DISSOLVED ORGANIC
MATTER IN NEW ZEALAND
NATURAL WATERS

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Abstract

Dissolved organic matter (DOM) is the most dynamic and least understood part of the global oceanic carbon cycle and the molecular composition of DOM is largely unknown. This study focused on the distribution pattern, removal processes and molecular characterisation of DOM in a range of estuaries and coastal zones in New Zealand. Doubtful Sound, the longest fjord in Fiordland National Park, South Island, New Zealand was of particular interest, because of the combination of extreme rainfall, enhanced production of DOM within the temperate rainforest, and the formation of an up to 5 m thick low salinity layer (LSL) at the fjord surface. A typical river estuary (Freshwater River) located in Stewart Island, New Zealand was also investigated. Optical water properties such as the UV/Vis absorption coefficient at 355 nm ($a_{CDOM}(355)$) and Excitation-Emission Matrix fluorescence (EEM) were determined for samples from freshwater and across the LSL into water with full salinity (open ocean salinity). These optical properties showed a marked decrease with salinity and highest levels of EEM fluorescence and $a_{CDOM}(355)$ in the brackish surface water. In addition to the observed changes in the optical properties, ultrahigh resolution Electrospray Ionisation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS) determination of molecular formulae revealed that in the fjord about 20 % of these formulae changed along a vertical salinity gradient across the LSL between the brackish surface water and the saline water at 5 m depth. This trend was even more pronounced along the salinity gradient of the Fresh Water River Estuary in Stewart Island, where 60 % of all assigned molecular masses changed from freshwater over the mixing zone to ocean water. Associated with these changes was a
marked increase in aromaticity with increasing salinity. A comparable be-
behaviour with increasing salinity was also found for samples from the Cape
Fear River system, North Carolina, USA.

In contrast, only minor changes were determined in molecular formulae
for surface water samples collected along a transect off the Otago Coast
and across the Subtropical Convergence (STC) into Subantarctic Water
(SAW). Some additional molecular formulae for neritic waters compared to
open ocean Subantarctic Water (SAW) were found. However, a comparison
of the molecular masses assigned to the DOM pool for the STC water
and a freshwater stream in Doubtful Sound, revealed that 75 % of all the
assigned masses for the open ocean sample were common to these two
markedly different types of natural waters. This seemingly refractory DOM
contained nearly 600 masses, which were all very similar (only spaced by
two hydrogen and CH₂ groups) in their molecular masses and could be
explained with 9 general molecular formulae. However, the comparison
of all assigned formulae for the freshwater sample suggested that about 90 %
of the terrestrially-derived DOM changed as it moves from rivers to the
open ocean and that only 10 % remained the same.

Photodegradation processes were confirmed to be responsible for a signifi-
cant destruction of CDOM. Samples collected from different salinity waters
showed major differences in wavelength-dependent photo-decay of CDOM
suggesting that the rate of photodegradation in the UV range decreased
with increase in salinity whereas it was enhanced for wavelengths ≥400
nm compared to freshwater. Additionally, the predominantly unsaturated
molecular masses produced during estuarine mixing were found to be highly
photolabile and were either destroyed or new unsaturated masses were pro-
duced within 21 h of solar irradiation.
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Chapter 1

Introduction

1.1 General Introduction

Dissolved organic matter (DOM) plays an important role in the global carbon cycle and Hansell et al. (1998) estimated the mass of the marine dissolved organic carbon (DOC) pool to be 680-700 Pg which is approximately equal to the carbon content of atmospheric carbon dioxide. An additional DOM mineralisation rate of only 1% annually would be equal to the annual emission of CO$_2$ generated by fossil fuel combustion. DOM is also the most dynamic/reactive carbon reservoir worldwide and the changes of DOM on its journey from freshwater to the open ocean appear to be dramatic, but are poorly understood. It is not surprising that the focus and subsequent research on DOM has intensified during the last decades and a comprehensive review is given in Hedges (2002). As a result, the importance of DOM and its cycling became more clear on a global scale (Hansell, 2002) and Benner (2004) estimated a substantial annual flux of organic matter (~0.5 Pg) from terrestrial sources to the open ocean. In recent times, the importance of photochemical reactions and mineralisation of DOM have been addressed and increasing UV radiation intensities at sea level arising from stratospheric ozone depletion and atmospheric carbon dioxide are two issues now associated with current DOM research.

The fundamental processes which might account for the fate of DOM in natural waters are still not completely understood (Benner, 2002). The open questions concerning the role of DOM in marine and global cycles indicate a need to further investigate the distribution, characteristics and molecular structural changes of DOM species. An ongoing part of DOM research is to evaluate the sources and sinks of marine and terrestrial DOM.
Most of the DOM in the open ocean is remarkably old and very different from terrestrial DOM (Hedges, 2002). However, the DOM input from rivers into the ocean is enormous (0.25 Gt) (Cauwet, 2002). Therefore, the mixing zone in coastal waters has to be the most reactive area in terms of degradation of terrestrially-derived DOM (Del Vecchio and Blough, 2002). The amount of dissolved organic carbon (DOC) in the open ocean is much less than one would expect considering this high terrestrial input. Hedges and Oades (1997) have concluded that either the global estimates for the amounts of particulate and dissolved organic matter in the ocean are not correct or there are some significant sinks for terrestrial natural organic matter (NOM) in coastal mixing zones. This suggestion raises the question: what are these sinks for terrestrially-derived DOM? This question has been studied extensively and a comprehensive review is given by Blough and Del Vecchio (2002). The following sinks for terrestrial DOM are currently being investigated:

1.1.1 Photodegradation

There is new interest in the chromophoric fraction of DOM, i.e. the light absorbing fraction (CDOM) following the discovery of ozone depletion in the stratosphere above Antarctica and globally which has led to enhanced UV-B radiation intensities on the Earth’s surface (Solomon, 1990; Erickson et al., 2000). In the past, CDOM was referred to by several other names, for example Gelbstoff, yellow substance, gilvin and humic substance (Blough and Del Vecchio, 2002). This CDOM mostly absorbs light in the UV-B and UV-A spectral region (Blough and Del Vecchio, 2002). CDOM is photolabile and enhanced UV-B radiation would possibly increase the photodegradation of CDOM (Kramer, 1979; Kouassi and Zika, 1990; Kouassi and Zika, 1992; De Haan, 1993; Skoog et al., 1996; Reche et al., 1999; Whitehead et al., 2000; Moran et al., 2000; Twardowski and Donaghay, 2001). These resulting changes in the structure and concentration of CDOM could in turn lead to enhanced penetration of UV radiation in the water column with possible impacts on aquatic environments (Blough and Zepp, 1990; Williamson et al., 1996; Haeder et al., 1998; Zepp et al., 1998; De Mora et al., 2000; Neale and Kieber, 2000). Additionally, the interaction of UV radiation with CDOM in natural waters may lead to higher mineralisation rates of CDOM and increasing CO$_2$ (Miller and Zepp, 1995; Mopper et al., 2000).

Kouassi et al. (1990) have reported that sunlight decreases the fluorescence of the CDOM. The process of photobleaching refers to photochemical changes which lead to the destruction of at least some of the chromophoric groups in CDOM and therefore
reduce the ability of the CDOM to absorb light.

The main photodegradation products are believed to be inorganic carbon monoxide (CO) and carbon dioxide (CO$_2$) (Miller and Zepp, 1995; Zuo et al., 1998). Two photooxidation pathways leading to this mineralisation are discussed in the literature (Mopper and Kieber, 2002). Firstly, the main loss of DOC is caused by an abiotic pathway leading to CO$_2$ and CO. Secondly, the DOM can be photooxidised to form low molecular weight compounds which are readily consumed by microorganisms (biotic pathway) and stored in the biomass. Miller and Moran (1997) stated that the biotic pathway accounts for the same amount of DOC loss as the abiotic pathway. Mopper and Kieber (2002) concluded that a minimum of 20-30 % DOC can undergo photooxidation via both of the above pathways.

There have been numerous reports showing that sunlight-induced photochemical reaction of CDOM leads to the destruction of CDOM (Vodacek et al., 1997; Blough and Del Vecchio, 2002; Del Vecchio and Blough, 2002) and these authors concluded that photobleaching is potentially the most important sink of CDOM. The extensive experimental research undertaken on photochemical reactions of CDOM are numerous and has been reviewed in detail by Mopper and Kieber (2002).

Lignins are complex organic compounds only produced by vascular plants (Sarkanen and Ludwig, 1971) and represent a major fraction of terrestrially-derived CDOM (Ertel and Hedges, 1984; Ertel et al., 1986). Opsahl and Benner (1998) examined the sensitivity of lignins to photolysis and showed that about 75 % of a lignin sample from the Mississippi River, USA was degraded after 28 days incubation in sunlight and the molecular weight of the lignin sample decreased during the irradiation. Brinkmann et al. (2003) investigated the photobleaching of DOM in a bog lake and postulated that hydrophilic DOM underlies photooxidation processes. In contrast, photooxidation lead to the formation rather than the degradation of the hydrophobic fraction of DOM.

The importance of reactive oxygen species (ROS) such as singlet oxygen ($^1$O$_2$), the superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (•OH) has been noted in photobleaching processes associated with CDOM (Aguer et al., 1999; Goldstone et al., 2002; Goldstone and Voelker, 2000). Singlet oxygen is an excited-state oxygen species formed from ground-state oxygen which exists in a triplet state. Zepp et al. (1977) developed a method to determine the steady-state concentration of singlet oxygen in natural waters involving the use of 2,5-dimethylfuran (DMF) as a trap for singlet oxygen. The authors suggested that singlet oxygen is produced by photochemically excited-state humic and fulvic acids which transfer energy to triplet
oxygen. Wolff et al. (1981) used the same detection method and were able to show a significant correlation between steady-state singlet oxygen levels and DOC content and this result supports the hypothesis of the energy transfer from excited-state humic and fulvic acid to form singlet oxygen.

The superoxide anion ($O_2^-$) is formed photochemically from excited-state CDOM, which reacts with dissolved oxygen to form $O_2^-$ in natural waters exposed to solar radiation. Goldstone and Voelker (2000) have shown that $O_2^-$ is not only removed by dismutation to form hydrogen peroxide (Zafiriou, 1990) and reactions with metal species, but also by reaction with CDOM. Furthermore, these researchers found a strong correlation between the pseudo first order rate constant of the $O_2^-$-decay and CDOM levels measured by UV/Vis absorbance at 300 nm. Hence, the superoxide anion seems to be involved in CDOM photochemistry and may also play an important part in photodegradation of CDOM.

The major source of hydrogen peroxide ($H_2O_2$) is believed to be photo-activated DOM which reacts with dissolved oxygen to form the superoxide radical ($O_2^-$) (Cooper and Zika, 1983). As noted above this superoxide anion can then disproportionate to yield hydrogen peroxide $H_2O_2$ and oxygen (Petasne and Zika, 1987). The connection between DOC and hydrogen peroxide was first noted by Cooper and Zika (1983) and has been the subject to subsequent studies such as Scully et al. (1996).

Hydroxyl radicals ($\cdot$OH) are highly reactive radicals in the environment largely due to their unpaired electron. They are mainly formed by photolysis of nitrate, nitrite and hydrogen peroxide (Takeda et al., 2004). However, Zhou and Mopper (1990) stated that the sum of hydroxyl radical production from all these sources does not account for the high hydroxyl radical levels in sea water and that photolysis of CDOM provides an additional major source of hydroxyl radicals.

### 1.1.2 Flocculation

The importance of flocculation as a removal pathway for DOM is debatable. Early studies pointed out that a large portion of humic acids was removed by flocculation during estuarine mixing (Sholkovitz, 1976; Sholkovitz et al., 1978), but these authors also mentioned that these humic acids account for only 3-6 % of the DOM pool and that the DOC behaved in a conservative manner. In 1983, Mantoura and Woodward (1983) described the DOC flux in the Severin Estuary, Wales as conservative and postulated a DOC flux model with less than 10 % removal of DOC caused by flocculation, which would be in agreement with the results of Sholkovitz et al.. Recently, Benner and
Opsahl (2001) suggested the loss of humic substances due to flocculation and also indicated that only a minor fraction of the DOC was removed. Chen and Gardner (2004) observed evidence for the flocculation of DOM in the Mississippi and Atchafalaya River plume, but did not mention the importance of flocculation in terms of DOM removal from estuarine systems. In the present study, flocculation was therefore not investigated due to its potentially minor effect of the removal of DOM and despite the fact that earlier studies suggested that flocculation can be a significant process for the removal of high molecular weight humic substances with increasing salinity and in high DOM level estuarine regions.

1.1.3 Changes in DOM related to Salinity, pH and Ionic Strength

The mixing of natural water end-members characterised by their differences in salinity is considered as conservative, if a linear correlation exists between their salinity and parameters such as e.g. dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) levels.

The physico-chemical processes involved in mixing of freshwater and seawater are still not well understood and they may well affect the properties of the CDOM. Benner and Opsahl (2001) investigated molecular biomarkers (e.g. combined natural sugars, lignin derivatives) and emphasised the importance of non-conservative physico-chemical as well as biological processes which were not reflected in the conservatively behaving DOC levels in estuarine systems. Boyd and Osburn (2004) suggested CDOM conformational changes during estuarine mixing and increasing salinity based on changes in the fluorescence of CDOM and that these changes influence the biodegradability of CDOM. Esteves et al. (1999) described a shift in fluorescence maxima of CDOM related to salinity also indicating conformational changes in the CDOM chromophores. Ionic strength has been demonstrated to have only minor effects on the fluorescence of humic and fulvic acids, whereas pH significantly influences the fluorescence (Mobed et al., 1996). Del Vecchio and Blough (2004) suggested the concept of intramolecular electron charge-transfer interactions between hydroxy-aromatic groups and quinoid structures. Based on this concept, it can be assumed that changes in the intramolecular structure (caused by an increase in salinity) of highly polar compounds such as lignins may lead to a very different e.g. photochemical behaviour.
1.1.4 Biological Degradation

Direct biological consumption of CDOM appears to be very small (Moran et al., 2000). However, it is not well known if the change in bacterial communities from freshwater to seawater affects the microbiological degradation of CDOM either with, or without, photochemically altered terrestrially-derived CDOM. Allochthonous (terrestrially-derived) CDOM was suggested to be more utilised by estuarine and marine bacteria (Boyd and Osburn, 2004) compared to freshwater bacteria, which would be an indication of the importance of the shift in bacterial communities along an estuarine mixing gradient. Marine bacterioplankton assimilated an average of 2.4 fold more terrestrially-derived dissolved organic nitrogen (DON) compared to freshwater bacteria (Stepanauskas et al., 1999) suggesting the more efficient biodegradation of DON at higher salinities. Stubbins et al. (2003) showed that microbial uptake and remineralisation of DOC were enhanced after exposure to sunlight. Hence, synergistic effects (e.g. changes in bacterial communities, photodegradation) can have a significant effect on the biological degradation of CDOM. The contribution of biological degradation to the behaviour of DOM was not investigated in the present project which was restricted to just abiotic effects.

1.2 Objectives and Hypothesis of the Present Study

In view of the realisation of the increasing importance of aquatic DOM as the most dynamic component of the global carbon cycle and yet the existence of many unanswered questions particularly concerning the physico-chemical behaviour and the molecular composition of this material, the objectives of the present study are:

- Investigate the changes in the chemical nature of aquatic DOM across a salinity gradient
- Investigate the influence of solar irradiation on the chemical degradation of aquatic DOM
- Investigate the specific role of the reactive oxygen species, singlet oxygen, on DOM behaviour in natural waters
- Compare the chemical nature of DOM derived from freshwater sources with DOM isolated from different oceanic masses
• Identify the chemical nature of the refractory component of DOM in natural waters

The lack of any published reports of DOM behaviour measured in any New Zealand natural waters also suggests a further objective:

• To compare these results for DOM collected from a range of New Zealand aquatic environments with those for DOM sampled overseas

These objectives are based on the general hypothesis: The chemical nature and associated physico-chemical properties of aquatic DOM change along a salinity gradient, between different sources of DOM and under the influence of solar radiation.

In Chapter 2, the importance of fjord systems in terms of DOM and CDOM dynamics is addressed. At present, there are no information available about DOM dynamics in New Zealand fjords. Furthermore, fjord systems have been largely ignored in terms of DOM cycling worldwide. However, temperate fjord environments, such as Doubtful Sound, Fiordland National Park, New Zealand are characterised by temperate rain forest, high annual rain fall and steep mountain slopes and the combination of these factors may account for a substantial DOM input into the fjords. Additionally, the New Zealand fjords and in particular, Doubtful Sound have a stable low salinity layer at the fjord surface, which is a common characteristic of fjords. It is well established that terrestrially-derived DOM contains much higher levels of the chromophoric fraction of DOM. Hence, CDOM is lost on the way from rivers and streams to the open ocean. One aim of the present study is to investigate the distribution pattern of the CDOM in detail to show the fate of CDOM laterally from the inner fjord toward the open ocean, but also on a vertical scale with depth. Additionally, molecular changes of the CDOM may occur and can be evaluated using parameters obtained from optical properties such the spectral slope $S$ and the fluorescence index $FI$ measured in natural water samples.

In Chapter 3, the molecular changes of DOM across freshwater/seawater interfaces is addressed. In earlier studies, it has been shown that open ocean DOM is very different compared to terrestrially-derived DOM and hence DOM undergoes dramatic changes in coastal regions. Estuarine systems exhibit the most reactive zone in terms of DOM changes. To understand the processes underlaying DOM cycling, a molecular understanding of DOM is required. In the past, it was not possible to look at the molecular composition of the bulk DOM, due to its complex mixture of organic compounds. Only recently, the ultrahigh resolution Electrospray Ionisation Fourier Transform Ion
Cyclotron Mass Spectrometry (ESI-FT-ICR-MS) technique has been applied to analyse DOM samples and results published in the last few years are promising. This technique is applied in the present study to characterise the molecular composition of DOM in water samples taken along a salinity gradient in the Freshwater River Estuary, Stewart Island and on different salinities samples collected in Doubtful Sound. The present research aims to address the long standing question on the molecular changes of DOM across estuarine mixing gradients and to investigate the processes responsible for changes of DOM on the molecular level.

In Chapter 4, the photochemical reactivity of CDOM is addressed. Several removal pathways of CDOM have been discussed in the literature and by far the most important one appeared to be the solar-induced photodegradation. Therefore, photodegradation experiments are included in this study to evaluate the importance of this removal pathway in natural waters around New Zealand. The wavelength-dependent photodegradation of CDOM is investigated and the changes related to sunlight irradiation time and also salinity are addressed. An additional research location in North Carolina, USA is also chosen to examine photochemical reactions of CDOM induced by solar radiation and its changes at the molecular level are analysed by ESI-FT-ICR-MS.

In Chapter 5, one of the reactive oxygen species, singlet oxygen, is examined in detail, because it is involved in photochemical processes associated with CDOM and its removal at least in CDOM-rich rivers and estuarine systems. This Chapter aims to investigate the direct correlation of steady-state concentrations of singlet oxygen and levels of absorbance and fluorescence of CDOM.

In Chapter 6, the molecular composition of marine DOM samples collected across the Subtropical Convergence (STC), a major oceanic current in Southern New Zealand using ESI-FT-ICR-MS is investigated. Neritic waters should be more influenced by terrestrially-derived DOM compared to offshore waters such as the Subantarctic Water (SAW). ESI-FT-ICR-MS is used to obtain molecular evidence for this suggestion. Using this technique, it may be also possible to evaluate the refractory DOM pool in the open ocean if compared to ESI-FT-ICR-MS results obtained from freshwater samples.

1.3 Study Sites

The study sites were in general chosen to be within reasonable proximity to laboratory facilities at Otago University in Dunedin, New Zealand and show different characteristics likely to influence the level and chemical composition of their DOM content.
including vegetation, geology and climate factors.

Doubtful Sound has been chosen as a study site to represent the fjords in the southwest of the South Island, New Zealand. Additionally, the Freshwater River Estuary, located in Stewart Island, New Zealand represents a typical estuarine system of a black water river. The proximity of the global oceanic Subtropical Convergence (STC) to the Otago Coast also provided an opportunity to collect and study DOM collected along a transect extending from neritic waters across this front into Subantarctic Water (SAW). Research was also carried out in North Carolina using samples from a major river basin, the Cape Fear River. This river is located in an urban region possibly heavily influenced by human activity, which is in contrast to the rivers and streams examined in New Zealand. Some further information concerning these study sites are given below and more details will be discussed in the subsequent chapters.

1.3.1 Doubtful Sound

The west coast of the South Island, New Zealand receives an average annual rainfall of approximately 6,200 mm (Stanton and Pickard, 1981) which is one of the highest precipitation rates in the world. Doubtful Sound represents a typical fjord within this region and is located between 45° 10’ and 45° 30’ S and between 166° 50’ and 167° 10’ E (see Figure 1.1). Most of the catchments of Doubtful Sound are characterised by temperate rain forest and the area is protected within the Fiordland National Park. Doubtful Sound is the longest fjord in Fiordland reaching 43 km inland and also has some side arms such as Hall Arm (9 km inland from main channel), Crooked Arm (16 km inland) and First Arm (6 km inland).

There is minimal human impact on this study site and hence it is suitable for the study of DOM produced under natural conditions. Doubtful Sound is however strongly influenced by the additional input of very high volumes of freshwater arising from discharge from the Tail Race of the Manapouri hydroelectric power station. Throughout the year, Doubtful Sound exhibits a stable low salinity layer (LSL) at the surface (Gibbs, 2001) and the depth of this layer decreases with distance toward the open sea. This LSL is superimposed upon an underlying saline layer and gives extraordinary opportunities to study the behaviour of DOM with depth as well as along a horizontal profile, extending from the Tail Race to the fjord mouth.
1.3.2 Freshwater River, Stewart Island

Stewart Island is located between 46° 40′ and 47° 18′ S and between 167° and 168° 20′ E. The area of the island is approximately 1,735 km² (see Figure 1.2). The annual rainfall of approximately 1,500 mm is moderate and relatively evenly distributed over the year. The Freshwater River on Stewart Island is located at the head of Paterson Inlet and drains a fault-bounded trough which divides the island into two distinct mountain areas. The swampy Freshwater River Valley is a large alluvial lowland characterised by low altitude vegetation such as swamps, heath and lowland scrub. The hillsides of the catchment area are covered by rain forest and tall sub alpine scrub, whereas boggy meadowland and short scrub are located at the tops of the surrounding mountains. This diversity of vegetation leads to the hypothesis that the DOM pool in the Freshwater
River shows major differences compared to Doubtful Sound, which is only influenced by mountain runoff and temperate rain forest. Furthermore, the Freshwater River, Stewart Island exhibits a pH of about 5.6 in contrast to the rivers and streams in Doubtful Sound, which have a neutral pH of 7.

1.3.3 Transect of the Subtropical Convergence

The Subtropical Convergence (STC) is a global oceanic front that passes close to the east coast of the South Island of New Zealand. It is characterised by a warm oceanic current, the Southland Current. The border between the Southland Current (SC) in the north-west and the Subantarctic Water (SAW) in the south-east is characterised by a decrease in temperature and salinity and is known as the Southland Front (SF), which also indicates the south-east border of the STC. The warm Southland Current is quite narrow (20-30 km) and it is of great interest to characterise the changes in DOM from coastal (neritic) waters across the STC and then into the colder SAW. Sites were

Figure 1.2: Stewart Island, New Zealand (NASA, Landsat 7 satellite: ETM+)
selected along this transect to obtain representative DOM samples from neritic (close to shore), STC and SAW waters (see Chapter 6).

1.3.4 Black River and Cape Fear River Estuary

The Cape Fear River basin is the largest drainage area in North Carolina, USA. The confluence of the Haw and Deep River forms the Cape Fear River, which is the longest river in North Carolina. The watershed is principally rural and strongly influenced by agriculture. The Black River is a smaller tributary and flows directly into the Cape Fear River 16 km upstream from Wilmington. Near Cape Fear, the Cape Fear River discharges into the Atlantic Ocean. An overview of the Cape Fear River basin is given in Figure 1.3.

![Figure 1.3: Cape Fear River basin, North Carolina, USA (NASA, Landsat 7 satellite: ETM+)](image-url)

Figure 1.3: Cape Fear River basin, North Carolina, USA (NASA, Landsat 7 satellite: ETM+)
1.4 Chemical Characteristics of DOM

About 80-90 % of DOM in the ocean still remains uncharacterised at the molecular level (Benner, 2002). However, some of the classes of organic compounds that have been identified in DOM include:

- **Carbohydrates:** These compounds make up from 10-25 % of DOC. Borsheim et al. (1999) pointed out that 19-21 % of the DOC in the surface water of a Norwegian fjord, is related to carbohydrates, but in deeper waters, the levels are slightly lower (15-16 %). Pakulski and Benner (1994) observed carbohydrate levels in surface water of the Atlantic and Pacific Oceans in the range of 7 to 33 µM, which accounted for up to 21 % of the total DOC.

- **Amino acids:** The concentration of total hydrolysable amino acids (THAA) is widely used to quantify the levels of amino acids in water. At the surface, THAA levels range between 200 and 500 nM (1-3% DOC) whereas in deep water they can range from 80-160 nM (0.8-1.8 % DOC) (Benner, 2002). In the northwestern Pacific (137° E), Yamashita and Tanoue (2003b) reported THAA levels of 260-818 nM in coastal waters and 256-372 nM in oceanic surface water.

- **Lignin:** Lignins are excellent biomarker for DOM of terrestrial origin. Between 8 and 11 different copper-oxidised lignin-derived phenols are used for characterisation of lignins (Louchouarn et al., 2000). Typically, the levels of lignin phenols decrease along a salinity gradient with a greater loss in the low salinity range (Benner and Opsahl, 2001). Opsahl and Benner (1997) calculated that about 0.2-0.7 % DOM in the Pacific and 0.5-2.4 % DOM in the Atlantic is from terrestrial origin.

- **Other functional groups found in DOM:** A range of different chemical instrumental techniques have been used to investigate the presence of other functional groups in DOM. For example, ¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopy has provided valuable information for the identification and quantification of different functional groups within the DOM. Table 1.1 is a summary of the functional groups associated with different fractions of DOM.
Table 1.1: Average chemical characteristics of DOM isolated from surface and deep ocean waters (adapted from Benner (2002))

<table>
<thead>
<tr>
<th>Chemical characteristics</th>
<th>Surface ocean</th>
<th>Deep ocean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humic substances (% DOC)</td>
<td>5-25 1–5</td>
<td>15-25 1–5</td>
</tr>
<tr>
<td>Humic substances (C/N atom)</td>
<td>36-56 3,4</td>
<td>39-57 3,4</td>
</tr>
<tr>
<td>Humic substances (% C-C)</td>
<td>44 4</td>
<td>46 4</td>
</tr>
<tr>
<td>Humic substances (% C-O, O-C-O)</td>
<td>19 4</td>
<td>17 4</td>
</tr>
<tr>
<td>Humic substances (% C=C)</td>
<td>19 4</td>
<td>19 4</td>
</tr>
<tr>
<td>Humic substances (% COO, CNO)</td>
<td>15 4</td>
<td>15 4</td>
</tr>
<tr>
<td>Humic substances (% C=O)</td>
<td>3 4</td>
<td>3 4</td>
</tr>
<tr>
<td>Total hydrolysable amino acids (%HS-DOC)</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Total hydrolysable amino acids (%HS-DON)</td>
<td>5-11 6,7</td>
<td>5-9 6,7</td>
</tr>
<tr>
<td>HMW DOM (% DOC)</td>
<td>25-40 8–10</td>
<td>20-25 8–10</td>
</tr>
<tr>
<td>HMW DOM (C/N atom)</td>
<td>15-18 8,10</td>
<td>18-20 8,10</td>
</tr>
<tr>
<td>HMW DOM (% C-C)</td>
<td>25 8,11</td>
<td>30 8,11</td>
</tr>
<tr>
<td>HMW DOM (% C-O, O-C-O)</td>
<td>54 8,11</td>
<td>28 8,11</td>
</tr>
<tr>
<td>HMW DOM (% C=C)</td>
<td>5 8,11</td>
<td>21 8,11</td>
</tr>
<tr>
<td>HMW DOM (% COO, CNO)</td>
<td>13 8,11</td>
<td>16 8,11</td>
</tr>
<tr>
<td>HMW DOM (% C=O)</td>
<td>3 8,11</td>
<td>5 8,11</td>
</tr>
<tr>
<td>Total hydrolysable neutral sugars(%HMW DOC)</td>
<td>6-13 12,13</td>
<td>1.5-2.5 12,13</td>
</tr>
<tr>
<td>Total hydrolysable amino acids (%HMW DOC)</td>
<td>3-4 12</td>
<td>3-4 12</td>
</tr>
<tr>
<td>Total amino acids (%HMW DOC)</td>
<td>3-4 12</td>
<td>3-4 12</td>
</tr>
<tr>
<td>Total hydrolysable amino sugars (%HMW DOC)</td>
<td>1.3-2.6 14</td>
<td>0.5-0.6 14</td>
</tr>
<tr>
<td>Total % DOC</td>
<td>10.3–19.6 1213</td>
<td>5.0-7.1 12,13</td>
</tr>
<tr>
<td>Total hydrolysable amino acids (%HMW DON)</td>
<td>17-29 12</td>
<td>12-28 12</td>
</tr>
<tr>
<td>Total hydrolysable amino sugars (%HMW DON)</td>
<td>3.6-7.1 14</td>
<td>1.4-1.9 14</td>
</tr>
<tr>
<td>Total %DON</td>
<td>20.6-36.1 12</td>
<td>13.4-29.9 12</td>
</tr>
</tbody>
</table>

References: 1, (Gagosian and Stuermer, 1977); 2, (Harvey et al., 1983); 3, (Druffel et al., 1992); 4, (Hedges et al., 1992); 5, (Ishiwatari and Editor, 1992); 6, (Hubberten et al., 1994); 7, (Hubberten et al., 1995); 8, (Benner et al., 1992); 9, (Guo et al., 1995); 10, (Benner et al., 1997); 11, (McCarthy et al., 1993); 12, (McCarthy et al., 1996); 13, (Skoog and Benner, 1997); 14, (Benner and Kaiser unpublished)
1.5 Isolation of DOM

Due to the general very low DOM levels in natural waters, it was essential to isolate the DOM material. A literature review and comparison studies between several DOM isolation techniques have been given elsewhere (Aiken and Leenheer, 1993; Peuravuori et al., 2005) and the most important techniques are summarized below.

1.5.1 XAD-8 and XAD-2 Resins

The Amberlite XAD resin series have been widely used in a nonionic solid-phase extraction method for the isolation of DOM in natural waters (Aiken, 1985). The advantage of this extraction method is the ability to process large volumes of water. However, the extreme pH changes (from pH = 8.5 to pH = 1) during the isolation process can potentially alter the structure of at least some DOM components. There is also a risk of sample contamination during the isolation steps, due to potentially remaining hydrophobic compounds in the resin and insufficient cleaning procedures. For these reasons, this method has been largely replaced by ultrafiltration or C-18 solid-phase extraction.

1.5.2 Reverse Osmosis

If two solutions with a gradient in dissolved molecules are separated using a semipermeable membrane, which allows only water to penetrate, a pressure (osmotic pressure) is generated and this pressure forces water to leave the solution with the lower concentration of dissolved molecules until an equilibrium is reached. This process can be reversed by applying a higher pressure compared to the osmotic pressure which forces water to leave the higher concentrated solution. This process is referred to as reverse osmosis and has been used to concentrate DOM from natural waters (Serkiz and Perdue, 1990). Kilduff et al. (2004) pointed out that reverse osmosis has several advantages compared to other widely used DOM isolation techniques with mass recovery of carbon being over 90%. In particular, it is possible to concentrate large volumes of water in a short time. Furthermore, it is believed that reverse osmosis leads to a low chemical alteration of DOM. However, reverse osmosis is not suitable for marine samples, because the salt content will also increase in the concentrate (Serkiz and Perdue, 1990). A promising new extraction technique, where reverse osmosis is coupled with electrodialysis has recently been developed, leading to up to 90% of the DOC
being extracted and also desalinated (personal communication with Michael Perdue, Georgia Institute of Technology, Atlanta, USA).

1.5.3 Ultrafiltration

Tangential-flow or cross-flow ultrafiltration is probably the most common concentration technique currently used to isolate DOM material (Benner et al., 1992). Briefly, the ultrafiltration is based on forcing the sample solution through a membrane using a powerful pump. The membranes are designed to only allow molecules with a molecular weight smaller than their cutoff size (pore size) to pass. Hence, it is possible to separate different molecular size fractions using ultrafiltration and appropriate membranes. An efficient way to avoid a concentration gradient at the membrane surface is to use a tangential-flow of the sample alongside the membrane. With this method, high flow rates can be used without the disadvantage of increasing polarisation at the membrane surface and it enables rapid concentration. This technique has the advantage of been able to isolate DOM from large water samples without drastic pH changes and also not any solvents are used. However, the salt content in at least brackish and seawater samples remain problematic.

Benner and Opsahl (2001) determined a 49% recovery of terrestrially-derived DOC and 22% recovery of marine-derived DOC using tangential-flow ultrafiltration for samples collected in the Mississippi river plume.

1.5.4 Silica-based C-18 Solid-Phase Extraction

The solid phase extraction (SPE) procedure using C-18 molecules bound to silica is based on the interaction of the C-18 molecules with compounds in solution (mobile phase). Compounds with low to mid polarity would adsorb to the solid phase resin and hence are isolated from the water. The adsorbed molecules on the solid phase resin can than be eluted using an organic solvent such as methanol. The solid phase extraction efficiency of DOM can be enhanced with an adjustment of the pH to below 4. At this pH most carbonic acids are protonated and therefore have a more hydrophobic character. Hence, these protonated acids would adsorb to the solid phase.

C-18 solid-phase extraction of DOM largely overcomes the potentially serious problems of contamination and large pH changes. However, it is still necessary to acidify the sample to a pH of 4 or lower in order to optimise the adsorption efficiency (Amador et al., 1990). Furthermore, Louchouarn et al. (2000) evaluated the solid-phase extrac-
tion method and showed that this procedure yields good reproducibility and recovery for lignin in various natural waters. The C-18 solid-phase extraction (SPE) procedure to isolate DOM is quick, easy, independent from salinity and reproducible, but unfortunately lacks the ability to quantitatively isolate DOM from natural waters.

1.6 Fractionation of DOM

The chemical composition of DOM (e.g. molecular weight distribution) is complex and fractionation techniques are widely used to separate different DOM fractions with the hope of simplifying the overall structural characterisation of the DOM. However, in this study these techniques were not available, but they are important in the overall aim to determine the molecular composition of DOM and are summarized below.

1.6.1 Ultrafiltration

Ultrafiltration has been used not only for concentration of DOM but also to help in the characterisation of DOM by enabling fractionation of different molecular weight ranges using various cutoff filters. Several research groups have successfully used ultrafiltration for fractionation of DOM (Ogura, 1974; Carlson et al., 1985; Mannino and Harvey, 2000). For example, Mueller et al. (2004) used membrane cutoff filters in the range of 1-30 kDa to give five different fractions. However, it is necessary to take into account electrostatic and hydrophobic interactions with the membranes, which could slightly change the molecular weight distribution within the fractions.

1.6.2 Size Exclusion Chromatography

Analytical size-exclusion columns with high pressure pumps have been applied to determine the molecular weight distribution of DOM and this method is known as high-performance (HPSEC) size exclusion chromatography. Several research groups have used this technique to separate DOM fractions based on their molecular weight. For example, Zhou et al. (2000) suggested that standardisation procedures lead to excellent reproducibility of the DOM molecular weight distribution established using this technique. Her et al. (2002) used HPSEC coupled with an online DOC detector to analyse the molecular weight distribution of DOM in natural waters. Nagao et al. (2003) used this method coupled with fluorescence detection to characterise humic substances sampled directly without isolation from the Kuji River in Japan. Her et al. (2003) have
described the use of a combination of HPSEC and EEM fluorescence spectroscopy to characterise the molecular weight fractions of DOM.

1.6.3 High Performance Liquid Chromatography (HPLC)

High Performance liquid chromatography (HPLC) is based on the separation of molecules in a liquid phase (mobile phase). The analytes in solution are forced through a column containing a specific stationary phase (e.g. C-18 bond on silica) and the separation of a mixture of compounds depends on the different chemical interactions of each compound with the stationary phase as they traverse the length of the column.

HPLC techniques have been widely used to separate different DOM fractions (Morelli et al., 1993; Namjesnik-Dejanovic and Cabaniss, 2004). For example, Pallanti et al. (2002) have used a Shodex ASAHIPAK ODP-50 4E (250 mm L x 4.6 mm ID) polymeric column to characterise DOM from different natural waters and have obtained a separation with peaks distributed over a 30 min retention time. A UV/Vis detector was used and the resulting spectra contained extensive information and appeared to be more comprehensive than results obtained from monomeric C-18 reversed phase columns (Grasso et al., 1990), which only showed a weak resolution of two major peaks over a short retention time.

1.6.4 Capillary Electrophoresis

Electrophoresis is a separation technique which is based on the movement of analytes through a conductive medium in response to an applied electrical field. Cationic species will travel toward the cathode and ionic species toward the anode with the rate of migration being based on the charge to size ratio. Egeberg and Bergli (2002) reported that capillary zone electrophoresis is a suitable technique to characterise natural organic matter (NOM) in at least Norwegian natural waters. Moreover, it can be used as a fingerprint for different sites. Schmitt-Kopplin and Kettrup (2003) developed a combination of capillary zone electrophoresis with electrospray ionisation mass spectrometry (CZE-ESI-MS) for the analysis of DOM.
1.7 Methods of the Characterisation of the Bulk DOM

The isolation of DOM involves a series of treatment steps and each treatment may influence the structural composition of DOM. Therefore, it is a great advantage to use techniques which can be used for direct measurements of CDOM in water samples without any prior extraction steps. These techniques include:

1.7.1 Dissolved Organic Carbon Analysis

The determination of the dissolved organic carbon (DOC) content can provide a good reference parameter typically expressed in units of $\mu$M C. The total amount of carbon has been measured in natural waters for more than a century (Natterer, 1892) using a variety of methods which changed very frequently in order to improve accuracy. The currently accepted method is based on high temperature catalytic combustion. A raw natural water sample is first filtered and then the dissolved inorganic carbon component is removed by acidification to convert it to carbon dioxide which is removed by sparging with nitrogen. The remaining organic carbon component is then oxidised using high temperature catalytic oxidation (HTCO) and the resulting carbon dioxide quantified using a non-dispersive infrared analyser. The accuracy of this method has been tested by comparison of measurements of standard sample in a broad range of laboratories (Sharp et al., 2002). To get most accurate results, four measurements are carried out on the same sample aliquot and the best three results are averaged to obtain the final DOC content.

1.7.2 UV/Vis Absorbance Spectroscopy

Absorbance spectroscopy is an easy and fast technique to quantify the light-absorbing fraction of DOM. This technique is based on the ability of molecules present in natural waters to absorb light in the wavelength range between 280 and 700 nm. The extent of absorbance is a function of pathlength and since a variety of cuvettes with wavelengths ranging from typically 1 cm to 10 cm have been used in previous studies, it is sometimes difficult to compare literature absorbance values. Therefore, Blough and Zepp (1995) converted the raw optical density ($A(\lambda)$) (or absorbance) measured at a specific wavelength ($\lambda$) in a cell of path length $l$, into a CDOM absorption coefficient.
\( a_{\text{CDOM}}(\lambda) \) using the expression:

\[
a_{\text{CDOM}}(\lambda) = 2.303A(\lambda)/l
\]  

(1.1)

It should be noted that an \( a_{\text{CDOM}}(\lambda) \) value calculated for a CDOM sample is not a true absorption coefficient, because it is not related to a specific concentration. However, in biogeochemistry this definition of an absorption coefficient is well established, because the relation of concentration to a specific absorbance is generally not known. The most commonly used absorption coefficient is that calculated from absorbance measurements at the wavelength of 355 nm. Blough and Del Vecchio (2002) have published a review of literature \( a_{\text{CDOM}}(355) \) values ranging from \( >15 \text{ m}^{-1} \) for coastal and fresh water to \( >0.1 \text{ m}^{-1} \) for oligotrophic seawater. The authors also provided a detailed summary of optical properties of CDOM for various geographical areas and from different research groups. Recently published absorption coefficient data from the Eastern US Atlantic Coast and the Baltic Sea are reported elsewhere (Kowalczuk et al., 2003; Kowalczuk et al., 2005).

The spectral slope \((S)\) is another parameter that can be calculated from UV/Vis absorbance data to describe the unusual absorbance behaviour of CDOM in natural waters. This spectral slope can be used to describe changes of the absorbance during solar radiation experiments. It can be calculated either using a linear fit to the logarithmic absorption coefficients over the wavelength range 280 - 500 nm (Blough et al., 1993; Nelson et al., 1998), or with an appropriate non-linear fit (NLF) approach over a broad wavelength range (Stedmon et al., 2000).

### 1.7.3 Fluorescence Spectroscopy

Excitation emission matrix fluorescence spectroscopy provides a three dimensional plot which can be used to distinguish between three or four different peaks. The peaks have been associated with distinct fluorescent CDOM classes: humic-like and fulvic-like compounds of terrestrial origin (A and C), marine fulvic-like (M) and aromatic amino acids (e.g. tryptophan, tyrosine)(T) as shown in Table 1.2.

A problem with EEM fluorescence is the occurrence of Raleigh and Raman scattering peaks of first and second order in the raw, unprocessed fluorescent spectra. Zepp et al. (2004) have developed a collection of software tools to correct EEM spectra from these undesired peaks and incorporated them into a MATLAB\textsuperscript{©} toolbox called "FLToolbox 1.91".
Table 1.2: Excitation and emission maxima for classes of fluorescent CDOM

<table>
<thead>
<tr>
<th>Type of fluorophore</th>
<th>λ_{ex}/λ_{em}</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein-like</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>230, 275/305</td>
<td>B</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>230, 275/340</td>
<td>T</td>
</tr>
<tr>
<td>Humic-like</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV-humic</td>
<td>230/430</td>
<td>A</td>
</tr>
<tr>
<td>UV-humic</td>
<td>260/400-460</td>
<td>N</td>
</tr>
<tr>
<td>Unknown</td>
<td>280/370</td>
<td></td>
</tr>
<tr>
<td>visible-marine humic</td>
<td>290-310/370-410</td>
<td>M</td>
</tr>
<tr>
<td>Intermediate marine-terrestrial</td>
<td>310/412</td>
<td>Intermediate C-M</td>
</tr>
<tr>
<td>Visible-terrestrial humic</td>
<td>320-360/420-460</td>
<td>C</td>
</tr>
<tr>
<td>Chlorophyll-like</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>398/660</td>
<td>P</td>
</tr>
</tbody>
</table>

(Modified by Blough and Del Vecchio (2002) from Coble et al. (1998). Table 2, p. 2208)

Kowalczuk et al. (2003) used this technique to characterise samples from the Cape Fear River and Onslow Bay on the East Atlantic Coast of the US. Similar research was undertaken in the Baltic Sea by Kowalczuk et al. (2005). These authors integrated the area of characteristic fluorescent peaks, compared it to the integrated area of the whole EEM and in this way estimated the percentage contributions of the different peaks to the overall fluorescent intensity.

The fluorescence index (FI) is another parameter, defined as the ratio of the emission at 450/500 nm and an excitation wavelength of 370 nm, and has been used to show differences in the composition of CDOM (McKnight et al., 2001; Jaffe et al., 2004). An FI value of 1.9 was associated with microbially-derived CDOM, whereas an FI values of 1.4-1.5 was indicative of terrestrially-derived CDOM. Hence, this FI values can be used as a tool to characterise sources of CDOM.

Recently, parallel factor analysis (PARAFAC) has been applied to investigate different fluorophore moieties apparent in EEM spectra (Stedmon and Markager, 2005; Hua et al., 2007). PARAFAC is based on a trilinear relationship between emission, excitation and the concentration of a fluorophore. PARAFAC builds a 3-way model to simulate as close as possible the data set made up of individual fluorescent chro-
mophoric species. A set of virtual components is created, which are indicative for each sample.

1.8 Methods of the Characterisation of Isolated DOM

Most information about the bulk molecular composition of DOM has historically been obtained using NMR. However, the combination of separation techniques such as liquid and gas chromatography combined with mass spectrometry can lead to a more detailed characterisation of DOM fractions. Ultra-high resolution Electrospray Ionisation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS) has been applied to the determination of exact molecular masses of DOM. Some recently used techniques are summarised here:

1.8.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared spectroscopy (FTIR) works on the fact that specific bonds vibrate after the absorption of infrared (IR) light by the molecule at a characteristic frequency. The solid sample is exposed to an IR beam and the transmittance and reflectance of the IR light at specific frequencies caused by various types of bonds of the molecule is translated into an IR absorption spectrum.

Chen et al. (2003) reported FT-IR spectra in conjunction with EEM fluorescence of various DOM fractions of waste water and compared these fractions to portable water based on differences in specific aromatic compounds. Kalbitz et al. (2003) described the FT-IR characteristics (amongst a variety of other analytical results) of 13 soil-derived DOM before and after incubation experiments to investigate the mechanisms and controlling factors of biodegradation. Li et al. (2004) commented that the FT-IR spectra determined for 8 different ultrafiltered fractions of DOM were similar.

1.8.2 Carbon Isotope Ratio Measurements

Stable $^{12}$C and $^{13}$C isotopes

Isotopes are measured using an isotope ratio mass spectrometer (IRMS) which allows for the precise determination of the carbon isotopes ($^{12}$C, $^{13}$C) whose abundance ratio is the most common one used to describe biological processes. The changes in this
isotope ratio is small and is commonly expressed in parts per thousand and defined as:

\[ \frac{(^{13}\text{C}_{\text{sample}}/^{12}\text{C}_{\text{sample}})}{(^{13}\text{C}_{\text{standard}}/^{12}\text{C}_{\text{standard}})} - 1 \times 1000 = \delta^{13}\text{C} \ \permil \] (1.2)

The \( \delta^{13}\text{C} \) values typically range between -28 \( \permil \) and -18 \( \permil \) for DOM. Terrestrially-derived riverine DOM typically exhibits low \( \delta^{13}\text{C} \) values of around -28 \( \permil \), compared to a \( \delta^{13}\text{C} \) value for phytoplankton of around -20 \( \permil \) (Cifuentes and Eldridge, 1998). The \( \delta^{13}\text{C} \) values are typically used to determine information about the sources, cycling and turnover times of DOM.

Harvey and Mannino (2001) reported values of \( \delta^{13}\text{C} \) for DOM as a function of seasonal changes for Chesapeake Bay and Delaware Estuary on the US Atlantic Coast. Kracht and Gleixner (2000) measured the isotope ratios for \( \delta^{13}\text{C} \) for DOM in a bog lake in the Black Forest in Germany and used the results to investigate the origin of DOM and the humification process of peat. Other reports of the use of stable isotope ratios for CDOM studies include various locations around the globe (Benner et al., 1997; Goni et al., 2003; Guo et al., 2003; Kaiser and Sulzberger, 2004; Bianchi et al., 2004; Wang et al., 2004).

Radioactive \( ^{14}\text{C} \) isotope

Radiochemical measurements of the levels of the radioactive \( ^{14}\text{C} \) isotope have been used to calculate the age of DOM. However, DOM pools are very dynamic and the input of carbon from various sources and therefore potentially various ages needs to be considered in the interpretation of \( ^{14}\text{C} \) results. The \( ^{14}\text{C} \) content is typically measured as the \( ^{14}\text{C}/^{12}\text{C} \) ratio using accelerator mass spectrometry (AMS) (Vogel et al., 1995) and in biogeochemistry or related fields, the \( ^{14}\text{C} \) content is defined as (Stuiver and Polach, 1977):

\[ \Delta^{14}\text{C} = \delta^{14}\text{C} - 2(\delta^{13}\text{C} + 25.0)(1 + (\delta^{14}\text{C} \times 10^{-3})) \] (1.3)

A detailed description about this calculations and the standardisation using oxalic acid is given elsewhere (Stuiver and Polach, 1977; Goh, 1991). Raymond and Bauer (2001) pointed out that the \( \Delta^{14}\text{C} / \delta^{13}\text{C} \) ratios can supply important information on the sources and turnover times of DOM in natural waters. The dynamic range of the \( \Delta^{14}\text{C} \) is quite large (-1000 to +200 \( \permil \)) compared to the \( \delta^{13}\text{C} \) (-32 to -12 \( \permil \)) and hence it may be more useful in the determination of smaller changes of the isotopic composition of DOM. However, the \( \Delta^{14}\text{C} \) and \( \delta^{13}\text{C} \) values serve different purposes: \( \Delta^{14}\text{C} \) is used for aging whereas \( \delta^{13}\text{C} \) is a measure for the fractionation of carbon in
biological processes. An excellent review of available δ\(^{13}\)C and Δ\(^{14}\)C data is given in Bauer (2002).

1.8.3 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance Spectroscopy (NMR) records the magnetic properties of certain nuclei. NMR has become a powerful tool in characterising the molecular structure of DOM (Benner, 2002). Extensive information on functional groups of DOM determined by NMR is available in the literature (Lu et al., 2003; Brown et al., 2004; Peuravuori and Pihlaja, 1998) and an overview of functional groups of DOM obtained from NMR analysis has already been given in Table 1.1. Aluwihare et al. (2002) used \(^1\)H-NMR to investigate the chemical composition of marine DOM from the Middle Atlantic Bight. The major peaks correspond to carbohydrates (δ 4.95.5 ppm (anomeric; OCHOH), δ 3-4.0 ppm (CHOH), δ 1.3 ppm (CH\(_3\))), acetate (δ 2.0 ppm (CH\(_3\)CO)), and lipids (δ 0.9 ppm (CH\(_3\)), δ 1.3 ppm (CH\(_2\))). The authors postulated a common macromolecular structure, which they have termed acylated polysaccharides (APS). Kim et al. (2003) reported the use of a two dimensional solution NMR using Total Correlation Spectroscopy (TOCSY) to characterise DOM. However, a disadvantage of the NMR technique is that only bulk information of functional groups can be determined and changes in the DOM pool have to be substantial to make an observable difference in NMR spectra.

1.8.4 Mass Spectrometry (MS)

Mass spectrometry has become an important analytical tool for structural characterisation of organic compounds. Over the last decades, several ionisation techniques have been developed and have been applied to characterise humic substances. Pyrolysis Field Ionisation Mass Spectrometry (Py-FI-MS) and Curie Point Pyrolysis Gas Chromatography/Mass Spectrometry (Py-GC/MS) have been successfully applied to study the DOM component in water from a bog lake in Germany (Schulten and Gleixner, 1999). Both methods are based on the pyrolysis of freeze-dried water samples. Furthermore, Schulten and Gleixner (1999) described the combination of Py-GC with isotope ratio mass spectrometry for DOM analysis to give rise to a technique referred to as Curie Point Pyrolysis Gas Chromatography Combustion Isotope Ratio Mass Spectrometry (Py-GC-C-IR-MS). These authors concluded that alkyl aromatics form the skeleton for humic substances and the importance of organic nitrogen hetero-
cyclic compounds was also emphasised. Results for size exclusion fractions of a swamp water analysed by Py-GC/MS and solid state NMR are reported by Sihombing et al. (1996) and indicated that the low and high molecular weight fractions contain aliphatic chains which are relatively unbranched compared to the intermediate molecular weight fraction. Additional Py-GC/MS results for DOM isolated from natural waters in Alaska (Guo et al., 2003; Lu et al., 2003) suggested a decrease in polysaccharide levels with decreasing size fraction. This technique has also been used in water samples collected from marshes in Florida and results suggested an increase in carbohydrates which was related to an autochthonous production of DOM (Lu et al., 2003).

**Ultrahigh Resolution Electrospray Ionisation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry**

Ultrahigh resolution Electrospray Ionisation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS) has been used to study the structure of large biomolecule ions (Williams, 1998; Lorenz et al., 1999).

The electrospray is a soft ionisation technique and it is believed that molecules do not undergo fragmentation. After the ionisation, the formed ions enter the first focusing octopole followed by a quadrupole and the accumulation octopole. In this octopole, ions are accumulated before they are pulsed out through the last two transfer octopoles into the Penning trap ICR cell (see Figure 1.4).

**Schematic NHMFL 9.4 T ESI-FT-ICR Mass Spectrometer**

![Schematic ESI-FT-ICR-MS apparatus](image)

Figure 1.4: Schematic ESI-FT-ICR-MS apparatus
In the ICR cell, ions are forced into a circular motion plane perpendicular to the magnetic field and the frequency of rotation of these ions is dependent on their m/z ratio. However, the radius of the ICR motion of ions was originally found to be too small and no signal was produced. Therefore, the rotating ions are now pulsed with a radio frequency which leads to a coupling of the natural motion of the ions and this frequency. As a result, the ions are excited to a higher orbit. This excitation and relaxation of the ions induces an alternating current between the detector plates and generates a frequency, which is the same as the cyclotron frequency. A Fourier Transformation is applied to deconvolute these signals to give a frequency versus intensity mass spectrum, which is then converted to a mass versus intensity spectrum (Marshall et al., 1998).

This technique was applied for the first time to analyse natural organic matter (NOM) by Brown and Rice (2000) who characterised several Humic Acid International Substances Society (IHSS) reference materials. However, the experimental conditions and instrumental performance reported in this study did not allow for the determination of exact molecular formulae of these materials. The ESI-FT-ICR-MS instrument at the National High Magnetic Field Lab (NHMFL) in Tallahassee, Florida was further optimised and Kujawinski et al. (2002) reported that they were then able to obtain m/z ratios accurate enough to determine exact molecular formulae of the components of Suwannee River fulvic acids, and humic acids from a degraded wood sample from Mt Rainier, USA. Stenson et al. (2002) showed that virtually every ion formed by ESI was singly charged and that fragmentation did not occur, at least in the case of DOM. This research group went on to use the technique to determine the exact molecular masses and molecular formulae of the components of Suwanee River Fulvic acid (SRFA) reference material (Stenson et al., 2003). Since that time, ultrahigh resolution ESI-FT-ICR-MS analysis has become the technique of choice for the molecular characterisation of DOM and has been applied to a variety of DOM samples (Dittmar, 2004; Koch et al., 2005; Koch and Dittmar, 2006; Koch et al., 2007).
Chapter 2
Spectral Characterisation of Chromophoric Dissolved Organic Matter in a Fjord (Doubtful Sound)

Fjord systems (e.g. Fiordland, New Zealand) have been mostly ignored in terms of biogeochemical cycles including DOM dynamics. This fact is surprising giving that fjord environments are often exposed to extreme levels of annual precipitation, due to steep mountain ranges rising from sea level and that the vegetation is characterised by a dense temperate rain forest, which leads to the enormous production of DOM. Additionally, fjordic environments with similarities to Fiordland, New Zealand exist in southwest Chile and the west coast of Canada. These regions cover a substantial coastal area. The present study in Doubtful Sound, Fiordland, New Zealand was undertaken to investigate DOM dynamics and unique processes associated with fjords.

In the present study, it is hypothesised that terrestrially-derived CDOM generated in fjordic environments can have a significant contribution to the CDOM pool in the coastal zone and potentially the open ocean. We also suggest that processes involved in the transformation from CDOM to non-chromophoric DOM can be studied in a unique manner in a fjord compared to an estuary, because of the extreme stratification of the extra surface freshwater and underlaying oceanic water.

To test this hypothesis, the present study was undertaken in Doubtful Sound which is the largest fjord (extending 43 km inland) within the Fiordland National Park. It receives an annual rainfall of approximately 6,000 mm (Stanton and Pickard, 1981) and therefore belongs to one of the wettest temperate rain forest areas on earth. Due to this high annual rainfall and an artificial freshwater input from the discharge of
a hydroelectric power plant, Doubtful Sound demonstrates an outflowing permanent and stable low salinity layer (LSL) extending some metres in depth (Gibbs et al., 2000; Gibbs, 2001), which overlays a saline layer (SL) flowing in from the open ocean.

The surface water in this LSL is particularly rich in CDOM and protects the marine environment from harmful UV irradiation. The distribution and cycling of CDOM in New Zealand fjords has not been characterised and indeed there appears to have been little research undertaken in these extreme environments related to CDOM and cycling of DOM in general. Although there have been some reports of optical effects in a fjord (Davies-Colley, 1992) and the consequences for photochemical production of species such as hydrogen peroxide (Peake and Mosley, 2004), there appears to have been no studies on the fluorescent properties of fjordic CDOM.

The fluorescence technique involving single wavelength excitation and emission has been used to monitor CDOM distribution in different aquatic systems (Hoejerslev, 1989). However, in fjords this fluorescence technique has been restricted to the monitoring only of chlorophyll a levels in relation to phytoplankton abundance (Glud et al., 2002). More recently, the fluorescent technique has been extended to allow 3-dimensional excitation emission matrix (EEM) fluorescence spectroscopy (Coble et al., 1990; Coble, 1996). This extension provides extra information on the relative contribution of terrestrial and marine sources of CDOM as well as identifying fluorescent peaks that can be assigned to specific proteins such as tryptophan and tyrosine (Coble et al., 1990; Coble, 1996; Coble et al., 1998; Kowalczuk et al., 2003; Kowalczuk et al., 2005; Yamashita and Tanoue, 2003a; Yamashita and Tanoue, 2004; Stedmon and Markager, 2005).

The aim of the present study was to use the EEM fluorescence technique together with UV/Vis absorbance measurements to characterise the CDOM content of a fjord as a function of depth and season and to address changes in the optical properties during vertical mixing of the upper freshwater with the underlying seawater.

### 2.1 Materials and Methods

Doubtful Sound has a deep basin of over 400 m depth with a shallow sill at the entrance of the fjord (see Figure 2.1). The present work was undertaken across the low salinity layer which varied in depths between 0 and 5 m. Samples were collected at 15 sample stations along the main fjord and within the major arms.
Figure 2.1: Sample stations and bathymetry, Doubtful Sound, Fiordland, New Zealand
Sampling was carried out in early spring (September 2004), summer (January 2005) and autumn (May 2005). At each station, water samples were collected in 500 mL acid-cleaned amber glass bottles at the surface and at depths of 1, 2, 3 and 5 m. Initially, samples from 10 m depth were also collected and the EEM fluorescence as well as the absorption coefficients determined, but the spectra showed no differences between the results for this depth at 5 m in most cases (data not shown) and so collection of samples from 10 m depth was discontinued.

Prior to sample collection, the amber glass bottles were rinsed several times with Milli-Q water and dried. The bottles were mounted on a sampling device which allowed for sampling at precise depths while minimising the risk of potential sample contamination. All samples were filtered within 24 hours through Millipore GV 0.22 µm filters. The salinity and temperature gradients were measured using a Seabird™ 19 CTD profiler and associated software.

A Cary Varian 500 dual beam UV/Vis spectrophotometer was used to determine the UV/Vis absorbance between 280-700 nm using a 1 nm slit width. The baseline was determined using a 50 mm quartz cuvette filled with re-distilled Milli-Q water. All samples were baseline-corrected and the baseline sample was measured at least twice a day to monitor any possible instrumental shift of the baseline. The absorbance was converted into the absorption coefficient at the wavelength of 355 nm: $a_{CDOM}(355)$.

In the present study an exponential function was chosen to calculate the spectral slope ($S$) between 280 and 700 nm using the single exponential model (SEM) described by Twardowski et al. (2004), but a different equation:

$$ y = e^{a + (-S)x} $$

Both variables $a$ and $S$ were determined using the Levenberg-Marquardt Method and appropriate iteration processes to find the best fit for the absorption coefficients between 280 and 700 nm. This equation [2.1] gave the best fit compared to other exponential functions, evaluated in the present study (data not shown). The non-linear fit is shown in Figure [2.2]. The values calculated for the spectral slope $S$ are critically dependent on the choice of the fitting procedure. Twardowski et al. (2004) described the problems involved in achieving accurate values for the spectral slope and stated that the actual variability of the spectral slope is less than currently thought. In the present study, the spectral slope $S$ was accurately calculated using the non-linear fit approach and the variation of $S$ was low ($R^2 \geq 0.99$).
Excitation emission matrix (EEM) fluorescence measurements were undertaken using a Jobin Yvon SPEX FluoroMax-3 fluorescence spectrometer. The emission was recorded over the range from 280-600 nm for excitation wavelengths ranging from 250-500 nm at 5 nm intervals. The spectra were corrected for the Raman and Raleigh scattering and EEM peak integrals were calculated using the FLToolbox 1.91 (Zepp et al., 2004). Quinine sulfate standards were used to calibrate the EEM spectra and fluorescence intensities were expressed in units of quinine sulfate equivalents (QSE), which facilitated a comparison of the present data with other EEM fluorescence data published in the literature (Coble et al., 1998; Yamaguchi et al., 2002; Chen et al., 2003; Zepp et al., 2004; Kowalczuk et al., 2005).

Coble (1996) has interpreted marine EEM fluorescent traces in terms of the following classes:

- A-peak: humic-like (terrestrial)
- C-peak: fulvic-like (terrestrial)
- M-peak: fulvic-like (marine)
- T-peak: protein-like
Based on these classification of EEM fluorescent peaks, the integrals for the humic-like peak (A) and the protein-like peak (T) were calculated. The two fulvic-like peaks (C and M) were combined to calculate the integral, because in this study the pattern of the EEM spectra did not allow one to distinguish between these peaks.

The Fluorescence Index (FI) was applied in this study as a tool to investigate potential molecular changes in CDOM (e.g. terrestrially-derived versus marine-derived CDOM). Briefly, the FI is defined as the ratio of the emission at 450/500 nm and an excitation wavelength of 370 nm (see Chapter 1.7.3 for more details).

Contour maps of various parameters were prepared by interpolating between data points using the Geographic Information System (GIS) open-source Geographic Resources Analysis Support System (GRASS). This GRASS-GIS system can be used for geospatial data management and analysis, image processing, graphics and maps production (Neteler and Mitasova, 2004). The present salinity, absorption and fluorescence data were interpolated using the GRASS 6.3 software package (Team, 2007) by application of the Inverse Distance Weighted (IDW) interpolator. This interpolator assumes that each data point has a local influence that becomes less important with distance (Isaaks and Srivastava, 1989). In the present study, a measure of true distance through the water mass was used instead of a simple Euclidean distance in order to account for geophysical barriers in the fjord such as islands and narrow channels (Wing et al., 2004). The interpolation was based on the consideration that the area within 3-5 km of any given sample station has sufficient proximity to reflect the geospatial data measured in the present study. The resolution of the raster map was set to 50 m, which was appropriate for the distance between the sampling sites.

### 2.2 Results and Discussion

#### 2.2.1 Rain Events in Doubtful Sound

The high annual precipitation recorded for this region arises mainly from intense distinct rain events. In austral autumn 2005 the rain intensity occasionally reached extremes of over 15 mm h$^{-1}$, but in general it was no more than 5-10 mm h$^{-1}$ (data not shown). The daily rainfall within the two weeks period prior to sampling varied dramatically between the sampling events (Figure 2.3). The sampling in May 2005 was carried out directly after a major rain event with elevated rain intensities of about 50 mm d$^{-1}$. In September 2004 and January 2005, the amount of rainfall was considerably
less compared to the amount of rain in May 2005 during the week prior to sampling.

Figure 2.3: Daily rainfall within a 2 weeks period prior to sampling, Doubtful Sound, New Zealand

2.2.2 Salinity and Temperature profiles

Within the fjord, the LSL can extend to several meters depth and this depth varies significantly with heavy rain events (Figure 2.4). Additionally, the surface salinity measured in the first 0.5 m depth increased dramatically from the head of the fjord and arms (e.g. Deep Cove, Hall Arm, Crooked Arm) toward the open ocean due to mixing with the incoming seawater from the ocean (Figure 2.5).

The surface water temperature in early spring was uniformly distributed along the fjord (9 °C). In summer, water in the main arms (Hall Arm, Crooked Arm and First Arm) was warmer (18 °C) compared to the main fjord (15 °C), whereas in autumn, the water temperatures within the arms (10 °C) were slightly lower (12 °C) than in the main fjord due to a more pronounced cold water run off from the mountains in the arms compared to the main channel. These spatial and temporal trends in the salinity and temperature profiles are similar to those reported in previous studies of this fjord (Peake et al., 2001).
Figure 2.4: Seasonal variation of vertical salinity and temperature gradients at three sample sites during the three sampling periods in Doubtful Sound, New Zealand
Figure 2.5: Interpolated surface salinity, May 2005, Doubtful Sound, New Zealand
2.2.3 Quantitative Distribution of CDOM within the fjord

Doubtful Sound exhibits characteristic fjordic behaviour with freshwater mixing with saltwater vertically down the water column as well as a horizontally along the fjord. The absorption coefficients \( a_{CDOM}(\lambda) \) between 280 and 700 nm were calculated and in the present study, the behaviour of CDOM with depth and along the fjord was determined using the absorption coefficient at 355 nm \( a_{CDOM}(355) \) and EEM spectroscopy. A major focus of this research was the extraordinary vertical decrease in the level of CDOM within the entire fjord and the associated changes in the salinity gradient.

**UV/Vis absorption spectroscopy**

An example of the typical decrease of the absorption coefficients \( a_{CDOM}(\lambda) \) between 280 and 700 nm with increasing depth is given in Figure 2.6.

![Figure 2.6: Depth dependence of \( a_{CDOM}(\lambda) \) at sample station CA02 (May 2005), Crooked Arm, Doubtful Sound, New Zealand](image)

Such a wide variation in the level of CDOM absorption between the surface LSL and at 5 m depth does not appear to have been previously reported for a fjord or any other variable salinity waters. Furthermore, at some of the sample stations located in Crooked Arm (CA01, CA02 and CA03), the absorption maxima were higher than the levels previously reported for many coastal environments and estuaries (Blough and Del Vecchio, 2002). However, much higher absorption coefficients have been reported in
freshwater rivers and lakes (Miller and Zepp, 1995; Moran et al., 2000). Apart from the earlier study in Doubtful Sound by Peake and Mosley (2004), values for the absorption coefficient have only been reported for a few other fjords and sounds: Danish fjords, (Stedmon et al., 2000) and East Sound, Washington State, USA (Twardowski and Donaghay, 2001)). The lowest absorption values measured at 5 m depth in the present study were comparable to surface absorption levels reported by Stedmon et al. (2000) in Danish fjords and by Obernosterer and Herndl (2000) for the northern Adriatic Sea. In the present study, the absorption coefficient \( a_{CDOM}(355) \) also decreased horizontally from the head of the fjord toward the open ocean as shown in Figure 2.7 for surface water.

![Figure 2.7: Interpolated \( a_{CDOM}(355) \) at the surface, May 2005, Doubtful Sound, New Zealand](image)

However, Crooked Arm always showed much higher absorption coefficients compared to the main channel. This observation can be explained by a much higher terrestrially-derived CDOM input from streams (high \( a_{CDOM}(\lambda) \)) feeding into this arm.
compared to the lower $a_{CDOM}(\lambda)$ found in the freshwater entering the main channel of the fjord from the Tail Race. Hence lower $a_{CDOM}(\lambda)$ levels indicate a much lower absorbance water from Lake Manapouri used in the hydro-electrical power station (Figure 2.8).

Figure 2.8: Wavelength-dependent absorption coefficients $a$ of different source waters feeding into Doubtful Sound, New Zealand

The differences in absorption coefficients from different source waters may be explained by the type of vegetation in the watersheds. Catchments from streams feeding into Crooked Arm are characterised by low altitude temperate rain forest, whereas the water from the Tail Race is derived from Lake Manapouri with catchments characterised by beech forest. Furthermore, Crooked Arm is potentially not influenced by the surface waterflow along the fjord generated by the extremely high freshwater input (approx. 400 m$^3$/sec) from the Tail Race. The marked changes of the absorption coefficient over the sampling periods in the present study may be related to intense rain events and leaching of humic material from the surrounding temperate rain forest soil. This hypothesis is supported by the rain data supplied for a two week period prior to each sample collection (see Figure 2.3). The rainfall was strongly elevated within a few days prior to the sampling in May 2005, compared to the sampling period in September 2004 and January 2005.

If the rain data and the average spectral slope $S$ of 0.014 nm$^{-1}$ for freshwater running into Doubtful Sound are taken into account, it becomes apparent that the
sampling period with the highest freshwater input (May 2005) also corresponded to the period with the lowest values for the spectral slope. Without extreme rainfall prior to sampling as occurred in January 2005, the spectral slope for all samples at levels of $a_{CDOM}(355) \geq 2 \text{ m}^{-1}$ within the fjord showed higher values ($S$ range: 0.015-0.016) compared to those observed for freshwater and for samples taken shortly after a major rain event in Doubtful Sound (Figure 2.9).

Figure 2.9: Behaviour of the spectral slope versus $a_{CDOM}(355)$ in samples collected in January and May 2005, Doubtful Sound, New Zealand

Additionally, the spectral slope values were not linearly correlated with the $a_{CDOM}(355)$ levels and behaved not in a conservative manner. The higher $S$ values at high $a_{CDOM}(355)$ levels for samples collected in January 2005 compared to the sampling undertaken in May 2005 suggest a change in the chromophores related to the increased residence time (caused by relatively low rainfall) of the CDOM in the LSL of the fjord.

**Excitation Emission Matrix (EEM) fluorescence**

A typical EEM spectrum for Doubtful Sound is given in Figure 2.10. The total integrated EEM intensities of the fluorescent peaks associated with humic and fulvic-like compounds were found to decrease with depth comparable to the decrease in the absorption coefficient $a_{CDOM}(355)$ (Figure 2.11).
Figure 2.10: Typical EEM fluorescence plot observed for surface water at sample station CA02 (May 2005) in Doubtful Sound, New Zealand
Figure 2.11: Depth dependence of EEM_{total} at sample station CA02, DS05 and DS07 in Doubtful Sound, New Zealand
At 5 m depth, all total EEM fluorescence intensities ($EEM_{\text{total}}$) at any site within the fjord were similar and comparable to the levels measured at the entrance of the fjord. Additionally, the overall integrated EEM fluorescence intensities (A- and (C+M)-peak) with the exception of the T-peak, decreased from the head of the fjord towards the ocean, again in agreement with the trends observed for $a_{CDOM}(355)$ (Figure 2.12).

![Figure 2.12: Interpolated $EEM_{\text{total}}$ for surface water (May 2005) in Doubtful Sound, New Zealand using geostatistics](image)

In general, the excitation and emission maxima of the peaks apparent in the present EEM fluorescent spectra were similar to those reported previously in the literature (Coble, 1996; Coble et al., 1998; Kowalczuk et al., 2003; Boehme et al., 2004; Kowalczuk et al., 2005). The absorption coefficient, A-peak and (C+M)-peak integrals were strongly correlated (Table 2.1) and this result suggested a direct relationship between $a_{CDOM}(355)$ and the EEM A- and (C+M)-peak fluorescence derived from terrestrial origin which is also in agreement with the previous literature (Green and Blough, 1994; Kowalczuk et al., 2003).

The relation between $EEM_{\text{total}}$, EEM A-peak and EEM (C+M)-peak integrals to $a_{CDOM}(355)$ was found to fit an asymptotic model better compared to a linear model (see Figure 2.13).
Figure 2.13: Asymptotic fit of the $a_{CDOM}(355)$ absorption coefficient to the humic-like EEM$_{A-peak}$ fluorescence

$$EEM_{A_{peak}} = b - c \cdot d^{(K_{sw})}$$

$R^2 = 0.97$

- $b = 168295 \pm 7259$
- $c = 162802 \pm 6917$
- $d = 0.87 \pm 0.01$
Table 2.1: Results of Pearson correlation analysis between $a_{CDOM}(355)$ and the intensities of the EEM A-peak, (C+M)-peak, T-peak integrals

<table>
<thead>
<tr>
<th></th>
<th>$a_{CDOM}(355)$ (n=220)</th>
<th>EEM A-peak (n=215)</th>
<th>EEM (C+M)-peak (n=215)</th>
<th>EEM T-peak (n=215)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_{CDOM}(355)$</td>
<td>1</td>
<td>0.968**</td>
<td>0.959**</td>
<td>0.445**</td>
</tr>
<tr>
<td>EEM A-peak</td>
<td>1</td>
<td>0.990**</td>
<td>0.539**</td>
<td></td>
</tr>
<tr>
<td>EEM (C+M)-peak</td>
<td></td>
<td>1</td>
<td>0.487**</td>
<td></td>
</tr>
</tbody>
</table>

** correlation is significant at the 0.01 level

The equations for the asymptotic model between various EEM fluorescence peak integrals and $a_{CDOM}(355)$ are given in Table 2.2 and show different behaviour for the T-peak integral compared to the EEM A-peak and EEM (C+M) peak integrals. However, the EEM T-peak integral contributes very little towards the EEM total and hence the EEM total show a very good agreement with the model.

Table 2.2: Regression analysis using an asymptotic fit between $a_{CDOM}(355)$ and respective EEM fluorescence peak integrals

<table>
<thead>
<tr>
<th>Variables</th>
<th>Equation</th>
<th>$R^2$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_{CDOM}(355)$ versus EEM total</td>
<td>$EEM_{total} = 819538 - 794025 \times 0.89^{a_{CDOM}(355)}$</td>
<td>0.96</td>
<td>215</td>
</tr>
<tr>
<td>$a_{CDOM}(355)$ versus EEM A-peak</td>
<td>$A_{peak} = 168295 - 162802 \times 0.87^{a_{CDOM}(355)}$</td>
<td>0.97</td>
<td>215</td>
</tr>
<tr>
<td>$a_{CDOM}(355)$ versus EEM (C+M)-peak</td>
<td>$(C+M)<em>{peak} = 111627 + 110025 \times 0.87^{a</em>{CDOM}(355)}$</td>
<td>0.94</td>
<td>215</td>
</tr>
<tr>
<td>$a_{CDOM}(355)$ versus T-peak intensity</td>
<td>$T_{peak} = 7143 - 4380 \times 0.70^{a_{CDOM}(355)}$</td>
<td>0.27</td>
<td>215</td>
</tr>
</tbody>
</table>

In the present study, low levels of both $a_{CDOM}(355)$ and EEM fluorescence and high levels of the spectral slope $S$ were associated with high salinity water and *vice versa*. Hence, changes in the optical properties of the CDOM are associated with changes in salinity. Whether or not this finding is related to the pure physical mixing of two end-members, with different fluorescence to absorbance ratios or is due to molecular changes during mixing across the LSL is not clear from the present data.

In contrast to the A- and (C+M)-peak integrals, the T-peak was not apparent in the EEM fluorescent spectra of any surface water samples, but appeared in the EEM spectra at random sites along the fjord at a water depth of 1 m or greater. As a result, the T-peak integrals did not correlate strongly with either the $a_{CDOM}(355)$, the
A-peak or the (C+M)-peak integrals, suggesting a different origin for the protein-like fluorescence. Furthermore, in Austral summer and autumn, the T-peak intensities were high at some sample stations, but in winter/early spring no distinct T-peak was observed anywhere in the fjord. Mostofa et al. (2005) described a linear relationship between increased DOC levels in summer and protein-like fluorescence and attributed this correlation to an autochthonous input of DOM. We therefore assume that the appearance of the T-peaks in Austral summer and autumn may be related to the biological activity within the fjord at these times.

The marked changes of the EEM fluorescence and of the absorption coefficients over the sample periods in the present study, may be related to intense rain events and leaching of humic and fulvic material from the surrounding temperate rain forest soil.

### 2.2.4 Non-conservative Behaviour of CDOM during Mixing in the Freshwater-Seawater Interface

From a plot of the integrated intensity of the A-peak versus salinity (Figure 2.14), it can be seen that 60-70 % of the decrease in EEM fluorescence (except the protein-like fluorescence) could be explained by conservative mixing. Additionally, toward lower salinity, the variability in the correlation between EEM fluorescence and salinity increased substantially as observed during all three sampling periods.

The flow rate of rivers and streams feeding into Doubtful Sound are highly variable depending on the amount of rain (personal observation). Hence, the supply of terrestrially-derived CDOM into the fjord from the source is also highly variable. The whole region is characterised by small and distinct catchments, which can receive huge amounts of water, leading to poorly developed soils and a limited capability to retain water. As a result, during extreme rain events a major portion of CDOM produced in soils is transported into the fjord via runoff into streams and rivers. This assumption is based on observations during a major rain event, where the $a_{CDOM}(355)$ measured in a stream discharging into the fjord, decreased with the duration of the rain event, without any apparent changes in the stream flow rate. Furthermore, toward the end of such rain events, the water at the fjord surface showed lower $a_{CDOM}(355)$ values compared to the underlying water (data not shown). However, the strong stratification of CDOM with highest $a_{CDOM}(355)$ values at the surface was restored within 24 h after the rain event. This observation cannot be explained by just conservative mixing. It appeared that the freshwater seawater interface somehow has an impact on the
transport of CDOM with the mixing occurring in less saline water.

Figure 2.14: Correlation of the EEM A-peak integral with salinity for three sampling periods: September 2004, January 2005 and May 2005, Doubtful Sound, New Zealand

2.2.5 Potential Changes of the CDOM Composition in the Freshwater-Seawater Interface

To evaluate chemical changes in CDOM with increasing salinity, the fluorescence index (FI) and the spectral slope $S$ were used as indicators for changes in the chromophores of CDOM (Figure 2.15 and 2.16). The fluorescence index calculated for all samples increased with depth, suggesting either an increasing influence of marine-derived CDOM located in the SL or chemical changes of CDOM occurred across the LSL/SL interface. In May 2005, the increase in FI across the first 5 m of the LSL was small, which could have been due to the input of large amounts of rain and hence freshwater into the fjord prior to sampling. Also a deeper vertical transport of freshwater into the fjord occurred and the depth of the LSL exceeded the maximum sampling depth of 5 m.
A more pronounced change in the spectral slope was observed for high salinity (> 25) sites during the sample period in January and May 2005 (Figure 2.17). This phenomenon has previously been reported as the inflection point by Del Castillo et al. (2000) and described by several other groups (Blough et al., 1993; Del Castillo et al., 1999; Kowalczuk et al., 2003). Blough and Del Vecchio (2002) noted that an increase in spectral slope $S$ could be caused by photochemical processes and / or biological transformations. However, Stedmon and Markager (2003) showed that just the “conservative” mixing of two different end-members could also lead to an increase in spectral slope $S$. Highly degraded terrestrially-derived CDOM and also marine-derived CDOM do show high values of the spectral slope and hence a molecular change of terrestrially-derived CDOM cannot be concluded in this study.
Figure 2.16: Behaviour of the spectral slope $S$ with depth in Doubtful Sound, New Zealand: (a) January 2005 and (b) May 2005

Figure 2.17: Seasonal behaviour of the spectral slope versus salinity in Doubtful Sound, New Zealand
2.3 Summary

A range of spectral analytical techniques have been used in this study to determine the distribution and chemical nature of the chromophoric fraction of the dissolved organic matter in a fjordic environment. The level of CDOM as measured by $a_{CDOM}(355)$ and the A- and (C+M)-peak EEM fluorescence decreased from the head of the fjord toward the ocean.

Information on the vertical mixing of the CDOM across the LSL/SL interface was provided from observations of changes in the optical properties across this interface. These changes included the 90% decrease of the CDOM absorption coefficient $a_{CDOM}(355)$ and the EEM fluorescence intensities such as the A-peak and (C+M)-peak within a vertical salinity gradient of 5-34. Such a strong vertical gradient characterised by changes not only in salinity, but also in CDOM absorbance and fluorescence does not appear to have been previously reported for any coastal environments or fjords. A very pronounced gradient of the $a_{CDOM}(355)$ and EEM fluorescence with depth developed after a few days with less rainfall in September 2004 and January 2005, whereas with strong rain events, this gradient can be disturbed giving rise to a subsurface maxima for the $a_{CDOM}(355)$ such as occurred in May 2005.

The results suggest that apart from conservative mixing, non-conservative processes are also affecting the CDOM. In this specific fjord environment, it would appear that the changes in the fluorescence index (FI) and spectral slope S suggest either the increasing influence of marine-derived CDOM with depth or the chemical changes of the chromophores of CDOM across the LSL and into the underlying SL. An asymptotic relationship between the CDOM absorption coefficient and CDOM fluorescence intensity has been demonstrated. Photochemical reactions of the CDOM and hence molecular transformation caused by sunlight in Doubtful Sound is unlikely because the penetration of sunlight through the water column is restricted due to the high absorbance of the fjord surface water. Therefore, either physico-chemical processes within the mixing or a shift from terrestrially-derived CDOM toward marine-derived CDOM seem more likely to cause the non-conservative changes of CDOM.

The T-peak intensities have shown a very different distribution compared to the A-peak or (C+M)-peak. In fact, the T-peak has a very different appearance at different sites within the fjord and at this stage, it suggested that the T-peak is related to in situ production within the fjord. Further research is needed to investigate the origin of the T-peak at least in such a fjordic environment.
Chapter 3

Salinity-dependent Changes in the Chemical Composition of DOM

3.1 Introduction

The coastal zone and in particular the freshwater-seawater interfaces in estuaries are the most reactive areas in terms of molecular changes of DOM. Despite the fact that DOM is the most dynamic and labile component of the carbon cycle, little is known about the processes responsible for these changes.

The fate of terrestrially-derived DOM in the oceans is poorly understood due in part to the complex molecular composition of fulvic and humic acids and the lack of adequate analytical techniques in the past. Recently, ultrahigh resolution ESI-FT-ICR-MS at high magnetic fields (9.4 T) (Marshall et al., 1998) has shown promise as a tool for advanced molecular characterisation of DOM (Kujawinski, 2002; Stenson et al., 2002). Despite the resolving power and therefore ability to obtain exact molecular masses, the ESI-FT-ICR-MS technique does not appear to have been applied to the study of DOM samples collected from water at different salinities.

However, solid-phase extracted DOM from marine samples such as the Weddell Sea (Antarctica) and terrestrially-derived samples from mangrove pore-water have been successfully characterised using this method (Koch et al., 2005). Koch et al. demonstrated the complexity of information which can be obtained from application of the ESI-FT-ICR-MS technique.

The focus of the present study was to investigate the molecular changes of DOM along salinity gradients during freshwater-seawater mixing in two distinct marine environments in the south of New Zealand. A major aim in modern biogeochemistry is
to elucidate the decomposition pathways during the transport of terrestrially-derived DOM from freshwater to the ocean. Since the first molecular evidence of terrestrially-derived DOM in the open ocean (Meyers-Schulte and Hedges, 1986), information on the molecular changes in DOM from freshwater toward seawater has become increasingly important. The transformation of terrestrially-derived DOM into a refractory DOM pool in the open ocean is also of great interest, because the budget of the organic carbon cycle in the ocean is based on such information.

In addition to assigning molecular formulae containing just carbon, hydrogen and oxygen (C:H:O), nitrogen- (C:H:O:N) and sulphur- (C:H:O:S) containing formulae have also been assigned in the present study from the ESI-FT-ICR-MS data. These three groups of molecular formulae have been evaluated separately, because of the very different molecular transformations of compounds containing neither nitrogen nor sulphur, nitrogen compounds and sulphur compounds that became apparent across freshwater-seawater interfaces.

Nitrogen is a major nutrient element and may be a limiting factor in autochthonous production (Stepanauskas et al., 1999). Meybeck (1993) calculated the amount of dissolved organic nitrogen (DON) entering the coastal zone from rivers to be 10^{13} g N/year. In general, the DON content progressively decreases as it passes from rivers, via estuaries and coastal zones until it reaches a minimum in the open ocean (Bronk, 2002). It is believed that the bioavailable fraction of the DON pool may get consumed within weeks in estuaries (Seitzinger and Sanders, 1997). Additionally, DON seems to be more readily consumed in seawater compared to freshwater (Stepanauskas et al., 1999). Therefore, it is important to evaluate the changes in the molecular composition of nitrogen-containing DOM in freshwater-seawater interfaces. DON can contain a variety of different compounds e.g. urea and other specific nitrogen groups such as dissolved combined amino acids (DCAA), dissolved free amino acids and fulvic and humic acids. However, a substantial fraction of these nitrogen compounds is still unidentified.

In the present study, the solid-phase extraction of natural water samples using C-18 cartridges did not allow for the extraction of the entire DON pool. However, it should be possible to use such solid-phase extraction techniques to extract nitrogen incorporated into humic and fulvic acids. Unfortunately, these compounds contain only a small fraction of the nitrogen pool and the average nitrogen content is 2-6 % nitrogen in humic acids and 1-3 % in fulvic acids (Hedges, 1988). Whitehead and Vernet (2000) showed that mycosporine-like amino acids have a 7.4 % extraction efficiency using C-18 based solid-phase extraction cartridges, suggesting that at least a small fraction of
the amino acid pool is extracted. Despite the fractionation of DON due to extraction and difficulties in analysing these compounds in negative ion mode ESI-FT-ICR-MS, it nevertheless seemed worthwhile in the present study to examine the changes in the extractable DON pool. A more effective extraction procedure for nitrogen compounds would have been desirable, but could not be applied in the present study.

Sulphur is another important nutrient element and humic acids in soils have been estimated to contain between 0.58 and 0.78 % sulphur (Krupskii et al., 1971). The low sulphur content in humic acids are unlikely to account for all C:H:O:S formulae assigned in the present study. Therefore, another source of sulphur is suggested. Dissolved organic sulphur in the ocean is produced in situ through phytoplankton metabolisms and contains mostly dimethylsulfide (DMS) (Kiene, 1992). In freshwater and coastal zones, the bulk of the sulphur seems to be incorporated in larger hydrophilic molecules, which can be easily extracted using C-18 SPE resins (Ruttinger and Binde, 1998). It has been shown that most sulphur compounds in surface freshwater are related to organic sulfonic acids (Schullerer and Frimmel, 1993). Additionally, sulfonic acids would be easily ionisable in the electrospray of the ESI-FT-ICR-MS. Therefore, a plausible source of the assigned sulfur-containing formulae in the present study may be organic sulfonic acids.

Two study sites, the Freshwater River on Stewart Island and Doubtful Sound were chosen to measure the molecular changes of DOM across the freshwater-seawater interfaces. These study sites have been described in Chapter 1 and showed very distinct differences in their freshwater-seawater interfaces. The entire catchments of the Freshwater River, Stewart Island were subject to very little anthropogenic influence and can therefore be considered as pristine environments. Terrestrially-derived DOM is generated within the Freshwater River Valley and it then enters the river before ultimately discharging into Paterson Inlet (see map in Figure 3.1). The estuary at the mouth of the river is shallow and allows for turbulent mixing. All these conditions are ideal for the study of potentially conservative estuarine horizontal mixing processes.

In Doubtful Sound, freshwater accumulates at the surface and mixes vertically with the underlying seawater. A detailed description of the vertical salinity gradient across the low salinity layer (LSL) in Doubtful Sound has been given in Chapter 2.

Lignin derivatives are excellent tracers for terrestrially-derived DOM (Dittmar et al., 2001; Dittmar and Lara, 2001). These compounds are only produced by vascular plants (Sarkanen and Ludwig, 1971) and contain mainly phenolic groups. Hence, lignin derivatives are difficult to metabolise by microorganisms and are decomposed
only slowly (Hedges and Parker, 1976). A significant amount of these lignins accumulate in soil leachate and then enter the streams and rivers. Lignin seems to be relatively stable in freshwater, but as soon as freshwater mixes with marine water, the lignin composition changes, which may be caused largely by photooxidation (Opsahl and Benner, 1998; Benner and Opsahl, 2001) and processes related to bacterial decomposition (Hernes and Benner, 2003) in the marine environment. Beside the ESI-FT-ICR-MS and lignin analyses, the following parameters were also measured either in the field or on return of the samples to the laboratory in order to provide further details of the processes across the freshwater-seawater interface:

- salinity
- absorption coefficient $a$ at 355 nm
- spectral slope $S$
- excitation emission matrix (EEM) fluorescence
- DOC content

3.2 Materials and Methods

In February 2006, 25 L water samples were collected at four stations within the Freshwater River Estuary in Stewart Island (Figure 3.1). The salinity at the four sample stations varied considerably: sample station FWR01 (salinity: 0) was considered to be the freshwater end-member and sample stations FWR02 (salinity: 5), FWR03 (salinity: 14) and FWR04 (salinity: 28) were located within the estuary.

In October 2005, a 25 L water sample was collected from a stream located in Crooked Arm close to sample station CA02, Doubtful Sound (see Figure 3.2). Two more samples were collected from the fjord surface and at 5 m depth at sample station CA02. Salinity, temperature and pH were measured using a Horiba U-10 Water Quality Checker for samples collected in the Freshwater River, Stewart Island. In Doubtful Sound, salinity and temperature were measured using a Seabird-19$^{TM}$ CTD profiler. All samples were filtered through Millipore GV 0.22 µm filters before further analyses. For lignin and ESI-FT-ICR-MS analysis, an established solid-phase extraction procedure for DOM has been followed (Louchouarn et al., 2000), where 25 L water samples were acidified to pH 2 and solid-phase extracted using C-18 Varian Mega Bond Elut cartridges (10 g C-18 resin).
Figure 3.1: Sample stations along the Fresh Water River Estuary, Stewart Island, February 2006

The cartridges were conditioned with 50 mL HPLC-grade methanol and then washed with 100 mL acidified Milli-Q (pH 2) prior to extraction. The extraction flow rate was adjusted to 50 mL/min using a peristaltic pump. After the extraction of 10-25 L sample (depending on the DOC content), each cartridge was washed with 500 mL acidified Milli-Q water to desalt the extraction cartridge and then the solid-phase extracted DOM was eluted with 50 mL HPLC-grade methanol. Each sample was divided into two: one sample was dried using a Speed-Vac evaporator for lignin analysis and the other sample was stored at -18 °C for subsequent ESI-FT-ICR-MS analysis.
Figure 3.2: Sample station CA02 in Doubtful Sound, Fiordland, New Zealand used in October 2005
Characterisation of lignin derivatives

Chemicals for lignin oxidation:

- 2 M NaOH, SODOX, AR grade
- CuO, BDH, AR grade
- Fe(NH$_4$)$_2$(SO$_4$)$_2$ · 6H$_2$O, AJAX, AR grade
- Phenylacetic acid, RIEDEL DEHAEN AG, AR grade
- 6 M HCl, BDH, AR grade
- Ethyl Acetate, BDH, AR grade
- Methanol, BDH, HPLC-grade
- SPE C18 Mega Bond Elute Varian cartridges and SPE C-18 Prevail cartridges

For the characterisation of lignin derivatives, the following phenolic compounds were used as standards:

- 4-hydroxybenzoic acid, BDH, AR grade
- 4-hydroxy-3-methoxybenzoic acid (vanillic acid), Merck, 97%
- 3,5-dimethoxybenzaldehyde (syringic acid), Aldrich, 97%
- 4-hydroxybenzaldehyde, Cambrian Chemicals LTD, AR grade
- 4-hydroxy-3-methoxybenzaldehyde (vanillin), BDH, AR grade
- 3,5-dimethoxy-4-hydroxybenzaldehyde (syringaldehyde), Aldrich, 98%
- 4-hydroxycinnamic acid (p-coumaric acid), Sigma
- 4-hydroxy-3-methoxycinnamic acid (ferulic acid), Aldrich, 99%
- acetovanillone, Aldrich, 98%
- acetosyringone, Aldrich, 97%
- p-hydroxyacetophenone, Aldrich, 99%
These phenolic compounds have been successfully identified as specific lignin derivatives by different research groups (Opsahl and Benner, 1995; Louchouarn et al., 2000).

1-2 mg sample from the dried C-18 solid-phase extracted sample was redissolved in 5 mL of 2 M NaOH and placed in a Teflon microwave digestion vial. The headspace was purged with nitrogen for 1 minute and closed. The digestion was carried out in a microwave digestion oven at 150 °C for 90 minutes. Samples were allowed to cool to room temperature, before 1 mL aliquots were neutralised using concentrated HCL and placed in an autosampler vial for analysing on an HPLC system. The identity of the peaks were confirmed using retention time as well as the absorption spectrum generated by the diode array detector. The phenolic standards mentioned above were used to develop calibration curves. The CuO oxidation is highly dependent on temperature and reaction time. However, since the relative ratio of different phenolic compounds, e.g. the syringyl to vanillyl phenol ratio (S/V), is used to evaluate the change in lignin composition, it is not critical to obtain the same CuO oxidation efficiency between different samples. Nevertheless, it is important to maintain the same oxidation rate for all determined phenols, so that the relative peak intensities of all measured phenols are affected in the same manner. The definition of phenol ratios and parameters used in lignin derivative analysis are summarised in Table 3.1. The meaning of these parameters will be discussed in the result section.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/V</td>
<td>Molar ratio of total syringyl phenols to vanillyl phenols</td>
</tr>
<tr>
<td>C/V</td>
<td>Molar ratio of total cinnamyl phenols to vanillyl phenols</td>
</tr>
<tr>
<td>(Ad/Al)v</td>
<td>Molar ratio of vanillic acid to vanillin</td>
</tr>
<tr>
<td>P/(V+S)</td>
<td>Molar ratio of total p-hydroxyl phenols to the sum of vanillyl phenols and syringyl phenols</td>
</tr>
<tr>
<td>Sum8</td>
<td>Sum of the three vanillyl, the three syringyl and the two cinnamyl phenols</td>
</tr>
</tbody>
</table>

**ESI-FT-ICR-MS analysis of riverine DOM**

The mass spectra were measured using a home-built ESI-FT-ICR-MS equipped with a superconducting 9.4 Tesla magnet located at the National High Magnetic Field Laboratory (NHMFL), Tallahassee, Florida (Figure 3.3).
Figure 3.3: ESI-FT-ICR-MS instrumentation at the National High Magnetic Field Lab, Tallahassee, Florida

100 µl Milli-Q water and 10 µl ammonium hydroxide (78 %) were added to 100 µl of the methanol extract of each DOM sample. The flow rate for the electrospray injection was set to 0.3 µl/min using a fused-silica transfer line. The electrospray was run in negative ion mode with a needle voltage set to 2,600 volts to form negatively charged macroions. The negative ions then passed through a tube lens and skimmer set to -13 V. A dual spray injection was used to spray the sample separately from the calibration standard and the generated negative ions were accumulated with accumulation times of 25 sec for the sample compared to 0.3 sec for the standard. This big difference in accumulation time was necessary, because otherwise the signal of the standard would have obscured the sample signal. To enhance the precision of the signal, 200 individual
measurements were typically averaged.

The individual m/z peaks were calibrated using a Hewlett Packard calibration mixture with m/z peaks at 431.9828; 734.00726 and 1033.98810 Da. The accuracy of the ESI-FT-ICR-MS was high with less then 1 ppm error for most assigned masses and a mass resolving power between 600,000 and 800,000. The mass resolving power was defined as the ratio between the mass (m) and the mass spectral peak full width at half-maximum peak height (∆m_{50\%}) (Stenson et al., 2003). The signal to noise ratio (S/N) varied slightly depending on the sample and apparatus performance, however complete mass resolution was always achieved.

The wealth of information obtained from a single ultrahigh resolution mass spectrum (see Figure 3.4 for an example of a typical DOM mass spectrum) made it necessary to develop a variety of algorithms to simplify the data analysis and to assist in the interpretation of such complex data sets. Stenson et al. (2003) have used the following parameters to aid in the data analysis: the Kendrick Mass (KM):

\[ KM = \text{IUPAC mass}_{\text{measured}} \times [14.00000/14.01565] \]  

(3.1)

where:

IUPAC mass_{measured} is the neutral mass (addition of 1 H for negative ionisation) obtained from the mass spectra

was developed to identify ions in a homologous series which differ by a number of CH\(_2\) groups.

the Kendrick Mass Defect (KMD):

\[ KMD = (NM - KM) \]  

(3.2)

where:

NM = nominal IUPAC mass

was established to find all the members of the same homologous series as they all exhibit the same KMD value.
Figure 3.4: Example of an ESI-FT-ICR-MS spectrum of DOM
the parameter $z^*$:

$$z^* = \text{modulus}(NM/14) - 14$$  \hspace{1cm} (3.3)

where:

modulus is remainder of the division of nominal mass by 14

is independent from $KMD$, and used to cross-validate the individual homologous series assigned by the $KMD$ values.

the Double Bond Equivalent ($DBE$):

$$DBE = 1 + 1/2(2C - H + N + P)$$  \hspace{1cm} (3.4)

where:

C = carbon; H = hydrogen; N = nitrogen and P = phosphor

is a measure of double bonds and aromatic rings in one specific molecular formula.

The Kendrick Mass ($KM$) was defined by Kendrick (1963) and basically converts the exact value of 14.01565 (based on the definition of $^{12}\text{C}$ as an exact value of 12.00000) for the CH$_2$ group into the value of 14.00000. The advantage of using this approach is that members of one specific homologous series would show the same $KMD$. A graphical explanation of the relationship of IUPAC mass, $NM$, $KM$ and $KMD$ is given in Figure 3.5. $KMD$s can be similar for different homologous series and to distinguish between homologous series but having the same $KMD$ value, the parameter $z^*$ was established as an independent parameter. $z^*$ is based on the division of the nominal mass by 14. The remainder of this division must be the same for any given homologous series, because of the given 14 mass unit spacing (corresponding to a CH$_2$ unit) between members of a homologous series. After sorting the molecular formulae by $z^*$ and $KMD$, the members of one homologous series showed the same $z^*$ and $KMD$. The homologous series can be obtained by adding CH$_2$ groups to the lowest molecular weight member of the series. A much more detailed description of $KM$, $KMD$, $z^*$ and $DBE$ is given in Stenson et al. (2003).

Graphical tools for the visualisation of ESI-FT-ICR-MS data have become increasingly important, due to the wealth of information contained in one mass spectra. Kendrick plots, where the nominal mass is plotted against the $KMD$, proved to be a valuable fingerprinting tool for complex mass spectra and can also give information
Figure 3.5: Graphical explanation of the relationship of IUPAC mass measured, Nominal Mass (NM), Kendrick Mass (KM) and Kendrick Mass Defect (KMD) about the degree of saturation. Kendrick plots for identical \( z^* \) are less complex and give detailed information about specific members of homologous series and the degree of saturation. Within one specific \( z^* \), all homologous series are visible and spaced by a KMD of 0.0036. Each row contained one distinct homologous series with compounds spaced by CH\(_2\). Furthermore, each \( z^* \)-specific Kendrick plot contains up to 4 different series, which are characterised by the amount of carbon-carbon double bonds. The degree of saturation (carbon double bonds) is indicated by the parameter DBE-O, in which the oxygen content is subtracted from the DBE.

The Van Krevelen or elemental plots (Van Krevelen, 1950; Van Krevelen, 1984), where the oxygen to carbon (O/C) ratios are plotted against the hydrogen to carbon (H/C) ratios, have also become increasingly important in graphical data analysis of mass spectra obtained from DOM samples. The simplicity of the plot makes the Van Krevelen diagram a popular fingerprinting tool for complex organic mixtures. Processes such as oxidation, chemical reduction, decarboxylation, or dehydration are represented.
by straight lines. This diagram can also be extended to three dimensions to incorporate a variety of additional parameters such as molecular weight, DBE, DBE-O, C/N ratios, C/S ratios etc. (Koch et al., 2005).

The resolution achieved using this ESI-FT-ICR-MS technique was sufficient to calculate exact molecular formulae from the m/z peaks, providing the molecules were singly charged. Calculations for the exact molecular formulae were based on the following chemical elements: C, H, O, N, S and P as well as the $^{13}$C isotope. Any numbers of carbon, hydrogen and oxygen atoms were allowed, but sulphur and nitrogen were limited to 5 atoms and $^{13}$C isotopes to 1 atom. In the present study, all molecular formulae were calculated manually to achieve the most accurate assignment. Only homologous series with $\geq 3$ members were considered. The measured masses were sorted using the $z^*$ and KMD values. With this presorting of masses, several homologous series were determined and molecular formulae could be assigned for all masses of one specific homologous series using a molecular mass calculator. However, within the mass window of $\leq 1$ ppm several molecular formulae are theoretically possible and the number of theoretically possible molecular formulae increases with molecular mass. Some of these theoretical formulae are highly unlikely, but cannot be ruled out. In the present study, the most likely molecular mass has been chosen, but unfortunately this judgement does not prevent false molecular formula assignments. Nevertheless, the purpose of the present study was to show differences between ESI-FT-ICR-MS spectra taken from various water samples and if all assignments are based on the same criteria to chose the most appropriate molecular formula, a comparison between samples is possible and even potentially wrong assigned formulae would not influence the results based on differences between samples. A few remaining masses, which could not be assigned using specific criteria were found and have not been considered in the present study. However, these masses made up for less than $1\%$ of all measured masses. A comprehensive description of the difficulties and appropriate rules to accurately assign molecular formulae of ultrahigh resolution ESI-FT-ICR-MS data is given in Koch et al. (2007). As mentioned above, ESI-FT-ICR-MS spectra obtained from DOM contained several hundred homologous series characterised by spacing corresponding to CH$_2$ (see sample DOM mass spectrum in Figure 3.6). The peak heights of individual members of one specific homologous series were often similar.

A spacing of 2.0157 Da was equivalent to the mass of two hydrogen atoms and therefore an indication for a change in the degree of saturation. The molecular mass change from a double bond to a single bond or vice versa accounts exactly for this
Figure 3.6: Homologous series of an ESI-FT-ICR-MS spectrum with CH$_2$ spacing measured for a DOM sample (FWR01) from Stewart Island

2 H spacing (Stenson et al., 2002). This hydrogen spacing was observed in all spectra obtained during the present study (see sample mass spectrum measured for DOM extracted from the Freshwater River (FWR01), Stewart Island in Figure 3.7). Additionally, a mass spacing of 0.0364 Da indicates the exact mass difference between CH$_4$ and an oxygen atom and allows one to distinguish between them (Figure 3.8).

Instrumental parameters were held constant for all samples. A major problem was to maintain a constant electrospray over the analysis time of 200 runs required for each sample. Changes in the performance of the electrospray caused slight differences in m/z peak intensities from one sample to the next. However, the resolving power and mass accuracy could be maintained with uncertainties of < 1 ppm, which allowed complete peak resolution.
Figure 3.7: Example of an extended ESI-FT-ICR-MS with molecular mass intervals of two hydrogen atoms, Freshwater River, Stewart Island.

Figure 3.8: Example of an extended ESI-FT-ICR-MS with molecular mass differences of CH$_4$ versus oxygen, Freshwater River, Stewart Island
EEM fluorescence and absorbance measurements

The absorbance was measured between 280 and 700 nm using the Cary Varian 500 UV/Vis spectrophotometer and a 5 cm quartz cuvette (see Chapter 2). Excitation emission matrix fluorescence was also measured in the same manner already mentioned in Chapter 2.

Dissolved organic carbon and total dissolved nitrogen measurements

Prior to analysis, water samples were filtered through Millipore 0.22 µm filters and acidified with concentrated HCl. Dissolved organic carbon (DOC) and total dissolved organic nitrogen (TDN) levels were measured using the high temperature combustion oxidation method and a Shimadzu 5000A TOC/TDN Analyser. Four standards were used for calibration and also the Suwannee River Reference Material was included to ensure the precision of the measurements.

3.3 Results and Discussion

3.3.1 Temperature, Salinity and pH Changes in the Freshwater River Estuary and Doubtful Sound

In the Freshwater River Estuary, the temperature increased slightly from 12 °C at sample station FWR01 (freshwater) to 14 °C at sample station FWR04 in Paterson Inlet. The freshwater sample FWR01 exhibited a low pH of 5.6, indicative of the acidic character of the freshwater flowing out of the Freshwater River catchments. The low pH may be caused by the very small buffer capacity of the weathered granite, which is the major rock substrate in Stewart Island. The few remaining basic compounds in granite are easily leached out during weathering and the remaining substrate is not capable of buffering the organic acids generated by microbial decomposition and mosses such as sphagnum, which are present in the Freshwater River watershed. The pH increased with increasing salinity from station FWR01 (pH: 5.6 and salinity: 0) towards FWR04 (pH: 8.5 and salinity: 28) caused by the mixing with incoming seawater from Paterson Inlet.

In Doubtful Sound at sample station CA02, the surface temperature was 14.0 °C and decreased to 12.6 °C at 5 m depth. The salinity changed from 10.3 at the surface to 34.2 at 5 m depth indicating the strong stratification across the low salinity layer.
(LSL) into the saline layer (SL). A neutral pH was measured for the freshwater river discharging into Crooked Arm close to sample station CA02.

### 3.3.2 Conservative Behaviour of DOM and CDOM during Estuarine Mixing, Freshwater River, Stewart Island

The total EEM fluorescence (EEM<sub>total</sub>) decreased rapidly with increasing salinity (Figure 3.9). This behaviour was expected due to a much lower CDOM content in the seawater sample (FWR04). Parameters such as DOC, TDN, a<sub>CDOM</sub>(355) and EEM<sub>total</sub> showed a strong correlation with salinity and therefore indicated conservative behaviour within the estuarine mixing of the Freshwater River, Stewart Island (Figure 3.11). The relatively high a<sub>CDOM</sub>(355), DOC, TDN and EEM<sub>total</sub> values at the highest salinity sample FWR04 (salinity: 28) supported a remaining high level of terrestrially-derived CDOM/DOM in this sample and hence the effect of the incoming marine-derived DOM seemed minor. The spectral slope S values did not change in all four examined samples and also emphasised that there was no significant effect of marine-derived DOM on the examined DOM pool (see Table 3.2).

<table>
<thead>
<tr>
<th>sample</th>
<th>salinity</th>
<th>spectral slope S</th>
</tr>
</thead>
<tbody>
<tr>
<td>FWR01</td>
<td>0</td>
<td>0.0152 ± 0.00006</td>
</tr>
<tr>
<td>FWR02</td>
<td>5</td>
<td>0.0147 ± 0.00006</td>
</tr>
<tr>
<td>FWR03</td>
<td>14</td>
<td>0.0155 ± 0.00005</td>
</tr>
<tr>
<td>FWR04</td>
<td>28</td>
<td>0.0150 ± 0.00003</td>
</tr>
</tbody>
</table>

The analysis of lignin and the similarities in their ratios also suggested that there were no major changes in the composition of the measured phenolic compounds along this estuary (Table 3.3). Increased acid to aldehyde ratios ((Ad/Al)v) are caused by propyl side chain oxidation and are therefore indicative of the degradation of lignin associated with the phenolic compounds such as vanillic acid and vanillin (Crawford, 1981; Ertel and Hedges, 1984; Ertel et al., 1986). Methoxylated phenols are affected by demethylation, whereas nonmethoxylated phenolic compounds remain intact. Hence, the ratio of non-methoxylated to methoxylated phenols (P/(V+S) can be used as another indicative parameter for lignin degradation (Dittmar et al., 2001).
Figure 3.9: EEM spectra for sample stations along the Freshwater River Estuary, Stewart Island, February 2006
Figure 3.10: Parameters indicating conservative mixing behaviour in the Freshwater River Estuary, Stewart Island, New Zealand
Table 3.3: Lignin analysis, Freshwater River, Stewart Island

<table>
<thead>
<tr>
<th>sample</th>
<th>S/V</th>
<th>C/V</th>
<th>(Ad/Al)v</th>
<th>P/(V+S)</th>
<th>Sum8 (nM/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FWR01</td>
<td>0.59±0.00</td>
<td>0.03±0.00</td>
<td>1.28±0.02</td>
<td>0.34±0.02</td>
<td>7.8±0.9</td>
</tr>
<tr>
<td>FWR02</td>
<td>0.59±0.02</td>
<td>0.03±0.00</td>
<td>1.47±0.08</td>
<td>0.29±0.00</td>
<td>8.5±0.1</td>
</tr>
<tr>
<td>FWR03</td>
<td>0.60±0.03</td>
<td>0.02±0.00</td>
<td>1.31±0.08</td>
<td>0.35±0.02</td>
<td>7.9±0.6</td>
</tr>
<tr>
<td>FWR04</td>
<td>0.57±0.01</td>
<td>0.02±0.01</td>
<td>1.14±0.01</td>
<td>0.32±0.01</td>
<td>8.0±0.2</td>
</tr>
</tbody>
</table>

3.3.3 Molecular Transformation of DOM in the Estuarine Mixing of the Freshwater River, Stewart Island

ESI-FT-ICR-MS data obtained for all four Stewart Island samples (salinity: 0, 5, 14 and 28) showed changes in the molecular composition of terrestrially-derived DOM during estuarine mixing and did not support the findings of parameters such as DOC, TDN, EEM$_{\text{total}}$ and $a_{\text{CDOM}}$(355), which indicated conservative mixing.

3-dimensional elemental plots (Van Krevelen diagrams), where the O/C ratio is plotted against the H/C ratio and also showing the colour-coded molecular weight range or Double Bond Equivalents (DBE), indicated large changes in the DOM molecular composition during mixing (Figure 3.11). There were an increased number of molecular formulae with decreased O/C and H/C ratios and higher DBE from sample FWR01 to FWR04, indicating an increase in unsaturated bonds. Beside the increase in unsaturated compounds, the increase in aromaticity was also confirmed using the most conservative aromaticity index (AI) established by Koch and Dittmar (2006). The AI is indicative for the carbon-carbon double bonds and the amount of π-bonds related to heteroatoms:

$$\text{AI} = 1 + \frac{C - O - S - 0.5H}{C - O - S - N - P}$$

(3.5)

An $\text{AI} \geq 0.5$ is an unambiguous minimum criterion for aromatic compounds. In the present study, an increase in numbers of molecular formulae with an $\text{AI} \geq 0.5$ with salinity was found (data not shown). Furthermore, the number of unique formulae in each sample increased largely from 305 (FWR01) to 2882 (FWR04) with increasing salinity (Figure 3.12).

1569 identical C:H:O formulae could be detected in all samples which represent the refractory portion during estuarine mixing and this number of formulae accounts for approximately 40 % of the total assigned C:H:O formulae (Figure 3.13).
Figure 3.11: 3-D Van Krevelen diagrams for samples collected in the Freshwater River Estuary, Stewart Island, February 2006 (Ellipsoids indicate O/C and H/C ratios in the freshwater sample FWR01)

This result also means that the remaining 60% of all determined molecular formulae changed during mixing. The increase in unsaturation can also be seen after combining all the DBE or the DBE minus oxygen (DBE-O) values from each sample and a comparison of all stations (Figure 3.14). The DBE-O value only accounts for double bonds formed by carbon and not oxygen and therefore it is a better indication of unsaturation/aromaticity. To show the changes in aromaticity in more detail, $z^*$-specific Kendrick plots were constructed from the ESI-FT-ICR-MS data (see 3.15 and Appendix A). The advantages of these diagrams lie in the detailed information they provide on the various homologous series within one $z^*$ value. The homologous series are spaced by 0.0036 Da in the vertical axes and by 14.0156 Da (corresponding to CH$_2$) in the horizontal axes. Furthermore, within one specific $z^*$ value, different series characterised by different DBE-O values were found.
Figure 3.12: Unique C:H:O molecular formulae from sample (a) FWR01, (b) FWR02, (c) FWR03 and (d) FWR04, Freshwater River Estuary, Stewart Island.
Figure 3.13: Shared molecular formulae of all four Freshwater River samples (FWR01-04), Stewart Island

Figure 3.14: Box plot of DBE and DBE-O values for the molecular formulae assigned for all four samples across the Freshwater River Estuary, Stewart Island

These DBE-O series were valuable in indicating a trend of increasing unsaturation with increasing salinity.

Molecular masses characterised by $z^*=−2$ showed three DBE-O series (DBE-O=−5; 2 and 9) at lower salinities (s=0-14) and a fourth series (DBE-O=16) in the most saline sample (FWR04, s=28). Furthermore, the first DBE-O series (highly saturated compounds) disappeared with increasing salinity and a fourth DBE-O series indicative of highly unsaturated compounds appeared. Additionally, the highest molecular weight homologous series (as indicated by high $KMD$) within the DBE-O=2 series, disappeared with increasing salinity and new low molecular weight homologous series (low $KMD$) appeared (Figure 3.15).
All z*-specific Kendrick plots showed exactly the same trends as described for z*=-2. However, different DBE-O series appeared indicating differences in saturation (see Appendix A).

The increase in aromaticity during estuarine mixing was surprising and results from open ocean samples showed that DOM in the open ocean is highly saturated (see also Chapter 6). The only possible explanation is the formation of labile aromatic compounds during estuarine mixing. These aromatic compounds must undergo a rapid oxidation toward highly saturated compounds in the coastal zone. These aromatic compounds are potentially highly photosensitive which has been shown on a sample from the Cape Fear River, North Carolina (see Chapter 4). Hence the dilution of these highly aromatic compounds in the coastal zone and therefore the enhanced exposure to sunlight may well be the most important degradation pathway of these compounds.

Figure 3.15: Kendrick plots for z* = -2 and the molecular formulae assigned for samples taken in the Freshwater River, Stewart Island
Nitrogen Compounds

Only molecular formulae containing one nitrogen have been considered and ESI-FT-ICR-MS data from the Freshwater River, Stewart Island did show 433 (FWR01), 340 (FWR02), 298 (FWR03) and 301 (FWR04) nitrogen-containing formulae (C:H:O:N). The low C/N ratio compounds (C/N range 12-25) apparent in the freshwater sample FWR01 (salinity) also characterised by low DBEs have disappeared by the time the salinity had only reached a value of 5 (FWR02). The high C/N ratio compounds (C/N ratio range: 19-33) seemed to show less changes with salinity, but shifted towards higher DBEs for the most saline sample (FWR04). Additionally, very high C/N ratio compounds (C/N ratio range: 37-43) appeared at intermediate salinities in sample FWR02 and FWR03 (Figure 3.16). Interestingly, low C/N ratios were found for formulae in sample FWR01 and these results differ substantially from literature values for this ratio in freshwater samples (Smith et al., 1991; Gordeev et al., 1996; Seitzinger and Sanders, 1997; Hopkinson et al., 1998; Stepanauskas et al., 1999; Stepanauskas et al., 2000). However, most nitrogen-containing compounds in the FWR sample did show high C/N ratios which were expected for natural organic matter in freshwater. The loss of the low C/N ratio compounds by the time the salinity of 5 had been reached indicated that this fraction of the DON pool was very labile.

Sulphur Compounds

Sulphur-containing compounds were much more abundant compared to those containing nitrogen and all assigned molecular formulae only contained one sulphur atom. 1030 sulphur-containing formulae were found for the FWR01 sample and the number of assigned formulae decreased with increasing salinity (FWR02: 697, FWR03: 609 and FWR04: 537). Two groups characterised by their H/C and O/C ratios appeared in the FWR01 sample and the group with low H/C and O/C ratios decreased with increasing salinity and disappeared in sample FWR03 and FWR04 (salinity > 14). Furthermore, the group which disappeared with mixing (increasing salinity) was characterised by elevated C/S ratios in the range of 23-39 and also higher DBEs. The remaining group of sulphur-containing compounds showed C/S ratios in the range of 15-30 throughout estuarine mixing (Figure 3.17). Interestingly, sulphur-containing compounds with high DBE and high C/S ratios appeared to be labile during mixing.
Figure 3.16: 3-D Van Krevelen diagrams of nitrogen-containing molecular formulae and mass-dependent C/N ratios derived from FT-ICR-MS analysis of samples from the Freshwater River, Stewart Island, February 2006
Figure 3.17: 3-D Van Krevelen diagrams of sulphur-containing molecular formulae and mass-dependent C/S ratios derived from FT-ICR-MS analysis of samples from the Freshwater River, Stewart Island, February 2006
3.3.4 Molecular Transformation of DOM in Doubtful Sound

ESI-FT-ICR-MS analyses were undertaken on samples collected in October 2005 at sample station CA02 in Doubtful Sound. No significant rain had fallen in the 3 days prior to sample collection and so it was expected that there would be pronounced changes in the molecular composition determined from the mass spectra measured in samples collected from stream water, surface fjord water and water at 5 m depth. EEM fluorescence (EEM$_{total}$) as well as the absorption coefficients ($a_{CDOM}(355)$) decreased dramatically with increasing depth and salinity in a similar pattern as already mentioned in Chapter 2 and indicated the vertical mixing gradient at station CA02. However, the spectral slope $S$ did not change across the LSL. The values for $S$ were low across the LSL (Table 3.4) and hence an important influence of marine-derived CDOM was unlikely.

Table 3.4: The spectral slope values at sample station CA02 within the first 5 m depth, Doubtful Sound, New Zealand

<table>
<thead>
<tr>
<th>sample</th>
<th>depth (m)</th>
<th>salinity</th>
<th>spectral slope $S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA02-0</td>
<td>0</td>
<td>10.3</td>
<td>0.0149 ± 0.00002</td>
</tr>
<tr>
<td>CA02-1</td>
<td>1</td>
<td>16.7</td>
<td>0.0146 ± 0.00002</td>
</tr>
<tr>
<td>CA02-2</td>
<td>2</td>
<td>30.2</td>
<td>0.0150 ± 0.00002</td>
</tr>
<tr>
<td>CA02-3</td>
<td>3</td>
<td>32.9</td>
<td>0.0145 ± 0.00002</td>
</tr>
<tr>
<td>CA02-5</td>
<td>5</td>
<td>34.2</td>
<td>0.0144 ± 0.00002</td>
</tr>
</tbody>
</table>

**Molecular Formulae containing only Carbon, Hydrogen and Oxygen**

3D-Van Krevelen diagrams of all compounds containing only carbon, hydrogen and oxygen for the three samples from Crooked Arm indicated molecular changes related to salinity (Figure 3.18). A detailed data analysis of the shared and unique molecular formulae revealed that approximately 80 % of these formulae were exactly the same for all salinity regions (Figure 3.19) and that 20 % changed (Figure 3.20) with increasing salinity. An increase in unsaturated compounds was only found between the freshwater source and the sample from the fjord surface water, but not for the high salinity sample at 5 m depth. A trend toward highly saturated compounds could be already observed in the fjord surface sample and suggested the potentially metastable character of the generated unsaturated compounds during estuarine mixing. The vertical mixing across
the LSL in Doubtful Sound is much slower compared to the estuarine mixing in the Freshwater River, Stewart Island due to the strong density differences and the resulting stratification within the LSL and the simultaneous formation and destruction of this potentially metastable aromatic compounds generated during estuarine mixing may explain the trends shown in Figure 3.20.

(a) CA02-freshwater stream, salinity: 0

(b) CA02-0, surface LSL, salinity: 14

(c) CA02-5, fjord at 5 m depth, salinity: 34

Figure 3.18: 3-D Van Krevelen diagrams for samples collected along a vertical salinity gradient in Crooked Arm (CA02), Doubtful Sound, October 2005
Figure 3.19: 3-D Van Krevelen diagrams of molecular formulae shared by all three Doubtful Sound samples, Crooked Arm (CA02)

(a) CA02-freshwater stream, salinity: 0
(b) CA02-0, fjord surface, salinity: 14
(c) CA02-5, fjord at 5 m depth, salinity: 34

Figure 3.20: 3-D Van Krevelen diagrams of the unique molecular formulae from (a) freshwater stream, (b) fjord surface water and (c) water from 5 m depth, Doubtful Sound, October 2005
Molecular Formulae containing Nitrogen

Similar, to ESI-FT-ICR-MS analysis of the Freshwater River, Stewart Island, molecular formulae containing only one nitrogen have been analysed in the present study. The ESI-FT-ICR-MS data measured for the freshwater stream in Doubtful Sound showed only 86 nitrogen-containing formulae and these compounds had high C/N ratios ranging from 21-30 (Figure 3.21), which were in the range expected for freshwater systems (Smith et al., 1991; Gordeev et al., 1996; Seitzinger and Sanders, 1997; Hopkinson et al., 1998; Stepanauskas et al., 1999; Stepanauskas et al., 2000). With increasing salinity, the number of nitrogen-containing formulae increased to n=127 in the fjord surface water (salinity: 14) and to n=549 in water at 5 m depth (salinity: 34). Additionally, four groups characterised by their C/N ratios appeared within the fjord surface water, which were not present in the freshwater stream (Figure 3.22).

The origin of these four distinct groups of nitrogen compounds is speculative, but it would seem to be related to processes which do not appear in the freshwater source. One such process might involve microbiological activity but the confirmation of such processes would require further evidence derived from the use of other techniques such as controlled incubation experiments with microorganisms. It was surprising that all nitrogen-containing formulae in this fjord surface water could be grouped on the basis of their narrow C/N ratios. The existence of low C/N ratio groups also suggested that these compounds might be degraded more quickly by microorganisms and may explain why this group was not present in the 5 m depth sample (CA02-5).

The nitrogen compounds at 5 m depth showed a much more uniform distribution in the van Krevelen diagram and just one distinct group of nitrogen compounds was apparent with a broad C/N ratio range (9-32). However, if the C/N ratio was plotted against the molecular mass, two distinct groups appeared, which were characterised by low C/N ratios of 8-12 and higher C/N ratios of 15-33 (Figure 3.23).

In general, groups with low C/N ratios appeared with increasing salinity. The mean C/N ratio in water samples collected in the open ocean tends to be around 14 (Hansell and Waterhouse, 1997; Ogawa et al., 1999; Hansell, 2000; Loh and Bauer, 2000), and hence these ratios are much lower compared to those reported for river water. In general, literature values supported a decrease in C/N ratios from the freshwater source toward the open ocean (Bronk, 2002).
Figure 3.21: (a) 3-D Van Krevelen diagram of nitrogen-containing molecular formulae and (b) mass-dependent C/N ratios and double bond equivalent (DBE) in the sample collected from the stream discharging into Crooked Arm, Doubtful Sound
Figure 3.22: (a) 3-D Van Krevelen diagram of nitrogen-containing formulae and (b) mass-dependent C/N ratios and double bond equivalent (DBE) derived from ESI-FT-ICR-MS data measured for water collected in the low salinity layer in Crooked Arm (CA02), Doubtful Sound, October 2005
Figure 3.23: (a) 3-D Van Krevelen diagram of nitrogen-containing formulae and (b) mass-dependent C/N ratios and double bond equivalent (DBE) derived from ESI-FT-ICR-MS data measured for water collected in the saline layer at 5 m depth in Crooked Arm (CA02), Doubtful Sound, October 2005.
Molecular formulae containing sulphur

The ESI-FT-ICR-MS results for the freshwater stream sample showed that 175 sulphur-containing molecular formulae were assigned (Figure 3.24) and that this number increased with increasing salinity (330 formulae (CA02-0) and 444 formulae (CA02-5)). Only two groups are apparent in the 3D-Van Krevelen diagram of the fjord surface water, one at low H/C ratios (0.8) and one at the double H/C ratio (1.6), but similar O/C ratios (Figure 3.25). At 5 m depth, all sulphur-containing formulae were located around H/C ratios 1.4 and O/C ratios of 0.5 (Figure 3.26). The C/S ratios did not change dramatically from sample to sample and ranged from 16-32.

Figure 3.24: (a) 3-D Van Krevelen diagram of sulphur-containing molecular formulae and (b) mass-dependent C/S ratios and double bond equivalent (DBE) in the sample collected from the stream discharging into Crooked Arm, Doubtful Sound
Figure 3.25: (a) 3-D Van Krevelen diagram of sulphur-containing molecular formulae and (b) mass dependent C/S ratios and double bond equivalent (DBE) derived from ESI-FT-ICR-MS data measured for water collected in the low salinity layer in Crooked Arm (CA02), Doubtful Sound, October 2005.
Figure 3.26: (a) 3-D Van Krevelen diagram of sulphur-containing molecular formulae and (b) mass-dependent C/S ratios and double bond equivalent (DBE) derived from ESI-FT-ICR-MS data measured for water collected in the saline layer at 5 m depth in Crooked Arm (CA02), Doubtful Sound, October 2005
3.4 Summary

The Freshwater River is characterised by a high load of CDOM, which is reflected in the high $a_{CDOM}(355)$ and EEM fluorescence levels. The $a_{CDOM}(355)$, EEM$_{total}$ and DOC measurements indicated conservative behaviour during mixing of the freshwater and the incoming seawater from Paterson Inlet. Furthermore, the influence of marine-derived CDOM was not apparent as indicated in stable spectral slope $S$ values across a broad salinity range.

In the estuarine mixing of the Freshwater River, Stewart Island, ESI-FT-ICR-MS results indicated that the unsaturation and aromaticity of 60% of the assigned molecular formulae containing just carbon, hydrogen and oxygen changed and assigned formulae showed a strong increase in aromaticity with increasing salinity. However, 40% of the C:H:O formulae remained the same during mixing. Additionally, the results from the lignin analysis supported the hypothesis that most lignin components stayed intact and hence make up part of the DOM pool which remained unaltered during this estuarine mixing. These results also suggest that the molecular changes occurred in compounds unrelated to lignin and with unknown molecular structures.

The results for ESI-FT-ICR-MS analysis suggested that assigned molecular formulae across the freshwater-seawater interface in samples from Doubtful Sound did not change as much as in samples during estuarine mixing in the Freshwater River, Stewart Island and 80% of the molecular formulae remained the same in all samples from Doubtful Sound.

In Doubtful Sound, the increase in aromaticity for C:H:O formulae was observed only between the freshwater stream and the fjord surface water, but not in the underlying saline layer sample (salinity: 34) at 5 m depth and indicated a labile aromatic fraction at the fjord surface and it seemed that potentially metastable aromatic compounds generated at the fjord surface may have already transformed into higher saturated compounds at 5 m depth.

Nitrogen- as well as sulphur-containing molecular formulae also indicated big differences in the transformation of terrestrial DOM in these two very different mixing environments. Nitrogen compounds appeared to be very labile in both sample locations. In the Freshwater River, Stewart Island, low C/N ratio nitrogen compounds disappeared during estuarine mixing, whereas in Doubtful Sound, a much more complex behaviour of nitrogen compounds was observed. Nevertheless, assigned nitrogen-containing molecular formulae found in the freshwater stream, at the fjord surface and
at 5 m depth in Doubtful Sound were all very different and also indicated the lability of these compounds.

Highly unsaturated sulphur-containing compounds disappeared along the Freshwater Estuary, Stewart Island, also indicating a labile fraction of the sulphur-containing DOM pool. In Doubtful Sound, the assigned sulphur-containing formulae were much more uniform, but additional compounds appeared with increasing salinity. This result would support the \textit{in situ} production of sulphur-containing compounds caused by marine biota. However, in the Freshwater Estuary, Stewart Island, \textit{in situ} production of sulphur-containing compounds could not be found, possibly due to the different mixing within the estuary and the high amount of terrestrially-derived DOM, which could easily obscure marine-derived sulphur compounds.

The vegetation of the two examined sample locations (Freshwater River, Stewart Island and Doubtful Sound) showed marked differences and may account for the differences seen in the molecular composition of the terrestrially-derived DOM. Furthermore, the more extreme pH change during estuarine mixing in the Freshwater River, Stewart Island compared to Doubtful Sound may also play a significant role.
Chapter 4

Photosensitivity of CDOM

4.1 Introduction

Photochemically-induced processes may well be the most important removal pathway of terrestrially-derived CDOM (Mopper and Kieber, 2002). Blough and Del Vecchio (2002) mentioned a loss in absorbance of up to 50% during 100 hours or more of simulated solar irradiation time in laboratory experiments, which they attributed to photochemical effects and these results were also in agreement with field studies (Vodacek et al., 1997; Nelson et al., 1998). Of these photochemical effects, photodegradation seemed to be the most important factor in influencing the biogeochemical cycling of chromophoric DOM (CDOM). Furthermore, the photodegradation of CDOM may also lead to the production of smaller molecules which may undergo further microbiological degradation. This aspect has been subject to varying opinions in the literature: some research groups have found that photodegradation of CDOM leads to products which can be consumed by bacteria (Kieber et al., 1989; Kieber et al., 1990; Wetzel et al., 1995), while others have reported exactly the opposite behaviour (Benner and Biddanda, 1998; Tranvik and Kokalj, 1998; Moran et al., 2000). This contradiction may be explained in part by differences in the molecular composition of the CDOM pool. The composition of CDOM is potentially highly dependent on the type of vegetation and the microbiological communities which are responsible for the degradation of plant material. Therefore, it is important to determine the molecular composition of CDOM and which fractions are influenced by solar-induced photodegradation in order to understand the role of this type of process in the overall biogeochemical cycle of CDOM.

ESI-FT-ICR-MS was applied in this study to reveal the molecular changes after
21 h of simulated solar irradiation. The strength of this ESI-FT-ICR-MS technique lies in the exact assignment of molecular masses and the comparison from the irradiated sample versus the untreated sample based on exact molecular masses and the changes associated with photodegradation. The molecular characterisation of the photochemically-influenced CDOM is a major goal in modern photobiogeochemistry and may help to understand the rather unique spectral properties of CDOM. Del Vecchio and Blough (2004) proposed that intramolecular electron donor-acceptor interactions are responsible for the photochemical changes in CDOM and that a superposition of independent chromophores cannot account for the observed photochemical alteration of CDOM. The present study aimed to supply additional information of the changes in the molecular composition during irradiation.

Furthermore, the time-dependence of the photodegradation was evaluated. The time-dependent photo-decay during irradiation should be dependent on the photolabile fraction of the CDOM pool, which means that with increasing radiation time less CDOM is photobleached. An exponential fitting procedure suggested a 1st order exponential decay function and hence the slope of the decay function would be an experimental measure for the photolability of CDOM (i.e. decrease in $a_{CDOM}(\lambda)$ of CDOM over time).

Additional irradiation experiments were undertaken to evaluate the effect of salinity on CDOM photodegradation and for the first time, ESI-FT-ICR-MS was used to observe the effect of different salinities on the changes of exact molecular masses during irradiation. A new index was also developed to quantify the extent of photolability of CDOM.

### 4.2 Materials and Methods

Water samples were collected from the Black River (sample station BR, salinity: 0), North Carolina, USA and from the Cape Fear River Estuary (sample station HB, salinity: 12.3 and M61, salinity: 13.7) into which the Black River discharges via the Cape Fear River. One litre from each sample was irradiated for 21 h using a 1000 W solar simulator. DOC levels, TDN levels, EEM fluorescence and UV/Vis absorbance were determined for each sample before and after irradiation. Additionally, samples taken from station BR and M61 were analysed using ESI-FT-ICR-MS. Sample preparation and settings for the ESI-FT-ICR-MS measurements were similar to those described in Chapter 3, but the solid-phase extracted water volume for each sample was only
approx. 500 mL.

Additional samples were collected in Doubtful Sound, January 2006 and along a salinity gradient in the Freshwater River Estuary in Stewart Island, February 2006. Time-dependent photodegradation studies were undertaken using UV/Vis measurements of samples collected from Doubtful Sound: CA02-riv (freshwater river), CA02-0 (fjord surface water, salinity: 10.3) and CA02-1 (fjord water from 1 m depth, salinity: 16.7), DS-stream (high CDOM stream water, collected close to Deep Cove), soil pore-water (collected within the catchment of the Crooked Arm river) and the Freshwater River Estuary, Stewart Island (FWR01, salinity: 0; FWR02, salinity: 5.3 and FWR03, salinity: 12.7). These samples were irradiated using a 500 W solar simulator (SCI-ENCETECH) and a xenon arc lamp. The UV/Vis absorbance was measured every 8-12 h during the radiation time of 60 h.

The extent of photodegradation was quantified using the relative decrease in the absorption coefficient (∆λ = (aₖ,λ/a₀,λ)*100), where aₖ is the absorption coefficient at a specific wavelength.

4.3 Results and Discussion

After 21 h irradiation, the relative decrease in the absorption coefficients of the Black River (BR) sample (freshwater end-member) was less compared to the Cape Fear River Estuary samples (HB, salinity: 12.1 and M61, salinity: 13.7) (Figure 4.1 and Table 4.2), indicating photobleaching in both samples, but a more pronounced photodegradation in the Cape Fear River Estuary samples (higher salinity).

The total EEM fluorescence (EEM_{total}) for the Black River samples decreased upon irradiation by 20% and for the Cape Fear River Estuary samples by approximately 50% (sample HB) and 40% (sample M61) (Table 4.2). Hence, the relative decrease in the EEM fluorescence was much more compared to the relative change in the UV/Vis absorbance. The relative decrease in the EEM fluorescence was more pronounced for the more saline samples analogous to the absorbance results.

The DOC and TDN values did not decrease during irradiation for either the Black River and Cape Fear River Estuary samples. The absorption coefficient at 355 nm (a_{CDOM,355}), EEM fluorescence and the DOC and TDN levels before and after the 21 h irradiation time are summarised in Table 4.1 and the relative decreases for these parameters are listed in Table 4.2.
Figure 4.1: Relative decrease in $a_{CDOM}(\lambda)$ after 21 h solar irradiation, Cape Fear River Estuary (sample HB and M61) and the Black River (sample BR), North Carolina, USA, April 2006.

For the irradiation time of 21 h, the transformation of photo-labile CDOM into other organic compounds was more likely compared to mineralisation to form CO and CO$_2$ as suggested in the abiotic pathway published by Mopper and Kieber (2002). However, some contamination during the irradiation period may have occurred as indicated by the slight increase in DOC and TDN levels and may have obscured changes in DOC and TDN potentially caused by mineralisation. It is easy to contaminate samples with DOC and in the present study samples were exposed for 21 h not only to the simulated solar sunlight but also to the surrounding air.
Table 4.1: $a_{\text{CDOM}}$355, EEM fluorescence, DOC and TDN levels before and after 21 h irradiation time of samples from the Black River (sample BR) and Cape Fear River Estuary (sample HB and M61), April 2006

<table>
<thead>
<tr>
<th>sample ID</th>
<th>$a_{\text{CDOM}}$355 (m$^{-1}$)</th>
<th>DOC ($\mu$M C)</th>
<th>TDN ($\mu$M N)</th>
<th>EEM$_{\text{total}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>before irradiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR</td>
<td>32.10</td>
<td>930±21</td>
<td>56 ± 1.0</td>
<td>3168592</td>
</tr>
<tr>
<td>HB</td>
<td>20.20</td>
<td>769±22</td>
<td>69 ± 0.5</td>
<td>2543501</td>
</tr>
<tr>
<td>M61</td>
<td>16.32</td>
<td>630±4</td>
<td>52 ± 0.4</td>
<td>2025070</td>
</tr>
<tr>
<td>after irradiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BR</td>
<td>31.98</td>
<td>965±8</td>
<td>62 ± 0.3</td>
<td>2557021</td>
</tr>
<tr>
<td>HB</td>
<td>18.11</td>
<td>774±7</td>
<td>72 ± 0.5</td>
<td>1701701</td>
</tr>
<tr>
<td>M61</td>
<td>14.81</td>
<td>651±4</td>
<td>57 ± 0.2</td>
<td>1448700</td>
</tr>
</tbody>
</table>

Table 4.2: Relative decrease in $a_{\text{CDOM}}$355, EEM$_{\text{total}}$, DOC and TDN after 21 h irradiation, Black River and Cape Fear River Estuary, April 2006

<table>
<thead>
<tr>
<th>decrease in:</th>
<th>Black river (BR)</th>
<th>Cape Fear (HB)</th>
<th>Cape Fear (M61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_{\text{CDOM}}$355 (m$^{-1}$)</td>
<td>0.4 %</td>
<td>11.0 %</td>
<td>9.3 %</td>
</tr>
<tr>
<td>EEM$_{\text{total}}$</td>
<td>23.9 %</td>
<td>49.5 %</td>
<td>39.8 %</td>
</tr>
<tr>
<td>DOC ($\mu$M C)</td>
<td>-3.6 %</td>
<td>-0.7 %</td>
<td>-3.3 %</td>
</tr>
<tr>
<td>TDN ($\mu$M N)</td>
<td>-10.7 %</td>
<td>-4.3 %</td>
<td>-9.6 %</td>
</tr>
</tbody>
</table>

4.3.1 Photobleaching induced Molecular Changes in DOM

In an earlier study, the ESI-FT-ICR-MS analysis of the Suwannee River Fulvic Acid standard (SRFA) revealed substantial changes in the mass spectrum after polychromatic irradiation and demonstrated the utility of ESI-FT-ICR-MS in evaluating molecular changes during photodegradation experiments (Kujawinski et al., 2004). In the present study, the effect of a 21 h irradiation experiment on two samples from the Cape Fear River basin (M61, salinity: 13.6 and BR, freshwater end-member) were examined using ESI-FT-ICR-MS. The assigned molecular formulae obtained from the ESI-FT-ICR-MS analysis were divided into three different sets:

- molecular formulae containing only carbon, hydrogen and oxygen (C:H:O)
- molecular formulae containing carbon, hydrogen, oxygen and sulphur (C:H:O:S)
- molecular formulae containing carbon, hydrogen, oxygen and nitrogen (C:H:O:N)
This separation into different groups of molecular formulae simplified the data analysis and enabled the evaluation of changes in the molecular formulae related to not just C:H:O compounds, but also to sulphur and nitrogen-containing compounds. No molecular formulae containing both, sulphur and nitrogen were assigned for masses apparent in the mass spectra.

**Irradiation-induced changes in C:H:O molecular formulae**

The 3D-Van Krevelen diagrams for the assigned C:H:O formulae in the untreated and irradiated samples showed substantial changes after a 21 h irradiation experiment (Figure 4.2). Additionally, the higher saline sample (M61) compared to the freshwater sample (BR) was much more affected by the polychromatic irradiation. The irradiation of the freshwater end-member sample (BR) led to an overall decrease in the number of assigned molecular formulae. The oxygen-subtracted double bond equivalent (DBE-O) of these compounds that disappeared ranged from -5 to 9, with the majority of formulae lost in the high DBE-O range (highly unsaturated compounds). After irradiation, new formulae appeared characterised by low DBE-O values (more saturated compounds) (Figure 4.3). This change of highly unsaturated compounds towards saturated compounds during solar irradiation was much more pronounced in the more saline sample from the Cape Fear River Estuary (M61, salinity: 13.6). Clearly, highly unsaturated species were destroyed during solar irradiation to be replaced by much more saturated molecules. However, the newly assigned molecular formulae in the irradiated samples could not entirely account for the lost formulae (e.g. sample M61, lost formulae: 1800 and gained formulae: 800), suggesting a removal or at least a transformation of molecules, which are no longer easily ionise in the electrospray. Additionally, the molecular formulae assigned for the more saline M61 sample initially showed much more highly unsaturated compounds compared to the freshwater end-member sample of the Black River (BR) (Figure 4.4). This observation is in agreement with a trend of increasing aromaticity with increasing salinity during estuarine mixing observed in the Freshwater River Estuary on Stewart Island, NZ (see Chapter 3). Similar to the stable $S$ values shown for the samples taken along the Freshwater River, Stewart Island, the $S$ values of the samples collected in the Cape Fear River did not change significantly either (sample BR: $S = 0.0151$; sample M61: $S = 0.0158$) indicating little influence of marine-derived CDOM.
Figure 4.2: 3D-Van Krevelen diagrams (DBE-O) of the Black River (BR, freshwater end-member) and Cape Fear River (M61, salinity: 13.6) sample before and after 21 h solar irradiation.
Figure 4.3: 3D-Van Krevelen diagrams (DBE-O): The effect of photodegradation (21 h irradiation) on the Black River (BR, freshwater end-member) and Cape Fear River Estuary (M61, salinity: 13.6) sample
Figure 4.4: Kendrick plots for $z^* = -14$: Black River (BR, freshwater end-member) and Cape Fear River (M61, salinity: 13.6) sample before and after 21 h solar irradiation.
Irradiation-induced changes in the sulphur-containing molecular formulae C:H:O:S

No molecular formulae containing more than one sulphur atom were apparent from analysis of the mass spectra. There were far fewer molecular formulae containing a sulphur atom than there were C:H:O masses. The sulphur-containing formulae for both samples (BR and M61) were strongly affected by solar irradiation (Figure 4.5) and sulphur-containing formulae assigned to the irradiated samples showed an increase in aromaticity, which was in contrast to the behaviour of C:H:O formulae discussed above, where the opposite behaviour was observed. Besides the trend towards higher aromaticity, the majority of the sulphur-containing formulae disappeared during irradiation, suggesting photochemically-induced changes in the molecular composition of these sulphur species. The increase in more aromatic or at least more unsaturated character was surprising and suggested a chemical reduction of compounds rather than their expected photochemical oxidation. A more detailed analysis of the sulphur-containing compounds that were unaltered, destroyed or created is given Figure 4.6 and Figure 4.7 and shows that just a small core fraction of sulphur-containing compounds remained unaltered. Different photochemically-induced degradation pathways may explain the observed decrease of sulphur-containing formulae during irradiation. Firstly, sulphur-containing compounds may get converted in compounds which do not easily ionise in the electrospray, hence do not appear in the mass spectrum. Secondly, sulphur-containing compounds are mineralised into inorganic sulphur compounds (e.g. DMS) and thirdly, a variety of sulphur-containing compounds may be converted into sulphur-containing compounds which have the same molecular formula.
Figure 4.5: 3D-Van Krevelen diagrams (DBE-O) of sulphur-containing masses of the Black River (BR, freshwater end-member) and Cape Fear River (M61, salinity: 13.6) sample before and after 21 h solar irradiation
Figure 4.6: 3D-Van Krevelen diagrams (DBE-O): The effect of photodegradation on sulphur-containing formulae assigned for the Black River sample
Figure 4.7: 3D-Van Krevelen diagrams (DBE-O): The effect of photodegradation on sulphur-containing formulae assigned for the Cape Fear River Estuary sample (M61)
Irradiation induced changes in the nitrogen-containing formulae C:H:O:N

Only molecular formulae containing one nitrogen atom have been considered in the present study. The Black river sample showed many more nitrogen-containing compounds compared to the sample from the Cape Fear River Estuary. The nitrogen-containing compounds were also strongly influenced by the 21 h irradiation experiment (Figure 4.8). During irradiation of the Black River sample, a majority of the nitrogen-containing compounds were converted from lower H/C ratios (H/C $\leq 1$) towards higher ratios ((H/C $\geq 1$), whereas the O/C ratios were not affected (Figure 4.9). This trend would also lead to more saturated compounds. Another possible explanation would be the saturation of carbon-carbon or carbon-nitrogen double bonds induced by irradiation. The trend observed during irradiation of the Cape Fear River Estuary sample was slightly different and showed a clear shift from unsaturated compounds towards more saturated compounds in both the H/C and the O/C ratios. This observation was also apparent in the change of the DBE-O values from values ranging between 5-10 before irradiation towards values in the range 2 to (-3) after irradiation (Figure 4.10).
Figure 4.8: 3D-Van Krevelen diagrams (DBE-O) of nitrogen-containing masses of the Black River (BR, freshwater end-member) and Cape Fear River (M61, salinity: 13.6) sample before and after 21 h solar irradiation
Figure 4.9: 3D-Van Krevelen diagrams (DBE-O): The effect of photodegradation on nitrogen-containing molecular formulae assigned for the Black River sample
Figure 4.10: 3D-Van Krevelen diagrams (DBE-O): The effect of photodegradation on nitrogen-containing molecular formulae assigned for the Cape Fear River Estuary sample (M61)
4.3.2 The Photo Lability Index

Photodegradation is potentially compound-specific and therefore any compound which can undergo photochemical reactions should have a different photodegradation rate constant. Therefore, the rate of the overall photodegradation or the photolability of CDOM depends on the composition of all CDOM compounds that are sensitive to photochemical reactions. This assumption is based on distinct chromophores, but Del Vecchio and Blough (2004) hypothesised that photobleaching of CDOM cannot be explained by the photodegradation of independent chromophores, but instead it is controlled by intramolecular electron-transfer processes between hydroxy/phenolic-aromatic and quinoid functional groups. Either way, it is not possible to quantify compound-specific and/or charge-transfer-related processes, because the molecular structures of individual CDOM compounds are largely unknown. In the present study, a new parameter: the Photo Lability Index (PLI) is introduced to quantify the time-dependent photobleaching behaviour of CDOM.

The observed decreases in the absorption coefficient at a specific wavelength ($a_{CDOM}(\lambda)$) could be satisfactorily fitted to a first order decay function (Eqn. 4.1) and the resulting slope ($k_{decay}$) of this function was used to describe the time dependent photo-induced decay for a given sample. $R^2$ was greater than 0.998 for wavelengths ranging from 280 to 500 nm. This function has also been successfully used in a previous study for modelling photobleaching of CDOM (Del Vecchio and Blough, 2002).

$$a(\lambda) = a_0 + K_{decay} \times exp(t \times \lambda)$$ (4.1)

with:

- $a(\lambda) =$ absorption coefficient at wavelength $\lambda$
- $a_0 =$ offset
- $K_{decay} =$ slope of the decay function

The slope of this function is indicative of the decay kinetics of CDOM and is used in the present study to evaluate the photolability of CDOM. An increase in the slope would result in an enhanced photo-decay, hence this CDOM must be more photolabile. However, it should be noted that it is difficult to directly compare samples with different initial absorption coefficient values, if the absorbance measurements are not carried out in optically thin layers. In optically thin layers, the differences in the initial $a_{CDOM}(\lambda)$ values should be negligible (Del Vecchio and Blough, 2002). However, dilution experiments of a water sample from a stream in Doubtful Sound (DS-stream) indicated that
the pathlengths of the 5 cm cuvettes used in the present study cannot be considered as optically thin and results showed that with increasing initial \( a_{CDOM}(\lambda) \) values, the relative decrease in \( a_{CDOM}(\lambda) \) does decrease (Figure 4.11). Hence the photo-decay slope \( (k_{decay}) \) is highly dependent on the concentration of absorbing compounds in the irradiated samples.

Figure 4.11: Dilution dependency of the decrease of \( a \) during 60 h solar irradiation for a sample taken from a stream in Doubtful Sound, May 2006

However, experimental results showed that the slope decreased linearly with decreasing initial \( a_{CDOM}(\lambda) \) values (Figure 4.12). Based on this linear correlation between dilution factor and \( k_{decay} \), the Photo Lability Index (PLI) is developed in the present study, where \( k_{decay} \), calculated between 360 and 370 nm at 1 nm intervals, is divided by the initial \( a_{CDOM}(\lambda) \) at the same wavelength. The median from the ten calculated \( k_{decay}/a_{CDOM}(\lambda) \) ratios (for each wavelength between 360 and 370 nm) was determined to obtain a more robust measure for PLI (see Eqn. 4.2).

\[
PLI = \text{median} \sum_{\lambda=360}^{370} K_{decay}(\lambda)/a_{CDOM}(\lambda) \tag{4.2}
\]

with:

- PLI = Photo Lability Index
- \( K_{decay}(\lambda) \) = slope of the decay function measured at wavelengths between 360 and 370 nm corresponding to \( a_{CDOM}(\lambda) \)
- \( a_{CDOM}(\lambda) \) = initial absorption coefficient at a wavelength between 360 and 370 nm measured at 5 nm intervals
Figure 4.12: Dilution dependency of the decrease in the photo-decay slope during 50 h solar irradiation for a sample collected from a stream in Doubtful Sound, May 2006

There appeared to be a wavelength-dependency of the PLI, but these changes occurred over a broad wavelength range and therefore changes over the wavelength range between 360 and 370 nm were negligible and justified the use of the median value for the calculation of the PLI at this wavelength range (Figure 4.13). The PLI is a simple experimentally determined parameter, which is entirely based on the slope of the decrease in the absorption coefficients during solar irradiation and the initial absorption coefficient values and does not take into account any parameter that describes the light intensities, which unfortunately makes it difficult to compare to samples irradiated with a different light source.

In Doubtful Sound, a river and a stream discharging into the fjord were examined (samples: CA02-riv and DS-stream) and also a soil porewater sample, which was collected using a home-built vacuum tensiometer apparatus in less than 24 hours within the catchment for the river discharging into Crooked Arm. Additionally, the PLI for samples from the Freshwater River Estuary in Stewart Island were measured (sample: FWR01, FWR02 and FWR03). The PLI determined for the soil pore water showed a significantly lower value compared to all other samples. A summary of all calculated PLI values is given in Table 4.3.
Figure 4.13: PLI values calculated at each wavelength between 360 and 370 nm for a river sample (CA02-riv) and a soilpore water sample, Doubtful Sound, New Zealand, May 2006

Table 4.3: The Photo Lability Index (PLI) for samples collected in Doubtful Sound in May 2006 and in the Freshwater River Estuary, Stewart Island in September 2006

<table>
<thead>
<tr>
<th>sample</th>
<th>PLI</th>
<th>stdev</th>
<th>salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA02-0, Doubtful Sound</td>
<td>0.63</td>
<td>0.05</td>
<td>16.64</td>
</tr>
<tr>
<td>CA-riv, Doubtful Sound</td>
<td>0.58</td>
<td>0.02</td>
<td>0</td>
</tr>
<tr>
<td>DS-stream, Doubtful Sound</td>
<td>0.52</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>soil pore water, Doubtful Sound</td>
<td>0.37</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>FWR01, Stewart Island</td>
<td>0.61</td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td>FWR02, Stewart Island</td>
<td>0.57</td>
<td>0.02</td>
<td>5.25</td>
</tr>
<tr>
<td>FWR03, Stewart Island</td>
<td>0.58</td>
<td>0.03</td>
<td>12.72</td>
</tr>
</tbody>
</table>

4.3.3 Salinity Effects on the Photodegradation of CDOM

During estuarine mixing, parameters such as salinity, pH, ionic strength and also the bacterial communities can change dramatically and these changes in the physical, chemical and biological properties of the water can have synergistic effects on photodegradation of the complex mixture of CDOM. However, there were no conclusive differences found in the PLI values observed in the present study which could be related to salinity. Nevertheless, the PLI provides a measure of the overall photobleaching ability, which does not account for potential wavelength-dependent differences in photodegradation, which may occur over a broad wavelength range during solar irradiation of samples at different salinities.
Samples from the Freshwater River Estuary, Stewart Island (FWR01, salinity: 0; FWR02, salinity: 5.3 and FWR03, salinity: 12.7) and from Doubtful Sound (DS02-R, salinity: 0; DS02-0, salinity: 16.6 and DS02-1, salinity: 20.9) were irradiated, but this time the wavelength-dependent changes between freshwater and saline samples have been evaluated. The relative decrease in $a_{CDOM}(\lambda)$ showed that with increasing salinity, the CDOM absorbing UV light (300-400 nm) was more stable to irradiation, whereas at longer wavelengths ($\geq 400$ nm), the photodegradation was enhanced with increasing salinity. This effect seemed to be independent of the initial $a_{CDOM}(\lambda)$ values (Figure 4.14). The lower photodegradation in the UV range apparent with increased salinity has been reported in a previous study (Minor et al., 2006), but the range of wavelengths $\geq 400$ nm was not specified. These authors concluded that the lower photodegradation in the UV range with increasing salinity may due to changes in the conformation of DOM or changes in iron-DOM photochemistry. However, they did not undertake a comparison of the salinity-dependent photodegradation between the UV range and longer wavelengths, but this comparison was evaluated in the present study, which will be discussed below.

Irradiation time did affect the wavelength-dependent photodegradation. After irradiation of samples from the Black River and Cape Fear River Estuary for 21 h, the photodegradation induced by UV light was relatively extensive for the more saline samples if compared to the photodegradation for wavelengths longer than 400 nm (sample station HB and M61, see Figure 4.1). In contrast, samples which have been irradiated for 60 h showed much more pronounced differences in the relative decrease of $a_{CDOM}$ between the UV range (280-400 nm) and wavelengths longer than 400 nm. One explanation could be that some compounds absorbing in the UV range are rapidly ($\leq 21$ h) photodegraded, whereas other compounds of this UV-absorbing CDOM are more stable in saline water. To confirm a time-dependent photodegradation, the ratio of $a_{CDOM}(315 \text{ nm})$ to $a_{CDOM}(455 \text{ nm})$ ($a_{315}/a_{455}$ ratio) was calculated at different times during irradiation and the results suggested that this ratio steadily increased for all saline samples, whereas for all freshwater samples, this ratio initially decreased during the first 5 hours of irradiation and then remained constant for the remainder of the irradiation (Figure 4.15). Clearly, irradiation time is an important factor in the wavelength-dependent photodegradation of CDOM and salinity does have a significant influence on the photodegradation behaviour. For saline water samples, the photodegradation of compounds absorbing at wavelengths longer than 400 nm compared to UV-absorbing compounds also increases with increasing irradiation time.

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Figure 4.14: Decrease in $a_{CDOM}(\lambda)$ after 56 h of solar irradiation of samples at different salinities from the Freshwater River, Stewart Island (FWR1-3) and Doubtful Sound (DS02-R, DS02-0 and DS02-1)
These results suggest that in saline water, the photodegradation of compounds absorbing at longer wavelengths ($\geq 400$ nm) is faster compared to UV-absorbing compounds. In the first hours of irradiation, some UV-absorbing compounds in freshwater were photobleached more rapidly compared to compounds absorbing at longer wavelengths, but after this initial decrease a constant photodegradation rate was observed for UV radiation compared to wavelengths longer than 400 nm.

Figure 4.15: The time-dependent photodegradation illustrated by using the $a_{315}/a_{455}$ ratio for saline and freshwater samples from Doubtful Sound, May 2006 and the Freshwater River estuary, Stewart Island, September 2006

4.4 Summary

The present study confirms that photodegradation provides an important pathway leading to structural changes in the molecular composition of CDOM and that CDOM from different sources, show different rates of photodegradation mostly dependent on the initial absorption coefficients. High initial $a_{CDOM}(\lambda)$ values lead to a reduced relative decrease compared to CDOM with lower $a_{CDOM}(\lambda)$ values. Hence, high CDOM levels in rivers would protect the underlaying water from photodegradation. Photodegradation would just occur within an upper thin surface layer. However, as soon as this water with high CDOM content is diluted in coastal zones, photodegradation would be enhanced. This finding is in agreement with the literature, where it has been hypothesised that the dilution of riverine CDOM in coastal zones would lead to enhanced photobleaching due to a higher penetration of solar irradiation into the water.
Fluorescence decreased more extensively compared to the absorbance during irradiation time suggesting a rapid photodegradation of particularly sensitive fluorescent compounds. In a previous study, Del Vecchio and Blough (2002) also reported a faster decrease in the fluorescence compared to the absorbance and attributed this observation to the presence of fluorescent chromophores sensitive to photochemical degradation.

Ideally, freshwater and saline samples should have the same initial absorption coefficients in order to better measure the relative rates of photodegradation, but this situation does not generally occur for natural samples. However, the photodegradation behaviour of CDOM in several samples ranging from freshwater to more saline conditions and with different initial $a_{CDOM}(\lambda)$ values, was successfully modeled in the present study using a first order exponential decay function. The photo-decay slope ($k_{decay}$) calculated from this decay function is a measure for the decrease in $a_{CDOM}(\lambda)$ and confirmed that photodegradation decreased with irradiation time in agreement with previous studies (Vodacek et al., 1997; Gao and Zepp, 1998; Moran and Zepp, 2000). Additionally, a new Photo Lability Index (PLI) was developed in the present study, which is independent of the values of the initial absorption coefficient values. Only preliminary values for the PLI were determined in the present study but these values showed that differences occur in this new parameter at least between soil pore water and water collected in rivers and estuaries. These results suggest that soil porewater is less photo-labile compared to river water, which was interesting. One might expect that the sunlight-protected soil water would be more quickly photodegraded compared to river water, because the most photo-labile components of the CDOM have not been exposed to sunlight, but that did not seem to be the case at least for the present samples. More extensive investigations of the PLI parameter are required using samples of different origin. At present, the PLI is highly dependent on the intensity of the irradiation and an extended version of the PLI could include a normalisation to allow for different light levels which would enable the comparison of results obtained under different light regimes.

Wavelength-dependent changes during irradiation time can be also evaluated regardless of the initial $a_{CDOM}(\lambda)$ values, because differences in the initial absorption coefficients do not change the overall wavelength-dependent photodegradation within the wavelength range of 280-500 nm. Compounds absorbing at wavelengths in the UV range appeared to be more stable in saline water while those absorbing at $\geq 400$ nm were less stable in saline water compared to freshwater. The comparison of the
$a_{315}/a_{455}$ ratio between freshwater and saline water samples for different periods of irradiation showed that the wavelength-dependent photodegradation was also dependent on irradiation time suggesting a change in the chromophores during irradiation. In freshwater, a rapid decrease occurred in the UV-absorbing compounds for $\leq 5$ h irradiation, whereas after this initial enhanced photodegradation, compounds absorbing shorter wavelengths (315 nm) compared to longer wavelengths (455 nm) seem to behave in the same manner. In contrast, the $a_{315}/a_{455}$ ratios of saline samples during irradiation were constantly increasing suggesting an increase in the photodegradation of compounds at greater wavelengths ($\geq 400$ nm) and/or a decrease in photodegradation of the UV-absorbing compounds.

Salinity or processes associated with it clearly play an important role in changing the photodegradation behaviour of CDOM and therefore more detailed studies should be undertaken to investigate the photodegradation of samples across other freshwater-seawater interfaces.

ESI-FT-ICR-MS analysis confirmed that solar irradiation has a significant effect on the molecular composition of DOM. During estuarine mixing, a production of highly photolabile (metastable) compounds is suggested in the present study (see also Chapter 3). Additionally, results confirmed that independent unsaturated compounds were altered and converted to more saturated species. Hence, the proposed photo-induced electron charge-transfer model by Del Vecchio and Blough (2004) cannot entirely account for this observation. Therefore, we propose a combination of effects associated with both individual chromophores and charge-transfer processes. There is clearly no single molecular explanation for photochemical alteration of CDOM and further research is needed to distinguish photochemical reactions either associated with potential intramolecular charge-transfer processes or changes of individual chromophores. These results confirmed that salinity plays an important role in photodegradation of CDOM and that molecular changes occurring during estuarine mixing and increasing salinity are highly underestimated in the present literature in terms of photochemical reactions of CDOM.
Chapter 5

The Influence of CDOM on Singlet Oxygen Production in Natural Waters

5.1 Introduction

The solar irradiation of any natural water can lead to the formation of a number of transient chemical species particularly involving oxygen. One of these reactive oxygen species (ROS) is excited state singlet oxygen (\( ^1\text{O}_2 \)) formed by excitation of ground triplet state oxygen (\( ^3\text{O}_2 \)). It has been shown that singlet oxygen can be formed by the absorption of light at 1269 nm, which corresponds to an energy of 92 kJ mol\(^{-1}\) (Herzberg, 1950). However, there is little radiation of this wavelength reaching the Earth’s surface, because most infrared (IR) radiation is scavenged by gas molecules such as CO\(_2\), H\(_2\)O or CH\(_4\) in the atmosphere (Coyle et al., 1982). Hence, direct photoexcitation is unlikely to form \( ^1\text{O}_2 \).

Instead, \( ^1\text{O}_2 \) is formed indirectly by the initial absorption of light by a sensitizer (Sens) in natural waters (Allen et al., 1996). Many organic molecules have been shown to function as a sensitizer (Gellerstedt and Pettersson, 1975; Crestini and D’Auria, 1996). Organic molecules function as sensitizers by absorbing light at much shorter wavelengths and then undergoing intersystem crossing (ISC) from the singlet excited state to a triplet excited state (see Eqn. 5.1).

\[
\text{Sens} + h\nu \rightarrow (\text{Sens}^*)^1 \xrightarrow{\text{ISC}} (\text{Sens}^*)^3 + ^3\text{O}_2 \rightarrow ^1\text{O}_2 + \text{Sens} \tag{5.1}
\]

The resulting \( ^1\text{O}_2 \) can decay back to \( ^3\text{O}_2 \) by physical quenching with H\(_2\)O releas-
ing energy as heat, or it can react with an organic acceptor molecule (A) leading to photooxidation:

\[ ^1\text{O}_2 + \text{H}_2\text{O} \rightarrow ^3\text{O}_2 + \text{H}_2\text{O} \quad (5.2) \]

\[ ^1\text{O}_2 + \text{A} \rightarrow \text{AO}_2 \quad (5.3) \]

The lifetime of \(^1\text{O}_2\) is very short in water and Schmidt and Afshari (1990) reported it to be less than 4.4 µs. Hence steady state concentrations of \(^1\text{O}_2\) ([\(^1\text{O}_2\)]\(_{ss}\)) are typically very low in water (10\(^{-14}\) M) and difficult to measure directly. Indirect methods have been developed to measure the [\(^1\text{O}_2\)]\(_{ss}\) using either 2,5 dimethylfuran (DMF) (Zepp et al., 1977) or furfuryl alcohol (FFA) (Haag et al., 1984) as trapping agents.

The absorption of light by CDOM leads to excited singlet state organic molecules (\(^1\text{CDOM}^*\)), which can return to the ground state by emission of light as fluorescence or relaxing via a radiationless heat transfer. Additionally, \(^1\text{CDOM}^*\) can follow the mechanism given in Eqn. 5.1 resulting in an intersystem crossing to form the triplet \(^3\text{CDOM}^*\) and finally undergo an energy transfer to ground-state \(^3\text{O}_2\) to form \(^1\text{O}_2\) (see mechanisms in Eqn. 5.4). In this way, CDOM can act as a sensitizer for the formation of \(^1\text{O}_2\) (Zepp et al., 1985).

\[ ^1\text{CDOM} \xrightarrow{hv} ^1\text{CDOM}^* \quad (5.4) \]
\[ ^1\text{CDOM}^* \xrightarrow{fluorescence} ^1\text{CDOM} \]
\[ ^1\text{CDOM}^* \xrightarrow{ISC} ^3\text{CDOM} \]
\[ ^3\text{CDOM} + ^3\text{O}_2 \rightarrow ^1\text{O}_2 + ^1\text{CDOM} \]

The involvement of \(^1\text{O}_2\) in the photooxidation of its sensitizer resulting in the destruction of CDOM (see Eqn. 5.3) is suggested, but has not been confirmed (Sandvik et al., 2000). However, Gellerstedt and Pettersson (1975) reported the formation of intramolecular endoperoxides triggered by singlet oxygen. These endoperoxides are unstable and undergo a carbon-carbon bond cleavage. Different phenolic cleavage products have been reported for the aromatic ring opening caused by the \(^1\text{O}_2\) endoperoxide formation (Vanucci et al., 1988; Castellan et al., 1989; Castellan et al., 1991). Furthermore, Crestini and D’Auria (1996) were able to show that for specific model compounds the sunlight-induced photodegradation would not occur in the absence of \(^1\text{O}_2\). Hence, these results suggest that \(^1\text{O}_2\) may play a role in CDOM photooxidation.

The first measurements of \(^1\text{O}_2\) were reported by Zepp et al. (1977) for samples in the south-eastern US and demonstrated that beside sunlight intensities, DOC levels affected \(^1\text{O}_2\) production. Since that time, many other publications have supported
the correlation of DOC levels and $^{1}\text{O}_2$ production at a constant light intensity (Wolff et al., 1981; Haag and Hoigne, 1986; Shao et al., 1994; Sandvik et al., 2000).

However, the level of DOC is not an ideal predictor for $[^1\text{O}_2]_{ss}$, because it does not contain information about the efficiency of the DOM to absorb light. Therefore, it is hypothesised that absorbance and fluorescence measurements of CDOM are much more valuable in predicting the $[^1\text{O}_2]_{ss}$ in natural water.

The aim of this study was to investigate the ability of the light-absorbing CDOM, characterised by UV/Vis absorbance and EEM fluorescence measurements, to function as a sensitizer for $^{1}\text{O}_2$ production and to support the hypothesis of fluorescence being a much better predictor of $[^1\text{O}_2]_{ss}$ compared to DOC levels. Also, the production of $^{1}\text{O}_2$ has never been reported before in New Zealand waters and the wide range of levels in absorbance and excitation emission matrix fluorescence in Doubtful Sound, Fiordland and within the Freshwater River Estuary in Stewart Island are ideal to study the relation of UV/Vis absorbance and EEM fluorescence measurements to $[^1\text{O}_2]_{ss}$.

5.2 Materials and Methods

Surface water samples were collected in October 2005 at 15 study sites, Doubtful Sound, Fiordland. The sample stations and the sampling procedure have been previously discussed in Chapter 2. Additional to the samples from Doubtful Sound, four samples along a salinity gradient were collected within the Freshwater River Estuary, Stewart Island. The location of these sample stations in Stewart Island and further information are given in Chapter 3. Briefly, samples were collected in acid-cleaned 500 ml amber bottles and then filtered through Millipore GV 0.22 µm filters to remove particulate material. The filtered samples were stored at 4 °C in a refrigerator prior to analysis.

Measurements of Singlet Oxygen Steady-State Concentrations

The lifetime of $^{1}\text{O}_2$ in water is 4.4 µs (Schmidt and Afshari, 1990) and therefore a trap for $^{1}\text{O}_2$ has to be used to determine the steady-state concentrations. In the present study, furfuryl alcohol (FFA) was used as the trapping reagent for $^{1}\text{O}_2$ using the method described in detail by Haag et al. (1984).

In a quartz tube, 1 mL FFA stock solution ($10^{-2}$ mol/L) was added to 99 mL of a sample of filtered natural water to achieve a final FFA concentration of $10^{-4}$ mol/L. The sample was then irradiated for 3 h using the Scienctech solar simulator equipped with a 500 watt Xenon arc lamp. Before and after irradiation, the FFA concentration was
determined using C-18 reversed-phase column and a GBC HPLC system. The mobile phase contained 40 % HPLC-grade methanol in Milli-Q water and was run isocratically at a flow rate of 1 ml/min. FFA was detected using a Jasco HPLC UV/Vis detector set to 220 nm. The decrease in peak area over time was used to calculate the singlet oxygen steady-state concentration.

The loss of the acceptor (loss in FFA) should be first order, which is a requirement fulfilled by using low enough concentrations of the acceptor A (Zepp et al., 1977). The rate expression for loss of A is given by the following Eqn. 5.5:

$$-\frac{d[A]}{dt} = k_r [^1O_2]_{ss} [A]$$ (5.5)

where:

$k_r$ is the rate constant for the reaction rate of the acceptor (A) with $^1O_2$

A value for $k_r$ of $1.2 \times 10^8$ L mol$^{-1}$ s$^{-1}$ was determined by Opriel et al. (1989). The loss of the acceptor (A) is first order with respect to the acceptor concentration, which leads to Eqn. 5.6:

$$k_r [^1O_2]_{ss} = k_{exp}$$ (5.6)

where:

$k_{exp}$ is the experimentally determined rate constant

The combination of Eqn. 5.5 and 5.6 gives the expression in Eqn. 5.7:

$$\ln \left[ \frac{[A]}{[A]_0} \right] = -k_{exp} \times t \quad \text{or} \quad \ln[A] = \ln[A]_0 - k_{exp}$$ (5.7)

From this linear expression, a plot of $\ln[A]$ versus time should give a straight line with a gradient $k_{exp}$. This value can then be rearranged in Eqn. 5.6 to give Eqn. 5.8:

$$[^1O_2]_{ss} = \frac{k_{exp}}{k_r}$$ (5.8)

Measurements of the Optical Properties of CDOM

The absorption coefficients ($a_{CDOM}$355) as well as the EEM fluorescence were determined in the same way described in Chapter 2. Briefly, the absorbance was measured between 280 and 700 nm using a Varian Cary 500 UV/Vis spectrophotometer. The absorption coefficient $a_{CDOM}$(355) was calculated using the absorbance data at 355 nm and a pathlength of 5 cm for the length of the cuvette. EEM fluorescence spectra were determined using a Jobin Yvon SPEX FluoroMax-3 fluorescence spectrometer and a 1
cm pathlength cuvette. The total EEM fluorescence (EEM$_{\text{total}}$) was expressed as the total integral area from 280-600 nm emission and 250-500 excitation wavelengths and calculated using the FLToolbox 9.1. Data were normalised to quinine sulfate fluorescence using this toolbox and expressed as quinine sulfate equivalents. Different areas within the EEM spectra such as the humic-like (A-peak) and fulvic-like fluorescence showed exactly the same trend as did the EEM$_{\text{total}}$ (data not shown).

5.3 Results

In Doubtful Sound, values for $[^1\text{O}_2]_{ss}$ gradually decreased from 6.25 $10^{-14}$M to 3.2 $10^{-14}$M along the main channel of the fjord towards the open ocean (Figure 5.1). This trend in $[^1\text{O}_2]_{ss}$ demonstrated a similar pattern to the corresponding values of the absorption coefficient $a_{CDOM}(355)$ and the total EEM fluorescence (EEM$_{\text{total}}$) (Figure 5.2). A maximum $[^1\text{O}_2]_{ss}$ values of 9.36 $10^{-14}$M was observed at sample station CA02, Crooked Arm and again these values in this arm of the fjord closely followed the trends in $a_{CDOM}(355)$ and EEM$_{\text{total}}$. The results for $[^1\text{O}_2]_{ss}$, $a_{CDOM}(355)$ and EEM$_{\text{total}}$ are summarised in Table 5.1.

![Figure 5.1: Singlet oxygen steady-state concentrations, October 2005, Doubtful Sound](image)
Table 5.1: Summary of results from Doubtful Sound, October 2005: singlet oxygen, $\alpha_{\text{CDOM}}(355)$ and $\text{EEM}_{\text{total}}$

<table>
<thead>
<tr>
<th>sample ID</th>
<th>$[^1\text{O}<em>2]</em>{\text{ss}} \times 10^{-14}$ M</th>
<th>$\alpha_{\text{CDOM}}(355)$ (m$^{-1}$)</th>
<th>$\text{EEM}_{\text{total}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC01</td>
<td>6.25</td>
<td>2.46</td>
<td>233351</td>
</tr>
<tr>
<td>HA01</td>
<td>6.43</td>
<td>2.72</td>
<td>256062</td>
</tr>
<tr>
<td>HA02</td>
<td>6.08</td>
<td>3.05</td>
<td>253232</td>
</tr>
<tr>
<td>DS01</td>
<td>6.45</td>
<td>2.44</td>
<td>224161</td>
</tr>
<tr>
<td>DS02</td>
<td>6.85</td>
<td>2.45</td>
<td>245459</td>
</tr>
<tr>
<td>DS03</td>
<td>6.05</td>
<td>2.19</td>
<td>193451</td>
</tr>
<tr>
<td>DS04</td>
<td>6.28</td>
<td>2.17</td>
<td>190594</td>
</tr>
<tr>
<td>DS05</td>
<td>5.74</td>
<td>1.63</td>
<td>156012</td>
</tr>
<tr>
<td>DS06</td>
<td>5.56</td>
<td>1.52</td>
<td>148209</td>
</tr>
<tr>
<td>DS07</td>
<td>3.9</td>
<td>0.86</td>
<td>109340</td>
</tr>
<tr>
<td>CA01</td>
<td>7.42</td>
<td>2.97</td>
<td>227779</td>
</tr>
<tr>
<td>CA02</td>
<td>9.36</td>
<td>3.22</td>
<td>256313</td>
</tr>
<tr>
<td>CA03</td>
<td>8.35</td>
<td>4.06</td>
<td>330533</td>
</tr>
<tr>
<td>FA01</td>
<td>6.38</td>
<td>2.62</td>
<td>223980</td>
</tr>
</tbody>
</table>

The correlation coefficients ($r$) between $[^1\text{O}_2]_{\text{ss}}$ and $\text{EEM}_{\text{total}}$ fluorescence, and the absorption coefficient $\alpha_{\text{CDOM}}(355)$ were greater than 0.85 in both cases (Figure 5.3). Similar relationships between $[^1\text{O}_2]_{\text{ss}}$ versus $\alpha_{\text{CDOM}}(355)$ and EEM fluorescence were determined for water samples collected in the estuary of the Freshwater River, Stewart Island. Values for $[^1\text{O}_2]_{\text{ss}}$ ranged from $8.53 \times 10^{-14}$ M in the freshwater end-member at sample station FWR01 and $4.6 \times 10^{-14}$ M at station FWR04 (salinity 28) in Paterson Inlet. The correlations between $[^1\text{O}_2]_{\text{ss}}$ to $\alpha_{\text{CDOM}}(355)$, $\text{EEM}_{\text{total}}$ and DOC levels are given for these samples in Figure 5.4. The $\alpha_{\text{CDOM}}(355)$, $\text{EEM}_{\text{total}}$ and DOC levels showed conservative mixing along the Freshwater River Estuary, Stewart Island as discussed earlier in Chapter 3 and hence the highly significant correlation of $[^1\text{O}_2]_{\text{ss}}$ with these parameters suggested also a conservative mixing behaviour of $[^1\text{O}_2]_{\text{ss}}$. 

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Figure 5.2: Absorption coefficient $a_{CDOM}(355)$ and EEM A-peak fluorescence, October 2005, Doubtful Sound.
Figure 5.3: Absorption coefficient $a_{CDOM}(355)$ and EEM fluorescence versus $1^O_2$, Doubtful Sound

(a) $a_{CDOM}(355)$ versus $1^O_2$

(b) EEM versus $1^O_2$

Figure 5.4: Linear relationship of $1^O_2$ and $a_{CDOM}(355)$, EEM fluorescence and DOC, Freshwater River Estuary, Stewart Island

(c) DOC versus $1^O_2$
5.4 Discussion

The $[^1O_2]_{ss}$ values measured for water samples collected from sites in both Doubtful Sound and the Freshwater River were within the range previously reported by Shao et al. (1994). However, it is difficult to make too detailed a comparison because some of these other previous studies used different trapping agents (e.g. DMF (Zepp et al., 1977; Wolff et al., 1981)) and different experimental conditions. In particular, $[^1O_2]_{ss}$ values are obviously highly dependent on the radiation source intensity and in the case of field measurements, on the geographical location and season.

Results also suggest the direct relationship of $[^1O_2]_{ss}$ and the optical properties of the water samples such as $a_{CDOM}(355)$ and EEM$_{total}$. Hence, both parameters seem to be very good predictors for $[^1O_2]_{ss}$ in natural waters for a given intensity of solar radiation.

The high correlation of $[^1O_2]_{ss}$ with DOC has been noted in many previous studies beginning with (Zepp et al., 1977) and summarised by (Cooper et al., 1989). In the samples from the Freshwater River, Stewart Island, there was no difference found between the correlation of DOC, $a_{CDOM}(355)$ and the total EEM fluorescence and therefore fluorescence measurements seemed not to be a better predictor of $[^1O_2]_{ss}$ compared to DOC levels in the present study.

5.5 Summary

The fluorescence intensities and absorption coefficients were strongly correlated with $[^1O_2]_{ss}$ suggesting that singlet oxygen is involved in the photochemistry of CDOM.

The present study confirmed that singlet oxygen is an important reactive oxygen species (ROS) strongly linked to CDOM levels in natural surface waters. Waters exhibiting high levels of CDOM, such as Doubtful Sound, resulted in elevated levels of $[^1O_2]_{ss}$.

During conservative mixing, e.g. the Freshwater River Estuary, Stewart Island, the $[^1O_2]_{ss}$ decreased conservatively with increasing salinity also suggesting the strong correlation of terrestrial derived CDOM with $[^1O_2]_{ss}$. Further investigations are needed to quantify the importance of singlet oxygen and its ability to change the chromophoric character of CDOM due to the formation of endoperoxides or similar reactions which would lead to a molecular change of CDOM.
Chapter 6

DOM Changes across the Subtropical Convergence off the South Island, New Zealand

6.1 Introduction

The Southern Ocean around New Zealand is characterised by a variety of ocean circulations. An overview of the different water masses affecting the oceanic environment surrounding New Zealand has been published by the National Institute of Water and Atmospheric Research (NIWA), New Zealand (see Figure 6.1).

A narrow band of the Subtropical Convergence (STC) stretches out from the Otago coast in the north-west toward the Subantarctic Water (SAW) in the south-east. The boundary between the warm waters on the south-east coast of the South Island and the Subantarctic Water is known as the Southland Front (SF). The STC was first characterised by Deacon (1937) using surface currents and temperature fronts as well as the subsurface salinity maximum. Later, the STC was delineated using a 10 °C isotherm in winter and a 15 °C isotherm in summer or the 34.7 - 34.8 surface isohaline (Garner, 1959; Health, 1975; Health, 1981; Health, 1985).

The direction of current flow within the STC around southern New Zealand (see 6.1) would suggest that a substantial amount of water enriched in terrestrially-derived DOM gets transported from the south-west coast of the South Island via the Southland Current (SC). The combination of high rainfall and dense temperate rainforest on the west coast seems responsible for a large input of terrestrially-derived organic matter into the coastal zone (see also Chapter 2). Therefore, terrestrially-derived DOM, or at
Figure 6.1: Ocean Circulation, New Zealand: Summarising the characteristics of the surface current systems, oceanic fronts and main water masses including Subtropical Water [STW]; Subtropical Front [STF]; Subantarctic Water [SAW]; Subantarctic Front [SAF]; Antarctic Circumpolar Current [ACC], the northern limit of which is defined by the SAF; Circumpolar Surface Water [CSW]. Currents are annotated as follows: East Australian Current [EAC]; East Auckland Current [EAUC]; North Cape Eddy [NCE]; East Cape Eddy [ECE]; East Cape Current [ECC]; Wairarapa Eddy [WE]; Southland Current [SC]; Southland Front [SF]; Westland Current [WC], and D’Urville Current [DC]; Tasman Front [TF]. Ref.: Carter, L., Garlick, R. D., Sutton, P., et al., 1998. Ocean Circulation New Zealand. NIWA Chart Miscellaneous Series 76.
least the refractory portion of it, could potentially be transported by the Southland Current to the east of the South Island across the Foveaux Straight and would be a characteristic of the STC. The concentration of this DOM should also decrease toward the Subantarctic Water (SAW). This DOM transport seems plausible, but has not been supported by any experimental measurements. Additionally, the Clutha River discharges a large amount of freshwater into the coastal zones south of the Otago Peninsula. However, this river is relatively clear throughout the year and hence it does not appear to be a potential source of significant amounts of at least chromophoric DOM (CDOM). Overall, the terrestrially-derived DOM pool within the STC is potentially influenced by a combination of river discharge and land runoff. The Subantarctic Water is from a different origin and is not influenced by the Southland Current, hence SAW may exhibit changes in the molecular composition of the DOM pool compared to the STC.

The main focus of the present study was to characterise the changes of the optical properties as a measure for terrestrially-derived CDOM across a major oceanic current (the SC) and to investigate the differences in the molecular composition of DOM from different origins using the ESI-FT-ICR-MS technique. The molecular composition of the refractory DOM pool in the SAW was also investigated and compared to freshwater DOM from Fiordland, New Zealand to support the hypothesis of a terrestrially-derived refractory DOM pool being present in freshwater and also in the open ocean.

6.2 Materials and Methods

In November 2004 and February 2006, surface water samples were collected using the University of Otago research vessel *R/V Munida* at 8 sample stations along a transect crossing the STC and reaching Subantarctic Water 60 km offshore (Figure 6.2). Water samples for ESI-FT-ICR-MS and lignin analysis were taken in February 2006 at sample stations STC01, STC04 and STC08.

Salinity and temperature were continuously recorded using the Seabird Thermo-salinograph SBE 21™. Samples were collected and filtered through Millipore GV 0.22 μm within 24 hours. DOC, EEM and lignin measurements were undertaken in the same way as described in Chapter 3. Briefly, DOC levels were measured on acidified samples using the Shimadzu 5000A TOC/TDN Analyser at the University of North Carolina, Wilmington (UNCW).
Excitation-Emission-Matrix (EEM) fluorescence spectra were measured using a 1 cm pathlength cuvette and emission spectra measured in the range of 300-600 nm for excitation in the range from 250-500 nm at 5 nm intervals. Lignin derivatives were determined using the CuO oxidation method and analysis of the lignin derivatives was undertaken using reversed-phase HPLC and a diode-array detector.

Additionally, three 25 L carboys were filled with surface water collected at three different locations (sites: STC01, STC04 and STC08) across the STC in February 2006. These bulk water samples were also filtered through Millipore GV 0.22 μm filters, acidified to pH 2 and solid-phase extracted using C-18 Varian Mega Bond Elut cartridges (details are given in Chapter 3). One portion of the C-18 extract was freeze-dried for lignin analysis and the remaining methanol extract was stored at -18°C prior to ESI-FT-ICR-MS analysis. ESI-FT-ICR-MS analysis was undertaken in the same manner as described in Chapter 3.
6.3 Results and discussion

6.3.1 Salinity and Temperature

The STC, extending from the outer edge of neritic (coastal) water to the SAW at the SF, is characterised by the change in salinity and/or the temperature. The salinity and temperature across the STC for November 2004 (spring) and February 2006 (summer) did show the expected changes at the Southland Front at approximately 30 km distance from the Otago Coast (Figure 6.3). In summer, the temperature dropped by about 2 °C across the SF and the salinity increased from 33.8 to 34.4.

![Salinity and temperature plots across the STC in spring 2004 and summer 2006](a) Spring 2004  (b) Summer 2006

Figure 6.3: Salinity and temperature plots across the STC in spring 2004 and summer 2006

6.3.2 EEM fluorescence and DOC Levels across the STC

The EEM fluorescence technique was used to estimate the terrestrial humic contribution (EEM A-peak) and the marine and terrestrial fulvic-like component ((C+M)-peak) of the fluorescence across the STC. A decrease in the EEM A-peak as well as the EEM (C+M)-peak became apparent across the STC (Figure 6.4), with no obvious seasonal trends. The EEM fluorescence did not vary much within the Subtropical Convergence (sample STC01-STC04), but changes occurred at the Southland Front, between the STC and the Subantarctic Water (sample ST04-STC08), suggesting that the Subtropical Convergence has considerably higher terrestrially-derived CDOM levels (Figure 6.5) compared to SAW.

The DOC values behaved differently and showed a seasonal trend with elevated values of about 150 μM C at the Southland Front and in the SAW in spring 2005. In
summer 2005, the DOC values reached an average of 75 µM C and did not change along the transect.

Figure 6.4: A-peak, (C+M)-peak fluorescence integrals and DOC across the STC in spring 2004 (a) and summer 2005 (b)

Figure 6.4: A-peak, (C+M)-peak fluorescence integrals and DOC across the STC in spring 2004 (a) and summer 2005 (b)
Figure 6.5: EEM fluorescence across the Subtropical Convergence and at the Southland Front on samples collected in February 2006
6.3.3 Lignin Analysis across the STC

Analyses of lignin derivatives as biomarkers for terrestrially-derived DOM were used to characterise the refractory DOM pool across the STC. The individual amounts of all eight lignin derivatives determined using the CuO oxidation method decreased across the STC (Table 6.1). The acid/aldehyde ratios were high, which is an indication of a high degree of decomposition caused by propyl side chain oxidation (e.g., white-rot decay) (Eriksson et al., 1990). Additionally, the ratio of non-methoxylated phenols (p-hydroxyphenols) to methoxylated phenols (vanillyl and syringyl phenols) (P/(V+S)) is also a measure for the degree of degradation (Dittmar et al., 2001). Demethylation caused by e.g., brown-rot decay, may lead to the degradation of methoxylated phenols resulting in an increase in this ratio. The samples collected across the STC showed high values for this P/(V+S) ratio, which was also indicative of a high degree of degradation. The results are summarised in Table 6.1 and compared to typical values for freshwater samples from a stream in Doubtful Sound (sample CA02-riv) and the Freshwater River, Stewart Island (sample FWR01).

Clearly, the amount of recognisable lignin (Sum8 in Table 6.1) is very low in all samples across the STC compared to the freshwater samples indicating either a very low amount of lignin present or highly degraded lignin, which is no longer recognised by the analytical technique as lignin. Furthermore, the total recognisable lignin levels also decreased from the coastal zone (sample STC01) across the STC and reached a minimum value in the SAW (sample STC08), which was 10-fold lower compared to the water closest to the coast.

<table>
<thead>
<tr>
<th>sample</th>
<th>S/V</th>
<th>C/V</th>
<th>(Ad/Al)v</th>
<th>P/(V+S)</th>
<th>Sum8 (nM/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STC01</td>
<td>n.d.</td>
<td>n.d.</td>
<td>1.57±0.13</td>
<td>2.06±0.24</td>
<td>1.59±0.16</td>
</tr>
<tr>
<td>STC04</td>
<td>n.d</td>
<td>n.d</td>
<td>1.59±0.23</td>
<td>2.83±0.88</td>
<td>0.87±0.23</td>
</tr>
<tr>
<td>STC08</td>
<td>n.d</td>
<td>n.d</td>
<td>2.35±0.23</td>
<td>4.57±0.92</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td>CA02-riv</td>
<td>0.71±0.003</td>
<td>0.06±0.002</td>
<td>0.88±0.06</td>
<td>0.19±0.01</td>
<td>24.05±1.04</td>
</tr>
<tr>
<td>FWR01</td>
<td>0.59±0.004</td>
<td>0.02±0.001</td>
<td>1.28±0.02</td>
<td>0.34±0.02</td>
<td>7.82±0.93</td>
</tr>
</tbody>
</table>

\(^1\text{n.d.: not detectable}\)
6.3.4 ESI-FT-ICR-MS Analysis of Samples from the STC

The ESI-FT-ICR-MS spectra determined for samples at station STC01, STC04 and STC08 were very similar, but the number of assigned molecular formulae containing C, H and O for each spectra decreased slightly across the STC (STC01: 994, STC04: 841 and STC08: 804). Most assigned formulae showed high O/C and H/C ratios indicating a high degree of saturation (Figure 6.6). This result is also emphasised in the 3D-Van Krevelen diagram showing the oxygen-subtracted Double Bond Equivalent (DBE-O) values. However, some molecular formulae with low O/C and H/C ratios were also present. The C:H:O compounds, which were only apparent in the sample closest to shore and disappeared across the STC (Figure 6.7) did not follow any obvious pattern and it was not possible to relate the decrease in EEM fluorescence in samples collected across the STC to the changes in the molecular composition.
Figure 6.6: 3D-Van Krevelen diagrams across the Subtropical Convergence for samples collected in February 2006
Figure 6.7: 3D-Van Krevelen diagram of compounds, which disappeared across the STC

(a) molecular weight

(b) DBE-O
Nitrogen-containing molecular formulae across the STC

Only molecular formulae containing only one nitrogen were considered in the present study. Similar to the C:H:O compounds reported above, the nitrogen-containing compounds showed small changes across the STC. Two distinct groups characterised by their O/C and H/C ratios were present. The group with high O/C (0.7-1.2) and H/C (1.5-2.1) ratios showed lower C/N ratios compared to the group with lower O/C (0.4-0.6) and H/C (1.1-1.5) ratios (Figure 6.8). The few nitrogen-containing compounds, which disappeared across the STC were mostly located within the first group, in the 3D-Van Krevelen diagrams characterised by a high degree of saturation and relatively low C/N ratios (Figure 6.9). The similarity between the assigned nitrogen-containing formulae in all samples (sample STC01, STC04 and STC08) may indicate a single source for these compounds, but further research is needed to support this hypothesis. These compounds may be generated by marine biota and this suggestion is supported by the observation that no nitrogen-containing compounds similar to the molecular formulae assigned for samples from the STC were detected in the freshwater stream in Doubtful Sound (see below). However, there does seem to be processes involved, which change or utilise a small fraction of the nitrogen-containing compounds across the STC until a minimum of assigned nitrogen-containing molecular formulae is reached in the SAW.
Figure 6.8: 3D-Van Krevelen diagrams of nitrogen-containing compounds across the Subtropical Convergence

(a) STC01, molecular weight distribution
(b) STC01, C/N ratios
(c) STC04, molecular weight distribution
(d) STC04, C/N ratios
(e) STC08, molecular weight distribution
(f) STC08, C/N ratios
Figure 6.9: 3D-Van Krevelen diagram of nitrogen-containing compounds lost across the STC

(a) molecular weight

(b) C/N ratios
The similarity between the sulphur-containing compounds obtained for STC01, STC04 and STC08 was also very high (Figure 6.10). Nevertheless, some sulphur-containing compounds with low C/S ratios and a high degree of saturation disappeared between STC01 and STC08 (Figure 6.11) and hence these compounds may make up at least part of the labile fraction of DOM. It seems plausible that compounds with a relatively low C/S ratio are labile, but the reason for the loss of low C/S ratio sulphur-containing compounds across the STC until a minimum is reached in the SAW remains unclear. In general, three different groups with different H/C and O/C ratios were present. Each of these groups also exhibited a different range of C/S ratios. Similar to the nitrogen-containing compounds, it can be also assumed that the sulphur-containing compounds from samples at each station are derived from closely related sources given the very similar appearance of the assigned sulphur-containing compounds in all samples.
Figure 6.10: 3D-Van Krevelen diagrams of sulphur-containing compounds across the Subtropical Convergence for samples collected in February 2006
Figure 6.11: 3D-Van Krevelen diagrams of sulphur-containing compounds lost across the STC
6.3.5 The Refractory DOM Pool in the Open Ocean

A comparison of all ESI-FT-ICR-MS data obtained from samples across the STC with the mass spectrum measured for a sample (CA02-riv) taken from a freshwater stream discharging into Crooked Arm, Doubtful Sound, revealed that there were 594 molecular formulae containing carbon, hydrogen and oxygen common to this samples (Figure 6.12). Hence, 75 % of all assigned formulae for the Subantarctic Water (sample station STC08) were also found in the freshwater stream in Doubtful Sound. The high degree of saturation and high oxygen content would support the hypothesis of an highly degraded refractory pool of terrestrially-derived DOM. Therefore, in the present study, these compounds are referred to as the refractory fraction of the terrestrially-derived DOM pool. Interestingly, this refractory pool seemed to be already present in the freshwater sample.

The individual compounds of the refractory fraction of the DOM pool were remarkably similar and can be expressed using nine general molecular formulae (Table 6.2). Even these nine general formulae differ only by a spacing of two hydrogen atoms. Additionally, all members of one specific homologous series can be obtained by adding or subtracting CH2 groups to the individual molecular formula (see also Chapter 3). An overview of all homologous series and assigned molecular formulae is given in Appendix 3.

<table>
<thead>
<tr>
<th>general molecular formula</th>
<th>n</th>
<th>DBE-O</th>
<th>z*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C26H38O14 + O_n H_{(-2n)}</td>
<td>0 - 4</td>
<td>-6</td>
<td>-14</td>
</tr>
<tr>
<td>C26H36O14 + O_n H_{(-2n)}</td>
<td>(-2) - 4</td>
<td>-5</td>
<td>-2</td>
</tr>
<tr>
<td>C26H34O14 + O_n H_{(-2n)}</td>
<td>(-3) - 3</td>
<td>-4</td>
<td>-4</td>
</tr>
<tr>
<td>C26H32O14 + O_n H_{(-2n)}</td>
<td>(-4) - 2</td>
<td>-3</td>
<td>-6</td>
</tr>
<tr>
<td>C26H30O14 + O_n H_{(-2n)}</td>
<td>(-5) - 2</td>
<td>-2</td>
<td>-8</td>
</tr>
<tr>
<td>C26H28O14 + O_n H_{(-2n)}</td>
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<td>-1</td>
<td>-10</td>
</tr>
<tr>
<td>C26H26O14 + O_n H_{(-2n)}</td>
<td>(-5) - 1</td>
<td>0</td>
<td>-12</td>
</tr>
<tr>
<td>C26H24O14 + O_n H_{(-2n)}</td>
<td>(-5) - 1</td>
<td>1</td>
<td>-14</td>
</tr>
<tr>
<td>C26H22O14 + O_n H_{(-2n)}</td>
<td>(-4) - 0</td>
<td>2</td>
<td>-2</td>
</tr>
</tbody>
</table>

Table 6.2: General molecular formulae of the refractory DOM pool assigned for one member of a specific homologous series
The given similarity of all assigned compounds of the refractory DOM, where the general molecular formulae only show differences of 2 hydrogen, may well be an indication that these compounds are closely related.

Lignin do not contain nitrogen and the degradation of lignin would lead to a high degree of readily-ionisable functional groups such as carboxyl groups. These compounds would also be quantitatively extracted from the water using the present solid phase extraction procedure (Louchouarn et al., 2000). Additionally, the degradation of various lignin would lead to highly-oxidised lignin derivatives which may show very similar molecular formulae compared to the assigned formulae in the present study. The enzymatic mechanisms of lignin degradation pathways involving peroxidases, oxidases and laccases have been extensively studied (Cullen and Kersten, 2004). The core structure of lignin is characterised by three main monomeric building blocks (see Figure 6.13).

Depolymerisation is the first step of lignin degradation, followed by side chain oxidation of hydroxyl groups into carbonyl groups. This side chain oxidation (e.g. white-rot decay) would lead to a decrease of two hydrogen atoms for each given hydroxyl group creating homologous molecules with a difference of two hydrogens. Demethylation is possible on methoxylated lignin subunits (e.g. guaiacyl, syringyl) and the brown-rot decay is one example of this degradation pathway. Demethylation would cause a loss of a CH$_2$ group for any given methoxy functional group.
Phenolic lignin subunits can also be degraded and would lead an aromatic ring opening. This process would add two oxygen atoms to the molecular formula and would create an additional carboxyl-group and carbonyl-group. The lignin degradation pathways are summarised in Figure 6.14.

The molecular formulae listed in Table 6.2 differ from each other by $O_n \cdot H_{(−2n)}$ and CH$_2$. These differences can all be explained by the lignin degradation pathways. Most of the compounds of the refractory DOM are highly saturated with high numbers of oxygen. The given saturation and number of oxygen does not always support lignin degradation products which would still contain double bonds and additional side chain aryl-groups. However, additional oxidation of the two remaining double bonds after the aromatic ring opening would seem plausible.

Hypothetical molecular structures for one of the molecular formulae classified as refractory DOM are given in Figure 6.15. These structures are only feasible, if the hypothesis of the appearance of refractory lignin derivatives in the open ocean is accepted. Meyers-Schulte and Hedges (1986) confirmed for the first time the evidence of terrestrially-derived lignins in the open ocean. Later, highly degraded lignin derivatives have been determined in the open ocean in numerous studies (Opsahl and Benner, 1997; Hedges et al., 1997; Hernes and Benner, 2002; Benner, 2004). In the present study, the analysis of lignin derivatives also suggested a high degree of decomposition, which is in agreement with the literature. With further degradation of lignin, the fraction of not chemically-recognisable lignin derivatives would also increase. Hence, the levels of chemically-recognisable lignin would be very low. Very low levels of lignin derivatives were found in the present study and support this suggestion.
On the other hand, chemically unrecognisable lignin degradation products would be easily determined using ESI-FT-ICR-MS. Molecular structures for most of the molecular formulae found in the refractory DOM pool could be constructed using the hypothetical structures given in Figure 6.15. The most saturated compounds with DBE-O values of -5 and -6 could not be explained keeping all aryl-groups intact. However, molecular structures with aliphatic side chains can easily explain the observed highly saturated compounds (see Figure 6.16).

In contrast to the assigned molecular formulae only containing carbon, hydrogen and oxygen, there were no molecular formulae including sulphur or nitrogen which were common to the STC and the river examined in Doubtful Sound. This result is quite surprising and suggests that all sulphur and nitrogen compounds in freshwater are not apparent anymore in the oceanic DOM pool and therefore are not part of the refractory DOM pool either.
Figure 6.15: Hypothetical structures for the molecular formulae $C_{26}H_{28}O_{14}$

Figure 6.16: Hypothetical structure for the molecular formulae $C_{26}H_{38}O_{14}$
6.4 Summary

Salinity and temperature can be used to accurately characterise the boundaries of the STC, which is consistent with the previous literature (Deacon, 1937; Garner, 1959; Health, 1975; Health, 1981; Health, 1985). The appearance and decrease of the terrestrially-derived humic-like EEM fluorescence (A-peak) across the STC was another characteristic feature of this oceanic current.

DOC values at the Southland Front were elevated in spring, but in summer the DOC level were steady across the STC with values of 75 µM C.

ESI-FT-ICR-MS analysis for samples collected across the STC showed minor changes in the molecular composition of the DOM with distance from shore. The comparison of ESI-FT-ICR-MS data from the STC with that for freshwater in Doubtful Sound, Fiordland, New Zealand revealed that 75 % of all assigned molecular formulae for samples from sample station STC08 located in the Subantarctic Water were also present in the freshwater sample from Doubtful Sound. These formulae also found at STC01 and STC04 sites in the STC were defined as the refractory DOM pool. This refractory DOM pool could be described by nine general formulae and their homologous series, and hence was remarkably uniform. Additionally, the refractory fraction of the open ocean DOM may be explained by degradation pathways for lignin and plausible structures have been suggested. Surprisingly, the refractory DOM pool did not include any nitrogen- and sulphur-containing compounds suggesting that all compounds containing these heteroatoms were labile or at least semi-labile.
Chapter 7

Overall Conclusions

Research undertaken during the last decades in the broad field of dissolved organic matter has emphasised that processes responsible for changes of DOM or even “just” the knowledge of its composition are not trivial and remain a real scientific challenge. Despite the difficulties and problems in the characterisation of DOM, research in this area continue to be a very important part in the investigation of global biogeochemical cycles particularly for carbon as discussed earlier in Chapter 1. Previous studies and the present study have shown that DOM is greatest altered in estuaries and coastal zones.

DOM from a range of natural aquatic environments in south-western New Zealand and North Carolina, USA were isolated and additionally their physico-chemical properties were measured including salinity, DOC, TDN, absorbance and fluorescence. The molecular mass composition of DOM isolates were determined using ESI-FT-ICR-MS and samples were compared with each other. Furthermore, the influence of salinity on the molecular composition of DOM was emphasised.

In Chapter 2 the presented research contributed to the database of distribution pattern of CDOM in a fjord environment and emphasised the importance of CDOM production in fjords. Modern analytical techniques such as EEM fluorescence combined with UV/Vis absorbance measurements proved to be useful in the characterisation of the levels of CDOM along the fjord as well as with depth within the fjord. The presented data of CDOM distribution in Doubtful Sound, a major New Zealand fjord, is the first of its kind in a fjord and showed in detail the fate of about 90 % CDOM within the first 5 m depth at the fjord surface. The lateral decrease along the fjord could also be demonstrated. Seasonal measurements of CDOM revealed the highly dynamic CDOM cycling in this fjord with highest CDOM levels ever recorded for coastal environments.
Molecular changes as indicated by changes in the spectral slope $S$, fluorescence index $FI$ and EEM fluorescence peak ratios seemed to have occurred with increasing salinity and depth in Doubtful Sound, but these changes may also be explained by the change of terrestrially-derived dominated CDOM to a marine dominated CDOM. Therefore, a molecular change of the terrestrially-derived CDOM with increasing salinity and depth in this fjord cannot be concluded based on the optical properties of the examined samples in Doubtful Sound. Beside the interesting features of the fjords such as the permanent low salinity surface layer (LSL) and very high CDOM levels within this LSL, the research undertaken in New Zealand waters and in the present study has implications far beyond local characteristics and raised the question of the importance of fjord systems worldwide in terms of DOM production and cycling.

In Chapter 3, the major objectives have been the molecular characterisation of DOM derived from terrestrial sources and the changes which occurred during estuarine mixing. For the first time, measurements on water samples across freshwater-seawater interfaces were undertaken using ultrahigh resolution Electrospray Ionisation Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS) and provided evidence that the aromaticity of DOM increased with increasing salinity. This increase in aromaticity appeared to be directly related to salinity in typical estuaries such as present in the Freshwater River Estuary, Stewart Island. These findings were surprising and may lead to a new discussion about processes responsible for an increase in aromaticity of DOM within the salinity gradient of an estuary. The mechanisms behind the increase in aromaticity with salinity are not clear and are subject to further research. In the present study, ESI-FT-ICR-MS analyses proved to be the best available analytical technique to evaluate DOM composition and changes at the molecular level and this study also confirmed that ESI-FT-ICR-MS is a powerful and important technique in DOM research.

In Chapter 4, Sunlight-induced photochemical reactions were confirmed to be a major removal pathway of CDOM. Photodegradation experiments of samples at different salinities suggested that salinity affected the wavelength-dependent photodegradation of CDOM. Freshwater CDOM seemed to be more affected by photodegradation in the UV range compared to saline water, but at wavelength greater 400 nm the opposite seemed to be the case. These results indicate that salinity needs to be considered in any photodegradation studies and also that wavelength-dependent differences occur in CDOM photodegradation caused by irradiation of samples at various salinities. A surprising result from the ESI-FT-ICR-MS analysis and solar irradiation experiments...
on samples taken from the Cape Fear River indicated that the highly aromatic molecular masses of the DOM pool generated during estuarine mixing are also photolabile and can be entirely photobleached in 21 h of solar irradiation. Furthermore, these highly aromatic masses are converted into much more saturated masses indicated by lower oxygen-subtracted double bond equivalents (DBE-O). Hence, the present study has provided evidence for the photochemically-induced destruction of highly unsaturated compounds and the transformation toward more saturated compounds. Additionally, sulphur- and nitrogen-containing masses showed substantial changes during solar irradiation. Nitrogen-containing masses changed to a higher saturation during irradiation similar to the C:H:O molecular formulae, whereas the sulphur-containing formulae showed a shift toward an increase in unsaturation/aromaticity. These different behaviours of sets of compounds which only containing carbon, hydrogen and oxygen and sets of molecules containing either sulphur or nitrogen clearly demonstrated that there are significant advantages to consider these sets separately and treat them individually.

In Chapter 5, a strong correlation between the steady-state concentration of singlet oxygen (\( ^1\text{O}_2 \)) and CDOM levels, characterised by using the optical properties \( a_{\text{CDOM}}(355) \) and Excitation Emission Matrix (EEM) fluorescence, was confirmed and was in agreement with the previous literature. Differences in the correlation of \(^1\text{O}_2\) to DOC and correlations to the optical properties could not be found. Hence, DOC, EEM fluorescence and absorbance seemed to be good predictors of \(^1\text{O}_2\) steady state concentrations in water at a given sunlight intensity.

In Chapter 6, the molecular composition across the Subtropical Convergence (STC), a major oceanic current in the south east of New Zealand, were successfully determined using ESI-FT-ICR-MS. Results from water samples collected across the STC were remarkably similar with only minor differences in the assigned molecular formulae. However, the EEM fluorescence across the STC decreased with the biggest changes occurring at the Southland Front, the boundary between the Southland Current and Subantarctic Water suggesting a decrease in terrestrially-derived CDOM across the STC. Approximately 75 % of the molecular formulae assigned for the open ocean DOM were also found in a freshwater sample collected from a river discharging into Crooked Arm, Doubtful Sound, hence a substantial fraction of this potentially refractory open ocean DOM was already present in the freshwater end-member. However, over 90 % of the assigned formulae in the Doubtful Sound freshwater river sample could not be found in samples collected across the STC. Additionally, there were no sulphur- or
nitrogen-containing formulae that were similar between the freshwater and open ocean samples indicating that all these compounds found in freshwater were labile or at least semi-labile. These results provided a detailed molecular view of the changes in the molecular composition of DOM during the transport from rivers to the open ocean and new evidence for the molecular composition of the terrestrially-derived refractory DOM in the open ocean.

The present study represents the first detailed research combining DOM dynamics, characterisation and photoreactivity assessments for the New Zealand coastal zone and addresses the molecular composition and changes in estuarine as well as open ocean samples using the modern ESI-FT-ICR-MS technique and confirmed that the chemical nature and associated physico-chemical properties of aquatic DOM change along a salinity gradient, between different sources of DOM and under the influence of solar radiation.
Chapter 8

Suggestions for Future Work

A major challenge in modern biogeochemistry and DOM research is the extraction efficiency of DOM. Even with modern solid-phase extraction cartridges such as the newly-available PPL resin, the extraction efficiency is still not greater than 50% in terms of extracted DOC. Recently, new techniques became available to enhance the extraction efficiency of DOM. Of particular interest is the combination of a reverse osmosis system coupled with electrodialysis, which could extract up to 90% of the DOM present in natural waters. This isolation technique combined with the analysis of the molecular composition of DOM should be subject to future research.

New derivatisation procedures need to be developed to enable the analysis of DOM components which are currently impossible to analyse using the ESI-FT-ICR-MS, because of the present limitations of using the electrospray ionisation method to ionise the sample. Such components would include e.g. mono- and polysaccharides and alcohols.

ESI-FT-ICR-MS analysis of DOM is still a very new analytical technique and very few results are available in the literature. Further analysis of water samples using this technique is needed to gain information about the molecular composition of DOM in a variety of environments. A major focus of this new research should point toward rivers (e.g. Amazon River) that are responsible for discharging most of the terrestrially-derived DOM into the world’s oceans. The combination of quantitative analysis of DOM or CDOM using techniques such as DOC, TDN, lignin, fluorescence and absorbance measurements and qualitative analysis such as ESI-FT-ICR-MS may lead to a better understanding of DOM-cycling on a global scale. Fjord systems in conjunction with extreme rainfall and dense vegetation such as that present in the north-west coast of Canada, the south-west coast of Chile and to a lesser extend the south-west coast of New Zealand, have been largely ignored. The present study aimed to emphasise
the importance of fjord systems based on the study undertaken in Doubtful Sound, Fiordland, New Zealand. Therefore, research could be extended to include other fjord systems.

Fundamental knowledge about the refractory DOM pool in the open ocean is also needed to better understand the global biogeochemical cycling of carbon and it has been shown in the present study that ESI-FT-ICR-MS analysis can supply detailed information about this DOM pool. However, spatial information on the molecular composition of DOM in the world oceans based on ESI-FT-ICR-MS analysis does not exist and extended investigations are needed to support the suggestion of a terrestrially-derived refractory DOM pool in the open ocean, which appears to be related to highly degraded lignin.

Further research is needed to investigate the processes responsible for the suggested increase in aromaticity during estuarine mixing. A variety of additional estuaries should also be examined to see if the present observations in the Freshwater River Estuary, Stewart Island and the Cape Fear River Estuary also hold within other estuaries.

The processes involved in making terrestrially-derived DOM bioavailable need to be examined and the contradiction in the literature between photochemically-induced changes in the DOM composition which lead to enhanced or restricted bioavailability needs to be addressed. Future ESI-FT-ICR-MS analysis may provide an insight into the fraction of readily available DOM using incubation experiments. Additionally, the same DOM pool can be exposed to solar irradiation to see which exact masses are influenced and if further incubation experiments do have an effect on the newly produced molecular masses.

The most promising analytical techniques such as ESI-FT-ICR-MS and $^{13}$C NMR should be combined to investigate possible structures of DOM components.
References


Appendix A

z* specific 3D-Van Krevelen diagrams, Freshwater River, Stewart Island
(a) FWR01, salinity: 0

(b) FWR02, salinity: 5

(c) FWR03, salinity: 14

(d) FWR04, salinity: 28

Figure A.1: Kendrick plots for $z^* = -4$, Freshwater River, Stewart Island
(a) FWR01, salinity: 0  
(b) FWR02, salinity: 5  
(c) FWR03, salinity: 14  
(d) FWR04, salinity: 28

Figure A.2: Kendrick plots for $z^* = -6$, Freshwater River, Stewart Island
(a) FWR01, salinity: 0
(b) FWR02, salinity: 5
(c) FWR03, salinity: 14
(d) FWR04, salinity: 28

Figure A.3: Kendrick plots for $z^* = -8$, Freshwater River, Stewart Island
Figure A.4: Kendrick plots for $z^* = -10$, Freshwater River, Stewart Island
Figure A.5: Kendrick plots for $z^* = -12$, Freshwater River, Stewart Island

(a) FWR01, salinity: 0

(b) FWR02, salinity: 5

(c) FWR03, salinity: 14

(d) FWR04, salinity: 28
(a) FWR01, salinity: 0

(b) FWR02, salinity: 5

(c) FWR03, salinity: 14

(d) FWR04, salinity: 28

Figure A.6: Kendrick plots for $z^* = -14$, Freshwater River, Stewart Island
Appendix B

All homologous series and molecular masses of the recalcitrant DOM pool
Figure B.1: Molecular masses of the recalcitrant DOM pool, DBE-O: (-6) - (-3)

(a) Masses with DBE-O: -6 and z*: -14

(b) Masses with DBE-O: -5 and z*: -2

(c) Masses with DBE-O: -4 and z*: -4

(d) Masses with DBE-O: -3 and z*: -6

Figure B.1: Molecular masses of the recalcitrant DOM pool, DBE-O: (-6) - (-3)
Figure B.2: Molecular masses of the recalcitrant DOM pool, DBE-O: (-2) - 2

(a) Masses with DBE-O: -2 and $z^*$: -8

(b) Masses with DBE-O: -1 and $z^*$: -10

(c) Masses with DBE-O: 0 and $z^*$: -12

(d) Masses with DBE-O: 1 and $z^*$: -14

(e) Masses with DBE-O: 2 and $z^*$: -2

Figure B.2: Molecular masses of the recalcitrant DOM pool, DBE-O: (-2) - 2