Using Contaminant Photoreactivity as a Holistic Indicator to Monitor Changes in Wetland Water Characteristics

Thesis

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ABSTRACT

The light-induced transformation of agricultural products in wetlands is strongly influenced by chromophoric dissolved organic matter (CDOM). Although the absorption of photons directly by contaminant molecules is not a primary transformation pathway in waters such as wetlands, indirect photolysis is a well-studied and important process by which absorbed light causes contaminant transformation in illuminated, aerated waters. The chemical and optical properties of CDOM determine the light absorbance of wetland water and therefore dictate which reactive species will form. In particular, light-induced loss of chromophores (photobleaching) of CDOM generates reactive species according to the concentration and character of chromophoric moieties present.

I collected water from four U.S. wetlands: an agricultural wetland in Ohio (YCF), Kawai Nui Marsh in Hawaii (KNM), the Everglades in Florida (EVG), and Okefenokee Swamp in Georgia (OKS). I investigated the photolysis of two broadleaf herbicides, acetochlor (AC) and isoproturon (IP), which have distinct known or suspected predominant indirect photolysis pathways. The purpose of my study was a) to characterize the process of photobleaching in each wetland by monitoring changing optical properties as well as photochemical oxygen consumption during irradiation in a solar simulator; b) to relate photobleaching parameters with contaminant loss in the same waters; and c) to use both of these dynamic properties to assess changes in sampled waters over short (hours) and long (months) timeframes in situ and in storage.

Wetland waters samples were split after filtration and half were acidified to investigate the effects of acidification on CDOM. Samples were brought to field pH, spiked with the target herbicides, and irradiated in a solar simulator (Suntest CPS+). Analyte concentrations, UV-vis absorbance and dissolved oxygen (DO) were monitored at
intervals throughout the experiment. This procedure was undertaken as soon as possible after sampling and was repeated for some samples after various durations of storage. For one wetland (YCF), initial samples were collected at intervals of ~3 hr throughout the course of one day to investigate the changes in CDOM properties and reactivity over short timeframes.

My results demonstrate that indirect photolysis of AC was primarily due to reaction with hydroxyl radical (OH•). IP indirect photolysis has been shown to be dependent on triplet-state CDOM (3DOM). Here, IP was observed to contribute to photobleaching without being consumed, suggesting that IP intermediates may act as reactive species. The changes in CDOM properties and photoreactivity for YCF wetland over the course of one day were small (in most cases statistically negligible) and were comparable to changes observed over the course of months of storage. Acidified samples had a somewhat lower rate of forming precipitates and lost slightly less [DOC] loss over time, but displayed significantly altered CDOM absorbance. Rate constants for both herbicides in these wetland waters do not correlate strongly to any one chemical or optical property of the sampled waters, indicating that it is difficult to predict the photochemical behavior of these type of herbicides based upon the chemical constituents in natural waters.
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CHAPTER 1: INTRODUCTION

1.1 Scope and Aims of Research

Photochemical reactions that occur in sunlit natural waters play an important role in biogeochemical cycling as well as contaminant fate. Chromophoric dissolved organic matter (CDOM) in surface water is the most light-absorbing constituent and is therefore the primary determinant of photochemical reactions (Brinkmann et al. 2003). The absorption of UV light by CDOM in natural waters both protects aquatic organisms (Bracchini et al. 2006) and contributes significantly to the breakdown and cycling of organic matter in watersheds (Huang and Chen 2009, Yamashita et al. 2010). The presence of CDOM isolates alone can also accelerate contaminant transformation in sunlit waters (Lam et al. 2003, Guerard et al. 2009a).

Understanding the fate of surface water contaminants is increasingly important as the detection of synthetic organic compounds in surface waters has become commonplace in recent years (Kolpin et al. 2002, Focazio et al. 2008). Although various biotic and abiotic pathways exist by which contaminants are transformed, sequestered, or transported in natural systems, sunlight-mediated reactions have been shown to be important relative to e.g. biodegradation in the transformation both of contaminants (Nelieu et al. 2009, Liu et al. 2009) and CDOM (Vahatalo and Wetzel 2004, Moran et al. 2000). To the extent that changes in CDOM including photobleaching—loss of chromophores during light exposure—drive the overall photochemical reactivity of natural water, CDOM dynamics are likewise important for contaminant fate. The broad aims of the research presented here are to relate the chemical and optical properties of natural waters with the
photoreactivity of CDOM during light exposure and to investigate the role that these processes play in the photochemical fate of organic contaminants.

1.2 Photochemistry of Natural Waters: An Overview

Solar-driven chemical reactions may be direct or indirect. The sunlight available at the earth’s surface contains a small proportion of photons energetic enough to cause transformation (direct photolysis) in contaminant molecules that absorb them ($\lambda \approx 295$-$380$ nm). The rate of direct photolysis of a contaminant $C$ in polychromatic light can be described by eq. 1.1,

$$\frac{d[C]}{dt} = \phi k_d [C] \quad (1.1)$$

where $\phi$ is the quantum yield and $k_d$ is the reaction rate constant, equal to the sum of $k_{d,\lambda}$ for all wavelengths absorbed by the contaminant (Zepp and Cline 1977). Light screening by CDOM is a significant limiting factor for contaminant direct photolysis in natural waters where $[C] \ll [CDOM]$. In aerated waters, direct photolysis of CDOM leads to the formation of reactive oxygen species (ROS; eq. 1.2) and triplet excited states via intersystem crossing (IS; eq. 1.3) (Dalrymple et al. 2010, Sulzberger and Durisch-Kaiser 2009). Complexation with iron allows for direct photolysis of DOM, essential to the photo-Fenton pathway in natural waters (eq. 1.4; (Southworth and Voelker 2003, White et al. 2003).

$$\text{CDOM} + \text{O}_2 \xrightarrow{hv} \text{CDOM}^* + \cdot\text{HO}_2/\text{O}_2^- \quad (1.2)$$

$$1^\text{CDOM} \xrightarrow{hv} 1^\text{CDOM}^* \xrightarrow{IS} 3^\text{CDOM}^* \quad (1.3)$$

$$\text{Fe(III)}\text{-DOM}_m \xrightarrow{hv} \text{Fe(II)}\text{-DOM}_{m-1} + \text{DOM}_{ox}^+ \quad (1.4)$$

Dissolved oxygen plays a major role in photobleaching CDOM as well as in the indirect photolysis of DOM in general. The products of eqs. 1.2-1.4 are short-lived, unstable
species that react readily with oxygen, in turn producing various ROS and other excited species (eqs 1.5-1.8; Sulzberger and Durisch-Kaiser 2009).

\[ ^3\text{DOM}^* + \text{O}_2 \rightarrow \text{DOM} + ^1\text{O}_2 \]  (1.5)

\[ ^3\text{DOM}^* + \text{O}_2 \rightarrow \text{DOM}^+ + \text{O}_2^- \]  (1.6)

\[ \text{DOM}^+ + \text{O}_2 \rightarrow \text{DOMO}_2^+ \]  (1.7)

\[ \text{DOM}^- + \text{O}_2 \rightarrow \text{DOMOO}^- \rightarrow \text{DOM}_{\text{ox}} + \text{HO}_2^- \]  (1.8)

One of the most important ROS in natural waters is the hydroxyl radical, OH· (Vione et al. 2006). OH· is formed via a) hydrolysis of DOM-derived organic radicals (eq. 1.9), b) direct photolysis of nitrate/nitrite (eqs. 1.10-1.11), and c) the photo-Fenton pathway (eqs. 1.12-1.14). This pathway in particular has been shown to be a dominant mechanism of both photobleaching and contaminant transformation in highly colored, acidic waters (Brinkmann et al. 2003, Gao and Zepp 1998).

\[ \text{DOM}^- + \text{H}_2\text{O} \rightarrow \text{DOM-H} + \text{OH}^- \]  (1.9)

\[ \text{NO}_3^- + \text{H}^+ \xrightarrow{hv} \text{OH}^- + \text{NO}_2^- \]  (1.10)

\[ \text{NO}_2^- + \text{H}^+ \xrightarrow{hv} \text{OH}^- + \text{NO}^- \]  (1.11)

\[ \text{Fe(III)} + \text{O}_2^- \xrightarrow{hv} \text{Fe(II)} + \text{O}_2 \]  (1.12)

\[ 2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]  (1.13)

\[ \text{Fe(II)} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(III)} + \text{OH}^- + \text{OH}^- \]  (1.14)

The steady-state concentration of hydroxyl radicals and other reactive species is also determined by the concentrations of quenchers such as carbonate species, nitrite and DOM. DOM in particular is an important quencher of radical species including OH· (eq. 1.15; Vione et al. 2010) and \(^1\text{O}_2\) (eq. 1.16; Cory et al. 2010).

\[ \text{DOM} + \text{OH}^- \rightarrow \text{DOM}^- + \text{H}_2\text{O} \]  (1.15)

\[ \text{DOM} + ^1\text{O}_2 \rightarrow \text{H}_2\text{O}_2 + \text{DOM}_{\text{ox}} \]  (1.16)
1.3 Predicting Contaminant Photochemical Fate

Although the kinetics of many individual photochemical reactions are known in isolation, the prediction and modeling of contaminant photofate is hampered by uncertainty (Vione et al. 2006, Brezonik and Fulkerson-Brekken 1998). In addition, recent research has also shown that DOM inhibits the completion of triplet-induced transformation of contaminant molecules in sunlit waters (Canonica and Laubscher 2008, Wenk et al. 2011), implying that contaminants themselves (or their energized intermediate states) may be able to catalyze photobleaching. Chapter 2 presents research investigating the role of CDOM/contaminant interactions in determining photochemical fate in wetland waters.

In addition to the physical and chemical heterogeneity of DOM, a diversity of operational definitions exists that limits the comparability of research results. This is partially due to the fact that standard sample handling and storage procedures do not exist for the use of natural water samples in environmental photochemical research. Chapter 3 compares several sample handling and storage techniques in the interest of developing a set of best practices for increased comparability and applicability of results.

1.4 References


Lam, M.W., Tantuco, K. and Mabury, S.A. (2003) PhotoFate: A new approach in accounting for the contribution of indirect photolysis of pesticides and


CHAPTER 2: CONTAMINANT-ACCELERATED PHOTobleaching of Colored Dissolved Organic Matter during Solar Irradiation

2.1 Introduction

Wetlands have the potential to attenuate mobile contaminants transported by runoff from land operations (such as agriculture). Relatively long retention times (relative to streams) and a generally favorable aspect ratio with respect to sun exposure make wetlands well suited as photoreactors for contaminant degradation via direct or indirect photolysis. Sunlight striking wetland waters may cause transformation directly, but a more efficient series of events is initiated when sunlight photoactivates other wetland water constituents that react with contaminants via indirect photolysis. These photosensitizing species include nitrate/nitrite, iron and chromophoric dissolved organic matter (CDOM) and lead to the formation of reactive oxygen species (ROS) such as OH·, \( ^1\)O\(_2\), O\(_2^−/HO_2^− \) or other excited species including triple-excited state DOM (\( ^3\)DOM) and organic radicals. Species such as these are unstable and quickly react with other water constituents including dissolved organic matter (DOM), carbonate species and contaminant molecules.

Photobleaching, the depletion of CDOM via both direct and indirect photolysis, is an important physicochemical process in wetland chemistry and contributes to the cycling of organic matter in watersheds. The direct absorbance of sunlight, particularly at \( \lambda < 400 \) nm, can excite CDOM, causing it to react with other species including H\(_2\)O, O\(_2\), and other DOM, ultimately leading to the destruction of chromophores. For this reason, CDOM is depleted in sunlit waters more rapidly than DOM in general (Brinkmann et al. 2003, White et al. 2003, Vahatalo et al. 2002, Andrews et al. 2000). Over time, sunlight exposure decreases the average size of DOM molecules, measured both in terms of lower
molecular weight and changes in light-absorbance (Helms et al. 2008). In wetlands the processing of DOM into smaller forms has been attributed more to photolytic than microbial processes (Moran et al. 2000, Yamamoto et al. 2009) and has been shown to affect the subsequent photolability and bioavailability of DOM (Vahatalo and Wetzel 2004, Cory et al. 2010, Vione et al. 2009).

The extent to which CDOM photobleaching occurs and facilitates the transformation of organic contaminants depends on water chemical and optical properties in addition to physical factors such as wetland dimensions and flow. The concentrations of known photosensitizers and quenchers present in natural waters affect the steady-state concentrations of reactive species generated during exposure to sunlight and can be used to some extent to predict the photofate of contaminants. Many attempts have been made in recent years to model these processes in surface water bodies using water chemical characteristics (Lam et al. 2003, Brezonik and Fulkerson-Brekken 1998, Gerecke et al. 2001), but much is still unknown about these complex mechanisms (Wallace et al. 2010). This is partially due to the fact that while some of the measurable chemical constituents of wetlands such as NO$_3^-$ play well-defined roles in the generation of radicals in the presence of sunlight, others such as CDOM are both sources and sinks of reactive species with highly variable efficiencies (Halladja et al. 2009). Further, recent work showing that DOM can quench contaminant-excited intermediates (Canonica and Laubscher 2008, Wenk et al. 2011) implies that contaminant molecules themselves may act as reactive species accelerating CDOM photobleaching in irradiated waters.

Measuring optical parameters of the CDOM pool in a natural water body has been used to predict photofate in surface water (e.g. Sulzberger and Durisch-Kaiser 2009, Vione et al. 2010), but little work has been done to look at wetland water in particular (Miller and Chin 2002, 2005). Further, to my knowledge, the mechanisms by which contaminants may alter CDOM photooxidation have not previously been explored. The motivation for this study was to investigate (1) the role of various wetland water constituents and optical
characteristics in contaminant photodegradation, and (2) the link between contaminant degradation and the coincident CDOM photooxidation.

In order to compare the water chemistry effects of various wetlands in this study, two probe compounds were chosen, acetochlor (AC) and isoproturon (IP). Both compounds are pre-emergent broadleaf herbicides used in large-scale agriculture and both have chemical properties that make them suitable for use in laboratory studies (low volatility, negligible hydrolysis rates at ambient temperatures, log$K_{ow} < 4.0$). Both are intended for use in spring before field crops emerge from the ground and are therefore at particular risk for exiting their intended target zones via various means including field runoff, tile drainage and groundwater infiltration. AC (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide; Fig. 2.1) is a member of the chloracetanilide family of herbicides. It was registered by the EPA in 1994 in exchange for an industry pledge to effect a substantial reduction of total herbicide use (including then widely used alachlor, atrazine and metolachlor) over the subsequent years (Newcombe et al. 2005). Usage of AC has become widespread since then in the U.S. and elsewhere (Vecchia et al. 2009, Gustafson et al. 2004). The herbicide is fairly mobile in soil (Lengyel and Foldenyi 2003), and has been seen in many hydrologic stocks since then, including surface waters (Kolpin et al. 1996, Clark and Goolsby 1999), groundwater (Hancock et al. 2008), rain (Vogel et al. 2008) and air (Coscolla et al. 2010).

IP (N,N-dimethyl-N,4-(1-methylethyl)phenyl]urea, Fig. 2.1), a phenylurea herbicide, is commonly used on cereals (IFEN 2004). In certain soils, it has been shown to leach readily (Sorensen and Aamand 2003, Doerfler et al. 2006). Both AC and IP are slow to mineralize if they infiltrate to groundwater (Janniche et al. 2010), so wetlands might be very relevant buffers for the transformation of these contaminants. AC has been shown to undergo both aerobic and anaerobic microbial transformation in natural settings (Loor-Vela et al. 2003), relevant in wetlands, especially where useful light is attenuated. Likewise, the rate of mineralization of IP has been shown to increase in the presence of plant residues and relatively unprocessed organic matter (Alletto et al. 2006), and in a
field trial, two-thirds of IP injected into the inlet of a Danish riparian wetland was degraded during residence time (Kidmose et al. 2010). IP has also been observed to photodegrade *in situ* in Lake Greifensee, Switzerland (Gerecke et al. 2001), and a different phenylurea herbicide (chlortoluron) was degraded in mesocosms where photodegradation was shown to be more important than biodegradation (Nelieu et al. 2009). However, it is not known whether photochemical degradation would be likely to play an important role in the transformation of these compounds in wetlands.

AC and IP are useful probe compounds for this study because of their known differences in light-induced degradation. Although AC is susceptible to substantial direct photolysis at UV wavelengths (Zheng and Ye 2003, Xu et al. 2007), it has been shown to degrade relatively slowly by direct photolysis in simulated solar irradiation (Brezonik and Fulkerson-Brekken 1998) and has not to my knowledge been studied in sunlit natural waters. Other chloroacetanilide herbicides have been shown to exhibit low absorbance above $\lambda = 300$ nm, slow direct photolysis rate and resistance to interaction with photosensitized water constituents beyond the most generic, e.g. OH- (Miller and Chin 2005, Wilson and Mabury 2000). In sunlight, IP has been shown to undergo minimal direct photolysis, but unlike AC, is readily degraded by indirect photolysis, notably $^3$DOM (Gerecke et al. 2001, Canonica et al. 2006). The use of both AC and IP as probe compounds allows for comparison of the ability of different natural waters to generate various types of excited species. This may both shed light on the mechanisms underlying photobleaching in different wetland waters and may facilitate prediction of the photochemical fate of these and similar compounds in wetlands.

The objectives of the current study, therefore, are 1) to quantify the rates of change of CDOM and of the probe compound herbicides AC and IP during the course of solar irradiation in various wetland waters, and 2) to relate these changes to water chemical and optical characteristics, and 3) to determine the extent to which photoinduced changes in CDOM and in the herbicides are interconnected.
2.2 Materials and Methods

2.2.1 Site descriptions
Water was collected from four freshwater wetland sites in the U.S. between June 2010 and January 2011. Yocom farm wetland, Champaign County, Ohio (YCF) is a small (<1 ha) modified forested wetland receiving tile drainage from corn and soybean fields in central Ohio. Kawai Nui Marsh (KNM) is a restored freshwater marsh on Oahu, Hawaii (335 ha). The Everglades Wildlife Management Area Water Conservation Area 2B (EVG), located near Ft. Lauderdale, FL, is positioned at the upstream end of the Okeechobee/Everglades system that covers much of southern Florida. Samples were taken from a channel emerging from an expansive stretch of vegetated marsh (USGS EDEN station S11A-H, upstream). The Okefenokee Swamp (OKS) is a 1.77 x 10^5-ha, peat-filled “blackwater” swamp that is the source of the Suwannee River. Samples were taken from the edge of designated open marsh area accessed from the eastern entrance to the Okefenokee National Wildlife Refuge, Georgia. Dates of sampling are shown in Table 2.1.

In all cases, grab samples were taken from the shore of inundated wetland areas with minimal emergent plant shading. Samples were collected with a glass container on a 3-m extender rod from a depth of 30-50 cm and were vacuum-filtered as soon as possible (<2 h) with pre-combusted Gelman A/E (1-µm) glass filters and stored in the dark on ice until being refrigerated at 4°C. In the case of OKS samples, high turbidity of the water necessitated splitting the sample and filtering only half of the sample in the field (split OKS_{FF}) and the remaining half in the lab two days later (split OKS_{LF}) with storage on ice in the meantime. The rest of the samples were also split to determine the effects of sample treatment (discussed in Chapter 3).

2.2.2 Water chemical component characterization
Parameters measured in the field at each sampling time include water temperature and dissolved oxygen (YSI EcoSense DO200, YSI Environmental, Inc. Yellow Springs, OH). The pH, conductivity, and total dissolved solids of the filtrate were measured immediately after filtering (Thermo Orion model 130 conductivity meter, Beverly, MA). Laboratory characterization of the samples included UV-visible absorbance (Varian Cary 13 UV-visible spectrophotometer), [DOC] (Shimadzu TOC-V CPN, non-purgeable organic carbon mode), total iron (Varian Vista AX CCD ICP-AES) and inorganic ions (Dionex DX-120, Sunnyvale, CA, U.S.A. and Skalar San++ Nutrient Analyzer, Breda, Netherlands).

2.2.3 Water optical characterization

UV-vis absorbance values were corrected for the absorbance of ultrapure water (Millipore MilliQ water purification system, Billerica, MA). Specific UV absorbance (SUVA$_{254}$) values were calculated in the manner of Weishaar et al. (2003) using equation 2.1,

$$SUVA_{254} = \frac{A_{254}}{[DOC]}$$  \hspace{1cm} (2.1)

where $A_{254}$ = the ultrapure water-corrected absorbance of the wetland water at $\lambda =$ 254 nm expressed with reference to the path length of my instrument (1 cm). [DOC] is expressed in moles C per L (M) such that SUVA$_{254}$ is expressed in M$^{-1}$ cm$^{-1}$. To determine SUVA$_{254}$, samples were diluted if their absorbances were greater than 1 and [DOC] was that of the diluted samples.

To calculate spectral slope, Napierian absorption coefficients for $\lambda =$ 250-500 nm were calculated as follows for each water,

$$a_\lambda = \frac{(2.303 A_\lambda)}{l}$$  \hspace{1cm} (2.2)

where $a_\lambda$ = the absorption coefficient (m$^{-1}$) at $\lambda$, $A_\lambda$ = corrected UV-vis absorbance at $\lambda$, and $l$ = path length (0.01 m for my instrument). A plot of ln $a$ as a function of $\lambda$ yields a relatively linear curve whose slope (S) in various ranges were determined; here S$_{275-295}$,
$S_{350-400}$ and $S_R$, the ratio of $S_{275-295}/S_{350-400}$ were calculated in the manner of Helms et al. (2008).

Absorbance was also used to calculate a screening factor (SF) for each water sample. The UV-vis absorbance spectra for the constituents of a given natural water may overlap with the absorbance spectrum for a contaminant of interest, screening out photons that might otherwise have facilitated direct photolysis. AC and IP absorb weakly in the UV-B range ($\lambda = 290$ to $320$ nm, Fig. 2.1), so SF was calculated for each water sample over that range following the method of Miller and Chin (2002). Briefly, SF at any given $\lambda$ is calculated as follows,

$$SF = \frac{(1-10^{-A\cdot l})}{(2.303A\cdot l)}$$

(2.3)

where the variables are the same as defined above. SF is plotted as a function of wavelength and fitted to a fourth-order polynomial ($R^2 > 0.98$), which is then integrated over the wavelengths of interest and subtracted from the value without light screening. SF values can be thought of as a percentage of UV-B light that is able to penetrate the solution compared to a clear solution.

2.2.4 Photolysis experiments

Photolysis experiments were conducted to investigate the change in optical and chemical characteristics of each water sample as well as to determine transformation rates for two contaminants, AC and IP. Both herbicides were supplied by Chem Service, Inc. (West Chester, PA) at highest available purity. Wetland water samples were adjusted to field pH using HCl or NaOH of highest available grade, spiked with aqueous stock solutions of IP or AC to form a $5 \mu M$ reaction solution, and equilibrated in room conditions for 2-3 hours. Fulvic acid solutions containing Suwannee River, Pony Lake, or Old Woman Creek fulvic acids (SRFA, PLFA, OWCFA respectively) were prepared at pH 8 with 1 mM bicarbonate buffer in ultrapure water and were equilibrated in the same manner.
Quartz photoreaction tubes (path length = 0.9 cm, volume = 8 mL) were then filled with reaction solution and fitted with air-tight caps in the dark. Up to 18 tubes at a time were laid on their sides inside the solar simulator (Suntest CPS+, Atlas MTT LLC, Chicago, IL) allowing collimated light to irradiate all tubes. The light source is a xenon lamp modified with coated quartz/window glass filters to approximate the spectral distribution of solar light at the earth’s surface, especially in the region $\lambda = 299-400$ nm most relevant to photochemistry. Light samples were irradiated for $\sim 8$ hr (IP) or for $\sim 25$ hr (AC) to adequately determine degradation rate constants. Dark controls consisted of foil-wrapped tubes exposed to the same conditions. Direct photolysis rates were determined by irradiating herbicides at the same concentrations in ultrapure water.

Overall light intensity of the solar simulator was determined using both $p$-nitroanisole/pyridine and $p$-nitroacetophenone/pyridine actinometry (Dulin and Mill 1982; Fig. A.3-A.4), which is calibrated to the known quantum yield of the reaction at 313 and 366 nm. The light emitted by my solar simulator (on the manufacturer’s 550 W/m$^2$ setting) was determined to be $\sim 2.0$ times as strong as the summer sun at 40°N latitude (Leifer 1988). Irradiance over the course of experiments was continuously monitored using a UV-light radiometer (VWR International, Bridgeport, NJ) that integrates signal over $\lambda = 320-390$ nm. The temperature of the solar simulator was maintained at $27\pm 3^\circ$C.

For a subset of photolysis experiments, UV-vis absorbance and dissolved oxygen (DO) were measured at time-points during irradiation (see Fig. A.5 for sample data). DO was determined using a micro-DO probe (Lazar Research Laboratories, Inc., Los Angeles, CA) dry-calibrated in 100% Ar and in open air ($\sim 21\%$ O$_2$) and wet-calibrated using air-saturated water (analogous to initial conditions for the mixed reaction solution). DO was measured in most cases within $\sim 1$ hour of removal from the solar simulator and after the tubes had equilibrated to room temperature (slightly cooler than the solar simulator). Influx of atmospheric O$_2$ into the tubes during measurement was determined to be
minimal during initial readings (acquisition time 2-3 min), but interfered with measurements over longer spans of time, precluding multiple readings of each sample tube. Herbicide concentrations were analyzed using a Waters reversed-phase HPLC (Milford, MA) equipped with a Nova-Pak C18 column (3.9 x 150 mm). With a mobile phase of 55:45 ACN:H$_2$O (v:v), detection parameters for IP were $\lambda = 240$ nm, r.t. $\approx 4.7$ min, and for AC, $\lambda = 220$ nm, r.t. $\approx 6.5$ min.

In order to explore the indirect photolysis mechanisms at work in these waters, $t$-butanol was added to EVG water spiked with AC such that the final concentrations were 25 mM and 5 µM, respectively. Another set of mechanistic experiments involved bubbling EVG with Ar (1 min/mL solution) to displace atmospheric gases and then preparing photolysis solutions in a glovebox filled with N$_2$/H$_2$ (95%/5%). Initial readings on these low-O$_2$ solutions showed that this treatment yielded DO of 35-40% of atmospheric O$_2$ saturation.

2.2.5 Data analysis
Linear regression of herbicide concentration data obeyed pseudo first-order reaction kinetics in most cases ($R^2>0.90$). Samples with curvilinear kinetics plots are discussed below as special cases. Indirect photolysis reaction rate constants ($k_{ind}$) were determined as follows:

$$k_{ind} = k_{obs} - k_{dir}$$

(2.4)

where $k_{obs}$ is the observed $k$ from each experiment and $k_{dir}$ is the rate constant for photolysis of the compound in ultrapure water multiplied by the SF for any given wetland water.

2.3. Results

2.3.1 Initial chemical and optical characteristics of collected wetland waters
Results of field and laboratory chemical analyses are shown in Table 2.1. The sampled wetlands represented a broad spectrum of acidity regimes (Table 2.1), from the alkaline
YCF (pH = 8.9) and EVG (pH = 7.9), which both sit on limestone bedrock, to the circumneutral KNM (pH = 6.1) and the acidic OKS (pH = 3.5). Filtered water samples were scanned for UV-visible absorbance in order to determine their overall light-absorbing properties, expected to largely be due to CDOM. Differing iron concentrations (Table 2.1) may also account for some of the coloration difference, although previous work has shown that at this [Fe$_{tot}$] range, the effect of iron on sunlight-relevant UV-visible absorbance is likely to be small (Weishaar et al. 2003).

TOC analysis showed a wide range of [DOC] (Table 2.1), from 7.2 ± 0.2 mg C/L for YCF to 139.1 ± 0.7 mg C/L for OKS$_{LF}$ (0.6-11.6 mM). Both OKS splits showed high [DOC], consistent with the high level of evident turbidity that necessitated splitting the sample in the first place.

The heterogeneity of CDOM structure and composition precludes a simple determination of concentration of the colored fraction (as opposed to total [DOC]). UV-vis absorbance spectra for each water sample were used to derive a screening factor (SF), a measure of the proportion of sunlight striking the water that is available for interaction with contaminant molecules. Screening factors for all sampled waters aligned inversely with [DOC] (Table 2.1), with the highest [DOC] sample (OKS$_{LF}$) absorbing nearly 50% of ambient sunlight.

Non-colored organic matter does not absorb sunlight to a meaningful extent but may participate in indirect photolysis reactions, including acting as quenchers of reactive species. Higher SUVA$_{254}$ values have been strongly correlated with the aromatic carbon (C$_{ar}$) content of DOM (Weishaar et al. 2003), which may indicate source material (autochthonous or allochthonous; Chin et al. 1994). Here, the SUVA$_{254}$ values of the wetland waters fell into two pairs (Table 2.1), with YCF and EVG at ~360 M$^{-1}$ cm$^{-1}$ (~2.95 L mg C$^{-1}$ m$^{-1}$) and KNM and OKS$_{FF}$ at ~225 M$^{-1}$ cm$^{-1}$ (~1.85 L mg C$^{-1}$ m$^{-1}$). High
aromaticity has been reported in the northern Everglades previously (Yamashita et al. 2010).

2.3.2 Changes to CDOM during light exposure

During the course of irradiation experiments in a solar simulator, EVG and OKS water samples were periodically monitored for UV-visible absorbance and DO levels. In all cases, the absolute UV-visible absorbance decreased with light exposure, indicating loss of chromophores over time. This trend is consistent with numerous previous observations of photobleaching (Brinkmann et al. 2003, Vahatalo and Wetzel 2004, Moran et al. 2000).

To assess any qualitative changes to the CDOM as a result of light exposure, spectral slopes of the linearized UV-vis absorbance spectra were determined for the ranges $\lambda = 275-295$ nm and $\lambda = 350-400$ nm. Initial spectral slopes are shown in Table 2.1, and changes in spectral slope values during irradiation are shown in Table 2.2. $S_{275-295}$ increased steadily with light exposure for all waters tested, implying greater loss of absorbance at the lower $\lambda$ (higher energy) end of this range. This increase in $S_{275-295}$ concurs with findings of previous studies of photobleaching in natural waters (Helms et al. 2008, Galgani et al. 2011). While $S_{275-295}$ changed at similar rates among measured waters (mean rate of change = $1.42 \times 10^{-4}$ hr$^{-1}$, SD = $2.78 \times 10^{-5}$ hr$^{-1}$), the $S_{350-400}$ parameter was more variable. In EVG water, $S_{350-400}$ decreased at about the same absolute rate (opposite direction) as $S_{275-295}$, but in OKS water, $S_{350-400}$ either remained constant or slightly increased with light exposure. Helms et al. (2008) also observed this variability in $S_{350-400}$.

In most of the irradiation experiments, the waters were spiked with AC or IP (5 µM). Compared to no AC or IP solutions, the presence of both AC and IP affected the rate of spectral slope change during irradiation for EVG water. Control experiments verified that the initial spectral slopes for EVG water with and without herbicide were identical.
(Fig. A.1, Appendix), indicating that this altered pattern of spectral slope change is not due simply to the decreasing absorbance of herbicide as it is transformed. Therefore, the presence of herbicide alters the way in which the CDOM changes over the course of irradiation.

The presence of each herbicide caused a different effect on spectral slope with time. With AC, S\textsubscript{275-295} increased ~30% more rapidly than with no herbicide present, and the usual rate of decrease for S\textsubscript{350-400} was slowed to almost no change. However, the slope ratio S\textsubscript{R} remained fairly constant. By contrast, the presence of IP considerably accelerated the rate at which S\textsubscript{350-400} decreased, causing a rate of change of S\textsubscript{R} over twice that of no-herbicide or AC-containing solutions.

Interestingly, reducing the initial DO concentration in reaction solutions somewhat mitigated the effects of herbicides on spectral slope behavior during irradiation. In experiments in which herbicide-spiked EVG solutions were prepared in a low-O\textsubscript{2} glovebox, the changes in spectral slope were of somewhat lower magnitude than with normal, air-saturated conditions (Table 2.2). It is interesting to note in this case that the reduction in O\textsubscript{2} did not seem to affect the rate of change in S\textsubscript{275-295}, perhaps implying that photobleaching of chromophores absorbing lower-wavelength (higher energy) photons is not as directly dependent on O\textsubscript{2} and perhaps more due to indirect photolysis. Additional experiments would be necessary to determine whether low-O\textsubscript{2} conditions in fact “mitigated” the effects of herbicides on photobleaching or whether these conditions affected the CDOM itself. The results of Gao and Zepp (1998), who observed lower spectral slope change in anoxic solutions, would seem to support the latter interpretation.

In OKS water, all experiments were performed in the presence of herbicides. Compared to irradiation with AC, for both OKS\textsubscript{FF} and OKS\textsubscript{LF} the presence of IP resulted in greater absorbance changes than those observed in EVG (Table 2.2). Although the absolute S\textsubscript{R} rates of change are different between irradiated OKS\textsubscript{FF} and OKS\textsubscript{LF} waters, in both splits,
the presence of IP caused both $dS_{275-295}/dt$ and $dS_R/dt$ to double compared to AC. The factor of two increase in $S_R$ rate of change caused by the presence of IP was consistent among EVG, OKS_{FF} and OKS_{LF} waters despite their disparate water characteristics.

2.3.3 Oxygen consumption during light exposure
Irradiated EVG and OKS waters were also monitored for changes in DO over the course of irradiation as shown in Fig. 2.2 and Table 2.2. O$_2$ is a participant in many known indirect photolysis reaction pathways including photobleaching of CDOM (Cory et al. 2010, Andrews et al. 2000), the formation of singlet oxygen (Haag and Hoigne, 1986) and photo-Fenton reactions (Voelker et al. 1997) such that photobleaching (loss of chromophores) has been shown to be largely due to photooxidation. From a starting value of 100% atmospheric saturation, DO was observed to decrease steadily during all irradiation experiments compared to dark controls, in which no DO change was observed. The rate of DO loss was relatively consistent for each water sample over successive trials. Additionally, measurements of pH in samples after irradiation showed little (<0.5 units) to no change from the starting value.

The acceleration of photobleaching in the presence of the herbicides AC or IP observed in spectral slope changes was mirrored by accelerated DO loss in herbicide-containing solutions compared to wetland water alone (Table 2.2). With AC, DO decreased ~30% faster than in wetland water alone, intriguingly parallel to the accelerated change in $S_{275-295}$ of about the same relative magnitude. With IP, the herbicide effect was larger (~70%). Experiments conducted in EVG water under low-O$_2$ conditions resulted in little change in DO level over the course of the irradiation, although readings exhibited a greater standard deviation compared to air-saturated irradiations (results not shown). The rate of DO loss was correlated to both [DOC] and [Fe$_{tot}$] for the wetland waters (Fig. 2.3), consistent with direct and Fenton-mediated photooxidation.
For all waters, the presence of IP caused substantially higher rates of DO loss compared to AC. Comparing DO loss rates in Table 2.2, IP enhancement was a factor of 1.3, 2, and 2.5 higher for EVG, OKS_{FF} and OKS_{LF}, respectively. This enhancement mirrors the increased photobleaching of waters containing IP versus AC described above. Interestingly, the same pattern emerges in the dependency of DO loss rate on [DOC] and [Fe_{tot}] shown in Fig. 2.3: i.e., twice the DO loss per unit DOC or Fe_{tot} for solutions containing IP versus AC.

For both of the OKS splits, the last DO measurements (taken after ~24 h of irradiation) actually indicate an increase in DO over the previous measurement (Fig. 2.2b, 2.2c). This phenomenon was observed in about half of the replicates of these experiments with OKS water; otherwise, the DO was observed to decrease at a steady rate to 0% well before 24 hr or to taper off to a non-zero plateau level (data not shown). The reason for the variability in this final reading is not evident; consequently, DO loss rates for OKS splits were calculated with the initial, more linear measurements.

2.3.4 Rates of contaminant loss during light exposure

Direct photolysis

The rates of direct photolysis of AC and IP were measured by irradiating the herbicides in ultrapure water. The optical properties of the herbicides themselves (Fig. 2.1) determine whether they are susceptible to direct photolysis at wavelengths relevant to solar exposure (λ>299 nm). The direct photolysis reaction rate constant for AC in ultrapure water (k_{UP}) was 7.5 ± 0.6 x 10^{-3} hr^{-1}, translating to a half-life of ~3.9 d. This value is consistent with a previous determination of k_{UP} in simulated sunlight: 8.2 x 10^{-3} hr^{-1} (Brezonik and Fulkerson-Brekken 1998). The k_{UP} for IP was 2.7 ± 0.9 x 10^{-3} hr^{-1} or a half-life of ~10.6 d. The lower k_{UP} of IP versus AC accords with its lower absorbance of light at λ > 299 nm (Fig. 2.1) and is consistent with previous observations (Millet et al. 1998).
Indirect photolysis

Observed herbicide loss rate constants ($k_{obs}$) were measured in irradiated wetland waters and in buffered fulvic acid solutions. The observed rate of loss in these more complex mixtures is attributed to both direct and indirect photolysis. To determine the rate constant that describes direct photolysis occurring in a given wetland water ($k_{dir}$), the rate constant for direct photolysis in ultrapure water ($k_{UP}$) was multiplied by the screening factor (SF$_{290-370}$; Table 2.1). Indirect reaction rate constants ($k_{ind}$) were then calculated by subtracting $k_{dir}$ from $k_{obs}$ as described in equation 2.4. In these experiments, all dark controls showed negligible (<1%) herbicide loss.

Acetochlor

For AC, wetland water caused a 2-3 fold increase in reaction rate constant relative to ultrapure water, even when accounting for light screening (Table 2.3, Fig. 2.4). Indirect photolysis rate constants ($k_{ind}$) were nearly the same for YCF, KNM, EVG and OKS$_{LF}$, with a mean of 1.2 ± 0.1 x 10$^{-2}$ hr$^{-1}$, suggesting a fairly uniform level of photosensitization even among significantly different waters. The dramatic exception to this trend was OKS$_{FF}$, in which indirect photolysis occurred at a rate 4-5 times higher than all other waters. Similarly, the presence of SRFA at low concentration (2.5 mg C/L) only slightly enhanced the degradation rate of AC, and the presence of OWCFA caused no enhancement over direct photolysis. Increasing concentrations of PLFA (7, 14 and 21 mg C/L) corresponded to increasing $k_{ind}$, although the mean was nearly the same (~1.1 x 10$^{-2}$ hr$^{-1}$) as with wetland waters (data not shown).

Isoproturon

Unlike AC, IP readily degrades via indirect photolysis, with an enhancement factor of 30-200 compared to $k_{dir}$ in wetland water (Table 2.3, Fig. 2.4). These results agree with previously reported ratios of direct to sensitized (SRFA) photolysis for IP (Gerecke et al. 2001). The irradiation of IP in PLFA solutions of increasing [DOC] yielded linearly increasing rate constants (Fig. 2.5), consistent with the involvement of $^3$DOM in IP
photolysis as shown by others (Gerecke et al. 2001, Canonica et al. 2006). However, the link between [DOC] and IP $k_{obs}$ does not neatly extend to the group of sampled wetland waters nor to other FA solutions.

2.3.5 Probing mechanisms of indirect photolysis

Low-$O_2$ conditions

To determine the extent to which $O_2$ was a player in indirect photolysis, herbicide-spiked solutions of EVG were prepared under low-$O_2$ conditions before irradiation in the solar simulator. Initial readings on the resulting photolysis solutions indicated that they contained 35-40% of the usual DO resulting from atmospheric equilibration. These solutions were monitored for DO and UV-vis absorbance (both discussed above) as well as herbicide concentration at intervals throughout irradiation. Herbicide loss rates are shown in Fig. 2.6. For AC, low DO conditions did not significantly alter the reaction rate constant, although the kinetics became curvilinear at later time points rather than strictly linear in accordance with pseudo-first order decay (Fig. 2.6a). This deviation from linear was more dramatic for IP (Fig. 2.6b). Curvilinear herbicide degradation kinetics may be caused by several factors, including the depletion of one or more reactants during irradiation, including $O_2$ (which competes with IP for $^3$DOM) or CDOM, and/or the increase in concentration of oxidized DOM involved in scavenging hydroxyl radicals.

Addition of $t$-butanol

To determine the extent to which AC indirect photolysis involves OH-, AC was irradiated in wetland water (EVG) spiked with 25 mM $t$-butanol, a known OH- scavenger. The resulting $k_{obs}$ was lower than $k_{dir}$, suggesting that the quencher shut down both direct and indirect pathways. Because $t$-butanol does not does not directly absorb chromophores, the reduction in the photolysis rate below the direct rate suggests that $t$-butanol quenches photoexcited AC intermediates incompletely transformed via direct photolysis (Fig. 2.6a). DO loss in the $t$-butanol-spiked solution was lower than an identical solution without the quencher, and more closely approximated the DO loss of EVG water alone.
(Table 2.2). The rate of change for $S_{275-295}$ was nearly the same as with AC alone, but the $S_R$ rate of change was somewhat lower than even wetland water alone, suggesting some inhibition in photobleaching with $\tau$-butanol.

2.4. Discussion

2.4.1 Contaminant acceleration of photobleaching

A few important ideas emerge from the results described above. First, the wetland waters studied here exhibited rates of spectral slope change and DO loss indicative of photooxidation that were fairly consistent for the same water and correlated with [Fe$_{\text{tot}}$] and [DOC] of the water (Table 2.2, Fig. 2.3). Moreover, the presence of either herbicide tested in this study (AC or IP) accelerated both the spectral changes and DO loss associated with irradiation, with IP having roughly double the effect of AC, although this herbicide effect may be somewhat reversed when starting with low-O$_2$ conditions. Although previous studies have shown that the presence of CDOM in irradiated waters enhances contaminant photolysis, to my knowledge, the opposite has not previously been demonstrated: that the presence of contaminants enhances CDOM photobleaching.

The probe compounds used in this study—AC and IP—were chosen because they are known or suspected to have different dominant pathways for indirect photolysis. For AC, no previous data is available for indirect photolysis in sunlit natural waters, but other chloroacetanilide herbicides have been shown to be largely dependent on non-specific ROS species such as OH$^\cdot$, including metolachlor (Sakkas et al. 2004, Dimou et al. 2005), alachlor (Miller and Chin 2005) and propachlor and butachlor (Benitez et al. 2004). The results of my experiments, in particular the elimination of indirect photolysis in the presence of the OH$^\cdot$ quencher $\tau$-butanol (Fig. 2.6), confirm that OH$^\cdot$ is the primary player in AC indirect photolysis in wetland waters. For IP, on the other hand, indirect photolysis has been shown to be largely due to reaction with $^3$CDOM (Gerecke et al. 2001, Canonica et al. 2006). The acceleration of IP reaction rate in low-O$_2$ conditions observed here (Fig. 2.6), as well as a strong increasing trend between fulvic acid
concentration and reaction rate constant (Table 2.3, Fig. 2.5), agree with these previous studies.

Despite the dissimilar mechanisms by which these herbicides undergo indirect photolysis, the presence of both AC and IP accelerated two measures of photobleaching monitored in this study—spectral slope and DO change—beyond the rates observed in wetland water alone. This suggests that the herbicides themselves are involved in the formation of an additional pool of reactive species that react with CDOM. Phenylurea herbicides such as IP are known to form radical cations (\(\text{IP}^{\bullet^+}\)) by reacting with triplet aromatic ketones in DOM (\(^3k\text{DOM}\)) as shown in eq. 2.5 (Canonica et al. 2006, Canle et al. 2005). In addition, Canonica and Laubscher (2008) showed that the triplet-sensitized photolysis of IP and other contaminants decreased in the presence of DOM. This suggests that the \(\text{IP}^{\bullet^+}\) can be reduced back to the parent form, \(\text{IP}\) (eq. 2.6) by DOM, rather than proceeding via oxidation by another water constituent, \(M\), to form \(\text{IP}_{\text{ox}}\), the measured end point in this study (eq. 2.7).

\[
\begin{align*}
^3k\text{DOM} + \text{IP} & \rightarrow k\text{DOM}^{\bullet^-} + \text{IP}^{\bullet^+} \quad (2.5) \\
\text{IP}^{\bullet^+} + \text{DOM} & \rightarrow \text{IP} + \text{DOM}^{\bullet^+} \quad (2.6) \\
\text{IP}^{\bullet^+} + M & \rightarrow \text{IP}_{\text{ox}} + M^{\bullet^+} \quad (2.7)
\end{align*}
\]

This reaction scheme is supported by the results presented here. First, the transfer of charge to DOM in eq. 2.6 would explain increased photobleaching of CDOM in the presence of IP. In effect, the intermediate form of the herbicide acts as a reactive species capable of participating in the cascading mechanisms of indirect photolysis of CDOM. Second, to the extent that the pathway described in eq. 2.6 is favored over the pathway in eq. 2.7, the rate of successful transformation of IP decreases. In the high-[DOC] solutions OKSFF and OKS_LF, the presence of IP causes an acceleration in both the change in spectral slopes and in consumption of \(\text{O}_2\) compared to AC. However, the rate of IP degradation itself is lowest in OKS water (Fig. 2.4, Table 2.3), which is contrary to the general rule that IP reaction rate roughly increases with increasing [DOC] observed for
solutions such as YCF, KNM and EVG (Fig. 2.5) and by others (Gerecke et al. 2001). The fact that IP increases photobleaching without being consumed to a greater extent implies a predominance of a scenario similar to that in eq. 2.6 over eq. 2.7.

Currently, there is less evidence supporting a similar mechanism of radical quenching in OH-mediated transformation, expected to dominate AC indirect photolysis (Wenk et al. 2011). The acceleration of photobleaching caused by the presence of AC was much smaller (EVG; Table 2.2). However, in the photolysis of AC in EVG (Table 2.2, Fig. 2.6), the presence of t-butanol cut the rate of AC degradation down to a level below direct photolysis but did not lead to a significant change in photobleaching (dS$_{275-295}$/dt and dDO/dt). These results hint at an incomplete transformation mechanism analogous to eq. 2.5-2.6; however, more experimentation would be necessary to further define this phenomenon.

2.4.2 Indirect photochemical mechanisms at play
Equations 2.5 – 2.7 illustrate a plausible generic scenario to explain the enhancement of photobleaching caused by the presence of IP, and to a lesser extent, AC. However, the mechanisms responsible for the various photochemical stages of indirect photolysis—from generating reactive species to the intertwined mechanisms of photobleaching and herbicide degradation—remain for the most part undifferentiated from the results of this study.

*Mechanisms likely to be important for acetochlor*
The most important reactive species with respect to AC transformation is likely to be OH$^-$. The major formation pathways of OH$^-$ in sunlit waters include (1) photolysis of NO$_3^-$ and NO$_2^-$ (2) the reaction of sensitized CDOM with H$_2$O, and (3) photo-Fenton reactions mediated by Fe(III) and DOM. Major sinks for OH$^-$ are DOM, carbonate, bicarbonate and, to a lesser extent, NO$_2^-$ (Vione et al. 2006). The balance of the rates of OH$^-$ production and consumption in irradiated natural waters determine the steady-state
concentration of OH· ([OH·]ss) at any given time. Given the second-order rate constant between AC and OH·, \(k_{OH} = 7.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}\); Breken and Brezonik 1998), [OH·]ss on the order of \(10^{-16} \text{ M}\) would be required to achieve the average \(k_{ind}\) of \(1.2 \pm 0.1 \times 10^{-2} \text{ hr}^{-1}\) that I observed for AC. This is a plausible scenario given that [OH·]ss of this magnitude has been observed in various wetland waters (White 2000; Jacobs et al. 2011).

Iron-rich, acidic, highly colored waters such as OKS are known to promote photo-Fenton chemistry (White et al. 2003, Gao and Zepp 1998). In addition, both OKS splits showed higher \([\text{NO}_3^-/\text{NO}_2^-]\) than the other waters. These factors may help to explain why AC photolysis was significantly faster in OKSFF than in any other water. Alachlor, a sister herbicide to AC, was shown to photodegrade substantially more rapidly in acidified (pH = 4) wetland waters versus unacidified (Miller and Chin 2005), and the same mechanism may be at work with AC. Although OKS_LF has the same pH as OKS_FF, it had nearly three times the [DOC]. OH·-quenching associated with this high [DOC] may explain why, rather than being enhanced in OKS_LF water, AC \(k_{ind}\) in OKS_LF was the slowest of any water.

Various models have been developed to predict the rate of OH· production (\(R_{OH}\)) and [OH·]ss in natural waters using known source and sink pathways of OH· (Brezonik and Fulkerson-Brekken 1998, Vione et al. 2010, among others). Inputs to these models consist of constituent concentrations and/or optical properties of natural water bodies. It is interesting to note that entering the parameters from my wetland waters into these models actually yields a fairly similar estimate of \(k\) for AC to my average observed \(k_{ind}\) (average: \(1.2 \pm 0.1 \times 10^{-2} \text{ hr}^{-1}\)). For water bodies comparable to the ones studied here, the model developed by Brezonik and Fulkerson-Brekken (1998) generates an AC \(k_{obs}\) of \(1.0-4.0 \times 10^{-2} \text{ hr}^{-1}\). (Actual values from Brezonik and Fulkerson-Brekken (1998) have been divided by 1.38 to account for the difference between the output of my solar simulator and the summer sun at 40°N.) Although the average \(k_{obs}\) reported here, \(1.6 \times 10^{-2} \text{ h}^{-1}\), falls well inside this range, the model does not account for the contribution of
direct photolysis and so is essentially an estimate of $k_{ind}$, which for my results would fall below the calculated range.

The model put forth by Vione et al. (2010) also ignores direct photolysis but includes a more sophisticated light-screening component. Using chemical parameter data for the wetland waters tested here, this model predicts $k_{ind}$ ranging from $1.6-4.2 \times 10^{-3}$ h$^{-1}$ (Fig. A.2). These predicted values are nearly one order of magnitude smaller than my observed values (Table 2.3), but they do capture the trend that I observed in which YCF, KNM, and EVG yield similar $k_{ind}$ with OKSFF ~4 times higher. The fact that both of these OH\'-based models are able to roughly predict the average reaction rate constants observed in these waters suggests that the relative consistency of AC degradation rate across waters may be limited by its $k_{OH}$. Therefore, although my experiments do not resolve the particular photochemical source or sink pathways at work for AC in these waters, it may in many cases be sufficient to rely on fairly well-known OH\'-mechanisms in order to arrive at a reasonable estimate for AC photochemical kinetics.

Mechanisms likely to be important for isoproturon

The primary reactive species responsible for IP transformation in natural waters have been shown to be $^3$DOM (Gerecke et al. 2001) and possibly DOM-derived organic radicals (Canonica and Freiburghaus 2001), although, IP also reacts with other reactive species such as OH\' (Galichet et al. 2002). The source of $^3$DOM is CDOM, and the rates of triplet-induced reaction have been shown to increase with [DOC], absorbance, and pH (Canonica and Freiburghaus 2001). DOM-derived organic radicals, on the other hand, may result from the photolysis of Fe-DOM complexes as well as from the interaction between DOM and other radicals (Sulzberger and Durisch-Kaiser 2009). Organic radicals were also produced at a higher rate with increasing pH, although the reason for this observation is not clear (Paul et al. 2006). This may confer a higher ratio of excited species to potential quenchers as a function of pH.
The wetland water in which IP exhibited the highest $k_{ind}$ was EVG, which also had one of the higher pH values among the sampled waters (7.9, second to YCF at 8.9). This water may provide the optimal combination of [DOC] and pH to maximize $^3$DOM (and possibly organic radicals). There may be an additional pH effect in completing the reaction in eq. 2.7 for IP (i.e., transitioning from an excited intermediate to the fully oxidized form of the parent molecule). Excited intermediates for IP and other phenylurea herbicides have low $pK_a$ values such that they would rapidly deprotonate at pH > 1 (Canonica et al. 2006). This process may be even more energetically favored at higher pH; specifically, the rate of collapse of the intermediate back to IP in presence of inhibitory DOM (Canonica and Laubscher 2008) may be lower in the presence of more strongly deprotonating conditions.

The idea that pH is correlated with greater IP phototransformation is superficially supported by Fig. 2.5, in which the two high-pH water samples (EVG and YCF) fall close to the trendline created by PLFA (buffered at pH 8). Interestingly, both EVG and YCF also happened to have more closely related SUVA$_{254}$ values (Table 2.1) compared to the other two waters. Guerard et al. (2009b) observed similar results to those reported here with the antibiotic sulfadimethoxine (also primarily degraded by $^3$DOM), with the highest $k$ in PLFA solution and the lowest in SRFA (pH 8). By contrast, Gerecke et al. (2001) include an analogous plot to Fig. 2.5 of $k$ as function of [DOC]. In this case, the trend line in $k$ was established by SRFA solution of increasing [DOC], and it correlated neatly with the reactivity in Lake Greifensee water also at pH ≈ 8. Since SRFA and PLFA have substantially differing SUVA values (Weishaar et al. 2003), it is clear that the reactivity of IP is not clearly predicted by a single factor such as [DOC], pH, or SUVA$_{254}$. The large scatter in Fig. 2.5 confirms that [DOC] is a poor predictor of reactivity.

One of the primary sinks of $^3$DOM in aerated waters is O$_2$, leading to the formation of $^1$O$_2$, the production of which has also been correlated with [DOC] in natural waters (Cory et al. 2010). The increased rate of IP photolysis in low-O$_2$ conditions accords with the
idea of decreasing competition with $O_2$ for $^3$DOM (Fig. 2.6). The reverse may be true for the wetland water with the lowest $k_{ind}$, OKS, in which $O_2$ may be more effectively out-competing IP for $^3$DOM. This is supported by observations that that the quantum yield for $^1O_2$ production in illuminated natural waters is higher at lower pH values (Dalrymple et al. 2010) and higher [DOC] (Cory et al. 2010), both of which apply to OKS water. In the case of $^1O_2$, the incorporation of O atoms into photooxidized DOM has also been shown to increase at higher [DOC] (Cory et al. 2010, Sandvik et al. 2000), which may help to explain the higher rates of photobleaching observed in OKS waters (especially in the presence of IP) compared to the other waters.

2.4.3 Implications for future work

The change in DO over the course of a long (8-30 hr) irradiation session is both illuminating and somewhat concerning as to the implications of employing a closed-tube photoreaction setup for determining the environmental photofate of contaminants. Solar simulators such as the Suntest are often used with air-tight, capped phototubes; a quick search of the past two years alone yields many examples (including Wallace et al. 2010, Shank et al. 2010, Goncalves et al. 2011). While studies have been made into the comparability of solar simulators and natural sunlight (e.g., Weber et al. 2009), little attention has been devoted to the representativeness of using closed phototubes to determine natural water photoreactivity given the potential for systemic interference caused by changing redox chemistry due to $O_2$ depletion. My observations of herbicide degradation kinetics in this study show that later timepoints deviate somewhat from pseudo-first order kinetics, with more parent compound left than would be predicted by a pseudo-first order model. This deviation is more pronounced in solutions such as OKS that contain high [DOC] and also more frequently with IP than AC—circumstances that are also correlated with higher rates of $O_2$ consumption as discussed above. This is despite the fact that IP irradiations were consistently shorter in duration than AC (IP: ~8 hr, AC: ~25 hr).
There are several possible explanations for curvilinear kinetics, including competition with daughter products, CDOM depletion, and reduced concentration of parent compound. Depleted CDOM is less able to produce OH· (White et al. 2003) and is mineralized less efficiently (Brinkmann et al. 2003). Indeed, in the case of IP, Canonica et al. (2006) observed this phenomenon and were able to determine that the extra parent product observed at later time points was the result of incompletely transformed reactive intermediates that collapsed back to their original form. They were able to both detect these species by HPLC as well as reverse this effect by lowering the temperature of samples waiting for analysis. It should be noted, however, that their photosystem also employs closed phototubes.

While the strong correlation between DO depletion and spectral slope changes observed over the experimental timeframes in this study renders the O₂ depletion explicable, it can be argued that low-O₂ conditions may not be representative of shallow wetland waters (assumed to be well-mixed and aerated at the top layers where photoreactivity is most relevant). Comparative studies between closed and open solar simulation systems are needed in order to determine whether differences in the mechanisms and rates of contaminant phototransformation exist and if they point to a preferred set of photofate testing procedures in natural waters. (For an example of such a comparison, see Fig. A.3 in the Appendix for the results of actinometry performed in an open and closed system).

2.5 Conclusions

The results presented here indicate that, whereas CDOM is known to be an important player in the processes leading to indirect photolysis of contaminants, the opposite is also true: contaminants can induce indirect photolysis in CDOM. Accelerated photobleaching was observed particularly in the presence of isoproturon (IP), an herbicide known to undergo indirect photolysis primarily mediated by triplet-state DOM, but was observed to a lesser extent in the presence of acetochlor (AC), which undergoes OH·-mediated
indirect photolysis. I hypothesize that contaminant molecules such as IP are able to act as reactants in a chain of events leading to indirect photolysis (photobleaching) of CDOM due to incomplete transformation of excited parent molecules of the contaminant.

Contaminant photoreactivity for AC and IP was mixed among the wetland waters. AC showed fairly consistent recalcitrance to indirect photolysis over a diverse set of wetland waters, likely due to quenching of OH· by DOM. A pH effect in colorful, acidic, iron-rich water from the Okefenokee Swamp was observed for this herbicide due to probable enhancement of photo-Fenton processes. The herbicide isoproturon (IP) was transformed much more readily by indirect photolysis in wetland water samples, although the most rapid transformation was at least partially dictated by an opposite pH effect (Everglades water, pH = 7.9). No single chemical constituent or optical parameter was able to accurately predict the varying rates of reaction.

Finally, the observation that DO can fall to below 25% (and in some waters to 0%) of atmospheric O₂ saturation during the course of 8-25 hour irradiations raises concerns over the appropriateness of using closed phototubes to investigate photofate of organic contaminants in shallow natural waters.
Table 2.1: Chemical constituent concentrations and optical characterization of collected wetland water samples

<table>
<thead>
<tr>
<th>Water Sample</th>
<th>Date collected</th>
<th>pH</th>
<th>( \text{Fe}_{\text{tot}} ) (µM)</th>
<th>NO\textsubscript{3}/NO\textsubscript{2} (µM N)</th>
<th>Total N (µM N)</th>
<th>DOC (mg C/L)</th>
<th>SUVA\textsubscript{254} (M\textsuperscript{-1} cm\textsuperscript{1})</th>
<th>SF\textsuperscript{(290-370)}</th>
<th>S\textsubscript{(275-295)} \textsuperscript{2}</th>
<th>S\textsubscript{(350-400)} \textsuperscript{2}</th>
<th>S\textsubscript{R} \textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>YCF</td>
<td>07/01/10</td>
<td>8.9</td>
<td>3.6</td>
<td>1.55</td>
<td>585.9</td>
<td>7.2 ± 0.2</td>
<td>372.0</td>
<td>0.921</td>
<td>0.0157</td>
<td>0.0155</td>
<td>1.017</td>
</tr>
<tr>
<td>KNM</td>
<td>12/18/10</td>
<td>6.1</td>
<td>28.8</td>
<td>bdl\textsuperscript{3}</td>
<td>119.4</td>
<td>32.2 ±</td>
<td>220.9</td>
<td>0.780</td>
<td>0.0123</td>
<td>0.0152</td>
<td>0.810</td>
</tr>
<tr>
<td>EVG</td>
<td>12/31/10</td>
<td>7.9</td>
<td>1.1</td>
<td>0.68</td>
<td>118.1</td>
<td>25.0 ±</td>
<td>351.1</td>
<td>0.814</td>
<td>0.0202</td>
<td>0.0205</td>
<td>0.987</td>
</tr>
<tr>
<td>OKS\textsubscript{FF}</td>
<td>01/01/11</td>
<td>3.5</td>
<td>4.3</td>
<td>2.64</td>
<td>73.7</td>
<td>52.5 ±</td>
<td>230.1</td>
<td>0.687</td>
<td>0.0138</td>
<td>0.0133</td>
<td>1.038</td>
</tr>
<tr>
<td>OKS\textsubscript{LF}</td>
<td>01/01/11</td>
<td>3.5</td>
<td>6.8</td>
<td>7.5</td>
<td>510.1</td>
<td>139.1 ±</td>
<td>173.6</td>
<td>0.513</td>
<td>0.0141</td>
<td>0.0151</td>
<td>0.935</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Screening factor calculated over \( \lambda = 290\text{-}370 \text{ nm} \). \textsuperscript{2} Spectral slope of linearized UV-vis absorbance over the ranges \( \lambda = 275\text{-}295 \text{ nm}, \lambda = 350\text{-}400 \text{ nm}, \) and the ratio of the two, after Helms \textit{et al.} (2008). \textsuperscript{3} below detection limit (~10 ppb N = 0.7 µM)
Table 2.2: Rates of change in optical characteristics and direct oxygen of wetland waters during irradiation.

Table 2.2: Rates of change in optical characteristics and dissolved oxygen of wetland waters during irradiation.

<table>
<thead>
<tr>
<th>Wetland Waters</th>
<th>Condition</th>
<th>$d\text{DO}/dt$ (x10^{-3} % hr^{-1})</th>
<th>$dS/dt$ (x10^{-3} hr^{-1})</th>
<th>$dS/dt$ (x10^{-2} hr^{-1})</th>
<th>Correlation between $S$ and DO loss ($R^2$)</th>
<th>Correlation between $S$ and DO loss (qualitative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVG</td>
<td>alone</td>
<td>-1.04</td>
<td>1.077</td>
<td>-0.2635</td>
<td>68.55</td>
<td>0.81 0.74 0.87</td>
</tr>
<tr>
<td>AC only</td>
<td></td>
<td>-1.35</td>
<td>1.368</td>
<td>-0.0303</td>
<td>70.95</td>
<td>0.83 0.14 0.93</td>
</tr>
<tr>
<td>IP only</td>
<td></td>
<td>-1.71</td>
<td>0.945</td>
<td>-1.7778</td>
<td>142.72</td>
<td>0.36 0.45 0.46</td>
</tr>
<tr>
<td>EVG</td>
<td>AC only</td>
<td>-1.06</td>
<td>1.423</td>
<td>0.3480</td>
<td>52.08</td>
<td>0.93 0.72 0.95</td>
</tr>
<tr>
<td>AC only</td>
<td>IP only</td>
<td>-1.35</td>
<td>1.368</td>
<td>-0.0303</td>
<td>70.95</td>
<td>0.83 0.14 0.93</td>
</tr>
<tr>
<td>IP only</td>
<td>IP only</td>
<td>-1.71</td>
<td>0.945</td>
<td>-1.7778</td>
<td>142.72</td>
<td>0.36 0.45 0.46</td>
</tr>
<tr>
<td>AC, Low-IP</td>
<td>AC only</td>
<td>-2.96</td>
<td>1.777</td>
<td>1.0666</td>
<td>43.66</td>
<td>0.74 0.77 0.55</td>
</tr>
<tr>
<td>IP only</td>
<td>IP only</td>
<td>-5.76</td>
<td>2.097</td>
<td>0.0326</td>
<td>83.01</td>
<td>- - -</td>
</tr>
<tr>
<td>OKS_{FF}</td>
<td>AC only</td>
<td>-3.74</td>
<td>1.322</td>
<td>1.1372</td>
<td>15.09</td>
<td>0.7 0.8 0.08</td>
</tr>
<tr>
<td>OKS_{LF}</td>
<td>AC only</td>
<td>-9.42</td>
<td>2.633</td>
<td>2.3351</td>
<td>29.35</td>
<td>0.97 0.96 0.97</td>
</tr>
</tbody>
</table>
Table 2.3: Reaction rate constants for the photodegradation of AC and IP in various waters under simulated sunlight

<table>
<thead>
<tr>
<th>Water Sample</th>
<th>$^1 k_{\text{obs}}$ ($10^{-2}$ hr$^{-1}$)</th>
<th>$^2 k_{\text{dir}}$ ($10^{-2}$ hr$^{-1}$)</th>
<th>$^3 k_{\text{ind}}$ ($10^{-2}$ hr$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetochlor</td>
<td>Isoproturon</td>
<td>Acetochlor</td>
</tr>
<tr>
<td>Wetland Waters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YCF</td>
<td>1.9 ± 0.1</td>
<td>12.7 ± 0.4</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>KNM</td>
<td>1.6 ± 0.1</td>
<td>16.4 ± 0.9</td>
<td>0.58 ± 0.06</td>
</tr>
<tr>
<td>EVG</td>
<td>1.8 ± 0.1</td>
<td>30 ± 3</td>
<td>0.61 ± 0.06</td>
</tr>
<tr>
<td>OKS$_{FF}$</td>
<td>5.4 ± 0.5</td>
<td>6.7 ± 0.7</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td>OKS$_{LF}$</td>
<td>1.7 ± 0.1</td>
<td>4.1 ± 0.4</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>Fulvic Acid Slns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 mg C/L</td>
<td>1.6 ± 0.1</td>
<td>20.6 ± 1.8</td>
<td>0.75 ± 0.06</td>
</tr>
<tr>
<td>14 mg C/L</td>
<td>2.0 ± 0.2</td>
<td>25.5 ± 3.3</td>
<td>0.75 ± 0.06</td>
</tr>
<tr>
<td>21 mg C/L</td>
<td>2.2 ± 0.1</td>
<td>31.9 ± 3.7</td>
<td>0.75 ± 0.06</td>
</tr>
<tr>
<td>SRFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 mg C/L</td>
<td>1.0 ± 0.0</td>
<td>8.1 ± 0.5</td>
<td>0.75 ± 0.06</td>
</tr>
<tr>
<td>OWCFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5 mg C/L</td>
<td>0.7 ± 0.1</td>
<td>0.75 ± 0.06</td>
<td>0.0</td>
</tr>
</tbody>
</table>

$^1$ Observed experimental reaction rate constant  
$^2$ Direct photolysis rate constant corrected for light screening by $k_{\text{UP}} \cdot SF_{290-370}$  
$^3$ Indirect photolysis rate constant; $k_{\text{ind}} = k_{\text{obs}} - k_{\text{dir}}$  
$^4$ Screening factors from Ward (2010)  
$^5$ Fulvic acid solutions diluted from stock solution, distinguished by final [DOC].
Figure 2.1: UV-visible absorbance spectra and chemical structures for the herbicides acetochlor and isoproturon.

![Absorbance spectra and chemical structures](image)

Figure 2.1: UV-visible absorbance spectra and chemical structures for the herbicides acetochlor and isoproturon.
Figure 2.2: DO measured during light exposure in three wetland waters
Figure 2.3: DO loss rate as a function of [DOC] or total iron in irradiated wetland waters

\[ y = 0.0046x + 0.0103 \]
\[ R^2 = 0.9526 \]

\[ y = 0.0086x + 0.0234 \]
\[ R^2 = 0.9869 \]

\[ y = 0.0002x + 0.0158 \]
\[ R^2 = 0.8835 \]

\[ y = 0.0004x + 0.0333 \]
\[ R^2 = 0.9129 \]
Figure 2.4: Indirect photolysis reaction rates for IP and AC in various wetland waters

![Graph showing indirect photolysis reaction rates for IP and AC in various wetland waters]

Fig. 2.4: Indirect photolysis reaction rates for IP and AC in various wetland waters
Figure 2.5: Indirect photolysis reaction rate constants for IP as a function of [DOC] in various wetland waters

![Graph showing indirect photolysis reaction rate constants for IP as a function of [DOC] in various wetland waters (field pH, see Table 1) and fulvic acid solutions (pH = 8.0).]

Fig. 2.5: Indirect photolysis reaction rate constants for IP as a function of [DOC] in various wetland waters (field pH, see Table 1) and fulvic acid solutions (pH = 8.0).
Figure 2.6: Herbicide photolysis in EVG water under low-O$_2$ and t-butanol

Fig. 2.6: Change in herbicide concentrations (a: AC, b: IP) during light exposure in EVG water under three conditions: standard (equilibrated in air, △), low-O$_2$ (○), or with 25 mM t-butanol (AC only, □). Filled symbols indicate dark controls.
2.6 References


CHAPTER 3: EFFECTS OF SAMPLE TREATMENT ON REPRESENTATIVENESS OF COLLECTED NATURAL WATERS FOR ENVIRONMENTAL FATE STUDIES

3.1. Introduction

The manner in which water samples are collected and handled from natural water bodies influences their ex situ reactivity. In many instances water samples are used in both the analysis of chemical and optical properties as well as laboratory experiments that mimic reactions of environmental importance. This practice has been commonly used in studies of various transformation pathways for organic contaminants in surface water bodies, including photochemical fate, adsorption and biodegradation (Liu et al. 2009, Vahatalo and Wetzel 2004, Vione et al. 2006, Yamamoto et al. 2009, Gerecke et al. 2001, Wallace et al. 2010, Dimou et al. 2005, Bartels and von Tumpling 2008). The environmental fate for any given contaminant is dependent on solution chemistry i.e. concentrations of relevant chemical species, and various optical properties of the sample matrix (for photochemical reactions). The physical, chemical and biological changes that take place in collected samples at various timescales e.g., hydrolysis, aggregation/disaggregation and biodegradation of DOM, pH-induced changes in speciation of metals and ions will invariably influence experimental results using these samples. Further sampling artifacts such as adsorption of water constituents onto container walls or leaching of undesirable chemicals into the sample could also result in altered experimental outcomes.

Various measures are commonly employed during sampling to minimize changes to chemical constituents in water samples. Many practices are intended to inhibit processes that would alter the chemistry of the samples including biodegradation, photodegradation
and abiotic transformation. Physical measures such as filtration and temperature adjustment, including freezing, can inhibit biodegradation. Nonetheless there exists no standard operating procedure regarding the selection of optimal filter material and pore size, timing of filtration and the optimal storage temperature. Filtration of water samples substantially alters its resemblance to in situ conditions, but this practice is widely employed in photochemical fate studies such as those cited above due to the dual benefit of removing most microbial biomass and particles that can scatter light.

Chemically altering collected samples via acidification or the addition of preservatives including sodium azide and mercuric chloride provides a way to suppress microbial activity without removing possibly reactive constituents via filtration or dramatically altering its temperature. The presence of preservatives or acidification may affect other chemical constituents in the samples or even initiate chemical processes not at work in situ (Hakala et al. 2009). To complicate matters further, sample preservation techniques do not affect all water properties uniformly. For example, freezing has been shown to preserve ion concentrations (Kotlash and Chessman 1998) but increase the likelihood of organic matter precipitation (Fellman et al. 2008). Consequently, researchers concerned with sample integrity and representativeness are potentially faced with dilemmas regarding best practices for each parameter of interest.

Isolated studies in the literature exist that compare the efficacy of various sample-handling methods for very specific analyses. For the analysis of inorganic species, studies have looked into the effect of sample treatment on the detection of N species (Kotlash and Chessman 1998, Corriveau et al. 2008), ions in general (Rinne and Schmitt 2004), metals (e.g. Hudson et al. 2009) and trace organics (Aboulfadl et al. 2010, Vanderford et al. 2011) in collected waters. Best practices for the filtration of collected natural water samples have also been the subject of comparative research, including the effects of filter media composition (Khan and Subramania-Pillai 2007, Danielsson 1982, Kaplan 1994) and pore size (Fellman et al. 2008).
Fewer studies have investigated the effects of sample handling on the optical properties of the dissolved organic matter (DOM) in the water. Chromophoric dissolved organic matter (CDOM) is an important chemical component that influences the photochemical fate of contaminants in natural waters—in particular, its optical (i.e. light-absorbing) properties. Fellman et al. (2008) and Hudson et al. (2009) investigated the effect of freezing natural water samples on SUVA and fluorescence properties, respectively. While other information about optical changes resulting from sample handling and storage can be gleaned from long-term photochemical studies (e.g. Vahatalo et al. 2002), few comprehensive assessments of the optical effects of sample handling have been performed.

Standard operating procedures (SOP) exist for isolated aspects of contaminant fate research, but they are not universally accepted. Most best-practice SOPs deals with the detection of contaminants associated with water quality monitoring. Governmental agencies including USEPA, USGS, UNESCO and OECD as well as standards bodies such as ISO and ASTM have published SOP for water quality analyses but they are not uniformly in agreement (for a review, see Sliwka-Kaszynska et al. 2003).

A much more informal SOP exists for the collection and use of natural water samples in the investigation of contaminant photochemical fate. One important pathway by which contaminants in sunlit natural waters are transformed is through photolysis. Photolysis can either occur via direct pathways when a contaminant molecule absorbs a photon capable of causing chemical transformation or indirectly via interaction with some other constituent in the water excited by a photon. The USEPA has published guidelines for the determination of indirect photochemical rate constants for aqueous contaminants (EPA 1998); however, these protocols dealt with “synthetic” natural water prepared with humic acid rather than collected natural waters. A draft OECD guideline for the same purpose allows for the option of using natural water in photolysis experiments in lieu of simulated
natural water, but does not specify protocols for acquiring and processing water. A de facto set of practices has emerged in the literature for photochemical fate studies. This informal SOP consists of: (1) grab sampling from boat or shore, (2) filtration to 0.2-1.0 µm, (3) cold/dark transport and storage, (4) laboratory chemical analysis and (5) experiment execution in a solar simulator with added target contaminants. Within the practitioners of the field, however, there exists a vast diversity in the details of these basic practices—from filter size to sterilization method to storage time before experimentation.

This lack of a SOP has resulted in inconsistencies in experimental protocols that render cross comparison of data from different research groups difficult. A more unified approach to sample handling procedures would be beneficial for many reasons e.g., data comparisons from different investigators, meta-analysis for decision-making, etc. Furthermore, there is a need to be able to assess the cost effectiveness of procedures. For example, knowledge of factors that may introduce systemic error and the magnitude of that error can help researchers more accurately weigh cost/benefit decisions.

In this study, I examined four best-practice dilemmas encountered while studying the photochemical fate of two herbicides. Sample handling issues that emerged (one accidentally and three intentionally) are as follows: 1) how quickly samples are filtered (one wetland), 2) acidification versus unacidified cold storage for split samples of three of the wetland waters, 3) the timing of sampling events, and, 4) the duration of sample storage. All samples underwent the same analysis: determination of water constituent concentrations, characterization of optical properties and measurement of photolysis rates for two target analytes.

This study is a holistic attempt to test sample integrity and its influence on the photoreactivity of the two target compounds. For compounds whose photodegradation behavior is fairly well characterized, the relative rates of photodegradation in the same natural water samples can shed light on the effects of sample handling. In this case, two
herbicides known to react via different pathways were used as probe compounds. Acetochlor (AC) is a commonly used pre-emergent broadleaf herbicide of the chloroacetanilide family of compounds (Vecchia et al. 2009). The indirect photolysis mechanisms of other chloroacetanilides in sunlit natural waters have been shown primarily to involve the hydroxyl radical OH• (Miller and Chin 2005, Sakkas et al. 2004, Dimou et al. 2005, Benitez et al. 2004). By contrast, isoproturon (IP), a phenylurea herbicide, has been shown to undergo indirect photolysis predominantly mediated by triplet-excited state DOM (3DOM; Canonica et al. 2001, 2006, Gerecke et al. 2001). Comparing the reaction kinetics of these two compounds in the same water under different treatments can help to elucidate the photochemical effects of various sample-handling regimes.

3.2. Methods
3.2.1 Sample collection and preservation
Water was collected from four freshwater wetlands between June 2010 and January 2011: Yocom farm wetland, Champaign County, Ohio (YCF); Kawai Nui Marsh, Oahu, Hawaii (KNM); Everglades Wildlife Management Area Water Conservation Area 2B, Florida (EVG) and Okefenokee National Wildlife Refuge, Georgia (OKS). Sampling dates are shown in Table 3.1. Grab samples (~4L) were taken from shore in acid-washed, DI-rinsed glass containers and vacuum-filtered as soon as possible (in all cases except OKS, within 2 h) with ashed Gelman A/E (1 µm pore size) filters. Blanks were collected by passing DI water through the filtration apparatus in the field and stored in glass for later analysis in the lab. These “field blanks” for all samples showed negligible increases in UV-vis absorbance compared to DI water alone.

Filtered samples were stored on ice during transport back to the Ohio State University laboratory with travel times roughly proportional to site distance from Ohio (maximum time: KNM sample, 5 days) after which they were stored in glass in the dark at 4°C. For one sample (OKS) high suspended solids precluded filtration of the sample at the field
site, necessitating the transport of the unfiltered whole water on ice back to the laboratory ~48 hours later. In the lab, the sample was prefiltered through an ashed Millipore glass filter apparatus prior to final filtration with the 1-µm pore size filters used for the other samples. To delineate the two filtration schemes (field- vs. lab-filtered), the following notation will be used: field-filtered, OKS\textsubscript{FF}, lab-filtered, OKS\textsubscript{LF}.

After filtration, samples of EVG and KNM were split and half were acidified to pH 2-3 with concentrated HCl (BDH Aristar, ACS grade). In the case of OKS, acidification of half of the unfiltered water was undertaken before transport (and therefore filtration). Acidified splits are denoted with the prefix $a$: $a$EVG, $a$KNM, $a$OKS\textsubscript{LF}.

3.2.2 Chemical characterization of water samples

Field-measured parameters included pH, dissolved oxygen, temperature and conductivity (the latter measured after filtration). For total iron analysis, all samples were acidified in plastic containers with concentrated HNO\textsubscript{3} (Fisher, ACS Plus grade) and analyzed using inductively coupled plasma atomic emission spectroscopy (Varian Vista AX CCD Simultaneous ICP-AES). Dissolved organic carbon [DOC] was measured using a Shimadzu TOC-5000 Total Organic Carbon Analyzer in non-purgeable organic carbon (NPOC) mode. Concentrations of inorganic ions (NO\textsubscript{3}/NO\textsubscript{2}, NH\textsubscript{3}, PO\textsubscript{4}\textsuperscript{3-}, SO\textsubscript{4}\textsuperscript{2-}) were determined using ion chromatography (Dionex DX-120, Sunnyvale, CA) with confirmation and total N analysis using a Skalar San++ Nutrient Analyzer (Breda, Netherlands). Reproducibility for all ion analysis was >95% (Welch \textit{et al.} 1996).

3.2.3 Optical characterization

The UV-visible absorption spectra (250-700 nm) was taken for all samples initially and at various points after storage using a double-beam Varian Cary 13 UV-visible spectrophotometer blanked with ultrapure water (Millpore) in the reference cell. For samples with absorbance $>1.0$, diluted samples were also scanned to determine deviations from Beer’s Law. SUVA\textsubscript{254} was calculated by dividing the corrected A at 254
nm by the [DOC] (for diluted samples, A_dil/[DOC]_dil). Spectral slopes were calculated from the linearized absorbance spectra of each water from 250-500 nm following the technique of Helms et al. (2008) for the following regions: S_{275-295}, S_{350-400}, and S_{R}, the ratio of S_{275-295} to S_{350-400}.

3.2.4 Irradiation experiments
Samples used in photolysis experiments were adjusted to field pH using HCl or NaOH (Fisher, certified ACS grade) and spiked to make 5 µM solutions with either acetochlor (AC) or isoproturon (IP) of highest available purity (Chem Service, Inc., West Chester, PA). Solutions equilibrated loosely covered at room temperature on a stir plate for several hours with slight pH adjustments as needed. Quartz phototubes (8-mL capacity, 0.9-cm path length) were filled with reaction solution in the dark and clamped shut with no headspace. Select tubes were wrapped in foil as dark controls. Tubes were placed horizontally in the solar simulator (Suntest CPS+, Atlas MTT LLC, Chicago, IL) and irradiated at the 550-W setting for either 7-9 hr (IP) or 24-26 hr (AC) in order to achieve sufficient degradation to accurately determine reaction rate constants of herbicide degradation. Temperature was maintained at 27 ± 3°C and fluctuations in light intensity during irradiations were <1% as monitored by a radiometer on the floor of the solar simulator chamber (VWR International).

The same setup was used to determine the direct photolysis reaction rate constant for each herbicide (via irradiation in Milli-Q water) as well as to measure the light output of the system using both p-nitroanisole (PNA) and p-nitroacetophenone (PNAP) actinometry after the method of Dulin and Mill (1982). Briefly, the known quantum yield of the reaction between pyridine and either PNA or PNAP allows for calibration of the light output based on the rate of change of the reactant concentrations over time. [Pyridine] in my actinometry solutions was 5 mM. Results of actinometry for two solar simulators are shown in Appendix Figs. A3-A4. Manufacturer-supplied specifications and the results of actinometry show my solar simulator to have an irradiance spectrum
closely aligned in shape with the solar energy received at Earth’s surface and to exert an average irradiance \(\sim 2.0\) times that of the summer sun in Columbus, OH (40°N; \(\lambda = 300-400\)).

After various intervals of light exposure, tubes were removed in duplicate or triplicate and the contents analyzed for herbicide and DO concentrations and UV-vis absorbance. Herbicide concentrations were quantified via RP-HPLC (Waters Corporation, Milford, MA) using a Nova-Pak C18 column (3.9 x 150 mm), mobile phase 55:45 ACN:H\(_2\)O (v:v), and UV detection (\(\lambda_{AC} = 220\) nm, \(\lambda_{IP} = 240\) nm). DO concentrations in the quartz tubes were determined using a micro-DO probe (Lazar Research Laboratories, Inc., Los Angeles, CA) calibrated using air-saturated water (ASW) and plunged to the bottom of the tube immediately after uncapping. Raw values were normalized to the average DO concentration of \(t = 0\) samples (always very near the ASW reading) and therefore represent percent O\(_2\).

3.2.5 Summary of Treatment groups

Four aspects of sample collection and treatment were considered in this study.

1. **Timing of filtration.** An unintentional treatment group was created due to the need to filter part of the OKS sample in the field and the rest upon arrival in the laboratory two days later. Two splits emerged from this situation: field-filtered (OKS\(_{FF}\)) and lab-filtered (OKS\(_{LF}\)).

2. **Acidification of samples for storage.** Three of the filtered waters (EVG, KNM, OKS) were split and acidified as described above. Non-acidified splits served as controls. All samples were adjusted back to field pH for analysis.

3. **Time of day of sampling.** YCF samples were collected at the same location in the Yocom wetland at seven times throughout one sunny day in June 2010, with the first and last timepoints \(\sim 1\) hr before and after daylight. Water was pulled from precisely the same position in the wetland throughout the day. Although the retention time of the wetland is not known precisely, there was no visible current
during any of the sampling times. The sampled area had patchy plant and algae
growth throughout the water column but was open on the surface and exposed to
the sunlight ~9 hours of the day (water depth ~1 m). Unlike the other wetland
waters collected, YCF had no acidified group.

4. Viability of stored samples. All collected samples were stored at 4°C in glass in
the dark. As they were tapped for experimental use, the properties of samples
were monitored to detect any changes in specific (e.g. [ions]) or holistic (e.g.
photoreactivity) properties over storage.

3.3. Results and Discussion

3.3.1 Timing between sample collection and filtration

Chemical and optical characterization of water samples

Higher-than-anticipated suspended solids levels in the OKS water necessitated splitting
the whole water sample into field-filtered (OKS_{FF}) and lab-filtered (OKS_{LF}) portions,
allowing us to explore the importance of aging prior to filtration on the reactivity of the
photosensitizers. The delay in filtration caused significant differences in the
concentration of chemical constituents (Table 3.1). Inorganic ion concentrations except
\([\text{SO}_4^{2-}]\) were all higher in OKS_{LF} compared to OKS_{FF}, and [DOC] was nearly three times
higher at 139.1 ± 0.5 mg C/L.

OKS_{LF} also showed dramatically higher UV-visible light absorbance than OKS_{FF} (Fig.
3.1a). The spectral slope for the wavelength ranges \(\lambda = 275-295\) nm and \(\lambda = 350-400\) nm
were calculated as well as the ratio of the former to the latter (\(S_{275-295}\), \(S_{350-400}\), \(S_R\),
respectively; Table 3.1). \(S_R\) is lower for OKS_{LF} than OKS_{FF} (0.93 v. 1.04), as is
\(\text{SUVA}_{254}\). A decrease in \(S_R\) for the same water has been correlated to a decrease in the
proportion of low molecular weight DOM (<1000 Da; LMW-DOM) comprising the
DOM pool (Helms et al. 2008). Previous studies have shown that this type of change
occurs as a result of microbial degradation (Vahatalo and Wetzel 2004, Moran et al.
2000). In fact, Helms et al. (2008) observed a nearly identical decrease in \(S_R\) (1.02 to
in unfiltered Elizabeth River water samples that were incubated with aerobic bacteria for two weeks. LMW organic compounds provide a relatively easy C source for microbes in stored lake and river waters (Karlsson et al. 1999, Kaiser and Sulzberger 2004) and in this case appear to have been disproportionately depleted during travel.

**Light-induced changes in CDOM**

Changes in spectral slope parameters that have been most often associated with photobleaching of CDOM in sunlight consist of an increase in S_{275-295} and S_R a decrease in S_{350-400}, caused by more rapid destruction of chromophores that absorb high-energy (low wavelength) photons (White et al. 2003, Helms et al. 2008, Sulzberger and Durisch-Kaiser 2009). While both of the OKS water samples followed this pattern, the absolute changes in spectral slope parameters for OKS_{LF} over the same period of irradiation were smaller than in OKS_{FF} (Table 3.2). This decreased photoreactivity is consistent with the lower SUVA_{254} (and therefore likely aromatic C content) of OKS_{LF} compared to OSK_{FF} (Weishaar et al. 2003). However, it is not clear from these results whether the CDOM in OKS_{LF} is less capable of direct photolysis or whether it is less efficient at producing reactive species that lead to indirect photolysis.

**Herbicide photolysis rates in water samples**

Photodegradation kinetics for AC and IP obeyed pseudo-first order rate laws over their respective periods of irradiation. The reaction rate constant k_{obs} comprises both direct and indirect photolysis components, with k_{dir} equal to the direct photolytic rate constant for each herbicide multiplied by a screening factor to correct for light attenuation. Calculation of screening factor is described at greater length in Chapter 2 and follows the procedure detailed in Miller and Chin (2002). The indirect rate constant, k_{ind}, represents the difference between k_{obs} and k_{dir}.

Indirect photolysis of AC proceeded nearly four times faster in OKS_{FF} than in any other water, including OKS_{LF} (Figure 3.2 and Table 3.2). Given the acidic nature of water from
the Okefenokee and other high DOC “blackwater” sites, I would expect that photo-
Fenton processes and OH•-mediated indirect photolysis would be important in AC’s
photofate (White et al. 2003, Meunier et al. 2005). This anticipated mechanism was
observed for OKS_{FF}, however, the \( k_{\text{ind}} \) was lower for OKS_{LF}, possibly due to fewer
LMW-DOM components in OKS_{LF} (based upon its lower \( S_R \)). Irradiated LMW-DOM
has been shown to be more effective than high molecular-weight (HMW) DOM at
generating the Fe(II) ions necessary for the photo-Fenton pathway (Meunier et al. 2005).
In addition, the higher [DOC] of OKS_{LF} sample is expected to be more efficient at
scavenging OH• (Brezonik and Fulkerson-Brekken, 1998, Vione et al., 2006).

Discussion
My data show that the timing of filtration can greatly impact the resulting chemistry of
the sample. The original sample chemistry was altered in the two days between
collection and filtration despite precautions undertaken to minimize changes. The delay
seemed to cause some level of change in the composition of the DOM pool and inorganic
species resulting in substantially higher ion and DOC concentrations in lab-relative to
field-filtered samples. Further, the herbicide rate data corroborate that the composition of
the CDOM phase was significantly different between the two samples. Spectral slope
shifts between the two splits imply depletion of LMW-DOM that may in part be
explained by microbial activity, but physical or abiotic processes may also be important.

3.3.2 Acidification for Storage of Samples

Initial chemical and optical characteristics of water samples
Acidification produced relatively little change in the initial water chemistry for the
Everglades sample (Table 3.1). Light absorbance was slightly affected in the visible
region, with a higher \( S_{350-400} \) for \( a\text{EVG} \) versus EVG resulting in a lower \( S_R \) (\( S_{275-295} \) was
nearly identical). Chemical and optical comparisons of acidified versus unacidified
samples are less straightforward for KNM and OKS. For KNM samples, a precipitate
formed in \( a\text{KNM} \) within 5 days of sampling but before laboratory-based analyses could
be performed, including TOC, Fe\textsubscript{tot} and UV-visible absorbance. Both samples were immediately re-filtered and stored as before in the dark at 4°C. However, the likely changes to both the organic and inorganic constituent concentrations as well as UV-visible absorbance resulting from this precipitate are most likely caused by the acidification process.

For OKS the order of filtration and acidification was reversed, with acidification of the whole OKS water first, followed by two days on ice and then lab filtration. Consequently, aOKS\textsubscript{LF} is not directly comparable to OKS\textsubscript{LF} as an acidified split of the same filtered water sample. Nevertheless all of the differences between OKS\textsubscript{FF} and OKS\textsubscript{LF} (described above) are even more pronounced for aOKS\textsubscript{LF}. This sample has an even higher [DOC], an even lower SUVA\textsubscript{254}, and spectral slope values that are even more strongly indicative of depleted LMW-DOM (Table 3.1). Since microbial action may have been inhibited by acidification, much of this change in DOM may be due to physical or pH-induced changes in DOM aggregation and/or conformation. In lower [DOC] samples, Kaplan (1994) found [DOC] decreases of 11-29% for acidified samples compared to unacidified.

*Initial photoreactivity of acidified v. unacidified samples*

Acidification had little effect on photoreactivity for most samples in terms of change in spectral slope characteristics or herbicide degradation kinetics (Fig. 3.2). Despite the formation of a precipitate in aKNM shortly after sampling, both AC and IP had statistically identical $k_{ind}$ in aKNM compared to KNM. This indicates that the rates of formation of reactive species relevant to indirect photolysis (including OH– and $^3$DOM) were also unaffected by acidified storage. No comparison of spectral slope changes during irradiation is available for KNM.

For OKS, AC $k_{ind}$ was lower in aOKS\textsubscript{LF} than OKS\textsubscript{LF} as well as all other waters studied (Fig 3.2a). Fig. 3.3 shows that the AC $k_{ind}$ decreases as a function of [DOC] for all OKS.
samples (including photolyses of OKS$_{FF}$, OKS$_{LF}$ and $a$OKS$_{LF}$ at various stages of storage) consistent with increased quenching of [OH•]. IP rates of indirect photolysis in $a$OKS$_{LF}$ were statistically identical to OKS$_{FF}$, whereas OKS$_{LF}$ showed lower reactivity, and could be related to changes in spectral slope during irradiation whereby $a$OKS$_{LF}$ and OKS$_{FF}$ showed comparable levels of change in $S_R$ over the course of irradiation (Table 2), while OKS$_{LF}$ underwent much less change. These results suggest that chromophoric DOM capable of creating reactive species leading to both photobleaching and IP transformation was present to a greater extent in the $a$OKS$_{LF}$ compared to OKS$_{LF}$.

Although acidifying whole OKS water during transport may have caused a greater release of aggregated DOM into the operationally defined dissolved fraction ($<1$-$\mu$m), this treatment may have preserved CDOM to a greater extent than by icing.

The greatest difference in photoreactivity due to acidification was observed for IP in EVG, (Fig. 3.2). For both AC and IP, $a$EVG was more extensively photobleached during the course of irradiation than EVG, based on change in $S_R$. Since AC $k_{ind}$ was identical between the two samples, acidification apparently had no effect on OH$^•$ production. By contrast, IP indirect photolysis most likely relies on the availability of $^3$DOM, and possibly on the absence of species that can quench excited IP intermediates, as discussed in Ch. 2. There, I conjectured that rapid IP indirect photolysis is facilitated in waters where both of these conditions are present: enhanced $^3$DOM production and reduced quenching of excited IP intermediates (Canonica and Laubscher 2008, Wenk et al. 2011) based upon the observed increase in photobleaching. The rate of dissolved oxygen loss between the IP and AC experiments in $a$EVG shows that 2-3 times more O$_2$ was consumed when IP was present (Table 3.2). This observation, combined with the fact that accelerated spectral slope ($S_R$) change occurred in the IP solution (1.1 v. 1.6 x $10^{-2}$ hr$^{-1}$ for AC v. IP respectively), shows that excited IP intermediates were interacting with and transforming CDOM, thereby leading to lower IP degradation in the acidified versus nonacidified split. The reason why acidification caused this change is unclear from these experiments.
3.3.3 Diurnal effects

Wetland diurnal effects have been studied in situ for various parameters—greenhouse gases (Juutinen et al. 2004, Wang and Han 2005, Chen et al. 2010), oxygen (Cornell and Klarer 2008, Dong et al. 2011, Nikolausz et al. 2008) and others (Naftz et al. 2011)—but rarely in the context of photochemistry other than photosynthesis. Others have investigated the effects of diurnal trends in optical parameters of water on dissolved metal concentrations including Hg (Garcia et al. 2005) and Fe (Vermilyea and Voelker 2009). However, to my knowledge, this is the first study to investigate the photoreactivity and photochemically relevant fluctuations of wetland water over very short timeframes.

Chemical and optical characterization of water samples

The effect of the diurnal cycle on photoreactivity was investigated by taking seven different samples from the same wetland (Yocom Farm wetland, Ohio) throughout the course of one sunny day near the summer solstice 2010. Diurnal trends were discernible in temperature, pH, and DO, likely due to solar warming and biological productivity, as has been observed in other wetlands (Cornell and Klarer 2008, Blossfeld et al. 2011), rivers (Vermilyea and Voelker 2009) and streams (Borman et al. 2010).

Nitrogen species ranged widely among the samples, with [NO\textsubscript{3}⁻/NO\textsubscript{2}⁻] from below the detection limit (0.01 µM N) to 8.32 µM N and [Total N] from 45 to 1197 µM N (Table 3.3). These extremes were not observed for the same samples (i.e., highest [total N] did not correlate with highest [NO\textsubscript{3}⁻/NO\textsubscript{2}⁻]). N species are subject to chemically mediated transformations based on pH and redox conditions as well as biological cycling by microbes and photosynthesizers. Although N species have been shown to have diurnal cycles in wetland systems that are relevant to contaminant photodegradation (Nelieu et al. 2009), they would be unlikely to be resolved on this short timeframe. [Fe\textsubscript{tot}] exhibited a smaller range of values, from 0.191 to 0.366 mM Fe, with no distinct diurnal trend.
visible. Dissolved concentrations and the speciation of iron have been shown to be photochemically mediated in natural waters as solar radiation affects water temperature, pH, and Fe oxidation state (Vermilyea and Voelker 2009, Borman et al. 2010, Shaked 2008), but the myriad other biological and chemical sources, sinks, and transformations involving Fe in wetlands may have masked solar-mediated cycling.

[DOC] values were more consistent among the samples, ranging with no discernible temporal trend from 6.9-7.1 mg C/L for all but one sample (6:30 a.m.; 7.4 mg C/L). Likewise, raw UV-visible absorbance spectra revealed no temporal changes with spectral slopes and SUVA<sub>254</sub> (SD ≤ 3% of mean). Consistent with its slightly higher [DOC], the 6:30 a.m. sample had the highest S<sub>350-400</sub> (and therefore the lowest S<sub>R</sub>) as well as the lowest SUVA<sub>254</sub> value of the samples. The random distribution of optical values indicates no detectable evidence of photobleaching in the small temporal (18 h) and spatial timeframe investigated here. This observation is consistent with other studies that have shown that the timeframe of discernible photobleaching in situ is on the order of weeks to months, even at the height of summer (Vahatalo and Wetzel 2004).

*Herbicide photolysis rates in water samples*
For both AC and IP, the majority of samples (at least 4 of the 7) showed statistically identical photolysis rate constants, with the two nonidentical samples (6:30 a.m. and 8:45 p.m.) being only slightly lower (Fig. 3.4). Variations in reaction rates were not correlated to any chemical constituents with the exception of a statistically positive relationship (R<sup>2</sup> = 0.78) between [N<sub>tot</sub>] and k<sub>obs</sub> for AC (not shown). Linear regressions of k<sub>ind</sub> as a function of [NO<sub>3</sub> - NO<sub>2</sub> - ] showed no significant correlation between the two. Changing optical parameters during laboratory irradiation were not measured for YCF water samples.

Discussion
The consistency of the [DOC], optical properties, and photochemical reactivity of water taken throughout the day from the same wetland suggest sufficient homogeneity in the DOM pool on short timescales (< 24 hours) that a grab sample provides an accurate “snapshot” of in situ DOM conditions. However, the variation observed here in some inorganic parameters including Fe_{tot}, pH and N species concentrations indicates that a single sampling time may yield a biased measurement of a system constantly in chemically and biologically mediated redox flux.

3.3.4 Storage effects

Changes in chemical and optical properties

After initial analysis and experiments were performed, wetland water samples were periodically monitored for changes in DOM that might be occurring during storage. All samples were stored at 4°C in the dark; some samples were split with acidified and unacidified portions as described above. All [DOC] measurements after storage were lower than initial measurements, although in several cases this can be explained by aggregation/precipitation processes (Fig. 3.5). Precipitates formed in the following samples: aKNM (within 5 d of sampling, before initial analysis), KNM and OKS_{FF} (after ~2 mo storage) and YCF-6:30 a.m. (after ~6 mo storage). The reason for precipitate formation may be due to a variety of biotic and abiotic processes beyond the scope of this paper ((Fellman et al. 2008),(Giesy and Briese 1978)). In solutions where no precipitate was observed, [DOC] loss was smaller, ranging from <1 mg C/L (aEVG) to 5.2 mg C/L (OKS_{LF}). Based on these few samples (EVG and OKS only, since KNM formed precipitates), acidified solutions lost less [DOC] than unacidified samples.

Over the course of 1-9 months of storage, the spectral slopes (S_{275-295} and S_{350-400}) of nearly all stored samples slightly increased (Table 3.4). If biodegradation were occurring, S_{275-295} would be expected to decrease and S_{350-400} to increase (Vahatalo et al. 2002, Helms et al. 2008). However, a slight increase in both parameters does not point to
any one known process; the more salient observation may be the fact that change was so minimal over the course of 3-9 months.

Changes in photoreactivity of stored waters
Table 3.4 shows several measures of photoreactivity by which I measured storage effects in the sampled waters, including $k_{ind}$ for AC and IP, rates of change for DO and spectral slope during irradiation, and initial and final objective values of $S_R$. Although there was some variation among these parameters during successive trials, the variation among trials was not greater than the variation between samples. No consistent trends emerged that indicated that one storage regime was superior to another across water samples or that after a discernible length of time, sample integrity deteriorated such that photoreactivity was significantly impacted.

Discussion
My data suggest that the photochemical properties of DOM in collected natural water samples may be relatively stable during cold, dark storage for at least a few months. These observations are supported by Andrews et al. (2000), who observed no change in CDOM absorption of stored estuarine and marine water samples (0.2-µm filtered) over one year of storage. However, changes in specific DOM properties e.g., spectral slope and photoreactivity certainly lead to a measure of uncertainty when attempting to predict contaminant fate in environmental waters. Fellman et al. (2008) found a loss of [DOC] during freezing of water samples as well as a corresponding decrease in SUVA$_{254}$ values, although some of this was due to aggregation/precipitation.

DOM changes observed over months-long cold storage are a great deal smaller than those observed from a delay in filtration (OKS samples). In the case of the field- versus lab-filtered OKS splits, and to a much lesser extent fresh versus stored samples, the shift in DOM size (mw) distribution implied by spectral parameters is a concern for the representativeness of samples for determining photofate. Small organic molecules have
been observed to disappear relatively quickly in stored samples (Karlsson et al. 1999, Aboulfadl et al. 2010, Vanderford et al. 2011) for various possible reasons including biodegradation, hydrolysis and DOM-metal complex formation.

However, with the exception of samples that formed precipitates during storage, the changes observed in stored samples are of comparable magnitude to those observed over one day in the YCF wetland. This suggests that the primary driver of wetland water photoreactivity—CDOM—remains fairly viable for weeks to months in cold, dark storage, even when concentrations of other potential photosensitizers (e.g. iron) may vary.

The case of the three OKS splits makes clear that the question of storage being addressed in this study relates more to the stability of the sample once it has made any initial changes from field conditions than to sample stability in the first few minutes to hours after collection. True representativeness would need to be assessed with a combination of field/lab comparison studies both on the sampling side (comparing in situ conditions, immediately fresh samples and those that have been processed) and on the experimentation side with laboratory photolyses run in tandem with in situ solar experiments (in the manner of e.g. Vahatalo and Wetzel 2004, Gerecke et al. 2001, Dimou et al. 2005, Shankar et al. 2008).

It is not clear from these results whether acidification represents in situ conditions relative to unacidified samples. In the short term, acidification has an evident impact on the resulting chemistry of samples, including altering metal/ion speciation and DOM properties. The fact that there was some difference in the herbicide photodegradation rates between acidified and unacidified samples, particularly for IP, which undergoes DOM-mediated oxidation indicates that acidification imposes disproportional changes to various fractions of CDOM. For analysis over longer timescales, however, acidified samples did show somewhat greater stability than unacidified samples in terms of [DOC]
and consistency of observed reaction rates over time (although no greater stability in spectral slope or SUVA) and showed a lower tendency to form precipitates.

Changes in the chemistry of water samples becomes complex when doing comparisons over the course of a day, season or year relative to changes that occur during storage. The collection of water samples from this study show that the DOM pool in terms of both quantity and quality is relatively stable. Nonetheless, I observed changes in my samples that cannot be easily attributed to a specific process e.g. biodegradation v. hydrolysis or even to natural versus researcher-imposed conditions.

3.4 Conclusions
The timing of certain sample processing steps including filtration can significantly impact the chemistry of water samples. Here, the comparison of field- and lab-filtered splits of water sampled from the Okefenokee Swamp indicated dramatic differences in DOM concentration and character as well as [NO$_3^-$/NO$_2^-$], [N$_{tot}$], [SO$_4^{2-}$],[PO$_4^{3-}$] and [Fe$_{tot}$]. By contrast, in a small, low-flow agricultural wetland in Ohio, the actual time of day that sampling occurred did not significantly impact the chemical properties or photoreactivity of the resulting samples, although the variation in these samples gives some sense of the inherent error associated with studying the dynamic properties of complex natural systems.

Acidification of samples is a practice that appears to confer sample stability over storage in terms of DOM optical properties and photoreactivity (as well as lowering the likelihood of precipitate formation). However, the benefit of greater stability may not outweigh the cost of the initial alteration of sample chemistry from in situ conditions that included changes in inorganic species, DOC concentrations, optical properties and herbicide photoreactivity. Finally, over several months of storage, samples showed little change in spectral slope or SUVA$_{254}$. In most cases (with the exception of those that formed precipitates), I observed small to moderate changes in herbicide degradation rates
and progressively higher rates of photochemical oxygen consumption in the highest [DOC] sample. Storing samples for the duration of repeated experiments (days to weeks) does seem to involve inherent chemical changes, but these are likely to be relatively small compared to *in situ* changes occurring in surface water bodies. Preserving filtered samples unacidified in the cold and dark over this timespan may provide the best compromise in studying complex and heterogeneous systems. The results of this study show that these sample processing measures allow for some measure of consistency for *ex situ* analyses and experiments.
Table 3.1: Characteristics of wetland waters stored with and without acidification

<table>
<thead>
<tr>
<th>Source Water</th>
<th>Split</th>
<th>DOC (mg C/L)</th>
<th>Fe&lt;sub&gt;tot&lt;/sub&gt; (uM)</th>
<th>NO&lt;sub&gt;3&lt;/sub&gt;^-/NO&lt;sub&gt;2&lt;/sub&gt;- (uM N)</th>
<th>NH&lt;sub&gt;3&lt;/sub&gt; (uM N)</th>
<th>Total N (uM N)</th>
<th>SO&lt;sub&gt;4&lt;/sub&gt;^2- (uM)</th>
<th>PO&lt;sub&gt;4&lt;/sub&gt;^3- (uM)</th>
<th>SUVA&lt;sub&gt;254&lt;/sub&gt; (M cm⁻¹)</th>
<th>S&lt;sub&gt;275-295&lt;/sub&gt; (x 10⁻²)</th>
<th>S&lt;sub&gt;350-400&lt;/sub&gt; (x 10⁻²)</th>
<th>S&lt;sub&gt;R&lt;/sub&gt; (x 10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kapa Nui Marsh, HI</td>
<td>aKNM</td>
<td>24.5</td>
<td>32.5</td>
<td>0.25</td>
<td>68.1</td>
<td>118</td>
<td>1.21</td>
<td>1.27</td>
<td>248.8</td>
<td>1.555</td>
<td>2.175</td>
<td>71.5</td>
</tr>
<tr>
<td>EVG</td>
<td>aEVG</td>
<td>24.3</td>
<td>1.0</td>
<td>&lt;0.01</td>
<td>1.54</td>
<td>0.18</td>
<td>0.43</td>
<td>355.4</td>
<td>2.050</td>
<td>2.051</td>
<td>99.9</td>
<td></td>
</tr>
<tr>
<td>Everglades, FL</td>
<td>EVG</td>
<td>25</td>
<td>1.1</td>
<td>&lt;0.01</td>
<td>1.1</td>
<td>0.18</td>
<td>0.58</td>
<td>351.1</td>
<td>2.020</td>
<td>2.047</td>
<td>98.7</td>
<td></td>
</tr>
<tr>
<td>Okefenokee Swamp, GA</td>
<td>aOKS&lt;sub&gt;LF&lt;/sub&gt;</td>
<td>193.6</td>
<td>9.1</td>
<td>2.02</td>
<td>87.8</td>
<td>1440</td>
<td>0.04</td>
<td>1.71</td>
<td>81.9</td>
<td>1.702</td>
<td>2.205</td>
<td>77.2</td>
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<tr>
<td></td>
<td>OKS&lt;sub&gt;LF&lt;/sub&gt;</td>
<td>139.1</td>
<td>6.8</td>
<td>7.53</td>
<td>77.3</td>
<td>510</td>
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### Table 3.2: Photoreactivity of wetland water after storage

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<th>Storage (mo)</th>
<th>Water</th>
<th>$k_{1,\text{in}}$ ($x$ 10^{-2} hr$^{-1}$)</th>
<th>$dQ/dt$ ($x$ 10^{-2} % hr$^{-1}$)</th>
<th>$S_r^\text{initial}$</th>
<th>$S_r^\text{final}$</th>
<th>$dS_{275-295}/dt$ ($x$ 10^{-3} hr$^{-1}$)</th>
<th>$dS_{350-400}/dt$ ($x$ 10^{-3} hr$^{-1}$)</th>
<th>$dS^3/dt$ ($x$ 10^{-3} hr$^{-1}$)</th>
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Table 3.3: Water characteristics for Yocom Farm wetland samples taken throughout the course of one day

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<th>Time of sampling</th>
<th>Temperature (°C)</th>
<th>Dissolved O₂ (mg/L)</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>TOC (mg C/L)</th>
<th>Fe₅₇₆ (mM)</th>
<th>NO₃/NO₂ (µM N)</th>
<th>Total N (µM N)</th>
<th>SUVA₂₅⁴ (M⁻¹ cm⁻¹)</th>
<th>S₂₇₅-₂₉₅ (x10⁻²)</th>
<th>S₃₅₀-₄₀₀ (x10⁻²)</th>
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Sampling date: June 30, 2010. ¹ Measured in freshly sampled whole water in the field. ² Measured just after filtration (<1 h after sampling).
Table 3.4: Spectral slope measurements for collected wetland waters stored in the dark at 4°C after filtration either acidified (prefix a) or unacidified.

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<th>KNM</th>
<th>aEVG</th>
<th>EVG</th>
<th>aOKS&lt;sub&gt;14&lt;/sub&gt;</th>
<th>OKS&lt;sub&gt;14&lt;/sub&gt;</th>
<th>YCF-5:00 a.m.</th>
<th>YCF-6:30 a.m.</th>
<th>YCF-9:00 a.m.</th>
<th>YCF-1:15 p.m.</th>
<th>YCF-4:00 p.m.</th>
<th>YCF-6:45 p.m.</th>
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Figure 3.1: UV-visible absorbance spectra of $\text{OKS}_{\text{LF}}$ versus $\text{OSK}_{\text{FF}}$ samples.

Figure 3.1. Comparison of UV-visible absorbance spectra of diluted (1:3 v:v) $\text{OKS}_{\text{LF}}$ versus $\text{OSK}_{\text{FF}}$ samples. Inset shows scan of undiluted samples.
Figure 3.2: Herbicide $k_{ind}$ for wetland waters stored acidified v. unacidified

![Graph showing herbicide $k_{ind}$ for different wetland waters](image)

Fig. 3.2. Indirect photolysis reaction rate constants of two herbicides, AC (a) and IP (b) in various wetland waters under two storage treatments, acidified (light grey) and unacidified (white). There was no acidified split of the sample OKSFF.
Figure 3.3: AC $k_{ind}$ as a function of [DOC] for OKS waters
Figure 3.4: Herbicide $k_{ind}$ in Yocom Farm wetland samples taken over course of one day

Fig. 3.4: Indirect photolysis reaction rate constants for two herbicides, AC (plain bars) and IP (patterned bars), in wetland water taken from Yocom Farm wetland (40°N) at different times throughout the course of day
Figure 3.5: Wetland water [DOC] before and after storage

Fig. 3.5: Changes in [DOC] for wetland water samples from initial measurements just after collection (plain bars) to measurement after storage of various durations: EVG and OKS waters (3 mos), KNM (4 mos), YCF (9 mos). Samples were either stored acidified (indicated by prefix a) or unacidified in the dark at 4°C.
3.5 References


The presence of wetland water constituents accelerated the light-induced transformation of the model contaminants that were studied here, acetochlor (AC) and isoproturon (IP). As both compounds are commonly used in agriculture and have been detected in surface waters (Gerecke et al. 2001, Kolpin et al. 1996), the fact that they are transformed in wetland waters is of environmental significance. “Transformation” in this context indicates the disappearance of the parent form of the compound, however, and although toxicological assessment was beyond the scope of this study, it must be noted that primary photoproducts of both compounds may exhibit greater ecotoxicity than the parent compounds (Galichet et al. 2002, Rebich et al. 2004). The potential for photochemical reactions to transform daughter products of known contaminants may be a crucial area of study in surface waters increasingly exposed to a diverse cocktail of both parent and daughter compounds (Focazio et al. 2008).

I also showed in this work that the reverse of the above observation is true i.e., in sunlit waters, the presence of contaminants can have an effect on CDOM. This observation presents greater implications for photochemistry research than for surface water bodies (since at environmentally relevant concentrations, this effect would be negligible). The fact that certain contaminants such as IP can themselves act as reactive species may help elucidate the primary reaction mechanisms by which CDOM photobleaching occurs.

My research corroborated several pervasive assumptions in the field of environmental photochemistry. By sampling from the same wetland seven times in the same day, I showed that the DOM pool exhibited sufficiently quantitative and qualitative stability over a short timeframe to be a representative snapshot of the wetland DOM at that time.
In addition, I showed that the DOM in wetland samples stored for weeks or months is also fairly stable. In wetlands, CDOM is the most colorful water constituent and DOM in general one of the highest-concentration constituents. As such, DOM is the primary water constituent controlling the photochemical reactions and dominant reactive species in sunlit wetland waters. For the purposes of research, it is useful to revisit the common assumption that DOM is stable and monolithic, if complex.

However, the methods assessment included in this study also revealed some potentially troublesome gaps between in situ wetland water and ex situ research conditions. The DOM-rich Okefenokee Swamp provided an example of the limitations of an operational definition for DOM. Many researchers define DOM with a filtration cutoff (e.g. the dissolved organic matter that can pass through a 0.7 or 0.45 µm filter). Although I did not in this study investigate filter pore size, I did show that the timing of filtration of water pulled from the wetland at the same moment can have dramatic implications for photoreactivity. This result all the more emphasizes the need for standard protocols for the sampling, processing and storage of natural waters for the purpose of environmental photochemistry research.

Finally, while the study of these four wetlands provided useful information about environmental chemistry systems and practices, the photoreactivity of the herbicides I measured defied prediction or explanation by one or two single parameters, mirroring the results of Wallace et al. (2010). Given that wetlands have much higher average [DOC] than other surface water bodies, and to the extent that CDOM is the dominant factor determining the photoreactivity of wetland water, these confounding results serve to emphasize the importance of understanding the dynamics of DOM in order to be able to predict contaminant fate.
References


Katonga and Bunjako Bays (Victoria Lake; Uganda). Chemosphere 63(7), 1170-1178.


Figure A.1: Spectral slopes for EVG with and without herbicide
Figure A.2: Predicted v. actual herbicide reaction rates for AC in wetland waters

Fig. A.2: Predicted indirect photolysis reaction rate constants ($k_{\text{ind}}$) for AC in four wetland waters based on Vione et al. (2010) compared to experimentally observed $k_{\text{ind}}$. 
Figure A.3: Pyrdine/p-nitroanisole (PNA) chemical actinometry performed in two solar simulators: a stirred, 500-mL photoreactor with ports open to the atmosphere (“Hitchcock”) and closed 8-mL quartz phototubes (“Mendenhall,” two trials), after the technique of Dulin and Mill (1982). The slopes of the lines are the pseudo first-order photolysis reaction rate constants for the reaction between pyridine and PNA (PNA is the measured reactant) from which solar reaction rates can be calculated (Fig. A.4)
Figure A.4: Chemical actinometry calculations

PLFA or SRFA solutions. Samples were irradiated in a Suntest CPS+ (Atlas Devices) solar simulator with a 500 W Xenon lamp over a length of two to three half-lives to determine both the reaction order and rate constants. Actinometer experiments showed that the Suntest operated at ~4.5 times the intensity of average sunlight at 40°N at noon in June (Leifer, 1988). A radiometer was used to ensure that no significant changes in irradiance occurred during photolysis, and temperature was maintained at 25° C. Dark
Figure A.5: Sample experimental data

Figure A.5. Data taken during one photolysis of wetland water. Top left, linearized UV-vis absorbances taken at different time points during irradiation (axes are \( \ln a \) and nm). Top right, spectral slopes taken from linearized absorbances for select \( \lambda \) regions. Bottom left, herbicide concentrations at various timepoints in light and dark samples. Bottom right, DO readings after irradiation.