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Understanding the photochemistry of coastal hypoxic blackwater

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Understanding the Photochemistry of Coastal Hypoxic Blackwater

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A thesis submitted in fulfilment of the requirements of the degree of Doctor of Philosophy

July 2019
I certify that the work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part, for a degree at this or any other university.

I acknowledge that I have read and understood the University's rules, requirements, procedures and policy relating to my higher degree research award and to my thesis. I certify that I have complied with the rules, requirements, procedures and policy of the University (as they may be from time to time).

Signed .................................................. Date .................................
Abstract

Deoxygenation is the major process behind generation of hypoxia and so-called blackwater in coastal waterways. However, the fundamental geochemistry of deoxygenation induced by solar irradiation in such systems is largely unknown. This thesis aimed to investigate the potential for photochemical deoxygenation of surface waters in a coastal floodplain system using laboratory and field studies.

Simulated solar irradiation experiments were initially conducted with three standard dissolved humic substances (HS): one aquatic fulvic acid (Suwannee River Fulvic Acid, SRFA) and two soil humic acids (Pahokee Peat Humic Acid, PPHA; and Leonardite Humic Acid, LHA). Dissolved oxygen (DO) was measured as a function of irradiation time to determine the magnitude and kinetics of DO consumption. The optical properties $E_2/E_3$ and $SUVA_{280}$, and redox state of the DOM were also measured.

A study of natural hypoxic blackwater was performed by sampling surface waters from the Richmond River system after a flood in 2017. Associations between various physico-chemical parameters showed that pH, chemical oxygen demand and Fe were significantly correlated with the occurrence of hypoxic blackwater. Thus, the impact of pH and Fe on photochemical deoxygenation potential was subsequently studied using 50 mg/L LHA in the presence of Fe concentrations from 0 to 175 µmol/L and pH from 4 to 8. The photochemical deoxygenation potential of surface water samples from the Richmond River system was also measured.

Standard HS solutions and natural water samples all consumed DO when irradiated with simulated sunlight, mostly in the initial 7 h of irradiation. The magnitude of DO consumption varied with DOM type and concentration. $SUVA_{280}$ decreased slightly after 3 h of irradiation for all DOM solutions; $E_2/E_3$ increased during irradiation; while changes in the electron donating capacity (EDC) of the DOM were variable. In the presence of LHA, photochemical DO consumption increased with increasing Fe concentration and decreasing pH, especially with Fe concentration $> 75$ µmol/L.

Overall the results from this thesis suggest that photochemistry may be a significant contributor to deoxygenation potential in shallow waters with high HS concentrations, however there is not a simple relationship between the chromophoric properties of DOM and DO consumption potential. Further studies involving varying
irradiation wavelengths, different temperature and added biota in both simulated and natural blackwater systems are required to fully understand the role of photochemistry in these environments.
Acknowledgements

Above all, I would like to deeply thank my principal supervisor, Professor Andrew Rose. Without him, it would have been impossible for me to come here and get this project done. He is not only a dedicated academic mentor, but also an inspiring guru with a balanced macroscopic life wisdom. I appreciate all of his support from the application stage until the last minute of thesis submission. The encouragement and knowledge he provided during meetings helped me out with tough equipment issues, he was also tireless working with me and giving me detailed comments on my thesis. The completion of my PhD was only possible due to his help.

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Last but not least, I thank my dear family back home at Inner Mongolia, who love me unconditionally and support me always. Particularly my loving Mum who brought me to this world, and my beautiful grandma who showed me how to be generous and open minded.

This PhD journey is a bitter and sweet magic. It had put me down to the black coloured water with no oxygen to breath and lifted me up to the eternity sunshine to feel as warm as Spring at Lismore. Finally I built a bridge between these two and formed into this thesis. It is a priceless treasure to live these four years in such a beautiful green town with this group of lovely people with huge brains and big hearts. My gratitude is beyond words.
“You were my North, my South, my East and West,
My working week and my Sunday rest,
My noon, my midnight, my talk, my song;
I thought this PhD journey would last forever: I was wrong.”

– adapted from collected poems by W. H. Auden.
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## Abbreviations

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<th>Full Form</th>
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<tr>
<td>As</td>
<td>Arsenic</td>
</tr>
<tr>
<td>ASS</td>
<td>Acid sulfate soil</td>
</tr>
<tr>
<td>CDOM</td>
<td>Chromophoric dissolved organic matter</td>
</tr>
<tr>
<td>CO</td>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>CO2</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CO3(^-)</td>
<td>Carbonate ion</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
</tr>
<tr>
<td>DCPIP</td>
<td>2,6-dichlorophenol-indophenol</td>
</tr>
<tr>
<td>DER</td>
<td>Direct electrochemical reduction</td>
</tr>
<tr>
<td>DIC</td>
<td>Dissolved inorganic carbon</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>DOM</td>
<td>Dissolved organic matter</td>
</tr>
<tr>
<td>(^3)DOM*</td>
<td>Excited triplet state dissolved organic matter</td>
</tr>
<tr>
<td>DON</td>
<td>Dissolved organic nitrogen</td>
</tr>
<tr>
<td>DOP</td>
<td>Dissolved organic phosphorus</td>
</tr>
<tr>
<td>(E_a)</td>
<td>Activation energy of a chemical reaction</td>
</tr>
<tr>
<td>EAC</td>
<td>Electron accepting capacity</td>
</tr>
<tr>
<td>EC</td>
<td>Electrical conductivity</td>
</tr>
<tr>
<td>EDC</td>
<td>Electron donating capacity</td>
</tr>
<tr>
<td>ESR</td>
<td>Electron spin resonance</td>
</tr>
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<td>Fe</td>
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<td>HCO3(^-)</td>
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<td>H(_2)CO(_3)</td>
<td>Carbonic acid</td>
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<tr>
<td>H(_2)O(_2)</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>HMW</td>
<td>High molecular weight (&gt; 1000 Da)</td>
</tr>
<tr>
<td>IHSS</td>
<td>International Humic Substances Society</td>
</tr>
<tr>
<td>ISC</td>
<td>Intersystem crossing</td>
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<tr>
<td>LHA</td>
<td>Leonardite humic acid</td>
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<tr>
<td>LMW</td>
<td>Low molecular weight (&lt; 1000 Da)</td>
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<td>LMCT</td>
<td>Ligand-to-metal charge transfer</td>
</tr>
<tr>
<td>N/A</td>
<td>Not applicable</td>
</tr>
<tr>
<td>NH4(^+)</td>
<td>Ammonium</td>
</tr>
<tr>
<td>Ni</td>
<td>Nickel</td>
</tr>
<tr>
<td>NO3(^-)</td>
<td>Nitrate</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>NO\textsubscript{2}\textsuperscript{-}</td>
<td>Nitrite</td>
</tr>
<tr>
<td>NOM</td>
<td>Natural organic matter</td>
</tr>
<tr>
<td>O\textsubscript{2}</td>
<td>Oxygen molecule</td>
</tr>
<tr>
<td>\textsuperscript{1}O\textsubscript{2}</td>
<td>Singlet oxygen</td>
</tr>
<tr>
<td>O\textsubscript{2}\textsuperscript{-}</td>
<td>Superoxide anion</td>
</tr>
<tr>
<td>•OH</td>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>ORP</td>
<td>Oxidation-reduction potential</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>PB</td>
<td>Phosphate buffer</td>
</tr>
<tr>
<td>PO\textsubscript{4}\textsuperscript{3-}</td>
<td>Phosphate</td>
</tr>
<tr>
<td>PPHA</td>
<td>Pahokee Peat humic acid</td>
</tr>
<tr>
<td>Pb</td>
<td>Lead</td>
</tr>
<tr>
<td>RFU</td>
<td>Relative fluorescence units</td>
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<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>S</td>
<td>Spectral slope (nm\textsuperscript{-1})</td>
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<td>SRFA</td>
<td>Suwannee River fulvic acid</td>
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<tr>
<td>SUVA\textsubscript{280}</td>
<td>Specific UV absorbance at 280 nm</td>
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<td>TOC</td>
<td>Total organic carbon</td>
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<tr>
<td>UV</td>
<td>Ultraviolet light</td>
</tr>
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<td>UVA</td>
<td>Ultraviolet radiation 320-400 nm</td>
</tr>
<tr>
<td>UVB</td>
<td>Ultraviolet radiation 280-320 nm</td>
</tr>
<tr>
<td>UVC</td>
<td>Ultraviolet radiation &lt; 280 nm</td>
</tr>
<tr>
<td>Zn</td>
<td>Zinc</td>
</tr>
<tr>
<td>(\phi\lambda)</td>
<td>Apparent quantum yield spectrum</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>Wavelength (nm)</td>
</tr>
<tr>
<td>(\Delta)</td>
<td>Magnitude of variation</td>
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</table>
Chapter 1 – Introduction

1.1 Deoxygenation in hypoxic blackwater

Blackwater events in water bodies are defined as the occurrence of elevated dissolved organic carbon (DOC) concentrations which generate a dark tea colour [1]. These events occur irregularly in main river channels or on the floodplains. When a blackwater event is associated with a decrease in dissolved oxygen (DO) concentrations to less than 2 mg L\(^{-1}\) and a decrease in pH due to the presence of organic acids and polyphenols from terrestrial organic matter, it thus may reduce growth rates, cause mortality and disturb the distribution of aquatic biota, and it becomes hypoxic blackwater [2].

Despite their short-term harmful influences, the floods which are the main trigger of the blackwater formation are significant for macroscopic renewal of riverine systems, floodplains and ecosystems. Breaking down organic material supplies additional nutrients to increase the productivity of rivers and swamps, which can thus support and maintain the food chain [3-5]. Even during hypoxic blackwater events, downstream components of the system can receive organic matter and nutrients that may be beneficial once the system is reaerated. The harmful economic and social impacts of hypoxic blackwater are mainly short term, including using public resources for water treatment and the financial loss due to the interruption of commercial and recreational uses such as water activities and fishing [6].

However, severe hypoxic blackwater events can cause widespread and significant social and economic effects. For example, in the United States, it is estimated that hypoxia directly causes 5.3% of fish kills; in Australia, the Richmond River experienced hypoxia in 2008 which was associated with the death of 2,000,000 fish and resulted in more than 6 months of fishing limitations [7]. Globally, hypoxic blackwater is a rapidly growing environmental crisis in estuarine, coastal and marine ecosystems [2]. Hypoxic blackwater events have occurred in rivers ranging from the Paraguay River, Brazil [8], the Atchafalaya River, Louisiana, USA [9] and rivers of the Murray–Darling Basin, Australia [1], among others. Hypoxia issues have affected a range of coastal areas globally, and their influence extends into other aquatic systems due to hydraulic connections. Fig 1 shows the global distribution of coastal hypoxic systems that have been reported in the scientific literature [2].
Figure 1 Global distribution of 400-plus coastal hypoxic systems associated with eutrophication that have been reported in the scientific literature [2].

Normally, hypoxic blackwaters occur in aqueous systems with a low gradient such as floodplains or capacious wetlands with a host stock of plant litter [10]. River–floodplain connectivity is a key driver to mobilise DOC from the floodplain to the river, which could then induce severe degradation of riverine water quality [11]. DOC is input into natural water bodies and transported downstream during the original flood flush, however additional inputs may continue from slow release leaching processes, and also with chemical, microbial and photochemical transformations of natural organic matter (NOM) that occur on floodplains as well as in water bodies [12]. The introduction of artificial drainage and flood mitigation strategies has resulted in much faster flood drainage from backswamps into estuaries, which has enhanced the accumulation of oxygen depleting compounds, as well as increased the flux of blackwater to downstream estuarine areas [4, 13, 14].

DO concentration is a key identifier of hypoxic blackwater events. DO plays an essential role for aquatic biota via a number of biological and chemical processes [15]. In natural waters, the principal controls of DO concentration include: (i) the diffusion of oxygen from the atmosphere into water bodies across the water-air interface; (ii) oxygen production by photosynthesis; (iii) oxygen demand by chemistry in the water column; and (iv) oxygen demand from microbial respiration processes [16]. When oxygen demand dominates, then a drop in DO may establish hypoxic or anoxic conditions. In terms of oxygen demand, microorganisms can respire NOM to produce CO₂ and H₂O, coupled with oxygen consumption. Photochemical reactions can also result in photooxidation of NOM to CO₂ and H₂O,
but photochemical reactions in blackwater are mainly confined to surface waters because light penetration is reduced with depth [17]. Consequently, high DOC concentrations might trigger rates of DO consumption which surpass the rate of oxygen replenishment in the water by diffusion from the atmosphere. The inundation duration and the type, quantity and quality of plant tissue and litter of the floodplain are important factors as well [1].

Elevation of water temperature is another vital factor impacting DO and DOC concentration in four main respects: (1) oxygen solubility decreases, resulting in lower DO saturation concentrations; (2) microbial metabolism is enhanced, resulting in oxygen consumption increasing [18]; (3) enzymatic reactions are more active and might enhance DOC decomposition rates; and (4) the solubility of some DOC constituents vary with temperature, thus the leaching rate changes [19]. These processes jointly enhance the development of hypoxic blackwater occurring at elevated temperatures. Thus ephemeral inundation and drying of floodplains under high temperatures can augment the risk of hypoxic blackwater [20].

Formation of hypoxic blackwater on coastal floodplains is tightly related with system deoxygenation processes. A previous study by Gieskes et al. [21] indicated that total photochemical oxygen consumption is approximately 12×10^12 g O_2 per year in the surface of the oceans and coastal water bodies across the world. Other studies have found mean photochemical oxygen consumption rates between 0.1 and 0.3 µM O_2 per hour, over the 12 hours of daylight in the open subtropical Atlantic Ocean [22-24]. This aligns with the findings by Laane et al. [24] that consumption of O_2 by heterotrophs was about equal to photochemically consumed O_2 during the day time. Nonetheless, the influence of photochemistry on deoxygenation in coastal rivers and backswamps, which are the focus of this thesis, has not yet been reported.

1.2 Natural organic matter (NOM)

Natural organic matter (NOM) contains an extremely complex group of organic compounds and complexes. The term NOM excludes living organisms as well as anthropogenic compounds. The general composition of NOM includes aliphatic and chromophoric aromatics, consisting of hydrophilic and hydrophobic components. The latter fraction is mainly phenolic structural aromatic carbon, whilst hydrophilic components have a considerable percentage of nitrogenous compounds and aliphatic carbon, like proteins, carbohydrates, amino acids and sugars. The primary fraction of aquatic NOM is hydrophobic acids, and those hydrophobic acids are mainly humic
substances [4, 10-16]. Humic substances (HS) possess a supramolecular structure with various functional groups such as lignin derivatives, organic acids, condensed cyclics, heterocyclics and hydrocarbons. HS are often used as typical NOM in laboratory investigations [25]. The major inherent properties of NOM that relate to their photochemical reactions include: (i) the capacity to transfer solar energy to the interior functional fractions or aqueous system components, including absorption properties and fluorescence properties; and (ii) the ability to transfer electrons in photo-induced reactions as an electron acceptor or donor. For this reason, NOM plays a role of redox buffering in natural waters, which primarily depends on the redox properties of the NOM.

Dissolved organic matter (DOM) is the dissolved fraction of NOM and anthropogenic organic matter, and is the major component of DOC in natural surface waters. It consists of a complicated group of organic compounds generated through plant decomposition (known as allochthonous material) and generated through microbial activity (known as autochthonous material) [26]. The composition of DOM varies with the nature of the parent organic matter and geochemical processes [27]. As with NOM, the complexity of the variation and diversity makes it extremely difficult to separately identify the properties of DOM. Coloured dissolved organic matter (CDOM) is the chromophoric fraction of DOM, i.e. the fraction that absorbs visible light, and thus dominates the absorption properties of DOM. It is of significance for natural aquatic systems via reaction with trace metals, sorption of organic pollutants, and supporting microbial metabolism as a nutrient base by undergoing chemical and biological reactions [27].

The synthesis and degradation properties of NOM in natural aquatic systems are highly diverse, depending on the origin, environmental conditions, and the separation procedure used. The environmental significance of NOM functional properties is better understood from the perspective of the whole group rather than by attempting to identify each pure compound. Distinct techniques for analysis of NOM properties are often hard to apply practically or the resulting data are difficult to interpret. In spite of all the difficulties, NOM related measurements have been used in numerous different areas from basic laboratory research to management and policy controls, with different fields often using different approaches for studies of NOM. Recently, advances in many bulk analytical methods such as chromatography and spectroscopy have improved NOM characterisation. There is an expanding interest to apply UV
and fluorescence spectroscopy to identify the properties of DOM, because these methods are reliable qualitatively and quantitatively. These approaches usually involve filtering as the only significant sample treatment before analysis, and thus can reveal a whole picture of DOM composition.

1.2.1 Absorption properties of CDOM

CDOM is the dominant fraction in terms of contribution to the optical properties of NOM. CDOM is vital in natural water ecosystems because it can alter the sunlit zone depth and facilitate photochemistry that influences the fate of elements and contaminants. Also, it plays important roles in protecting aquatic microbes from damage from UV radiation [28]. CDOM typically has quite a broad absorption spectrum, which decreases exponentially over the whole ultraviolet and visible wavelength range, normally without readily discernible absorption bands. This general spectrum has been fitted to the following equation [29]:

\[
a(\lambda) = a(\lambda_0) e^{-S(\lambda - \lambda_0)}
\]

(Eq 1)

where \(a(\lambda_0)\) and \(a(\lambda)\) (typically in m\(^{-1}\)) represent the absorption coefficients at reference wavelength \(\lambda_0\) and some other wavelength \(\lambda\), respectively, and \(S\) is the spectral slope (nm\(^{-1}\)), which is discussed further below. \(a(\lambda)/DOC\) ratios can vary over a large range in many natural water systems, especially in fresh water columns, and the value of \(a(\lambda)\) is related to DOC concentrations [30]. Hestir et al. [31] concluded that local conditions are significant for estimation of freshwater DOC concentrations by absorption. Several spectral parameters have been defined to summarise CDOM properties from spectra, and those ratios are broadly independent of the concentration of CDOM, which is useful for comparison of various water samples.

Spectral Slope (\(S\))

The spectral slope (\(S\), nm\(^{-1}\)) is a key parameter derived from the CDOM absorption spectrum equation. \(S\) is a general indicator that can identify the rate at which absorption increases with decreasing wavelength: higher \(S\) values indicate a faster decrease and less contribution to absorption in the visible wavelengths. Spectral slopes are widely independent of CDOM concentration [34], and have been used to monitor CDOM degradation processes [35]. \(S\) values of terrestrial CDOM typically range from 0.01 to 0.02 nm\(^{-1}\) [29].
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**E₂/E₃, E₄/E₆**

Despite increases as molecular size increases, and is considered to be a useful indicator of high molecular weight (HMW) CDOM. E₄/E₆ is the ratio of absorption at 465 nm and 665 nm. It is regarded as being related to the aromaticity of CDOM [37], and recently has been considered as a general tracer for humification processes. These parameters aim to find a relationship between spectral line-shape and CDOM source and structural properties resulting from various transformation processes [38].

**SUVA₂₈₀**

SUVA₂₈₀ is defined as UV absorption at 280 nm when normalised to DOC concentration. SUVA₂₈₀ is highly related to DOM aromaticity for most of the isolated humic substances [39].

Finally, it is essential to consider that not all carbon compounds can absorb UV-visible radiation, while other inorganic species and metal complexes may absorb UV-visible radiation [40], which might make the interpretation of these parameters complicated.

1.2.2 Redox properties of DOM

An oxidation-reduction (redox) reaction is defined as a chemical reaction in which electrons are transferred between molecules, atoms or ions. The redox potential controls the availability of electrons for reaction and the overall oxidising or reductive tendency in an aquatic system. The oxidation-reduction potential (ORP) is measured in millivolts (mV) using an ORP electrode and reflects the redox state of the aquatic system. Previous studies in drinking water systems demonstrated that the ORP increased with decreasing pH for any oxidant species or concentration, and ORP increased rapidly with increasing oxidant dose, especially when DO was low (< 1 mg/L) [41]. Redox processes are also strongly related to groundwater quality, and studies have suggested that the interpretive power of system redox state is high while measurement of ORP is relatively inexpensive and simple [42].

The redox properties of NOM have been broadly investigated by numerous methods such as electron spin resonance (ESR) [43], electrochemical oxidation and reduction [44], and fluorescence spectroscopy [45]. Considerable interest has been focused on the redox properties of HS. For example, previous work suggested HS electron
accepting capacities (EAC) are positively correlated with the aromaticity of HS [44]. Also, HS show analogous reactivities to electron-transferring mediators of reductive processes transforming organic pollutants [46]. It is evident that the presence of quinone functional fractions lead to these redox properties. Quinone moieties may exist in different redox states which lead to a noticeable photosensitivity. In particular, quinone functional groups can form reactive oxygen species (ROS) through electron transfer reactions, a process that is also at least partly responsible for NOM optical properties [47].

DOM shows similar redox activity, with the capability to mediate the redox reactions of biogeochemically important compounds, such as triggering electron transfer between biota and metal oxides [26], and between abiotic reagents and organic pollutants. Besides, DOM could be a significant redox buffer, accepting electrons in anoxic environments via microbial respiration, then donating electrons to oxygen when oxic conditions are re-established.

Previous studies mainly focused on the analysis of HS-related electron transfer in reducing environments, considering quinones as the principal reducible HS moieties [44]. Whether the photochemically reactive fraction of NOM is primarily due to quinone moieties is still under debate, however NOM displays noticeable similarities in redox behaviour, with quinone-like properties including a tight linkage of EAC with the aromaticity of HS [44], the existence of at least three redox states [48], the ability to induce reversible redox transformations [49], and the ability to generate quite long-lived (lifetimes ranging from minutes to days) free radicals [50]. However, some distinct differences are noticed: (1) NOM possesses a broad group of aromatic constituents with potential conjugation which leads to possible delocalisation of electrons, which is related to the charge transfer formation and stability of free radicals [51]; and (2) compared with the whole constituents of NOM, the quinone-like fractions occupy just a small part, although they are still considered to play a significant role as electron acceptors in charge transitions [52].

In contrast to the EAC of quinones in HS, the phenolic fractions are considered as electron donating moieties. Phenolic groups, which are thought to be derived from precursors in higher plants, can offer a protective role for other functional groups that could be oxidised, and consequently are important for HS stability in natural waters [18, 20]. In the photic zone of natural waters, dissolved HS may quench ROS as well
as induce electron donation to form radical intermediates in the degradation of pollutants, thus being reduced back to their original compounds [21,22].

1.3 **Photochemistry of NOM in the sunlit zone**

Photochemical reactions are of vital significance during the transformation of aqueous NOM [54]. They are also important in many redox reactions and affect the bioavailability of many inorganic nutrients, whilst photo-mineralisation processes are important for the carbon cycle [55]. Photochemistry represents a major influence of solar radiation on the hydrosphere; the illuminated portion, which is known as photic area or sunlit zone, is particularly active and diverse, where material exchange, energy transport and biological activity are quite intense [56]. In the sunlit zone of natural waters, the photo-induced transformations of organic and inorganic components include two key pathways: direct photolysis and indirect photoreactions. Direct photolysis is induced by a molecule absorbing solar radiation, which transfers a photon into energy required for photolysis. For direct photolysis, the ability of a compound to absorb a photon is a major prerequisite. Direct photolysis is primarily controlled by radiation intensity, wavelength and the solution pH [57]. In contrast, indirect photoreactions occur when photosensitisers absorb solar radiation then generate reactive transient species that lead to various subsequent reactions. The dominant photosensitisers in the sunlit zone are nitrate, nitrite and CDOM [58]. CDOM could influence sunlight penetration depth, the key study zone for photochemistry in natural waters, by absorbing ultraviolet and visible radiation [27]. As previously outlined, the chromophoric properties of CDOM are mainly generated by HS, including humic acids (HA) and fulvic acids (FA) [59].

CDOM is of great consequence to the photochemistry of sunlit natural waters [47]. In the last 30 years, extensive investigations have provided significant exploration of the photochemical properties of CDOM, and a few widely accepted theories have been established [56]. By absorption of solar radiation, CDOM can be excited to a singlet electronic state (1CDOM*) that rapidly undergoes intersystem crossing to the triplet state (3CDOM*). 3CDOM* can react with DO to generate ROS, including singlet oxygen (1O2) [60], hydroxyl radicals (•OH) [61], superoxide anions (O2−) and hydrogen peroxide (H2O2) [47]. 3CDOM* is considered of great importance for photoinduced reactions in aquatic systems, and may influence up to 75% of aquatic system photochemistry [62]. Photogenerated ROS have considerable instability and chemical reactivity, with lifetimes ranging from µs to several days. They can undergo
a range of different reactions in the sunlit zone, including thermal deactivation and reaction with other chemical constituents. As a result, the ROS typically reach steady concentrations relatively rapidly in the sunlit zone [63]. Fig 2 illustrates the basic conception of CDOM photolysis and photochemistry reactions involving ROS.

![Conceptual model of CDOM photolysis and photochemical transformations with ROS](image)

Figure 2  Conceptual model of CDOM photolysis and photochemical transformations with ROS (adapted from [33]).

In most natural aqueous systems, DOM is the main scavenger for the reactive transients formed by indirect photochemistry. Other scavengers include dissolved inorganic carbon, bromide, and (for $^3\text{CDOM}^*$) dissolved oxygen [64]. Thus the composition of the sunlit zone waters plays an important role in mediating the reactions of ROS [65]. From the general conception of ROS formation, it is obvious that DO is a vital impacting factor, both as a reactant and product. Therefore the processes of direct photolysis and reactions of ROS with DOM are suggested to be significant influences on DO conditions in natural water bodies. To study photochemical DO depletion in the natural waters, understanding ROS reaction mechanisms is crucial.

1.4  DOM photo-generated Reactive Oxygen Species

ROS are normally produced through photolysis, energy or electron transfer reactions [56]. They have been hypothesised to be significant in DOM photooxidation, as they can potentially oxidise the DOM fraction which cannot undergo solar radiation-induced direct photochemical degradation. In the natural environment, $^3\text{CDOM}^*$, $^1\text{O}_2$, •OH, and $\text{O}_2^-$ can oxidise a large number of molecules relatively non-selectively [56], and have thus been implicated in degradation of contaminants and transformation of DOM in natural waters [66]. $\text{O}_2^-$ is more selective than other ROS when reacting with aqueous organic compounds, while its reduction potential
overlaps those of some biologically important metals which could also affect DOM oxidation [67]. \( \text{H}_2\text{O}_2 \) is a strong oxidant thermodynamically however its reactions are typically slower than reactions involving free radicals. In the following sections, the photo-generated transients that occur in surface waters (•OH, \(^3\text{CDOM}^*\), \(^1\text{O}_2\), \text{H}_2\text{O}_2\), and \( \text{O}_2^- \)), as well as related photochemical processes involving metals and optical properties of CDOM, will be discussed.

1.4.1 **CDOM triplet states \((^3\text{CDOM}^*)\)**

CDOM can be excited into singlet states \((^1\text{CDOM}^*)\) by absorption of solar radiation. Then if the conditions are favourable, the singlet state can transform to a triplet state \((^3\text{CDOM}^*)\) by intersystem crossing (ISC) [68].

\[
\text{CDOM} + \hnu \rightarrow ^1\text{CDOM}^*(\text{ISC}) \rightarrow ^3\text{CDOM}^* \tag{Eq 2}
\]

Reactions between \(^3\text{CDOM}^*\) states and other dissolved compounds can be triggered by redox reactions. \(^3\text{CDOM}^*\) act as oxidants in reactions involving electron or hydrogen transfer, and thus could influence the degradation of some pollutants, like phenols, phenylurea herbicides, and sulfonamide antibiotics in the sunlit zone [62].

DOM can inhibit the reaction of organic compounds with \(^3\text{CDOM}^*\) (Fig 3) [69], since DOM contains antioxidant moieties like phenolic compounds that can reduce oxidised compounds back to the initial substrate, consequently suppressing the transformation.

![Figure 3](image_url) **Reactions of \(^3\text{CDOM}^*\) involving oxygen (adapted from [70]).**

It is suggested that the capability of organic matter to degrade substrates hinges on the chromophoric fractions of NOM. Thus the related reactions are based on the properties of DOM as well as the substrate [68]. There is also the possibility of reactions which inhibit the transformation of substrate \( S \), following a recombination/deactivation process with the substrate [70]:
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\[ ^3\text{CDOM}^* + S \rightarrow \text{CDOM}^- + \text{S}^+ + \text{S} \] \hspace{0.5cm} (Eq 3)

\[ \text{CDOM}^- + \text{S}^+ \rightarrow \text{CDOM} + S \] \hspace{0.5cm} (Eq 4)

In aerated systems, the back reaction of \( \text{CDOM}^- \) to \( \text{CDOM} \) by substrate \( S \) can be shut down by oxygen scavenging of \( \text{CDOM}^- \), forming superoxide [70]:

\[ \text{CDOM}^- + \text{O}_2 \rightarrow \text{CDOM} + \text{O}_2^- \] \hspace{0.5cm} (Eq 5)

Thus in anoxic systems, there might be an enhancement of triplet-sensitised degradation of organic compounds [71]. It has been found that formation of \( ^3\text{CDOM}^* \) is most efficient when smaller CDOM fractions are irradiated, though the larger CDOM constituents and particles also can generate \( ^3\text{CDOM}^* \) [72]. The hypothesis for this phenomenon is that the HMW moieties undergo rapid internal transformation and return to the ground state, thereby suppressing both fluorescence emission and photoreactivity [73].

1.4.2 Singlet oxygen \( (^1\text{O}_2) \)

The singlet state of oxygen \( (^1\text{O}_2) \) can be produced via energy transfer reactions involving DOM or by a chemical pathway like the reaction of hypohalites with \( \text{H}_2\text{O}_2 \) [74]:

\[ \text{CDOM} + h\nu \rightarrow ^3\text{CDOM}^* \] \hspace{0.5cm} (Eq 6)

\[ ^3\text{CDOM}^* + \text{O}_2 \rightarrow \text{CDOM} + ^1\text{O}_2 \] \hspace{0.5cm} (Eq 7)

\[ \text{OCl}^- + \text{H}_2\text{O}_2 \rightarrow \text{Cl}^- + \text{H}_2\text{O} + ^1\text{O}_2 \] \hspace{0.5cm} (Eq 8)

In the sunlit zone, \( [^1\text{O}_2] \) is from \(~10^{-12} \) to \(~10^{-13} \) M. The reaction between \( ^3\text{CDOM}^* \) and \( \text{O}_2 \) is the major generation process of singlet oxygen in the presence of oxygen [75]. The deactivation of \( ^1\text{O}_2 \) primarily occurs by collisions with \( \text{H}_2\text{O} \), and this deactivation is normally much faster than the reaction of \( ^1\text{O}_2 \) with DOM [76]. Singlet oxygen might be important in the transformation of chlorophenoates [77] including a few phenolic pesticides, and of various labile oxidised amino acids [55].

Singlet oxygen reacts not only in the aqueous phase, as it is found at noticeable concentrations associated with hydrophobic sites in DOM. Such sites could be microreactors for \( ^1\text{O}_2 \) chemistry, and hence these sites could facilitate active transformations of the hydrophobic pollutants [78]. The \( ^1\text{O}_2 \) generated in
hydrophobic sites would be unlikely to diffuse considerably into the surrounding solution where it could induce other transformation reactions [78]. The reaction kinetics of $^1$O$_2$ in hydrophobic sites with DOM are not understood yet; nor are the reaction rate constants of the main functional moieties involved in this process. These factors are significant for the hypothesis that hydrophobic sites might shield $^1$O$_2$ from deactivation.

1.4.3 Superoxide anion (O$^2_-$)

Superoxide (O$^2_-$) is produced by reduction of O$_2$ through one-electron transfer. Superoxide radicals can be generated by photo-degradation of DOM, photo-induced reactions, O$_2$ reduction by organic radicals, or reactions between H$_2$O$_2$ and •OH [62].

\[
\text{DOM} + \text{O}_2 + h\nu \rightarrow \text{DOM}^+ + \text{O}_2^- \quad \text{(Eq 9)}
\]

\[
\text{H}_2\text{O}_2 + \cdot\text{OH} \rightarrow \text{HO}_2\cdot + \text{H}_2\text{O} \quad \text{(Eq 10)}
\]

\[
\text{HO}_2\cdot \rightleftharpoons \text{O}_2^- + \text{H}^+ \quad \text{(Eq 11)}
\]

Bacterial metabolism is another important source of extracellular superoxide production [2].

Although many investigations have been carried out during the past thirty years, the mechanism of the photochemical reduction of O$_2$ to O$^2_-$ by HS and CDOM has not been confirmed yet. The possible reactions are as follows:

\[
\text{CDOM} + h\nu \rightarrow \text{CDOM}^\pm + \text{e}^- \quad \text{(Eq 12)}
\]

\[
\text{e}^- + \text{O}_2 \rightarrow \text{O}_2^- \quad \text{(Eq 13)}
\]

\[
\text{CDOM} + h\nu \rightarrow 3\text{CDOM}^* + \text{O}_2 \rightarrow ^1\text{CDOM} + ^1\text{O}_2 \quad \text{(Eq 14)}
\]

\[
^1\text{O}_2 + \text{CDOM} \rightarrow \text{CDOM}^+ + \text{O}_2^- \quad \text{(Eq 15)}
\]

\[
\text{CDOM} + h\nu \rightarrow ^1\text{CDOM}^* + \text{O}_2 \rightarrow \text{CDOM}^+ + \text{O}_2^- \quad \text{(Eq 16)}
\]

\[
\downarrow
\]

\[
3\text{CDOM}^* + \text{O}_2 \rightarrow \text{CDOM}^+ + \text{O}_2^- \quad \text{(Eq 17)}
\]

\[
\text{CDOM} + h\nu \rightarrow ^1\text{CDOM}^* \rightarrow \text{CDOM}^\pm + \text{O}_2 \rightarrow \text{CDOM}^+ + \text{O}_2^- \quad \text{(Eq 18)}
\]
where CDOM represents an intermolecular charge transfer product of CDOM.

Superoxide can react with organic compounds [56]. The redox reactions of O$_2^-$ are usually able to vary the local pH by consumption or release of H$^+$ in sunlit aqueous systems, with the pH variation potentially comparable to that induced by atmospheric CO$_2$ dissolution. In contrast to other ROS, O$_2^-$ can act as an oxidant as well as a reductant in redox reactions [79]. Consequently, O$_2^-$ redox chemistry might be an important factor in the quantification of acidification of natural waters [80].

1.4.4 Hydrogen peroxide (H$_2$O$_2$)

Hydrogen peroxide (H$_2$O$_2$) is ubiquitous in surface waters. It influences redox chemistry and biological reactions in natural waters and acts as an indicator for DOM photochemical oxidation and as a photic zone tracer in the ocean. It has been known for several decades that photochemical reactions involving HS and CDOM can produce H$_2$O$_2$ in natural waters [47]. Previous works suggested that the photochemical production of H$_2$O$_2$ primarily occurs via O$_2$ reduction through photo-produced transients to generate O$_2^-$ [81], which can subsequently form H$_2$O$_2$ through disproportionation.

In natural waters, O$_2^-$ and H$_2$O$_2$ exert important controls on ambient redox reactions, influence the speciation of trace metals [82], and are involved in production of hydroxyl radicals and other oxidants related to Fenton reactions (see section 1.4.5 below for details) [61]. Previous investigations showed that at least in the UVA and visible regions, hydrated electrons are not involved in the majority of H$_2$O$_2$ production [83], while production by singlet dioxygen reduction is also minor [84]. Work by Zhang et al. [85] has suggested that H$_2$O$_2$ is likely formed via a less efficient intramolecular charge transfer producing reduced intermediates which can react with O$_2$ to generate O$_2^-$ and H$_2$O$_2$.

1.4.5 Hydroxyl radicals (•OH)

Hydroxyl radicals have high second-order kinetic constants, thus the reactions of many persistent xenobiotics with •OH are normally limited by diffusion phenomena,
and the steady-state concentration of \( \cdot \text{OH} \) is quite low in surface waters (from \( 10^{-17} - 10^{-15} \text{ M} \)) [86]. As the most reactive ROS with nonselective oxidant properties in the sunlit zone, hydroxyl radicals are easily scavenged by some components of aquatic systems, most notably DOM [52]. \( \cdot \text{OH} \) is also known to have a significant role in transforming many organic pollutants [52]. As a result, its role during photo transformations in the sunlit zone depends on many factors like environmental conditions and substrate species, which is one of the current research hotspots [70].

Recently, the photochemical formation of \( \cdot \text{OH} \) by CDOM has been a highly active area of research, with some hints that CDOM might be the primary source of \( \cdot \text{OH} \) in most sunlit zone systems [64]. With the effective capability of sunlight absorption, CDOM might be the most important source of \( \cdot \text{OH} \), however the \( \cdot \text{OH} \) yielding process has not yet been confirmed because of the structural complexity of CDOM [87]. The whole process of \( \cdot \text{OH} \) photo-generation by CDOM is suggested to contain at least two primary pathways: \( \text{H}_2\text{O}_2 \) dependent processes and \( \text{H}_2\text{O}_2 \) independent processes, which are more or less equally significant [88].

The \( \text{H}_2\text{O}_2 \)-dependent path for \( \cdot \text{OH} \) formation is suggested to be related to Fenton or Fenton-like reactions [89], while the involvement of \( \text{H}_2\text{O}_2 \) photolysis is still under debate [89]. The Fenton reaction (Eq 21) can occur in surface waters, and both \( \text{H}_2\text{O}_2 \) and Fe(II) can be produced via photochemistry, while Fenton-like reactions can occur when semiquinone radicals are present (Fig 4), and the reduction of Fe(III) to Fe(II) by semiquinones tends to take place and contribute to Fenton reactions as well [90].

\[
\text{H}_2\text{O}_2 + \text{Fe(II)} \rightarrow \text{Fe(III)} + \cdot \text{OH} + \text{OH}^- \quad \text{(Eq 21)}
\]

When electron rich compounds like phenols exist, semiquinone radicals could be formed from quinone fractions of CDOM by photochemical reactions or dark reduction under anoxic conditions [70]. When anoxic systems are aerated, such as when the anoxic water column is in contact with oxygen, semiquinones generated by dark reduction could produce \( \cdot \text{OH} \), which could be significant for transformation of dissolved compounds [91].
In the case of \( \text{H}_2\text{O}_2 \)-independent \( \cdot \text{OH} \) formation by irradiated CDOM, the exact pathways are not clear yet. Many excited triplet states can produce \( \cdot \text{OH} \) by oxidising water or \( \text{OH}^- \), but it is not a general process [70]. The environmental influences on water oxidation need to be elucidated. When \( \text{H}_2\text{O}_2 \)-independent \( \cdot \text{OH} \) formation occurs in the low molecular weight (LMW) fraction of CDOM, \( \cdot \text{OH} \) generation is more efficient, and considered together with the observation that LMW fractions of CDOM yield higher fluorescence quantum yields, a recent assumption is that the internal conversion of HMW fraction CDOM might deactivate the excited states [73].

In surface freshwaters, DOM accounts for \(~90\%\) of \( \cdot \text{OH} \) scavenging [54]. Carbonate and bicarbonate are other important scavengers of \( \cdot \text{OH} \), reacting to produce \( \text{CO}_3^{2-} \) [70]:

\[
\text{HCO}_3^- + \cdot \text{OH} \rightarrow \text{CO}_3^{2-} + \text{H}_2\text{O} \tag{Eq 22}
\]

\[
\text{CO}_3^{2-} + \cdot \text{OH} \rightarrow \text{CO}_3^{3-} + \text{OH}^- \tag{Eq 23}
\]

Overall, the steady state \([\cdot \text{OH}]\) is extremely low, and decreases with increasing concentrations of dissolved organic carbon (DOC), carbonate, and bicarbonate [64]. However, DOC includes both DOM (\( \cdot \text{OH} \) scavenger) and CDOM (\( \cdot \text{OH} \) source); thus the net effect of DOM on reactions involving \( \cdot \text{OH} \) requires more research. Normally, the influence of DOM as a scavenger is stronger than its influence as a source for \( \cdot \text{OH} \) in surface waters, so \( \cdot \text{OH} \) concentrations are generally not correlated with DOC concentrations [93].
1.5 Photo-induced transformation of DOM by iron

Iron species in natural waters significantly impact on global carbon cycles, oceanic primary production and nitrogen fixation [94, 95]. Previous investigations demonstrated that almost 99% of dissolved Fe is associated with DOM, forming various metal bridged complexes [96, 97]. Besides having significant effects on the transformation and function of Fe in natural waterbodies, Fe-DOM complexes also affect the speciation and transformations of various pollutants. A range of environmental factors can potentially influence the complexation and concentration of Fe and DOM in natural waters [98-100].

In hypoxic blackwaters, iron can form stable complexes with HS, resulting in high concentrations of soluble iron complexes that can change the light-absorbing properties of HS in the sunlit zone. It has also been demonstrated that the oxidation of Fe(II) by DO and reduction of Fe(III) is influenced strongly by the presence of various organic compounds including HS [101-105]. Thus Fe(II) and Fe(III) can act as electron transfer catalysts for the oxidation of DOM by DO in natural waters:

\[
\text{Fe(II)} + \text{DOM} + \text{O}_2 \rightarrow \text{Fe(III)} \quad \text{(Eq 24)}
\]

\[
\text{Fe(III)} \rightarrow \text{Fe(II)} + \text{DOM}_\text{ox} \quad \text{(Eq 25)}
\]

Iron species also could influence the reading of in situ fluorescence sensors used in natural water systems, because dissolved Fe(II) and Fe(III) oxyhydroxide colloids can weaken the excitation and emission signals by absorption and light scattering effects. Also, iron quenching can decrease fluorescence emission by DOM [106, 107]. Previous studies with various types of DOM demonstrated a linear relationship between Fe(II) concentration and absorption properties [108].

The photoreduction of Fe(III)-organic complexes generates Fe(II)-organic complexes, and thus light could accelerate the redox cycling of Fe species, thereby inducing O\(_2\) consumption [92]. In the sunlit zone, photo-induced transformation of iron species such as Fe(III) compounds and iron-organic complexes is regarded as a significant factor affecting the bioavailability of iron. The primary processes for photo-induced Fe(III) reduction involve: (i) superoxide-mediated iron reduction (SMIR), in which Fe(III) is reduced by photo-generated superoxide [82]; (ii) ligand-to-metal charge transfer (LMCT) induced by Fe(III), as shown in Fig 5; (iii) direct electron transfer to Fe(III) by organic donors within CDOM; and (iv) direct Fe(III)
reduction by reduced products of NOM photolysis [109]. Some works have showed that SMIR was the major route of photo induced Fe(III) reduction, however this is limited to near neutral or alkaline pH, with SMIR being less important under acidic conditions [40, 110]. Although there have been a few suggestions, the significance of LMCT or short-lived organic intermediates for the reduction of Fe(III) has not been well elucidated at low pH.

![Figure 5](image)

**Figure 5**  Mechanisms for transformation of iron compounds by irradiation of HS with sunlight at pH 4 (adapted from [111]).

The persistence of photochemically generated Fe(II) in sunlit water is also related to oxidation processes [103]. Recently, it has been suggested that long-lived semiquinone-like organic species generated during irradiation of HS are significant Fe(II) oxidants in acidic solutions [102]. Whilst other oxidants possibly involve ROS [112], the roles of short-lived oxidising organic species (such as phenoxyl radicals and organic peroxides) under typical natural conditions are still far from well-known.

Due to its impact on hydrolysis, polymerisation and precipitation, pH is a significant impact factor for the speciation of Fe in aquatic systems [113, 114]. DOM, pH and redox potential determine the photochemical reactions of Fe [115]. For example, the reactions of deoxygenation and cycling between Fe(II) and Fe(III) were found to be strongly related to pH and redox potential [115]. A few studies have investigated the photochemical properties of CDOM and the influence of Fe concentration on these properties [39, 116, 117], and it has also been revealed that pH is highly influential for these processes [110, 118-120]. Still, little is understood about the association between Fe and DOM and the resulting photochemical properties [117]; thus more studies at different pH values are required to understand the influence of pH on the light-induced redox reactions of Fe when NOM is present.
1.6 **Study aims**

The above review demonstrates that absorbance of solar radiation by DOM impacts photic zone depths [121-125], and generates various photochemical reaction products which can in turn generate low molecular weight compounds and/or inorganic carbon [124-130], alter trace metal (such as Fe and Mn) redox status [131, 132], change the forms of aquatic components [78, 133-135], and influence aquatic oxygen consumption [22-24, 136]. The optical properties and photochemical reactivity of DOM change with season, DOM source, and chemical properties of the aquatic system [36, 83, 137-139]. Previous studies suggested that the properties of DOM are highly relevant to electron transfer reactions, and therefore the electron donating or accepting capacity of DOM becomes a significant parameter for study [59]. Because of the complex correlations between environmental factors, optical properties of CDOM, electron transfer, and related photochemical reactions, it is essential to understand how these processes vary and interact to impact deoxygenation during post flood hypoxia blackwater events.

Hypoxic blackwater formation is obviously associated with deoxygenation processes and high DOM concentration. The optical properties of CDOM have been found to be influenced by a variety of physical, chemical and biological environmental factors such as season, temperature, salinity, CDOM source and concentration, and chlorophyll concentration [32, 33]. However, few studies have investigated the photochemical consumption of DO under these specific conditions. The few previous studies of the photochemical DO consumption in natural surface waters have measured rates varying from about 17 µM O₂ h⁻¹ to 0.2 µM O₂ h⁻¹ [22, 24, 120]. The impact of this on DO concentration in healthy natural waters, which ranges from 187.5 to 437.5 µM L⁻¹ [140], will vary depending on the timescale on which these processes apply. However, photochemical oxygen consumption would be expected to be more significant in low DO conditions, such as during hypoxia blackwater events which have less than 62.5 µM L⁻¹ DO. Furthermore, the relationships between spectral properties, electron donating properties, and the photochemical deoxygenation potential of DOM have not been thoroughly studied either. In particular, the influence of photochemistry on these processes remains very poorly understood. In this context, this thesis aimed to study the role of photochemistry in generating hypoxia in coastal blackwaters.

This thesis mainly focused on investigating the photochemical DO consumption by NOM in irradiated surface floodwaters, with regard to the influence of NOM...
properties and environmental factors. Selected properties of NOM were also studied to gain insight into the processes involved.

Three primary results chapters are presented, as follows:

Chapter 2: Deoxygenation potential of model humic substances under simulated solar irradiation

This study aimed to characterise the magnitude of DO consumption associated with photo-induced reactions of DOM, and to examine how this varied with different DOM type and concentration under a controlled range of experimental conditions representative of those found in blackwaters.

The study used three well characterised standard humic substances from the International Humic Substances Society (IHSS) as model DOM, and a low pressure xenon lamp solar simulator as the irradiation source. Temperature and pH were controlled. Dissolved oxygen concentration was monitored essentially continuously during irradiation, while DOM optical properties including SUVA_{280} and E_{2}/E_{3} and DOM redox state were measured at regular intervals.

Chapter 3: Post-flood hypoxic blackwater field study

This chapter aimed to study the associations of optical properties, redox properties and deoxygenation capability in natural hypoxic blackwater. The Tuckean Swamp, Rocky Mouth Creek, and Bungawalbyn/Sandy Creek wetlands in the Richmond River floodplain system were selected as the study sites. Field measurements were performed, and water samples acquired for analysis, on 13 April 2017 and 18 May 2017 after a 1 in 30 year flood in the Richmond River system.

In situ measurements used portable probes and water samples were analysed for key geochemical parameters. Spectral properties and redox state and were also measured on all water samples.

Chapter 4: Influence of Fe and pH on photochemical DO consumption by NOM

As the results of the field study in Chapter 3 indicated correlations between deoxygenation, organic carbon, pH and iron, this chapter aimed to gain insights into the influence of Fe concentration and pH on photo-induced deoxygenation during irradiation of DOM. Similar experimental methods were employed to those in Chapter 2. In the first part of the experiment, Fe(III) was added into solutions containing 50 mg/L of a standard HS from the IHSS and pH adjusted over a range of
values. In addition, photochemical DO consumption was measured in water samples collected as part of the field study presented in Chapter 3.

The study implemented Central Composite Design (CCD) optimised combinations of pH and Fe concentration to describe the dependency of deoxygenation across the range of variability considered using a multivariate linear regression. The chapter also compared photochemical deoxygenation, optical and redox properties of Fe-DOM solutions prepared from the standard HS with field study results to link the laboratory studies under highly controlled conditions with observations in the field.
Chapter 2 – Deoxygenation processes of model humic substances under simulated solar irradiation

2.1 Introduction

Dissolved oxygen (DO) is a critical parameter related to a range of processes in natural aquatic systems including, for example, the rate of primary production in the oceans, and organic matter oxidation in sunlit waterbodies and sediments [141-143]. DO concentration directly and indirectly influences various chemical and biological reactions and is essential for most aquatic life. In human society, drinking water containing sufficient DO is better for taste and health as well [140]. Thus, understanding processes related to DO production and consumption in aquatic systems is of fundamental importance.

Low DO concentration is a key characteristic of coastal hypoxic blackwater, an environmental problem which is attracting growing attention globally. Blackwater formation is considered a natural post-flood occurrence in coastal river systems, especially after dry seasons with high temperatures [9, 20, 144]. The mobilisation of high loads of dissolved organic carbon (DOC) from terrestrial plants and forests can stimulate DO consuming processes such that they outstrip the rate of DO production and replenishment, resulting in a low DO concentration defined as hypoxia [9, 144, 145]. Blackwater created on floodplains can enter rivers during flood recession, potentially seriously impacting riverine water quality [9]. Thus, the quality of surface water can be strongly impacted by the interactions between floodplains and coastal rivers via organic matter decomposition during episodic floods [1, 4, 8].

Sudden DO depletion in severe hypoxic blackwater events can pose a significant threat to ecosystem health and water quality [4]. In the short-term, hypoxic blackwater is detrimental for native fish or other organisms, while at the same time having social and economic influences including high costs for treating water and the suspension of commercial and recreational fishing [9, 146]. In the Richmond River system in north-eastern NSW, Australia, which is the focus of this thesis, low DO concentration (< 2 mg/L) has typically been found to develop approximately 6 to 10 days after the flood peak in summer or early autumn [3, 114]. Previous investigations of deoxygenation during blackwater events have considered a range of factors...
including impacts of temperature [147], and dissolved organic matter (DOM) related reactions with bacterioplankton [148], phytoplankton [149], macrophytes [149] and heavy metals [4, 82, 101, 104, 105, 109, 111, 112, 132, 139, 150-158]. Related consequences of hypoxic blackwater have also been studied, such as fish mortality [9, 159], zooplankton emergence [160], and the corresponding coastal area management [1, 5, 12, 144, 161, 162].

While DO dynamics are known to be strongly influenced by biological processes such as photosynthesis and respiration, abiotic photochemical processes may also be important in some systems [163-165]. A number of studies have indicated that the oxygen demand induced by photochemical processes in the surface sunlit section of ocean is of the same order as oxygen production through photosynthesis [166]. Photochemical oxygen consumption processes in natural surface waters could thus be a significant portion of the global oxygen budget but have so far been largely ignored.

Very few studies have examined the magnitude or kinetics of photochemical DO consumption during irradiation of NOM. Reported photochemical oxygen loss measurements in natural water systems were generally limited by the DO measuring methods because of the relatively small DO changes. In one of the earlier studies on the subject, Miles and Brezonik [113] demonstrated that the oxygen consuming process occurred at a ratio of one mole of oxygen consumed per two moles of CO$_2$ produced. Miles and Brezonik [113] proposed a decarboxylation mechanism to support this ratio corresponding to the stoichiometry of:

$$2RCOOH + O_2 \rightarrow 2ROH + 2CO_2 \quad \text{(Eq 26)}$$

This mechanism assumes that oxygen is not consumed by any other photochemical reactions. This ratio might vary due to the redox state of DOM (e.g. more oxidised DOM may consume less O$_2$ to produce CO$_2$), other possible photochemical O$_2$ consuming processes [23, 81, 85, 167, 168] and microbial processes [169, 170]. Other studies in natural waters found an approximate molar ratio of O$_2$ consumption to CO$_2$ production ranging from 0.8 to 1.2 [22, 23, 120, 171, 172], while Estapa and Mayer [173] found values of about 1.3 for irradiated suspensions of marine sediments. These measured values were more than the theoretical stoichiometric ratio, which is 0.5 according to Eq 26. This suggested that oxygen consumption also occurred via processes other than the photochemical pathway shown in Eq 26, and
that these processes generated less CO₂. Amon and Benner [22] showed that the molar ratio of DOC to O₂ consumed during photochemical processes in a main tributary of Amazon River system was about 1.11–1.14 in all photooxidation experiments. This suggested that DOC underwent additional reactions to that shown in Eq 26.

Most previous studies of the photochemistry of natural organic matter (NOM) and dissolved organic carbon (DOC) primarily examined relationships between optical, electrochemical, and photochemical properties, including absorption spectral slopes, fluorescence indices and electron donating capacities [174-176]. Photochemical dissolved oxygen consumption by irradiation of NOM has been broadly neglected, and there is a noticeable knowledge gap about the magnitude and kinetics of DO depletion in natural waters by this process.

This study aimed to better constrain the magnitude of photochemical DO consumption during irradiation of NOM induced by simulated sunlight using International Humic Substances Society standard and reference humic substances (HS) as model dissolved organic matter. A previous study by Sharpless et al. [174] demonstrated that photooxidation decreased electron donating capacities but had less impact on the electron accepting capacities. The correlation between optical and redox properties varied between aquatic and soil HS [174]. In order to investigate the influence of HS type on photochemical deoxygenation processes, one aquatic fulvic acid and two soil humic acids were used in this study.

DO consumption was monitored during irradiation under controlled pH and temperature. Optical and redox properties of the NOM were also analysed to gain insight into the chemical mechanisms involved. The Clark electrode method was chosen for measurement of DO concentration in this study, because it is subject to minimal interferences in the presence of simulated solar irradiation and humic substances [177], and offers advantages over other common methods for DO measurement (see Table 1). Based on the sensor specifications from the supplier and as verified experimentally, the microelectrode probe used in this study has a very small DO consumption rate during operation. As such, DO consumption by the sensor was expected to be negligible compared to photochemical DO consumption rates under the conditions investigated.
Table 1  Methods for dissolved oxygen measurement

<table>
<thead>
<tr>
<th>Method</th>
<th>Detection principle</th>
<th>Limitations</th>
<th>Required sample volume</th>
<th>Detection limit</th>
<th>Response time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winkler’s method</td>
<td>Iodometric colorimetry</td>
<td>Not continuous; interference from NOM, nitrites, iron, reducing and/or oxidising agents.</td>
<td>~1 mL</td>
<td>3.0 μM</td>
<td>1 min (operation time not included)</td>
</tr>
<tr>
<td>Gas chromatography</td>
<td>Gas chromatography</td>
<td>Excessive time &amp; cost</td>
<td>~2 mL</td>
<td>0.05 mg/L</td>
<td>~3 min (operation time not included)</td>
</tr>
<tr>
<td>Clark electrochemical sensor</td>
<td>Polarographic type or galvanic cell type</td>
<td>Consumes oxygen (4 x 10^-4 – 5 x 10^-3 nmol/h with the sensor used in this study)</td>
<td>Enough to submerge the probe tip</td>
<td>0.3 μM</td>
<td>1 min</td>
</tr>
<tr>
<td>Optical sensor</td>
<td>Oxygen molecular Fluorescence quenching</td>
<td>Interference from light, NOM (from this study)</td>
<td>Enough to submerge the probe tip</td>
<td>0.004% oxygen at 1 atm</td>
<td>&lt; 1 s</td>
</tr>
</tbody>
</table>

2.2  Experimental Section

2.2.1  Reagents

All reagents used in this study were prepared with analytical grade (> 99% purity) chemicals and high purity water from a Milli-Q Academic system (18.2 MΩ.cm resistivity, Millipore, USA). Leonardite humic acid standard (LHA, 1S104H), Suwannee River fulvic acid standard (SRFA, 2S101H) and Pahokee Peat humic acid standard (PPHA,1S103H) were purchased from the International Humic Substances Society (St Paul, MN). For each type of HS, a solution of 100 mg/L HS was made in 0.1 M phosphate buffer (made from NaH₂PO₄ and NaH₂PO₄), adjusted to pH 7.00 (± 0.02 pH units) by addition of 0.01 M NaOH, and equilibrated at room temperature for approximately 24 h. All experiments reported in this chapter were performed in 0.1 M phosphate buffer at pH 7. Additional HS solutions were prepared at 75 mg/L, 50 mg/L and 10 mg/L by dilution of the 100 mg/L solution with 0.1 M phosphate buffer at pH 7.00 ± 0.02 prepared as described above. All HS solutions were filtered through a 0.45 μm pore size polyether sulfone filter membrane before use in experiments. A 500 μM 2,6-dichlorophenol-indophenol sodium salt hydrate (DCPIP; Sigma) solution was also prepared in 0.1 M phosphate buffer at pH 7.00 ± 0.02 as described above, then equilibrated at 25°C for 24 h before use.
2.2.2 Photochemical experimental system

The photochemical experimental system consisted of three main parts: a light source, reaction cell and temperature control system. The reaction cell was a water-jacketed cylindrical quartz cuvette of 1.0 cm length and 2.5 cm diameter. The irradiation beam was applied perpendicular to the circular face, and the illuminated depth of sample was 1 cm. However, the effective optical pathlength was determined to be 3.4 cm based on actinometry measurements (see Appendix 1). The cell had a Teflon stopper through which was drilled a small hole with a diameter just large enough to insert the DO probe, and an outer water jacket connected to the temperature-controlled water bath. The cell was illuminated by a 150 W ozone free Xe lamp (Oriel 6255) equipped with an AM1 air mass filter to produce a spectrum in the UV-visible region similar to that of midday solar radiation at the Earth’s surface. The lamp was also fitted with a water filter (Oriel 61945) to decrease the intensity of infrared wavelengths that mainly induce heating instead of photochemical reactions.

Irradiation experiments were carried out in triplicate using the solar simulator system. Temperature was maintained at 25°C with a recirculating water bath. The Xenon lamp was positioned immediately in front of the quartz reactor cell to illuminate a 1 cm deep section of sample. Initially, long duration (72 h) irradiation experiments were performed to identify the optimal duration over which to monitor DO consumption. Subsequently, 7 h was chosen to use as the irradiation duration for all the experiments in this study. Aliquots of solution were sampled before and after each run experiment to study the influence of irradiation on optical properties and electron donation capacity (EDC) of standard HS. Any possible effects of non-photochemical processes were observed in parallel using a foil-covered control cell installed in series immediately behind the sample cell to ensure the temperature was the same as that in the irradiated reaction cell.

DO concentrations were measured using a Clark-type needle electrode (Unisense model OX-NP). The detection limit of the DO sensor used in this study is 0.3 μM with 1 min response time. The probe was calibrated using MQ water that had been vigorously purged with air for 10 min as a 21% DO standard, and standard zero dissolved oxygen solution (5% sodium sulfite in deoxygenated water with a trace of cobalt chloride, Australian Scientific) as 0% DO solution. Before each experiment, the two rubber tubes connecting the water bath to the joints on the water filter attached to the lamp were switched to make sure the water bath circulation system
operated smoothly. Before turning on the lamp, the probe was pre-polarised in the HS sample for about 20 h. Subsequently, the lamp was ignited for an irradiation duration of 7 h and DO concentration recorded every 20 s. The recorded data were averaged in 20 min intervals for subsequent analysis. The metal jacketed side of the DO probe tip was set towards the irradiation beam while DO was recorded to minimise any influence of light on the signal of the DO sensor (although no influence was expected). After each run, the probe was again put in 0% DO standard solution and air saturated MQ water to verify the calibration.

Dark oxygen loss rates due to oxygen consumption by the probe were measured before and after irradiation experiments and found to be negligible over the irradiation duration. Thus, oxygen consumption by the probe was ignored during calculations of oxygen consumption rates in all irradiation experiments.

2.2.3 Rate and magnitude of DO consumption

DO consumption data were modelled by non-linear regression using a first-order decay function (one phase exponential decay equation) with GraphPad Prism 7.03. The fitting routine calculated the initial DO concentration, ultimate DO concentration, and first-order decay constant. The magnitude of DO consumption, ΔDO, was then calculated as the difference between the fitted initial and ultimate values of DO concentration.

The ultimate value of DO concentration calculated from the first-order decay model fit did not always equal the measured DO concentration after 7 h irradiation, since at high HS concentrations the DO concentration did not reach its ultimate value after 7 h.

2.2.4 Optical property measurement

Samples were taken before irradiation commenced and immediately after the irradiation finished for analysis of UV-visible absorbance and EDC. The UV-visible absorbance of the samples was measured from 200 nm to 600 nm in 1 nm intervals with a Clariostar microplate spectrophotometer (BMG Labtech), using the absorbance spectrum of MilliQ water as a reference. Analysis was performed in 96 well microplates (Corning® 96 Well Microplates, with UV transparent flat bottom, sterile acrylic), with 0.1 M pH 7 phosphate buffer as blanks. Samples were scanned three times and the average calculated to determine absorbance. Absorbance values
Chapter 2 – Deoxygenation processes of model humic substances under simulated solar irradiation

Understanding the Photochemistry of Coastal Hypoxic Blackwater

at each wavelength were converted into absorption coefficients, $a(\lambda)$, using the Beer-Lambert law:

$$a(\lambda) = 2.303 \frac{\text{Abs}(\lambda)}{L}$$

(Eq 27)

where $\text{Abs}(\lambda)$ is the measured absorbance at wavelength $\lambda$ and $L$ is the optical path length in metres.

Spectral parameters were calculated based on the following equations [28]:

$$\frac{E_2}{E_3} = \frac{a(254 \text{ nm})}{a(365 \text{ nm})}$$

(Eq 28)

$$\text{SUVA}_{280} = \frac{\text{Abs}(280 \text{ nm})}{[\text{TOC}]}$$

(Eq 29)

2.2.5 Electron donation capacity analysis

The EDC of samples was determined with a spectrophotometric assay using the nonradical organic mediator 2,6-dichlorophenol-indophenol (DCPIP) based on a procedure adapted from Aeschbacher et al. [178]. Oxidised DCPIP is blue ($\lambda_{\text{max}} = 603 \text{ nm}$), while reduced DCPIPH$_2$ is colourless. For each sample, a dilution series was prepared in duplicate in the wells of a 96 well microplate (Corning® 96 Well Microplates, with UV transparent flat bottom, sterile acrylic) by diluting the sample with various volumes of phosphate buffer at pH 7 to create a concentration gradient for DCPIP analysis, as shown in Table 2, resulting in total volumes of 270 µL in each well. Two additional wells (well number 11 and 12) were also filled with 300 µL of undiluted sample, to determine $\frac{E_2}{E_3}$ and SUVA$_{280}$ in the same microplate.

Table 2: Volumes of sample and various reagents (in µL) added to microplate wells for determination of the optical properties and redox state of HS solutions

<table>
<thead>
<tr>
<th>Well number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>150</td>
<td>150</td>
<td>200</td>
<td>200</td>
<td>270</td>
<td>270</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>pH buffer</td>
<td>270</td>
<td>270</td>
<td>170</td>
<td>170</td>
<td>120</td>
<td>120</td>
<td>70</td>
<td>70</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DCPIP</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The absorbance spectrum of each well was measured from 400 to 800 nm using a Clariostar microplate spectrophotometer (BMG Labtech) to determine the absorbance resulting from the HS alone. Subsequently, 30 µL of 500 µM DCPIP was added to each well, resulting in a final concentration of 50 µM DCPIP. After waiting 10 min for the reaction between the HS and DCPIP to occur, the absorbance spectrum in each well was again measured from 400 to 800 nm. The electron donating capacity
was then determined from the absorbance data in the absence and presence of DCPIP based on the following theory.

The reaction between HS and DCPIP is as follows:

$$2\text{HS}_{\text{red}} + \text{DCPIP} \rightarrow 2\text{HS}_{\text{ox}} + \text{DCPIP}_{\text{red}}$$  \hspace{1cm} (Eq 30)

where the subscripts ox and red denoted the oxidised and reduced form of the compound, respectively, and the coefficient 2 represents the stoichiometry of electron transfer (two electrons transferred per molecule of DCPIP reduced).

The relationship between EDC and DCPIP reduction is thus:

$$\text{EDC} = 2[\text{DCPIP}]_{\text{red}}/[\text{HS}]_{\text{T}}$$  \hspace{1cm} (Eq 31)

where the subscript T denotes the total concentration in mg/L.

The measured absorbance at 603 nm, $A_{\text{ST}}$, in the wells containing HS and DCPIP is given by:

$$A_{\text{ST}} = A_{\text{HS}} + A_{\text{DCPIP}}$$

$$= \varepsilon_{\text{HS}} [\text{HS}]_{\text{T}} + \varepsilon_{\text{DCPIP}} [\text{DCPIP}]_{\text{ox}}$$

$$= \varepsilon_{\text{HS}} [\text{HS}]_{\text{T}} + \varepsilon_{\text{DCPIP}} ([\text{DCPIP}]_{\text{T}} - [\text{DCPIP}]_{\text{red}})$$

$$= \varepsilon_{\text{HS}} [\text{HS}]_{\text{T}} + \varepsilon_{\text{DCPIP}} [\text{DCPIP}]_{\text{T}} - \varepsilon_{\text{DCPIP}} 0.5\text{EDC} [\text{HS}]_{\text{T}}$$

$$= \varepsilon_{\text{DCPIP}} [\text{DCPIP}]_{\text{T}} + (\varepsilon_{\text{HS}} - \varepsilon_{\text{DCPIP}} 0.5\text{EDC}) [\text{HS}]_{\text{T}}$$ \hspace{1cm} (Eq 32)

where $A_{\text{HS}}$ is the absorbance (at 603 nm) of the HS in the well, $A_{\text{DCPIP}}$ is the absorbance (at 603 nm) of the DCPIP in the well, $\varepsilon_{\text{HS}}$ denotes the absorption coefficient of HS, and $\varepsilon_{\text{DCPIP}}$ denotes the absorption coefficient of oxidised DCPIP. Hence, linear regression of $A_{\text{ST}}$ against $[\text{HS}]$ in samples containing DCPIP yields $(\varepsilon_{\text{HS}} - \varepsilon_{\text{DCPIP}} 0.5\text{EDC})$ as the slope from the regression and $\varepsilon_{\text{DCPIP}}[\text{DCPIP}]_{\text{T}}$ as the intercept. The EDC was therefore calculated from the measured absorbance data as follows:

1. Linear regression of the absorbance at 603 nm against the HS concentration in the dilution series prior to addition of DCPIP was performed to determine $\varepsilon_{\text{HS}}$ as the slope from the regression.
2. Linear regression of the absorbance at 603 nm against the HS concentration in the dilution series after addition of DCPIP was performed to determine ($\varepsilon_{HS} - \varepsilon_{DCPIP0.5EDC}$) as the slope and $\varepsilon_{DCPIP}[DCPIP]_T$ as the intercept.

3. EDC was calculated as:

$$EDC = 2[DCPIP]_T \left( \frac{\text{slope}_2 - \text{slope}_1}{\text{intercept}_1} \right)$$

where slope$_1$ is the slope from the linear regression in step 1, slope$_2$ is the slope from the linear regression in step 2, and intercept$_1$ is the intercept from the linear regression in step 1.

2.2.6 Kinetic model for DO consumption in natural waters

To understand the potential significance of the laboratory measurements for photochemical DO consumption in river waters, a kinetic model was constructed to estimate the apparent diel-averaged rate constant for DO consumption as a function of HS concentration and water depth. The model assumed that the absorbance values were constant over the entire irradiation duration for a particular type of NOM and the measured output from the solar simulator lamp is equivalent to midday sun with sinusoidal variation over a 12 hour daylight cycle (i.e. sunrise at 6 am, sunset at 6pm) for incident light calculation, and only photons < 400 nm contributed measurably to photochemical DO consumption. The rate constants for photochemical DO consumption used in the model were those calculated from the non-linear regression fit in irradiated DO consumption experiments. The apparent rate constant for a given water depth and time of day was then calculated by multiplying the measured rate constant by the ratio of the absorbed photons at the actual water depth of interest to that for a 3.4 cm optical pathlength at midday.

2.3 Results and Discussion

2.3.1 DO consumption during irradiation

DO concentration decreased during a 7 h exposure to simulated solar irradiation for all HS types and concentrations investigated (Figure 6). The magnitude of DO consumption ($\Delta$DO) determined from the fit of the first-order decay model by non-linear regression ranged from about 13.9 $\mu$mol/L to 144.6 $\mu$mol/L over the 7 h irradiation for all three standard humic substances (Table 3).

All HS solutions exhibited greater $\Delta$DO with increasing TOC concentration (Figure 7A). For the same TOC concentration, LHA displayed the highest DO consumption,
while SRFA showed the lowest DO consumption. The relationship between TOC and ∆DO was well described by an exponential equation for each HS and for all HS solutions overall. However, statistical testing using an $F$-test indicated that fitting a single equation to all data for all HS types was the best model (Table 4). This suggested that the HS type had less influence than TOC concentration. The best fit model was given by the relationship:

$$\Delta DO = 14.81 e^{0.03413[TOC]} \quad \text{(Eq 34)}$$

where $\Delta DO$ is the magnitude of DO consumption ($\mu$mol/L).

For LHA and PPHA, the highest rate constants for DO consumption were 0.45 and 0.51 h$^{-1}$ with 50 mg/L HS (equivalent to 31.0 mg/L and 21.9 mg/L TOC, respectively), while for SRFA the highest rate constant for DO consumption was 0.29 h$^{-1}$ with 75 mg/L HS (equivalent to 39.5 mg/L TOC). The rate constants for photochemical DO consumption tended to decrease at HS concentrations above or below these values (Figure 7B), implying the existence of an optimum. Table 21 in Appendix 1 shows that 24.8 to 99.7% photons were absorbed by HS in the wavelength range from 300 to 400 nm over a 3.4 cm effective optical path length for HS concentrations from 10-200 mg/L, respectively. Thus, the total absorbed photon dose becomes approximately constant at high HS concentrations (>100 mg/L) when all the photons are absorbed. This is expected to be the main cause of decreasing rate constants at higher HS concentrations. Therefore, the optimum in Figure 7B represents the combined effects of photon absorption and photochemical DO consumption.
Table 3  Irradiation induced DO consumption in LHA, SRFA and PPHA solutions

<table>
<thead>
<tr>
<th>HS concentration (mg/L)</th>
<th>TOC (mg/L)</th>
<th>ΔDO (μmol/L)</th>
<th>First order rate constant for DO consumption (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6.38</td>
<td>13.9 ± 7.7</td>
<td>0.09 ± 2.01 x 10⁻²</td>
</tr>
<tr>
<td>50</td>
<td>31.9</td>
<td>60.0 ± 0.9</td>
<td>0.45 ± 1.47 x 10⁻⁴</td>
</tr>
<tr>
<td>75</td>
<td>47.9</td>
<td>94.2 ± 0.7</td>
<td>0.32 ± 8.25 x 10⁻⁷</td>
</tr>
<tr>
<td>100</td>
<td>63.8</td>
<td>144.6 ± 6.1</td>
<td>0.21 ± 5.04 x 10⁻⁷</td>
</tr>
<tr>
<td>SRFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.26</td>
<td>18.8 ± 4.2</td>
<td>0.20 ± 4.55 x 10⁻⁷</td>
</tr>
<tr>
<td>50</td>
<td>26.3</td>
<td>30.8 ± 4.5</td>
<td>0.17 ± 3.79 x 10⁻⁷</td>
</tr>
<tr>
<td>75</td>
<td>39.5</td>
<td>43.9 ± 0.7</td>
<td>0.29 ± 7.46 x 10⁻⁷</td>
</tr>
<tr>
<td>100</td>
<td>52.6</td>
<td>69.4 ± 3.4</td>
<td>0.25 ± 6.19 x 10⁻⁷</td>
</tr>
<tr>
<td>PPHA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5.64</td>
<td>17.5 ± 0.6</td>
<td>0.31 ± 8.21 x 10⁻⁷</td>
</tr>
<tr>
<td>50</td>
<td>28.2</td>
<td>43.3 ± 0.3</td>
<td>0.51 ± 1.89 x 10⁻⁶</td>
</tr>
<tr>
<td>75</td>
<td>42.3</td>
<td>68.4 ± 0.9</td>
<td>0.34 ± 9.32 x 10⁻⁷</td>
</tr>
<tr>
<td>100</td>
<td>56.4</td>
<td>85.0 ± 1.5</td>
<td>0.28 ± 7.23 x 10⁻⁷</td>
</tr>
</tbody>
</table>

* Best fit value and standard error from non-linear regression fit of first-order decay model

Table 4  Exponential relationships (Y = Y₀eᵏˣ) between TOC and ΔDO for each HS solution and for all HS solutions considered together. Best fit parameters were determined by non-linear regression using GraphPad Prism. An F-test indicated that the relationship was best described by a single equation for all HS types together.

<table>
<thead>
<tr>
<th>HS type</th>
<th>Best-fit parameters for ΔDO vs TOC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y₀</td>
</tr>
<tr>
<td>LHA</td>
<td>16.70</td>
</tr>
<tr>
<td>SRFA</td>
<td>15.10</td>
</tr>
<tr>
<td>PPHA</td>
<td>17.95</td>
</tr>
<tr>
<td>All HS</td>
<td>14.81</td>
</tr>
</tbody>
</table>
Chapter 2 – Deoxygenation processes of model humic substances under simulated solar irradiation

Figure 6  Measured DO concentration during 7 h irradiation of standard HS solutions with simulated sunlight. A: LHA. B: PPHA. C: SRFA. In each panel, symbols represent the mean from at least three replicate experiments and lines represent the best fit first-order decay model from non-linear regression (---x: 10 mg/L HS, ---x: 50 mg/L HS, ---x: 75mg/L HS, ---x: 100 mg/L HS).
Figure 7  Relationship between rates and magnitude of DO consumption and TOC in standard HS solutions after 7 h irradiation with simulated sunlight. A: Magnitude of DO consumption. B: first order rate constant for DO consumption. In all panels, symbols represent different HS types (○:LHA, ◇:PPHA, □:SRFA) and error bars represent the 95% confidence interval (CI) of the mean (for most points, error bars are smaller than the size of the symbols). Lines in panel A represent the best fit of linear regression for each HS type (—:best fit trend line for SRFA, —:best fit trend line for PPHA, —:best fit trend line for LHA). The line in panel B represents the best fit to all data of an exponential relationship.

2.3.2  Relationship between DO consumption and optical properties
Exposure to simulated solar irradiation caused photobleaching in all the standard HS over time, as indicated by a decrease in SUVA$_{280}$ (Figure 8). This is consistent with previous work, which demonstrated that irradiation caused the SUVA$_{280}$ to decrease and E$_2$/E$_3$ to increase [34, 180-182]. Differences were found between the influence of irradiation on the optical properties of the two terrestrial HS (LHA and PPHA).
and the aquatic HS (SRFA). Specifically, after 7 h irradiation, SUVA\textsubscript{280} of LHA changed from 0.050 ± 0.0013 to 0.046 ± 0.0008, SUVA\textsubscript{280} of PPHA changed from 0.051 ± 0.0028 to 0.048 ± 0.0025, and SUVA\textsubscript{280} of SRFA changed from 0.030 ± 0.0021 to 0.029 ± 0.0023.

SRFA displayed the smallest decrease in SUVA\textsubscript{280} after 7 h irradiation in absolute terms for DO consumption (Figure 8). LHA and PPHA showed similar magnitude and range in terms of absolute decrease in SUVA\textsubscript{280}. Changes in SUVA\textsubscript{280} with different TOC concentrations were also greater for LHA and PPHA, with greater change in SUVA\textsubscript{280} at lower TOC concentrations. In contrast, SUVA\textsubscript{280} did not vary much in relation to TOC concentration for SRFA. These data suggest that SRFA might be more resistant to photooxidation than LHA and PPHA. However, this effect could be exaggerated by inner-filtering in solutions with higher HS concentration and for HS types with more chromophores, since this can lead to a higher fraction of energy being absorbed in the visible wavelength range, and visible radiation is known to induce considerably less photobleaching than UV radiation [181-183].

All three HS solutions showed decreases in SUVA\textsubscript{280} and increases in $E_2/E_3$ associated with photobleaching, which can be related to loss of aromatic groups and decreasing molecular weight. It is generally accepted that the molecular size of DOM can be estimated based on the ratio of absorption coefficients $E_2/E_3$, with decreasing ratios indicating increasing molecular size. Lou et al. [184] found that the relationship between molecular weight and $E_2/E_3$ can be well fitted by a quasi-exponential function, such that the $E_2/E_3$ ratio could serve as a proxy to roughly estimate the molecular weight change induced by irradiation.

The association between molecular weight and $E_2/E_3$ has been considered to derive from an increased potential for electronic effects between the chromophores of larger DOM molecules. In particular, intramolecular charge transfer complexes, which possess electron donor and acceptor capability, generate broad absorbance bands in the visible and near UV range [185-187]. SUVA\textsubscript{280}, which is obtained by normalising absorption to organic carbon concentration, has been applied to characterise HS and DOM in terms of how strongly these materials absorb light on a mass-carbon basis. For isolated HS, SUVA has been reported to increase with increasing molecular weight and percent aromaticity, and is generally larger for HA than FA [37, 39, 181, 188]. These relationships have been used to infer the molecular size of DOM in
different environments [180, 188-190]. In this study, the increase in $E_2/E_3$ after irradiation suggests that destruction of charge transfer complexes may have been associated with a decrease in molecular weight as well as with photooxidation of acceptor/donor moieties in DOM. Humic acids contain more aromatic components and higher molecular weights than fulvic acids, which suggests that the terrestrial DOM may form radicals more efficiently or have longer lifetimes than microbial DOM [59]. Measured absorbance values of SRFA in this study were < 1, while those for PPHA and LHA were less than 1 for concentrations less than 10 mg/L. The absorbance values of PPHA and LHA at higher concentrations were > 1 which meant that > 90% light had been absorbed. This led to less accurate results.

Previous studies have found a reasonable linear relationship between $E_2/E_3$ and measured quantum yield $^1\text{O}_2$, regardless of pH, for various natural water samples [57, 188]. This trend is due to the relationship between increasing photosensitisation efficiency and decreasing molecular weight in DOM [188]. The decrease in molecular weight during irradiation is consistent with the fact that DOM photodegradation may involve reactions between DOM and reactive oxygen species, thus consuming $\text{O}_2$ [164]. Energy transfer and electron transfer are two pathways through which DOM could consume $\text{O}_2$ to produce $^1\text{O}_2$, or $\text{O}_2^-$, as discussed in Chapter 1.
Figure 8  Relationships between the change in SUVA$_{280}$ in standard HS solutions after 7 h irradiation with simulated sunlight and A: ΔDO and B: TOC. ○: LHA, ◇: PPHA, □: SRFA. Error bars representing the 95% confidence interval (CI) of the mean are smaller than the symbols for all data points.
Figure 9  Relationships between the change in $E_2/E_3$ in standard HS solutions after 7 h irradiation with simulated sunlight and A: $\Delta DO$ and B: TOC. ○:LHA, ◇:PPHA, □:SRFA. Error bars representing the 95% confidence interval (CI) of the mean are smaller than the symbols for all data points.

2.3.3  Relationship between DO consumption and electron donation capacity

The EDC and irradiation induced EDC changes of all standard HS solutions are shown in Table 5, Figure 10 and Figure 11. The EDC value for LHA before irradiation (mean ± standard deviation of all HS concentrations, excluding outliers) was $30.0 \pm 19.1 \mu$mol/g HS, while values for SRFA and PPHA were $7.3 \pm 1.9 \mu$mol/g HS and $31.5 \pm 13.2 \mu$mol/g HS, respectively.

The measured EDC value for LHA in this study compares well with the EDC value of $42.3 \mu$mol/g LHA measured by Aeschbacher et al. [178] using direct electrochemical reduction (DER). Their findings showed that the quantification of redox state by DCPIP was approximately 65% of that measured by DER [178], which is therefore consistent with the EDC value determined in this study. While the EDC
of the different HS solutions varied slightly, all were relatively similar in magnitude, particularly when the uncertainty in the measured values is taken into account. EDC values for the various HS solutions after irradiation (mean ± standard deviation of all HS concentrations, excluding outliers) were 21.6 ± 3.3 µmol/g HS, 9.4 ± 2.5 µmol/g HS and 18.5 ± 17.4 µmol/g HS for LHA, SRFA and PPHA, respectively. Overall there were no obvious trends in the magnitude of irradiation induced change in EDC among all three HS solutions, and no significant difference between values before and after irradiation when the uncertainty in the measured values is considered.

### Table 5  EDC of HS solutions before and after 7 h irradiation with simulated sunlight.

<table>
<thead>
<tr>
<th>HS concentration (mg/L)</th>
<th>LHA EDC (µmol e⁻/g LHA)</th>
<th>SRFA EDC (µmol e⁻/g SRFA)</th>
<th>PPHA EDC (µmol e⁻/g PPHA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EDC&lt;sub&gt;Before&lt;/sub&gt;</td>
<td>EDC&lt;sub&gt;After&lt;/sub&gt;</td>
<td>EDC&lt;sub&gt;Before&lt;/sub&gt;</td>
</tr>
<tr>
<td>10</td>
<td>53.2*</td>
<td>91.5*</td>
<td>117.5*</td>
</tr>
<tr>
<td>50</td>
<td>10.8</td>
<td>17.8</td>
<td>7.4</td>
</tr>
<tr>
<td>75</td>
<td>30.3</td>
<td>23.3</td>
<td>9.1</td>
</tr>
<tr>
<td>100</td>
<td>49.0</td>
<td>23.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Mean ± SD*</td>
<td>30.0 ± 19.1</td>
<td>21.6 ± 3.3</td>
<td>7.3 ± 1.9</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation across all HS concentrations for each HS type. Values marked with a * were excluded as outliers. This included all values obtained with 10 mg/L HS, which were considered unreliable as the small quantity of electrons transferred was very close to the method detection limit, and one negative value that is likely spurious.

The redox properties of HS have been broadly ascribed to quinone-hydroquinone moieties [44, 45, 49, 191, 192], enabling HS to act as redox buffer under varying environmental conditions by accepting or donating electrons [193-195], and also mediating other electron transfer processes [193, 194]. While quinones have commonly been considered as the major electron acceptor moieties in HS, recent works have shown that aromatic ketone/aldehyde acceptors are even more significant in determining DOM optical properties [71, 186, 196, 197]. Furthermore, other studies have shown that optical properties appear to involve rapid photophysical relaxation through electron transfer interactions of the chromophores [71, 73, 85, 170, 198, 199]. However the results of this study do not show significant effects of irradiation on EDC in any of the three standard HS solutions. In contrast, a strong correlation between optical properties and EDC was observed. This is consistent with the donor-acceptor model proposed in various earlier studies in which, on irradiation, the acceptor molecule forms ROS via electron transfer while altering NOM optical properties [46, 47].
Figure 10 Relationships between EDC and TOC in standard HS solutions before and after 7 h irradiation with simulated sunlight. A: LHA, B: SRFA, C: PPHA. ●: LHA before irradiation, ○: LHA after irradiation; ■: SRFA before irradiation, □: SRFA after irradiation; ◆: PPHA before irradiation, ◇: PPHA after irradiation. Error bars representing the 95% confidence interval (CI) of the mean are smaller than the symbols for all data points.
Understand the Photochemistry of Coastal Hypoxic Blackwater

The EAC of the three standard HS has been determined by Aeschbacher et al. [178] as shown in Table 6. The correlation between the EAC determined by Aeschbacher et al. [178] and magnitude of DO consumption measured in this study is shown in Figure 12, illustrating a positive correlation between these parameters (Pearson product moment correlation coefficient of the linear regression fit is 0.696). The higher EAC values of LHA and PPHA compared to that of SRFA may be indicative of a higher proportion of aromatic reducible moieties (generally recognised as quinone like moieties) based on the strong correlation between these parameters found by Aeschbacher et al. [178]. Compared to terrestrial HS, aquatic HS has less oxidative transformations of phenolic precursors and less processed aromatic-rich moieties, therefore it has lower EAC [200]. Lignin is acknowledged as an essential
precursor of HS with a low EAC value, and previous studies found that precursor materials are degraded more in terrestrial HS compared to aquatic HS [201].

Recent studies have suggested that the intermediate formed during electron transfer from the excited state of HS to O₂ is a long-lived charge-separated species created by electron transfer from singlet excited donor to ground state acceptor moieties [85]. The observation of minimal EDC loss as a result of irradiation in this study suggests that the labile electron donor moieties that exist under dark conditions are unlikely to be involved in photochemical electron transfer to DO. This is also supported by the fact that no clear trends were observed between the change in EDC and parameters related to photochemical DO consumption, including the magnitude of DO consumption (Figure 11).

### Table 6 Correlation between EAC and DO consumption in 50 mg/L HS solutions after 7 h irradiation with simulated sunlight. Values are reported as the mean ± standard error from triplicate measurements.

<table>
<thead>
<tr>
<th></th>
<th>EAC (µmol e⁻/g HS)</th>
<th>△DO (µmol/L)</th>
<th>First order rate constant for DO consumption (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHA</td>
<td>1711 ± 33</td>
<td>60.0 ± 0.9</td>
<td>0.45 ± 1.47 x 10⁻⁶</td>
</tr>
<tr>
<td>SRFA</td>
<td>671 ± 8</td>
<td>30.8 ± 4.5</td>
<td>0.17 ± 3.79 x 10⁻⁷</td>
</tr>
<tr>
<td>PPHA</td>
<td>1684 ± 30</td>
<td>43.3 ± 0.3</td>
<td>0.51 ± 1.89 x 10⁻⁶</td>
</tr>
</tbody>
</table>

![Relationships between EAC and DO consumption rate in standard HS solutions after 7 h irradiation with simulated sunlight.](image)

**Figure 12** Relationships between EAC and DO consumption rate in standard HS solutions after 7 h irradiation with simulated sunlight.

### 2.3.4 Kinetic model for DO consumption with water depth and HS concentration

The modelled apparent diel-averaged rate constant for photochemical DO consumption as a function of HS concentration and water depth is shown in Figure 13, 14 and 15. As expected the photochemical DO consumption rate constant varied
with HS concentration as well as water depth. The photochemical DO consumption rate reached a maximum value at 0.01 m depth for all three standard HS types, and decreased to near zero and became negligible over 0.5 m depth, which can be attributed to the fact that nearly 100% of incident photons are absorbed at depths > 0.5 m. This is consistent with the expectation that natural sunlight absorption is significant for a water column over 0.5 m deep [82, 202].

A previous investigation of the Richmond River blackwater issues during the floods in February 2001 showed that non-photochemical O₂ consumption rates by inundated floodplain vegetation ranged from approximately 0.1 to 1 mg/L O₂ per hour [203]. This equates to about 6 to 60 µmol/L O₂ per h, which equates to a first-order rate constant for DO consumption of about $7 \times 10^{-6}$ to $7 \times 10^{-5}$ s⁻¹. This spans a similar range to the predicted magnitude of photochemical O₂ consumption over the range of water depths and HS concentrations shown in Figure 13 to Figure 15, and implies that photochemical DO consumption rates may be of a similar order of magnitude to that measured Richmond River floodwater DO consumption rates. Assuming the post flood deoxygenation rate was similar in the 2001 and 2017 floods, this demonstrates the potential for photochemical DO consumption to contribute significantly to post flood deoxygenation processes in the Richmond River.

![Figure 13](image-url)  

**Figure 13** Modelled apparent diel-averaged rate constant for photochemical DO consumption as a function of LHA concentration and water depth.
Figure 14 Modelled apparent diel-average rate constant for photochemical DO consumption as a function of SRFA concentration and water depth.

Figure 15 Modelled apparent diel-averaged rate constant for photochemical DO consumption as a function of PPHA concentration and water depth.
2.4 Conclusions and Implications

All DOM solutions consumed DO during irradiation, and the majority of DO consumption occurred in the initial 7 h of irradiation. The magnitude of DO consumption over this duration was found to vary with DOM concentration, ranging from around 15 µmol/L with 10 mg/L HS, up to nearly 150 µmol/L with 100 mg/L. Neither the rate nor magnitude of photochemical DO consumption showed a strong relationship to the type of HS used. Hence, the relationship between the magnitude of DO consumption and TOC was best fit by a single exponential equation for all HS types, represented in Eq 34.

Rates of photochemical oxygen consumption in previous studies involving for HS-containing freshwaters with DOC concentrations from 2 to 68 mg C/L ranged from 0.6 to 2.8 µM O$_2$ h$^{-1}$ [113, 120], which is comparable to the rates measured in this study.

SUVA$_{280}$ decreased slightly and E$_2$/E$_3$ increased after 7 h of irradiation for all DOM solutions. Specifically, after 7 h irradiation, SUVA$_{280}$ of LHA changed from 0.050 ± 0.0013 L m$^{-1}$mg$^{-1}$ HS to 0.046 ± 0.0008 L m$^{-1}$mg$^{-1}$ HS, SUVA$_{280}$ of PPHA changed from 0.051 ± 0.0028 to 0.048 ± 0.0025 L m$^{-1}$mg$^{-1}$ HS, and SUVA$_{280}$ of SRFA changed from 0.030 ± 0.0021 to 0.029 ± 0.0023 L m$^{-1}$mg$^{-1}$ HS. This showed that SRFA exhibited lower photosensitivity than LHA and PPHA. Irradiation appeared to have no significant effect on the EDC of the HS solutions, and there were no apparent correlations between the EDC and any parameters related to photochemical oxygen consumption. The EDC value for LHA before irradiation (mean ± standard deviation of all HS concentrations, excluding outliers) was 30.0 ± 19.1 µmol/g HS, while values for SRFA and PPHA were 7.3 ± 1.9 µmol/g HS and 31.5 ± 13.2 µmol/g HS, respectively. EDC values for the various HS solutions after irradiation (mean ± standard deviation of all HS concentrations, excluding outliers) were 21.6 ± 3.3 µmol/g HS, 9.4 ± 2.5 µmol/g HS and 18.5 ± 17.4 µmol/g HS for LHA, SRFA and PPHA, respectively. Previous work has reported correlations between the quantum yields of photochemical reactions and absorption coefficients, specific absorption coefficients and E$_2$/E$_3$ for DOM. This association could be used for predicting the efficiency of photochemical reactions in natural water systems, although the robustness of this approach would depend on having sufficient understanding of the underlying principles [75, 83, 127, 129].
Kinetic modelling of photochemical DO consumption with water depth and HS concentration showed that the maximum value of photochemical DO consumption rate using the standard HS in this study would be expected at 0.01 m depth, while the modelled rates decreased to near zero and became insignificant when depth was > 0.5 m. Compared with previous study results at the same location, the photochemical DO consumption rate was similar in magnitude to measured rates of DO consumption in Richmond River floodwater.

Previous studies of the Richmond River system indicated that DOC concentrations in hypoxic blackwaters were around 50 mg/L, which is within the range of the standard HS solutions examined here, and that terrestrial humic acids are the major components of post-flood blackwater DOM [3, 114]. Although the experiments conducted in this study are greatly simplified compared to the photochemistry of coastal hypoxic blackwaters, this work nonetheless demonstrates the potential for substantial photochemical deoxygenation to occur with exposure to a few hours of solar irradiation. However, there are evidently more environmental factors involved in photochemical reactions in natural waters, which could potentially cause more DO consumption. The results suggest that in hypoxic blackwater systems, there may not be a simple relationship between chromophoric properties of DOM and DO consumption potential. Further studies involving other components of real blackwater systems such as trace metals or varying pH are required to fully understand the role of photochemistry in these environments. Further studies comparing the reactivity and properties of standard HS solutions and real hypoxic blackwaters is also required to better understand these relationships.
Chapter 3 – Post flood hypoxic blackwater field study

3.1 Introduction

Hypoxic blackwater is defined by a low dissolved oxygen (DO) concentration accompanied by an elevated concentration of dissolved organic carbon (DOC). Hypoxia can be stressful or fatal to many aquatic organisms, and hypoxic blackwater has been claimed to be responsible for some fish kills and other events that are detrimental to aquatic biota [1, 159]. Aquatic biota may be impacted when DO concentration is below 4 mg/L, and DO below 2 mg/L is considered lethal for many biota [5, 9], including Australian native fish [1].

Most hypoxic blackwater events occur in lowland rivers with forested or agricultural floodplains [1, 144]. Unseasonal inundation caused by extended rainfall has typically been the main trigger [10, 162]. The main river streams normally develop hypoxic blackwater due to drainage from surrounding floodplains [3, 204]. This has been confirmed by previous study findings that the deoxygenation of natural coastal rivers is impacted by hydraulic connectivity between floodplains and rivers during floods [1, 3, 4, 8, 11]. Flood mitigation works and drainage systems, along with agricultural expansion, have increased the hydrological interactions between main rivers and backswamps, decreasing the inundation time of floodplain backswamps and potentially decreasing the timescale on which deoxygenated water flows into the main river channels [4].

DOC transfer from floodplains to natural waters is significant for the sustainability of riverine ecological systems, however changes in landuse and hydrology can impact the quantity and type of DOC in rivers. The DOC composition and concentration in rivers experiencing hypoxic blackwater events is affected by the accumulation of dissolved organic matter (DOM) and redox sensitive inorganic chemical species on floodplains prior to inundation [13, 14, 205, 206]. Previous studies of water chemistry at key locations in the Richmond River estuary (northeastern NSW, Australia) indicated elevated concentrations of redox sensitive species coincide with the occurrence of hypoxic blackwater [3]. Changes in DOC type and concentration can also change the optical properties of sunlit surface waters [11], which is a key focus of this PhD.
The Northern Rivers area, and in particular the Richmond River system, is one of the most flood prone areas of Australia. The Richmond River and its tributaries, including the Wilsons River upstream from Lismore through to Coraki (Figure 16), are periodically subject to major flooding. Particularly prolonged rainfall often occurs during late summer, autumn and early winter (January–April), with the mean annual rainfall of 1300-1700 mm across the lower catchment (BOM, 2009). Nearly 360 km² of the lower Richmond River floodplain has elevation < 1 m AHD (Australian Height Datum, where 0 m AHD is approximately sea level), which also leads to a significant influence of sea level variations on flooding [204].

Previous studies of Richmond River flood events showed that hypoxic blackwater formed in floodplain backswamps and surrounding areas around 6-10 d after the flood peak [3, 204]. High DOC and dissolved Fe concentrations coincided with the flood recession, when hypoxic waters drain from the floodplain backswamps to the main channel, and DOC concentrations were found to subsequently decrease when streams later disconnected from the adjacent floodplains [1, 3, 4, 207]. This is consistent with previous studies showing that DOC increases simultaneously with the rising limb of the flood hydrograph [208], or after the flood peak during recession [209]. While the primary source of DOC generation in other systems is often wetlands [210], DOC in the Richmond River estuary is principally generated on the floodplain backswamps.

![Figure 16 Richmond River floodplain and catchment (source: http://www.environment.nsw.gov.au/ieo/Richmond)](image)
On 31st March 2017, a 30-year average return interval (ARI) flood occurred in the Richmond River system after 24 h of extreme rainfall associated with ex-tropical cyclone Debbie. A post-flood hypoxic blackwater event subsequently occurred. In this study we investigated changes in *in situ* DO and DOC, as well as related geochemical, optical and redox properties, of surface waters in the Richmond River and its tributaries after this 30 year ARI flood event. This chapter therefore aims to investigate the relationships between deoxygenation potential and the geochemical, optical and redox properties of flood waters following this flood event.

### 3.2 Experimental Section

#### 3.2.1 Ethics statement

The field study locations were not on privately-owned or protected land, and the *in situ* measurements did not involve any protected or endangered species. Thus, no permits were required.

#### 3.2.2 Field measurements and sampling

Field measurements and sampling were performed at the 10 sites shown in Table 7 and Figure 17 two weeks after the flood peak on 13 April 2017 and seven weeks after flood peak on 18 May 2017 (see Figure 18 for water levels in the Richmond River at Bungawalbyn). DO, pH, temperature, oxidation-reduction potential (ORP) and electrical conductivity (EC) were measured *in situ* using freshly calibrated portable meters. Different portable meters were used on each of the two sampling days: on 13 April 2017, a HQ40D Portable Multi Meter (Hach) was used, while on 18 May 2017, YSI Professional Plus multifunction portable probes were used. The units of EC were different for the two different meters, and were later transformed into consistent units of EC25 using the nonlinear temperature compensation model described in ref [212].

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Sampling location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lismore River</td>
<td>28°48'43.09&quot;S</td>
<td>153°16'12.70&quot;E</td>
<td>Main river channel</td>
</tr>
<tr>
<td>2</td>
<td>Ruthven</td>
<td>28°53'17.65&quot;S</td>
<td>153°17'55.17&quot;E</td>
<td>Main river channel</td>
</tr>
<tr>
<td>3</td>
<td>Codrington</td>
<td>28°57'13.67&quot;S</td>
<td>153°16'03.11&quot;E</td>
<td>Tributary</td>
</tr>
<tr>
<td>4</td>
<td>Coraki</td>
<td>28°59'15.53&quot;S</td>
<td>153°17'14.54&quot;E</td>
<td>Tributary</td>
</tr>
<tr>
<td>5</td>
<td>Bungawalbyn Creek</td>
<td>29°4'35.89&quot;S</td>
<td>153°14'44.18&quot;E</td>
<td>Backswamp</td>
</tr>
<tr>
<td>6</td>
<td>Woodburn</td>
<td>29°4'15.81&quot;S</td>
<td>153°20'36.04&quot;E</td>
<td>Backswamp</td>
</tr>
<tr>
<td>7</td>
<td>Rocky Mouth Creek</td>
<td>29°5'39.15&quot;S</td>
<td>153°19'43.97&quot;E</td>
<td>Backswamp</td>
</tr>
<tr>
<td>8</td>
<td>Tuckean Barrage</td>
<td>28°59'21.96&quot;S</td>
<td>153°24'42.78&quot;E</td>
<td>Backswamp</td>
</tr>
<tr>
<td>9</td>
<td>Wardell</td>
<td>28°57'12.91&quot;S</td>
<td>153°27'55.23&quot;E</td>
<td>Main river channel</td>
</tr>
<tr>
<td>10</td>
<td>Ballina River</td>
<td>28°52'5.53&quot;S</td>
<td>153°32'52.91&quot;E</td>
<td>Main river channel</td>
</tr>
</tbody>
</table>
Figure 17  Field study sampling sites (adapted from Sammut et al. [213], refer to Table 7 for details)

Figure 18  Watercourse level in the Richmond River at Bungawalbyn from 24 March 2017 to 20 May 2017 (Data resource: http://www.bom.gov.au/waterdata/)
A total of 30 surface water samples were collected: two at each of the sites, and at the same times when access was possible during the flood recession. Samples were collected in clean sterile plastic bottles supplied by Southern Cross University’s Environmental Analysis Laboratory (EAL), and the bottles were rinsed three times in the surface waters prior to sample collection. The bottles were submerged in the field water until fully filled and then were capped with minimal headspace. Samples were taken between the surface and a depth of 10 cm below the surface, using long arm pliers to hold the bottles when collecting surface water. After bringing all water samples back to the laboratory, samples for DOC analysis were filtered through 0.45 μm pore size polyether sulfone filter membranes (Sigma Aldrich) and kept at 4°C in the fridge, while samples for total metal analysis were preserved by adding 60% HNO₃ to adjust to pH < 2 and kept at 4°C prior to sending for analysis. Samples for determination of DOC, BOD, COD and total metals were sent for analysis at the EAL on each sampling day. Total metal analysis was performed on all samples on 18 May 2017. Analyses were performed according to APHA (2005) [214], as described in Table 8.

**Table 8**  EAL analysis methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method [214]</th>
<th>Method</th>
<th>Detection limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-day biological oxygen demand (BOD₅)</td>
<td>APHA 5210-B</td>
<td>5-Day BOD Test</td>
<td>2 mg/L</td>
</tr>
<tr>
<td>Chemical oxygen demand (COD)</td>
<td>APHA 5220-D</td>
<td>Closed Reflux, Colorimetric Method</td>
<td>12~20 mg O₂/L</td>
</tr>
<tr>
<td>DOC</td>
<td>APHA 5310-B</td>
<td>High-Temperature Combustion Method</td>
<td>1 mg C/L</td>
</tr>
<tr>
<td>Total metals (Ag/Al/As/Cd/Cr/Cu/Fe/Mg/ /Ni/Pb/Se/Zn/Hg)</td>
<td>APHA 3125</td>
<td>ICP-MS(Inductively Coupled Plasma Mass Spectrometry)</td>
<td>1~100 ng/L</td>
</tr>
</tbody>
</table>

The electron donating capacity (EDC) of field water samples was analysed using the DCPIP method as described in Chapter 2, except that the pH of all samples was adjusted to 7.0 by addition of 0.1 M phosphate buffer (made from NaH₂PO₄ and NaH₂PO₄) prior to analysis to avoid effects of variable pH. Optical properties including E₂/E₃, Abs₂₈₀ and absorbance at 603 nm were also measured concurrently with this procedure, without pH adjustment. Outliers were excluded based on the expected linearity of the relationship between HS concentration and absorbance.

Data were analysed using GraphPad Prism 7.02. To identify possible associations between all the measured parameters, analysis of the nonparametric Spearman’s
rank-order correlation was undertaken. Correlation analysis was conducted on log-transformed values for all parameters, to enable identification of any generalised power law dependence between variables, rather than linear dependence only. The statistical significance of each correlation pair was determined by calculating a two-tailed \( p \) value. Correlation analysis was conducted on data from each sampling day separately and on all data combined.

The bivariate correlation analysis illustrated the complexity of the system and the existence of multiple inter-correlated variables after the flood event. Unravelling these inter-correlations required use of a multidimensional statistical analysis approach. The data set of post flood water in this study, which contained 20 individual water samples described by 17 variables, was subjected to Principal components analysis (PCA) using Rstudio software (https://www.rstudio.com) and coding (http://www.sthda.com/). PCA has been previously applied in studying natural aquatic environments [215-217]. PCA is a non-parametric method of classification, and it does not make assumptions about the underlying statistical distribution of the data. This analysis was also able to compress the dataset size and extract the most significant information, thus allowing a more global analysis the experimental results. The methods of performing PCA in R in this study included spectral decomposition, which examines the covariances and correlations between variables, and singular value decomposition, which examines the covariances and correlations between individuals [218]. As the R function used in this study standardises the data automatically during the PCA by default, data were not independently transformed before the PCA.

3.3 Results and Discussion

3.3.1 Spatial and temporal variations in surface water parameters
Values of measured parameters at all sites on each of the sampling dates are shown in Figure 19 to Figure 21. The sites are arranged from upstream to downstream (left to right on the graph axes) to allow visualisation of spatial trends along the floodplain.

DO
The minimum DO concentrations measured were 0.55 mg/L on 13 April 2017 and 2.78 mg/L on 18 May 2017, both at Rocky Mouth Creek, while the highest DO concentrations measured were 7.92 mg/L on 13 April and 7.46 mg/L on 18 May 2017, both at the Lismore River site. On 13 April 2017, two weeks after the flood peak,
four sites showed hypoxic conditions including Woodburn (DO concentration = 2.24 mg/L), Rocky Mouth Creek (DO concentration = 0.55 mg/L), Tuckean Barrage (DO concentration = 1.96 mg/L) and Wardell (DO concentration = 2.08 mg/L). In contrast, on 18 May 2017 only Rocky Mouth Creek showed hypoxic conditions (DO concentration = 2.78 mg/L). At this time, DO concentrations were > 6 mg/L at all other sites.

Water temperature is potentially an important influence DO concentrations, as increasing temperature can decrease oxygen solubility, increase rates of respiration by microorganisms, and also increase the solubility of various DOC types which are potentially involved in DO consuming processes [18, 19, 144]. Consequently, elevated temperature might increase the development or severity of hypoxic blackwater in floodplain water systems when inundated occurs in the warm season. In this study, the surface water temperature on 13 April 2017 averaged 17.5°C, while the water temperature on 18 May 2017 averaged 23°C, as shown in Figure 19A.

**DOC**

DOC concentrations in the upper catchment sites (including Lismore River, Ruthven, Codrington and Coraki) were generally below 10 mg/L throughout this post flood blackwater event. On the lower floodplain from Bungawalbyn Creek to Ballina River, the waters had high DOC concentration two weeks after the flood peak on 13 April 2017. DOC concentrations above 15 mg/L commonly existed at these sites whilst the DOC concentrations at Bungawalbyn Creek and Rocky Mouth Creek reached about 25 mg/L. In this situation, low DO combined with high DOC can be fatal for aquatic animals [10]. Seven weeks after the flood peak on 18 May 2017, the DOC at downstream sites had decreased to < 10 mg/L for almost all sampling sites besides Bungawalbyn Creek which had 13.4 mg/L DOC. DOC values measured on 13 April 2017 were higher than those measured on 18 May 2017 at all sites on the floodplain backswamps including Rocky Mouth Creek, Woodburn and Bungawalbyn Creek.

The high DOC concentration post flood is typical of hypoxia blackwater [3, 114]. The inundated areas were largely agricultural or forested land types, and when flooded the vegetation could input an enormous amount of DOC into the water [219]. The DOC in the hypoxic blackwater samples collected in this study ranged from 10 mg/L to 25 mg/L, which is within the typical range of water samples from previous floods. Wong et al. [3] measured DOC ranging from 3.58 mg/L to 25.03 mg/L in a
study on surface waters in the same area one month after a 1 in 10 year ARI flood peak. Johnston et al. [114] measured DOC ranging from 5 mg/L to 70 mg/L in post flood water samples from Everlasting Swamp and Shark Creek during floods in February and March 2001, and in samples from Wardell during a 2008 flood. In contrast, the DOC of non-hypoxic blackwater samples in this study was < 10 mg/L.

Organic carbon mobilisation from floodplains to the main river and the subsequent reactive processes consuming DOC and DO are major factors contributing to hypoxic blackwater development. Previous studies found that DOC decomposition and release from forested or agricultural floodplain backswamps had a significant influence on water quality downstream [10, 162], and the low elevation of the backswamps on the Richmond River floodplain might also contribute to the generation and persistence of hypoxic blackwater [204, 220].

**pH**

The lowest in situ pH on 13 April 2017 was 6.2 at Rocky Mouth Creek and the highest in situ pH was 7.48 at Lismore River. In contrast, the lowest in situ pH on 18 May 2017 was 5.2 at Tuckean Barrage and highest in situ pH was 7.64 at Ballina River. The pH values at Rocky Mouth Creek and Tuckean Barrage on 18 May 2017 were lower than those measured on 13 April 2017, while the pH at other sites did not exhibit big changes between 13 April 2017 and 18 May 2017.

Previous studies by Wong et al [204] using soil cores from the Richmond River floodplain showed pH decreased during a 15 d inundation with artificial seawater and ranged from 3.66 to 5.45. This suggests that the low post flood pH values observed in some surface waters (e.g. at Rocky Mouth Creek and Tuckean Barrage) might be due to inputs of H+ from acidic porewaters in inundated surface sediments on the Richmond River floodplain, which include substantial acid sulfate soils (ASS), however there might also be a possible contribution of organic acids [204, 220]. ASS typically contain high concentrations of acidic metal cations, which can induce pH decrease by proton exchange, hydrolysis and redox reactions [220]. A post flood study at Firewood Creek, which is located near ASS in the north of Australia, similarly found the pH ranged from 3.5 to 6.5 while decreasing approximately 10 days after flood [3, 4].
ORP
The lowest in situ ORP on 13 April 2017 was -146.6 mV at Rocky Mouth Creek. All other values measured on 13 April 2017 were > 85.1 mV, with the highest being 133.1 mV at Woodburn. The highest ORP on 18 May 2017 was 393 mV at Tuckean Barrage, then 277.8 mV at Rocky Mouth Creek, while the lowest value was 87.7 mV at Ballina River. No negative values were recorded on 18 May 2017. The negative ORP measured at Rocky Mouth Creek one week after the flood peak indicates strongly reducing conditions in the surface water in this location. All other sites displayed values from 85.1 mV to 133.1 mV, which are indicative of oxidising conditions (although less so than air saturated pure water). The ORP increased at almost all sites from 13 April 2017 to 18 May 2017, with the largest change occurring at Rocky Mouth Creek from -146.6 mV to 277.8 mV, indicating a large shift from reducing to oxidising conditions.

ORP represents the net status of all the oxidation and reduction reactions in a water sample, indicating the overall potential to donate or gain electrons. Surface waters with sufficient DO concentration to decompose organic matter efficiently typically exhibit ORP values between 300 and 500 mV, while negative ORP values are indicative of reducing conditions with low DO, which is typically not conducive for decomposition of organic matter [49, 221, 222].

EC25
The minimum EC25 of all sampling sites on 13 April 2017 was 87.4 µS/cm at Bungawalbyn Creek, while the maximum EC25 was 233.1 µS/cm at Codrington. On 18 May 2017, the maximum EC25 was 1018 µS/cm at Rocky Mouth Creek and the minimum was 358.8 µS/cm at Bungawalbyn Creek. The EC25 values at all sites on 18 May 2017 were higher than those measured on 13 April 2017, with particularly large increases at the Rocky Mouth Creek and Tuckean Barrage sites, as expected due to increasing influence of marine waters in the lower reaches after the flood recession. The EC of post flood surface waters in the same area measured in a previous study ranged from 228 µS/cm to 2080 µS/cm [3], which is similar to results from this study.

Optical properties (E2/E3 and SUVA280)
The maximum E2/E3 on 13 April 2017 was 3.79 at Ballina River and the minimum was 2.96 at Ruthven. On 18 May 2017, the maximum E2/E3 was 3.49 at Rocky Mouth Creek and the minimum was 2.97 at Woodburn. E2/E2 values were comparatively
stable spatially and temporally for all field study sites. As an indicator for the relative abundance of aromatic compounds in DOM, this suggests that the type and source of DOM was likely similar at all sites on both days. In comparison the $E_2/E_3$ values of the standard HS obtained in Chapter 2 ranged from 2.5 to 2.8 for LHA, 3.6 to 4.9 for SRFA and 2.7 to 3.1 for PPHA.

The maximum SUVA$^{280}$ on 13 April was 0.058 m$^{-1}$L/mg at Ruthven and the minimum SUVA$^{280}$ was 0.026 m$^{-1}$L/mg at Ballina River. On 18 May 2017, the maximum SUVA$^{280}$ was 0.056 m$^{-1}$L/mg at Lismore River and the minimum was 0.030 m$^{-1}$L/mg at Rocky Mouth Creek. SUVA$^{280}$ values measured on 13 April 2017 were higher than those measured on 18 May 2017 for most of the study sites, besides Lismore River, Ruthven and Rocky Mouth Creek. This implies that bleaching of CDOM occurred during the month after the flood peak. This could be due to input of DOM into the main river channel via discharge from the backswamps, where DOM may have been subjected to photobleaching over the previous few weeks. However, the spectral quality of the DOM inputs to the river may also have changed due to non-photochemical processes. By comparison, SUVA$^{280}$ values of standard HS obtained in Chapter 2 ranged from 0.044 to 0.054 m$^{-1}$L/mg for LHA, 0.025 to 0.037 m$^{-1}$L/mg for SRFA and 0.044 to 0.059 m$^{-1}$L/mg for PPHA with constant pH and temperature and varying HS concentrations.

The similarity between $E_2/E_3$ and SUVA$^{280}$ of natural surface water samples and the standard HS solutions suggests that HS type compounds are likely major components of NOM in the natural surface waters. These two optical parameters have been broadly applied as indicators for molecular size and aromatic content in DOM [73, 176, 223-225]. This suggests that DOM in the post flood surface waters experienced a decrease in molecular size from 13 April 2017 to 18 May 2017. Previous studies showed that DOM generated on floodplains during post flood inundation conditions normally contained high aromaticity [226], and DOM exported from wetland sediments generally has high molecular weight and high aromatic content [37, 227]. This suggests that in the natural post flood waters, the aromatic components might mainly derive from lignin and surface soils on the floodplain [114, 228].

**EDC**

The only positive EDC on 13 April 2017 was 11.8 mmol e/g DOC at Tuckean Barrage. All the other sites had negative EDC ranging from -10.5 mmol e/g DOC at
Rocky Mouth Creek to -116 mmol e/g DOC at Lismore River. Positive EDC was measured on 18 May 2017 at Rocky Mouth Creek (253 mmol e/g DOC) and Tuckean Barrage (290 mmol e/g DOC), which indicated the surface water had substantial electron donating or reductive capability. EDC values at the other sampling sites were negative and ranged from -32.7 mmol e/g DOC at Ruthven to -99.7 mmol e/g DOC at Codrington. This unexpected result suggests a possible source of experimental error or method interference, and this issue requires further study in the future. Nonetheless, the EDC values at all field sites were generally consistent except for two strongly positive values on 18 May 2017 at Rocky Mouth Creek and Tuckean Barrage (Figure 20), which are at the outlet of the backswamps on the Richmond River floodplain, and which have experienced blackwater issues before [204, 220].

Quinones have been generally considered as the dominant redox reactive components in NOM such as HS [59, 229], with quinone sites being responsible for 44 to 79% of the redox activity [44, 230]. However, in anaerobic coastal floodplains, other redox active species such as Fe, Mn and SO$_4^{2-}$ can also act as terminal electron acceptors during organic matter decomposition [193, 207], and their reduced forms could potentially also contribute to the measured EDC in the surface water samples.

**BOD and COD**

The maximum BOD on 13 April 2017 was 8.6 mg/L O$_2$ at Rocky Mouth Creek and the minimum BOD was 0.9 mg/L O$_2$ at Lismore River. The maximum BOD on 18 May 2017 was 2.6 mg/L O$_2$ at Woodburn and the minimum BOD was 0.5 mg/L O$_2$ at both Tuckean Barrage and Ballina River. Rocky Mouth Creek and Tuckean Barrage experienced a decrease in BOD between the two sampling dates, while the BOD at the other field sites did not change substantially. The peak in BOD values coincided with development of hypoxia at the lower floodplain sites two weeks after the flood peak, and subsequently went back to < 4 mg/L O$_2$ after another month.

The maximum COD on 13 April 2017 was 76 mg/L O$_2$ at Rocky Mouth Creek and the minimum was 7 mg/L O$_2$ at Ruthven. On 18 May 2017, the maximum COD was 25 mg/L O$_2$ at Rocky Mouth Creek, and the minimum was 1 mg/L O$_2$ at Ruthven. For all field samples, COD values measured on 13 April 2017 were higher than those measured on 18 May 2017, especially at sites in the backswamps, including Bungawalbyn Creek, Woodburn, Rocky Mouth Creek, and Tuckean Barrage. This flood event thus greatly increased the COD in the Richmond River system, especially
on the floodplain backswamps, immediately after the flood, while the COD returned to < 40 mg/L O\textsubscript{2} one and a half months after the flood peak.

Previous studies of post flood surface waters in the Richmond River system reported COD values ranging from 25 mg/L O\textsubscript{2} to 175 mg/L O\textsubscript{2} at Everlasting Swamp and Shark Creek during the February/March 2001 flood events, and from 15 mg/L O\textsubscript{2} to 105 mg/L O\textsubscript{2} at Wardell during the January 2008 flood event [114]. A previous study on the baseflow water quality post flood in 2009 in the same area as this study measured COD of < 0.002 mg/L O\textsubscript{2}, while the overall post flood surface water COD ranged from < 0.002 to 62 mg/L O\textsubscript{2} [3]. The COD values measured in this study are thus generally of similar magnitude as those measured in previous studies of surface water quality in the Richmond River system, and BOD values were typically around 10% of COD values at each site.

**Total metals (Al, Cu, Fe, Mg, Ni, Zn)**

Al concentrations ranged from 5.89 to 121.8 μmol/L, Cu concentrations ranged from 0.009 to 0.056 μmol/L, Fe concentrations ranged from 23.1 to 532.1 μmol/L, Mg concentrations ranged from 0.87 to 9.63 μmol/L, Ni concentrations ranged from 0.019 to 0.19 μmol/L, and Zn concentrations ranged from 0.025 to 0.58 μmol/L (all total metal concentrations). The total metal concentrations were generally similar on both sampling days, with no major spatial trends, except for a large spike in Fe near Rocky Mouth Creek and Tuckean Barrage on 13 April 2017, and elevated Al, Ni and Zn near Rocky Mouth Creek and Tuckean Barrage on 18 May 2017.

A previous study conducted in the same area outside of any floods, which was considered representative of baseflow water quality, measured Fe concentrations ranging from 4.7 to 18.9 μmol/L [3], while Fe concentrations in surface water samples from the backswamps after Richmond flood in January 2008 ranged from 12.7 to 297.8 μmol/L and displayed enhancement in the postflood hypoxic blackwater [3]. Previous field studies during the 2008 and 2009 flood events showed that dissolved Fe concentrations reached a maximum about 6 to 14 days after the flood peak in the main channel of the Richmond River [114]. Previous comparisons of total dissolved Fe concentrations between the 2008 and 2009 flood events have demonstrated considerable variation in the extent of Fe mobilisation during warm season floods [114].
Concentrations of total metals were noticeably higher at hypoxic blackwater sites compared to the sites that were not experiencing hypoxia. Total metal results combined with the high COD values at the hypoxic blackwater sites suggest the post flood waters were likely impacted by reductive mobilisation of redox active species, which may have contributed to deoxygenation potential. Previous studies in the same area demonstrated that the backswamps in this study were typical ASS basins containing accumulations of Fe(III) minerals in the surface soil [207], which could be mobilised under flood conditions, particularly when waters had low pH (< 6). When combined with low DO, solubilisation of Fe(III) minerals can be further enhanced by reduction to ferrous iron. These results support the findings of previous studies on the Richmond River floodplain that increased floodplain-channel connectivity during floods contributes to the enhancement of hypoxic blackwater occurring in the estuary and main streams[3, 220].
Figure 19 General physical and chemical parameters of surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: Temperature, B: ORP, C: pH, D: EC25, E: DO.
Properties of organic carbon in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: $E_2/E_3$, B: EDC, C: SUVA$_{280}$, D: BOD, E: COD, F: DOC.

Figure 20
Figure 21  Total metal concentrations in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (∙) and 18 May 2017 (□). A: total Al, B: total Mg, C: total Fe, D: total Cu, E: total Ni, F: total Zn.
3.3.3 Bivariate correlations between measured surface water parameters

Heat maps showing the significance of correlations between the measured parameters on each sampling day separately, and for all data together, are shown in Figure 22. The heat map for the samples taken on 13 April 2017 displayed a greater number of significant ($p < 0.001$) correlations than did the heat map for samples taken on 18 May 2017, while the correlations for the entire dataset displayed more complex relationships. In water samples taken on 13 April 2017, seven significant ($p < 0.001$) correlations were identified, namely DO with pH, DO with EDC, DO with COD, DO with DOC, ORP with Fe, pH with COD, pH with DOC, BOD with Fe, and DOC with COD (Figure 22A). In the samples taken on 18 May 2017, four significant ($p < 0.001$) correlations were identified, namely ORP with pH, and Mg with Zn (Figure 22B). When considering all samples taken on both sampling days together, 12 significant correlations were identified, namely DO with pH, DO with COD, DO with Fe, EC25 with DOC, EC25 with ORP, $E_2/E_3$ with DOC, $E_3/E_3$ with COD, $E_3/E_3$ with SUVA$_{280}$, COD with DOC, DOC with EC25, Al with Fe, Fe with Mg, and Mg with Zn (Figure 22C). Correlations between each parameter therefore changed temporally and spatially.

On the basis of this analysis, relationships between DO, pH, COD, DOC and Fe were identified as being particularly influential in the sampled surface waters on 13 April 2017. The chemical characteristics of the surface waters on this sampling date were most typical of those expected for a hypoxic blackwater event (low DO and elevated DOC, BOD and COD), and therefore these correlations may be considered most important in terms of the geochemical behavior of blackwaters. In contrast, there were fewer correlations between parameters measured in the surface waters on 18 May 2017, which are more representative of non-blackwater conditions. Fe showed more significant correlations than other metal elements in both the blackwater samples (13 April 2017) and overall, which is consistent with the importance of Fe in this system identified by previous studies in the same area, and with the potential for Fe reduction under anaerobic conditions leading to elevated dissolved Fe concentrations [3, 114, 204, 220]. No correlations between optical properties and parameters related to deoxygenation potential (DO concentration, ORP, DOC, COD or BOD) were identified in the overall dataset except between $E_2/E_3$ and DOC, and $E_2/E_3$ and COD.
To assess the relationships between significantly correlated parameters related to hypoxic blackwater, the correlations between DO, pH, DOC, BOD, COD, temperature and the other parameters are shown in Figure 23 to Figure 40 with linear regression trend lines fitted and associated Pearson product moment correlation coefficient ($R^2$) values reported. In this study, parameters correlated with $R^2 \geq 0.8$ were considered strongly correlated and those with $0.5 \leq R^2 < 0.8$ were considered as somewhat correlated, while correlations with $R^2 < 0.5$ were considered weak and are not generally discussed in detail.

**Relationship between DO and DOC, COD and BOD**

For the field surface water samples collected on 13 April 2017, DO was strongly negatively correlated with DOC ($R^2 = 0.830$, Figure 24F), and somewhat negatively correlated with COD ($R^2 = 0.775$, Figure 24E) and BOD ($R^2 = 0.576$, Figure 24D). DOC and COD were strongly positively correlated ($R^2 = 0.921$, Figure 27E), while BOD displayed weak positive correlations with DOC ($R^2 = 0.452$, Figure 27D) and COD ($R^2 = 0.475$, Figure 33D). The results of this study agree with previous studies in the same area that also found strong positive correlations between DOC and COD in post flood surface water samples [114]. A few studies found that the drier weather caused a change in local vegetation, which then significantly impacted the decomposition rate and rate of DO consumption by inundated vegetation during flooding events [114, 219]. The source of the organic carbon has been suggested to be more influential than the carbon concentration during deoxygenation of post flood water systems; for example, the simpler structured carbon from fresh organic matter would be more reactive during decomposition compared with non-labile carbon species [228, 231-233], and the lability of the carbon type is more significant than the total carbon concentration in this process [203, 234]. Previous studies suggested the strong correlation between DOC and COD implied DO consumption by inundated vegetation [114, 235], mainly triggered by the microbial respiration and organic carbon reactions generated during inundation [236].

For the water samples collected on 13 April 2017, ORP was significantly negatively correlated with BOD ($R^2 = 0.62$, Figure 32B). EDC was somewhat positively correlated with DOC ($R^2 = 0.579$, Figure 27B), COD ($R^2 = 0.509$, Figure 36B) and BOD ($R^2 = 0.630$, Figure 33B). EDC was strongly negatively correlated with DO ($R^2 = 0.821$, Figure 24B) and was somewhat negatively correlated with DO ($R^2 = 0.500$, Figure 24B) for the water collected on 18 May 2017. Previous studies in the same
area or in similar post flooding water samples showed that decreasing DOC size generated more low molecular components which were more readily available for decomposition and bacterial production [237, 238], and that this process accelerated variations in redox state in hypoxic blackwater systems when DO concentrations were low. As an indicator of reducing capacity, EDC partly reflects the change in redox properties associated with post flood hypoxia, as EDC displayed a strong negative correlation with DO and a somewhat positive correlation with DOC. In situ ORP measured at Rocky Mouth Creek on 13 April 2017 was strongly negative, which implies strongly reducing character, consistent with the EDC results.

Relationship between DO and metals
For the water samples collected on 18 May 2017, DO was somewhat negatively correlated with Mg ($R^2 = 0.559$, Figure 25B), Ni ($R^2 = 0.602$, Figure 25E) and Zn ($R^2 = 0.503$, Figure 25F). For the water samples collected on 13 April 2017, Al was somewhat negatively correlated with DO ($R^2 = 0.565$, Figure 25A) and somewhat positively correlated with DOC ($R^2 = 0.7083$, Figure 28A) and COD ($R^2 = 0.505$, Figure 37A). Fe was strongly positively correlated with BOD ($R^2 = 0.9496$, Figure 34C). Generally, the less reactive metals such as Mg, Ni and Zn showed less of a relationship with hypoxic blackwater, whereas Al and Fe displayed stronger correlations with DO, while Al also showed a positive correlation with organic carbon concentrations. The strong positive correlation between Fe and BOD for the water samples collected on 13 April 2017 suggests that under hypoxic conditions, Fe might be involved in biological oxygen consumption, for example via the Fenton reaction [61, 110]. Previous studies have identified the ability of some bacteria to use exogenous electron shuttles like HS for anaerobic oxidation, with the redox properties of the HS influencing microbial iron transformations in anoxic environments [151].

Fe, Al, Mg, Ni and Zn were negatively correlated with DO, while Cu was positively correlated with DO. The maximum concentration of Fe obtained in this study was at Rocky Mouth Creek on 13 April 2017, and was several orders of magnitude higher than values typically reported in the previous studies at similar sites [204, 217, 239]. The increased metal concentrations likely originate from ASS in floodplain soils and sediments by flood flushing [3, 220].
Influence of pH

The pH was strongly or somewhat positively correlated with DO on both sampling days ($R^2 = 0.642$ for the samples collected on 13 April 2017; $R^2 = 0.847$ for the samples collected on 18 May 2017, Figure 23C). For the water samples collected on 13 April 2017, pH was strongly or somewhat negatively correlated with DOC ($R^2 = 0.8117$, Figure 26C), EDC ($R^2 = 0.627$, Figure 30B) and COD ($R^2 = 0.778$, Figure 30E), while pH was somewhat positively correlated with EC25 ($R^2 = 0.591$, Figure 29C). For the water samples collected on 18 May 2017, pH was somewhat negatively correlated with EDC ($R^2 = 0.785$, Figure 30B) and EC25 ($R^2 = 0.576$, Figure 29C), while pH was strongly negatively correlated with ORP ($R^2 = 0.901$, Figure 29B). The pH was somewhat or strongly negatively correlated with all total metals including Al ($R^2 = 0.541$, Figure 31A), Mg ($R^2 = 0.880$, Figure 31B), Ni ($R^2 = 0.782$, Figure 31E), Fe ($R^2 = 0.850$, Figure 31C) and Zn ($R^2 = 0.851$, Figure 31F). Generally, pH exhibited correlations with more parameters in water samples without hypoxia. However under the hypoxic conditions, pH exhibited correlations with DO and organic carbon properties such as DOC and COD, and redox properties such as ORP and EDC. These findings suggest that under hypoxic conditions typical of post flood hypoxic blackwater events, reactions involving organic carbon and other redox processes likely impact pH, as electron transfer is often coupled to uptake or release of $H^+$ [240]. This could also explain the correlations observed between redox properties such as EDC and ORP with pH.

Other relationships

The relationships between temperature and other parameters are shown in Figure 38 to Figure 40. The in situ temperature was approximately 24°C at all sites on 13 April 2017 and about 18°C at all sites on 18 May 2017. DO, ORP and EC25 were typically lower at higher temperature (~23.5°C) on 13 April 2017 compared to 18 May 2017. In contrast BOD, COD and DOC were typically higher at higher temperature, while $E_2/E_3$ and SUVA$_{280}$ did not vary noticeably with temperature. Total metals did not show much change with temperature.

For the water samples collected on 18 May 2017, COD was somewhat positively correlated with $E_2/E_3$ ($R^2 = 0.667$, Figure 36A), while COD was somewhat negatively correlated with SUVA$_{280}$ ($R^2 = 0.532$, Figure 36C). Previous studies found that the DOM produced from wetland sediments normally has a higher molecular weight with a less labile character [133, 226, 227], suggesting it would take a longer time to
decompose during inundation. Organic carbon from this source also tends to decompose at a slower rate than the organic carbon from material like grass [228, 231]. Thus, the relatively stable SUVA$_{280}$ values observed in this study suggest that much of the organic carbon present in the surface waters likely originated from the floodplain sediments.

Figure 22 Heat maps of significance ($p$ values) of the correlation between surface water parameters using the nonparametric Spearman’s rank-order correlation. A. Correlation analysis of data from samples collected on 13 April 2017. B. Correlation analysis of data from samples collected on 18 May 2017. C. Correlation analysis for all data. Red shading corresponds to $p < 0.001$. 

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Figure 23  Relationships between DO and general physical and chemical parameters of surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: Temperature, B: ORP, C: pH, D: EC25. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 24 Relationships between DO and properties of organic carbon in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: E₂/E₃, B: EDC, C: SUVA₂₈₀, D: BOD, E: COD, F: DOC. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 25 Relationship between DO and total metals in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: total Al, B: total Mg, C: total Fe, D: total Cu, E: total Ni, F: total Zn. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 26 Relationships between DOC and general physical and chemical parameters of surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: Temperature, B: ORP, C: pH, D: EC25. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 27 Relationships between DOC and properties of organic carbon in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: E₂/E₃, B: EDC, C: SUVA₂₈₀, D: BOD, E: COD. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 28 Relationship between DOC and total metals in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: total Al, B: total Mg, C: total Fe, D: total Cu, E: total Ni, F: total Zn. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 29 Relationships between pH and general physical and chemical parameters of surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: Temperature, B: ORP, C: EC25. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 30 Relationships between pH and properties of organic carbon in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: $E_2/E_3$, B: EDC, C: SUVA$_{280}$, D: BOD, E: COD. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 31 Relationship between pH and total metals in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: total Al, B: total Mg, C: total Fe, D: total Cu, E: total Ni, F: total Zn. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 32 Relationships between BOD and general physical and chemical parameters of surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: Temperature, B: ORP, C: EC25. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 33: Relationships between BOD and properties of organic carbon in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: $E_2/E_3$, B: EDC, C: SUVA\textsubscript{280}, D: COD. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 34 Relationship between BOD and total metals in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: total Al, B: total Mg, C: total Fe, D: total Cu, E: total Ni, F: total Zn. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 35 Relationships between COD and general physical and chemical parameters of surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: Temperature, B: ORP, C: EC25. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 36 Relationships between COD and properties of organic carbon in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: $E_2/E_3$, B: EDC, C: SUVA$_{280}$. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 37 Relationship between COD and total metals in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: total Al, B: total Mg, C: total Fe, D: total Cu, E: total Ni, F: total Zn. Lines represent the best fit from linear regression, with Pearson product moment correlation coefficients also shown.
Figure 38 Relationships between temperature and general physical and chemical parameters of surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: DO, B: ORP, C: pH, D: EC25.
Figure 39 Relationships between temperature and properties of organic carbon in surface waters at field study sites in the Richmond River system measured on 13 April 2017 (○) and 18 May 2017 (□). A: E$_2$/E$_3$, B: EDC, C: SUVA$_{280}$, D: BOD, E: COD, F: DOC.
3.3.4 Multivariate correlation analysis

As shown in Figure 41, the eigenvalues of the two first principal components identified by PCA represent 64.6% (PC1 33.9%; PC2 30.7%) of the total variation of the post flood water samples. This increases to 76.1% when taking into account the first three principal components.
The factor loadings representing the correlation between the original variables and principal components 1 and 2 are shown in Figure 42. More than 75% of the variance of the first eight parameters (EDC, DOC, pH, COD, Zn, DO, Ni, ORP) is represented in the PC1-PC2 plane. In Figure 42B, positively correlated parameter values point to the same side of the plot, and negatively correlated parameter values point to opposite sides of the graph, while the distance between parameters and the origin shows the magnitude of the correlation coefficient between variances of the origin parameters and the first two principal components. Temperature, ORP and E2/E3 contribute to the construction of PC1, and pH, EC25, Mg and Al contribute to the construction of PC2. EDC, Zn, Ni and Cu, which are located next to the diagonal Y=X separating the positive values of PC1 and PC2, are characteristic of external impacts. DO and Fe next to Y=-X are characteristic of all allochthonous impacts. DO is positively correlated with SUVA280 and negatively correlated with Fe, DOC, BOD and COD.
Figure 42 Loadings of physico-chemical parameters in surface waters at field study sites in the Richmond River in principal components 1 and 2 resulting from PCA. A. Shown as a proportion of the total contribution to principal components 1 and 2. B. Shown in the PC1-PC2 axes plane. The quality of representation of each parameter is given by $\cos^2$ (square cosine, squared coordinates), which represents the importance of a principal component for a given observation (vector of original variables) [218]. The distance between parameters and the origin shows the correlation coefficient magnitude between variances and the two major principal components. Positively inter-correlated parameters are grouped together, while negatively inter-correlated parameters are positioned on opposite sides of the origin.
Figure 43 shows the loadings of each of the water samples in this study. The water samples from Wardell (13 April 2017) and Ballina River (13 April 2017) were strongly correlated to PC1; the water samples from Lismore River (13 April 2017), Codrington (13 April 2017), Coraki (13 April 2017), Wardell (18 May 2017) and Ballina River (18 May 2017) were strongly correlated to PC2. The other water samples all demonstrated correlations between PC1 and PC2. 10% to 27% of the properties of water samples from Rocky Mouth Creek (both 13 April 2017 and 18 May 2017) and Tuckean Barrage (18 May 2017) could be explained by PC1 and PC2. These three locations exhibited characteristics of hypoxic blackwater during the field study period, indicating strong correlations between these principal components and the properties of hypoxic blackwater.
Figure 43 Loadings of the field study sites in the Richmond River in principal components 1 and 2 resulting from PCA of the measured physico-chemical parameters. A. Shown as a proportion of the total contribution to PC$_1$ and PC$_2$, where the dashed line represents 5% contribution. B. Shown as a scatter plot indicating the relative contributions of PC$_1$ and PC$_2$ to the total loading for each site.
3.4 Conclusions and Implications

This study combined *in situ* and laboratory measurements of surface water samples from the Richmond River floodplain system, enabling observation of spatial and temporal variability after a 1 in 30 year ARI flood event in the area. DO measurements indicated that four sites exhibited hypoxic conditions two weeks after the flood peak on 13 April 2017, including Woodburn with 2.24 mg/L DO, Rocky Mouth Creek with 0.55 mg/L DO, Tuckean Barrage with 1.96 mg/L DO and Wardell with 2.08 mg/L DO. One site still exhibited hypoxic conditions seven weeks after the flood peak on 18 May 2017, namely Rocky Mouth Creek with a DO concentration of 2.78 mg/L. In the backswamps on the lower floodplain from Bungawalbyn Creek to Ballina River, the waters had high DOC concentrations (above 15 mg/L) two weeks after the flood peak on 13 April 2017, including DOC concentrations at Bungawalbyn Creek and Rocky Mouth Creek that were about 25 mg/L. On 18 May 2017, the DOC had decreased to < 10 mg/L for almost all sampling sites besides Bungawalbyn Creek with 13.4 mg/L DOC.

The lowest *in situ* pH measured on 13 April 2017 was 6.2 at Rocky Mouth Creek and the highest *in situ* pH was 7.48 at Lismore River. In contrast, the lowest *in situ* pH on 18 May 2017 was 5.2 at Tuckean Barrage and highest *in situ* pH was 7.64 at Ballina River. The pH values at Rocky Mouth Creek and Tuckean Barrage on 18 May 2017 were lower than those measured on 13 April 2017, while the pH at other sites did not exhibit big changes between 13 April 2017 and 18 May 2017. The ORP values were higher on 18 May 2017 at almost all sites compared with the ORP results on 13 April 2017, and the maximum change was Rocky Mouth Creek from -146.6 mV to 277.8 mV, indicating a large shift from reducing to oxidising conditions. The minimum EC25 at all sampling sites on 13 April 2017 was 87.4 μS/cm at Bungawalbyn Creek, while the maximum EC25 was 233.1 μS/cm at Codrington. On 18 May 2017, the maximum EC25 was 1018 μS/cm at Rocky Mouth Creek and the minimum was 358.8 μS/cm at Bungawalbyn Creek. The EC25 values at all sites on 18 May 2017 were higher than those measured on 13 April 2017.

E2/E2 values were comparatively stable spatially and temporally for all field study sites and ranged from 2.96 to 3.49, similar to the E2/E3 values of the three standard HS obtained in Chapter 2 which ranged from 2.5 to 4.9. Values of SUVA280 on 13 April 2017 were higher than those on 18 May 2017 for most of the sites, besides Lismore River, Ruthven and Rocky Mouth Creek. The range was from 0.026 m−1.
L/mg to 0.058 m⁻¹L/mg, similar to values for the standard HS in Chapter 2 which ranged from 0.025 m⁻¹L/mg to 0.059 m⁻¹L/mg. BOD ranged from 0.9 mg/L O₂ to 8.6 mg/L O₂ on 13 April 2017 and ranged from 0.5 mg/L O₂ to 2.6 mg/L O₂ on 18 May 2017. COD ranged from 7 mg/L O₂ to 76 mg/L O₂ on 13 April 2017 and ranged from 1 mg/L O₂ to 25 mg/L O₂. Concentrations of total metals were higher at hypoxic blackwater sites than non-hypoxic sites.

Principal components analysis (PCA) results showed that the eigenvalues of the two first principal components extracted from the dataset represent up to 64.6% (PC₁ 33.9%; PC₂ 30.7%) of the total parameters of the post flood water samples. DO was shown to be positively correlated to SUVA₂₈₀ and negatively correlated to Fe, DOC, BOD and COD. Strong correlations were established between these principal components and characteristics of hypoxic blackwaters, with a range from 10% to 27% of the water sample properties able to be explained by principal components 1 and 2.

The results indicated that DOC, pH, COD and Fe were significant parameters associated with hypoxic blackwater formation. The importance of these parameters reflects the important role played by backswamps on the Richmond River floodplain in contributing to geochemical conditions that can promote hypoxic blackwater formation. Leaching and subsequent decomposition of carbon from organic material on these floodplains results in development of hypoxic conditions [10], which can then extend into the main streams via hydrologic connectivity, potentially leading to expanding coastal hypoxia blackwater events as described in the Introduction. The results of this chapter indicated that DO concentration was very low at some sampling sites after 2017 flood. Statistical analysis showed that Fe and pH might also be important factors influencing development of hypoxia in the systems. However, this correlation does not indicate causality. For example, Fe (and its complexes with DOM) could consume DO via photochemical reactions, while at the same time Fe concentrations could also increases as a result of the low DO concentrations [150, 241]. Another issue was that the bivariate statistical correlations can include incidental relations among the various parameters that are not particularly meaningful, e.g. between COD and BOD.

Large scale hypoxic blackwater issues have previously occurred in the Richmond River system during warm season flooding, and the very low DO concentrations
recorded in the backswamps and streams could last for one month or more [3, 114]. Inflows of the hypoxic water into the main river increase the risk to water quality and biota. Based on this field study, the sites that were most strongly impacted by post flood hypoxic blackwater were Rocky Mouth Creek, Bungawalbyn Creek, Woodburn, Tuckean Barrage, and Wardell. Accurate knowledge of the locations of hypoxia is important for floodplain management processes, which might include *in situ* monitoring and drainage management plans.

The findings of Chapter 2 suggested that higher DOM concentrations could lead to higher photochemical DO consumption. The findings in Chapter 3 showed that the surface waters at the floodplain sites with higher DOC concentrations (Rocky Mouth Creek, Bungawalbyn Creek, Woodburn, Tuckean Barrage, and Wardell) experienced more severe post flood deoxygenation during the 2017 flood event. Previous studies on the Richmond River system have demonstrated that the DOC concentration of surface waters in this aquatic environment can be influenced by the type of inundated floodplain vegetation and soil composition; deoxygenation potential can also be enhanced by modification of floodplain drainage [4, 8, 204]. Thus, the investigations in this chapter have confirmed a major relationship between DOC and deoxygenation potential, as expected, while the findings in Chapter 2 have shown that photochemical DO consumption under these conditions could be a significant contributor to deoxygenation.
Chapter 4 – Influence of Fe and pH on photochemical DO consumption by NOM

4.1 Introduction

The role of iron in natural waters has been broadly studied due to its important impacts on global carbon cycles, nitrogen fixation, and oceanic primary production [94, 95, 150, 242]. Eastern Australian coastal floodplains often contain acid sulfate soils (ASS) and sulfidic sediments, which can release a considerable amount of reactive Fe and other metals into surface waters, inducing redox processes including dissolved oxygen (DO) consumption during flooding [4, 207, 243, 244]. Post-flood hypoxic blackwater normally exhibits high chemical oxygen demand (COD) and dissolved Fe concentrations, while Mn(IV), Fe(III) and SO$_4^{2-}$ are significant electron acceptors during organic matter decomposition [3, 4].

In natural waterbodies with pH from 4 to 9, Fe(III) typically associates with DOM in dissolved complexes and stable colloids [102, 115]. Previous studies showed that approximately 99% dissolved Fe exists in form of the complexes with DOM through metal-bridge complexation processes, which are a ubiquitous influence on Fe speciation in natural waters [96, 97, 118, 245, 246]. HS is considered to account for about 15% to 19% of aquatic DOM, and is the main component of DOM responsible for Fe complexation [51, 247]. Previous studies showed that Fe-DOM complexes appeared around pH 6, but were barely observed when pH was < 4 [150]. Complexation of Fe(III) can increase light absorption of CDOM, especially in the longer wavelengths of the visible spectrum [108, 117]. For example, the absorption coefficient of CDOM at 330 nm was previously observed to increase with rising Fe concentration [150]. Previous studies also showed that dissolved Fe in surface waters could enhance the UV absorbance of DOM [248, 249]. Photoreduction of Fe(III) to Fe(II) can also occur in Fe-DOM complexes, with increasing efficiency at shorter irradiance wavelengths, while photon flux densities of solar radiation are maximal in the near UV and short wavelength range of the visible spectrum [40, 102].

Fe can contribute to photoreactions in two main ways: (1) through photochemical decarboxylation of Fe(III)-carboxylate complexes; and (2) via photo-Fenton reactions. In the first mechanism, decarboxylation can occur through light-induced ligand-to-metal-charge-transfer (LMCT) to produce dissolved inorganic carbon
In the second mechanism, photoproduced Fe(II) can reduce O$_2$ to superoxide/hydroperoxyl radicals (HO$_2$/O$_2^{-}$). Dismutation of HO$_2$/O$_2^{-}$ produces hydrogen peroxide (H$_2$O$_2$), which can subsequently oxidise Fe(II) and generate hydroxyl radicals. Recent study by Gu et al. (2017) showed that at low pH (< 4), high Fe loading (> 3.5 µmol mg DOM$^{-1}$) had a stimulatory effect on light absorption under simulated solar irradiation, and increased the photobleach ing rate and spectral slope of CDOM by changing its optical properties [118].

In Chapter 2 of this thesis, all three model humic substances (HS) examined displayed a strong linear relationship between the magnitude of DO consumption and the optical parameter SUVA$_{280}$ ($\Delta$SUVA$_{280}$) over 7 h during exposure to simulated solar irradiation, highlighting the potential to use SUVA$_{280}$ as an indicator or predictor of photochemical deoxygenation potential in hypoxic blackwater. However, correlation analysis of field samples from hypoxic blackwater in Chapter 3 of this thesis showed that pH and Fe concentration were also significantly correlated with in situ deoxygenation of floodwaters. Low DO concentration (< 2 mg/L) was found to occur concurrently with high Fe concentration and low pH, especially in the backswamp area of the Richmond River system, during the post flood period. Optical properties including absorption and fluorescence have been used to study the composition and reactivity of DOM, but the relationship between optical properties and potential DO consumption rate in natural waters has not yet been investigated in detail as far as I know. The influence of Fe on photochemical deoxygenation also remains unclear due to limits of analytical methods and the complexity of DOM photoreactions in natural waters. The principal components analysis (PCA) in Chapter 3 showed that DO is positively correlated with SUVA$_{290}$ and negatively correlated with Fe, DOC, BOD and COD, which further highlighted the relationship between DO concentration and Fe as well as DOC. The findings of Chapter 2 and Chapter 3 suggest that HS concentration, pH and Fe concentration may all potentially contribute to photochemical deoxygenation in the Richmond River system.

In order to further understand this potential, Chapter 4 further investigates the relationships between DO consumption and HS concentration, Fe concentration and pH, specifically. This study aimed to determine the influence of Fe concentration and pH on the magnitude of photochemical deoxygenation in the presence of DOM. A combination of various Fe concentrations ranging from 0 to 175 µmol/L and pH
ranging from 4 to 8 was examined. These conditions span the typical range of natural river waters, including hypoxic blackwaters [111, 118, 250]. Additionally the photochemical deoxygenation potential of 20 natural field surface water samples obtained from the Richmond River system after a 1 in 30 year flood in 2017 was measured. Measurements of photochemical DO consumption and changes in optical properties were compared between the natural water samples and solutions prepared from standard HS, which allowed further evaluation of the relationship between SUVA$_{280}$ and deoxygenation potential. In this study, the association of Fe with DOM is abbreviated as Fe-DOM, which includes complexes between Fe and DOM as well as Fe(III) (oxy)hydroxide colloids stabilised by DOM.

4.2 Experimental Section

4.2.1 Preparation of solutions with standard HS

In order to study the influence of Fe and pH on photochemical deoxygenation in the presence of DOM, experimental conditions were based on a Central Composite Design (CCD) approach used by Gu et al. [118], as shown in Table 9. Five points close to various combinations of pH and Fe concentration represented in the form (pH, [Fe] in µmol L$^{-1}$) as (4.59, 150), (6, 90), (7.99, 90), (4.01, 90), and (6, 0) were duplicated to test the response variance. Irradiation experiments therefore involved 16 combinations of pH and [Fe] altogether.

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>pH</th>
<th>[Fe] (µmol L$^{-1}$)</th>
<th>Replicate experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.59</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>7.41</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>7.41</td>
<td>150</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>4.59</td>
<td>150</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>175</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>7.99</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>4.01</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>175</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

A single HS concentration of 50 mg/L was used based on a typical DOC concentration observed in previous blackwater studies. Because soil humic acid is expected to be the main DOC type in floodwater, standard LHA was used as the model DOM in this study. A 50 mg/L LHA stock solution was prepared by dissolving
standard LHA in 18 MΩ.cm resistivity MilliQ water, using 1 M NaOH and 0.1 M HCl to help with dissolution and final pH adjustment. A 17.5 mM Fe stock solution was made by dissolving ferric chloride hexahydrate (FeCl₃•6H₂O, Sigma) in 0.1 M HCl. Various Fe-DOM complex solutions were made by mixing 5 mL of 50 mg/L LHA solution with the required volume of FeCl₃ stock solution, and adjusting to the required pH using 1 M NaOH or 100 mM HCl. After reaching the required pH, Fe-DOM samples were kept in the dark at 25°C for 48 h to enable equilibration of the Fe and LHA before commencing irradiation experiments.

4.2.2 Natural water samples
On 13 April 2017 and 18 May 2017, 20 field surface water samples were collected in Richmond River system including backswamps and main riverine streams, as described in Chapter 3. These samples included 6 hypoxic blackwater samples from Rocky Mouth Creek, Bungawalbyn Creek, Woodburn, Tuckean Barrage, and Wardell on 13 April 2017, and a sample from Rocky Mouth Creek on 18 May 2017. The remaining water samples all contained > 4 mg/L DO measured in situ. Before experiments, all the natural water samples were filtered to remove microorganisms and prevent microbial respiration and/or photosynthesis. Samples were also kept at 25°C for 24 hours before irradiation to allow equilibration with atmospheric O₂.

4.2.3 Experimental measurements and analysis
Simulated solar irradiation experiments were performed over a 7 h duration with both field water samples and Fe-DOM solutions prepared using standard HS. Details of the irradiation procedure are the same as those described in Chapter 2. Measured parameters in this study included DO consumption, SUVA₂₈₀, E₂/E₃, and electron donating capacity (EDC), using the same procedures as described in Chapter 2.

The relationships between optical properties, EDC, photochemical DO consumption and combinations of Fe concentration and pH were investigated with multivariate linear regression implemented via the MATLAB code ‘Fast & Detailed Multivariate OLS Regression’ (downloaded from https://au.mathworks.com/matlabcentral/), again following the approach of Gu et al. [118]. For this analysis, [H⁺] (= 10⁻pH) was used as the variable instead of pH. The general form of the regression equation applied is:

\[ y = b_1 + b_2[Fe] + b_3[H^+] + b_4[Fe][H^+] \]  

(Eq 35)
where \( y \) is the dependent (measured) variable (e.g., DO consumption, optical properties and EDC) and \( b_1 \) to \( b_4 \) are the regression coefficients. For each dependent variable, six models (i.e., the general case where all coefficients were non-zero, and simpler models where at least one of the coefficients was zero) were analysed. In each case the adjusted coefficient of determination (adjusted \( R^2 \)), root mean squared error (RMSE), degrees of freedom (df), and corrected Akaike Information Criterion (AICc) values were calculated [251, 252]. The best model for each dependent variable was selected based on the lowest AICc value.

### 4.3 Results and Discussion

#### 4.3.1 Effects of Fe concentration and pH during irradiation of standard HS solutions

Table 10 shows the initial values for optical properties (\( E_2/E_3 \) and SUVA\(_{280} \)) and EDC, and change in these parameters along with DO consumption after 7 h irradiation.

<table>
<thead>
<tr>
<th>No.</th>
<th>Fe (µmol/L)</th>
<th>pH</th>
<th>( E_2/E_3 )</th>
<th>EDC (mole e⁻/g DOC)</th>
<th>SUVA(_{280} ) (m⁻¹L/mg DOC)</th>
<th>∆DO (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>initial</td>
<td>Δ( E_2/E_3 )</td>
<td>initial</td>
<td>ΔEDC</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>3.98</td>
<td>2.56</td>
<td>0.30</td>
<td>0.22</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>4.00</td>
<td>2.09</td>
<td>0.19</td>
<td>1.52</td>
<td>1.46</td>
</tr>
<tr>
<td>3</td>
<td>175</td>
<td>4.00</td>
<td>1.90</td>
<td>0.14</td>
<td>2.12</td>
<td>1.96</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>6.00</td>
<td>2.74</td>
<td>0.01</td>
<td>0.38</td>
<td>0.18</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
<td>7.99</td>
<td>2.55</td>
<td>0.30</td>
<td>0.42</td>
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</tr>
<tr>
<td>6</td>
<td>90</td>
<td>6.00</td>
<td>2.57</td>
<td>0.05</td>
<td>0.65</td>
<td>0.01</td>
</tr>
<tr>
<td>7</td>
<td>175</td>
<td>6.00</td>
<td>2.54</td>
<td>0.10</td>
<td>0.67</td>
<td>0.22</td>
</tr>
<tr>
<td>8</td>
<td>150</td>
<td>4.59</td>
<td>2.04</td>
<td>0.03</td>
<td>2.76</td>
<td>4.70</td>
</tr>
<tr>
<td>9</td>
<td>150</td>
<td>7.41</td>
<td>2.60</td>
<td>0.01</td>
<td>0.60</td>
<td>-0.02</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>4.59</td>
<td>2.64</td>
<td>0.12</td>
<td>0.72</td>
<td>-0.12</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>7.41</td>
<td>2.50</td>
<td>0.16</td>
<td>0.55</td>
<td>-0.18</td>
</tr>
</tbody>
</table>

When the same HS concentration (50 mg/L LHA) was associated with various Fe concentrations and different pH values, all the Fe-DOM solutions exhibited DO consumption after 7 h simulated solar irradiation. The magnitude of DO consumption as a function of pH and Fe concentration is shown in Figure 44, while Table 11 shows the fitted coefficients and calculated fit statistics for multivariate linear regression modelling. As shown, DO consumption increased with increasing Fe concentration,
but did not change much with pH. Previous studies showed that irradiation could lead to DOM mineralisation and bleaching [181, 253], and could cause the photochemical flocculation of DOM [152]. It was also suggested that the ROS generated by DOM photo reaction could react with Fe(II) and Fe(III) [253, 254]. Ligand-metal charge transfer reactions can contribute to significant photodegradation of DOM at low pH [112, 132, 255]. The investigation by Helms [152] found that photochemical flocculation accounted for approximately 90% of total iron remaining after long term irradiation, and was responsible for about 10% of the decrease in absorbance at 300 nm. Photochemical reactions of DOM are likely produce both dissolved and particulate forms of iron, such as dissolved or colloidal Fe-DOM complexes [245, 256]. Previous studies also found that the “bridge” between DOM and Fe was degraded in surface seawater and transformed Fe into inorganic complexes, which was considered to be induced via photochemical reactions within DOM [257]. The possible reactions are as follows:

\[
\begin{align*}
& \text{Fe}^{2+} \leftrightarrow \text{Fe}^{3+} + e^- \quad \text{(Eq 36)} \\
& \text{Fe}^{2+} + \text{O}_2 \rightarrow \text{Fe}^{3+} + \text{O}_2^- \quad \text{(Eq 37)} \\
& \text{Fe}^{3+} + \text{OH}^- \rightarrow \text{Fe(OH)}_3 \quad \text{(Eq 38)} \\
& \text{Fe}^{3+} + \text{DOM} \rightarrow \text{Fe-DOM} \quad \text{(Eq 39)} \\
& \text{Fe}^{2+} + \text{HOOH} \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH} \quad \text{(Eq 40)} \\
& \text{Fe(OH)}^{2+} + \hnu \rightarrow \text{Fe}^{2+} + \cdot\text{OH} \quad \text{(Eq 41)} \\
& \text{Fe}^{2+} + \cdot\text{OH} \rightarrow \text{Fe}^{3+} + \text{OH}^- \quad \text{(Eq 42)} \\
& \text{Fe(OH)}^{2+} + \text{Fe}^{2+} + \text{O}_2 + \hnu \rightarrow \text{Fe(OH)}^{2+} + \text{Fe}^{3+} + \text{O}_2^- \quad \text{(Eq 43)}
\end{align*}
\]

The initial SUVA\textsubscript{280} of Fe-DOM solutions is shown in Figure 45, and the ΔSUVA\textsubscript{280} after 7 h irradiation is shown in Figure 46. Table 12 and Table 13 show the fitted coefficients and calculated fit statistics for multivariate linear regression modelling of these two quantities, respectively. Figure 45 showed that the SUVA\textsubscript{280} values were influenced by both Fe concentration and pH: SUVA\textsubscript{280} decreased with higher Fe concentration and lower pH, especially when pH < 5. These findings are different from those in the previous study by Gu et al. [108] which found SUVA\textsubscript{280} had greater values with higher Fe concentration but did not vary with pH. Previous research
claimed that pH likely controlled the Fe(III) distribution between colloidal form and dissolved form, and that there may be more Fe(III) oxyhydroxide colloids at higher pH [256]. In this study, we used commercially available standard HS and applied different procedures for preparation of the Fe-DOM solutions compared Gu et al. [108]. It is generally acknowledged that the HS properties can change with experimental procedures (particularly in relation to isolation of NOM), and caution is needed to compare parameters between different studies [225, 258]. Previous work demonstrated that the different pathways for Fe-DOM formation could result in noticeable differences in absorption spectra even with the same Fe concentration [108].

For all Fe-DOM solutions, 7 h irradiation induced a decrease in SUVA\textsubscript{280} which suggests a decrease in the average molecular size and aromaticity of DOM, and/or destruction of Fe-DOM complexes given that lower SUVA\textsubscript{280} and higher E\textsubscript{2}/E\textsubscript{3} both indicate less aromaticity and smaller molecular weight [188]. This is consistent with the results of Chapter 2 and Chapter 3. However, the magnitude of this change appeared relatively independent of both pH and Fe concentration.

The initial E\textsubscript{2}/E\textsubscript{3} of Fe-DOM solutions is shown in Figure 47, and the ΔE\textsubscript{2}/E\textsubscript{3} after 7 h irradiation is shown in Figure 48. Table 14 and Table 15 show the fitted coefficients and calculated fit statistics for multivariate linear regression modelling of these two quantities, respectively. Fe concentration was negatively correlated with E\textsubscript{2}/E\textsubscript{3} for all samples regardless of the pH value. When pH < 4.5, the E\textsubscript{2}/E\textsubscript{3} value was influenced particularly strongly by Fe concentration, which is consistent with the study of natural hypoxic blackwater samples in Chapter 3. Previous studies found that light absorption at higher wavelengths decreased with Fe(III) concentration to a greater extent than at lower wavelengths [108], consistent with the decrease in E\textsubscript{2}/E\textsubscript{3} with higher Fe concentration observed in this study. Previous studies have also found that the way in which Fe-DOM solutions are produced also influences the spectral properties of the solution, which will result in a negative correlation between Fe concentration and change in E\textsubscript{2}/E\textsubscript{3}, along with a greater increase in \( a_{\text{365}} \) than \( a_{\text{250}} \) with decreasing Fe(III) concentration. The presence of dissolved would also lead to precipitation Fe(OH)\textsubscript{3} when Fe concentration is high [118, 259].

\[
\text{Fe}^{3+} + \text{OH}^- \rightarrow \text{Fe(OH)}_3 \quad \text{(Eq 44)}
\]
For all the Fe-DOM solutions, 7 h irradiation induced a decrease in $E_2/E_3$ which suggested a decrease in the average molecular size of the DOM. This was most pronounced at low pH ($< 4$) and low Fe concentration ($< 100 \mu\text{mol/L}$). This could potentially be due to the fact that low pH can stimulate photoproduction of HO•, which can attack chromophores in DOM thus increase photobleaching [61, 89, 119], although such an effect would be expected to be more pronounced at high Fe concentration, rather than low Fe concentration. It has been demonstrated that the production of hydroxyl radicals in sunlit waters occurs by various pathways including photolysis reactions of nitrite and nitrate, DOM photolysis and photo-Fenton reaction when dissolved Fe is present [89, 260]. Nitrite photolysis is considered to be unimportant for hydroxyl radical production when its concentration is less than 1% of nitrate concentration [89]. The Fe related photo reactions of DOM are limited by the solubility of Fe(III), which is impacted by Fe concentration as well as pH. Dissolved Fe complexes are more photochemically reactive than non-dissolved counterparts, and the precipitation of Fe in forms such as iron hydroxide could increase the internal light scattering in water.

Figure 49 shows the initial EDC of Fe-DOM solutions at different pH while Figure 50 shows the change in EDC after 7 h irradiation. Table 16 and Table 17 show the fitted coefficients and calculated fit statistics for multivariate linear regression modelling of these two parameters, respectively. EDC ranged from 0.22 mole/g DOC to 2.76 mole/g DOC, and increased with increasing Fe concentration and decreasing pH. The latter effect was not necessarily expected, given that samples were adjusted to pH 7 immediately prior to addition of DCPIP in order to be able to perform the assay for EDC. This suggests that the observed changes in EDC relate to changes in the chemical properties of DOM or Fe-DOM complexes that are not readily reversible on the timescale of the assay (~1 h).

Simulated solar irradiation for 7 h increased the EDC, especially at high Fe concentration ($> 150 \mu\text{mol/L}$) and low pH ($< 6$). In samples with low Fe concentration and high pH, the change in EDC was not significant. The EDC of DOM has been identified as arising mainly from phenols, which are also strongly involved in photochemical reactions of DOM [62, 68, 71, 261]. For aquatic HS, SUVA$_{280}$ is strongly correlated with EDC [46], and phenols are also the main contributor to both SUVA$_{280}$ and $E_2/E_3$. In contrast, for HS of terrestrial origin, quinones are considered as the main determinant of SUVA$_{280}$ [174]. Previous studies of the relationships...
between Fe species and DOM suggested that there are a range of reactions which can occur. For example ligand to metal charge transfer (LMCT), reactions between Fe(III) and superoxide, and reactions between Fe(III) and other organic moieties in HS including semiquinone and hydroquinone. All these reactions would produce Fe(II) [150, 251], which could potentially contribute to the EDC in DOM solution. This reaction is strongly influenced by Fe(III) concentration and pH: when Fe(III) concentration is higher, the rates of Fe(II) and H⁺ production are higher. Previous studies also found that EDC of SRFA increased with pH, which suggested that electron donating components in SRFA generated protons [53], or perhaps due to slower oxidation of phenolic than phenolate components in HA [178]. The results in this chapter do not agree with these previous findings, but rather suggest that EDC might be strongly related to Fe(III) concentration and pH. Possible reasons for this include more dissolved Fe(III) and slower oxidation kinetics of Fe(II) [251].
Table 11 Multivariate linear regression modelling for photochemical DO consumption in irradiated Fe-DOM solutions. The optimal model is marked with a * and highlighted in yellow.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>1</th>
<th>2*</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>b₁</td>
<td>111.75</td>
<td>88.09</td>
<td>100</td>
<td>80</td>
<td>101.90</td>
<td>89.90</td>
<td>103</td>
</tr>
<tr>
<td>[Fe]</td>
<td>b₂</td>
<td>0.257</td>
<td>0</td>
<td>0.20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[H⁺]</td>
<td>b₃</td>
<td>2.61 x 10⁵</td>
<td>2.06 x 10⁵</td>
<td>-5.13 x 10⁴</td>
<td>1.24 x 10⁵</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Fe][H⁺]</td>
<td>b₄</td>
<td>2.72 x 10⁵</td>
<td>1.77 x 10⁵</td>
<td>3.02 x 10⁵</td>
<td>8.70 x 10²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted R²</td>
<td></td>
<td>0.000</td>
<td>0.203</td>
<td>0.073</td>
<td>0.237</td>
<td>0.202</td>
<td>0.233</td>
<td>0.147</td>
</tr>
<tr>
<td>RMSE</td>
<td>1.0 x 10⁻⁶</td>
<td>7.2 x 10⁻⁵</td>
<td>9.7 x 10⁻⁵</td>
<td>6.6 x 10⁻⁵</td>
<td>7.2 x 10⁻⁵</td>
<td>6.6 x 10⁻⁵</td>
<td>8.2 x 10⁻⁵</td>
<td>7.5 x 10⁻⁵</td>
</tr>
<tr>
<td>df</td>
<td>16</td>
<td>15</td>
<td>15</td>
<td>14</td>
<td>15</td>
<td>14</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>AICs</td>
<td>7.14</td>
<td>6.97</td>
<td>7.12</td>
<td>6.98</td>
<td>6.97</td>
<td>6.98</td>
<td>7.09</td>
<td>7.08</td>
</tr>
</tbody>
</table>

Figure 44 Impact of [Fe] and pH on DO consumption in irradiated 50 mg/L LHA solutions. Coloured squares represent measured data and the mesh represents the fit of the optimal multivariate linear regression model shown in Table 11.
Table 12 Multivariate linear regression modelling for the SUVA$_{280}$ of Fe-DOM solutions. The optimal model is marked with a * and highlighted in yellow.

<table>
<thead>
<tr>
<th>Item</th>
<th>Coefficient</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Intercept $b_1$</td>
<td>0.066</td>
<td>0.065</td>
</tr>
<tr>
<td>[Fe] $b_2$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[H$^+$] $b_3$</td>
<td>-127.98</td>
<td>-136.88</td>
</tr>
<tr>
<td>[Fe][H$^+$] $b_4$</td>
<td>-0.916</td>
<td>-1.131</td>
</tr>
<tr>
<td>adjusted $R^2$</td>
<td>0</td>
<td>0.066</td>
</tr>
<tr>
<td>RMSE</td>
<td>6.1 x 10$^{-9}$</td>
<td>7.0 x 10$^{-9}$</td>
</tr>
<tr>
<td>df</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 45 Impact of [Fe] and pH on the initial SUVA$_{280}$ of 50 mg/L LHA solutions. Coloured squares represent measured data and the mesh represents the fit of the optimal multivariate linear regression model shown in Table 12.
Table 13  Multivariate linear regression modelling for the change in SUVA$^{280}$ (ΔSUVA280) of Fe-DOM solutions after irradiation. The optimal model is marked with a * and highlighted in yellow.

<table>
<thead>
<tr>
<th>Model Coefficient</th>
<th>Coefficient</th>
<th>Model</th>
<th>1*</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept $b_1$</td>
<td>-0.007</td>
<td>-0.007</td>
<td>-0.006</td>
<td>-0.006</td>
<td>-0.006</td>
<td>-0.007</td>
<td>-0.006</td>
<td>-0.006</td>
<td>-0.006</td>
<td>-0.006</td>
</tr>
<tr>
<td>[Fe] $b_2$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[H$^+$] $b_3$</td>
<td>-19.11</td>
<td>-19.17</td>
<td>-9.93</td>
<td>-7.91</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Fe][H$^+$] $b_4$</td>
<td>-0.16</td>
<td>-0.19</td>
<td>-0.11</td>
<td>-0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted $R^2$</td>
<td>0.000</td>
<td>-0.105</td>
<td>0.016</td>
<td>-0.010</td>
<td>0.030</td>
<td>-0.078</td>
<td>-0.070</td>
<td>-0.221</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMSE</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>df</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 46  Impact of [Fe] and pH on the ΔSUVA$^{280}$ in irradiated 50 mg/L LHA solutions. Coloured squares represent measured data and the mesh represents the fit of the optimal multivariate linear regression model shown in Table 13.
Table 14  Multivariate linear regression modelling for the E$_2$/E$_3$ of Fe-DOM solutions. The optimal model is marked with a * and highlighted in yellow.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
<th>Model 5</th>
<th>Model 6*</th>
<th>Model 7</th>
<th>Model 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept $b_1$</td>
<td>2.460</td>
<td>2.697</td>
<td>2.500</td>
<td>2.7</td>
<td>2.528</td>
<td>2.691</td>
<td>2.5</td>
<td>2.600</td>
</tr>
<tr>
<td>[Fe] $b_2$</td>
<td>-2.6 x 10$^{-3}$</td>
<td>-2 x 10$^{-3}$</td>
<td>0</td>
<td>-2 x 10$^{-3}$</td>
<td>0</td>
<td>-2 x 10$^{-3}$</td>
<td>0</td>
<td>-2 x 10$^{-3}$</td>
</tr>
<tr>
<td>[H$^+$] $b_3$</td>
<td>-2.93 x 10$^{-3}$</td>
<td>-2.01 x 10$^{-3}$</td>
<td>-7.36 x 10$^{-3}$</td>
<td>7.36 x 10$^{-3}$</td>
<td>4.38 x 10$^{-3}$</td>
<td>7.36 x 10$^{-3}$</td>
<td>4.38 x 10$^{-3}$</td>
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<tr>
<td>adjusted $R^2$</td>
<td>0.000</td>
<td>0.415</td>
<td>0.084</td>
<td>0.451</td>
<td>0.310</td>
<td>0.533</td>
<td>0.436</td>
<td>0.550</td>
</tr>
<tr>
<td>RMSE</td>
<td>4.5 x 10$^{-3}$</td>
<td>1.5 x 10$^{-3}$</td>
<td>3.8 x 10$^{-3}$</td>
<td>1.4 x 10$^{-3}$</td>
<td>2.1 x 10$^{-3}$</td>
<td>9.8 x 10$^{-4}$</td>
<td>1.4 x 10$^{-3}$</td>
<td>9.1 x 10$^{-4}$</td>
</tr>
<tr>
<td>df</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>12</td>
<td>11</td>
</tr>
</tbody>
</table>

Figure 47  Impact of [Fe] and pH on the E$_2$/E$_3$ of 50 mg/L LHA solution. Coloured squares represent measured data and the mesh represents the fit of the optimal multivariate linear regression model shown in Table 14.
Table 15  Multivariate linear regression modelling for the change in $E_2/E_3$ ($\Delta E_2/E_3$) of Fe-DOM solutions after irradiation. The optimal model is marked with a * and highlighted in yellow.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Model</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7*</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>$b_1$</td>
<td>0.126</td>
<td>0.165</td>
<td>0.100</td>
<td>0.100</td>
<td>0.124</td>
<td>0.167</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>[Fe]</td>
<td>$b_2$</td>
<td>-4.0 x 10^{-4}</td>
<td>0</td>
<td>-6.0 x 10^{-4}</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[H+]</td>
<td>$b_3$</td>
<td>1.06 x 10^{3}</td>
<td>1.05 x 10^{3}</td>
<td>1.84 x 10^{3}</td>
<td>1.73 x 10^{3}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Fe][H+]</td>
<td>$b_4$</td>
<td>1.01</td>
<td>4.36</td>
<td>-9.20</td>
<td>-7.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted $R^2$</td>
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<td>0.000</td>
<td>-0.019</td>
<td>0.131</td>
<td>0.121</td>
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<td>-0.091</td>
<td>0.189</td>
<td>0.078</td>
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<td>RMSE</td>
<td></td>
<td>1.2 x 10^{-4}</td>
<td>1.2 x 10^{-4}</td>
<td>8.5 x 10^{-5}</td>
<td>8.7 x 10^{-5}</td>
<td>1.4 x 10^{-4}</td>
<td>1.3 x 10^{-4}</td>
<td>7.4 x 10^{-5}</td>
<td>9.5 x 10^{-5}</td>
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<td>8</td>
<td>7</td>
</tr>
<tr>
<td>AICs</td>
<td></td>
<td>-4.28</td>
<td>-4.18</td>
<td>-4.31</td>
<td>-4.27</td>
<td>-4.10</td>
<td>-4.05</td>
<td>-4.35</td>
<td>-4.17</td>
</tr>
</tbody>
</table>

Figure 48  Impact of [Fe] and pH on the $\Delta E_2/E_3$ in irradiated 50 mg/L LHA solutions. Coloured squares represent measured data and the mesh represents the fit of the optimal multivariate linear regression model shown in Table 15.
Table 16  Multivariate linear regression modelling for the EDC of Fe-DOM solutions. The optimal model is marked with a * and highlighted in yellow.

<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6*</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>$b_1$</td>
<td>0.965</td>
<td>0.350</td>
<td>0.800</td>
<td>0.100</td>
<td>0.676</td>
<td>0.389</td>
<td>0.700</td>
<td>0.416</td>
</tr>
<tr>
<td>[Fe]</td>
<td>$b_2$</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>$[H^+]$</td>
<td>$b_3$</td>
<td>6.40 x 10^3</td>
<td>6.54 x 10^3</td>
<td>-4.23 x 10^2</td>
<td>-7.22 x 10^2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Fe][H+]</td>
<td>$b_4$</td>
<td>101.79</td>
<td>79.70</td>
<td>125.30</td>
<td>84.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted $R^2$</td>
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<td>0.000</td>
<td>0.266</td>
<td>0.030</td>
<td>0.340</td>
<td>0.434</td>
<td>0.471</td>
<td>0.340</td>
<td>0.396</td>
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<td>0.439</td>
<td>0.236</td>
<td>0.413</td>
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<td>0.141</td>
<td>0.123</td>
<td>0.158</td>
<td>0.160</td>
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<tr>
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<td>7</td>
</tr>
<tr>
<td>AICs</td>
<td></td>
<td>-0.143</td>
<td>-0.376</td>
<td>-0.097</td>
<td>-0.419</td>
<td>-0.635</td>
<td>-0.640</td>
<td>-0.513</td>
<td>-0.460</td>
</tr>
</tbody>
</table>

Figure 49  Impact of [Fe] and pH on the initial EDC of 50 mg/L LHA solutions. Coloured squares represent measured data and the mesh represents the fit of the optimal multivariate linear regression model shown in Table 16.
Table 17  Multivariate linear regression modelling for the change in EDC (ΔEDC) of Fe-DOM solutions after irradiation. The optimal model is marked with a * and highlighted in yellow.

<table>
<thead>
<tr>
<th>Item</th>
<th>Coefficient</th>
<th>Model</th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept b₁</td>
<td>0.749</td>
<td>-0.155</td>
<td>0.400</td>
<td>-0.500</td>
<td>0.368</td>
<td>-0.107</td>
<td>0.400</td>
<td>-0.300</td>
</tr>
<tr>
<td>[Fe] b₂</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
<td>0.01</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[H⁺] b₃</td>
<td>9.77 x 10³</td>
<td>9.98 x 10³</td>
<td>-3.04 x 10³</td>
<td>4.18 x 10³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Fe][H⁺] b₄</td>
<td>134.07</td>
<td>97.43</td>
<td>151.00</td>
<td>67.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted R²</td>
<td>0.000</td>
<td>0.135</td>
<td>-0.012</td>
<td>0.143</td>
<td>0.174</td>
<td>0.160</td>
<td>0.076</td>
<td>0.050</td>
</tr>
<tr>
<td>RMSE</td>
<td>4.83</td>
<td>3.62</td>
<td>4.95</td>
<td>3.55</td>
<td>3.30</td>
<td>3.41</td>
<td>4.13</td>
<td>4.37</td>
</tr>
<tr>
<td>df</td>
<td>10</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>AICs</td>
<td>1.06</td>
<td>0.99</td>
<td>1.14</td>
<td>1.04</td>
<td>0.94</td>
<td>1.02</td>
<td>1.12</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Figure 50  Impact of [Fe] and pH on the ΔEDC of irradiated 50 mg/L LHA solutions. Coloured squares represent measured data and the mesh represents the fit of the optimal multivariate linear regression model shown in Table 17.
4.3.2 Photochemical behaviour of natural water samples

Table 18 shows measured parameters associated with photochemical deoxygenation experiments using field water samples, including Fe concentration, pH, *in situ* DO, DOC, and irradiated DO consumption.

Samples 5 (from Bungawalbyn Creek on 13 April 2017), 6 (from Woodburn on 13 April 2017), 7 (from Rocky Mouth Creek on 13 April 2017), 8 (from Tuckean Barrage on 13 April 2017), 9 (from Wardell on 13 April 2017), and 17 (from Rocky Mouth Creek on 18 May 2017) exhibited characteristics typical of hypoxic blackwater samples. After 7 h simulated solar irradiation, almost all the hypoxic blackwater samples consumed substantially more DO than non-hypoxic water samples. High DO consumption was also observed in a few non-hypoxic samples, namely samples 3 (Codrington on 13 April 2017, 272 ± 218 μmol/L), 16 (Woodburn on 18 May 2017, 269 ± 1.3 μmol/L), 18 (Tuckean Barrage on 18 May 2017, 129 ± 21 μmol/L) and 20 (Ballina River on 18 May 2017, 267.0 ± 1 μmol/L), while low DO consumption was observed in the hypoxic sample 9 (Wardell on 13 April 2017, 39.4 ± 0.5 μmol/L). The large error associated with the non-linear regression model fitting to sample 3 suggests that this discrepancy can be attributed to experimental uncertainty, however the behaviour of the remaining samples is harder to rationalise. Considering the underlying chemical factors that can support high photochemical deoxygenation rates, the only real unknown factor is the quality of the organic matter, i.e. perhaps even though the DOC concentration was low, the DOC present was particularly photosensitive. However, the $E_2/E_3$ and SUVA$_{280}$ values are not particularly different to those in the other samples. This implies either the involvement of some other geochemical factors in photochemical deoxygenation that have not been considered or, more likely, a large experimental error.

In order to investigate the potential role of pH and Fe concentration in natural water samples more closely, predicted photochemical DO consumption at pH 7 in the absence of Fe was calculated using the relationship between DO consumption and DOC for standard HS determined in Chapter 2 as follows:

\[
\text{DO consumption} = 14.81e^{0.03499\text{DOC}}
\]

(Eq 45)

(This assumes that TOC = DOC for the standard HS solutions studied in Chapter 2). DO consumption calculated in this way is shown in Table 18 and compared to measured DO consumption in Figure 51.
Table 18 Irradiation induced DO consumption and associated parameters in natural water samples compared with calculated DO consumption using relationships developed in experiments involving standard HS. Samples with * represent hypoxic blackwaters. ΔDO values are reported as the best fit value ± standard error from non-linear regression as described in the methods section. Values highlighted with yellow shading represent the field sites with high photochemical deoxygenation capacity in surface waters, identified based on photochemical DO consumption experiments. Predicted values of ΔDO are calculated based on the relationship between DO consumption and DOC concentration in the standard HS solutions at pH 7 in the absence of Fe (Eq 45).

<table>
<thead>
<tr>
<th>No.</th>
<th>Field site</th>
<th>Fe (µmol/L)</th>
<th>pH</th>
<th>In situ DO (mg/L)</th>
<th>DOC (mg/L)</th>
<th>ΔDO (µmol/L)</th>
<th>Predicted ΔDO (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lismore River</td>
<td>37.7</td>
<td>7.5</td>
<td>7.9</td>
<td>6.4</td>
<td>29.4 ± 5.2</td>
<td>18.4</td>
</tr>
<tr>
<td>2</td>
<td>Ruthven</td>
<td>23.2</td>
<td>7.2</td>
<td>7.2</td>
<td>4.2</td>
<td>45.6 ± 0.3</td>
<td>17.1</td>
</tr>
<tr>
<td>3</td>
<td>Codrington</td>
<td>28.0</td>
<td>7.6</td>
<td>6.8</td>
<td>9.6</td>
<td>272.0 ± 218.0</td>
<td>20.6</td>
</tr>
<tr>
<td>4</td>
<td>Coraki</td>
<td>23.0</td>
<td>7.1</td>
<td>6.9</td>
<td>7.2</td>
<td>22.3 ± 0.2</td>
<td>18.9</td>
</tr>
<tr>
<td>5*</td>
<td>Bungawalbyn Creek</td>
<td>46.0</td>
<td>6.2</td>
<td>3.0</td>
<td>24.7</td>
<td>259.0 ± 227.0</td>
<td>34.4</td>
</tr>
<tr>
<td>6*</td>
<td>Woodburn</td>
<td>61.4</td>
<td>6.3</td>
<td>2.2</td>
<td>17.6</td>
<td>134.0 ± 8.0</td>
<td>27.0</td>
</tr>
<tr>
<td>7*</td>
<td>Rocky Mouth Creek</td>
<td>532.0</td>
<td>6.2</td>
<td>0.6</td>
<td>24.8</td>
<td>211.0 ± 0.5</td>
<td>34.5</td>
</tr>
<tr>
<td>8*</td>
<td>Tuckean Barrage</td>
<td>1803.0</td>
<td>6.5</td>
<td>2.0</td>
<td>17.4</td>
<td>270.0 ± 2.0</td>
<td>26.8</td>
</tr>
<tr>
<td>9*</td>
<td>Wardell</td>
<td>55.3</td>
<td>6.7</td>
<td>2.1</td>
<td>17.7</td>
<td>39.4 ± 0.5</td>
<td>27.1</td>
</tr>
<tr>
<td>10</td>
<td>Ballina River</td>
<td>32.2</td>
<td>6.7</td>
<td>5.0</td>
<td>14.6</td>
<td>51.7 ± 5.2</td>
<td>24.4</td>
</tr>
</tbody>
</table>

Sampled on 13 April 2017

<table>
<thead>
<tr>
<th>No.</th>
<th>Field site</th>
<th>Fe (µmol/L)</th>
<th>pH</th>
<th>In situ DO (mg/L)</th>
<th>DOC (mg/L)</th>
<th>ΔDO (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Lismore River</td>
<td>25.4</td>
<td>7.3</td>
<td>7.5</td>
<td>4.4</td>
<td>30.3 ± 0.7</td>
</tr>
<tr>
<td>12</td>
<td>Ruthven</td>
<td>20.1</td>
<td>7.3</td>
<td>6.3</td>
<td>3.4</td>
<td>56.8 ± 0.3</td>
</tr>
<tr>
<td>13</td>
<td>Codrington</td>
<td>11.3</td>
<td>7.2</td>
<td>6.1</td>
<td>4.2</td>
<td>43.4 ± 0.3</td>
</tr>
<tr>
<td>14</td>
<td>Coraki</td>
<td>13.8</td>
<td>6.9</td>
<td>6.1</td>
<td>3.6</td>
<td>68.4 ± 0.7</td>
</tr>
<tr>
<td>15</td>
<td>Bungawalbyn Creek</td>
<td>51.4</td>
<td>6.3</td>
<td>4.3</td>
<td>13.4</td>
<td>94.8 ± 5.2</td>
</tr>
<tr>
<td>16</td>
<td>Woodburn</td>
<td>43.9</td>
<td>6.7</td>
<td>6.1</td>
<td>4.8</td>
<td>269.0 ± 1.3</td>
</tr>
<tr>
<td>17*</td>
<td>Rocky Mouth Creek</td>
<td>53.5</td>
<td>5.4</td>
<td>2.8</td>
<td>9.5</td>
<td>177.0 ± 13.0</td>
</tr>
<tr>
<td>18</td>
<td>Tuckean Barrage</td>
<td>90.1</td>
<td>5.2</td>
<td>4.7</td>
<td>3.4</td>
<td>129.0 ± 21.0</td>
</tr>
<tr>
<td>19</td>
<td>Wardell</td>
<td>13.0</td>
<td>7.4</td>
<td>5.8</td>
<td>6.0</td>
<td>68.7 ± 0.4</td>
</tr>
<tr>
<td>20</td>
<td>Ballina River</td>
<td>5.5</td>
<td>7.6</td>
<td>6.1</td>
<td>4.8</td>
<td>267.0 ± 1.0</td>
</tr>
</tbody>
</table>

Sampled on 18 May 2017
Figure 51 Comparison between measured DO consumption and predicted DO consumption based on Eq 45 in natural water samples from the Richmond River system. Circles represent the best fit value and error bars the standard error from non-linear regression fitting of first order kinetics to the DO consumption data. The red dashed line represents a slope of 1, corresponding to the theoretical relationship if measured values were predicted exactly by Eq 45. The blue line represents the fit from linear regression of the measured data against the predicted values from Eq 45.

Measured DO consumption was higher than that predicted by Eq 45 for all natural water samples. This is not unexpected, given that a wider range of processes are expected to influence photochemical deoxygenation in natural waters compared to simplified laboratory systems, and the role of DOC in photochemical deoxygenation is likely only one contributor to the range of factors influencing DO consumption. Even so, measured and calculated DO consumption for all natural water samples were within the same order of magnitude, except for samples from sites 3, 5, 6, 7, 8, 16, 17, 18, and 20. The samples whose behaviour deviated strongly from that predicted by Eq 45 were mostly taken from the backswamps of Richmond River floodplain, whose hydrology and land use has been significantly modified [219, 220], and which consequently are likely supply DOC with different properties during flood inundation compared with the other sites examined. This again reinforces the conclusion that DOC type is a critical factor impacting photochemical deoxygenation potential. The reasonable agreement between measurements in field samples and values predicted by Eq 45 suggests that DOC might be a reasonable proxy to estimate photochemical deoxygenation potential for samples near pH 7 and containing relatively low Fe concentrations.
The largest differences between measured data and predicted DO consumption occurred in the water samples from hypoxic blackwater sites. Figure 51 shows a linear regression fit between measured photochemical DO consumption and DO consumption predicted from Eq 45 for all field water samples. Excluding points with [Fe] > 500 µmol/L and pH < 5.5, measured DO consumption was about 5 times predicted DO consumption. This ratio could be considered as a connection between optical parameters, which can be used as a proxy to quantify DOC concentration, and photochemical deoxygenation, meaning that it could be possible to predict photochemical deoxygenation potential by accurately monitoring optical parameters, at least for waters with relatively low Fe concentrations around neutral pH. However, this requires further study with samples from a wider range of natural water systems to establish its potential.

Although this suggests some relationship between the behaviour observed in the simplified experiments and the behaviour of natural hypoxic blackwaters, because of the more complex composition and variable environmental influences in the natural waters, the relationship is not necessarily straightforward. For example, the HS type was not characterised in the field water samples, even though this might be expected to play a significant role in photochemical deoxygenation potential based on the conclusion of Chapter 2 in this thesis. In the irradiation experiments involving model HS in this chapter, it was also observed that when Fe concentration was less than 100 µmol/L, DO consumption did not display clear trends with changing pH.

4.4 Conclusions and Implications

This study investigated the combined influence of Fe and pH on the photochemical deoxygenation potential of natural waters containing concentrations of DOM typical of those found in hypoxic blackwater. 50 mg/L LHA was used as a standard model HS. All the Fe-DOM solutions experienced DO consumption after 7 h exposure to simulated solar irradiation, ranging from 43.7 µmol/L to 155.9 µmol/L. DO consumption increased with increasing Fe concentration, but did not change much with pH.

All Fe-DOM solutions exhibited decreasing SUVA\textsubscript{280} during 7 h irradiation. Initial SUVA\textsubscript{280} ranged from 0.05 m\textsuperscript{-1}L/mg DOC to 0.08 m\textsuperscript{-1}L/mg DOC, and the ΔSUVA\textsubscript{280} ranged from -0.012 m\textsuperscript{-1}L/mg DOC to -0.0034 m\textsuperscript{-1}L/mg DOC. SUVA\textsubscript{280} values varied with Fe concentration and pH, and SUVA\textsubscript{280} decreased with increasing Fe and
decreasing pH, especially when pH < 5. This result was different from that in a previous study using natural HS isolates, and suggests further work is required to fully understand how Fe-DOM complex formation can influence optical properties. This study also found that the combination of high Fe concentration with low pH resulted in high SUVA$_{280}$, low E$_2$/E$_3$, and high EDC. The different magnitude of some parameters determined in this study compared with those determined in previous works suggests that the character and components of DOM are sensitive to many factors, and that different types of water samples, methodology, irradiation duration and analysis methods can lead to different results, even for the same parameters.

All the Fe-DOM solutions experienced increasing E$_2$/E$_3$ after 7 h irradiation, especially at low pH (< 4) and low Fe concentration (< 100 µmol/L). Initial E$_2$/E$_3$ ranged from 1.90 to 2.74, and ∆E$_2$/E$_3$ ranged from 0.01 to 0.30. The initial EDC ranged from 0.22 mole e-/g DOC to 2.76 mole e-/g DOC, and ∆EDC ranged from -0.18 mole e-/g DOC to 4.70. This indicates less aromaticity and smaller molecular weight along with the decrease in SUVA$_{280}$ and increase in E$_2$/E$_3$ after 7 hours irradiation, which agreed with the findings of Chapter 2 and Chapter 3.

Fe concentration was negatively correlated with E$_2$/E$_3$ for all samples regardless of the pH. When pH < 4.5, the E$_2$/E$_3$ value was influenced particularly strongly by Fe concentration, which is consistent with the study of natural hypoxic blackwater samples in Chapter 3.

Measured photochemical DO consumption in natural water samples from the Richmond River system was also compared with predicted DO consumption based on the relationship between DOC and photochemical DO consumption generated using model HS in Chapter 2. This comparison aimed to determine if the relationship developed in Chapter 2 could be used to provide a rough estimate of DO consumption in the natural post flood hypoxic blackwater. The results showed that the measured DO consumption was more than predicted for all natural water samples by a factor of about 5 times.

This study suggests that NOM (including Fe-DOM) complexes in natural waters have more complicated photochemical behaviour than is predicted by simplified models based on experiments with standard HS. The mixing of various DOM species, varying concentrations and speciation of iron, and varying pH could all significantly
impact the optical properties and photochemical reactivity of natural waters. In addition, the components of different HS types could generate very different relationships between optical properties and photochemical deoxygenation capacity. Further studies are required to relate the optical properties to photochemical deoxygenation potential in more natural systems and ideally provide a general basis for understanding the relationships between easily monitored optical parameters and the processes leading to hypoxic blackwater events.
Chapter 5 – Conclusions and outlook

5.1 Summary

DO consumption after large scale floods has not been extensively examined in literature, although there are some works on DOM photooxidation and related consumption of dissolved oxygen [3, 24, 113]. The detailed mechanism of DOM photooxidation has been examined in the last couple of decades, and DO consumption in high DOM water (especially seawater) has been studied [262, 263]. Previous studies showed that photochemical oxygen consumption could be considerable in the surface ocean, and even comparable with bacterioplankton respiration demand. Results from these studies were similar to the photochemical induced deoxygenation rates of 0.1 and 0.3 μmol L$^{-1}$ h$^{-1}$ found by Gieskes et al. [21], Laane et al. [24] and Obernosterer et al. [136] in the surface seawater of the subtropical and tropical Atlantic Ocean, and the world-wide average photochemical oxygen consumption in the ocean is around $12 \times 10^{12}$ gO$_2$ y$^{-1}$ [21]. In the last couple of decades, studies have focused on DO consumption via photosynthesis and/or the optical changes in DOM induced by photooxidation, as well as numerous investigations of the mechanism of oxygen reduction by specific components of DOM such as quinone and semiquinone [264, 265]. Oxygen consumption due to photochemical reactions has been difficult to quantify in laboratory experiments due to the typically small sample volume and irradiation duration, and because of O$_2$ measurement methods may lack the sensitivity to measure small changes against a large background concentration.

Coastal hypoxic blackwater issues have received global attention from the end of the last century; however, there remains a huge knowledge gap regarding the potential role of photochemically induced deoxygenation in these events, with very few studies investigating this issue to date. This thesis aimed to investigate the photochemical deoxygenation potential of surface waters in coastal floodplain systems, particularly during flood conditions that can give rise to blackwater events, and to determine the major influential factors. The findings of the main study chapters are summarised following.
Chapter 2: Deoxygenation potential of model humic substances under simulated solar irradiation

Results of this chapter demonstrated that representative standard humic substance (HS) solutions consumed DO during solar simulated irradiation in simplified model laboratory experiments with constant temperature of 25 °C and pH controlled at 7. Three types of standard HS from the International Humic Substances Society were examined: Leonardite humic acid (LHA), Suwannee River fulvic acid (SRFA) and Pahokee Peat humic acid (PPHA) standard. The majority of DO consumption happened in the first 7 h of irradiation, and ranged from 15 µmol/L to 150 µmol/L. The magnitude of DO consumed depended on the concentrations of HS, and this relationship appeared to be independent of humic substance type. The relationship between DO consumption and TOC concentrations was best fit with an exponential function:

\[
\text{DO consumption} = 14.32e^{0.03499\text{TOC}}
\]  
(Eq 46)

where DOC is concentration of dissolved organic carbon in mg/L.

Kinetic modelling based on this relationship showed that, over the range of HS concentrations typically found in natural rivers waters, the maximum rate constant for photochemical deoxygenation was expected when depth is 0.01 m for all three standard HS, and that photochemical deoxygenation was expected to be negligibly small when water is deeper than 0.5 m.

The rate constant for DO consumption by inundated vegetation from the Richmond River floodplain was previously found to range from \(7 \times 10^{-6}\) to \(7 \times 10^{-5}\) s\(^{-1}\) [203], and a previous field study in the same area during the floods in February 2001 showed that deoxygenation rate constants in the Richmond River ranged from \(7 \times 10^{-7}\) to \(3.3 \times 10^{-6}\) s\(^{-1}\) [113, 120]. These values are a similar order of magnitude to modelled values of photochemical DO consumption in solutions containing the model HS at similar concentrations to those found in Richmond River flood waters, suggesting that photochemical DO consumption might be significant in the high DOC shallow water areas of the Richmond River floodplain during inundation.

After 7 h irradiation, the optical parameters SUVA\textsubscript{280} decreased and \(E_2/E_3\) increased for all DOM solutions. SUVA\textsubscript{280} ranged from 0.029 to 0.051 L m\(^{-1}\)mg\(^{-1}\) HS, and EDC ranged from 7.3 to 31.5 µmol/g HS. The redox state parameter electron donating
capacity (EDC) did not change substantially during irradiation for any of the HS examined, suggesting that the EDC was decoupled from the photochemical deoxygenation potential. In contrast, the change in optical properties demonstrated that photochemical deoxygenation processes were associated with decreasing molecular size and aromaticity. Overall, the results of this chapter suggest that the chromophoric and redox properties of DOM may not have a simple relationship with DO consumption potential.

Although the experimental conditions examined in this chapter were highly simplified compared to real hypoxic blackwater, it was still clearly demonstrated that substantial photochemical deoxygenation of DOM solutions occurred within 7 hours of irradiation. The highest DO consumption rate in this study was lower than that for the data recorded for natural blackwater in previous studies. This is reasonable, however, as the factors impacting DO consumption in natural waters are likely to be more complex than in the simplified model systems studied here. Nonetheless the findings were generally in accordance with those from previous work. Overall it was demonstrated that photochemical deoxygenation of HS solutions may be substantial, suggesting that photochemistry may play a role in deoxygenation processes leading to hypoxic blackwater formation.

**Chapter 3: Post-flood hypoxia blackwater field study**

The results of this chapter suggested that DOC, pH, COD and Fe concentration were key parameters which were strongly correlated with the formation of hypoxic blackwater. The geochemical components of the backswamps in the Richmond River system significantly contributed to the formation of hypoxic blackwater not only in the backswamps themselves, but also could extend this influence to the broader aquatic system, including the main river channel, via hydrologic connectivity. This would increase the areas impacted by coastal hypoxic blackwater events as described in Chapter 1. This field study additionally showed that the most sensitive sites influenced by post flood hypoxic blackwater on the Richmond River floodplain included Rocky Mouth Creek, Bungawalbyn Creek, Woodburn, Tuckean Barrage, and Wardell. Principal components analysis (PCA) results showed that the eigenvalues of the two first principal components represent up to 64.6% (PC1 33.9%; PC2 30.7%) of the total parameters of the post flood water samples. DO was found
to be positively correlated to SUVA$_{280}$, and negatively correlated to Fe, DOC, BOD and COD.

**Chapter 4: Influence of Fe and pH on photochemical DO consumption by model humic substances under simulated solar irradiation**

All the Fe-DOM solutions experienced decreasing DO after 7 h exposure to simulated solar irradiation. The DO consumption ranged from 43.7 µmol/L to 155.9 µmol/L. DO consumption increased with increasing Fe concentration, but did not change much with pH. SUVA$_{280}$ values were lower with higher Fe concentration and lower pH, and the correlation was stronger when pH < 5. This did not agree with previous investigations using natural HS isolates, implying that further work is required in order to understand how the formation of a Fe-DOM complex could impact optical properties. It was also found that the combination of high Fe concentration with low pH resulted in relatively low E$_{2}$/E$_{3}$ and high EDC. The magnitude and trends of some parameters of this study compared with previous work suggested that many factors could impact the photochemical behaviour of DOM, such as types of water samples, experimental methodology, irradiation duration and analysis methods. This indicated less aromaticity and smaller molecular weight along with the decreasing SUVA$_{280}$ and increasing E$_{2}$/E$_{3}$ after 7 hours irradiation, which agreed with the findings of Chapter 2 and Chapter 3.

Measured DO consumption in natural water samples from the Richmond River system was compared with predicted DO consumption based on the relationship between DOC and photochemical DO consumption generated using model HS in Chapter 2. The comparison of ∆DO in irradiated field samples and predicted ∆DO aimed to provide a rough estimate photochemical DO consumption in the natural post flood hypoxic blackwater. The results showed that the measured DO consumption was more than predicted for all natural water samples by a factor of about 5 times, indicating photochemical deoxygenation in the natural water has more contribution factors besides HS which was used as model DOC in Chapter 2 of this study.

**5.2 Outlook**

The results from the simulated solar irradiation experiments could have significant implications for study of hypoxic blackwater in the Richmond River system. I have estimated the relative contribution of photochemical deoxygenation via photochemical reactions of DOM to the total deoxygenation potential of the post
flood blackwater. The measured DO consumption rate constants in this study were similar in magnitude to those from a field study during the 2001 flood as well as the DO consumption rate in a laboratory study using inundated floodplain vegetation from the same field study area of the Richmond River system. This study elucidated for the first time the relationship between DO consumption and TOC concentrations with a simple equation. However, more systematic control experiments are required to evaluate the validity and applicability of this model under different conditions. Due to limitations of the scope of this work, other factors that were not specifically considered may have significant impacts on natural water deoxygenation process. There is broad uncertainty around the role of factors such as nutrients, regional variation in UV irradiation and the influence of microorganisms, which are considered as potentially important driving factors for photochemical reactions, and the interactions between them have not been thoroughly studied [163, 249]. For example, increasing DOM in aquatic systems could increase the water surface temperature as well as decrease the surface water mixing depth, and this pattern would lead to more thermal stratification [163, 267]. In this work, thermal influences were controlled by use of a water filter on the solar simulator irradiation system and a temperature controlled water recirculation system on the cuvette. However, in order to understand the complex interactions between a wide ranges of influences in natural waters, larger scale investigations considering more variables, such as temperature, may be required in future.

Climate change is expected to cause global warming as well as changes in regional precipitation patterns, potentially leading to increasing floods, droughts and extreme storm events, which may have severe consequences for hypoxic blackwater generation [163]. At the same time, rising global temperatures and increased precipitation could accelerate DOM release into natural water systems such as lakes, rivers and coastal oceans [268, 269]. As a critical parameter influencing hypoxic blackwater generation, increasing concentrations of DOM in natural waters thus has the potential to impact water quality from a range of perspectives.

The findings of this thesis have provided an initial evaluation of the significance of photochemical deoxygenation in aqueous systems containing NOM, with particular focus on the role of such processes in hypoxic blackwater formation. However, to develop a more comprehensive understanding of these processes, and address some
of the additional questions arising from this work, further studies suggested to be performed include:

1. Comparing the relationships between chemical properties and photochemical reactivity in solutions containing a mix of model standard HS and natural waters with different types of DOM. In addition, to adequately understand the significance of photochemistry in real blackwater systems requires investigation of the influence of additional factors that are known to exist in such systems, such as different trace metals and varying temperature.

2. Accurately identifying sites with high deoxygenation potential in order to improve the management of hypoxia events, which might include *in situ* monitoring and drainage management plans.

3. Better quantifying the relationship between optical properties and photochemical deoxygenation potential in a broader range of natural systems, and ideally developing a general basis for understanding the relationships between easily monitored optical parameters and the processes leading to hypoxic blackwater events. These relationships could then be applied to develop remote monitoring methods or other monitoring tools for large scale floodplain management.
Chapter 6 – References

References


Appendices
Appendix 1  Photon fluxes and absorption during photochemical experiments using the solar simulator

A1.1  Determination of Xe lamp photon flux by spectroradiometry

The incident photon flux from the Xe lamp at the cuvette, \( Q_{i,\lambda} \), was measured across the wavelength range 300-1100 nm in 1 nm intervals using an Ocean Optics USB4000 spectrometer equipped with a cosine corrector lens (CC-3-UV-S). The spectrometer output was calibrated in terms of spectral irradiance (W m\(^{-2}\) nm\(^{-1}\)) using a reference deuterium/tungsten-halogen light sources DH2000-CAL), and subsequently converted to photon flux density (mol photons m\(^{-2}\) s\(^{-1}\) nm\(^{-1}\)) using the relationship:

\[
E = \frac{hc}{\lambda}
\]  

(Eq 47)

where \( E \) = photon energy, \( h \) = Planck’s constant, \( c \) = speed of light, and \( \lambda \) = photon wavelength.

The incident photon flux was measured at 10 separate points throughout the cross-section of the lamp output at the same distance from the lamp as the cuvette, and the average incident photon flux determined as the mean of the measured values, as shown in Figure 52.

![Solar simulator radiance density](image-url)
A1.2 Nitrite actinometry

Although the cuvette width was 1 cm, the apparent optical pathlength may be greater due to refraction and scattering of the incident photons by the cuvette walls. Nitrite actinometry was therefore used to calculate the actual absorbed photon flux, and hence calculate the apparent optical pathlength. In the nitrite actinometer, photolysis of nitrite yields hydroxyl radicals, which then react with benzoic acid (BA) to yield salicylic acid (SA):

\[
\text{NO}_2^- + h\nu + H_2O \rightarrow NO^- + OH^+ + OH^- \quad (\text{Eq 48})
\]

\[
\text{BA} + \text{OH}^+ \rightarrow \text{SA} \quad (\text{Eq 49})
\]

SA concentration can then be quantified by fluorescence. The nitrite actinometer was employed in this work using the method of Jankowski et al. [270].

The actinometer solution contained 1 mM sodium nitrite, 1 mM benzoic acid and 2.5 mM sodium bicarbonate as buffer. Standard solutions of 20 mM SA for calibration of the fluorescence quantification method were prepared in the same matrix actinometer solution. Actinometer solution was irradiated for 30 min with simulated solar irradiation in the same way as per the photochemical experiments in Chapter 2, and 100 µL of sample was taken from the cuvette at 5 min intervals.

SA concentrations were measured fluorometrically with a Clariostar microplate spectrophotometer (BMG Labtech). Analysis was performed in 96 well microplates (Corning® 96 Well Microplates, with dark flat bottom, sterile acrylic) at an excitation wavelength of 305 ± 10 nm and an emission wavelength of 410 ± 10 nm. Calibration was performed using standard solutions of SA prepared by dilution of the 20 mM SA standard in unirradiated actinometer solutions. The overall rate of SA formation was then determined from linear regression of SA concentration against irradiation time to be \(4.00 \times 10^{-10} \text{ mol L}^{-1} \text{ s}^{-1}\).

With absorption \(< 0.05 \) at \(\lambda \geq 310\text{nm}\), the photochemical production of SA from nitrite photolysis is described by the following equation [270]:

\[
\left( \frac{d[\text{SA}]}{dt} \right)_\lambda = 2.303 \varepsilon_\lambda \phi_\lambda E_\lambda [\text{NO}_2^-] \quad (\text{Eq 50})
\]

where [SA] is SA concentration (M), \(\varepsilon_\lambda\) is the wavelength dependent molar absorption coefficient of nitrite (cm\(^2\) mol\(^{-1}\)) at wavelength \(\lambda\), \(\phi_\lambda\) is SA quantum yield.
from nitrite photolysis \((\text{mol Einstein}^{-1})\) at wavelength \(\lambda\), \(E_\lambda\) is the spectral irradiance \((\text{Einstein cm}^{-2} \text{ nm}^{-1} \text{s}^{-1})\) at wavelength \(\lambda\), and \([\text{NO}_2^-]\) is the nitrite concentration in actinometer \((\text{M})\).

The expected SA formation rate was calculated in 10 nm bands over the wavelength range 300-420 nm from this equation, using values of \(E_\lambda\) as measured in section A1.1 and values of \(\varepsilon_\lambda\) and \(\varphi_\lambda\) reported by Jankowski et al. [270], and assuming a 1 cm optical pathlength. Integrating over the wavelength range 300-420 nm yielded an expected total SA formation rate of \(1.16 \times 10^{10} \text{ mol L}^{-1} \text{s}^{-1}\). Comparison with the measured total SA formation rate of \(4.00 \times 10^{10} \text{ mol L}^{-1} \text{s}^{-1}\) therefore implies that the effective optical path length was \(4.00/1.16 = 3.4 \text{ cm}\).

### A1.3 Absorbed photon fluxes

The absorbed photon flux by the HS solutions used in Chapter 2 at each wavelength, \(Q_{\text{abs},\lambda}\) \((\text{mol photons m}^{-2} \text{s}^{-1} \text{ nm}^{-1})\), was calculated as:

\[
Q_{\text{abs},\lambda} = Q_{i,\lambda} (1 - e^{-\alpha_\lambda L}) \quad (\text{Eq 51})
\]

Where \(Q_{i,\lambda}\) = incident photon flux at wavelength \(\lambda\) \((\text{mol photons m}^{-2} \text{s}^{-1} \text{ nm}^{-1})\) measured in section A1.1, \(\alpha_\lambda\) = absorption coefficient (measured as described in Chapter 2) and \(L = \text{optical path length} = 3.4 \text{ cm in this case}\).

The absorbed photon dose by each HS solution, \(Q_{\text{abs},\lambda,T}\) \((\text{mol photons m}^{-2} \text{ exposure}^{-1} \text{ nm}^{-1})\), was then calculated as:

\[
Q_{\text{abs},\lambda,T} = Q_{\text{abs},\lambda} \times \text{irradiation duration} \quad (\text{Eq 52})
\]

These calculations were performed over different wavelength ranges and different HS concentrations as shown in Table 19. The proportion of incident photons absorbed over different wavelength ranges and different HS concentrations is shown in Table 20 and Figure 53.
Table 19  Absorbed photon flux by HS (E) (mmol photons L\(^{-1}\).s\(^{-1}\))

<table>
<thead>
<tr>
<th>[HS] (mg/L)</th>
<th>Wavelength range (nm)</th>
<th>300–400</th>
<th>300-500</th>
<th>300-600</th>
<th>300-700</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2.7</td>
<td>2.7</td>
<td>3.8</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>4.9</td>
<td>7.0</td>
<td>8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>14.1</td>
<td>17.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>12.8</td>
<td>20.8</td>
<td>26.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>14.3</td>
<td>24.4</td>
<td>32.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>15.0</td>
<td>26.5</td>
<td>35.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 20  Proportion of incident photon flux absorbed by HS solutions (%)

<table>
<thead>
<tr>
<th>[HS] (mg/L)</th>
<th>Wavelength range (nm)</th>
<th>300–400</th>
<th>300-500</th>
<th>300-600</th>
<th>300-700</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>25.7</td>
<td>17.4</td>
<td>12.7</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>44.8</td>
<td>31.5</td>
<td>23.3</td>
<td>17.5</td>
<td></td>
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<tr>
<td>50</td>
<td>77.1</td>
<td>60.0</td>
<td>46.7</td>
<td>36.0</td>
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</tr>
<tr>
<td>100</td>
<td>94.6</td>
<td>82.7</td>
<td>68.9</td>
<td>55.4</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>98.7</td>
<td>92.1</td>
<td>80.8</td>
<td>67.2</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>99.7</td>
<td>96.2</td>
<td>87.6</td>
<td>75.1</td>
<td></td>
</tr>
</tbody>
</table>

Figure 53  Proportion of photons absorbed by HS solutions in different wavelength ranges during the irradiation experiments described in Chapter 2.
### Appendix 2  Post flood field water sample measurements

#### Table 21  Measured values of major physico-chemical parameters of post flood field water samples

| Site | Temp (C) | DO (mg/L) | EC25 (µS/cm) | ORP (mV) | pH | Eo/E3 | Abs280 (mol e/g DOC) | EDC (mg/L O2) | BOD (mg/L O2) | COD (mg/L) | DOC (mg/L) | Al (µmol/L) | Cu (µmol/L) | Fe (µmol/L) | Mg (µmol/L) | Ni (nmol/L) | Zn (nmol/L) |
|------|----------|-----------|--------------|----------|----|-------|---------------------|----------------|--------------|-----------|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-----------|
| 1    | 23.7     | 7.92      | 131.0        | 85.8     | 7.48 | 2.89  | 0.210               | -0.116         | 0.9          | 12         | 6.4        | 19.5        | 46.0        | 37.7        | 6.77        | 39.6        | 198        |
| 2    | 24.2     | 7.19      | 127.0        | 85.1     | 7.24 | 2.66  | 0.272               | -0.0982        | 1.2          | 7          | 4.2        | 15.5        | 22.6        | 23.2        | 1.99        | 27.8        | 76.4       |
| 3    | 23.9     | 6.78      | 233.1        | 86.9     | 7.57 | 3.11  | 0.491               | -0.0983        | 1.1          | 24         | 9.6        | 26.0        | 44.6        | 28.0        | 2.31        | 64.1        | 136        |
| 4    | 23.9     | 6.85      | 158.9        | 101      | 7.13 | 3.24  | 0.362               | -0.0956        | 1.1          | 18         | 7.2        | 21.7        | 31.4        | 23.0        | 1.57        | 41.7        | 68.8       |
| 5    | 24.2     | 3         | 87.4         | 93.6     | 6.23 | 3.52  | 0.904               | -0.0536        | 2            | 65         | 24.7       | 44.3        | 19.3        | 46.0        | 5.34        | 19.7        | 104        |
| 6    | 23       | 2.24      | 111.1        | 133.1    | 6.25 | 3.43  | 0.659               | -0.0504        | 2.9          | 48         | 17.6       | 36.1        | 28.6        | 61.4        | 2.01        | 42.4        | 95.9       |
| 7    | 23.6     | 0.55      | 98.3         | -146.6    | 6.2  | 3.34  | 0.996               | -0.0105        | 8.6          | 76         | 24.8       | 50.4        | 29.0        | 532         | 7.22        | 71.6        | 143        |
| 8    | 22.9     | 1.96      | 107.0        | 126.4    | 6.45 | 2.63  | 1.03                 | 0.0118         | 4.6          | 51         | 17.4       | 21.5        | 21.6        | 180         | 3.37        | 28.8        | 71.7       |
| 9    | 24.6     | 2.08      | 132.7        | 97.5     | 6.66 | 3.47  | 0.672               | -0.0456        | 1.9          | 46         | 17.7       | 38.1        | 33.2        | 55.3        | 1.16        | 37.7        | 90.4       |
| 10   | 23.7     | 4.9       | 114.7        | 107.9    | 6.73 | 3.65  | 0.418               | -0.0965        | 1.5          | 58         | 14.6       | 16.5        | 56.4        | 32.2        | 1.89        | 38.8        | 123        |
| 11   | 17.1     | 7.46      | 390.3        | 133      | 7.31 | 2.88  | 0.302               | -0.0503        | 1.1          | 10         | 4.43       | 13.6        | 21.0        | 25.4        | 1.42        | 20.1        | 60.5       |
| 12   | 17.3     | 6.32      | 410          | 143.9    | 7.3  | 2.79  | 0.186               | -0.0327        | 1.6          | 1          | 3.39       | 10.5        | 25.1        | 20.1        | 1.23        | 18.7        | 69.7       |
| 13   | 18       | 6.1       | 746          | 166.3    | 7.2  | 2.72  | 0.206               | -0.0997        | 1.9          | 5          | 4.21       | 7.07        | 21.8       | 11.3        | 1.65        | 26.8        | 150        |
| 14   | 17.9     | 6.14      | 518.4        | 184.8    | 6.93 | 2.68  | 0.190               | -0.0864        | 2            | 5          | 3.64       | 8.76        | 18.0       | 13.8        | 0.873       | 20.0        | 25.3       |
| 15   | 17.2     | 4.31      | 358.8        | 211.7    | 6.32 | 2.87  | 0.645               | -0.0532        | 0.8          | 24         | 13.43      | 54.5        | 20.7       | 51.4        | 2.54        | 26.1        | 116        |
| 16   | 18.3     | 6.06      | 477          | 232      | 6.71 | 2.83  | 0.253               | -0.0976        | 2.6          | 13         | 4.81       | 22.2        | 37.7       | 43.9        | 3.52        | 48.6        | 176        |
| 17   | 17.3     | 2.78      | 1018         | 277.8    | 5.43 | 2.91  | 0.412               | 0.253          | 1.8          | 25         | 9.53       | 17.1        | 9.1        | 53.5        | 8.63        | 193         | 475        |
| 18   | 16.2     | 4.66      | 978          | 393      | 5.2  | 2.58  | 0.164               | 0.290          | 0.5          | 8          | 3.41       | 121         | 15.6       | 90.1        | 9.64        | 172         | 579        |
| 19   | 18.6     | 5.75      | 21981*       | 139.4    | 7.43 | 3.07  | 0.230               | -0.0853        | 2.1          | 39         | 6.01       | 15.3        | 19.4       | 13.0        | 1.76        | 41.1        | 69.4       |
| 20   | 19       | 6.07      | 54586*       | 87.7     | 7.64 | 3.23  | 0.157               | -0.0800        | 0.5          | 40         | 4.82       | 5.89        | 35.5       | 5.54        | 1.08        | 28.7        | 34.5       |

a Samples 1-10 were collected on 13/4/2017 and samples 11-20 were collected on 18/5/2017 at the same locations. Site names are: 1/11: Lismore River, 2/12: Ruthven, 3/13: Codrington, 4/14: Coraki, 5/15: Bungawalbyn Creek, 6/16: Woodburn, 7/17: Rocky Mouth Creek, 8/18: Tuckean Barrage, 9/19: Wardell, 10/20: Ballina River. Refer to Chapter 3 for full site details.

Understanding the Photochemistry of Coastal Hypoxic Blackwater