The chemical and bio-optical characterisation of gelbstoff in southern African waters: a preliminary analysis

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Chapter 1

Introduction

As the global marine science community moves towards a new age of ocean colour sensors, the need has arisen for a greater understanding of the marine bio-optical processes that are fundamental to the remote sensing of ocean colour. One of the most pressing problems in environmental science is the need for a greater knowledge of large scale biogeochemical cycles. Remotely sensed ocean colour can potentially provide unique information on these cycles in the upper ocean, offering rapidly and frequently acquired synoptic biogeochemical data. However, the truly productive use of ocean colour sensors across a diversity of marine systems will only be achieved if the various classes of optically significant components in the marine environment can be effectively characterised. This is particularly necessary in view of the refinements made to the core chlorophyll algorithms, which have progressed from the empirical CZCS variety [Gordon & Morel 1983] to potentially more accurate semi-analytical variants such as those to be used with the more sensitive SeaWiFS sensor [Carder et al. 1991]. If these algorithms, dependent as they are on the application of idealised bio-optical parameters, are to be used in highly productive areas such as shelf seas then it is vital that they are not used on an indiscriminate basis. Significant bio-optical parameters, and their effects on the underwater light field, must be evaluated to discover if site and season specific variations from modelled algorithm parameters will cause unacceptable deviations in accuracy. If these bio-optical conditions do not correspond closely enough to the idealised parameters of a global algorithm, effective sensor use will require the construction of regional or seasonal alternatives [Carder et al. 1991].

The principle determinants of ocean colour in the world oceans, and hence the major marine bio-optical components, can be considered to be phytoplankton and their associated degradation products [e.g. Carder et al. 1991, Hoge et al. 1993]. A strong emphasis is thus placed on a thorough understanding of complex phycological processes from a bio-optical perspective. Phycologically related processes can generally be regarded as having three main bio-optical components - the phytoplankton itself, detritus (non-living particulate matter of a biological origin), and dissolved organic degradation products otherwise known as gelbstoff, chromophoric dissolved organic matter (CDOM), yellow substance, gilvin, aquatic humus and marine humic substances. Recent research [Carder et al. 1989,1991] has shown that if gelbstoff is not accounted for, it can significantly compromise remotely determined chlorophyll measurements through absorption in the lower wavelengths of the visible spectrum.

This research has made manifest several issues concerning the potential problems of gelbstoff with respect to ocean colour. Autochthonous formation of the marine fulvic and humic acids that comprise the majority fraction of gelbstoff [Carder et al. 1989] has been shown to be higher than previously thought, contrary to earlier thinking suggesting that the effects of marine gelbstoff are "negligible" in comparison to terrigenously derived material [Bricaud et al. 1981, Hojerslev 1988]. Evidence has also come to light suggesting that there can be a distinct lack of temporal covariance between concentrations of autochthonous gelbstoff and the source phytoplankton population [Peacock et al. 1988, Carder et al. 1989]. In certain situations the combination of these two factors can negate one of the central criteria for the application of the CZCS algorithm for Morel Case 1 waters, i.e. the global chlorophyll algorithm [Gordon & Morel 1983]. Morel Case 1 waters are defined as being dominated by the bio-optical processes of phytoplankton and its related degradation products [Morel 1980]. The important assumption is made that these degradation products covary with phytoplankton concentrations. Adverse effects resulting from the failure of this assumption appear to be highest in areas prone to episodic upwelling events, where the combination of relatively high and rapidly changing pigment concentrations in highly dynamic systems lead to dissimilar residence times for phytoplankton and the more refractive fractions of their degradation products, such as gelbstoff [Carder et al. 1989, Peacock et al. 1988]. The implication that gelbstoff typically has a long half life and accrual period relative to its precursive phytoplankton population could also result in an area having high ambient gelbstoff levels which have no direct biochemical link to present algal populations. However manifested, the lack of temporal covariance between gelbstoff and phytoplankton poses questions which can only be answered through a more complete understanding of their inherent association.

This has lead to the inclusion of spectral bands in the violet on future ocean colour sensors (e.g. the 412nm band on SeaWiFS), which will be sensitive to the higher absorption of gelbstoff at these wavelengths. However the effective use of this increased spectral resolution has necessitated the use of semi-analytical chlorophyll algorithms employing two waveband ratios, as opposed to the empirically derived CZCS variants based on a single blue/green ratio. The semi-analytical algorithm is designed to solve for two unknowns, the absorption of phytoplankton and gelbstoff, which are then used to explicitly calculate chlorophyll *a* concentrations. The determinative processes used are based upon the modelled values and

behavioural trends of these two core unknowns. Although this form of algorithm is potentially both more sensitive and accurate than the empirical variety, it is dependent upon the application of these idealised parameters, which may not be representative in areas dissimilar to those used for the derivation process. The central endeavours of any investigation into algorithm performance must therefore be the characterisation of the optical properties of phytoplankton and gelbstoff, the nature of the relationship between them, and the resultant effects upon the underwater light field.

This study will attempt to begin the bio-optical characterisation of gelbstoff in southern African waters. Gelbstoff is a collective term, in itself perhaps an indication of a poorly understood phenomenon, given to a complex group of macromolecular organic compounds. It is the common bio-optical properties of these compounds that cause such an association, specifically the exponential decrease of absorption with increase in wavelength, resulting in typical absorption spectra decaying exponentially from a maximum in the ultra-violet. It is the accurate measurement or inferral of these spectra that is the primary aim of any bio-optical investigation of gelbstoff. However, the *in vivo* measurement of gelbstoff is complicated by both its variable character and the typically very low concentrations in which it is present. The most common and effective approach to *in vivo* determination, given the optical framework of the problem, has been the direct spectrophotometric measurement of filtered seawater samples. This can also have the advantage of bypassing the sometimes unnecessary analysis of the highly variable and complex chemistry of marine humic compounds. Conversely, the amount of qualitative information available can be restrictive, confined as it is to only to the spectral slope of the absorption coefficient. However, given the sensitivity levels of most modern spectrophotometers and the low concentrations at which gelbstoff is optically significant, it can be extremely difficult to make direct absorption measurements. An example of this, taking the specific absorption figures of Carder et al. [1991], is a typical gelbstoff concentration of 0.25 g m^{-3}, corresponding to a chlorophyll concentration of 0.1 mg m^{-3}, which would have an associated absorption value of 0.005 m^{-1} at 450 nm.

Various alternative approaches have been taken, such as the extrapolation of measurements made in the more absorptive ultra-violet [Bricaud et al. 1981], chemical or physical isolation and concentration [Carder et al. 1989, Blough et al 1993, Green & Blough 1994], and the use of fluorescence based regressions [Ferrari & Tassan 1991, Hoge et al. 1993, Green & Blough 1994, Vodacek et al. 1995]. Extrapolative absorption techniques, whilst having the advantage of simplicity, offer little qualitative information and are dependent on simplified assumptions concerning the behaviour of absorption on a spectral basis. Isolation techniques, while being complex, time consuming and prone to selective behaviour [Green & Blough 1994], offer the advantages of sufficient quantities of isolated material for detailed optical and chemical studies. Fluorescent analyses, on both natural water samples and isolates, appear to promise both high sensitivity and qualitative information on structure, and can be well correlated to absorption values. However, site specific variations of the relationships between absorption, fluorescence and chemical character must be accounted for if quantifiable data is to be produced. In any event, it is clear that any comprehensive attempt at the bio-optical characterisation of gelbstoff must include the investigation and possible development of available methodologies.

Gelbstoff has been shown to play an important and historically understated role in the marine environment. The effective use of upcoming ocean colour sensors will require a more complete understanding of the formation, accrual and effects of this collective of complex substances. This study will attempt to set out a theoretical framework for gelbstoff formation and structure, investigate and possibly refine appropriate analytical methodologies, and employ these to determine the possible effects of gelbstoff on remotely sensed ocean colour in southern African waters.

Chapter 2

The formation and structure of gelbstoff

Gelbstoff : Chromophore containing or absorbing aqueous dissolved organic material

Gelbstoff is an equivocal term, and as such somewhat of an anachronistic one in the precise field of hydrological optics. This is reflected in the variety of terms that have been used for it: yellow substance, chromophoric dissolved organic matter, gilvin or even "gunk" [Hunter & Liss 1981]. It has arisen from both the ability and the need to treat aqueous dissolved organic matter as a collective entity from a bio-optical perspective. Since the early work of researchers such as James & Birge [1938] and Kalle [1961,1966], it has become apparent that the optical classification of natural water types must account for the ubiquitous presence of absorbing dissolved material. The characteristic shape of spectral absorption for this material (seen in comparison to a schematic phytoplankton absorption spectra in figure 1), through a wide variety of natural water types, has since facilitated a collective optical treatment. However, this composite approach is not suggestive of homogeneity - it must be remembered that the diverse collections of organic molecules which may constitute gelbstoff will reflect a huge variety of possible environments, pathways and stages of formation. In the marine environment, gelbstoff formation can either be considered autochthonous (primarily resulting from phytoplankton activity) or allochthonous (resulting from terrigenous discharges or anthropogenic activity). Whilst there is some evidence for the presence of a terrigenous component to oceanic gelbstoff [Meyers-Schulte & Hedges 1986], it is felt that this component will be small in comparison to autochthonously formed substances when considering the highly productive southern African marine environment. Evidence for this can be considered to be the extremely high episodic levels of phytoplankton biomass, generally high dynamic activity, and the lack of significant riverine input in the majority of the area under consideration i.e. the Agulhas Bank and Benguela [Chapman & Shannon 1985]. Marine systems dominated by terrigenous or anthropogenic gelbstoff must be considered on an individual basis, and

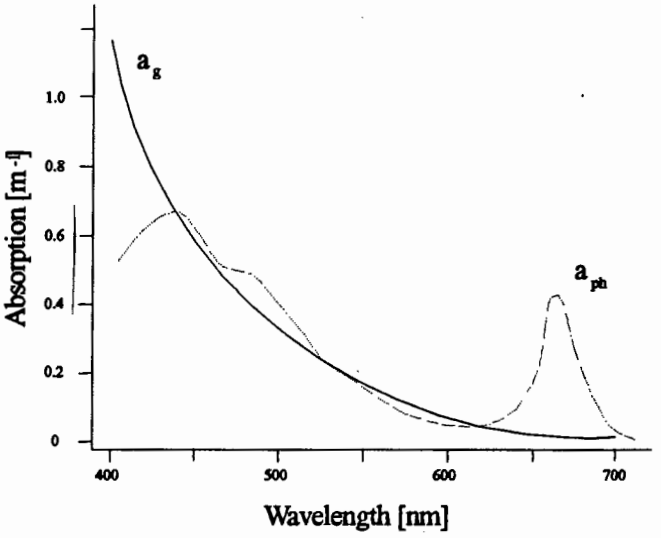


Figure 1. *Schematic absorption curves typical of gelbstoff (a_g) and phytoplankton (a_{ph}), normalised at 443nm.*

as such are beyond the scope of general studies [Gordon & Morel 1983, Carder et al. 1989]. It is more appropriate, when considering "...the problem of gelbstoff..." [Kalle 1966] in southern African waters, to examine the relationship of gelbstoff to the growth and decay of phytoplankton. Accordingly this study will focus, at least on a theoretical basis, on autochthonously formed gelbstoff.

The majority components of gelbstoff have often been assumed to be fulvic and humic acids [Zika 1981, Ehrhardt 1984, Hawes et al. 1992, Carder et al. 1989,1991], considered to be the most refractory component of marine dissolved organic matter (DOM) [Moran & Hodson 1990, Carlsson & Granelli 1993]. Given the predominance of humic substances (or HS) in much of the marine environment, and the labile nature of other chromophore containing compounds, regarding gelbstoff as exclusively composed of humic and fulvic acids is generally a reasonable indication of minimum absorption levels [Carder et al. 1989]. However, in extreme bio-optical environments, such as highly productive coastal areas, this assumption may have less validity - less refractory compounds such as pigments, flavins, proteins and lipids may form significant fractions of gelbstoff [Seritti et al. 1994]. Nevertheless, the importance of understanding the role and characteristics of the complex and poorly understood marine humic substances cannot be denied, particularly as regards the relationship between phytoplankton and humification.

2.1 Humic substances - fulvic and humic acids

Humic materials, of which fulvic and humic acids represent the soluble fractions, are derived from the breakdown and metabolism of plant material. They have a hugely complex and variable nature, to the extent of even being defined in a negative sense; as including all organic components of soil, water or sediment excluding proteins, polysaccharides and lipids [Buffle 1990]. They can be divided into three operationally defined categories: fulvic acids which are soluble in acid and base, humic acids which are soluble only in base, and insoluble humin or kerogen [e.g. Ishiwatari 1992]. Of these humin, as the insoluble fraction, is only of indirect interest here through its role in the humification process. Fulvic and humic acids are large acidic, complex, organic macromolecules ranging in molecular size from less than five hundred daltons to many hundreds of thousands of daltons, and are typically a yellow/brown colour in extracted form [Harvey et al. 1983, Hedges 1988]. Although they are examined here primarily from a bio-optical perspective, their properties are many: complexation of metals and other molecules, dissolved organic carbon (DOC) and nutrient reservoirs, acid-base buffering, surface active adsorbers, photoreactants...the list continues. The chemical, physical and optical properties of all humic substances are thought to be dictated by several factors: the nature of available precursive materials, the reactive environment including the humification process itself, and the characteristics of active degradative mechanisms [Hedges 1988, Hatcher & Spiker 1988, Ishiwatari 1992]. Given this, humic materials from environments such as different soil types, rivers, lakes and the oceans exhibit huge variety in structure, functionality, molecular weight and optical properties [Wilson et al. 1983, Ertel & Hedges 1983, Harvey & Boran 1985, Malcolm 1990]. Fulvic and humic acids, as the two dissolved humic fractions, also exhibit different properties, although these are generally less marked than those due to environment of formation [Malcolm 1990]. The following discussions relating to these variations in humic character will focus primarily on composition - detailed discussion of the core optical properties will appear later. The point should be made that these substances are complex and poorly understood, even to the point where analytical identification is questioned on a frequent basis. The relevant body of research embraces many conflicting opinions, concerning both structure and formation, and what follows is by no means an in depth review, rather a simple summary of some salient points.

In the marine environment, autochthonous fulvic acids are generally far more prevalent than their humic counterparts, typically accounting for 90% or more of dissolved HS [Harvey et al. 1983, Carder et al. 1989, 1991, Malcolm 1990]. Typical characteristics of marine fulvic acids (MFA) in comparison to marine humic acids (MHA) are: greater functionality and hydrophilicity [Hatcher & Spiker 1988, Ishiwatari 1992], lower molecular weight, and a less aromatic (or more aliphatic) structure [Harvey et al. 1983, Harvey & Boran 1985, Buffle 1990, Malcolm 1990]. The last point has important optical ramifications, as the optically resonant ring structures associated with aromaticity strongly influence molecular absorption characteristics [Wang et al. 1990]. This trend towards greater numbers of aromatic structures in the generalised molecular structure of MHA, as compared to MFA, is consistent with their differing absorption characteristics.

This important variation of aromatic character between humic fractions is also one of the distinguishable features between humic substances of different origins e.g. soils, lakes and oceans. Whilst the primary interests of this study are autochthonous marine humic substances, variation in the character of humic substances produced in different environments yields valuable insight to potential humification pathways. These differences are primarily associated with precursor materials, often based on the assumption that HS of terrigenous origin are derived from lignin or other polyphenols [Fischer & Schrader 1921, Waksman 1938, Flaig 1966, Ertel & Hedges 1983, Meyers-Schulte & Hedges 1986], compounds unique to vascular plants, which are generally absent in the marine environment. This hypothesis is consistent with the greater aromaticity of terrigenous HS, as compared to the aliphatic nature of marine compounds [Ertel & Hedges 1983, Harvey et al. 1983, Harvey & Boran 1985, Malcolm 1990]. Once again, the possible inclusion of aromatic structures in the form of optically resonant polyphenolic rings is likely to significantly affect humic absorption and fluorescence characteristics [Ertel & Hedges 1983, Malcolm 1990, Senesi 1990].

Another significant difference relating to the origin of HS can be found in carbon isotopic data, namely $\delta^{13}C$ values. Marine HS (-20 $‰$ to -24 $‰$) typically are relatively enriched in ^{13}C compared to their terrigenous counterparts (-25 $‰$ to -31 $‰$) [Skopintsev 1981, Ertel & Hedges 1983, Meyers-Schulte & Hedges 1986, Malcolm 1990]. This indicates that marine HS are primarily autochthonous by virtue of the similarities in their $\delta^{13}C$ values to those of intracellular phytoplankton compounds [Williams 1968, Degens et al. 1968, Skopintsev 1981].

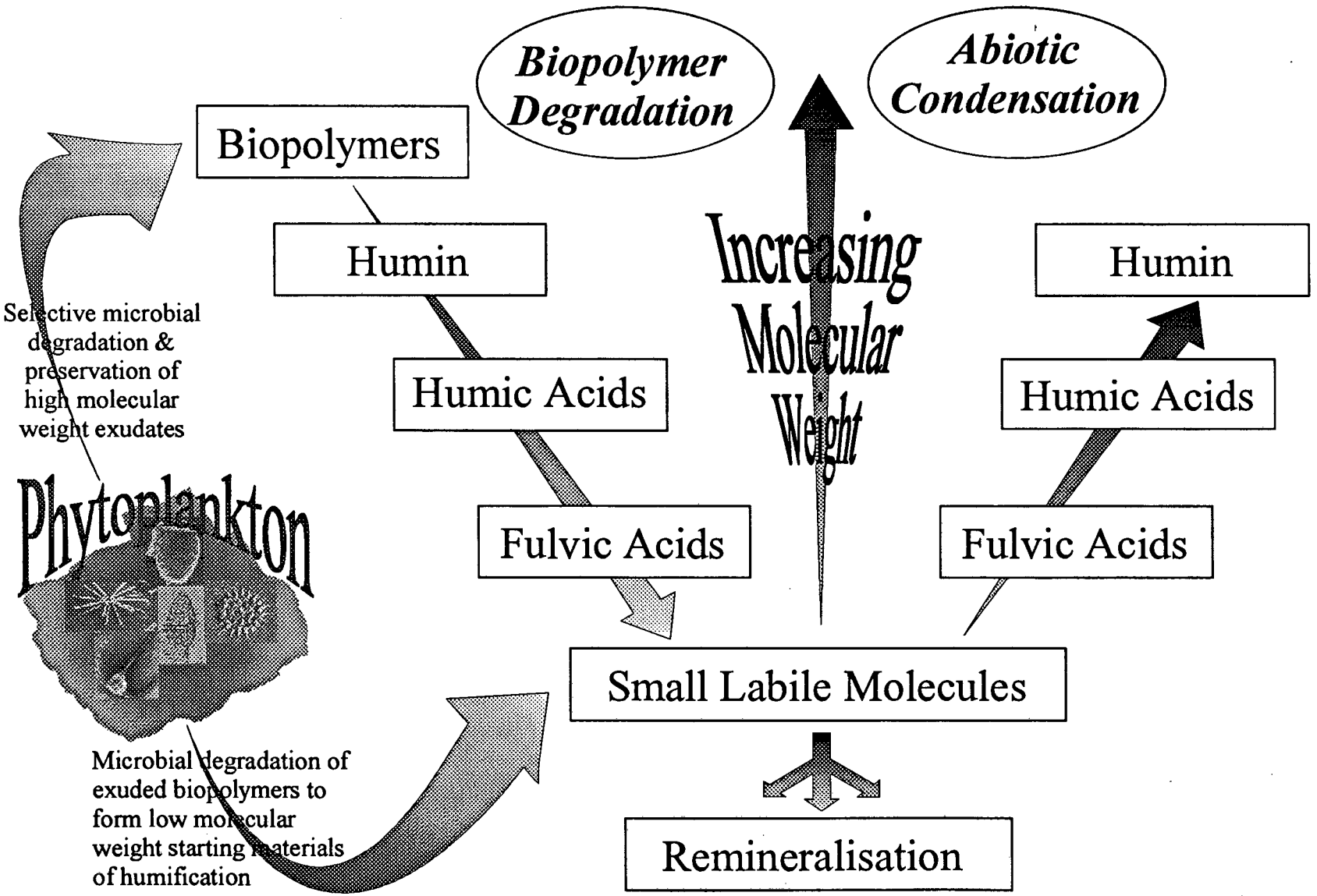
This is further supported by the trends found in elemental composition - marine HS are generally relatively enriched in both nitrogen and hydrogen, reflected by lower C/N and C/H ratios [Nissenbaum & Kaplan 1972, Stuermer & Payne 1976, Ertel & Hedges 1983, 1985, Meyers-Schulte & Hedges 1986]. Phytoplankton are known to have considerably lower C/N ratios than vascular plants, due to the latter having higher proportions of structural compounds [Lancelot & Billen 1985]. This nitrogenous enrichment is considered highly typical of marine HS, and the feasible incorporation of functionally appropriate nitrogen into proposed molecular structures is a central issue for formational models [Hatcher & Spiker 1988, Ishiwatari 1992].

2.2 The formation of fulvic and humic acids

The mechanism of formation for humic substances, commonly called humification, is the subject of much debate and speculation. It is fair to say that there is very little consensus as to a single proposed humification mechanism, which is perhaps consistent with the wide variety of attributes reported for extracted HS. It is quite possible that a variety of processes occur simultaneously, all contributing to "the dynamic continuum of formation and degradation that is humification" [Hatcher & Spiker 1988]. It must be borne in mind that the vast majority of research into HS has been conducted with a focus on soil biochemistry, which for the reasons presented above is likely to differ appreciably in character from chemical processes in the marine environment. Research into marine HS is further complicated by methodological problems, namely the problems of extracting sufficient quantities of truly representative substances from the very small quantities typically present in the marine environment [Benner et al. 1992, Green & Blough 1994]. A further complication, of particular optical significance, is the ability of HS to form complexes with substances ranging from metals [e.g. Mantoura 1981] to other dissolved organic molecules such as phaeopigments [Ertel & Hedges 1983]. This complexation may significantly alter optical properties in a variety of ways ranging from relatively subtle changes in electron transition energies to the inclusion of chromophoric structures. However, although an awareness of such idiosyncrasies is needed, they will be dealt with as the specific need arises.

Proposed humification mechanisms can generally be divided into two categories; one following essentially a selectively degradative pathway, the other reliant on hypotheses of condensation through polymerisation [Hatcher & Spiker 1988, Hedges 1988, Ishiwatari 1992]. These models shall be referred to as Biopolymer Degradation (BD) and Abiotic Condensation (AC), after the nomenclature of Hedges [1988]. They are discussed below with particular reference to autochthonously produced marine humic substances, with the assumption that phytoplankton and marine bacteria represent the main sources of precursive material. A schematic representation of the two models can be seen in figure 2. It must be realised that these proposed routes of humification represent huge simplifications of highly complex natural reactions, especially as concerns assumptions of well ordered and linear diagenetic processes. The opposing nature of the models does not make them mutually exclusive and some of the envisioned processes could in fact be common to both.

Figure 2. *Schematic representation of pathways for marine humification (after Hedges 1988)*



2.2.1 Biopolymer degradation models

There are a variety of proposed humification schemes based on degradative pathways such of those as Waksman [1938], Philip & Calvin [1976] and Poutanen & Morris [1983]. Two of the most contemporary and comprehensive general models are outlined below - those of Hatcher & Spiker [1988] and Ishiwatari [1992]. This does not imply disregard for other proposed models or their obsolescence, but is the simplest means of presenting an overview of a multifaceted subject.

A variety of biomolecular compounds are released from phytoplankton as extracellular exudates through a number of possible mechanisms. These might include metabolism, senescence, exudation for antiviral protection [Murray 1995], sloppy grazing by zooplankton and the egestion of fecal pellets [Nienhuis 1981, Senior & Chevolot 1991, Lee & Wakeham 1992]. Microbial utilisation of these biomolecules results in a selective process of preservation and degradation, with labile compounds being lost and refractory biopolymers eventually being incorporated into humin. This operationally defined humin, regarded as an intermediate step in the humification process, is likely to consist of partially modified plant macromolecules [e.g. Tegelaar 1989], and will reflect the characteristics of the incorporated components of the precursor microorganisms. According to Hatcher & Spiker [1988], further degradation of the insoluble humin results in a lowering of the molecular weight and greater functionality (hence greater solubility), resulting in the formation of humic acids. If the process is carried further, producing still greater functionality and lower molecular weights, fulvic acids are produced. The order of the humic fractions in diagenesis (figure 2) has important bio-optical ramifications, considering the differing absorption characteristics and typically highly weighted concentration ratios of humic and fulvic acids. The continual processes of preservation, degradation and cross linking allows for the incorporation of protein constituents, polysaccharides or even chromophoric molecules, such as pigment fractions, into the humic structure. This ability has been used to explain the relative nitrogenation of marine humics, through the "trapping" or "immobilisation" of reactive nitrogen compounds such as proteinaceous material. It has also been postulated that the generally aliphatic (parafinnic), nitrogen rich nature of marine humics result from the structural inclusion of parafinnic biopolymers, such as algaenan, originating from algae [Tegelaar 1989].

The scheme proposed above has also been presented in modified forms, particularly with respect to the important diagenetic positioning of the humic fractions. Ishiwatari [1992] considers a "biopolymer degradation and polymerisation model" where Maillard (amino-carbonyl) type reactions between biopolymers in various states of degradation are responsible for humification. The functionalities of the humic fractions are attributed in part to amino acid composition; humic acids are dominated by hydrophobic amino acids, whilst hydrophilic amino acids dominate in fulvic acids. This would seem to represent some possibility for parallel formation of the humic fractions; the characteristics defining a fraction would appear to depend more on the nature of the "reactants" than on diagenetic position. Despite this the evidence presented here, of a fairly general nature, is applicable to the Ishiwatari model at this level.

The concept of humification as microbially mediated is consistent with the $\delta^{13}C$ values of marine HS - typically more depleted in ^{13}C than their phytoplankton precursors. This is due to the loss through degradation of the labile fractions of plant matter, such as carbohydrates, which typically have more positive $\delta^{13}C$ values. As this fraction is lost, so the $\delta^{13}C$ values of the preserved material will decrease, reflecting the values of the refractory component of planktonic organic matter [Williams 1968, Degens et al. 1968, Skopintsev 1981]. The similar values of $\delta^{13}C$ values for marine HS and these resistant phytoplankton components are consistent with their position as humic building blocks in the BD model. Published rates of bacterial production on DOM, both humic and non-humic, are also consistent with the general BD model. Bacterial utilisation of DOM, in different aquatic environments, appear to show that whilst non-humic DOM supports considerably more bacterial growth, there is a small but significant contribution associated with the humic fraction [Moran & Hodson 1990, Norrman et al. 1995]. This appears to show both selective microbial utilisation of non-humics e.g. carbohydrates, and the presence of a minority fraction of biolabile HS. Lee & Wakeham [1992] also propose a biologically labile, but chemically refractory component of DOM, particularly associated with high molecular weight (HMW) compounds. This is consistent with both the mechanisms and diagenetic positioning of the BD model. As is the view that the majority of oceanic DOM is relatively small, slowly cycled and biologically unavailable, whilst HMW components are relatively biolabile [Amon & Benner 1994]. Interesting digressions concerning the refractory nature of DOM components arise from the possibilities of bacterial *production* of refractory bio-polymers from low molecular weight (LMW) compounds [Brophy & Carlson 1989], and photodegradation of otherwise refractory DOM components in the photic zone [Kieber et al. 1989, 1990, Chen & Bada 1992].

2.2.2 Abiotic condensation models

It is fair to say that polymerisation (or condensation) models of humification have been widely accepted by marine chemists over the past several decades [Stevenson 1982]. As the name suggests, these models attribute the formation of humic substances to a construction of large organic macromolecules through the use of smaller, relatively well defined molecules as building blocks. They therefore, in a simplistic sense, represent a reversed order of diagenesis to the BD models (see figure 2). Once again, in the interests of constructing an overview, the models presented below do not represent an exhaustive review of the topic, but are based on published schemes of a contemporary and general nature; in this case Hedges [1988] and Ishiwatari [1992]. It must be realised that the term abiotic refers to the actual constructive process of humification - precursive materials are essentially biologically formed and microbially degraded to form the starting points of humification. The origin for marine humification can be considered similar to that presented for the BD model - the release of biopolymers into the water column from microorganisms. These are then microbially degraded to form the starting compounds of condensation schemes; smaller molecules such as phenols, sugars, amino acids, ammonia and lipids.

Polyphenol models

In these models, humification occurs through the reaction of phenols or quinones with nitrogenous substances such as amino acids or ammonia [Flaig 1966, Martin & Haider 1971, Ertel & Hedges 1983]. Schiff base formation between reactants are thought to be responsible for polymerisation, the inclusion of nitrogen, and formation of chromophores [Laane 1984, Hedges 1988]. Whilst these reactions are considered feasible for terrigenous HS, where there is an abundance of polyphenolic and quinone precursors in vascular plants [Flaig et al. 1975, Kirk 1984], they are thought unlikely to occur in the marine environment. This is primarily due to the lack of phenols in phytoplankton, the major organic source - this is evidenced by the typically aliphatic nature of marine HS [Ertel & Hedges 1983, Hedges 1988, Malcolm 1990]. There are also problems associated with the small likelihood of condensation reactions occurring at such small concentrations, and instability of Schiff bases to photodegradation [Harvey 1984]. It is therefore improbable that polyphenol models are viable in the marine environment.

Melanoidin or "browning reaction" models

Polymerisation occurs from the reactions of sugars with amino acids or ammonia [Hoering 1973, Ertel & Hedges 1983]. These amino-carbonyl ("Maillard") reactions form Schiff bases, subsequently forming nitrogen substituted compounds such as glucosamines, which then polymerise in a complex series of reactions [Hedges 1988, Ishiwatari 1992]. The melanoidin model has several attractions from the marine perspective and has received much support over several decades [Kalle 1966, Gagosian & Lee 1981, Ertel & Hedges 1983, Laane 1984]. The carbohydrates and amino acids or ammonia necessary for these reactions generally comprise a majority fraction of phytoplankton released DOM [Lee & Wakeham 1992, Amon & Benner 1994]. Reactions of sugars with basic amino acids, of the type leading to melanoidins, are kinetically favoured over other amino acid types [Hedges 1988]. Isolated humic acids show many similarities to melanoidins, such as bulk chemical characteristics [Hoering 1973, Ertel & Hedges 1983], ^{13}C NMR spectra [Ikan et al. 1986], and fluorescence characteristics [Coble 1996].

However, various criticisms of melanoidin models have arisen. The concentrations of the starting materials in seawater, typically of the order of parts per million, are prohibitive to condensation reactions, which are dependent on intermolecular collisions [Gagosian & Lee 1981, Harvey & Boran 1985, Hedges 1988, Ishiwatari 1992]. It is therefore unlikely that polymerisation will be able to occur unless there is some form of aggregational reaction centre, such as the surface microlayer [Hunter & Liss 1981]. This is further emphasised by the slow reaction rates at typical oceanic temperatures [Hedges 1978], and highly biologically labile nature of free sugars and amino acids in seawater - further reducing the probability of intermolecular collisions [Hedges 1988]. There are also spectral mismatches between melanoidins and natural humic substances in ^{13}C NMR spectra [Hedges 1988 and references therein]. Finally, conclusive experimental evidence has yet to be produced that melanoidins exist in a natural state.

Polyunsaturated lipid condensation models

This class of model attributes the formation of humic substances to the autooxidative crosslinking of polyunsaturated fatty acids, such as unsaturated lipids [Harvey et al. 1983, Harvey 1984, Harvey & Boran 1985]. The resultant products have the ability to incorporate other functional groups, such as chromophoric structures and oxygen or nitrogen containing groups [Harvey et al. 1983, Hedges 1988]. This humification mechanism has been specifically constructed to explain the oxygen enriched, generally aliphatic nature of marine humic substances [Harvey et al. 1983, Harvey & Boran 1985]. Proposed structures are compatible with physical, chemical and spectroscopic properties of natural HS [Ishiwatari 1992], and the model has several other positive attributes. Concentration related problems of other polymerisation models are overcome by the consolidation of reaction centres - the reactive carbon double bonds associated with unsaturation are packed closely in typical fatty chains [Harvey & Boran 1985, Hedges 1988]. Polyunsaturated lipids are also common components of phytoplankton [e.g. Zika 1981].

However, once again there are problems associated with the model. It does not have clear mechanisms for the incorporation of nitrogen and is unlikely to incorporate organic amines [Hedges 1988, Ishiwatari 1992]. Incompatibilities also arise in the comparative $\delta^{13}C$ values of marine HS and phycological lipids, which are generally 3 to 5 $‰$ lower [Ishiwatari 1992]. Although laboratory synthesis of lipid derived humics [Harvey & Boran 1985] has shown feasible experimental evidence for this isotopic fractionation, the magnitude is considered too large in theory to be achieved with this form of reaction [Hedges 1988, Ishiwatari 1992]. It is worth noting that non lipid components of phytoplankton have similar $\delta^{13}C$ values to autochthonous humic and fulvic acids [Williams 1968, Degens et al. 1968, Skopintsev 1981].

Chapter 3

The absorption of gelbstoff

The term gelbstoff, and its equivalents, have come into existence through the need to categorise groups of highly absorbing organic compounds in the context of their colour and its effects. Colour, strongly dependent upon the spectral nature of light absorption, therefore becomes definitive when viewing the relevant constituent compounds of gelbstoff through its collective lens. Such a fundamental property has become the basis on which gelbstoff is quantified. It would be fair to say that the closest approximation to an index of gelbstoff "concentration" is its absorption coefficient, determined through the spectrophotometric measurement of a filtered water sample [Carder et al. 1989, Green & Blough 1994, Mueller & Austin 1995]. Alternative techniques, such as fluorescence and the analysis of isolates, can be considered either indirect routes to quantifying absorption, or the examination of gelbstoff *constituents*. Of course, gelbstoff is not only measured and defined by its absorption properties - the effect of gelbstoff absorption on remotely sensed ocean colour is the primary reason for its bio-optical importance.

3.1 The absorption coefficient as a physical variable and its effects on ocean colour

Before discussing the absorption properties of gelbstoff, it is important to understand the nature of absorption, or more correctly the volume absorption coefficient [Priesendorfer 1976], and the way in which absorption processes affect ocean colour. In any hydrosol, which can be considered the water and its constituents, the absorption coefficient a is considered an inherent optical property (IOP) [Priesendorfer 1976] i.e. a fundamental property that is independent of the geometry of the incident light field. This effectively means that any optical classification of a water type (e.g. as occurs through the application of the SeaWiFS semi-analytical algorithm) must account for all significant absorption processes in the target hydrosol. In ocean waters unaffected by either dissolved or suspended terrigenous material, which must be considered on a site specific basis [Gordon & Morel 1983, Carder et al. 1991], the principal absorption processes can be assumed to result from phytoplankton, gelbstoff, detritus (i.e. non-living particulate matter of biological origin), and the water itself [Gordon & Morel 1983, Gordon et al. 1988, Carder et al. 1986, 1989, 1991, Lee et al. 1994]. It is these processes, in conjunction with the various scattering processes of the water and its constituents, that are considered to be primarily responsible for the quality of the light leaving the sea surface. In certain circumstances, other optical processes may make significant contributions, such as the fluorescence of either gelbstoff (see Chapter 4) [Spitzer & Dirks 1985, Hawes et al. 1992, Vodacek et al. 1994, Lee et al. 1994] or photosynthetic pigments [e.g. Gower & Borstad 1981], and inelastic (Raman) scattering by the water [Stavn & Weidermann 1988, Marshall & Smith 1990, Lee et al. 1994]. However, owing to both the complexities of modelling these phenomena, and the fact that they are viewed as being only of occasional significance, these processes are not currently accounted for in the proposed SeaWiFS algorithm. A schematic of the processes contributing to the underwater light field can be seen in figure 3. The remotely sensed reflectance R_{rs} , equivalent to the corrected satellite received signal, can therefore be represented by equation 3.1 - expressed solely in terms of the inherent optical properties of the backscattering and absorption coefficients. [e.g. Carder et al. 1991, 1995]. The simplifications inherent in this expression are beyond the scope of this study, and are examined in closer detail by Preisendorfer [1976], Carder et al. [1986, 1989, 1991, 1995] Gordon et al. [1988], Morel & Gentilli [1993], and Lee et al. [1994], amongst others.

$$R_{rs}(\lambda) = \text{constant}(b_{bw}(\lambda) + b_{bp}(\lambda)) / (a_w(\lambda) + a_g(\lambda) + a_p(\lambda)) \qquad 3.1$$

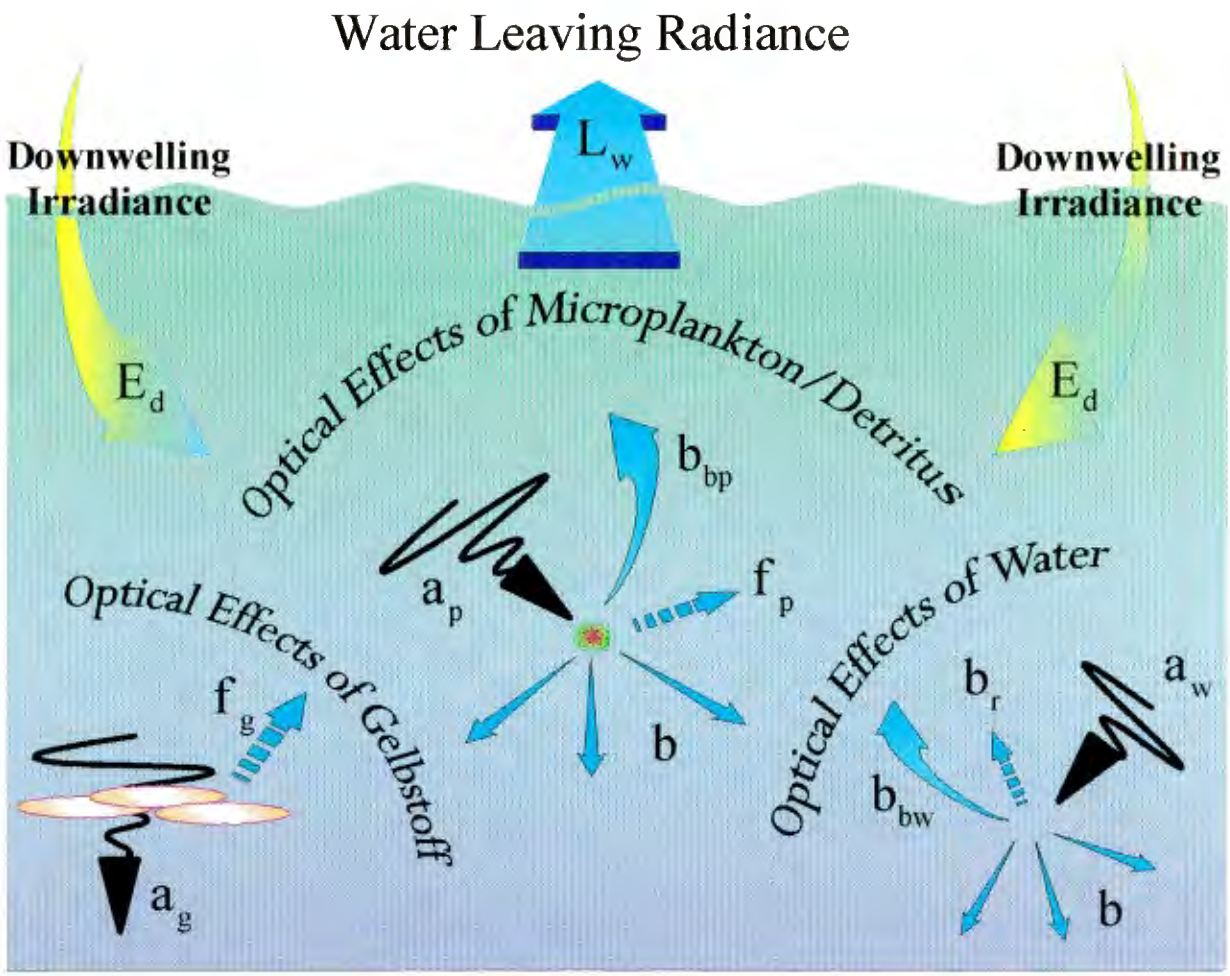


Figure 3. *Schematic representation of bio-optical processes potentially affecting the water leaving radiance. Equation 3.1 shown above is an expression in the same form as that used in the proposed semi-analytical SeaWiFS algorithm [Carder et al. 1995]*

Bio-optical Variables

$R_{rs}(\lambda)$ = remotely sensed reflectance
= $\pi L_w(\lambda) / E_d(\lambda, 0\text{-})$ [sr^{-1}]
$L_w(\lambda)$ = water leaving radiance [$W\ m^{-2}\ sr^{-1}$]
$E_d(\lambda)$ = downwelling irradiance [$W\ m^{-2}$]
$b(\lambda)$ = scattering coefficient [m^{-1}]
$b_b(\lambda)$ = backscattering coefficient [m^{-1}]
$a(\lambda)$ = absorption coefficient [m^{-1}]
f = fluorescence

Subscripts

p = particulate (microplankton and detritus)
g = gelbstoff or CDOM
w = water
r = raman

3.2 Characterisation of gelbstoff absorption

Gelbstoff absorption spectra tend to have very typical shapes - decreasing exponentially with wavelength from a maximum in the ultra-violet (as discussed earlier). This exponential shape lends itself to the following mathematical description [Zepp & Schlotzhauer 1981, Bricaud et al. 1981, Hayase & Tsubota 1985, Carder et al. 1986, 1989, 1991, Blough et al. 1993, Hoge et al. 1993, Green & Blough 1994, Nelson & Guarda 1995] :

$$a_g(\lambda) = a_g(\lambda_r)\exp[S(\lambda_r - \lambda)] \qquad 3.2a$$

or, if absorption is normalised to either mass or carbon :

$$a_g^*(\lambda) = a_g^*(\lambda_r)\exp[S(\lambda_r - \lambda)] \qquad 3.2b$$

where a_g =absorption coefficient (m^{-1})

a_g^* =specific absorption coefficient (m^{-2} g^{-1} for mass specific)

λ_r =reference wavelength (nm)

S =spectral slope (nm^{-1})

The application of this relationship to gelbstoff absorption gives the ability to characterise spectra by two variables: the reference wavelength λ_r and the spectral slope S. Unfortunately both the choice of λ_r, and the wavelength range chosen for modelling e.g. 350nm - 700nm [Bricaud et al. 1981], have not been constant amongst researchers. It is therefore important to have an appreciation of the constraints used by researchers when comparing modelled gelbstoff absorption parameters. Nevertheless this approach has been widely used and is employed by the SeaWiFS semi-analytical algorithm in its classification of gelbstoff absorption, which uses a reference wavelength of λ_r = 400nm. On a more basic level, such a relationship shows that typically featureless gelbstoff absorption spectra only really have two means of classification. Assuming wavelength dependence, these are the magnitude or absolute value - in this case the reference absorption $a(\lambda_r)$ - and the slope, or first derivative with respect to wavelength - in this case S. It is worth noting that earlier marine and soil science studies employ simplified variations of the slope parameter, based on the ratio of *absorbance* at two wavelengths e.g. k_{420}/k_{665} [Kalle 1966],

or the more usual E4/E6 (the ratio of absorbance at 465nm and 665nm respectively [Chen et al. 1977, Ertel & Hedges 1983].

The curves given by equations 3.2a and 3.2b have also been presented in modified form to represent the relative contributions of fulvic and humic acids to gelbstoff absorption [Carder et al. 1989, 1991]:

$$a_g(\lambda) = C_f a_f^*(\lambda_r)\exp[S_f(\lambda_r-\lambda)] + C_h a_h^*(\lambda_r)\exp[S_h(\lambda_r-\lambda)] \qquad 3.2c$$

where C =concentration of fulvic/humic acid (g m^{-3})

a* =specific absorption coefficient (m^{-2} g^{-1})

S =fulvic/humic spectral slope (nm^{-1})

f =fulvic acid

h =humic acid

Using this relationship it is possible to provide a measure of the effects of the two fractions of marine humic substances on both the magnitude and spectral slope of gelbstoff absorption. However, this equation is dependent on two assumptions: that gelbstoff is composed only of fulvic and humic acids, and that the absorption of combined fulvic and humic acids can be represented by summation of the isolated fractions. The first assumption has already been discussed [Chapter 2] - the second can be criticised as evidence exists that the summed absorption for different fractions of isolated humic substances is non-linear [Wang et al. 1990]. Wang and his co-researchers observed increases in the longer wavelength absorption, or a red shift, when fulvic acid fractions of different molecular weights are combined. This may effectively lead to over prediction in slope values if equation 3.2c is followed. Nevertheless, it is a useful mechanism for studying trends in behaviour related to the relative abundance of the fulvic and humic fractions. This may be of potential importance in scenarios where atypical fulvic/humic ratios might arise, such as periods around bloom senescence, as discussed below.

3.3 The variability of gelbstoff absorption

It is important to appreciate what factors are potentially responsible for variations in gelbstoff absorption, both from a remote sensing perspective and to promote understanding of the interrelationships between environmental influences and bio-optical properties. Once again the assumption that gelbstoff is comprised only of humic substances is central to the discussion, which will focus primarily on their attributes.

3.3.1 Effects of the structure of fulvic and humic acids

The spectroscopic properties of HS are determined primarily by their molecular structures, which have been seen to display a wide degree of variation [Chapter 2]. It follows that the absorption characteristics of HS vary widely, across both different environments of formation and the different humic fractions. Researchers have examined both extracts and natural water samples to determine the effects on absorption of such factors as molecular weight, aromaticity, acidity, and elemental composition. Secondary, or circumstantial factors, such as pH, concentration and salinity have also been examined. There are probably three primary structural phenomena affecting absorption [Chen et al. 1977, Choudry 1981, Choudry 1984] :

- Molecular weight or size.
- The relative abundance and configuration of chromophores and other functional groups within the molecular structure.
- Total carbon content.

One of the principle determinants of both the slope and magnitude of absorption is considered to be molecular weight or size [Chen et al. 1977, Stewart & Wetzel 1980, Hayase & Tsubota 1985, Carder et al. 1989, Wang et al. 1990]. Interestingly, fulvic acids appear to have different characteristics to humic acids, even when HS from a variety of origins are compared. As concerns the magnitude of absorption, fulvic acids appear to show a direct correlation i.e. increasing specific absorption with molecular weight, whilst the reverse is true of humic acids - specific absorption decreases with molecular weight [Ghassemi & Christman 1968, Chen et al.

1977, Hayase & Tsubota 1985, Carder et al. 1989, Wang et al. 1990]. Good correlations have also been found for the slope parameters of fulvic acids, revealing that slopes become smaller (or flatter) as molecular weight increases [Chen et al. 1977, Hayase & Tsubota 1985]. The slope parameters of humic acids, on the other hand, do not appear to *consistently* display such significant variation with molecular weight [Hayase & Tsubota 1985, Carder et al. 1989], although there is some evidence that they display similar trends to those of fulvic acids [Chen et al. 1977, Banerjee 1979]. It should be noted that secondary relationships exist between absorption parameters and pH, %O, %C, CO_2H (carboxyl) content, free radical concentration and total acidity - all of which are functions of molecular weight to some extent [Chen et al. 1977, Brown 1977 and references within, Choudry 1981, Wang et al. 1990]. These relationships, in particular the relative abundance of carboxyl groups, have been used to explain the stronger absorption in the blue part of the spectrum by LMW compounds - leading to steeper slopes [Butler and Ladd 1969, Chen et al. 1977, Wang et al. 1990]. [1]

The effects of chromophore abundance and configuration on absorption has generally been attributed to the following factors :

- the degree of condensation of the aromatic rings in the molecular structure [Choudry 1981 and references within].
- the degree of chromophore conjugation [Ertel & Hedges 1983, Wang et al. 1990].
- the ration of carbon in aromatic "nuclei" to carbon in aliphatic or alicyclic side chains [Choudry 1981 and references within].

It would thus appear that the abundance and nature of the aromatic structures within the molecule have the most significant effects on absorption.. Relatively aromatic molecules will tend to have

1. It must be remembered that humic chemistry is both complex and poorly understood, and this inhibits the formation of clearly defined cause-effect relationships between structural variables and absorption parameters. Many of the physical and chemical variations discussed here are interrelated to some degree, and to ascribe particular absorption effects to one of many covarying factors is difficult. An example of this is the apparent effect of steeper slope parameters associated with both low molecular weights and abundance of carboxyl groups for fulvic acids [Brown 1977 and references within, Chen et al. 1977, Wang et al. 1990]. As LMW fulvic acids appear to have a higher carboxyl content as a matter of course, it is difficult to describe either variable as "primary" as regards its effect on absorption. However, the "apparent" effects of either variable have been demonstrated.

both higher specific absorption coefficients and lower slope values. This again can be seen from a comparison of terrigenous and marine HS, and of the fulvic and humic fractions. The more aromatic terrigenous HS, products of their polyphenolic type precursors [Chapter 2], tend to absorb more strongly per unit carbon, and display flatter slope values than their marine counterparts [Zepp & Schlotzhauer 1981, Ertel & Hedges 1983, Malcolm 1990]. This tendency is not only evident from the analysis of extracted HS, but also from natural measurements of gelbstoff absorption, particularly in areas with strong terrigenous influences. Researchers have consistently shown both lowered absorption values and steeper slopes as the transition is made from systems dominated by terrigenous gelbstoff to those dominated by marine gelbstoff [Kalle 1966, Brown 1977, Hojerslev 1988, Blough et al. 1993, Nelson & Guarda 1995].

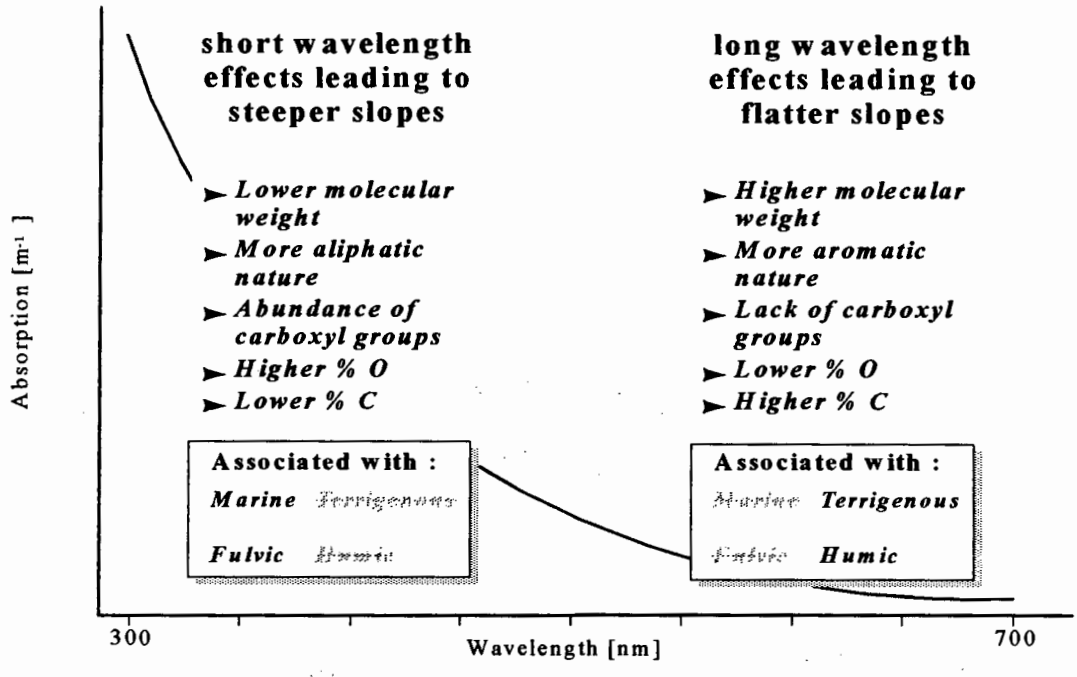


Figure 4. *Summary of probable structural effects of humic substances on absorption slope parameter S_g. Structural effects and their associations with humic fractions are given in a relative sense.*

Absorption variations related to the aromatic nature of the molecule are also displayed through a comparison of fulvic and humic acids of the same origin. The generally more aromatic humic acids [Chapter 2] consistently display higher specific absorption coefficients and lower slope parameters [Ghassemi & Christman 1968, Chen et al. 1977, Zepp & Schlotzhauer 1981, Hayase & Tsubota 1985, Carder et al. 1989]. The influence of chromophore configuration is

perhaps most striking when the effects of molecular size are negated by studying humic and fulvic fractions of comparable molecular weights [e.g. Hayase & Tsubota 1985]. However, it is extremely difficult to ascribe these effects to any particular structural characteristic of the humic molecule (if even necessary). Take, for example, the hypothesis that optical behaviour is primarily governed by the degree of condensation of aromatic structures - this would appear unlikely if the generally highly aliphatic nature of marine HS are considered [Chen et al. 1977]. On the other hand, the evidence presented above would appear to indicate that the presence of aromatic structures exerts a significant optical influence. A prudent conclusion, in the context of this study, might be that it is simply the relative abundance of aromatic groups that influences the nature of absorption.

3.3.2 Effects of the fulvic : humic ratio

As the perspective is changed from the theoretical influences of molecular structure to larger scale considerations of *in situ* gelbstoff absorption, it is apparent that the relative concentrations of the humic fractions is an important variable. Gelbstoff absorption can be regarded as an aggregate quantity, and in this context the relative abundance of fulvic and humic acids, sometimes expressed as the fulvic fraction [Carder et al. 1989], is perhaps likely to exert as much, if not more of an influence than relatively subtle changes in the character of an isolated fraction. In the marine environment, fulvic acids are generally far more prevalent than humic acids, typically comprising 85% or more of HS [Harvey et al. 1983, Carder et al. 1989, 1991, Malcolm 1990]. In effect this means that fulvic acids are often dominant contributors to gelbstoff absorption, despite their relatively small specific absorption coefficients. The generally steeper slope parameters of fulvics will also lend them greater significance at shorter wavelengths - the part of the spectrum targeted by SeaWiFS for gelbstoff detection through the 412nm band. However, whilst these statements are relevant in the vast majority of marine scenarios i.e. relatively oligotrophic environments, in highly productive areas significant deviation can be expected. The data of Harvey et al. [1983] shows a significant increase in the relative concentration of humic acid around a period of bloom senescence, specifically at their Cape San Blas station (a fulvic fraction of 0.63, as opposed to a typical oligotrophic value of 0.90). It would be possible to interpret such data as being consistent with the diagenetic positioning of the Biopolymer Degradation models discussed in chapter 2 - i.e. humic acids being precursors to

fulvic acids. If this is the case then it is quite possible that such temporary increases in the relative humic acid concentration (or decrease in the fulvic fraction) is not unusual as concerns bloom senescence. Carder et al. [1989] have shown that a_g is particularly sensitive to humic acid concentration, as a result of its typically high a_h^* values. This means that even a small increase in the humic fraction may have a significant effect on absorption parameters, increasing the magnitude of absorption and flattening the slope parameter. This can be seen in figure 5, which shows the changing effects of the fulvic fraction on gelbstoff absorption, through the use of equation 3.2c.

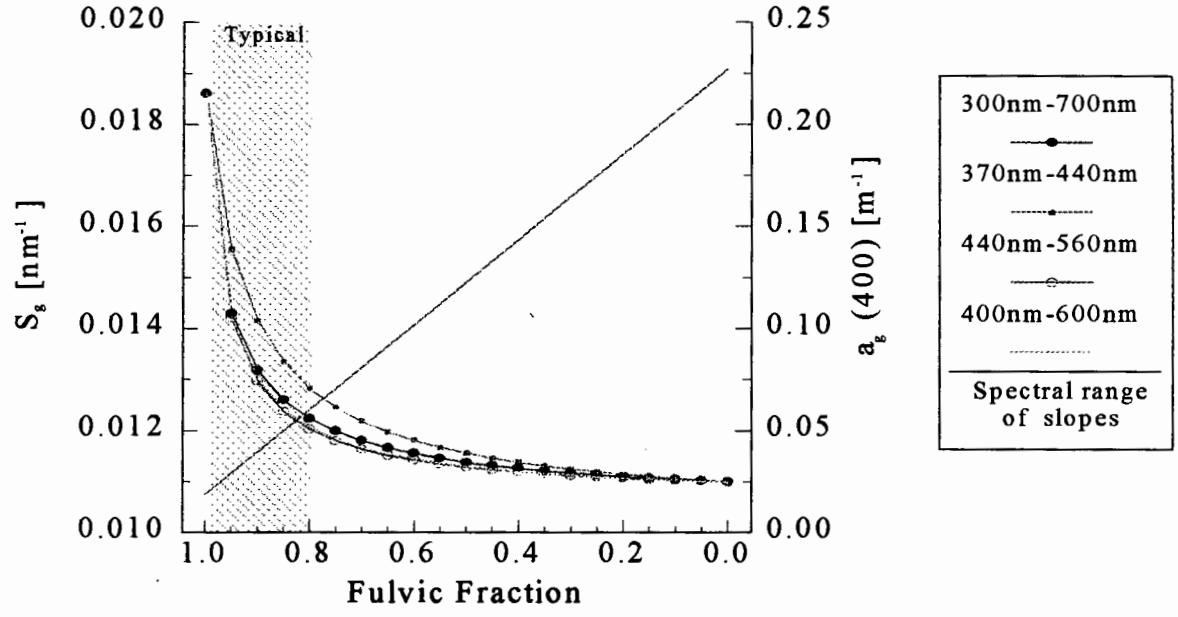


Figure 5. *The effects of the increasing relative concentration of humic acid on gelbstoff absorption parameters.* ***Note the sensitivity of S_g to changes in the fulvic fraction at typical values of 0.8 to 0.98, and the increase in a_g (400) with humic acid concentration.*** *S_g has been determined using various spectral ranges, showing the importance of consistent range selection when comparing published data. A total concentration of 1 g m^{-3} for HS is assumed, with absorption parameters based on the data of Carder et al. [1989].*

Based on this, and in the context of assessing the effects of the fulvic : humic ratio, it would seem likely that any significant deviations from assumed SeaWiFS slope values are likely to be humic acid induced reductions in value, particularly in highly productive areas subjective to rapid changes. However, it must be remembered that this is to some degree speculation, and that both the likelihood and possible effects of such changes must be assessed through measurement and modelling of the appropriate bio-optical parameters.

3.3.3 Effects of the environment

Environmental effects refer to those phenomena arising primarily from influences external to the HS themselves, such as the thermochemical properties of seawater or light induced reactions. Of these, the effects of changing salinity and photo-oxidation or bleaching will be focused on, as they may be considered the most significant apparent effects as concerns measured gelbstoff absorption in the marine environment [Brown 1977, Hojerslev 1988, Kieber et al 1990, Blough et al 1993, Esteves et al. 1995]. Whilst the effects of large changes in salinity may not be strictly relevant when considering what are essentially marine systems, the proposed causes of these effects are pertinent, and addressing the issue can be considered an indirect way of assessing some of these environmental influences.

There is no doubt that pH also exerts a considerable influence on the optical properties of gelbstoff [e.g. Chen et al. 1977, Ewald et al. 1988], but in view of the relatively small changes in pH generally found in marine surface waters [Millero & Sohn 1992], these effects can be considered either artefactual or relevant only to estuarine environments [Ferrari 1991] and will be considered as the need arises. It is possible that in extreme cases other factors such as HS concentration itself [Chen et al. 1977] or the presence of pollutants such as metals, pesticides or other organics [e.g. Wang et al. 1990, Ferrari 1991] may make an impact, but again these need to considered on a case specific basis.

In a more general bio-optical context it is apparent that photobleaching, and its associated loss of chromophoric activity, may have significant effects on absorption. Humic substances have been considered the major source of photochemically produced compounds in the ocean [Mopper et al. 1991], and very good correlations have been reported between photochemical production and photodegradative absorbance losses [Kieber et al. 1990]. However, the small amount of work in the field has produced conflicting reports, particularly as regards the spectral alteration of absorption properties. Whilst there seems little doubt that there can be significant losses in absorption (e.g. an approximate 10% loss in a_g at 300 nm after 4 hours of irradiation in sunlight [from the data of Kieber et al. 1990]), there are conflicting reports as to how S_g is affected, i.e. what portion of the spectrum is most changed. Slawinski et al. [1978] reports an increase in slope values with insolation, with most of the visible spectrum losing absorbance to some degree.

However, these results were obtained using Fluka humic acids derived from soils. Perhaps the findings of Kieber et al. [1990], obtained using various aqueous samples, are more applicable to this study. These indicate that the main loss of absorption occurs at ~300 nm, with little change in absorption after insolation for wavelengths longer than 400 nm. This would result in a lowering of slope values, a conclusion supported by Zepp [1992, as cited in Blough et al. 1993]. Whilst there is some possibility that bacterial activity, possibly influenced by insolation, may be indirectly responsible for spectral alteration, evidence has been presented showing negligible biological effects on photodegradation [Kieber et al. 1989, 1990]. Loss of absorbance also appears to be highly correlated with initial absorbance values [Kieber et al. 1990] - a possible ramification of this may be that humic acids, with their typically significantly higher specific absorption coefficients, are more prone to photodegradation than their fulvic counterparts. This would also hold true in a comparison of the generally more highly absorbing terrigenous HS with their marine counterparts, possibly a factor in the salinity related effects discussed below. It is also worth noting that the *absorption normalised* photochemical reactivity of HS from a wide range of environments appear to be very similar, perhaps indicating that photodegradative loss of gelbstoff absorption is relatively predictable.

Since the earliest studies of gelbstoff, coherent relationships have been found between salinity and gelbstoff absorption [Kalle 1966, Jerlov 1976, Brown 1977, Hojerslev 1988, Blough et al. 1993, Ferrari & Mingazzini 1995]. These have generally manifested themselves as the lowering of absorption coefficients and the steepening of slope parameters with increasing salinity (or marine influence). It appears unlikely that salinity changes have any direct effect of the nature of HS [Esteves et al. 1995] - observed absorption effects are perhaps better thought of as *associated* with salinity. Whilst it is possible to ascribe at least some of these variations to the transition from riverine to oceanic influence, and the resultant effects of HS of differing origins and concentrations, it is likely that the effects of changing salinity on gelbstoff absorption is somewhat more complex. A non-conservative relationship is suggested by the data of Brown [1977] and Blough et al. [1993], identifying non-linear effects on absorption parameters through different salinity regimes.

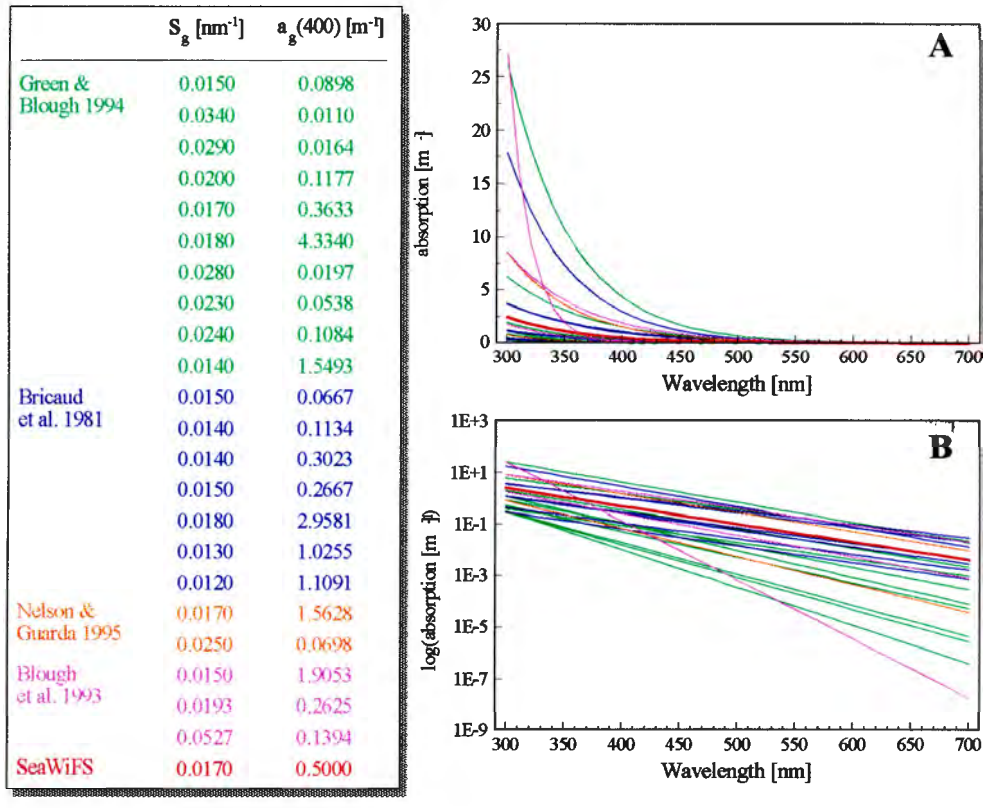
Various factors have been proposed as mechanisms for spectral alteration, specifically the prominent increases in S_g [Blough et al. 1993] :

- Changes in pH, ionic strength, or HS concentration with salinity.
- Preferential bacterial consumption of the longer wavelength absorbing components of HS at higher salinities.
- Preferential precipitation of longer wavelength absorbing components at higher salinities.
- Selective photobleaching of longer wavelength absorbing components at higher salinities

The first of these factors is disregarded by Blough, although in dissimilar riverine/estuarine environments pH effects have been demonstrated [Ferrari 1991, Millero & Sohn 1992]. However bacterial consumption appears, at least potentially, capable of spectral alteration. Although Brown [1977] suggests that bacterial activity lowers slope values, there is evidence for preferential bacterial consumption of the higher molecular weight fraction of DOM [Lee & Wakeham 1992, Amon & Benner 1994]. If this is the case then the removal of HMW humic substances, shown to be associated with lower slope values, could be consistent with observed increases in slope parameters. However, direct evidence for significant coherence between bacterial utilisation and salinity is lacking, although there is some evidence for an increased bacterial production at riverine plume boundaries [Kirchman et al. 1989]. The selective coagulation and precipitation of longer wavelength absorbing fractions is another possible reason for salinity related effects, particularly as the HMW components are thought to be more likely to precipitate [Brown 1977 and references within]. However, the major part of DOM removal is thought to occur at appreciably lower salinities than those at which slope modification is most significant [Blough et al. 1993 and references within]. As concerns the interactive effects of salinity and photobleaching, whilst there is no doubt that photoreactions can significantly alter the bio-optical properties of HS [Choudry 1984 and references within, Kieber et al. 1989, 1990, Mopper et al. 1991], it is difficult to comment on these effects in the context of salinity related spectral alterations. It seems unlikely that insolation significantly or coherently changes with salinity, inferring that a variation in photosensitivity associated with the changing structure of HS through salinity regimes would have to be responsible. In the light of the previous discussion of photobleaching, a purely speculative explanation might be that the more highly absorbing terrigenous HS, associated with lower salinities, are more prone to loss of lower wavelength absorbance, with resultant lower slope values [Kieber et al. 1990].

3.4 Reported values of gelbstoff absorption

A range of absorption data from the literature is displayed in figures 6 and 7, demonstrating the respective variations in gelbstoff and extracted humic substances. It should be noted that the data are selected to show the widest reported range for both $a_g(\lambda)$ and S_g in the marine environment, and it is fair to say that some of the values shown represent extreme bio-optical conditions. The value of S_g to be used by the SeaWiFS semi-analytical algorithm is shown for reference in both figures [Carder et al. 1995]. The data shown represent a variety of regions and seasons, including South Florida and the Gulf of Mexico [Carder et al. 1989, Green and Blough 1994], the Caribbean and Gulf of Pariah [Blough et al. 1993], the South Atlantic Bight [Nelson & Guarda 1995], and the Mauritanian upwelling region, the Gulf of Guinea, the coastal Mediterranean, the Baltic and the North Sea [Bricaud et al. 1981].



	S_g [nm^{-1}]	$a_g(400)$ [m^{-1}]
Green & Blough 1994	0.0150	0.0898
	0.0340	0.0110
	0.0290	0.0164
	0.0200	0.1177
	0.0170	0.3633
	0.0180	4.3340
	0.0280	0.0197
	0.0230	0.0538
	0.0240	0.1084
	0.0140	1.5493
Bricaud et al. 1981	0.0150	0.0667
	0.0140	0.1134
	0.0140	0.3023
	0.0150	0.2667
	0.0180	2.9581
	0.0130	1.0255
	0.0120	1.1091
Nelson & Guarda 1995	0.0170	1.5628
	0.0250	0.0698
Blough et al. 1993	0.0150	1.9053
	0.0193	0.2625
	0.0527	0.1394
SeaWiFS	0.0170	0.5000

Figure 6. *A selection of modelled absorption data from the literature, shown in respect to the value of a_g for the proposed SeaWiFS semi-analytical chlorophyll algorithm. Note that the above values represent "natural" measurements of gelbstoff absorption i.e. those performed on filtered water samples. A value of 0.5 m^{-1} for a_g (400) has been assumed for the SeaWiFS data, for the purposes of comparison.*

The only information regarding gelbstoff absorption for southern African waters is that of Walters [1986 or 1987]. However, the measurement protocols used for obtaining these data differed markedly from both the SeaWiFS measurement protocols [Mueller & Austin 1995], and those of other researchers [figure 6], and little would be gained from a comparison.

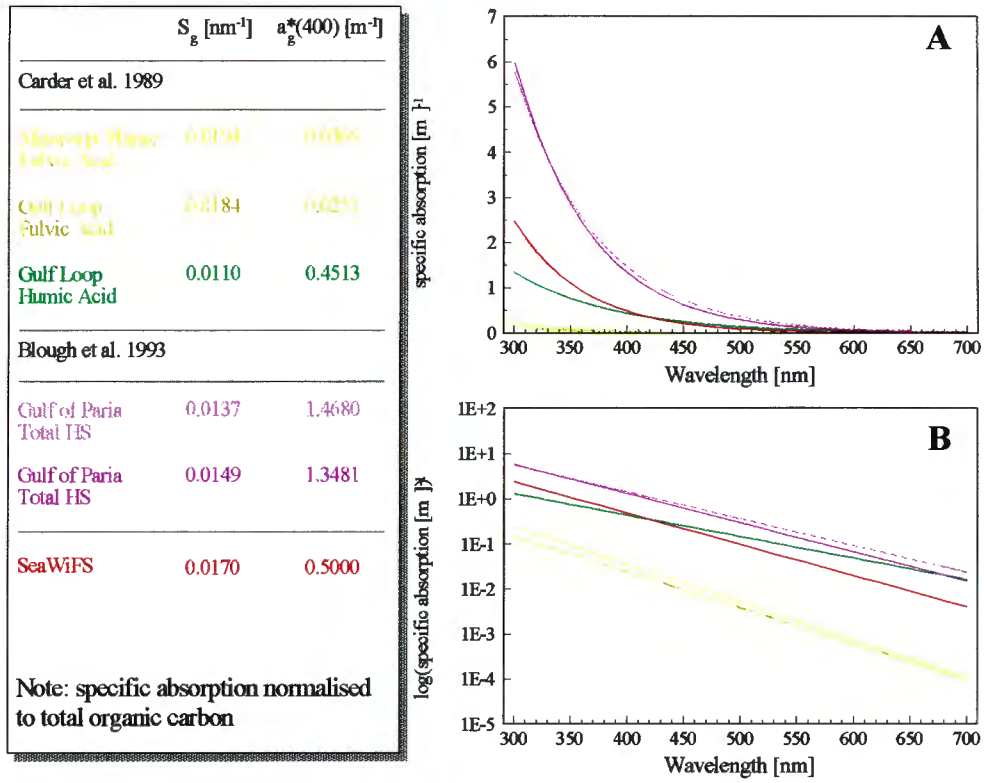


Figure 7. *A selection of modelled absorption data from the literature for extracted fulvic and humic acids, and total humic substances. These are shown in comparison to the value of a_g to be used by the proposed SeaWiFS semi-analytical algorithm, using the same constraints as figure 6.*

It can be seen from figures 6B and 7B that the slope value of the proposed semi-analytical SeaWiFS algorithm would appear to represent a reasonable "global" average, even in this set of highly varied absorption data. However, the data set also shows that there can be significant deviation from the SeaWiFS value, and in highly productive and rapidly changing systems, it is this point that is important.

Chapter 4

The fluorescence of dissolved organic matter

One of the first recognised properties of marine dissolved organic matter was that it had an ability to fluoresce when irradiated with ultra-violet or visible light [Kalle 1966 and references therein]. This fluorescence can have important consequences in the marine environment, arising both from "natural" (i.e. sunlight stimulated) fluorescence, or from the use of artificially induced fluorescence as an analytical tool. From the perspective of remotely sensed ocean colour, sunlight stimulated DOM fluorescence can make a significant contribution to the water leaving radiance signal under certain circumstances [Spitzer & Dirks 1985, Peacock et al. 1990, Hawes et al. 1992, Lee et al. 1994, Vodacek et al. 1994], a contribution that is currently unaccounted for in the SeaWiFS algorithm.

The main body of research on DOM fluorescence has concerned the use of artificially induced fluorescence as an analytical tool. Investigations have been made into several facets of DOM fluorescence - absorption/fluorescence relationships [Stewart & Wetzel 1980, Hayase & Tsubota 1985, Donald et al. 1987], alternative, more sensitive and simpler methodological routes to gelbstoff absorption through fluorescence [Ferrari & Tassan 1991, 1992, Hoge et al. 1993, Green & Blough 1994, Vodacek et al. 1995], the characterisation or structural elucidation of DOM and its precursors [Larson & Rockwell 1980, Visser 1983, Senesi 1990, Coble et al. 1990, 1993, Seritti et al. 1994, Ferrari & Mingazzini 1995], the fluorometric determination of chemically related parameters such as DOC [Smart et al. 1976, Laane & Koole 1982, Philpot & Vodacek 1989, Vodacek 1992], and the use of fluorescence to examine the distribution of dissolved components [Postma et al. 1976, Cabaniss & Schumann 1987, Hayase et al. 1987, 1988, Chen & Bada 1989, 1992, Hoge et al. 1993, Mopper & Schultz 1993, Determann et al. 1994].

The importance of DOM fluorescence to this study lies in two distinct areas - its potential impact on the water leaving radiance signal and the possible effects of this on the SeaWiFS algorithms, and the ability to use fluorescence analytically both to facilitate the measurement of gelbstoff absorption and to improve understanding of gelbstoff structural characteristics and its pathways of formation and degradation.

4.1 The characterisation of DOM fluorescence

Attributing *all* of the fluorescence emitted by seawater samples to gelbstoff would be somewhat misleading. Whilst the emission of fluorescence is obviously dependent on an input of energy through absorption, the quantum efficiencies of marine compounds vary significantly. It is possible that compounds such as tyrosine, with relatively high quantum efficiencies, may make little apparent contribution to net absorption effects whilst displaying significant fluorescence [Datta et al. 1971, Ferrari & Mingazzini 1995]. With this in mind, the discussions in this chapter will generally refer to DOM fluorescence, although in effect the majority of this can be attributed to HS or their precursors [Laane & Koole 1982, Hayase et al. 1987, 1988, Chen & Bada 1989, Ferrari & Mingazzini 1995].

An appreciation of the factors affecting DOM fluorescence is perhaps most easily achieved through a discussion of induced, or analytical fluorescence, rather than natural occurrences through sunlight stimulation. Induced fluorescence involves fewer variables than the complex natural phenomenon, making for a simpler framework in which to explore controlling factors. By virtue of its nature fluorescence must involve at least three variables - excitation wavelength, emission wavelength, and fluorescent intensity - and different analytical perspectives can be achieved through the appropriate control of variable combinations. Any discussion of fluorescence must therefore begin with an understanding of the analytical methodologies available.

Four main different types of fluorescence scanning are generally used for analysis: emission scans, excitation scans, synchronous scans, and excitation/emission scans. They are presented below with relevant examples from the literature - figures 8 to 11.

Excitation scans

Excitation spectra are generated by measuring fluorescent intensity at a constant emission wavelength whilst exciting at a range of wavelengths. Theoretically, if all absorbed energy is re-emitted as fluorescence, then the excitation spectra of a sample should have an identical shape to the absorption spectra - in practice this is unlikely to happen with HS, as can be seen in figure 8. This is partly due to the complex polychromophoric nature of HS, giving rise to possible spectral variations in quantum efficiency, internal quenching and inter-chromophore energy transfer [Donard et al. 1987, Senesi 1990].

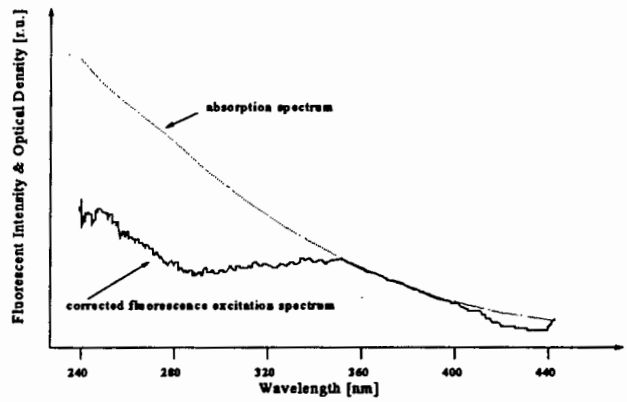


Figure 8. *An example of an excitation scan, in this case a comparison with the absorption spectra of an aqueous fulvic acid. (Reproduced from Donard et al. 1987)*

Emission scans

Emission spectra are generated by exciting at a fixed wavelength, whilst scanning a range of emission wavelengths. This is probably the most widely used simple form of fluorescence scanning, and scans can also be referred to as fluorescence spectra [e.g. Stewart & Wetzel 1980, Dujmov et al. 1992, Hoge et al. 1993, Green & Blough 1994]. The shape of an emission spectrum, *for a single fluorophore*, should be independent of excitation wavelength [Senesi 1990, Coble et al. 1990, Coble 1996]. However, due to the polychromophoric nature of DOM, this is unlikely to be true when considering typical emission spectra.

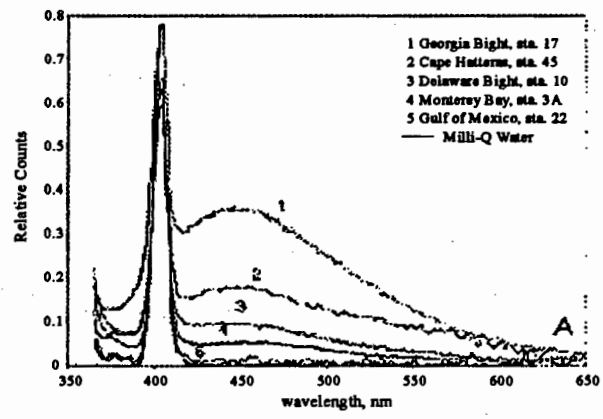


Figure 9. *Examples of emission scans, in this case for filtered seawater samples from various sites off the West Coast of the U.S.A. (reproduced from Hoge et al. 1993)*

Synchronous scans

Synchronous scans are produced by simultaneously scanning over both excitation and emission wavelengths, using a constant wavelength offset between the two, generally given as $\Delta\lambda$. This offers advantages over the single variable scanning techniques shown above, through increases in sensitivity, selectivity and resolution [Senesi 1990, Ferrari & Mingazzini 1995]. Figure 10 shows the advantages offered by synchronous scanning in terms of constituent differentiation.

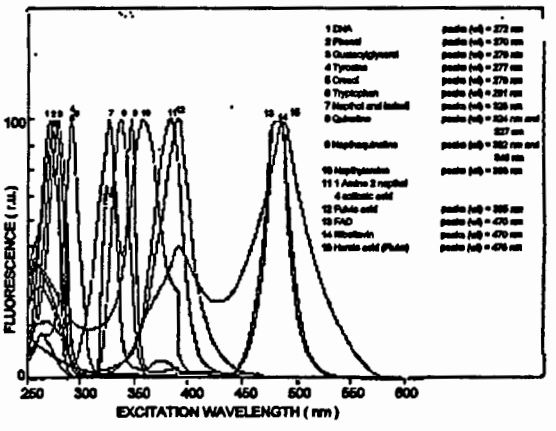


Figure 10. *Examples of synchronous scans, in this case for various compounds present in DOM, with $\Delta\lambda$=25nm. (reproduced from Ferrari & Mingazzini 1995)*

Excitation-emission scans

Excitation-emission matrices (or EEMs) can be viewed as 3D concatenations of excitation or (more usually) emission scans. They offer advantages in that the entire spectrum of fluorescence can be presented, particularly useful when investigating variations in chromophoric structure [Coble et al. 1990, 1993, Mopper & Schultz 1993, Coble 1996]. All of the above spectra can be extracted from EEMs, by choosing a cross section either parallel to the respective axes for single variable scans, or on an appropriate diagonal for synchronous scans [Coble 1996]. EEMs are also particularly useful when considering natural fluorescence, as they allow modelling of the full spectral behaviour of fluorescence [e.g. Hawes et al. 1992, Vodacek et al. 1994]

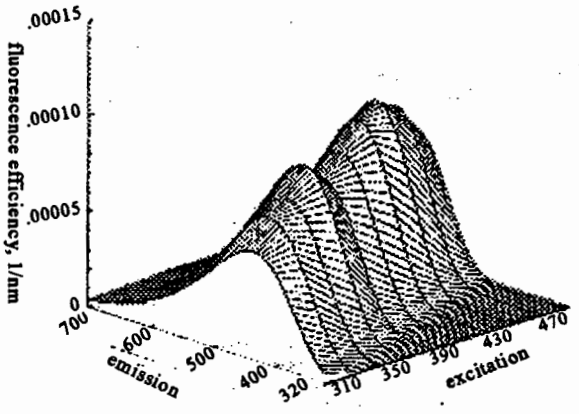


Figure 11. *An example of an excitation-emission matrix, in this case for a marine humic acid. (Reproduced from Hawes et al. 1992)*

The manner in which DOM fluoresces and its typical spectral characteristics vary not only with chemical structure and environmental effects, but is also dictated by the analytical constraints imposed by the investigator. Fluorescent characteristics reported in the literature will therefore be discussed as the need arises - in an examination of the causes of fluorescent character, and in comparison to the results of this study. What is important in a general sense is the polychromophoric nature of DOM i.e. the fact that it may typically contain several fluorophores associated with a number of compounds [Coble et al. 1990, 1993, Hawes et al. 1992, Mopper & Schultz 1993, De Souza Sierra et al. 1994, Determann et al. 1994, Vodacek et al. 1994]. Therefore, typical EEMs for DOM will display several maxima, ranging from the ultra-violet to approximately 500nm if considering both excitation and emission wavelengths. Thus the ability to characterise fluorescence is complicated not only by variations in the structure of the main fluorophores, but also by the interactions arising between them. However, DOM fluorescence, and the structural and environmental factors governing its behaviour, can still be well characterised.

4.2 The variability of DOM fluorescence

Fluorescence and absorption are both phenomena arising from the behaviour of chromophores contained within the molecule, thus similar factors may be expected to govern these two properties. They are distinct phenomena, however, and the added spectral dimension and sensitivity that fluorescence brings adds its own complexities. It must be borne in mind that one of the primary reasons for interest in fluorescence is its use as an alternative analytical tool in determining the core measurement of absorption. Thus whilst there are a number of marine compounds (e.g. proteins, pteridines and flavins) that will perhaps become more significant when considering fluorescence, the discussion will again focus primarily on the principal absorbing component of DOM - humic substances [Chapter 2].

For the purposes of this discussion, the factors responsible for fluorescent character have been divided into three expedient categories. It must be emphasised that it is not sought to imply that these effects are independent or isolated from one another - in reality it is probably rather difficult to separate one effect from another because there is a high degree of association between them. However, using a mixture of theoretical and experimental evidence, it is possible to isolate the main determinants of DOM fluorescent character, and gain some appreciation of their general effects. The first category concerns the effects of molecular structure and weight, with a perspective biased towards theoretical and isolated effects. The second deals with analytical or *in vitro* variations, focusing primarily on phenomena affecting the *measurement* of fluorescence, rather than those factors directly affecting the nature of DOM. The last category considers *in situ* phenomena - how *in vivo* DOM fluorescence varies in response to natural stimuli - and draws to some extent on an appreciation of molecular character.

4.2.1 The effects of molecular structure

As with absorption, the overall fluorescent properties of a compound must be considered as a cumulative effect of structural changes in the molecule [Senesi 1990]. There are probably three main determinants of fluorescent behaviour if considering the isolated effects of molecular structure on HS [Choudry 1981, Visser 1983, Senesi 1990] :

- The abundance of fluorophores in the molecule. These are primarily aromatic rings or a few highly unsaturated aliphatic groups.
- The nature of functional groups attached to fluorophores.
- Molecular weight or size.

Organic molecules will fluoresce with reasonable efficiency only if they contain extensive π electron systems i.e. aromatic or highly unsaturated aliphatic groups [Senesi 1990]. Whilst there is no doubt that aromaticity is an important determinant of fluorescent character, its specific effects appear to be prone to some variation. Concerning spectral behaviour, the study of relatively pure aromatic compounds suggests that a greater abundance of aromatic rings results in a red shift, or increase in wavelength of maximum fluorescence [Berlman 1965, Senesi 1990 and references within]. In the case of HS, conflicting reports have appeared as to fluorescent behaviour, particularly regarding HS of different origins. As might be expected from the trends of Berlman [1965] and Senesi [1990], there is some evidence to suggest that, for marine material, the generally less aromatic FAs fluoresce at shorter wavelengths (i.e. blue shifted) than HAs, even when molecular weight effects are accounted for [Visser 1983, Hayase & Tsubota 1985]. Several studies of gross HS (i.e. with no molecular weight related fractionation) have also shown that the fluorescence of autochthonous FAs is blue shifted relative to HAs [Ewald et al. 1983, Senesi 1990 and references within]. Consistent with this, synchronous scans of marine DOM appear to indicate that molecules relatively abundant in aromatic ring systems fluoresce at longer wavelengths [Dujmov et al. 1992, Ferrari & Mingazzini 1995 and references within]. However, there have been conflicting reports concerning the comparisons of marine or aquatic HS to those of a terrestrial origin. Whilst terrestrial HS are of a lesser direct interest to this study, it is well established that they are of a more highly aromatic nature [Chapter 2], and therefore provide an interesting point of comparison. It appears that marine HS tend to have blue shifted fluorescence maxima in

comparison to those of terrestrial origin [Ewald et al. 1983, Senesi 1990, Coble et al. 1990, De Souza Sierra et al. 1994, Coble 1996], but Visser [1983] indicates that terrestrial type HS fluoresce at shorter wavelengths than their aquatic counterparts. However, the main body of evidence would seem to be consistent with the argument that less aromatic HS fluoresce maximally at shorter wavelengths.

There is also a lack of consensus concerning the effects of aromaticity on fluorescent intensity. Ample evidence exists indicating that FAs from a variety of environments fluoresce more intensely, or have a greater fluorescent efficiency, than corresponding HAs [Ghosh & Schnitzer 1980, Hawes et al. 1992], even at comparable molecular weight ranges [Visser 1983, Hayase & Tsubota 1985]. This is possibly due to HAs having more absorbing centres and thus being more prone to both inner filter effects and intermolecular energy transfers, reducing fluorescent intensity [Ghosh & Schnitzer 1980]. The reasoning that the more highly absorbing aromatic molecules [Chapter 2] are more prone to self absorbing inner filter effects is borne out by the appreciably lower concentrations of them needed to achieve maximum fluorescence [Visser 1983]. However, data have been presented to the contrary, showing that HS with an expectedly high relative aromaticity fluoresce more intensely [Larson & Rockwell 1980, Visser 1983, Senesi 1990, De Souza Sierra et al. 1994]. But the character of fluorescence is determined by many complex factors, some of them associated to a certain degree, and attempting to distinguish one determinant from many for gross samples of HS is a difficult procedure. It is perhaps most sensible in this case to confine comparisons to the type of HS of interest i.e. those of marine origin. With this in mind the findings of Hayase & Tsubota [1985], as outlined above, are probably the most pertinent.

Variations caused by functional groups attached to the fluorophores are less well documented, as they are in other aspects of humic chemistry [Chapter 2, 3], and thus are of a lesser interest. In most cases, the attachment of substituents, such as hydroxyl, carbonyl and amino groups, to the fluorophore appears to increase the wavelength of maximum fluorescence [Visser 1983, Philpot & Vodacek 1989, Senesi 1990]. The complexation of cations such as metal ions appears in most cases to shift fluorescent maxima to shorter wavelengths and inhibit fluorescent intensity [Choudry 1981, 1984 and references within, Philpot & Vodacek 1989, Senesi 1990].

Well defined relationships between fluorescence and molecular weight have been reported with a relatively high degree of consistency by researchers. The principle effect appears to be that HS of a lower molecular weight fluoresce more intensely [Levesque 1972, Stewart & Wetzel 1980, Carlson & Shapiro 1981, Choudry 1981, Visser 1983, Hayase & Tsubota 1985, Ewald et al. 1988, Senesi 1990], and at longer wavelengths [Visser 1983, Ewald et al. 1988]. These effects are consistent with the apparent fluorescent character of FAs compared to HAs (as discussed above), in view of the generally smaller molecular weights of FAs [Chapter 2]. Again, however, some discrepancy arises as contrasting data shows that although fluorescent intensity is highly dependent on molecular weight for both FAs and HAs from marine sediment, the wavelength of maximum fluorescence is independent [Hayase & Tsubota 1985]. Nevertheless there is a significant body of evidence indicating that molecular weight is one of the prime determinants of fluorescent character.

4.2.2 Analytical (*in vitro*) effects

In vitro effects, such as solvent nature or pH, perhaps have a greater significance for fluorescence as compared to absorption. The determination of fluorescence is essentially the application of an analytical methodology rather than the measurement of an *in situ* process, such as absorption. Measurements of fluorescence therefore require a greater appreciation of both sampling and analytical conditions, and this is reinforced by its generally greater sensitivity [Donard et al. 1987, Ferrari & Tassan 1991, Hoge et al. 1993, Green & Blough 1994]. Further complexities arise from the difficulties of separating environmental and structural effects, which are associated to some degree. Examples can be found in the case of pH, which can affect the excitation energy of fluorophores through ionisation changes in substituent groups [Visser 1983, Senesi 1990]. Metal ions too can have variable effects, dependent on whether they directly affect structure through complexation, or produce external effects through fluorescent quenching [Senesi 1990].

There are a number of factors which could potentially affect the measurement of DOM fluorescence [Ghassemi & Christman 1968, Ghosh & Schnitzer 1980, Stewart & Wetzel 1980, Carlson & Shapiro 1981, Ewald et al. 1983, Visser 1983, Cabaniss & Schumann 1987, Donard et al. 1987, Philpot & Vodacek 1989, Senesi 1990, Ferrari & Tassan 1991, Dujmov et al. 1992, Vodacek 1992, Ferrari & Mingazzini 1995] :

- Concentration
- pH
- The presence of external quenchers, such as metal ions or dissolved oxygen
- Solvent polarity, viscosity, ionic strength, redox potential and temperature

Concentration effects are of significance only at relatively high DOM concentrations, as there appear to be well established linear relationships between fluorescence and either absorption or concentration at low DOM levels [Ghassemi & Christman 1968, Stewart & Wetzel 1980, Ferrari & Tassan 1991, Dujmov et al. 1992, Hoge et al. 1993, Green & Blough 1994]. It also appears that HS from dissimilar environments obtain maximal fluorescent intensity at different concentration ranges [Visser 1983], as might be expected from the above discussions on fluorescent character. At high concentrations, self absorption of emitted radiation leads to inner filtering effects, and concentration/fluorescence relationships of an exponential nature can be expected [Stewart & Wetzel 1980, Senesi 1990]. However, from an analytical perspective this can be dealt with by simply diluting samples to an appropriately low level of absorption [Vodacek et al. 1994, Green & Blough 1994], or applying an inner filter correction [Ferrari & Tassan 1991, Ferrari & Mingazzini 1995].

The effect of changes in pH can have a considerable and varied effect on fluorescence, whether they result from *in situ* environmental effects [Philpot & Vodacek 1989, Vodacek 1992] or artificially imposed analytical constraints [Ghassemi & Christman 1968, Ghosh & Schnitzer 1980, Stewart & Wetzel 1980, Ewald et al. 1983, Visser 1983, Cabaniss & Schumann 1987, Donard et al. 1987, Senesi 1990]. These effects can manifest themselves as changes in both fluorescent intensity and the wavelength of maximal fluorescence, and have been attributed to the ionisation of functional groups and molecular dissociation [Ghosh & Schnitzer 1980, Senesi 1990].

As concerns the pertinence of pH to this study, *in situ* variations are likely to be insignificant [Chapter 2], and analytical constraints will be discussed as necessary.

Fluorescent quenching can occur through a number of mechanisms, but it generally results from the presence of other solutes interacting with the target compound [Senesi 1990]. The most common quenchers are perhaps metal ions and dissolved oxygen [Senesi 1990]. As discussed previously, it can be difficult to ascertain the effects of metal ions on DOM fluorescence, as they affect fluorescence in both free and bound states. However, the phenomenon of quenching can be considered similar to in some respects to inner filtering effects, as it involves an absorption of emitted radiation or losses of fluorescent energy through other mechanisms of inter-molecular interaction [Senesi 1990]. Again, it will be treated as the specific need arises.

There are several other relatively minor environmental effects on fluorescence, such as solvent polarity, solvent viscosity, ionic strength, redox potential and temperature [Visser 1983, Senesi 1990, Dujmov et al. 1992]. Whilst an appreciation of these factors is needed, the effects of them will hopefully be kept to a minimum by replicating the analytical conditions of other researchers, such as Carder et al. [1989] and Green & Blough [1994].

4.2.3 *In situ* effects

Considering the evidence already presented concerning the complex and poorly understood pathways of formation and degradation of HS [Chapter 2, 3], it would be rather unlikely to expect a terse interpretation of the natural variations in DOM fluorescence. Such variations are likely to be highly dependent on the processes dictating the formation of HS, and it is not intended to revisit the territory explored in Chapter 2 from a marginal change in perspective. Whilst an appreciation of such factors is necessary, the present discussion will rather use a broader focus, exploring variations in fluorescence in relation to other natural variables such as depth, nutrient concentration and insolation. Principal variables can then be examined in greater detail to assess more clearly defined cause/effect relationships.

There are perhaps three main methodological approaches pertinent to the discussion. The first applies limited and consistent fluorescent techniques to the spatial and temporal examination of DOM fluorescence [Hayase et al. 1987, 1988, Chen & Bada 1989, 1992, Determann et al. 1994].

The second approach focuses more on the variations in fluorescence itself, utilising more spectrally dense fluorescence measurements, such as excitation-emission matrices, to relatively limited data sets [Coble et al. 1990, 1993, Mopper & Schultz 1993, De Souza Sierra et al. 1994, Ferrari & Mingazzini 1995, Coble 1996]. The last focuses on specific photophysical phenomena, principally the effects of photodegradation [Dunlap & Susic 1986, Kieber et al. 1990, Kouassi & Zika 1990a, Chen & Bada 1992].

The distribution of fluorescence with depth has been shown by several researchers to be relatively consistent through varied marine environments [Hayase et al. 1987, 1988, Chen & Bada 1989, 1992, Mopper & Schultz 1993]. Typical profiles reveal lowest fluorescence values in the surface mixed layer increasing to a maximum in the region of the thermocline, followed by a constant value in deep water at near maximal values, with occasional increases in bottom water values. The typically low values of fluorescence in surface waters have been ascribed to the effects of photodegradation [Hayase et al. 1987, 1988, Chen & Bada 1989, 1992, Kieber et al. 1990, Kouassi & Zika 1990a, Mopper et al. 1991] or the minor effects of biological uptake [Hayase et al. 1987]. It would therefore seem that photodegradation is a highly significant factor in the regulation of DOM fluorescence, as will be discussed below. It appears that the increase in fluorescence at greater depths is due to either some degree of reversal of photodegradation as material leaves the euphotic zone [Kouassi & Zika 1990a], or the slow formation of HS from sinking precursive material [Postma et al. 1976, Hayase et al. 1987, 1988, Chen & Bada 1992, Smith et al. 1992]. The last point would seem to be supported by the generally very good correlations found between DOM fluorescence and nutrients (i.e. nitrates, phosphates and silicates) through the water column [Hayase & Tsubota 1987, 1988, Chen & Bada 1992]. Interestingly, the point is made by Chen & Bada [1992] that such a fluorescence/nutrient relationship would support the hypothesis of microbially regulated humification processes, such as the Biopolymer Degradation models presented in Chapter 2. A minor point of interest, from the perspective of this study, are the increases in fluorescence observed in bottom waters, attributed to leaching of DOM from bottom sediments [Chen & Bada 1989, Mopper et al. 1991].

The use of more spectrally dense fluorescent techniques, such as excitation-emission matrices (EEMs), has provided more detailed information on variations in fluorescent structure through the water column. As well as confirming the general fluorescent distribution discussed

above, it appears that surface waters tend to be dominated by more recent, protein-like fluorophores with shorter wavelength excitation maxima e.g. 210/330 [2], whilst deeper waters show a predominantly older humic-like fluorescence, with longer wavelength excitation peaks e.g. 320/420 [2] [Coble et al. 1990, 1993, Mopper & Schultz 1993, Determann et al. 1994, Coble 1996]. This appears to be consistent with the hypothesis presented above - that there is significant humification arising from the degradation of sinking organic material. Further evidence can be considered that studies of the general fluorescent distribution outlined above appear to use excitation/emission wavelengths located close to the region of maximum humic-like fluorescence e.g. 320/420 [Hayase et al. 1987, 1988] or 325/450 [Chen & Bada 1989, 1992]. Again, the distribution of protein-like fluorescence through the water column show maxima in surface waters, corresponding with nitrite and chlorophyll maxima, thereafter decreasing with depth [Mopper & Schultz 1993]. Independent evidence from polar waters, based upon the chemical determination of amino acid compositions and extracted HS, would appear to show similar water column distributions of the relative fractions [Hubberten et al. 1995].

The location of protein-like fluorophores in productive surface waters has been used to argue that such molecules are relatively young and produced from recent biological activity [Traganza 1969, Coble et al. 1990, Mopper & Schultz 1993, De Souza Sierra et al. 1994, Coble 1996]. Whilst the synchronous spectra of Ferrari & Mingazzini [1995] employ different wavelength pairings, they would also appear to indicate a good relationship between wavelength of maximal fluorescence and DOM age. In this case, based on synchronous spectra of DOM exudates from various algal cultures, shorter wavelength fluorescence peaks appeared to decrease with age, whilst longer wavelength peaks increased in intensity [Ferrari & Mingazzini 1995]. Whilst the transition from "age" to "history" would obviously be rather difficult to make in the complex marine environment, it appears likely that the strong relationship between the nature of DOM and its history can be extended to include the spectral nature of fluorescence.

There appears to be little correlation between surface DOM fluorescence and productivity [Chen & Bada 1992] - a minor point that assumes greater significance in a study orientated to ocean colour. It is thought that upwelling could explain the higher surface fluorescence found in

2. This notation refers to a pair of excitation/emission wavelengths, in nm. The values of the protein-like and humic-like fluorescence peaks are taken from Mopper & Schultz [1993].

productive regions [Chen & Bada 1992], a plausible mechanism in the light of deep water maxima in DOM fluorescence and the significant effects of photodegradation. Chen & Bada [1992] offer an explanation in which sunlight affects DOC and fluorescence in opposite ways - DOC is increased through photosynthesis, whilst fluorescence is diminished through photodegradation. This is consistent with a comparison of the fluorescence profiles discussed and typical DOC profiles [e.g. Druffel et al. 1992], which show a decrease in depth from a maximum at the surface.

There appears be little doubt that photodegradation has a significant effect on DOM fluorescence - typical figures indicate an approximate 50% reduction in fluorescent intensity after 2 days of sunlight insolation for deep water samples [Kouassi & Zika 1990a, Chen & Bada 1992]. The effect of insolation on spectral behaviour is also of great interest, particularly given the variations in spectral structure through the water column discussed above. Kieber et al. [1990] show that photochemically induced fluorescence losses appear to occur across the visible spectrum, with the implication that insolation affects intensity to a far greater degree than spectral shape. However, Kouassi & Zika [1990a] show that HS with varying spectral shape react in different ways to insolation at various wavelengths. Whilst the majority of HS studied showed only changes in intensity, a marine HA with an exceptionally long wavelength emission maximum showed a decrease in wavelength of peak intensity after insolation, with resultant post insolation spectra showing greater similarity to other samples studied [Kouassi & Zika 1990a]. It may be possible to interpret such behaviour as indicating that, despite the complex variations in typical fluorophore configurations of HS, photodegradation promotes homogeneity in the fluorescence of affected material. Such a hypothesis is supported by the findings of Chen & Bada [1992], showing similar values of surface fluorescence from a variety of regions - with the exception of deep water enhanced upwelling regions. However the lack of knowledge relating to the fluorophoric structure of DOM, and fluorophoric responses to photochemical forcing [Kouassi & Zika 1990a, 1990b], prevent a good understanding of the possible influences of photochemical effects on typical marine fluorophore distributions. It may be possible that the more complex humic-like fluorophores, fluorescing at longer wavelengths, are more susceptible to photochemically induced degradation than simpler protein-like fluorophores functioning at shorter wavelengths [Slawinski et al. 1978]. Such speculation could be supported by the data of Kouassi & Zika [1990a], showing that fluorescent efficiency is increased by shorter wavelength insolation, whilst decreases are caused by longer wavelengths.

Irradiations of soil HAs also show a blue shift in emission peaks, with increases in intensity at shorter excitational wavelengths [Slawinski et al. 1978]. It is worth noting that sunlight irradiations resulted only in decreases in fluorescence [Hayase et al. 1988, Kouassi & Zika 1990a, Chen & Bada 1992]. There also appears to be a lack of consensus regarding the reversibility of fluorescence losses. Kouassi & Zika [1990a] indicate that fluorescence changes can be slowly reversed by dark processes, and use this to explain the regeneration of deep water fluorescence, whilst Chen & Bada [1992] indicate that photodegradative losses in fluorescence are irreversible.

Of particular interest to this study are any factors potentially affecting the nature of the relationship between fluorescence and absorption. In the light of this, and with an appreciation of the significance of photodegradative effects to the properties of DOM in surface waters, fluorescence/absorption relationships must be examined for potentially significant causes of divergence. Unfortunately there is little relevant information available, but the good correlations found between photoproduction and absorbance/fluorescence losses, and the generally similar figures of 50% losses in 2 days of insolation for both fluorescence and absorbance [Kieber et al. 1990, Kouassi & Zika 1990a, Chen & Bada 1992] would appear to indicate that gross photodegradative losses in absorption and fluorescence are similar. There is also evidence demonstrating that fluorescence and absorption are well correlated through the water column, indicating that DOM quantum yields remain constant despite the effects of photodegradation [Vodacek et al. 1995].The typically well correlated relationships established between DOM absorption and fluorescence for a wide variety of marine systems, as considered below, would also indicate that little divergence is caused by photochemical effects.

4.3 Gelbstoff absorption from fluorescence

One of the most powerful applications of DOM fluorescence is as an alternative methodological route to the determination of gelbstoff absorption. Direct spectrophotometric determination of the gelbstoff absorption coefficient can be a cumbersome affair, requiring strict adherence to rigorous and time consuming methodological protocols [Mueller & Austin 1995]. The filtration of samples in particular, apart from precluding any form of continuous measurement of absorption, is prone to error inducement by through either leaching of filter papers [Bricaud et al. 1981, Norrman 1993, Mueller & Austin 1995, unpublished results], scattering from residual particles in the filtrate [Bricaud et al. 1981, Ferrari & Tassan 1991] or the rupture of plankton cells under even modest vacuum [Mueller & Austin 1995]. Sensitivity problems can also arise, as levels of gelbstoff absorption in oligotrophic waters can approach the detection limits of most modern spectrophotometers [Ferrari & Tassan 1991, Hoge et al. 1993, Green & Blough 1994].

As an alternative route to absorption determination fluorescence can offer many potential advantages, such as rapidity, sensitivity, bypassing the need for filtration, and continuity [Ferrari & Tassan 1991, 1992, Hoge et al. 1993, Green & Blough 1994, Vodacek et al. 1995]. However, the establishment of quantitative fluorescence/absorption algorithms is dependent upon the consistency of the relationship between the two variables. Potential variations in this relationship must be assessed, particularly so given the well known diversity in the fluorescent character of DOM. Whilst good correlations between fluorescence and absorption might be expected for DOM of a given origin and nature, it may be unrealistic to expect such correlations to be consistent for a wide variety of marine systems, given the changing fluorophore configurations of DOM from differing origins and natural systems [e.g. Hoge et al. 1993]. Of particular importance is an understanding of variations in the fluorescence quantum yield ϕ [3], a measure of the efficiency with which absorbed energy is re-emitted as fluorescence, and thus the primary determinant of the nature of the relationship between fluorescence and abso

exhibit surprisingly little variation [Green 1993, Green & Blough 1994, Vodacek et al. 1995], and this is consistent with the good correlations between absorption and fluorescence reported by a number of researchers [Ferrari & Tassan 1991, Hoge et al. 1993, Green & Blough 1994, Vodacek et al. 1995].

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3. Also given as the fluorescence efficiency function η (nm^{-1}) [Hawes et al. 1992], or quantum efficiency ϕ [Senesi 1990].

One of the problems associated with the quantitative use of fluorescence is the determination of *absolute* fluorescence measurements i.e. producing data in comparable and common units. Given the variation in the optical configurations of typical instrumentation, this is most easily achieved through the use of a fluorescent standard, with quinine sulphate being the most commonly used [Zepp & Schlotzhaeur 1981, Hayase & Tsubota 1987, 1988, Kieber et al. 1990, Kouassi & Zika 1990a, Senesi 1990, Chen & Bada 1989, 1992, Mopper et al. 1991, Coble et al. 1993, Hoge et al. 1993, Green & Blough 1994, Mopper & Schultz 1993, Vodacek et al. 1994, 1995]. However, doubts have been expressed as to the suitability of using quinine sulphate as a standard for the entire fluorescent spectrum [Coble et al. 1993], given typical spectral variations in quantum yields of DOM and the fact that the quinine sulphate standardisation is generally based on a single wavelength value e.g. 350 nm [Green & Blough 1994].

Given the complex nature of chromophoric phenomena associated with DOM, research has taken an empirical approach to the determination of fluorescence/absorption algorithms. Sets of concurrent absorption and fluorescence data, or calculated quantum yields, have generally produced strong linear correlations [Ferrari & Tassan 1991, Hoge et al. 1993, Green & Blough 1994, Vodacek et al. 1995]. The majority of this research has concentrated on the determination of $a_g(\lambda)$ at various wavelengths, and to date there appears to have been only one attempt to calculate the absorption slope parameter S from fluorescence [Green & Blough 1994]. Apart from the question of fluorescent standardisation techniques, on which there has been some debate [Ferrari & Tassan 1991, Hoge et al. 1993], there appear to be several other constraining factors. At low levels of absorption e.g. $< 0.2\ m^{-1}$ at 355 nm [Green & Blough 1994] larger errors are associated with the measurement of absorption, as typical spectrophotometer detection limits are approached [Ferrari & Tassan 1991, Green & Blough 1994]. This affects any determination of $a_g(\lambda)$ and to a greater extent S, as calculation of the wavelength dependency of absorption requires at least one measurement at lesser absorbing longer wavelengths [Green & Blough 1994]. Variations in quantum yield can also produce marked scatter from modelled relationships, particularly so when data from diverse marine systems are used to establish "universal" regressions [Hoge et al. 1993, Green & Blough 1994]. Again the determination of S is affected in a slightly different manner to that of $a_g(\lambda)$, as quantum yields at multiple wavelengths are required in the calculation of wavelength dependency, resulting in sensitivity to a ratio of quantum yields [Green & Blough 1994]. It is emphasised that regional fluorescence/absorption algorithms perform with significantly greater accuracy [Hoge et al. 1993, Green & Blough 1994].

It would also appear that quantum yields of isolated DOM differ spectrally from natural water samples, as red shifts in the wavelength of maximum quantum yield have been observed for isolates [Green & Blough 1994, Vodacek et al. 1994 and references within]. This is thought to be due to the fact that the hydrophobic media used for extraction purposes have varying retention efficiencies for different DOM fractions [Amador et al. 1990, Coble et al. 1990, Green & Blough 1994, Esteves et al. 1995, Lara & Thomas 1994a, 1994b, 1995]. This phenomenon will be discussed in greater depth at a later stage [Chapter 5]. With respect to the determination of absorption parameters from fluorescence, it is possible that isolate-based regressions may vary from those of natural water samples [Green & Blough 1994].

4.4 Natural or sunlight stimulated fluorescence

One of the less well understood processes affecting ocean colour is the contribution made by the fluorescence of dissolved material to the water leaving radiance or reflectance (see figure 3). Perhaps this is due in part to the imbroglio of complex and interactive processes that must be incorporated into any viable model of reflectance [cf. Vodacek et al. 1994]. This is compounded by the difficulty of making any form of direct measurement of *in situ* DOM fluorescence, due primarily to the effects of overlapping scattered light [Peacock et al. 1990, Vodacek et al. 1994]. However, various models have been developed, and these have been used to assess both variations in *in situ* DOM fluorescence and its possible effects on the underwater light field [Spitzer & Dirks 1985, Peacock et al. 1990, Hawes et al. 1992, Lee et al. 1994, Vodacek et al. 1994].

While it is beyond the scope of this study to discuss the complexities of models developed to analyse the effects of DOM fluorescence on the underwater light field, it appears that there are several factors determining the significance of *in situ* fluorescence effects. The quantum yield of dissolved material is of obvious importance, in terms of both its magnitude and spectral distribution [Hawes et al. 1992, Lee et al. 1994, Vodacek et al. 1994]. Lee et al. [1994] found that water types with particularly high quantum yields had the greatest fluorescent contribution to the water leaving radiance. From a simplistic perspective this perhaps sounds obvious, but it should be remembered that other optical processes, such as absorption and scattering, are generally the predominant determinants of the underwater light field [Vodacek et al. 1994]. This is reinforced by the effect that particulate concentration has on fluorescent contributions. Increasing levels of particulate matter, using phytoplankton pigment concentrations as an index, cause an increase in scattering, thus inhibiting fluorescence effects [Spitzer & Dirks 1985, Hawes et al. 1992, Vodacek et al. 1994]. This has important considerations in highly productive coastal waters, such as the Benguela, as particulate levels are likely to be relatively high [Chapman & Shannon 1985, Brown & Cochrane 1991]. Another consideration is that of depth - there appears to be an exponential relationship between depth and solar stimulated fluorescence, due to both decreases in incident intensity and inhibition of emitted fluorescence by scattering processes [Vodacek et al. 1994]. This also raises several interesting questions, such as the effects of photodegradation and the surface microlayer. This last point in particular does not seem to have been addressed by researchers, and the fluorescent effects of surface microlayers are open to speculation.

If such phenomena are composed primarily of chromophoric compounds such as HS or their precursors [Frew & Nelson 1992], then it is possible that they could have fluorescent effects on ocean colour that are currently unaccounted for. The combination of high concentrations of fluorescent material, maximal intensity of incident irradiation and little inhibition of emitted fluorescence (due to the virtual absence of competing optical processes) might result in high fluorescent contributions to the water leaving radiance.

It is important to distinguish between the gross contribution of DOM fluorescence to reflectance, and the *relative* contributions in core regions of the spectrum i.e. the wavebands of ocean colour sensors. In the case of SeaWiFS, primary considerations are the effects of *in situ* fluorescence on the two waveband ratios employed in the semi-analytical chlorophyll *a* algorithm - R(412):R(443) and R(443):R(555). To begin with, it seems likely that contamination of core spectral regions from *in situ* DOM fluorescence will only be significant in water types with high levels of gelbstoff relative to particulate matter (i.e. phytoplankton or detritus) in areas of little terrigenous influence [Hawes et al. 1992, Vodacek et al. 1994]. Vodacek et al. [1994] calculate a 12% difference in R(445):R(555), and a 28% difference in R(415):R(445), for water types with $a_g(355) = 32.91\ m^{-1}$ and chlorophyll $a = 0.2\ mg\ m^{-3}$ (from a blackwater estuary in the Florida Everglades). Whilst these values would significantly compromise the SeaWiFS algorithm, it should be noted that it would be unlikely to find such extreme bio-optical conditions in areas such as the Benguela. This is particularly true of the very high gelbstoff absorption coefficient - such values are normally associated with highly terrigenously influenced systems [Hojerslev 1988, Green & Blough 1994]. In less extreme bio-optical conditions e.g. $a_g(355) = 0.44\ m^{-1}$ and chlorophyll $a = 4.0\ mg\ m^{-3}$, maximal fluorescent contributions to reflectance are thought to be in the region of 4%, which can be considered negligible [Hawes et al. 1992, Vodacek et al. 1994].

Chapter 5

Methodology

There can be little doubt that gelbstoff must be accounted for if ocean waters off the coast of southern Africa are to be effectively characterised from a bio-optical perspective. However, facing an almost complete dearth of knowledge for the region, with the added constraints of lacking appropriate instrumentation and expertise, it has been difficult to assess which methodological approaches offer the most significant returns. There is certainly a case for the argument of maximum coverage, for the gathering of a resource limited set of strictly relevant "sea truth" data, encompassing the most varied marine systems that ocean colour data is likely to be applied to. Such a rationale is fundamental to the validation of scientific tools such as ocean colour satellites, whose value lies in their synoptic capabilities. The application of such an approach to SeaWiFS from a "local" perspective would have to take the form of a well organised and regulated programme, sampling *at the very least* the core bio-optical variables of phytoplankton and gelbstoff absorption, and photosynthetic pigments. However, even such a minimal approach to validation, which could frankly be regarded as limited to the point of compromise, requires significant resources and the application of intricate methodological protocols in a consistent and rigorous manner. Considering the lack of knowledge concerning the behaviour of gelbstoff in the region, it is felt that a slightly different approach may be more appropriate, at least as a means of *initiating* an appreciation of gelbstoff characteristics. This does not seek to imply that more holistic validation programmes are unnecessary - to the contrary they are essential to any serious validation exercise - but rather that a more narrowly focused and in depth exercise in the analysis of gelbstoff represents a good springboard from both an educative and scientific perspective.

With a reliance on the literature necessitated by the lack of relevant knowledge for the region, several factors determined the experimental approach decided upon. Concerns over potentially low gelbstoff absorption levels and the associated sensitivity problems of direct spectrophotometric measurements [Carder et al. 1989, Ferrari & Tassan 1991, Hoge et al. 1993, Green & Blough 1994] were exacerbated by the lack of a *consistently* available and appropriately specified instrument. It was felt that this factor contributed to a strong argument for the derivation and application of fluorescence to absorption techniques similar to those discussed in Chapter 4. More significantly, potential sensitivity problems were also thought to support the idea of the concentration or isolation of the major dissolved absorbing components [Carder et al. 1989]. Isolation would also offer a far greater investigative scope, allowing a more flexible and in depth analysis of bio-optical parameters through the ability to study different gelbstoff fractions through

a range of concentrations and analytical parameters. The ability to undertake chemical and isotopic analyses on isolated fractions was considered a further advantage, as was the ability to conduct any form of future research such as further fractionation or photodegradation studies. It was also felt that isolates could be used as semi-quantitative standards, particularly for fluorescent techniques, although in hindsight this seems somewhat misplaced in the light of the large variations in gelbstoff chromophoric structure across marine systems [Chapter 2,3,4].

With this rationale, it was decided that the isolation of humic substances, as the majority components of gelbstoff [Chapter 2], represented the most beneficial means of gaining a greater initial understanding of gelbstoff characteristics in southern African waters. However, the benefits of hindsight, and publications subsequent to the initiation of the project, have revealed that significant problems can be associated with the optical characterisation of isolated HS. The hydrophobic resins generally used for such purposes have been to shown to adsorb and retain DOM fractions on a selective basis - consequently isolated HS may not be wholly representative of associated *in situ* material [Coble et al. 1990, Benner et al. 1992, Green & Blough 1994, Lara & Thomas 1994a, 1994b, 1995]. The most immediately significant consequences of this from an optical perspective are possible changes in the quantum yield of isolates as compared to natural water samples, shown to clear effect by Green & Blough [1994]. However, the full significance of these effects have yet to be understood, particularly as they relate to the optical properties of DOM, and it appears that the variety of media used by researchers (e.g. XAD-2, C_{18}) are biased to different degrees [Amador et al. 1990, Green & Blough 1994]. Nevertheless while some uncertainty might be introduced regarding the general application of methodologies derived from the quantum yields of isolates, the original rationale for isolation, as discussed above, retains a high degree of validity.

5.1 Humic substances isolation system

The design of the isolation system was based on well established methodologies, specifically those of Tokar et al. [1981] and Harvey et al. [1983]. The system common to these researchers was chosen as a design template for several reasons: it has been used for the optical analyses of isolated HS [Carder et al. 1989], it was felt to ensure maximum protection from contamination or sampling induced degradation, and it allowed large volumes of seawater to be processed. Several aspects of the system ensured minimal contamination, either from xenobiotic sources or degradation whilst sampling. Only metallic or glass components were used throughout the system, as plastic components were felt to be potential sources of contamination [Tokar et al. 1981]. At all stages of sampling, exposure to both light and air, possible causes of degradation, were avoided through the use of nitrogen pressure to drive a dark system [Harvey et al. 1983]. The system itself took the form of a nitrogen lift pump connected by flexible stainless steel hose to a pressurised stainless steel reservoir, with a controlled flow through to parallel XAD-2 adsorption columns. A schematic is shown in Figure 12.

The nitrogen lift pump was thought to be preferable over either on-board systems or mechanical pumps as potential sources of contamination were removed [Tokar et al. 1981]. Stainless steel flexible hose was used for both water and gas lines, with outside diameters of $^3/_4$" and $^1/_4$" respectively. These offered greater ease of deployment than the solid sections of pipe used by Tokar et al. [1981], and reduced the number of between section connectors needed. All hose sections had customised end pieces welded on of an appropriate size for the connectors used. These lines were attached to the hydrowire by a set of custom designed aluminium clamps. As the initial focus was to be on surface waters, the pump section was designed for the shallow water U-tube type deployment discussed by Tokar et al. [1981]. This type of set up is necessary at the surface if a suitably large head of pressure is to be created. To facilitate ease of deployment, all connectors were of the Swagelock "Quick Connect" type, allowing rapid coupling and decoupling. The gas line was connected to the main water line using an adapted connector with an elbow bend, at approximately one meter above the U-tube nadir.

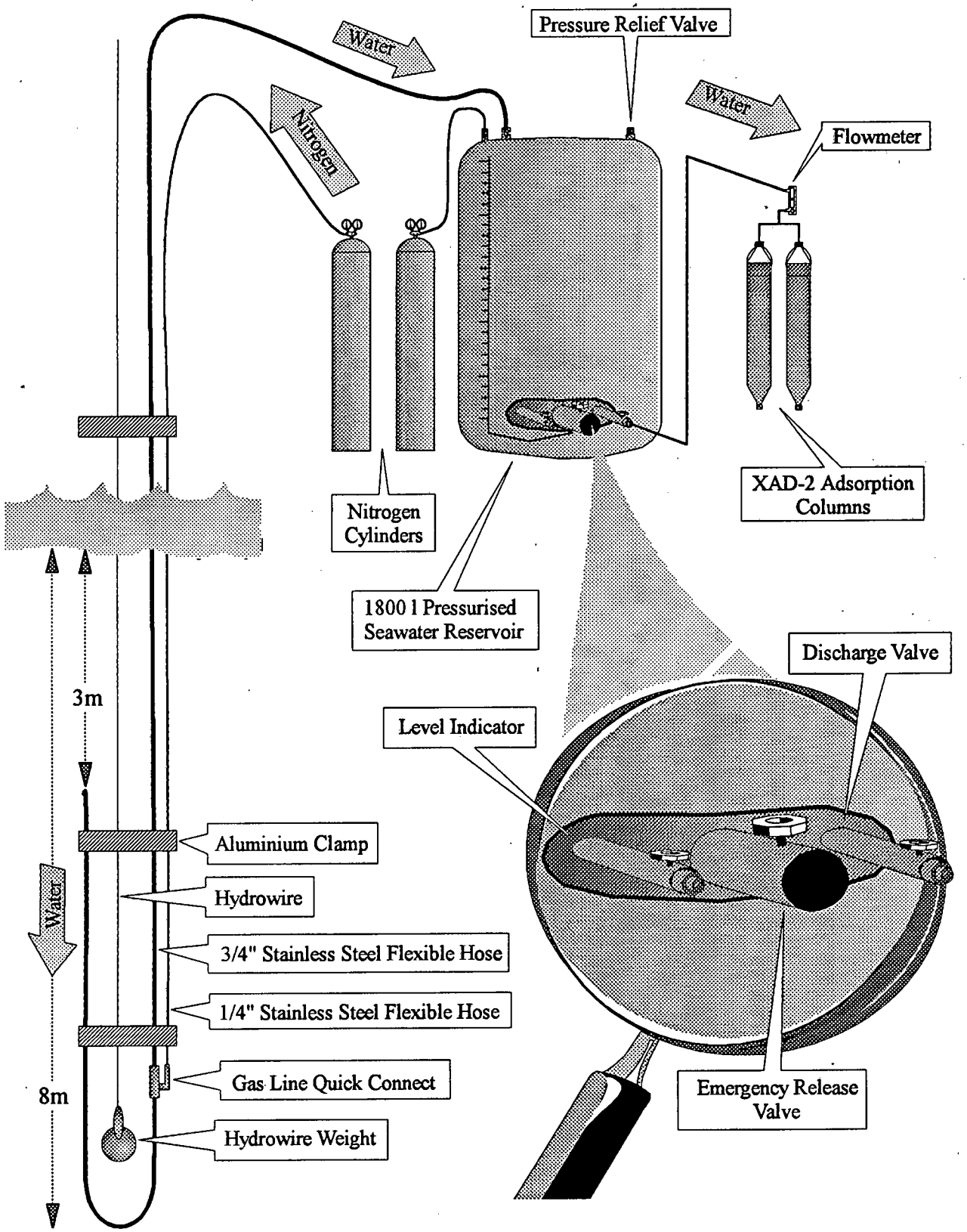


Figure 12. *Humic substances isolation system*

The seawater reservoir consisted of a 1750 litre pressurised tank, constructed of 316 grade stainless steel. A model commercially available from Consani Engineering (Pty) Ltd was modified by them according to the following specifications:

- A 100 mm diameter removable flange was added to the top of the tank, to allow inspection and cleaning.
- Gas inlet ($^1/_4$"), water filler ($^3/_4$") and water discharge ($^1/_4$") sockets were fitted to the top of the tank, with a siphon tube attached to the discharge socket to enable top discharge.
- A pressure relief valve rated at 0.7 bar was fitted to enable nitrogen pressurisation.

After initial deployment of the system, the reservoir was further modified as follows:

- An additional discharge tube was fitted to the sump of the tank, with a three way end point consisting of an emergency discharge valve ($1^1/_2$"), a bottom discharge valve ($^1/_4$"), and a sight glass ($^1/_4$") connection.
- The pressure relief valve was upgraded to a 1.4 bar rating to allow faster discharge.

All connectors attaching to the reservoir allowed either single or double end pressure shut off, enabling nitrogen pressure to be maintained during decoupling. Discharge to the XAD-2 columns was effected through $^1/_4$" stainless steel pipes, regulated by a Fischer & Porter Purgemaster needle valve flowmeter, with stainless steel end fittings.

The core component of any isolation system is obviously the medium of isolation itself, in this case the XAD-2 resin. Alternative isolation methods for HS can be considered :

- Ultrafiltration [Amador et al. 1990, Benner et al. 1992, Amon & Benner 1994, Bianchi et al. 1995].
- Hydrophobic solid-phase isolation media, such as C_{18} Sep-Pak cartridges [Amador et al. 1990, Coble et al. 1990, Blough et al. 1993, Green & Blough 1994].

- Alternative matrices or combinations of hydrophobic macroreticular resins, such as XAD-4, XAD-7, XAD-8 or combinations of these with XAD-2 [Druffel et al. 1992, Lara & Thomas 1994b, Esteves et al. 1995].

The most significant advantage offered by ultrafiltration is that material is isolated solely on a basis of molecular size, thus avoiding any form of bias connected with the hydrophobic based retention of other media, such as resins or cartridges [Benner et al. 1992, Green & Blough 1994]. Ultrafiltration thus represents one of the best means of isolation if a valid representation of the complete DOM fraction is to be obtained [Benner et al. 1992]. However, such size based selectivity may not be appropriate if chromophoric DOM or HS are to be specifically targeted, unless some further isolation procedure is performed on the concentrated DOM ultrafiltrate. Whilst a molecular size determined concentration of DOM will no doubt facilitate the measurement of optical characteristics through signal enhancement, it may be difficult to fully quantify these measurements if the chromophoric fraction of the concentrate is unknown. Perhaps this is the reason why there appears to be few applications of this method in studies of a bio-optical nature.

The use of hydrophobic solid-phase media, on the other hand, is widespread and relatively well documented [Amador et al. 1990, Coble et al. 1990, Hawes et al. 1992, Blough et al. 1993, Green & Blough 1994]. There is evidence showing that C_{18} cartridges are one of the more efficient isolators of CDOM, comparable even to ultrafiltration [Amador et al. 1990]. However, this study did not analyse the qualitative capabilities of isolation media, and there is evidence indicating that C_{18} cartridges isolate material in a biased manner i.e. the optical properties of isolates differ from those of their corresponding natural water samples [Coble et al. 1990, Green & Blough 1994]. Effects of this are a "red shift" in the wavelength of maximum quantum yield, and lower S values for some water types, implying that C_{18} isolates longer wavelength absorbing material with greater efficiency [Green & Blough 1994]. Flavins and their degradation products have been suggested as a possible source of these more efficiently isolated fluorophores [Green & Blough 1994, Seritti et al. 1994].

However, while similar problems of biasing may exist for XAD resins, they were chosen over C_{18} for this study primarily because they are possibly the most widely recognised media for the isolation of HS [Stuermer & Harvey 1977, Laane & Koole 1982, Ehrhardt 1984, Harvey et al. 1983, Meyers-Schulte & Hedges 1986, Carder et al. 1989, Malcolm 1990, Druffel et al. 1992, Hawes et al. 1992, Lara

& Thomas 1994a, 1994b, 1995, Esteves et al. 1995], and an XAD based isolation system [Harvey et al. 1983, Carder et al. 1989] was felt to most suitable for the purposes of this study.

There are a variety of differently specified XAD resins available, and it would appear that retention characteristics can be manipulated through the combination of available variants [Lara & Thomas 1994b, Esteves et al. 1995]. Resins most commonly used for the isolation of marine HS differ in both size specification and material, and include XAD-2, XAD-4, XAD-7 and XAD-8. Without entering into technical discussions, it appears that sequential arrays of resin types appear to offer the most effective means of isolation, by virtue of combining the different resin retention characteristics [Druffel et al. 1992 and references within, Lara & Thomas 1994b and references within, Esteves et al. 1995]. However, much of this information became available only after the initiation of the project, and the exclusive use of XAD-2 resins for the isolation of HS still has much validity [Hubberten et al. 1994, 1995, Lara & Thomas 1995].

The exclusive use of XAD-2 is not without its problems however. Of particular significance are potential changes in the character of XAD-2 isolates in relation to the original water sample. It is well known that XAD-2 can have a poor quantitative isolation efficiency [Stuermer & Harvey 1977, Amador et al. 1990, Lara & Thomas 1994b], but this is not of great significance in isolation procedures that typically are not regarded as quantitative *in themselves*. Of greater importance is the *qualitative* isolation efficiency, or the ability to recover material that can be considered representative of the water type sampled, from both an optical and chemical perspective. It appears that XAD-2 isolates can be fractionated in terms of their retention/elution characteristics, with typical fractions operationally defined as hydrophilic Hl (percolate), hydrophobic acid HbA (base eluate), hydrophobic neutral HbN(alcohol eluate), and hydrophobic bound (non recoverable fraction with base/alcohol elution) [Kukkonen et al. 1990, Hubberten et al. 1994, 1995, Lara & Thomas 1994a, 1994b, 1995][4]. From a chemical perspective, such a classification shows that evaluating the retention characteristics of XAD-2 resin is by no means straightforward, particularly as the structural characteristics of some fractions, such as the hydrophobic bound, are not well understood [Lara & Thomas 1994a, 1994b, 1995]. From a study of XAD-2 retention characteristics of labelled ^{14}C on the growth and decay of a diatom culture, elutable material

4. It should be noted that this eluate classification was not followed in this study, which rather followed the elution methods of Harvey et al. [1983] - a mixed base/alcohol elution followed by fulvic/humic fractionation, as will be discussed later.

(HbA and HbN) only comprised ~33% of total DOC, with ~50% unretained (Hl) and a further ~17% bound to the resin (HbB) [Lara & Thomas 1994a, 1995]. Whilst there are obvious dangers to assuming such *in vitro* results have a wide spread application, it is of concern that of the hydrophobic fraction retained, traditionally regarded as operationally defined HS [Lara & Thomas 1995], a significant portion is not completely recoverable using standard methodologies. However, given the intangible nature of "humic substances" and their ambiguous operational definitions, any query over the performance of XAD-2 *for the purposes of this study* can not be satisfactorily answered. In its simplest form the question remains whether the isolation procedure produces an *optically* representative sample.

There appears to be little information available regarding possible changes in quantum yield brought about by biased retention or elution of XAD-2 resins. Laane & Koole [1982] indicate that XAD-8 isolates have identical absorption and emission spectra to corresponding water samples, using only a base eluate. Whilst XAD-8 and XAD-2 are reported as having similar performances for marine humics [Fu & Pocklington 1983, Lara & Thomas 1994b and references within], the two resins have different material and size specifications [Druffel et al. 1992], and it is considered unlikely that they will have closely similar *qualitative* retention characteristics. The data of Green & Blough [1994] would seem to suggest that isolates prepared with XAD-2 resins do not exhibit the red shift in maximum quantum yield associated with C_{18}, although this effect could be due to sample differences. Finally, excitation-emission matrices presented by Coble [1996] show that XAD-2 isolates have blue shifted fluorescence maxima, although it is suggested that this phenomenon is not necessarily caused by isolation procedures, and may simply result from typical oligotrophic fluorescence characteristics [De Souza Sierra et al. 1994, Coble 1996]. It would therefore seem that discussions of optical biasing or fractionation by XAD-2 isolation remain inconclusive, although there does not appear to be firm evidence indicating that isolates produced by this method are *not* optically representative.

The XAD-2 columns were prepared using custom designed glass columns with approximate resin volumes of 850 cm^3. Glass wool plugs were inserted both above and below the resin to trap any large particles. The resin was prepared by washing with several bed volumes of acetone and methanol, followed by 5 l of 1.0 M NaOH, and 2 l of 1 M aqueous NH_3 in methanol to remove impurities [Stuermer & Harvey 1977, Woolard 1994]. Columns were then acidified to pH=2 using 12 M HCl and stored in the dark until use.

5.2 Deployment and sampling

The humic substances isolation system was constructed over a period of several months and deployed on two cruises of the Sea Fisheries Research Institute research vessel *RS Algoa*. These were cruise ALG010, taking place in March 1994 in the vicinity of the Agulhas Ridge, and cruise ALG013 in May 1994, in and around St. Helena Bay. Sampling locations and details are presented in figure 13 and table 5.2 respectively.

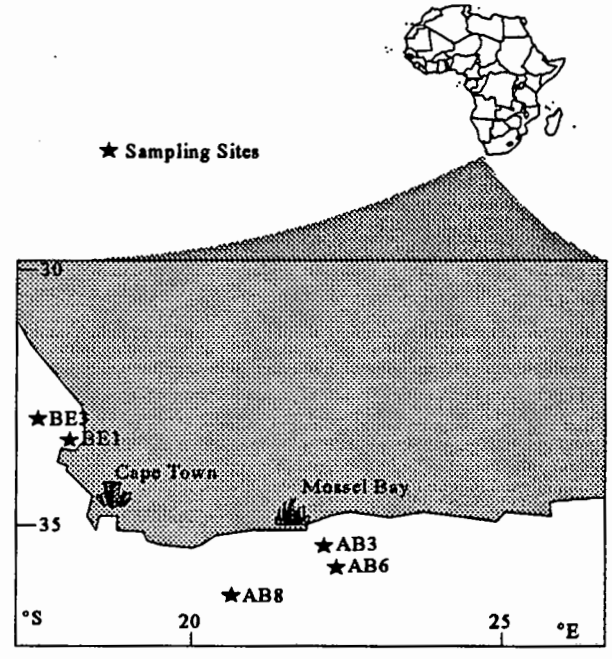


Figure 13. *Location of sampling sites*

Table 5.2 *Details of sampling sites*

Station No.	XAD-2 Column	Date	Pump Source	Sampling Time	Surface Chl *a* (mg m^{-3})	Surface Temp. (°C)
AB3	A	03/03/94	Lift pump	14:30→17:30	4.82	18.1
AB6	B	05/03/94	Lift pump	14:30→16:30	2.24	17.2
AB8	C, D	08/03/94	Ships pump	14:30→16:30	0.19	20.8
BE1	E, F	17/05/94	Lift pump	08:30→12:30	11.98✗	14.2
BE3	G, H	18/05/94	Ships pump	22:30→24:00	na	14.4

✗ Station BE1 was sampled during the senescent phase of a red tide

The system was deployed in the following manner. As the weighted hydrowire was slowly lowered overboard, the aluminium hose clamps were attached at 2-3 m intervals and the water and gas lines secured to them. Once the gas lift system had been assembled, it was swung out on an A-frame and adjusted so that the water intake was 3-4 m below the surface. Approximately the first

50 l of seawater were used to rinse the inside of the reservoir. It was discovered that the gas lift system was only capable of delivering ~5 l min^{-1} at maximum flow, compared to the 10 l min^{-1} expected [Tokar et al. 1981], thereby allowing less seawater to be collected than anticipated in the allocated sampling slot. In the light of the poor performance of the lift pump, it was decided to use the ships scientific seawater supply at several stations to collect close to the capacity of the reservoir. It was hoped that contamination from this source would be minimal, as the majority components were metallic, and the source had been run continuously since installation, thus minimising potential leaching. As chemical analyses were to be run on the isolates, it was felt that any contamination would be detectable, thereby also allowing an evaluation of the lift systems effectiveness. The ships pump delivered flows of ~20 l min^{-1} from a depth of ~4 m. When approximately half the anticipated sample volume had been collected, 12 M HCl was added to the reservoir using an acid/sample volume ratio calculated from Harvey et al. [1983], in order to achieve the necessary pH=2 required by the XAD-2 isolation procedure [Harvey et al. 1983]. Whilst routine checks of sample pH were not made, a subsample from station BE3 measured subsequent to sampling gave a pH=2 (±0.5).

Once the required volume of acidified seawater had been collected, the reservoir was put under maximum nitrogen pressure and discharged through either single or parallel XAD-2 columns, dependant on total sample volume. Discharge rates were kept between 250 and 400 ml min^{-1}, well within the maximum adsorption efficiency limits of 1 bed volume/minute determined by Stuermer & Harvey [1977]. Total discharge time for the reservoir varied between 12 and 40 hours. The columns were subsequently frozen and kept in the dark at -20°C until elution.

At some stations concurrent seawater samples were taken for the determination of optical properties. These were filtered through precleaned and ashed 47 mm GF/F filter papers under gentle vacuum pressure, and stored in amber glass bottles in the dark at 4°C until analysis.

5.3 Elution and purification

After defrosting, the columns were rinsed with 2 l of 0.01 M HCl to remove salts. Adsorbed HS were eluted with 2.5 l of 1 M aqueous NH_3 in methanol after an initial soak in the same solvent. A further elution with 2 l of methanol ensured a more complete recovery. The full methodology is described by Woolard [1994]. The two eluates were then combined and concentrated through rotary evaporation below 40°C. Once all methanol had been removed, the resultant crude solution of HS was redissolved in a minimal quantity of deionised water containing a few microlitres of NaOH in the case of columns A, B and D. These solutions were then acidified to pH=2 using 1 M HCl and refrigerated for 72 hours at -5°C to allow precipitation of the humic acid fraction. The precipitate was then removed by centrifugation and decantation. In the case of column H, the sample was not fractionated as it was to be used to check possible dissociative effects of treating the fulvic and humic fractions in isolation. Both the fulvic, humic and combined fractions were then freeze dried and weighed. The isolates produced varied widely in terms of their appearance - pale to dark yellow powders in the case of the fulvic acids, with the humic acid fraction tending to be dark brown platelets, often with an oilier consistency. Samples were kept in a desiccator in the dark until analyses were performed. Samples are referred to by their column label in all discussions e.g. fulvic acid A. The various analytical methods used on the samples are described below, with full results given in Chapter 6.

Fulvic acid samples were submitted for standard CHN analysis [Woolard 1994]. As the results of these analyses were unexpected [Chapter 6], further checks for contamination were made. Fulvic acid D was dry ashed, and sent for ICP analysis [Woolard 1994]. These revealed an unexpectedly high ash and cation content [Chapter 6]. Fulvic acid A was redissolved in 0.001 M NaOH, and passed through Amberlite IR 120, an ion exchange resin, to remove cations [Woolard 1994]. The percolated solution was freeze dried, and the process gave a recovery of 23.6%. A further analysis of cation contamination was performed by dialysing fulvic acid C. For this procedure 29 mg of the sample was dissolved in 50 ml of 0.001 M NaOH. This solution was then dialysed using Sigma dialysis tubing of cut off 12000 g mol^{-1} into 250 ml of distilled water [Woolard 1994]. The $\delta^{13}C$ values for the samples were determined using continuous flow mass spectrometry, employing a system consisting of a Carla Erba N1500 Elemental Analyser interfaced with a Finnegan Matt 252 Mass Spectrometer. A commercially available terrigenous humic acid sodium salt (Aldrich Chemical Company) was also analysed for reference purposes.

5.4 Bio-optical measurements

For the purposes of determining absorption and fluorescence measurements of the isolates, samples were redissolved in Milli-Q water adjusted to pH=9 with 1% NaOH [Carder et al. 1989]. Sample dissolution produced a drop in pH to ~8.7. The solutions were made up in amber glass bottles to minimise photodegradative effects, at concentrations of 20 mg/l, except for a concentration series of the combined fraction H. For this series a stock solution of 40 mg/l was made up and used to produce serial dilutions down to a minimum concentration of 1 mg/l. In some cases, most noticeably for the humic acid fractions, the samples did not dissolve easily and all samples were placed in an ultrasound bath for 20 minutes to aid dissolution. No particulate was observed after this procedure, and it was assumed that all samples had fully dissolved.

Absorption measurements were performed using a Hitachi U-2000 UV/VIS double beam scanning spectrophotometer, with 40 mm pathlength quartz cuvettes. The reference in all cases was Milli-Q water. Sample absorbance was measured from 200 nm to 650 nm using a 5 nm wavelength resolution at a scanning speed of 800 nm/min. An empirical correction factor, forcing absorbance at all wavelengths to zero at zero concentration, was determined and applied from absorbance data from the concentration series. For filtered water samples, a refractive index correction was applied by subtracting absorbance at 650 nm from all data [Green & Blough 1994]. Corrected data was then converted to absorption coefficients using the following equation [Blough et al. 1993, Hoge et al. 1993, Green & Blough 1994] :

$$a(\lambda) = 2.303 \frac{A(\lambda)}{l} \qquad 5.4$$

where $a(\lambda)$ = absorption coefficient [m^{-1}]

$A(\lambda)$ = absorbance

l = pathlength [m]

Absorption data from 250 nm to 650nm were then fitted to equation 3.2a [Chapter 3] using a non-linear regression technique (Statgraphics) to determine slope values.

Fluorescence measurements were determined using a Hitachi F-4000 fluorescence spectrophotometer operating in corrected mode. Both excitation and emission bandpasses were set to 10 nm, and a scanspeed of 120 nm/min was used. All samples were measured using the same 10 mm quartz cuvette. A blank of Milli-Q water was used to correct for Rayleigh and Tyndall scattering processes. Where scattering peaks were not completely removed by this procedure, linear interpolations were performed to remove aberrant data. It should be noted that in the case of fulvic acid E, scattering correction procedures produced negative fluorescence values, a factor accounted for in subsequent analyses. It was decided that excitation-emission matrices represented the most complete spectral coverage, and these were produced using a fluorescent scanning program designed for the purpose. Data was produced at 10 nm resolution on both excitation and emission matrices, with an excitation range of 220 nm to 400 nm and an emission range of 230 nm to 650 nm. Hardcopies of all scans were produced and transferred manually to spreadsheet software. Processing, filtering, removal of fluorescent scattering and plotting were all achieved through the use of Quattro Pro for Windows, and the procedures and routines used are discussed in Chapter 6.

It should be noted that it was attempted to redissolve and measure the optical characteristics of the samples using more stringent methodological protocols, approximately one year after making the initial measurements. This was considered desirable for several reasons: oligotrophic seawater was felt to have a greater validity as a solvent due to its more representative optical and buffering characteristics [Green & Blough 1994], there was some concern over fluorescent quenching effects due to the concentrations of the initial solutions [Green & Blough 1994] , and no form of error propagation procedure had been employed to determine the accuracy limits of both the dissolution and optical methodologies. Unfortunately it was not possible to complete these measurements due to the arisal of unforeseen problems with sample dissolution, compounded by time and instrumental limitations. When making up new solutions using filtered oligotrophic seawater, at concentrations of 10 mg/l, it appeared that few samples dissolved completely, even after several days on an orbital shaker and several hours in an ultrasound bath. This was apparent both visually and from the high degree of variation between sample absorption spectra at supposedly identical concentrations. It was decided to examine several solvents to determine if solvent character influenced the ability of samples to go into solution - 0.005 N NaOH (pH=11.50), filtered oligotrophic seawater (pH=7.91), and a borate buffer (pH=9.19) [Green & Blough 1994]. The high pH of the new Milli-Q/NaOH

solution relative to the initial solvent (pH=9) was intended to aid the dissolution of the humic acid fraction, as it was thought that this fraction may have been rendered insoluble by relatively low pH values. Five solutions were made up with each solvent, using combined fraction H, at higher concentrations of 150 mg/l to improve the signal/noise ratio. All samples were placed on an orbital shaker for 24 hours and immersed in an ultrasound bath for 30 minutes. Again, it was apparent that the samples had not dissolved completely, both visually and from the lack of reproducibility in absorbance measurements. The solutions were then filtered using pre-rinsed 0.2 μm Millipore PC membrane filters, as it was thought that recalcitrant particulate may have been responsible for absorption variations through scattering effects. This did not improve the reproducibility in absorption results. At this stage it was decided that the aluminium foil used to weigh and transfer the samples to solution, and filtering processes were probably at least partially responsible for the wide variations in absorbance. This was deduced from observed discolorations in the aluminium foil, and checks on leaching from the Millipore PC membrane filters used. These showed a significant increase in the absorbance of Milli-Q water filtrate after a 500 ml pre-filtration of Milli-Q.

The solvent comparison procedure was repeated using alternative weighing procedures. It was discovered at this stage that whilst consistency in a(450) was much improved, computed slope values were unexpectedly low, with mean S=0.009, and generally poor correlations with equation 3.2a (mean r^2=0.96). As the presence of particulates is expected to reduce slope values through scattering [Bricaud 1981, S. Hawes, pers. comm.], the samples were centrifuged for 15 minutes at 3000 rpm. This did not appear to have any significant effect on mean a(450) or slope values. At this stage time constraints and problems with baseline drift in the Shimadzu UV-160A spectrophotometer being used for absorption measurements forced further experimentation to stop.

It is of obvious concern that the optical results obtained initially were not reproducable, and that the isolates tended not to dissolve completely in any of the solvents tested. It was felt unlikely that the powdered HS would have degraded significantly during storage, but sample degradation appears to be the only plausible solution in the light of the initial success in redissolving the samples. However the effects of sample contamination and possible degradation will be discussed in further detail later [Chapter 6, 7], and this would appear to be the correct forum to analyse the failure of the later experiments.

Chapter 6

Results and discussion

6.1 Chemical and isotopic analyses

Table 6.1 *Quantity of isolates produced [Woolard 1994]*

Column I.D.	Station No.	Approximate Volume [l]	Fulvic acid mass[g]	Humic acid mass[g]	Total isolate mass [g]
A	AB3	650	1.0975	0.0305	1.1280
B	AB6	350	0.6401	0.0177	0.6578
C	AB8	750	0.3204✗	0.0177	0.3381
D	AB8	750	1.0587	0.0117	1.0704
E	BE1	800	1.0119	0.0602	1.0721
F	BE1	800	1.2015	0.0772	1.2787
G	BE3	800	0.5961✗	0.0134	0.6095
H	BE3	800	Unfractionated Sample		1.1018

✗ Unknown quantity of sample lost during freeze drying

The CHN analyses of the fulvic acid samples (Table 6.2) yielded unexpected results, as the typical composition of marine humic substances are %C ≈ 45%-55%, %H ≈ 3%-6% and %N ≈ 1%-5% [Ertel & Hedges 1983, Choudry 1984, Meyers-Schulte & Hedges 1986, Druffel et al. 1992]. It appears highly likely that the samples are contaminated, which is confirmed by the other analyses carried out on the samples. The ash content of fulvic acid D was determined at 54.7%, indicating the presence of a large fraction of inorganic material. The ICP analysis carried out on fulvic acid D (table 6.3) show the presence of relatively large amounts of silicon and calcium - particularly true of silicon, which if taken as SiO_2 accounts for 14.7% of the original sample [Woolard 1994].
The cation analysis of fulvic acid C shows a large cation contamination of the samples (table 6.4). Of particular significance is the high quantity of ammonium ions, which account for 67% of total nitrogen from the CHN analysis [Woolard 1994].

Table 6.2 *Uncorrected CHN analyses of fulvic acid samples [Woolard 1994]*

Fulvic acid I.D.	% Carbon	% Hydrogen	% Nitrogen	C/N Ratio	H/C Ratio
A	11.03	2.23	4.15	3.10	2.42
B	11.78	3.17	6.89	1.99	3.22
C	20.98	4.89	9.75	2.51	2.79
D	11.14	3.39	7.37	1.76	3.65
E	7.91	6.84	20.87	0.44	10.37
F	6.67	7.02	21.67	0.35	12.62
G	9.23	6.23	18.38	0.58	8.09
H	9.47	6.65	19.74	0.55	8.42

The high nitrogen content of the samples, evident from the CHN analyses, indicates that excess nitrogen has been incorporated into the samples in some way. The most likely source of this nitrogen is thought to be ammonium from the methanol eluent, as the ion chromatography analysis of fulvic acid C indicated that 67% of nitrogen was present as NH_4^+ [Woolard 1994]. The use of aqueous ammonia, rather than ammonia in methanol [Harvey et al. 1983], may have provided a proton source for the formation of NH_4^+ ions, which could then be complexed by HS. Stuermer & Harvey [1977] found that fulvic acids eluted with NH_4OH, as opposed to NaOH, had lower C/N ratios, indicating that aqueous ammonia can be a source of nitrogen contamination. However, the nitrogen contamination observed by Stuermer & Harvey (C/N=13.4) was far lower than that observed here (mean C/N=1.41), implying that ammonia protonation and complexation would have to be an order of magnitude higher to successfully account for observed contamination.

Table 6.3

ICP analysis of ashed fulvic acid D [Woolard 1994]

ash content = 54.7%

Metal	Concentration [ppm]	% of original sample
Mg	0.169	0.48
Ca	1.690	4.83
Al	0.065	0.19
Cu	0.010	0.03
Si	2.410	6.89
Zn, Mn	<0.01	trace
Pb,Fe,P	not detectable	

Table 6.4

Ion chromatography cation analysis of fulvic acid C [Woolard 1994]

Cation	Concentration [ppm]	% of original sample✗
Mg^{2+}	1.5	1.3
Ca^{2+}	7.9	6.8
Na^{+}	16-17	10.3
NH_4^{+}	9.5-10	8.4
K^{+}	1.4	1.2

✗ Corrected for sodium contribution in NaOH solvent.

It is possible that the isolates of Stuermer & Harvey [1977] were relatively hydrophobic, thereby having a low nitrogen affinity [Benner et al. 1992], as their HA:FA ratios were several orders of magnitude higher than those reported by other researchers [e.g. Carder et al. 1989]. This may have lead to a lesser degree of contamination from the use of NH_4OH as an eluent. If it is assumed that all NH_4^{+} in the sample results from contamination, and using the ash figure of fulvic acid D, it is possible to calculate a corrected C/N ratio of 5.6 for fulvic acid C. This value is still lower than any C/N value previously reported for marine HS [Stuermer & Harvey 1977, Ertel & Hedges 1983, Choudry 1984, Meyers-Schulte & Hedges 1986, Druffel et al. 1992]. This would imply that the ammonia eluent is not the only source of nitrogen contamination. However, given the large contamination of the samples from nitrogen, cations and siliceous material, the degree of which is unknown except for fulvic acid C, applying any further form of correction for the purposes of interpreting the CHN results is not feasible.

There are several possible causes of the large cation contamination of the samples. The acidification of the seawater prior to the isolation process serves not only to make target DOM more hydrophobic through protonation of carboxyl groups, but should release bound cations [Woolard 1994]. As the pH of processed seawater was not monitored on a regular basis, it is possible that pH=2 was not achieved, particularly as sampling occurred in productive areas and microorganisms may have provided a source of proton competition. However, given that at some stations (most noticeably AB8 or samples C and D - the samples that underwent cation analysis) chlorophyll *a* concentrations were relatively low i.e. 0.187 mg m^{-3}, this does not seem a feasible explanation for the unexpected CHN results of all the samples. It is worth noting that similar isolation methodologies have produced uncontaminated results from areas of comparable biological activity [Carder et al. 1989, Druffel et al. 1992]. However, given the high concentrations of Si and Ca observed in fulvic acid samples C and D, it does seem likely that detrital material resulting from planktonic degradation is likely to have been retained on the columns, as Si and Ca are known to form important structural components in some planktonic species e.g. diatoms and coccolithophores. Consistent with this, column discoloration was observed during sampling, and the glass wool plugs became clogged with particulate material at several stages during processing. Nevertheless, the CHN results indicate that all samples have a large degree of inorganic contamination, implying that even if this is partially caused by *in situ* effects there is some other overlying methodological problem. A possible cause of this is that salts on the column were not effectively removed by washing with 0.01 M HCl [Woolard 1994].

Despite the high levels of contamination, the $\delta^{13}C$ values of the samples (table 6.5 and figure 14) compare well with other published data. This is consistent with the likelihood of little inorganic carbon in the samples, given the removal of any potential dissolved sources. Excluding samples E and F, which as products of a red tide must constitute a uncommon case, there are several apparent trends in the data. The fulvic acid fractions, with values ranging from -21.82 ‰ to -23.11 ‰, compare particularly well with published data for marine HS, ranging from -20.8 ‰ to -23.50 ‰ [Ertel & Hedges 1983, Pempkowiak & Pocklington 1983, Meyers-Schulte & Hedges 1986, Druffel et al. 1992].

The humic acids, however, ranging from -23.92 ‰ to -26.00 ‰, appear to have consistently lower $\delta^{13}C$ values than those of the corresponding fulvic acid fractions. It is thought unlikely that terrestrial material, known to have lower $\delta^{13}C$ values [Ertel & Hedges 1983, Pempkowiak & Pocklington 1983, Meyers-Schulte & Hedges 1986, Malcolm 1990], will influence fractions of the same sample in different ways, unless terrestrial material was present predominantly as humic rather than fulvic acids. It is more likely that the carbon isotope values of the humic acids reflect a different range of phycological compounds in the humification process such as a higher proportion of lipids, which are relatively depleted in ^{13}C (-25 ‰ to -28 ‰) [Harvey & Boran 1985]. The persistence of this trend in the red tide samples E and F (see figure 14) is strong evidence for this hypothesis, as HS so uncommonly enriched in ^{13}C must have a recent phycological origin [Skopintsev 1981]. In comparison the Aldrich humic acid, which at -26.94 ‰ is typical of soil derived HS [Malcolm 1990], contains over 7 ‰ less ^{13}C.

Table 6.5 *$\delta^{13}C$ values of isolates*

Sample I.D.	Fulvic Acid Fraction	Humic Acid Fraction
A	-21.94	-23.92
B	-21.82	-25.70
C	-22.00	-25.84
D	-23.11	-26.00
E	-18.56	-19.46
F	-18.48	-19.72
G	-22.31	-24.73
H	-22.52	
Aldrich		-26.94

The $\delta^{13}C$ values of the two samples E and F, taken during a red tide, are extremely interesting. Unfortunately, concurrent pigment samples taken for HPLC analysis had not been processed at the time of writing, but from a descriptive standpoint the water was a dull red-brown colour, and smelt strongly of sulfurous, decaying organic matter. The relatively low isotopic values (see table 6.5) for both fractions are indicative of a very recent planktonic origin [Degens et al. 1968,

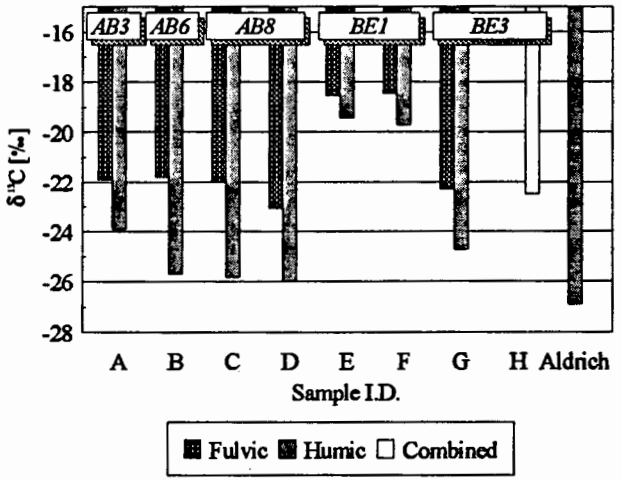


Figure 14. *$\delta^{13}C$ values for isolated HS and a terrigenou sample. Station numbers indicate parallel isolations.*

Williams 1968, Skopintsev 1981, Hatcher & Spiker 1988] as well as implying the existence of different humification pathways for the two fractions (see above).

The carbon isotope values of the samples may also be of use in identifying the different sample fractions. $\delta^{13}C$ values of isolates extracted in parallel from the same stations appear to duplicate well (figure 14), if inappropriate sample pairings are disregarded. These are fulvic acids C and D (colour differences indicate suspected humic acid contamination), and samples G and H (as column H material was not fractionated). The range of appropriately paired samples is only 0.7%, as compared to 17.6% for the whole data set. This implies a reasonable duplication between parallel samples, at least regarding the target organics, despite the inconsistent contamination of duplicates indicated by the CHN analyses (table 6.2). The consistency of carbon isotope values for the samples, and their good comparison with published values, indicates that despite the high levels of observed contamination in the samples the organic fraction has some characteristics of typical marine HS.

6.2 Absorption characteristics

Sample contamination and its effects on optical properties are of an obvious concern, particularly as the degree of contaminant complexation is not known. A high degree of cation complexation, or possible modification of nitrogenous structures within the molecule, could reasonably be expected to modify optical characteristics. It may also be possible that the nature of contaminant association with "humic" molecules has not resulted in any significant optical modifications. The lack of analytical knowledge concerning the nature of contamination and the complexity of humic chemistry precludes any real understanding of the problem.

Absorption characteristics of isolates and corresponding filtered water samples are presented in figures 15 to 18. Absorption spectra show typical exponential decay with wavelength, and generally are well described by equation 3.2c [Chapter 3]. Slight deviations from an exponential relationship appear in samples from station BE1, taken during a red tide, predominantly composed of the dinoflagellate *prorocentrum micans*.. Fulvic acids E and F display a shoulder in absorption at ~325 nm, a feature previously observed by Bricaud et al. [1981] in filtered water samples from the coastal Mediterranean. It is not known what the cause of this discontinuity is. Humic acids from the same station display a small peak centred at ~405 nm, ascribed by previous researchers [Ertel & Hedges 1983 and references within] to phaeopigments associated with HS. The $\delta^{13}C$ data for samples E and F indicate recent formation [Chapter 6.1], and it is likely that these features indicate the presence of partially decomposed molecular fragments in material at an early stage of diagenesis. For the purposes of calculating slope parameters in humic acids E and F, aberrant absorption peaks were removed using PeakFit software [Jandel Scientific] to fit exponential absorption values from 350 nm to 450 nm. Corrected data displayed reduced $a_h(400)$ values and steeper slopes, yielding better correlations (r^2 increased from 0.955 to 0.998).

Absorption	S_f [nm^{-1}]	$a_f(400)$ [m^{-1}]	r^2
A	0.0147	0.6698	0.990
B	0.0153	0.4395	0.994
C	0.0174	0.4971	0.994
D	0.0144	0.7274	0.993
E	0.0121	0.8425	0.997
F	0.0131	0.5546	0.984
G	0.0143	0.6698	0.994

Specific Absorption	S_f [nm^{-1}]	$a^*_f(400)$ [m^{2}gC^{-1}]	r^2
A	0.0147	0.3036	0.990
B	0.0153	0.1865	0.994
C	0.0174	0.1185	0.994
D	0.0144	0.3265	0.993
E	0.0121	0.5326	0.997
F	0.0131	0.4158	0.984
G	0.0143	0.3628	0.994

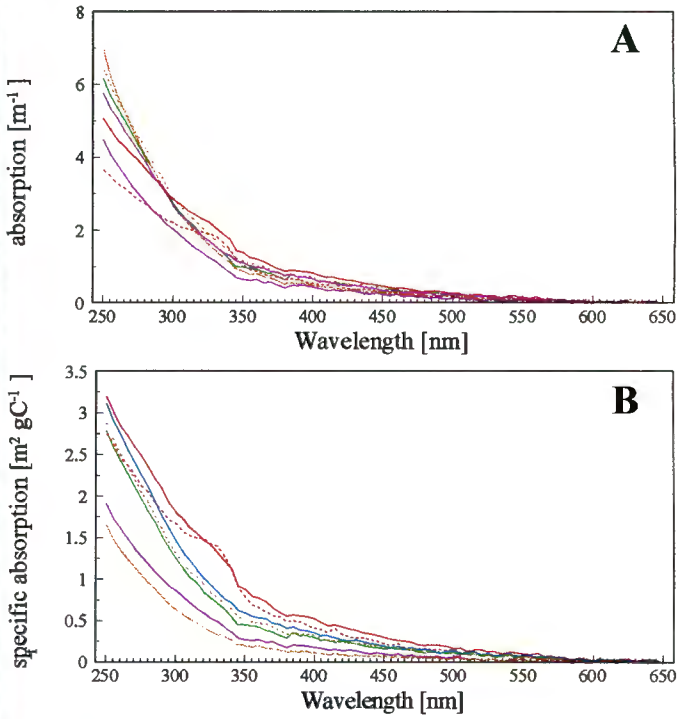


Figure 15. *Absorption characteristics of isolated fulvic acids.*

Absorption	S_h [nm^{-1}]	$a_h(400)$ [m^{-1}]	r^2
A	0.0104	5.6212	0.995
B	0.0151	1.0728	0.987
C	0.0137	2.3395	0.991
D	0.0145	1.0728	0.990
E	0.0111	3.4694	0.997
F	0.0119	2.5476	0.998
G	0.0122	1.9479	0.989

Specific Absorption	S_h [nm^{-1}]	$a^*_h(400)$ [m^{2}gC^{-1}]	r^2
A	0.0104	2.5482	0.995
B	0.0151	0.4553	0.987
C	0.0137	0.5575	0.991
D	0.0145	0.4815	0.990
E	0.0111	2.1930	0.997
F	0.0119	1.9098	0.998
G	0.0122	1.0552	0.989

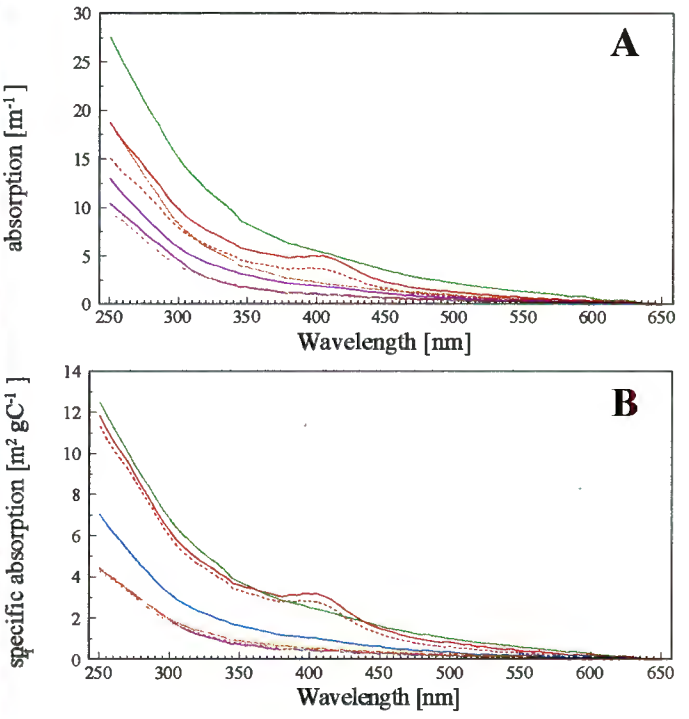


Figure 16. *Absorption characteristics of isolated humic acids.Slope parameters for samples E and F have been corrected for assumed phaeopigment absorption centred at 405nm*

Absorption

Concentration Series [mg/l]	S_c [nm^{-1}]	$a_c(400)$ [m^{-1}]	r^2
40	0.0144	1.2455	0.998
25	0.0138	0.7274	0.997
20	0.0147	0.6698	0.995
10	0.0147	0.2668	0.998
5	0.0139	0.2092	0.992
2	0.0145	0.0365	0.975

Specific Absorption

Concentration Series [mg/l]	S_c [nm^{-1}]	$a^*_c(400)$ [m^2gC^{-1}]	r^2
40	0.0144	0.3288	0.998
25	0.0138	0.3072	0.997
20	0.0147	0.3536	0.995
10	0.0147	0.2817	0.998
5	0.0139	0.4418	0.992
2	0.0145	0.1925	0.975

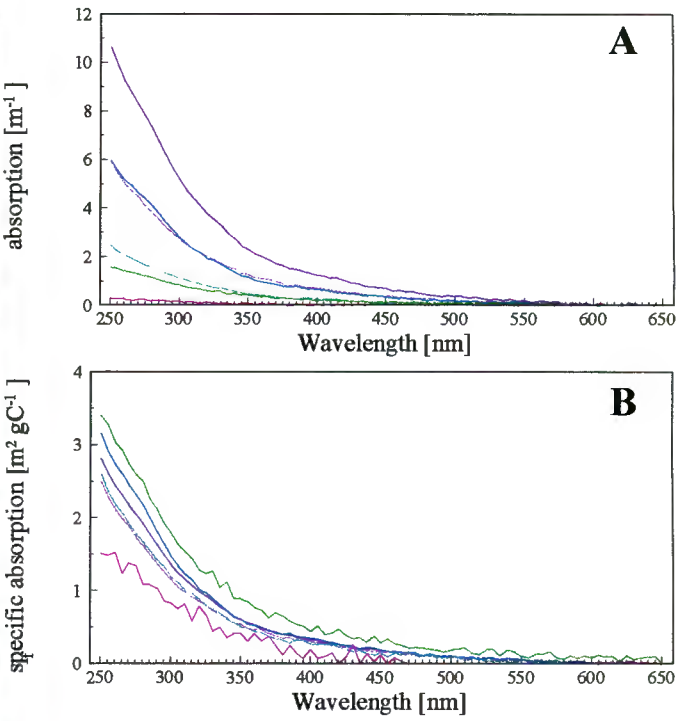


Figure 17. *Absorption characteristics of isolated gross humic substances from station BE3.*

Normalised Absorption

Station	Type	S_g [nm^{-1}]	$a_g(400)$ [m^{-1}]	r^2
AB3	FA+HA	0.0140	1.2091	0.989
	Water	0.0162	1.2091	0.999
AB6	FA+HA	0.0153	0.9788	0.993
	Water	0.0178	0.9788	0.998
BE1	FA+HA	0.0123	0.8061	0.997
	Water	0.0179	0.8061	0.999
BE3	FA+HA	0.0143	1.0939	0.993
	Combined	0.0147	1.0939	0.995
	Water	0.0161	1.0939	0.999

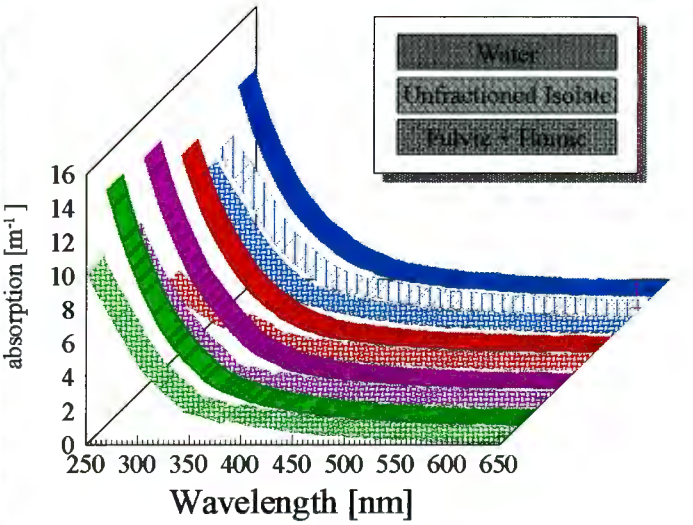


Figure 18. *A comparison of gelbstoff absorption from filtered water samples and corresponding isolates. Summed fulvic and humic acid absorption (FA+HA) has been calculated using equation 3.2c, normalised to the filtered seawater absorption at 400nm. Station BE3 shows absorption due to summed fulvic and humic acids (FA+HA), gross humic substances (combined) and filtered seawater (water).*

It appears that the fulvic acids have both steeper slopes and lower specific absorption coefficients than the humic acids (figures 15 and 16), consistent with previously published research [Zepp & Schlotzhauer 1981, Hayase & Tsubota 1985, Carder et al. 1989]. This is apparent even considering the lack of confidence in $a_h^*(\lambda)$ associated with the application of fulvic acid CHN data, necessitated by the lack of CHN analyses on the humic acids [Chapter 5]. However, the relative magnitude of the difference in slope between the two fractions (Table 6.6) is not as great as that observed by Carder et al. [1989], and some samples show little variation in slope between the two fractions (figures 15 and 16). The high levels of contamination observed in the samples may be responsible for this, as it thought unlikely that "true" humic and fulvic acid fractions would have similar slope parameters [Zepp & Schlotzhauer 1981, Hayase & Tsubota 1985, Carder et al. 1989]. Contaminant effects are also consistent with the lack of replication in data from isolates extracted in parallel from the same sites e.g. C and D. This point in particular would seem to indicate that contamination may be of optical significance, and furthermore its effects are potentially inconsistent.

Table 6.6. *Absorption characteristics of isolates*

Variable	Fulvic Acids	Humic Acids	Combined Series
mean a(400) [m^{-1}]	0.63(±0.13) (20mg/l)	2.58(±1.47) (20mg/l)	-
mean a*(400) [m^2gC^{-1}]	0.32(±0.12)	1.31(±0.82)	0.32(±0.08)
mean S ($\times 10^{-2}$) [nm^{-1}]	1.45(±0.16)	1.27(±0.16)	14.3(±0.04)
Coefficients of variation, determined from the combined concentration series, are: a*(400)=23%, S=3%			

Nevertheless, both slope values and specific absorption coefficients for the fulvic acids appear to be in a similar range to published data, if samples from a variety of marine systems are considered, and it is assumed that gross isolates (CDOM) are predominantly fulvic acids [Zepp & Schlotzhauer 1981, Hayase & Tsubota 1985, Carder et al. 1989, Blough et al. 1993, Green & Blough 1994]. The S_f values appear low relative to those of Carder et al. [1989] (mean S_f=0.0189 nm^{-1}, produced using a similar methodology with samples from the Gulf of Mexico), as are S values from corresponding water samples (figure 18). This indicates that some of the observed differences in slope values may arise from valid differences in sample character, supported by good comparisons of fulvic slope parameters with other data, although possibly from more disparate systems [Zepp & Schlotzhauer 1981, Hayase & Tsubota 1985, as cited in Carder et al. 1989, Blough et al. 1993]. Note that the use of a(400) here as a reference, rather than a(450) as used by

Carder, serves to *increase* slope values, further increasing possible discrepancies. The slope values of the majority of humic acids, S_h, show good comparison with published values [Zepp & Schlotzhauer 1981, Carder et al. 1989], although in this case some values (samples B, C and D) appear significantly higher than those of Carder's. Whether these discrepancies reflect sample differences or result from contamination is unclear.

The specific absorption coefficients of the fulvic acid isolates appear disproportionately high in comparison to those of Carder et al. [1989] and Hayase & Tsubota [1985], with relative values more than two orders of magnitude greater. Whilst the same is true of the specific absorption coefficients of the humic acids, there is a lesser degree of confidence in these results as they were not based upon direct CHN analyses [Chapter 5]. It appears that these relatively large $a^*(\lambda)$ values are more closely comparable to terrestrially influenced HS [Zepp & Schlotzhauer 1981, Blough et al. 1993]. In the light of the varied sampling conditions in this study, the low probability of terrestrial influences at the majority of sampling sites and the consistency in observed $a^*(400)$ values, it appears that these differences may be induced by contamination.

Despite possible contaminant effects, there is a good linear correlation between absorption and concentration of isolate solutions. Figure 19 shows this relationship, based upon a concentration series using the unfractionated sample from Column H. Such a good correlation indicates that dissolution of the samples is complete, at least as regards the core organic fraction. It also shows that the manipulation of concentration with respect to absorption e.g. as with equation 3.2c, has a high degree of validity.

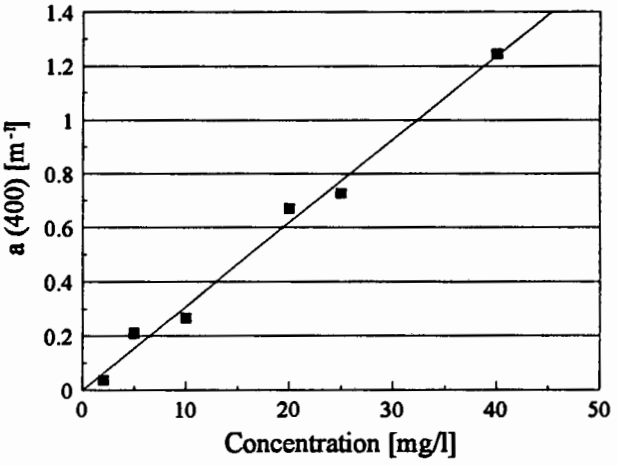


Figure 19. *Linear regression between concentration and reference wavelength a(400), for a series of solutions using sample H (r^2=0.99, n=6, p=6.5×10^{-6}).*

In the light of possible contaminant effects, comparisons between the absorption characteristics of isolates and corresponding filtered water samples are reduced in value, particularly as concerns potential optical biasing associated with XAD-2 resin [cf. Chapter 5].

The number of comparative samples (4) also precludes any form of statistical treatment. Nevertheless, such a comparison reveals that the slopes of isolates, as calculated from equation 3.2c, are typically flatter than those of corresponding water samples (figure 18). Note that these calculations used the relative concentrations of the fractions in conjunction with the appropriate absorption coefficients, thus bypassing uncertainties associated with the specific absorption coefficients. These relatively flattened slopes may indicate that, like C_{18} isolation media [Green & Blough 1994], XAD-2 resin preferentially extracts longer wavelength absorbing material. It should be noted that water samples for this study were filtered through GF/F filters (nominal retention size=0.7 μm) rather than through 0.2 μm membrane filters as recommended by the SeaWiFS optical protocols [Mueller & Austin 1995]. Calculated slopes therefore represent a *minimum*, as particles in the filtrate resulting from coarser filtration are expected to flatten slope values [Bricaud et al. 1981]. It is not possible to use the isolates to compare the magnitude of absorption, as the isolation efficiency of the XAD-2 resin was not quantified. However, the absorption parameters of the few filtered water samples presented here compare well with other researchers (cf. figure 4) and $a_g(\lambda)$ values can be considered relatively high for waters of an expectedly low terrestrial influence [Bricaud et al. 1981, Blough et al. 1993, Green & Blough 1994, Nelson & Guarda 1995].

6.3 Fluorescence characteristics

Fluorescence data for isolates and filtered water samples are presented in two formats: excitation-emission matrices (figures 20 and 21) and synchronous scans (figure 22). Whilst fluorescence data was not standardised, the consistency of analytical conditions for all samples allows a confident intra-comparison of fluorescence data, and qualitative comparisons to published material. In this regard the fluorescent characteristics of isolates generally compares well with published data for both isolates and water samples [Coble et al. 1990, 1993, Mopper & Schultz 1993, De Souza Sierra 1994, Seritti et al. 1994, Ferrari & Mingazzini 1995, Coble 1996]. Fluorescence peaks are described using the standard notation of $\lambda_{excitation}/\lambda_{emission}$.

Excitation-emission matrices

The four humic acid samples for which excitation-emission matrices were obtained appear to be the most well characterised of all samples as concerns spectral fluorescence (figure 20). Three features appear consistently in all humic acids: a major peak at 250/310 (*A*), a minor peak at 280/360 (*B*) and a shoulder/peak at 250/360-390 (*C*). Whilst ascribing these features to specific fluorophores is not possible, a comparison with similar features observed by other researchers allows a general identification. Peak *A* [5], which appears to be the dominant spectral feature for the humic acid fraction, has been attributed to a protein-like fluorophore [Coble et al. 1993, Mopper & Schultz 1993] and, based on emission wavelength, is most similar to the amino acid tyrosine [Seritti et al. 1994, Coble 1996]. The smaller feature *B* has also been attributed to a protein-like fluorophore, possibly tryptophan, in isolates from the Black Sea [Coble et al. 1990]. Similar features, with slightly blue shifted wavelengths (e.g. 275/340-350) have been observed for natural water samples and have been variously associated with tryptophan [Coble 1996] or recent phycological activity i.e.. bloom conditions or culture exudates [Traganza 1969, De Souza Sierra et al. 1994, Seritti et al. 1994].

5. Based on undisplayed data extending to 220 nm excitation wavelength, the feature at 250/310 is associated with a peak situated at 220/300, similar to that of Mopper & Schultz [1993] and Coble et al. [1993].

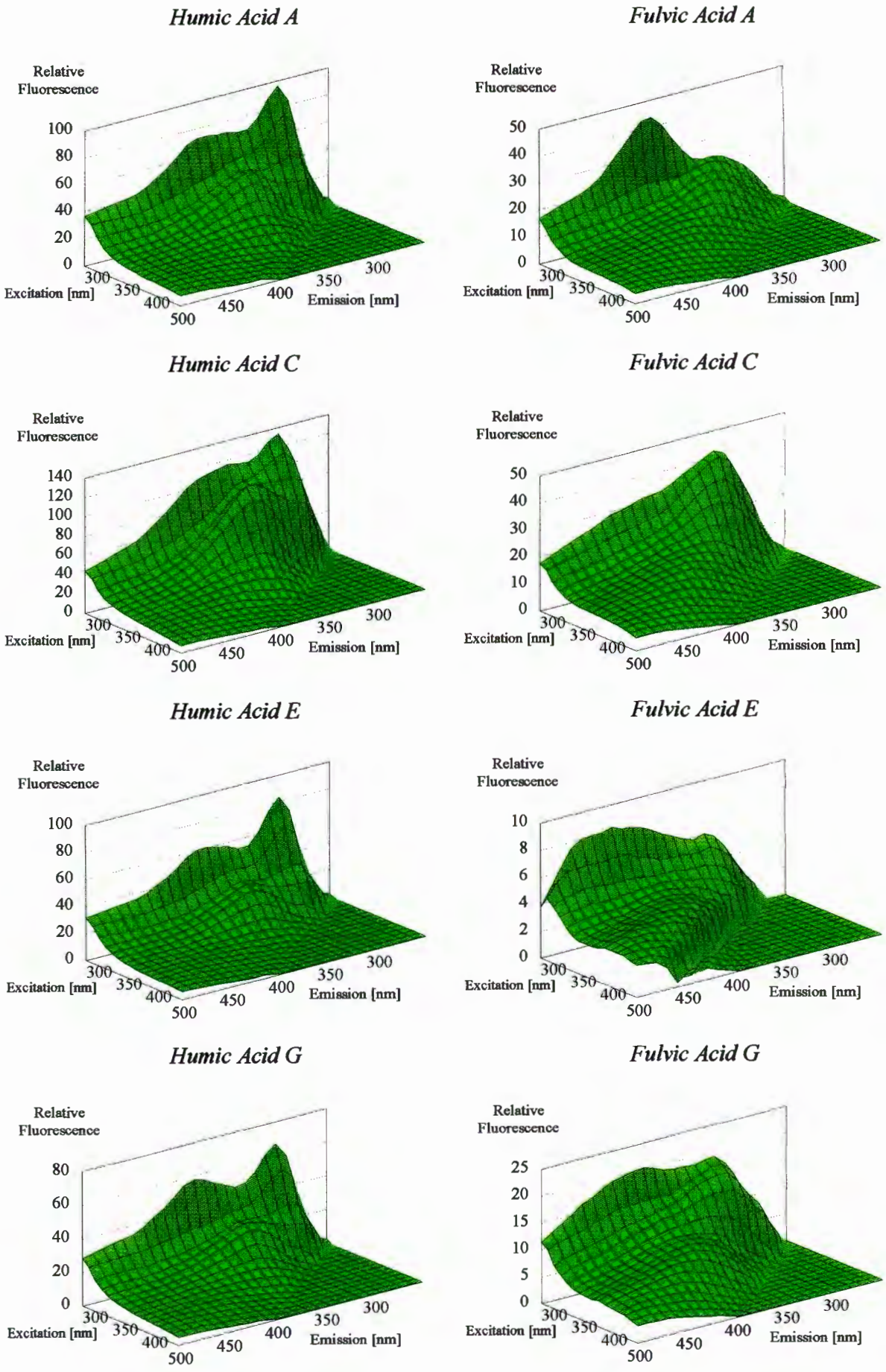
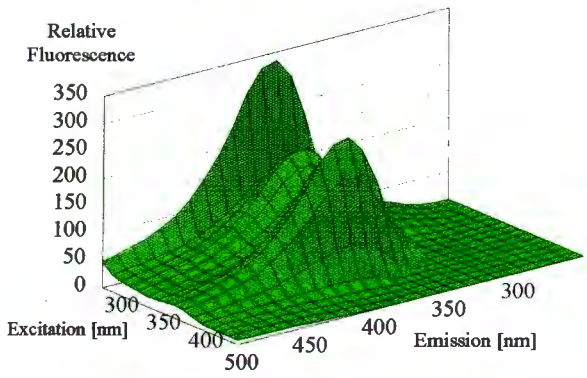


Figure 20. *Fluorescence excitation emission matrices of isolated fulvic and humic acids.*

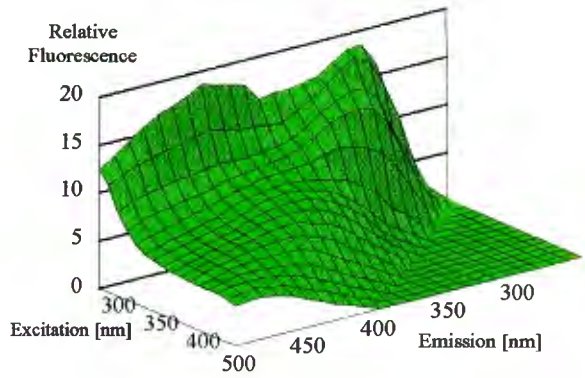
This significant presence of protein-like features in XAD-2 isolates is consistent with published data indicating that between 60%-100% of total dissolved amino acids are associated with XAD based "humic" fractions [Hubberten et al. 1995]. Such protein-like features are also considered typical of surface waters [Chapter 4.2.3], from which all samples were taken. The third peak, feature *C*, appears subject to the most variation in spectral position, and is most similar to humic-like features observed by other researchers [Coble et al. 1990, 1993, Mopper & Schultz 1993, Coble 1996]. In all other reported cases, feature *C* appears at longer wavelengths than observed here e.g. 250/380-460, with generally greater variation in spectral location relative to the previous features. Differences in spectral character with published data are also manifested in the lack of fluorescent activity at longer wavelengths i.e. from 400 nm onwards. This can be considered unusual, as published data typically displays a high degree of fluorescent activity in these longer wavelength regions [e.g. Traganza 1969, Coble et al. 1990, 1993, Mopper & Schultz 1993, De Souza Sierra et al. 1994, Coble et al. 1996]. However the lack of longer wavelength fluorescence is exaggerated here to some degree by the focus on the typically highly active ultra-violet region.

The fulvic acid fraction displays some striking differences to the humic acids, by virtue of their typically lower fluorescence intensities (<50% of humic acid maximum intensity), different spectral characteristics, and a higher degree of variation between fraction samples (figure 20). Fulvic acid E will be disregarded as problems with the blank correction may give spurious results [Chapter 5]. Feature *A*, red shifted slightly to 250/330, is significant in samples C and G, and appears as a shoulder in sample A. This red shift is well within the range of spectral locations observed by other researchers for the same feature [Coble et al. 1993, Mopper & Schultz 1993]. Feature *C*, also red shifted within acceptable ranges (see above) to 250/390-400, is the dominant feature in samples A and G, but appears only as a shoulder in sample C. Whilst feature *B* is no longer plainly visible, and is less prominent in comparison to the humic acid samples, it is possible that a red shifted feature *A* masks the feature slightly, as correctly located shoulders appear in all samples. Whilst there seems to be a greater amount of fluorescent activity at longer wavelengths, relative to the humic acid samples, there are still no clearly delineated maxima. Weak shoulders at 310/400-410 (*D*), a feature identified by Coble [1996] as "marine humic-like", can be tentatively identified in sample C and slightly more strongly in sample G.

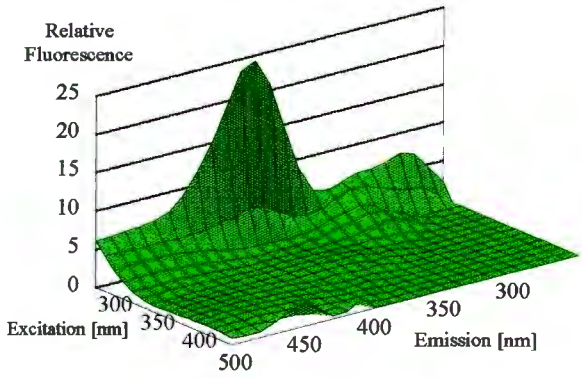
Combined Isolate H at 40 mg/l



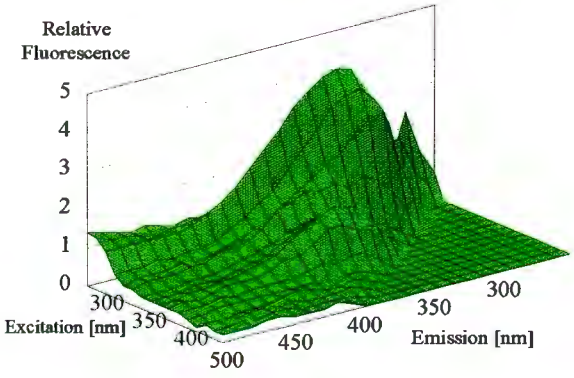
Combined Isolate H at 20 mg/l



Filtered Seawater - Station BE3



Combined Isolate H at 2 mg/l



Combined Isolate H at 10 mg/l

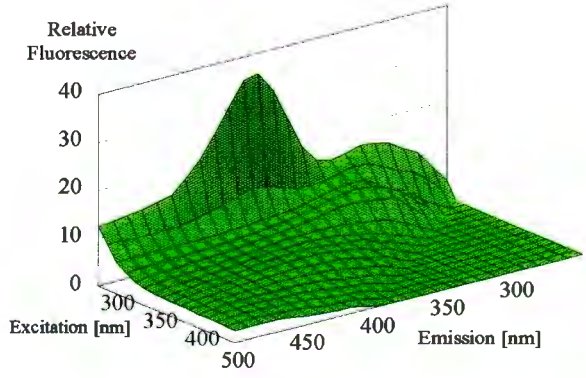


Figure 21. *Fluorescence excitation emission matrices of combined isolate H at various concentrations, in comparison to a filtered seawater sample from the same station.*

The observed variations in fluorescence between the two fractions give rise to some interesting observations. A coarse comparison of intensity can be effected using fluorescence intensity I_f (given in relative units) at 250/390, the location of feature *C*. I_f (250/390) ranges between 108 and 58 for the humic fraction as compared to 44 to 20 for the fulvic fraction. This conflicts with published information, showing that fulvic acids display a greater unit intensity [Chapter 4.2.1]. This reversal of the observed trend may be caused by differing degrees of quenching associated with cation contamination. It is possible that the humic fraction is either less contaminated i.e. has a relatively small degree of associated cation complexation, or the typical molecular structure of the humic fraction is less *prone* to internal quenching e.g. through rearrangement of intra-molecular bonding or inter-fluorophore energy transfers [Senesi 1990]. Unfortunately it is impossible to establish what may be causing this phenomenon, due to the lack of relevant analytical information - in particular CHN data for the humic acids. The greater degree of homogeneity in the spectral fluorescence of the humic acid samples is also curious, and may reflect a divergence in humification pathways or stages of diagenesis. It is possible that this homogeneity reflects either different formative/degradative processes, or a more advanced state of diagenesis culminating in convergence to a more common molecular structure.

The excitation-emission matrices of the concentration series for combined isolate H (figure 21) show that concentration effects on fluorescence are extremely important, at least in the context of this study. It is immediately apparent that the spectral character of fluorescence undergoes a marked change as sample concentration changes. This is of particular significance given the relatively good replication between the absorption characteristics of the concentration series [Chapter 6.2], and the possible effects of this will be discussed in the next section of this chapter. Concerning the identification of spectral features, the isolate at 40 mg/l (H40) appears to deviate to a relatively large degree from the fluorescent character of other isolates. Feature *A* , prominent in other samples, is not visible or is eclipsed by the much greater intensity of other peaks. Feature *C*, however, is visible in a similar location to other isolates, and if used as a common index of fluorescent intensity, is over three times greater than humic acid C, the most intensely fluorescing sample at 20 mg/l. A second peak in the EEM, located at 280/380, is difficult to positively identify, particularly as features in similar spectral locations are not immediately apparent at other concentrations of isolate H.

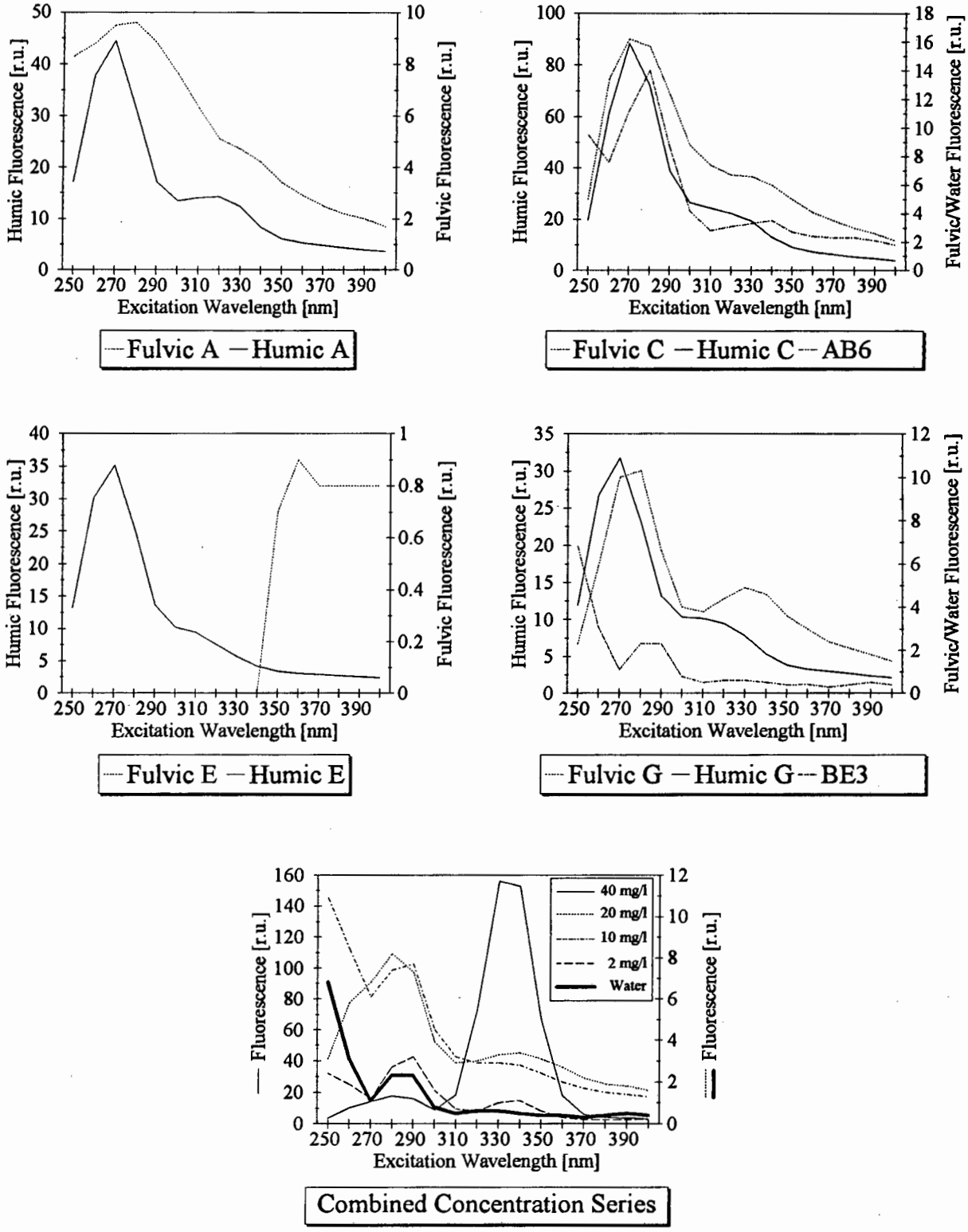


Figure 22. *Synchronous fluorescence scans of isolates and corresponding water samples. Note blank subtraction errors assciated with fulvic acid E. $\delta\lambda$=30 nm*

However, a similar feature has been observed by Coble [1996], also for an extraordinary highly fluorescing sample of filtered seawater (GOM-MLML 7m), identified as "marine humic-like" (*D*). A third peak for sample H at 40 mg/l appears at 330/380, which cannot be easily identified and is therefore assumed to be "humic-like" (*E*). It is possible that both the features appearing at 280/380 and 330/380 result from wavelength shifts in previously identified (or unidentified) fluorophores brought about by changes in inter-fluorophore energy transfers associated with concentration changes. The EEMs of sample H at other concentrations display previously identified features to varying degrees. Sample H at 20 mg/l (H20) compares well with fulvic acid G, isolated in parallel, both in terms of intensity and spectral behaviour. This is consistent with the high fulvic acid percentage of the total sample [Chapter 6.1]. Another good comparison can be made between the spectral characteristics of sample H at 10 mg/l (H10) and the corresponding water sample from station BE3. This implies that XAD-2 isolates may provide an unbiased representation of the original water's fluorescence characteristics, if studied in the appropriate concentration ranges. It also serves to reinforce the hypothesis that the spectral fluorescence of DOM is strongly linked to concentration, of great importance to establishing appropriate analytical constraints.

It is important to assess the possible causes of these fluorescence/concentration effects, particularly in the light of possible contaminative effects. To determine whether observed effects are due to *concentration* related quenching, or the "inner filter effect" [Senesi 1990], the relationship between concentration and fluorescence at a frequently used wavelength pairing, 320 nm/450 nm [Chen & Bada 1992], was examined (figure 23). A good exponential correlation, consistent with expected theoretical behaviour [Senesi 1990], indicates that quenching due to concentration effects is *not* significant, at least in the part of the spectrum examined. An examination of contaminant induced quenching is not possible, but the strong exponential correlation observed indicates any such processes do not vary with concentration. Nevertheless there is no doubt that concentration changes do induce some spectral

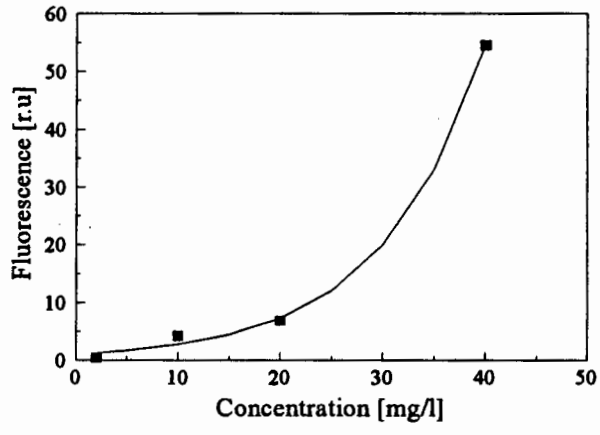


Figure 23. *Relationship between concentration and fluorescent intensity at 320/450 for isolate H.*

$$I_f = e^{(0.1 \times Concentration)} \quad (r^2=0.998,\ n=4.)$$

variation in fluorescence. Such a phenomenon has been observed before, using synchronous scanning techniques [Senesi 1990], and has been ascribed to changes in fluorophore association and configuration, possibly resulting from a higher collision rate of excited molecules [Senesi 1990].

Synchronous fluorescence spectra

Synchronous fluorescence data was culled from excitation-emission matrices using a wavelength offset of $\Delta\lambda$=30 nm, chosen to mimic as closely as possible the conditions of Ferrari & Mingazzini [1995], who used an offset of $\Delta\lambda$=25 nm. The slight increase in wavelength offset, necessitated by scanning resolutions of 10 nm, is felt to offer the best compromise between spectral separation and sensitivity [Senesi 1990, De Souza Sierra 1994, Ferrari & Mingazzini 1995], given the scattering corrections associated with fluorescence scanning configurations [Chapter 5].

Synchronously scanned fluorescence spectra are presented in figure 22, displaying comparative spectra for the fractions from each station and concurrent filtered water samples when available. Spectra compare well with published data [Serriti 1990, Dujmov et al. 1992, De Souza Sierra et al. 1994, Ferrari & Mingazzini 1995] with the majority displaying a major peak at ~270 nm, with occasional minor peaks at ~340 nm. These will be referred to as hA and hB respectively, after the nomenclature of Ferrari & Mingazzini [1995]. The other major peak observed by Ferrari & Mingazzini [1995], located at ~390 nm (hC), does not appear in any samples, consistent with the general lack of fluorescent activity at longer wavelengths in the EEMs. Also consistent with the EEMs is the typically higher fluorescence levels of the humic acids in comparison to the fulvics, evinced by the use of different fluorescence scales in figure 22. In general, the spectra of the humic acids are characterised by the strong and relatively well defined presence of feature hA, with an additional shoulder appearing at ~310-320 nm. This second feature does not appear in the spectra of Ferrari & Mingazzini [1995], although they do observe a peak at 310 nm which is attributed to contamination. It is also possible that this feature simply results from poor spectral separation, as it is also present in some fulvic acid spectra, and is similar in character to large offset ($\Delta\lambda$=100 nm) spectra for marine waters measured by De Souza Sierra et al. [1994]. Feature hB does not appear in any of the humic acid spectra.

The fulvic acids generally display similar spectral shapes to the humic acids, at least with regard to the dominance of hA. Differences can be observed in that hA is typically less well defined i.e. broader peak widths in the fulvic fraction, and the peak is marginally shifted towards longer wavelengths, appearing at 280 nm in fulvic samples A and G. Fulvic sample G is also the only isolate to display feature hB.

The concentration series of combined isolate H displays behaviour similar in some ways to that observed in the EEMs. Sample H40 has a maximal fluorescent intensity significantly greater than any other sample, and is the only sample to fluoresce most strongly at location hB. This shift in maximal fluorescent activity to longer wavelengths is consistent with the EEM for the same sample. The lower concentrations of sample H display spectra closer in shape to those of the other samples, with feature hB visible in all cases. Again, consistent with behaviour in the EEMs, samples H20 and H10 do not appear to display the expected differences in fluorescent intensity, although figure 23 indicates fluorescence at an index wavelength pairing behaves in an expected manner. Similarities in spectral shape between BE3 and H10 are also consistent with EEM patterns. It is worth noting the inconsistent appearance of feature hB at stations AB6 and BE3, alternatively present in either the water, fulvic or combined fractions. This indicates that the lack of feature hB in the majority of the isolates does not arise from XAD-2 biasing against this fluorophore.

The general trends in the synchronous spectra allow some commentary on the fluorescent character of the samples as a whole. The prominent appearance of feature hA is consistent with the significant presence of a monoaromatic amino acid such as tyrosine in the isolates [Ferrari & Mingazzini 1995], as evinced from the prominence of feature *A* in the EEMs. This is deduced from observations relating red shifts in peak wavelength to the complexity of the aromatic structure, specifically an increasing number of aromatic rings in the molecule [Dujmov et al. 1992, Ferrari & Mingazzini 1995 and references within]. Such logic is supported by the greater fluorescent activity above 400 nm for terrigenous samples [Cabaniss & Schumann 1987, Senesi 1990].The homogeneity of the humic acid spectra, in comparison to those for the fulvic acids, is also consistent with both EEM characteristics and the previously discussed implications of this concerning humification. The general shape of the synchronous spectra i.e. dominance of hA over hB may be indicative of relatively youthful material [Ferrari & Mingazzini 1995], as implied by the predominance of large protein-like peaks in the EEMs.

However, the spectra of H40 shows that concentration, seemingly unconsidered by Ferrari & Mingazzini [1995], is also capable of large effects on the hA:hB ratio.

The generally good qualitative comparisons between isolates produced here, and published data sampled from a range of environments, and based on a variety of methodological protocols, indicates that the organic fraction of the isolates is representative to a large degree of *in-vivo* DOM. This is supported by the similarity, *in some cases*, of the fluorescence characteristics of isolates and corresponding water samples. However, possible incongruities have also appeared, such as the apparent lack of longer wavelength fluorescence activity, and the questions posed by contamination and its effects still hang over the study. Nevertheless, the fluorescence data as a whole indicates that the organic substances isolated in this study are representative, perhaps in an appropriately equivocal manner, of "humic" substances.

6.4 Fluorescence/absorption relationships

Fluorescence to absorption algorithms were generated in several ways, using both published methodologies and a technique based upon the spectral location of major fluorophores, as indicated by the excitation-emission matrices of the previous section. Both published methodologies are based upon a linear regression between absorption and emitted fluorescence, with the important proviso that fluorescence must be excited at the same wavelength as measured absorption [Hoge et al. 1993]. This spectral juxtapositioning is necessitated by spectral variations in quantum yield, resulting from the typical polyfluorophoric structure of DOM [Hawes et al. 1992, Green 1993, Green et al. 1994, Vodacek et al. 1994]. Both methodologies employ a wavelength of 355 nm [Hoge et al. 1993, Green & Blough 1994, Vodacek et al. 1995], chosen for compatibility with laser fluorosensing applications. A similar wavelength of 350 nm is used here - the slight offset is dictated by the fluorescence spectral resolution of 10 nm.

The first methodology (*simple*) regresses a(350) against a single fluorescence wavelength pairing of 350/450, or fluorescence emission at 450 nm [Hoge et al. 1993a, Vodacek et al. 1995]. The use of a single fluorescence value facilitates rapid measurement, and excellent correlations with absorption have been reported for water samples from a variety of marine systems [Hoge et al. 1993, Vodacek et al. 1995]. The second methodology (*integrated*), based upon that of Green & Blough [1994], deviates only in that *integrated* (i.e. total) fluorescence at excitation=350 nm is used to base regressions on, providing an indication of the total number of emitted photons [Green & Blough 1994]. Integrated fluorescence was calculated from the full emission scan, of 360 nm to 650 nm, at $\lambda_{excitation}$=350 nm, culled from the corrected EEMs [Chapter 6.3].

The third methodology (*multi*), utilises fluorescence values based upon single wavelength pairings, determined from the main fluorescent features in the EEMs, i.e. features *A* (250/310), *B* (260/380), and *C* (250/390) [Chapter 6.3]. These are regressed against a(400), chosen as it is the absorption reference wavelength for the SeaWiFS sensor [Chapter 3]. It is felt that such a technique, by targeting the spectral location of core fluorophores, can make effective use of the intrinsic relationship between absorbing and fluorescing processes. The technique can be criticised, however, particularly in its disregard for concerns over spectral variations in quantum yield, as discussed above. If such a position is adopted, it might be argued that targeting equivalent features

in spectral quantum yield, as opposed to fluorescence, might better quantitavely describe absorption. Nevertheless, given the complex associations between fluorescent processes and humic chemistry, and the independence of the proposed variables, such a technique can be justified.

Linear regressions were performed on the data pertinent to each of the methods outlined above, employing several data sets. These were: the humic acid fraction, the fulvic acid fraction, the concentration series of combined isolate H, all data including the two filtered water samples, and more concise data set based on analytical consistency. Data are excluded from this last set with the intention of establishing the upper accuracy limits of predictive relationships, rather than as outliers. Such data are : fulvic acid E due to lack of confidence in fluorescence measurements, and any data other than isolates at 20 mg/l concentration. It should also be noted that the small sample size of some data sets (n=4) results in a low degree of confidence in calculated statistical parameters. Results of the various regressions can be seen in Table 6.7.

The absorption and fluorescence regressions are characterised by generally poor correlations. Other researchers, using a variety of similar techniques, have reported correlations of $r^2 \geq 0.98$ on a regular basis. Data for all samples, with a corresponding line of best fit for an isolate based regression using the *multi* technique, is shown in figure 24. It can be seen that while the expected linear trend between absorption and fluorescence is followed, there is a large amount of scatter, even if the data are constricted to isolates at the same concentration. Whilst correlations for the humic and fulvic acid fractions are generally better than other data sets, both the lack of consistent performance between regression techniques, and the small number of samples in these sets limit analysis.

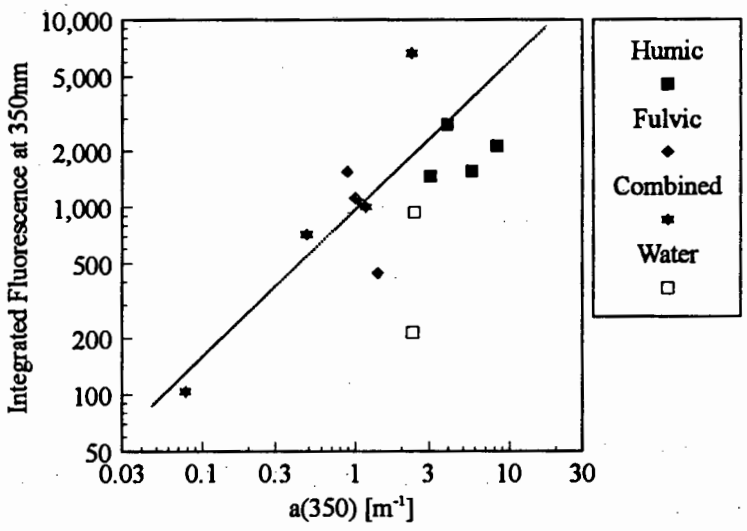


Figure 24. *The relationship between absorption at 350 nm and integrated fluorescence excited at the same wavelength. The regression line indicates that of the multi technique for isolates at 20 mg/l.*

Table 6.7 *Regression between absorption and fluorescence data sets*

Method		*simple*	*integrated*	*multi*	
Data Set	Parameter			*A* (250/310)	*B* (280/360)
Humic Acids	Slope	-0.13	19.69	0.24	-0.22
(n=4)	Intercept	11.06	1881.79	-6.90	
	r	-0.08	0.07	0.99 (r^2=0.98)	
	p	0.05	0.0006	0.008	
Fulvic Acids	Slope	-11.38	-2005.30	-0.005	-0.008
(n=4)	Intercept	19.09	3241.07	0.88	
	r	-0.97	-0.98	0.98 (r^2=0.89)	
	p	0.006	0.0004	0.69	
Combined Series	Slope	12.78	2903.20	0.03	0.003
(n=4)	Intercept	-2.93	-818.24	-0.05	
	r	0.96	0.93	0.96 (r^2=0.78)	
	p	0.22	0.21	0.87	
All data	Slope	0.86	195.48	0.04	-0.008
(n=14)	Intercept	6.13	1073.64	0.45	
	r	0.27	0.34	0.77 (r^2=0.52)	
	p	0.008	0.001	0.44	
Isolates [20 mg/l]	Slope	0.44	136.62	0.10	-0.11
(n=8)	Intercept	7.47	1149.79	0.46	
	r	0.40	0.61	0.94 (r^2=0.82)	
	p	0.001	4×10^{-6}	0.42	

It appears the *multi* regressions perform as well, if not better, than comparative methodologies, but confirmation of this would require further investigation with a suitably large data set. However, it was discovered that regressions of this type appeared almost completely insensitive to the inclusion of feature *C* as a variable, possibly indicating that the fluorophore responsible for this feature does not have a significant role in absorption processes. It should be noted that similar regression techniques were applied to the absorption slope parameter S, producing poor correlations in all cases. However, given the increased sensitivity to quantum yield variations arising from the multi-spectral nature of S [Green & Blough 1994], and the generally low correlations observed for the absorption coefficient, this is to be expected.

There are several factors that may explain the relatively poor correlations between fluorescence and absorption for the isolates. Due to the limited number of isolations performed, the small sample size is not conducive to establishing well correlated statistical relationships. This is particularly true given that humic and fulvic acids have been shown to display asymmetrical fluorescence/absorption relationships - humic acids are positively correlated while fulvic acids are negatively correlated [Hayase & Tsubota 1985]. Whilst small sample size and range precludes confirming the same is true for this study, it is apparent from figure 24 that the two fractions display different behavioural characteristics. Such factors are highly likely to adversely affect the degree of correlation, as a regression on all isolate fractions considers two potentially highly divergent sets of data as concerns quantum yield. It is also possible that the poor correlations are due to contaminant effects, given the high levels of contamination in the isolates, and the sensitivity of humic substance fluorescence to cation contamination [Choudry 1981, Tuschall & Brezonik 1983, Senesi 1990, Philpot & Vodacek 1989, Ferrari 1991]. However, given the generally good comparisons with published data, particularly as regards the fluorescence characteristics of the isolates [Chapter 6.3], it appears that there is little optically significant contamination. Unfortunately, the limited sample size, and analytical information available, does not allow any more conclusive examination of the optical characteristics of the isolates as regards contaminant effects. Nevertheless, the high degree of scatter in the fluorescence/absorption relationships of the isolates can be adequately explained through effects typical of uncontaminated humic substances.

Chapter 7

Conclusions

The chemical and optical characteristics of the isolates produced in this study provide valuable information on the nature of gelbstoff in southern African waters, and the methodologies available for its examination. It is apparent, not only from the obvious contaminant problems with isolated material, that the detailed study of such complex material requires the application of well considered and highly rigorous methodological protocols. The observed contamination of isolated humic substances, attributed to several possible sources, shows that even the application of well documented methodologies is highly prone to error. Sample contamination is particularly compounded in the sphere of humic chemistry, as the complexities inherent to these materials precludes a complete understanding of contaminant cause and, more importantly, effect. This is especially true of the core bio-optical properties of the isolates - contaminant induced changes in character appear to be relatively subtle, given that generally good comparisons can be made with published data. Nevertheless the data set as a whole provides a large amount of information concerning the formation and bio-optical nature of gelbstoff and its constituents in southern African waters.

From a chemical perspective the carbon isotope data provide the most significant results, as contamination of the isolates precludes any real examination of their elemental composition. The very good comparison of $\delta^{13}C$ data with published data indicates that the organic content of the isolates is typical of "humic substances". The less positive values of the humic acids relative to the fulvic acids can also be considered typical, and indicates a higher proportion of more refractory phycological compounds, such as lipids [Chapter 6.1]. Such a trend may indicate that the structural differences between the fulvic/humic fractions are produced from dissimilar starting materials, rather than different stages of diagenesis along similar formational pathways. Carbon isotope data for the "red tide" samples, products of an unusual formational environment, appear to support such reasoning. Material isolated from red tide conditions, a bloom of the dinoflagellate *Prorocentrum micans*, provides some of the most interesting isotope data from this study. The more positive $\delta^{13}C$ values of these samples, similar to more labile phycological compounds, are indicative of a predominance of recently produced autochthonous material [Chapter 6.1], an important consideration in a near-shore system. In the highly productive Benguela, prone to episodic bloom formation, such data indicate that the autochthonous production of gelbstoff is of major concern to ocean colour studies.

The general absorption characteristics of the isolates [Chapter 6.2] compare favourably with published data, in that they fall within the range of previously published values for isolated humic substances. The characteristics of the fulvic and humic acid fractions are consistent with previous

research, with the fulvic acids typically displaying higher slope values and lower specific absorption values. However there are several aspects of isolate absorption characteristics that are divergent to some degree from published information. The relatively small slope differences between the two fractions, and the seemingly high values of the specific absorption coefficients, may point towards contaminant induced optical effects. Yet given the wide range of reported absorption parameters from diverse waters [Chapter 3.4], and the complex nature of humic chemistry, it is quite possible that such effects are a reflection of in-water phenomena. Again the "red tide" samples appear to result from unusual formational conditions, displaying aberrant spectral features consistent with the inclusion of phaeopigments in the humic acid molecule. Such features support the theory that the chemical and optical differences between fulvic and humic acids are products of their respective starting materials, rather than a result of different stages in diagenesis. This is particularly true given that similar features have been observed in sedimentary humic acids [Ertel & Hedges 1983], surely indicating that the recent formation of these materials cannot solely account for such features. As concerns the ability of XAD based isolation procedures to produce optically representative absorption data, the typically lower slope values of isolated material in comparison to corresponding water samples [figure 18] may be indicative of some bias in isolation. Lower isolate slope values are consistent with the theory of Green & Blough [1994] - that longer wavelength absorbing components are preferentially isolated.

Good comparisons with published data can also be made with the fluorescence characteristics of the isolates, using both excitation-emission matrices and synchronous fluorescence scans. Protein-like fluorophores, typical of surface waters [Chapter 6.3], dominate excitation-emission matrices of the samples. The implication that much of the material isolated is linked to recent biological processes is supported by typical spectral shapes of the synchronous fluorescence scans. Interestingly, a far greater degree of homogeneity was observed in the fluorescence characteristics of the humic acids, indicating either similar humification processes or that greater homogeneity is associated with advanced stages of diagenesis. The general lack of longer wavelength fluorescent activity represents a degree of divergence to published data, although these effects are quite feasibly a reflection of in-water processes.

The necessarily small number of isolations performed, and the small range of associated data, prevented the development of statistically rigorous fluorescence-absorption algorithms. The division of isolates into their fulvic and humic acid fractions further impeded any such statistically based algorithm production, as the fluorescence-absorption relationships of these two fractions are typically highly dissimilar [Chapter 6.4]. It also appears that the use of multi-spectral fluorescence

to determine absorption, based on the spectral location of core fluorophores, offers some potential as an alternative means of measuring absorption, at least in surface waters typically dominated by protein-like fluorophores.

Despite sample contamination, the isotopic and optical characteristics of isolates can be considered representative of humic substances, given the equivocal definition of such material. However, the small data set and uncertainties associated with contamination, precludes further application of these results for the routine determination of gelbstoff. Attempting to overcome contaminant problems through further purification of isolates represents a possible means of increasing confidence in the data set. However, given the unknown degree of molecular modification caused by contaminants, any such process would have to be approached with extreme care.

Whilst the results represent a considerable advancement in the knowledge of gelbstoff formation and characteristics in southern African waters, they fall considerably short of what is required - the primary target of validating the performance of ocean colour sensors, such as SeaWiFS. Such a goal requires a considerable adjustment in attitude, from the specific problems of applying suitable methods to the need for the effective and astute management of large scale research projects. As concerns the suitability of isolation processes for *optically* based studies of organic material, the use of seawater samples would appear to effectively remove any potential for biasing. In highly productive regimes, such as the Agulhas Bank or Benguela upwelling system, sensitivity problems associated with low concentrations of dissolved organic material are likely to be minor in comparison to bloom related large scale autochthonous production. If biochemical analyses are to be focused on, then perhaps ultrafiltration, as a size based isolation procedure, is the most suitable available methodology. The isolation of chromophoric DOM for the purposes of "ground truthing" ocean colour sensors is perhaps always likely to be subject to some speculation regarding unrepresentative sampling. It would therefore appear that methodology selection must be determined by the broad objectives of a well defined research project.

The data produced here serves only to underline the need to promote the understanding of gelbstoff from a remote sensing perspective, particularly in the highly productive waters of southern Africa. The *effective* application of ocean colour sensors in this region surely can only be realised through the instigation of a bio-optical sampling programme, with the well defined objectives of raising the understanding of core bio-optical processes to acceptable levels.

Glossary

Symbols

$a\ [m^{-1}]$	Absorption coefficient
$a^*[m^{-1}]$	Specific absorption coefficient
A	Absorbance or optical density
$b\ [m^{-1}]$	Scattering coefficient
$b_b\ [m^{-1}]$	Backscattering coefficient
$\delta\,^{13}C\ [‰]$	Ratio of ^{13}C to ^{12}C
$\Delta\lambda$ [nm]	Synchronous scan wavelength offset
$C\ [g\ m^{-3}]$	Concentration
$E_d\ [W\ m^{-2}]$	Downwelling irradiance
f [r.u.]	Fluorescence
I_f [r.u.]	Fluorescence intensity
λ [nm]	Wavelength
λ_r [nm]	Reference wavelength
l [m]	Pathlength
$L_w\ [W\ m^{-2}\ sr^{-1}]$	Water leaving radiance
ϕ	Fluorescent quantum yield
R	Reflectance
$R_{rs}\ [sr^{-1}]$	Remotely sensed reflectance
$S\ [nm^{-1}]$	Spectral slope coefficient

Subscripts

g	Gelbstoff
f	Fulvic
h	Humic
p	Particulate
ph	Phytoplankton
r	Raman
w	Water

Acronyms

AC	Abiotic Condensation
BD	Biopolymer Degradation
CDOM	Chromophoric Dissolved Organic Matter
CZCS	Coastal Zone Color Scanner
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EEM	Excitation Emission Matrix
FA	Fulvic Acid
HA	Humic Acid
HMW	High Molecular Weight
HS	Humic Substances
ICP	Inductively Coupled Plasma
IOP	Inherent Optical Property
LMW	Low Molecular Weight
MFA	Marine Fulvic Acid
MHA	Marine Humic Acid
NMR	Nuclear Magnetic Resonance
SeaWiFS	Sea-viewing Wide Field-of-view Sensor

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