FRRF production parameters. The use of a new generation of FRRF type instruments, which can use wavelengths specific to these organisms, should be attempted within future oceanic work.
• It would be advantageous to increase the scope of measurements using these instruments, so that *Synecococcus* dominated samples can be effectively quantified.

9.2.2 HPLC pigment ratios

• A major unknown factor in this investigation was the relative concentration of chlorophyll *a* to accessory pigments in *Synecococcus*. It would therefore be useful to determine this, possibly through culture experiments.

• The phycobilin accessory pigments of *Synecococcus* cannot currently be measured by HPLC. Development of techniques to determine the concentration of these pigments would be desirable in order to enable their inclusion in pigment ratio relationships.

9.2.3 ac9+ absorption techniques

• Further developments of this method should attempt to quantify the package effect and the effects of absorption of material other than pigments so as to resolve the relationships between absorption and HPLC when compared to FRRF data.

• Absorption measures have been shown to be an effective method of determining primary production parameters. Consequently comparison of these absorption data with measurement of ambient light upwelling radiances would be useful.

• With relationships from upwelling radiances, determination of algorithms for the determination of primary production parameters by remote sensing would be possible.
Chapter 10

Conclusions

Hypothesis 1 - The FRRF is a viable instrument for measuring primary production parameters in oceanic waters.

- The FRRF was working at its detection limits in the subtropical gyres and therefore blanks are of great significance.

- Blanks must be taken and applied separately for both the light and dark chambers of the instrument.

- Instrument $F_m$ values appeared to be prone to unexpected values in the gyres due to either:
  - Instrument or blanking error
  - Different reaction of *Synechococcus* to the FRRF relative to the eukaryotes and *Prochlorococcus*

- Extreme care should be taken to quality assure data when using FRRFs in open water conditions.

- The 'out of the box' use of the FRRF in oceanic waters can therefore not be supported.

- However if blanking and data quality control are rigorously applied these data can be used.

Hypothesis 2 - Primary production parameters from the FRRF vary with measures of pigment concentration.

- Most major outliers in FRRF data could be attributed to taxonomic variation, mainly due to the dominance of *Synechococcus* in some samples.
• Photosynthetic quantum efficiency, $\frac{F_p}{F_m}$, varied as expected in a positive log linear fashion with chlorophyll $a$ measures.

• The effective cross sectional area of PSII, $\sigma_{PSII}$, also followed a positive log linear relationship with chlorophyll $a$ measures in contrast to the negative relationship suggested.

• FRRF production parameters were shown to co-vary with chlorophyll $a$ measures.

Hypothesis 3 - Pigment ratios are the causative factor of this variation.

• The concentration of chlorophyll $a$ was not found to be proportional to either total pigment or to total carotenoid concentration.

• The ratio of chlorophyll $a$ : total pigment showed a log linear relationship with chlorophyll $a$.

• Chlorophyll $a$ was found to make up a greater proportion of the pigment load of a sample as the chlorophyll $a$ concentration increases.

• The pigment ratio was found to be linearly correlated to FRRF $\frac{F_p}{F_m}$ and $\sigma_{PSII}$.

• The hypothesis that the pigment composition and TChl$a$ load of a sample is a causative factor in the photosynthetic ability of phytoplankton is supported.

Hypothesis 4 - Absorption ratios can be used as a proxy for pigment ratios and therefore for FRRF primary production parameters.

• The novel double cast methodology produced viable particulate absorption spectra.

• Absorption measures correlated well with other measures of pigment concentration.

• Absorption ratios were shown to be correlated with pigment ratios, suggesting that passive measurement of pigments can be used to determine primary production parameters.

• Absorption ratios equating to $a_{TChl_a: a_{Tpig}}$ showed positive linear correlations with FRRF $\frac{F_p}{F_m}$ in agreement with HPLC pigment ratios.
• These absorption ratios showed a negative linear correlation with FRRF $\sigma_{PsII}$ in line with those suggested but in contrast to those shown in HPLC measurements.

• This variation could be explained through examination of the functional absorption characteristic of pigments in situ or the suggestion that HPLC data may not have been high quality.

• Measurement of primary production parameters using passive IOPs is a viable methodology.

10.1 Conclusion

This thesis has shown that primary production parameters as measured by the FRRF co vary with chlorophyll $a$ concentrations. This relationship was explained by examining the pigment composition of samples and it was demonstrated that changes in pigment composition could be measured passively by absorption. It was then demonstrated that primary production parameters could be determined through passive measurements of absorption, an inherent optical property.
Appendix A

Symbols
<table>
<thead>
<tr>
<th>Quantity</th>
<th>Unit</th>
<th>Description</th>
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<tbody>
<tr>
<td>( \alpha )</td>
<td></td>
<td>Regression intercept</td>
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<tr>
<td>( a )</td>
<td>( m^{-1} )</td>
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<td>( m^{-1} )</td>
<td>Temperature and salinity corrected absorption</td>
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<tr>
<td>( a_{mts}(\lambda) )</td>
<td>( m^{-1} )</td>
<td>Absorption measured corrected for temperature and salinity</td>
</tr>
<tr>
<td>( a_{mts}(\lambda_{\text{ref}}) )</td>
<td>( m^{-1} )</td>
<td>Absorption measured at the reference wavelength (here 715nm) corrected for temperature and salinity</td>
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<td>( m^{-1} )</td>
<td>Raw measured absorption</td>
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<td>Total corrected absorption</td>
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<td>( m^{-1} )</td>
<td>Absorption of pure water</td>
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<tr>
<td>( b )</td>
<td>( m^{-1} )</td>
<td>Scattering coefficient</td>
</tr>
<tr>
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</tr>
<tr>
<td>( c )</td>
<td>( m^{-1} )</td>
<td>Attenuation coefficient</td>
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<td>( m^{-1} )</td>
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<td>$\mu$mol photons m$^{-2}$</td>
<td>Potential photochemical yield of PSII</td>
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<td>Photon flux</td>
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<td>mol O$_2$ mol photon$^{-1}$</td>
<td>Photon yield of electron transfer by PSII</td>
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Appendix B

Acronyms

ac9+  Absorption attenuation meter at 9 wavelengths with temperature sensor
AMT   Atlantic Meridional Transect.
CDOM  Coloured Dissolved Organic Matter
CTD   Conductivity Temperature Depth [meter]
DP    Diagnostic Pigment
Euk   Eukaryote
fmax  Fluorescence Maximum
FRRF  Fast Repetition Rate Fluorometer
HPLC  High Performance Liquid Chromatography
IOP   Inherent Optical Property
Lat   Latitude
LED   Light Emitting Diode
Lon   Longitude
Micro Microplankton
MLD   Mixed Layer Depth
Nano  Nanoplankton
PAR   Photosynthetically Available Radiation
Pico  Picoplankton
PMT   Photo Multiplier Tube
PPC   Photoprotectant carotenoids
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Appendix C

AMT station positions
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Appendix D

FRRF AMT 12 profiles

This appendix contains FRRF optics cast profiles from all data collected on AMT 12 after post-collection processing had been applied.
Figure D.1: AMT 12 FRRF optics cast profiles.
Figure D.2: AMT 12 FRRF optics cast profiles. Station 33 fmax data shows a localised $F_{m\text{ax}}/F_m$ minimum.
Figure D.3: AMT 12 FRRF optics cast profiles. Station 39 Surface Mixed Layer data missing.
Figure D.4: AMT 12 FRFF optics cast profiles.
Figure D.5: AMT 12 FRRF optics cast profiles.
Figure D.6: AMT 12 FRRF optics cast profiles. Station 62 Fmmax data shows a localised \( \frac{Fm}{Fm} \) minimum. Station 64, 66 and 68 \( \frac{Fm}{Fm} \) aberrations meant that these stations were not used.
Appendix E

FRRF AMT 13 profiles

This appendix contains FRRF optics cast profiles from all data collected on AMT 13 after post-collection processing had been applied.
Figure E.1: AMT 13 FRRF optics cast profiles.
Figure E.2: AMT 13 FRRF optics cast profiles. Station 11 showed zero $F_m$ in the surface waters so Surface Mixed Layer data were not used.
Figure E.3: AMT 13 FRRF optics cast profiles. Stations 15, 16 and 17m showed zero $F_m$ in the surface waters so Surface Mixed Layer data were not used.
Figure E.4: AMT 13 FRRF optics cast profiles. Stations 17n, 19m, 19n and 22 showed zero $F_m$ in the surface waters so Surface Mixed Layer data were not used.
Figure E.5: AMT 13 FRRF optics cast profiles. Station 23 showed zero $F_m$ in the surface waters so Surface Mixed Layer data were not used.
Figure E.6: AMT 13 FRRF optics cast profiles. Stations 31, 32, 33 and 35 showed zero $F_m$ in the surface waters so Surface Mixed Layer data were not used.
Figure E.7: AMT 13 FRRF optics cast profiles. Stations 36, 39 and 41 showed zero $F_m$ in the surface waters so Surface Mixed Layer data were not used.
Figure E.8: AMT 13 FRRF optics cast profiles. Stations 42, 43, 45 and 46 showed zero $F_m$ in the surface waters so Surface Mixed Layer data were not used.
Figure E.9: AMT 13 FRRF optics cast profiles. Stations 48, 51, 52 and 54 showed zero $F_m$ in the surface waters so Surface Mixed Layer data were not used.
Figure E.10: AMT 13 FRRF optics cast profiles. Stations 58m, 58n, 60m and 60n fmax data showed a localised \( \frac{F_m}{F_m} \) minimum.
Figure E.11: AMT 13 FRRF optics cast profiles. Station 63 showed aberrant Surface Mixed Layer data and a localised $F_{\text{m}}$ minimum at the $f_{\text{max}}$. Stations 66m and 66n showed aberrant $f_{\text{max}}$ data.
Figure E.12: AMT 13 FRRF optics cast profiles.
Appendix F

FRRF AMT 14 profiles

This appendix contains FRRF optics cast profiles from all data collected on AMT 14 after post-collection processing had been applied.
Figure F.1: AMT 14 FRRF profiles. Station 3 showed aberrant data in the Surface Mixed Layer and station 7 showed bad $F_{m}^*$ values throughout.
Figure F.2: AMT 14 FRRF profiles.
Figure F.3: AMT 14 FRRF profiles.
Figure F.4: AMT 14 FRRF profiles. Station 42 showed a localised $F_m$ minimum at the $f_{\text{max}}$.  

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Figure F.5: AMT 14 FRRF profiles.
Figure F.6: AMT 14 FRRF profiles. Stations 51 and 54 showed localised $\frac{F_m}{F_v}$ minima at the fmax.
Figure F.7: AMT 14 FRRF profiles. Station 62.0.1m was filtered through a 0.1µm filter and is therefore a blank profile as discussed in chapter 3.
Figure F.8: AMT 14 FRRF profiles. Station 62.0.2m was filtered through a 0.1μm filter and is therefore a blank profile as discussed in chapter 3.
Figure F.9: AMT 14 FRRF profiles.
Appendix G

ac9+ AMT 13 Spectra

This appendix contains ac9+ optics cast CDOM and particulate absorption spectra from all data collected on AMT 13 after post-collection processing had been applied.
Figure G.1: AMT 13 ac9+ particulate spectra. These data are shown prior to taking \( a_{715} \) as 0. Station 17 shows non viable data.
Figure G.2: AMT 13 ac9+ particulate spectra. These data are shown prior to taking $a_{715}$ as 0.
Figure G.3: AMT 13 ac9+ CDOM spectra. These data are shown prior to taking $a_{715}$ as 0.
Figure G.4: AMT 13 ac9+ CDOM spectra. These data are shown prior to taking $a_{715}$ as 0.
Appendix H

ac9+ AMT 14 Spectra

This appendix contains ac9+ optics cast CDOM and particulate absorption spectra from all data collected on AMT 14 after post-collection processing had been applied.
Figure H.1: AMT 14 ac9+ particulate spectra. These data are shown after taking $a_{715}$ as 0.
Figure H.2: AMT 14 ac9+ particulate spectra. These data are shown after taking $a_{715}$ as 0.
Figure H.3: AMT 14 ac9+ particulate spectra. These data are shown after taking $a_{715}$ as 0.
Figure II.4: AMT 14 ac9+ CDOM spectra. These data are shown prior to taking $\alpha_{715}$ as 0.
Figure H.5: AMT 14 ac9+ CDOM spectra. These data are shown prior to taking $a_{715}$ as 0.
Figure II.6: AMT 14 ac9+ CDOM spectra. These data are shown prior to taking $a_{715}$ as 0.
Appendix I

ac9+ AMT 13 Profiles

This appendix contains ac9+ optics cast profiles from all data collected on AMT 13 after post-collection processing had been applied.
Figure 1.1: AMT 13 ac9+ particulate profiles. Each line represents an absorption wavelength measured by the ac9+. Station 17 shows bad data.
Figure 1.2: AMT 13 ac9+ CDOM profiles. Each line represents an absorption wavelength measured by the ac9+.
Appendix J

ac9+ AMT 14 Profiles

This appendix contains ac9+ optics cast profiles from all data collected on AMT 14 after post-collection processing had been applied.
Figure J.1: AMT 14 ac9+ particulate profiles. Each line represents an absorption wavelength measured by the ac9+.
Figure J.2: AMT 14 ac9+ particulate profiles. Each line represents an absorption wavelength measured by the ac9+. 
Figure J.3: AMT 14 ac9+ CDOM profiles. Each line represents an absorption wavelength measured by the ac9+.
Figure J.4: AMT 14 ac9+ CDOM profiles. Each line represents an absorption wavelength measured by the ac9+.
Reference List


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