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Abiotic Release of Low Molecular Weight Nitrogen from Effluent Organic Nitrogen

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Abiotic Release of Low Molecular Weight Nitrogen from Effluent Organic Nitrogen

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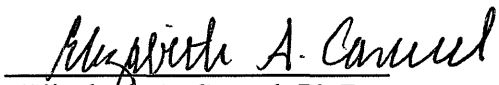

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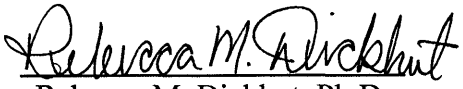

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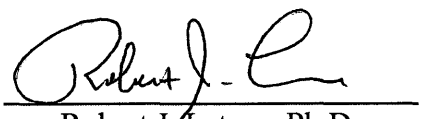

Robert J. Latour, Ph.D.

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CHAPTER 1: PROJECT OVERVIEW

Eutrophication in Chesapeake Bay

Eutrophication is a major concern for coastal systems and has been attributed to nutrient pollution. Excess nutrients, primarily nitrogen (N) and phosphorus (P), stimulate large algal blooms during spring and summer leading to a plethora of problems such as anoxia, reduced water clarity, and die off of marine organisms (e.g. Kemp *et al.* 2005; Diaz and Rosenberg 2008). Eutrophication has been particularly troublesome in Chesapeake Bay, the largest estuary in the United States (Kemp *et al.* 2005). A new regulation, the Chesapeake Bay Total Maximum Daily Loads (TMDL), is an attempt to coordinate all of the states included in the Bay's watershed to decrease the amount of nutrients that enters the Bay (CB TMDL 2010). Nutrients enter the Bay from both non-point and point sources. Non-point sources, such as land run-off and atmospheric deposition, are notoriously difficult to regulate. In contrast, point sources that originate from specific locations, such as wastewater treatment plant (WWTPs) or discharge from an industrial plant can be more easily quantified and controlled.

Effluent Nitrogen

Wastewater treatment plants contribute an estimated 20% of the total nutrient load to Chesapeake Bay (Chesapeake Bay Foundation) and, with the new regulation imposed by the Chesapeake Bay TMDL, WWTPs will be required to reduce their N load down to approximately 3 mg N L^{-1} to 8 mg N L^{-1} (Chesapeake Bay Program 2006). Effluent from WWTPs includes both inorganic N, composed of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+), and organic N. Wastewater treatment plants are well equipped to

remove dissolved inorganic nitrogen (DIN) using biological nutrient removal (BNR), which can remove up to 95% of DIN. The BNR system is made up of a series of biochemical reactions with microbial communities and conditions that result in a change in the oxidation state of the N, eventually leading to its removal in the form of N_2 gas. The first step in this process is nitrification, wherein NH_4^+ is oxidized to NO_2^- and then NO_3^-, under aerobic conditions. During the second step, denitrifying bacteria reduce NO_3^- to N_2O or N_2 gas under anoxic conditions (Metcalf and Eddy 2003). Dissolved organic N (DON) is less efficiently removed with this system, resulting in approximately 80% of total effluent N being made up of DON (Pehlivanoglu and Sedlak 2004, 2006). This large percentage of effluent DON, termed effluent organic nitrogen (EON), has created a great challenge to WWTPs when it comes to its removal. Dissolved organic N was once considered mostly refractory (i.e. cannot be used by microorganisms) and with this view it would be cost effective for WWTPs to exclude EON from the N release limit, under the assumption that the DON would not lead to eutrophication if released into the environment. A number of recent studies, however, have provided evidence that a substantial fraction of EON can be used by microorganisms and would contribute to eutrophication (Urgun-Demirtas *et al.* 2008; Sattayatewa *et al.* 2009; Bronk *et al.* 2010; Filippino *et al.* 2011). As a result, the study of EON has become important so that the data are available to inform the development of EON removal technologies in the future.

Bioavailability of Dissolved Organic Nitrogen

Within the past twenty years our knowledge of the bioavailability of DON has increased dramatically (reviewed in Bronk 2002; Aluwihare and Meador 2008). Riverine DON was once thought to be refractory due to its conservative mixing behavior in

estuarine systems (Thurman 1985). Regions that were thought to be N limited frequently had high DON concentrations, resulting in the view that DON was refractory. One issue in studying DON is that measured bulk changes in the pool is often impossible because the concentration of DON is so high and the labile fractions, such as urea or amino acids, are small fractions of the total. As a result it has been difficult to measure changes in DON concentration in a variety of systems (Bronk *et al.* 2007). More recently, however, bioassays in some systems have shown that 2 to 70 % of DON originating from natural and anthropogenic sources can be taken up in short-term (days) bioassays (Seitzinger and Sanders 1997; Seitzinger *et al.* 2002; Stepanauskas *et al.* 2002). This work has shown that the bioavailability of DON can be significantly influenced by its origin. For example, DON derived from urban and agriculture watersheds, has demonstrated to be more bioavailable than DON derived from wetland watersheds (Seitzinger *et al.* 2002). In another study, Wiegner *et al.* (2006) concluded that the bioavailability of DON is dependent on both the microbial community present and the chemical composition of DON. Another factor that affects the bioavailability of DON is the presence of other N sources, such as DIN (Perakis and Hedin 2002).

Determining the bioavailability of DON is challenging because so little is known about the chemical composition of the DON pool (Bronk 2002). Our biggest barrier in knowing more about the DON pool is attributed to analytical constraints. For example, relatively few compounds in the DON pool have been identified. Those that have been identified and routinely measured, such as urea, combined and free dissolved amino acids, humic and fulvic substances and nucleic acids, frequently make up a relatively small fraction of the total DON pool (Bronk 2002).

Chemical Composition of Effluent Organic Nitrogen

Analogous to the analytical constraints surrounding the DON pool, the majority of the chemical composition of EON is also unknown. Compounds that can be measured such as amino acids and a few consumer products have been identified but are small fractions of the total; approximately 15% of EON is amino acids with only 1% identifiable as consumer products like caffeine and drugs (Pehlivanoglu and Sedlack 2004, 2006). Since little is known about the chemical composition of the EON pool most studies of EON bioavailability look at changes in bulk DON concentrations in bioassay experiments.

Bioavailability of Effluent Organic Nitrogen

Similar to DON, results from bioassay experiments showed a range in EON bioavailability. The bioavailability of EON in tributaries of the lower Chesapeake Bay ranged from 31-96% (Filippino *et al.* 2011). Filippino *et al.* (2011) demonstrated that EON was used as a source of N that helped fuel the growth of microbial communities. Although the concentration of DIN was below detection limits after the second day of the experiment yet, there was still growth up to the seventh day, likely a function of DON utilization. The reason for observing a large range in bioavailability may be attributed to different microbial communities found at different salinities along the Elizabeth River (Filippino *et al.* 2011). In another bioassay experiment, effluent was added to four natural water samples collected along the salinity gradient of the James River, Virginia. In this study a narrower range in EON percent bioavailability, from 2- 23%, was observed (Bronk *et al.* 2010). Effluent from two WWTPs was used and uptake of both effluents was greater in incubations using waters from low salinity waters (0- 10) than from higher

salinities (22-30). These results suggest that the microbial community determines how much EON is consumed.

Uptake of Dissolved Organic Nitrogen by Microorganisms

Microorganisms are capable of taking up DON of different sizes. Low molecular weight (LMW) DON such as amino acids and urea can be taken up through active transport or facilitated diffusion (Mulholland and Lomas 2008). High molecular weight (HMW) compounds such as proteins, polypeptides and humic acids can be broken down using extracellular enzymes (Bronk *et al.* 2007). Many species of bacteria and phytoplankton have been shown to produce a variety of extracellular enzymes (Berges and Mulholland 2008). For example, amino acid oxidases can cleave off amines from organic molecules releasing peroxide, NH_4^+ and α-keto acid or aldehydes during the process (Palenik and Morel 1990). Subsequently, microorganism can take up the free amino acids and NH_4^+. Other types of extracellular enzymes can hydrolyze peptide bonds of proteins. For example, leucine amino-peptidase breaks the terminal N of non-polar amino acids, such as leucine (Landry *et al.* 2009). In order for these types of biological processes to occur it is important that the enzyme have access to the active site of the molecule.

Some organic molecules, however, appear to be resistant to microbial decay and are referred to as refractory. Defining a compound as labile or refractory is difficult because it depends on the time scale and microbial community under consideration. A compound may not be taken up by one microbial community but rapidly taken up by cells in an adjacent community. One reason that a compound may appear refractory is because the reactive sites, if present, are not physically accessible to microorganisms.

Another hypothesis is that after an organic molecule has been partially degraded by microorganisms the molecule is chemically transformed, making it unrecognizable to enzymes (Ogawa *et al.* 2001). Barber (1968) suggested that perhaps some organic molecules have been completely stripped of their functional groups and can no longer be further broken down for energy requirement. Other factors that control reactivity include association to other organic compound or the mineral phase. For example, Ransom *et al.* (1998) observed that aggregates of living and nonliving organic matter act as glue that adheres to the sediments surfaces. The placement of the organic matter coagulate on the sediment might then be physically protected from enzyme attack (e.g. Ransom *et al.* 1998; Mayer 1999). Even if organic compounds can no longer be degraded biotically, abiotic processes may enhance their lability.

Photochemistry of Dissolved Organic Matter

One abiotic condition, exposure to sunlight, has been shown to significantly impact organic matter transformation, influencing the biogeochemical processes in surface water. Chromophores and phenolic compounds are highly reactive in the light causing them to transform and break down, leading to the degradation of dissolved organic matter (DOM) (Mopper and Kieber 2002). It has also been shown that the rate at which DOM compounds break down in sunlight occurs relatively quickly (1-2 days) (Buffam and McGlathery 2003; Bushaw-Newton and Moran 1999). A significant amount of research has looked at the phototransformation of DON, where concentrations of NH_4^+, dissolved primary amines (DPA) and NO_2^- typically increase when exposed to sunlight (Koopmans and Bronk 2002; Wang *et al.* 2000; Grzybowski and Tranvik 2008). Not only has sunlight exposure been shown to increase the bioavailability of DON by

producing DIN, it has also been shown to make DON more bioreactive. Tranvik *et al.* (2000) demonstrated that when DON was exposed to sunlight, DON uptake by bacteria increased.

Salinity Effects on Dissolved Organic Nitrogen

Another abiotic condition is a change in salinity, which can alter interactions between molecules and cause conformational changes within molecules (Bushaw *et al.* 1996). One study that exposed humic substances to higher salinities resulted in an increase in NH_4^+ concentrations (See 2003). Humic substances are highly saturated with carboxylic acid and phenolic acid functional groups. When deprotonated, humic substances become weak bases that have a coloumbic attraction to cations such as NH_4^+ or positively charged amino acids. At higher salinities dissolved cations (i.e. calcium, sodium, magnesium) may have a higher affinity for the weak bases, causing the NH_4^+ or positively charged amino acids to dissociate from the humics. Ammonium can also absorb to the negatively charged binding sites of humic compounds at low salinities (Tipping 2002, Wang *et al.* 2001).

Thesis Objectives

The goal of this study was to quantify the role of abiotic conditions in breaking-down EON. The first objective of this study was to quantify the amount of LMW-N (NO_2^-, NO_3^-, NH_4^+ and DPA) that is released when EON is exposed to sunlight, increased salinity, changes in temperature and a combination of the three. Experiments were conducted using three effluents that were exposed to different methods of disinfection: no disinfection, UV disinfection and chlorine disinfection. Results for the different

disinfected effluents were compared to determine whether disinfection influences how much EON decays after exposing it to abiotic conditions. The second objective was to determine rates of LMW-N production and EON decay rates when exposed to sunlight, and to determine how changes in salinity affect rates of production or decay of various N compounds. Two experiments were run to investigate these two separate objectives. Experiment 1 quantified the amount of LMW-N released when exposed to abiotic conditions. Experiment 2, which differed from Experiment 1 by incorporating more time points, was used to determine LMW-N production and EON decay rates when exposed to abiotic conditions.

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CHAPTER 2: ABIOTIC RELEASE OF LOW MOLECULAR WEIGHT NITROGEN FROM EFFLUENT ORGANIC NITROGEN

ABSTRACT

To help restore water quality in Chesapeake Bay, the Chesapeake Bay Total Maximum Daily Load (TMDL) is designed to ensure that each state in the Bay's watershed only releases a certain amount of nutrients. Specific improvements address nutrient reduction in wastewater treatment plants (WWTPs) (CB TMDL 2010).Currently there are techniques to remove dissolved inorganic nitrogen (DIN) from effluent within a WWTP. Similar techniques do not yet exist for the dissolved organic nitrogen (DON) fraction, termed effluent organic nitrogen (EON). Within the past few years studies have tried to determine whether EON was refractory and thus would not contribute to eutrophication if released into the environment. In cases where EON is refractory, it could be excluded from N release limits applied to WWTPs. Most of the studies that have investigated the reactivity of EON have focused on the bioavailability of EON by performing bioassay experiments, and have concluded that some fraction of EON is bioavailable. Another important factor to consider is abiotic processes that may change and transform EON making it more bioavailable. The goal of the first part of this study was to quantify how EON was changed following exposure to sunlight, increases in salinity, temperature changes and a combination of all three. Three different effluent types (no disinfection, UV disinfected and chlorine disinfected effluent) were used to determine if any significant differences were observed in the amount of EON decay after exposing it to different abiotic conditions. Results indicated that ~1.8 ± 0.2 μmol N L^{-1} (8%) of the EON transformed into low molecular weight N (LMW-N) compounds after

exposing the effluent to 9 hours of sunlight and increasing the salinity from 0 to 33. Both UV and chlorine disinfection were shown to break down a portion (~9%) of EON during the disinfection process, thus there was less EON decay after abiotic exposure compared with the un-disinfected effluent. The second part of this study examined EON decay and low molecular weight N (LMW-N) production rates when EON was exposed to sunlight and a combination of sunlight and high salinity. Results suggest that the photoreactivity of EON was as high as observed for humic compounds, known to be highly reactive in the light. Salinity stimulated photobleaching of chromophoric dissolved organic matter (CDOM) and decay of EON. The overall results from this study indicate that abiotic factors, such as sunlight exposure and increases in salinity, cause a small percentage of EON to break down and transform into labile forms of N.

INTRODUCTION

Chesapeake Bay has experienced eutrophication problems for nearly half a century (Kemp *et al.* 2005). Eutrophication is defined as excess nutrients, particularly nitrogen (N) and phosphorus (P), entering a body of water that stimulates algal growth. High rates of primary production can reduce water quality and have negative impacts on commercial and recreational fish species (Diaz and Rosenberg 2008; Kemp *et al.* 2005). Despite 25 years of nutrient reduction efforts from government and private sectors there have been insufficient improvements to the Bay. A new stringent regulation established by the Environmental Protection Agency (EPA), the Chesapeake Bay Total Maximum Daily Load (TMDL), requires a 25% reduction in N and a 24% reduction in P. It is designed to ensure nutrient control measures are sufficient to fully restore the Bay by 2025 (CB TMDL 2010). These nutrient limits include both point and non point sources. In this study the point source of interest, wastewater treatment plants (WWTPs), contributes an approximate 20% to the nutrients to Chesapeake Bay (Chesapeake Bay Foundation). For most WWTPs a N load reduction down to approximately 3 to 8 mg N L^{-1} is necessary to meet the new requirements (Chesapeake Bay Program 2006). The majority of WWTPs are well-equipped to remove dissolved inorganic N (DIN) through a biological nutrient removal (BNR) system. The BNR system efficiently removes most of the DIN, which is made up of nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+), through a series of biochemical reactions that transform the DIN, eventually leading to its removal in the form of N_2 gas (Metcalf and Eddy 2003). In contrast, dissolved organic N (DON) is less efficiently removed than DIN.

Dissolved organic N in effluent, termed effluent organic nitrogen (EON), can represent a large fraction of N released from WWTPs, even as high as 98% (Bronk et al. 2010). Removing EON efficiently is currently not feasible and would require WWTPs to develop new and costly technology. This may not be necessary, however, if EON is not bioavailable. Compounds that are refractory (i.e. cannot be used by organisms) may not contribute to eutrophication. If EON is comprised of refractory components these should not count towards the effluent N release limit, which would save WWTPs a significant amount of money. Multiple studies have previously determined that 23 to 96% of EON can be taken up by microorganisms on times scales of days, therefore, not all EON is refractory (Urgun-Demirtas *et al.* 2008; Sattayatewa *et al.* 2009; Bronk *et al.* 2010; Filippino *et al.* 2011).

Similar to DON in natural waters, the chemical composition of EON remains relatively unknown. Prior research has characterized a portion of the EON pool and determined that a small fraction (~15%) is made up of amino acids; another one percent comes from consumer products, such as ethylenediaminetetraacetic acid (EDTA), caffeine, and pharmaceuticals (Pehlivanoglu and Sedlak 2004, 2006). Additional data also suggest that EON is made up of humic substances and partially polymerized biological fragments (Pehlivanoglu and Sedlak 2004, 2006). Since such a large portion of the chemical composition of EON is unknown, it is challenging to predict its bioavailability.

Another complicating factor in determining the bioavailability of EON is that refractory compounds may change abiotically once they are released into the environment and become increasingly labile as they travel along the estuarine gradient.

Organic compounds can be altered abiotically by both photochemistry and salinity. Photochemical processes stimulate organic matter transformation and can influence biogeochemical processes in surface waters. Phototransformation appears to be a major process in the degradation of dissolved organic matter (DOM) in surface waters, resulting in the loss of chromophores and phenolic compounds (Mopper and Kieber 2002). Phototransformation of DOM has also been shown to increase its bioavailability, increasing the rates of biomineralization as much as seven-fold (Obernosterer and Benner 2004). Many studies in natural waters have observed increases in concentrations of NH_4^+, NO_2^- and dissolved primary amines (DPA) when DON was exposed to UV radiation (Bushaw *et al.* 1996; Wang *et al.* 2000; Bronk 2002).

Rates of photochemical breakdown of organic matter, particularly terrestrial derived humic and fulvic substances, have been studied in detail (Buffam and McGlathery 2003; Bushaw *et al.* 1996; Bushaw-Newton and Moran 1999). These compounds appear to degrade quickly (1-2 days) with sunlight exposure. Bushaw *et al.* (1996) calculated that terrestrially derived organic matter can contribute an additional 20% of NH_4^+ to coastal ecosystems through photochemical processes. However, while several previous studies have focused on photochemical transformation of natural organic matter, the photochemical decay rate of organic matter derived from effluent has, to our knowledge, never been examined in detail.

Changes in salinity along the estuarine gradient are another abiotic factor that can influence the bioavailability of EON. Exposing organic matter to changes in salinity causes conformational changes within molecules, subsequently altering the interactions within and between molecules (Baalousha *et al.* 2006). See (2003) demonstrated that

NH_4^+ was released from humic substances when exposed to higher salinities. This may occur because, at lower salinities, there are columbic attractions between positively charged NH_4^+ or amino groups and negatively charged functional groups (Tipping 2002). At higher salinities it is thought that dissolved cations (i.e. calcium, sodium, magnesium) have a higher affinity for the weak bases (i.e. unprotonated carboxylic and phenolic functional groups), causing NH_4^+ and positively charged amino acids to dissociate and be released into the water column (Tipping 2002; Wang *et al.* 2001). It has long been known that rates of reactions generally increase with increasing temperature (Engel and Reid 2006), which could also affect how organic matter changes abiotically. Higher temperature increases the energy of the particles in the solution and subsequently increasing the collision of particles, thus the likelihood of a reaction to occur rises (Engle and Reid 2006).

One variable that can change EON and how it behaves when changing abiotic conditions in the environment is the disinfection method used in a WWTP. Disinfection is the final process used in WWTPs prior to releasing water to the environment and is used to kill bacteria and viruses associated with disease (US EPA F-99-064). The two main disinfection techniques used at WWTPs are chlorine disinfection and UV exposure. Most facilities use the chlorine disinfection method where chlorine gas, sodium or calcium hypochlorite is added to the effluent for 30 minutes, followed by the removal of the chlorine residuals with sodium sulfite. During this process, chlorine reacts and oxidizes and destroys cellular material (US EPA F-99-062). UV disinfection exposes the effluent to UV radiation from a mercury arc lamp with wavelengths that range from 250-270 nm, thereby penetrating and destroying the genetic material (DNA and RNA) of

microorganisms. UV disinfection is less common than chlorine disinfection as mercury arc lamps require a substantial amount of energy and are thus only used in smaller treatment plants (US EPA F-99-064). In addition to the ability to destroy pathogens within effluent, UV and chlorine disinfection may also be a useful method for breaking down EON. A few studies have demonstrated that some disinfection processes, such as ozone and UV radiation, can remove high molecular weight compounds (Beschkov *et al.* 1997; Wenzel *et al.* 1999).

Due to the limited ability to characterize the chemical nature of the EON pool, it has been challenging to determine its bioavailability. To date, only a handful of studies have conducted incubation experiments to try to answer this question (e.g. Urgun-Demirtas *et al.* 2008; Sattayatewa *et al.* 2009; Filippino *et al.* 2011). Typically these incubation experiments are set up by adding innocula (algae and bacteria) to bottles of effluent and then measuring a suite of nutrient species to determine what species have been utilized. The bioavailability of EON, however, cannot be determined solely by incubation experiments and measurement of EON uptake; abiotic factors that may affect EON in the environment must also be considered (Bronk *et al.* 2010). Exposing DON to changes in abiotic conditions has not only shown to increase concentrations of low molecular weight N (i.e. Bushaw *et al.* 1996), but it has also shown to increase the amount of DON that can be taken up by microorganisms (i.e. Tranvik *et al.* 2006). Therefore, the bioavailability of EON may be significantly underestimated. In addition, EON derived from effluent that has undergone different disinfection processes may behave differently under abiotic conditions, which could provide guidance about the capability of different disinfection methods to breakdown EON. This study evaluates how

certain abiotic factors influence the production of labile N compounds and gives insight about how EON may be broken down in WWTPs.

OBJECTIVES

The primary objective of this study was to determine the extent to which exposure to selected abiotic variables affects EON once it is released from a WWTP. Two separate experiments were run in order to address this objective.

The aim of Experiment 1 was to quantify how UV exposure (sunlight), increasing salinity and different incubation temperatures alter EON through the release of low molecular weight N (LMW-N), defined as NH_4^+, NO_3^-, NO_2^-, and dissolved primary amines (DPA), by measuring concentrations over time. The objectives of Experiment 1 were fourfold: 1) to determine the effect of sunlight on concentrations of LMW-N, 2) to determine the effect of increasing salinity on concentrations of LMW-N, 3) to determine whether sunlight and salinity have a synergistic affect on concentrations of LMW-N when combined, and 4) to determine whether the concentration changes listed above are affected by temperature. In addition, changes in concentrations of LMW-N were determined with effluent that had not been disinfected, effluent that had been disinfected by UV exposure and effluent that had been disinfected with chlorine to examine the effects of disinfection on N release from EON when exposed to variable abiotic conditions. We hypothesized that there would be significant abiotic release of LMW-N compounds from EON, which could contribute to eutrophication. We further predicted that pre-disinfected effluent would release more LMW-N compared to disinfected effluent under a range of abiotic conditions.

The aim of Experiment 2 was to determine the photochemical rate of release of LMW-N from EON at different salinities. The objectives of this experiment were threefold: 1) to determine the photo-production rates of LMW-N from EON, 2) to determine if changes in effluent concentration affect photo-production rates, and 3) to examine whether increases in salinity affect photo-production rates. We hypothesized that EON would have lower rates of photolysis than organic matter derived from terrestrial material (compared to literature values), that effluent concentration would not affect rates, and that changes in salinity would change photo-production rates.

METHODS

Experiment 1: Changes in the Concentration of LMW Nitrogen when EON was Exposed to Sunlight, Increasing Salinity, and Changes in Temperature

A five way nested factorial design was used to test the effect of selected variables on LMW-N concentrations. The variables examined were: 1) effluent disinfection type (pre and post UV disinfection and chlorine disinfection), 2) salinity (0, 17 and 33), 3) sunlight versus dark, 4) time duration (0, 4.5 and 9 hours), and 5) temperature (ambient at ~30°C and cooled at ~18°C) (Table 1).

Effluent was collected in mid-August 2010, from a small biological nutrient removal plant in Maryland, USA. The plant was chosen for its efficient removal of DIN, thus producing effluent that is largely comprised of DON. The facility releases approximately 18 million gallons of effluent per day into a small tributary of the Patuxent River (EPA CWNS 2008). Since the facility uses UV exposure as a means of disinfection, effluent samples were collected both pre and post UV disinfection and were treated as described below in the laboratory at the Virginia Institute of Marine Science

(VIMS). After collection, effluent samples were immediately transported to VIMS in coolers where they were filtered through a Whatman GF/F filter (0.7 μm) followed by a pre-rinsed (200 ml Barnstead water) Supor filter (0.2 μm) to remove microorganisms and particulates. Samples were then stored in a refrigerator (5°C) until used for the experiment.

Effluent Disinfection

For preparation of the three effluent types, the portion of the effluent collected pre-UV disinfection was split into two parts - one was untouched (not disinfected) and the other was disinfected with chlorine. Ten liters of the pre-UV disinfected effluent was treated with 1.05 mL of Chlorox bleach and mixed. After 10 minutes chlorine residuals, measured with a pool test kit, were approximately 2 ppm. After an additional 30 minutes 0.06194 g of sodium sulfite was added to the effluent to remove total chlorine residuals. Chlorine residuals were measured again and were 0 ppm (Charles Bott provided the chlorination method, personal communication). The sample of effluent that had passed through the UV disinfection stage at the WWTP received no further treatment.

Experiment 1 was conducted over three days - one day (8/29, 8/31 and 9/1) for each effluent type (not disinfected, chlorine disinfected, UV disinfected). All three days were sunny and cloudless and experiments started ~ 0900 and ended ~1800. There were three different solutions (A, B and C) made for each effluent type to reflect the three salinities tested (0, 17, 33), described below.

Salinity Assay

To imitate salinity changes along the estuarine gradient, salinity treatments included 0, 17 and 33. Solution A, with salinity 0 had no salt additions. Solution B, with salinity 17, contained 138.87 g of sodium chloride (NaCl), 30.80 g magnesium sulfate ($MgSO_4$) and 0.72 g sodium bicarbonate ($NaHCO_3$), all precombusted. Solution C, with salinity 33, contained 277.73 g NaCl, 61.60 g $MgSO_4$ and 1.45 g $NaHCO_3$ of precombusted salts. Salinity was measured using a refractometer.

Photochemical Assay

Aliquots (~160 mL) of each solution (A, B or C) were transferred into 12 quartz tubes and 12 high-density polyethylene (HDPE) bottles wrapped in aluminum foil to represent the light and dark treatments, respectively; each solution was run in triplicate for each time point. An aliquot (~160 mL) of water from each solution was then frozen for later analysis of nutrient concentrations for time 0. The light samples were placed in a water bath with flowing water under approximately 2 inches of water. The dark samples were placed underneath the light samples in the flow through water bath. Concentration changes in the light treatments were calculated by taking the average differences in concentration for all three replicates over the duration of the experiment (i.e. 9 hours) for the samples at light/salinity 0.

Temperature Baths

Two water baths were set up to control for temperature. One contained York River water on a flow-through system, with temperature ranging from 28-33°C. The second water bath was connected to a chiller (Fisher Scientific) set at 10°C, temperature

ranging from 16-20°C with fresh water. Due to the high ambient air temperature (~35°C), ice was occasionally added to the cold water bath in order to maintain a constant water temperature. The light and dark bottles for each salinity treatment were equally divided between the first and second water bath.

Analytical Methods

Nutrient analyses were determined as follows. Total dissolved N (TDN) samples were oxidized in triplicate using the persulfate oxidation method (Valderrama 1981). Subsequently NO_3^- concentrations were measured on a Lachat QuickChem 8500 autoanalyzer. Concentrations of NH_4^+ were analyzed using the manual phenol hypochlorite method (Grasshoff *et al.* 1999) with NH_4^+ concentrations corrected for salinity. Concentrations of $NO_3^-$and NO_2^- were also measured on the Lachat QuickChem 8500 autoanalyzer (Parsons *et al.* 1984). Concentrations of DON were calculated by taking the difference between TDN and DIN (i.e. NO_3^-, NO_2^-, NH_4^+) (Bronk *et al.* 2000); propagation of error was used to estimate the standard deviation. Concentrations of DPA were measured using the fluorometric o-phthaldialdehyde (OPA) method (Parsons *et al.* 1984). The absorbance of chromophoric dissolved organic matter (CDOM) was determined by measuring the adsorption coefficient at 300 nm on a Shimadzu UV-1601 spectrophotometer, using the following equation:

$$a_\lambda = 2.303 * A_\lambda / l$$

where a_λ is the absorption coefficient, A_λ is the wavelength, and l (m) is the path length of the quartz tube (Castillo *et al.* 1999; Kitidis *et al.* 2006). Solar radiation measurements were obtained from a buoy on the York River adjacent to VIMS, as part of the Virginia Estuary and Coastal Observing System (http://chsd.vims.edu/realtime/YRK005.67P).

Statistical Analysis

Data were analyzed using Statistical Analysis Software ® (SAS 9.2) and R statistical program (R Development Core Team, 2010). Five variables were tested to determine which combination of variables significantly affected the concentration of nutrients. The five variables tested were: effluent type (*e*), salinity (*s*), sunlight vs. dark (*l*), temperature (*p*) and time exposure (*t*). Fixed effect models were used to fit the data, shown below,

$$Y_{eslpt} = \mu + \alpha_e + \beta_s + \gamma_l + \delta_p + (\gamma\zeta)_{lt} + \varepsilon_{eslpt}$$

where Y_{eslpt} is the response variable being measured (the concentration of the nutrient species), α_e is the effluent type (post-disinfection, UV and chlorine disinfection), β_s is the salinity term (0, 17, 33), γ_l is the light vs. dark variable, δ_p is the temperature variable (18° or 30°C) and $(\gamma\zeta)_{lt}$ is the light and time interaction.

Samples that had the same salinity and effluent type were prepared from one batch of water and, as a result, the samples taken at different time intervals were not independent from one another. The model, therefore, incorporates the relatedness of the sample with multiple observations through time with a repeated measures model, where the underlying variance and covariance structure is noted. The underlying covariance structure used in this model was unstructured, meaning that each sample had its own variance and each pair of samples had its own covariance. In contrast, for a structured covariance model, such as Gaussian or compound symmetry, the variance and covariance structure for each sample is related by some mathematical pattern (e.g. exponentially) (Littell *et al.* 2006).

In addition, the variance (i.e. measure of spread between numbers) of the data was examined to determine homogeneity. Levene's Test was used to examine the variance within each set of treatments (Littell *et al.* 2006). Results indicate that the variance for samples within each effluent type was not equal, thus this was incorporated into the model by grouping by effluent. Grouping by effluent allowed effluent types to have different residual variance, where the error term is normally distributed with its own variance and covariance structure: $\varepsilon_{eslpt} \sim iid\ N(0,\sigma^2_{eslpt})$ (Littell *et al.* 2006).

The best fitting model (i.e. the model that incorporated only those variables that significantly changed the concentration of a specific nutrient) was determined by calculating Akaike's Information Criterion (AIC) in SAS. Akaike's Information Criterion allows one to determine the best fitting model because the equation is a balance between model fit and complexity. The AIC was corrected for small sample size (AIC_c). The full equation is as follows:

$$AIC_c = -2\ \ln(L) + 2p + ((2p\ (p+1)/\ (n-p-1))$$

where L is the estimated maximum likelihood value, p is the number of variables in the model, and n is the overall samples size (Burnham and Anderson 2002). The maximum ΔAIC_c was calculated to determine which model was the strongest fit:

$$\Delta\ AIC_c = AIC_c - \text{minimum}(AIC_c)$$

The model with the smallest ΔAIC_c was the stronger fit. In addition to calculating AIC, *p*-values for each variable were calculated to determine the weight and importance it had in the model.

Secondary analyses were performed in R to investigate significant difference within sets of vectors. To determine the significant difference of concentration changes

for different salinity intervals, a 2-way ANOVA was performed. Changes in nutrient concentrations as a function of effluent type and salinity intervals (e.g. 0-17 or 17-33) were tested. Some of the vectors had heterogeneous variance, thus the data were transformed using Box-Cox transformation, using the following equation:

$$T(Y) = (Y^{\lambda} - 1)/ \lambda$$

where Y is the response variable that needs to be transformed and λ is the transformation parameter. Lambda (λ) is determined from the maximum log-likelihood of the data (Crawley 2007). To investigate significant concentration differences before and after disinfection type, paired t-tests were performed.

Experiment 2: Release Rates of LMW-N after EON was Exposed to Sunlight and Increasing Salinity

A four way nested factorial design was used to test the effect of selected variables on nutrient concentrations and to determine rates of reactions. The variables examined were: 1) effluent concentration (40% and 90%), 2) salinity (0 and 30), 3) sunlight versus dark, and 4) time duration (0, 0.5, 1, 2, 4.5 and 9 hours) (Table 2). Experiment 2 differed from Experiment 1 in that there were a larger number of time points taken at smaller intervals, which allows more robust rates to be determined.

The effluent used in Experiment 2 came from the same WWTP as the effluent used in Experiment 1; it was collected after UV disinfection in July 2010. Samples were shipped to VIMS in coolers overnight and filtered through a Whatman GF/F filter (0.7 μm) followed by a pre-rinsed (200 ml Barnstead water) Supor filter (0.2 μm). The filtrate was then stored in a refrigerator (5° C) until use. Just prior to the start of the experiment,

four solutions were prepared with varying combinations of effluent concentrations and salinities.

Effluent Concentration

To examine how the concentration of effluent affects photochemical rates, two different solutions were created - one with 40% effluent and 60% Barnstead water and the other with 90% effluent and 10% Barnstead water.

Salinity Assay

To imitate the range of salinities frequently observed in an estuary, pre-combusted salts (following the recipe described for Experiment 1) were added to one set of each solution to increase the salinity to 30. The original 40% and 90% effluent solutions described above had a salinity of 0. The salinity of both solutions was measured using a refractometer.

Photochemical Assay

Each solution (0 salinity-40% effluent, 0 salinity-90% effluent, 30 salinity-40% effluent, 30 salinity-90% effluent) was distributed equally among 15 quartz tubes (for the light treatment) and 15 high-density polyethylene (HDPE) bottles; the latter were covered in aluminum foil (for the dark treatment). Light and dark treatments for each solution were run in triplicate for each time point, as described previously. Bottles were placed in a flow-through water bath with York River water (~30°C) under approximately four inches of water. The experiment started at 0900 and ended at 1800. Concentration changes due to increasing salinity were calculated by taking the average differences between samples at dark/salinity 0 and dark/salinity 33 at time 0.

Analytical Methods

Nutrients were measured in the same manner described for Experiment 1. In addition, pH was measured in one of each set of triplicates at each time point, using Orion pH/ISE meter model 710A.

Statistical Analysis

The rate of decay or production of N species were modeled and analyzed in R (R Development Core Team, 2010). Each variable of interest specifying: nutrient, effluent concentration, light and salinity, were plotted against time. That data were fit to two models, a linear model ($y= mx + b$) and an exponential model ($y = b * e^{mx}$), where b is the intercept and m is the slope of production or decay. Akaike's Information Criterion was calculated for both models to determine which equation best describes the data. The rates were modeled using the non-linear least square function in R (Crawley 2007).

RESULTS AND DISCUSSION

This study tested the affects of six different variables (sunlight, salinity, combined sunlight and salinity, effluent concentration, temperature, and disinfection method) on the abiotic release of LMW-N. Sections i, ii and iii focus on photochemical/sunlight, salinity and combined photochemical and salinity conditions, respectively. The first half of these sections discusses concentration changes calculated from Experiment 1; the second half discusses photochemical rates determined in Experiment 2. In Experiment 2 we further examined how effluent concentrations affect photochemical rates (section iv). Additional concentration data are presented from Experiment 1 on the effect of temperature on abiotic release of LMW-N (section v), changes in effluents due to disinfection (section vi), and the affect of disinfection on the changes in abiotic conditions studied (section vii). In the final section (section viii) we discuss likely ways these abiotic conditions could impact EON in the environment.

i -Photochemical Effects

Changes in Nitrogen Concentrations due to Photochemistry

Significant ($p < 0.05$) changes in the concentration of NO_2^-, NH_4^+, DPA, and DON were observed for the light treatments in Experiment 1 (Tables 3 and 4). There was a significant increase in NO_2^- concentration in our study, approximately 0.04 μM (Table 4). Another study has also seen an increase of NO_2^- when exposed to light (Keiber *et al.* 1999) and attributed this release to a dependence on the concentration of humic substances. When the concentration of humics is greater than NO_2^- there is a release of NO_2^-; when there is high NO_2^- and low humic concentrations, NO_2^- is lost (Keiber *et al.*

1999). In this study, the initial NO_2^- concentrations were low, and although humics were not measured here, some effluents have been shown to contain humic substances (Amy *et al.* 1987; Manka *et al.* 1982; Pehlivanoglu and Sedlak 2006). Approximately 0.40 μmol L^{-1} NH_4^+ was released from EON when samples were exposed to sunlight for 9 hours (Table 4). These results are similar to other studies that documented an increase in NH_4^+ (e.g. Bushaw *et al.* 1996; Bronk *et al.* 2010; Koopmans and Bronk 2002; Wang *et al.* 2000). When organic N is exposed to light, photo-production of NH_4^+, known as photoammonification, may occur through the photolysis of the amide or peptide linkages followed by the hydrolysis of the amide to NH_4^+ (Figure 1; Wang *et al.* 2000). Wang *et al.* (2000) also observed an increase in DPA; however, in our study DPA decreased by approximately 0.12 μmol N L^{-1}. A decrease in DPA could still be supported by the photoammonification mechanism if the decrease in DPA in our study was due to the second step of the reaction (hydrolysis of the amide to NH_4^+) occurring faster than the first step, where DPA is released from DON.

Significant decreases in DON concentration, of about 0.6 μmol N L^{-1}, were observed for samples exposed to light for 9 hours (Table 4). A study that looked at functionality of EON saw that amide was the dominant N functional group (Dignac *et al.* 2000). Amides, as discussed above, are key functional groups involved in photoammonification (Wang *et al.* 2000) and, therefore, it was surprising to see such small (0-8%) changes in DON concentration (Table 4). One explanation is that the 9 hour duration of the experiment was not long enough to cleave all amide functional groups. However, other photochemical experiments that ran longer than 10 hours have observed a decrease in NH_4^+ (Kieber *et al.* 1997; Kitidis *et al.* 2008; Koopmans and Bronk 2002). It

has been suggested that unsaturated fatty acids photo-oxidize to produce aliphatic aldehydes, which then react with ammonia (NH_3) to form amides; these fatty acids may condense together to produce humic substances (Kieber *et al.* 1997). Alternatively, NH_3 may be incorporated into DOM, possibly through oxidative processes or via a mechanism where NH_3 reacts with keto and quinone groups found on humic acids (Thorn and Mikita 1992; Jørgensen *et al.* 1999). It is difficult to predict the exact mechanism because the reaction is dependent on a number of variables, including the chemical makeup of the effluent and environmental conditions. Yet, there is strong evidence that the increases in DIN concentrations are being cleaved off of DON through mass balance calculations (Appendix 1).

The absorbance of CDOM is a good proxy to determine the amount of organic matter that is reactive with light. In these experiments, CDOM absorbance was higher for dark treatments than for light treatments (Figure 2). Chromophoric dissolved organic matter absorbance, however, was not correlated with the concentration of DON in the light, as was expected (open symbols, Figure 2). When the absorbance of CDOM was plotted against the amount of time with sunlight exposure, there was an inverse relationship (graph not shown); there was a higher CDOM absorbance at time 0 compared with 9 hours, indicating photobleaching of organic matter after 9 hours. When DON concentration was plotted against the length of time that samples were exposed to sunlight, DON concentrations decreased with time but the change was not significant ($p > 0.05$). As noted in Figure 2 (data for all effluent types) the range of DON concentrations for both the light and the dark were similar, but there were significant differences in CDOM absorption between the light and dark samples. This suggests that DON does not

make up a large portion of the organic matter that is reactive with light, which may be the reason for observing small changes of EON transforming to DIN.

Photochemical Rates

Significant ($p < 0.05$) rates and constants were calculated for a few of the treatments in Experiment 2 (Table 5). The photochemical trends in Experiment 2, were opposite from those observed in Experiment 1 (Table 4). For example, NH_4^+ exhibited a decline in concentration and DPA and DON were photo-produced with light exposure in Experiment 2 (Table 5). This could indicate that the conditions for these sets of samples varied (e.g. chemical makeup of the effluent, pH) resulting in different reactions and products. More information on photochemical production was obtained from the samples exposed to light/salinity 30 from Experiment 2, which is explained in the following sections.

ii -Salinity Effects

Changes in Nitrogen Concentrations due to Increasing Salinity

Increasing salinity (from 0 to 33) significantly increased the concentrations of all LMW-N (e.g. NH_4^+, NO_2^-, NO_3^-, DPA) compounds measured for Experiment 1 (Tables 3 and 4). Note that changes reported for NH_4^+ were only for pre and post UV disinfection; chlorine disinfected effluent had a decrease in NH_4^+ concentrations when exposed to higher salinities and are thus not included. These results are similar to other studies that have documented an increase in NH_4^+ when DON is exposed to higher salinity (Bronk *et al.* 2010; See 2003). Percent change of EON into NH_4^+ in the Bronk *et al.* (2010) study was much higher, 10-47%, than in this study where only a 0-4% conversion was

observed. In the See (2003) study, significant changes in NH_4^+ were observed when humics isolated from different estuaries on the east coast of the U.S. were exposed to salinity of 35, increasing NH_4^+ concentrations by approximately 15%. Other studies have examined the effect of NH_4^+ binding to sediments in freshwater and its removal when exposed to higher salinities (Boatman and Murray 1982; Gardner *et al.* 1991; Weston *et al.* 2010). It is thought that carboxylic acid and phenolic functional groups found attached to humic acids and the surface of sediments can become weak bases, which can then coloumbically bind to cations such as NH_4^+ or positively charged amino acids at low salinities (Tipping 2002; Wang *et al.* 2001). At higher salinities, dissolved salt cations (i.e. calcium, sodium, magnesium) have a higher affinity for the weak bases, causing the NH_4^+ or positively charged amino acids to dissociate from humics or sediments (Tipping 2002).

Although no studies have looked at changes in concentration of NO_3^- and NO_2^- when organic matter is exposed to higher salinities, the same principles likely apply to these compounds. These ions also form columbic attractions with positively charged functional groups. At higher salinities chlorine or sulfate may replace the inorganic N releasing NO_2^- and NO_3^- into the water column.

When examining mass balance calculations for these sets of samples there appears to be an increase in N concentration after the additions of salts. The positive change in DIN is greater than the negative change in DON, which could indicate that the addition of salts also added DIN into the solution. However, even after this addition of DIN is subtracted, the decrease in DON matches up well with the increases in DIN due to the effects of increasing salinity (Appendix 2).

Nitrate, NH_4^+, and DPA experienced significantly different concentration changes between the two salinity intervals tested (e.g. 0 -17 and 17- 33 salinity) (Figure 3). The changes, however, were not consistent for the different effluent types. For example, NO_3^- and NH_4^+ showed a significantly ($p<0.05$) larger concentration change between 0 and 17 salinity compared to the amount released between 17 and 33 salinity. This trend was observed for all three effluent types with the exception of chlorinated effluent, which showed a small decrease in NH_4^+ (only 2-3% change) (Figure 3). Following chlorine disinfection, NH_4^+ is thought to bind to chlorine forming chloramines (U.S. EPA 1999), which may explain the distinct trends observed for the chlorinated effluent. Dissolved primary amines showed a significantly ($p<0.05$) larger concentration change between 17 and 33 salinity. Again, the chlorinated effluent is an exception to this trend, where DPA was released to a greater extent between 0-17 salinity. Based on the results, it is clear that chlorinated effluent has different ionic interactions compared with the other two effluents. Since NH_4^+ and DPA (both positively charged groups) are released more abundantly at different salinities this could signify that DPA has a higher affinity for negatively charged particles, which require higher salinities to dissociate. These results suggest that different nutrients species may be released at different points along the salinity gradient.

Salinity Rates

Similar to what we observed in Experiment 1, all nutrients exhibited significant changes in concentration at higher salinities in Experiment 2. However, most of the regressions between time and concentration for samples in the dark/salinity 30 treatment were not significant (data not shown). This was expected, however, because salinity

reactions are not thought to be time dependent. When a sample is exposed to higher salinities, the dissociation between a pair of ions is replaced by a salt ion that has a higher affinity (Tipping 2002); this reaction likely occurs instantaneously. The one treatment that did show a significant ($p < 0.05$) relationship was DON in the 40% effluent samples; this treatment had a first-order rate constant of -0.027 ± 0.005 h^{-1} and an average change of about 23%.

As a possible explanation, the observed changes in DON over time when samples were exposed to salinity 30 could be due to conformational changes in molecular structure. Even though columbic associations and dissociations occur right away with salinity changes, salinity also has the ability to change the structure of organic molecules by causing repulsive or attractive forces within the organic molecules (i.e. folding and unfolding) or among various organic molecules (Baalousha *et al.* 2006). The unfolding or folding to obtain molecular stability may require time. Once that conformation change is made, it exposes more of the organic molecule, allowing it to release more N containing ions associated with it, thus the decrease in DON. If significant decreases in DON were observed, significant increases in DIN may also be expected. This was not the case here, however, likely because decreases in DON resulted in an even N conversion to DIN species causing concentration changes in NH_4^+, NO_2^-, and NO_3^- to be small and unnoticeable.

iii -Combined Photochemistry and Increasing Salinity

Changes in Nutrient Concentrations for Combined Photochemistry and Increasing Salinity

Nutrient concentration changes for combined affects were calculated by subtracting the concentrations of samples at 9 hours in the light/salinity 33 treatment from the concentration from samples at 0 hours in the dark/salinity 0 treatment in Experiment 1 (Table 4). There was a wide range in percent concentration changes for combined effects, which is primarily attributed to the different effluent types. Combined affects had a larger percent change than sunlight and increases in salt conditions alone, which increased the concentrations of LMW- N by approximately 1.8 ± 0.2 μmol N L^{-1}, while DON concentrations decreased by approximately 1.6 ± 0.3 μmol N L^{-1} (Table 4). This suggests that the DON may be lost by conversion to DIN when it is released into the environment; if this EON is refractory, abiotic conditions on EON will release labile forms of N into the water column. When taking a closer look at mass balances for these sets of samples, an increase in N is observed after the additions of salts, as explained in the salinity results section, but only for the UV and chlorinated effluents. Even after subtracting the increase in DIN due to the addition of salts, there is still an increase in DIN of 1.6 ± 0.7 μmol N L^{-1}, suggesting that photochemical exposure and increase in salinity causes some DON to break down into DIN (Appendix 3).

Changes in DPA concentration were very small for samples with combined affects. We hypothesize that the DPA lost during light effects was balanced out with the release of DPA from the salt effects (Table 4), therefore little overall change in nutrient concentration was observed.

The amount of DON transformed photochemically between different salinity intervals was significantly different for pre and post UV disinfected effluent only, which was also similar to changes observed for CDOM absorbance (Figure 4). The decrease in DON concentration in samples between light/salinity 17 and light/salinity 33 was significantly higher (1.46 to 2.63 μmol N L^{-1}; $p < 0.05$), than the decrease between 0 and 17 salinity (0.07 to 0.36 μmol N L^{-1}). The amount of photobleaching, or the decrease in CDOM absorbance, also decreased the most between light/salinity 17 and light/salinity 33, indicating that at higher salinities there is more photobleaching of EON. These results are consistent with other studies that also noted that photobleaching of CDOM increased with salinity (Osburn and Morris 2003; Osburn *et al.* 2009). Our results contrast with Minor *et al.* (2006) who found that at higher salinities there was less photobleached organic matter. The Minor *et al.* (2006) study, however, used DOM collected from a DOM-rich freshwater system, and so it was very different from the type of DOM used in this study. Both studies attributed the differences to conformational changes of the organic molecules caused by higher salinities. Minor *et al.* (2006) explained that the conformational changes of DOM at higher salinities made the compounds more resistant to photochemical decay, whereas, Osburn *et al.* (2009) argued that the conformational changes of DOM at higher salinities made the compounds more 'photo-labile'. These conflicting results are likely due to differences in the chemical composition of organic matter, causing them to react differently in the presence of salinity and photochemical exposure.

Similar to the DIN and DPA released in the dark at different salinity intervals (Figure 3), there were differences in DON concentration and CDOM absorbance for the

chlorinated effluent. The percent changes of DON concentration and CDOM absorbance for the chlorinated effluent from 0 to 17 and 17 to 33 were not significantly different, i.e. photobleaching was not affected by salinity changes for the chlorinated effluent.

Combined Photochemical and Salinity Rates

Significant regressions were observed for the majority of the nutrient species for samples that were exposed to 9 hours of light/salinity 30 in Experiment 2. These changes were similar to those observed in Experiment 1 except for DPA, which exhibited photo-production (Table 6). Ammonium concentrations increased by 12%, which was lower than the percent changes in Experiment 1, 14-93% (Table 4). Dissolved organic nitrogen concentrations decreased with combined effects by 8-20%, which was slightly higher than in Experiment 1, 1-13% (Table 4). Dissolved primary amine concentrations increased by 54-173% in Experiment 2, but decreased in Experiment 1 by 14-40% (Table 4). There was a zero-order production of NH_4^+ and DPA, while DON and CDOM exhibited first-order decay. As noted from the salinity rates, most nutrient concentrations increased with higher salinity instantaneously. Salinity also has the ability to cause conformational changes to molecules, which may cause slight changes in concentration over time, as mentioned previously. The rates and rate constants calculated for this section are mostly attributed to photolysis or photo production but may be slightly affected by salinity, with the exception of DON, which was mostly affected by salinity and will be discussed later.

The order and rate that effluent organic matter, measured here as CDOM absorbance and DON concentration, decays when exposed to UV radiation, and the production of LMW-N resulting from photolysis (Table 6) is comparable to photolysis of

humic and fulvic acids derived from coastal waters and estuaries (Buffam and McGlathery 2003; Bushaw *et al.* 1996; Bushaw-Newton and Moran 1999; Shank *et al.* 2010). For example, CDOM had first-order decay as noted by other studies (Wang *et al.* 2000; Miller and Zepp 1995). The rate constants are comparable to a study that looked at photobleaching of CDOM (a_{305}) derived from mangrove leaf litter with a half-life ranging from 29–50 hours (Shank *et al.* 2010). In our study, we determined a zero-order photo production of DPA with rates ranging from 0.024-0.032 μmol N L^{-1} h^{-1}, which are similar to photo production rates of DPA from humics isolated from a river estuary in Georgia, USA (Bushaw-Newton and Moran 1999). There was a zero-order photo production of NH_4^+ ranging from 0.025 - 0.063 μmol N L^{-1} h^{-1} in this study, similar to rates determined by experiments that isolated humics and fulvic acids from river water (Buffam and McGlathery 2003; Bushaw *et al.* 1996; Bushaw-Newton and Moran 1999). The similarity in rates and rate constants between our study and those cited above may indicate that effluent has similar photo-reactivity to samples comprised of a large percentage of humic acids. Humic acids are comprised largely of aromatic compounds that are known to be reactive with light (Nelson and Siegel 2002). The goal of many of these studies was to investigate the amount of DIN produced from photochemical breakdown of terrestrially derived DON, specifically humics. One study found that up to 20% of DON was photochemically transformed to DIN (Bushaw *et al.* 1996). The photochemical percent transformation of EON to DIN calculated in this study lies within 8-20% (Table 6).

iv - Effluent Concentration Effects on Rates

The different sets of samples (40% and 90% effluent) in Experiment 2 had significantly different rates and rate constants for NH_4^+, DON and CDOM (Table 6). Half

of the nutrient species had a greater rate or rate constants in the 40% effluent, with the exception of NH_4^+ and CDOM. These rates/ rate constants are in good agreement with the percent change of each nutrient species; samples that had higher rate constants had a larger percent change (Table 6). Based on the proposed mechanism by Wang *et al.* (2000) (Figure 1), one would expect that NH_4^+ production and DON photolysis would have opposing rates, however, this was only seen for the 40% effluent samples. Ammonium photo production was 2.5 times faster than DON decay in 90% effluent. This disagreement was also noted by Buffam and McGlathery (2003), who examined dissolved N transformation in UV light and noted that NH_4^+ photo production was better predicted by CDOM absorbance than by DON concentration. In our study NH_4^+ production was also better correlated with CDOM absorbance than for DON concentration (Figure 5). A few suggestions as to why there are different patterns between effluent concentrations are discussed below.

Concentration can also be thought of as how close molecules are to one another. The amount of sunlight hitting the 40% and 90% effluent samples was the same, but light photons had a higher chance of hitting an EON molecule in the 90% sample because there were more EON molecules in the same amount of space. Thus samples with higher concentrations should have a larger rate constant, however, this was not observed for DON and DPA. According to the proposed mechanism (Figure 1), DPA and NH_4^+ should have opposite percent changes since DPA is being consumed as NH_4^+ is being produced. During photoammonification DPA is produced in the first step and used as a reactant in the second step, therefore the rate of DPA is an average of both of these processes; a positive rate constant means that the first step occurs more rapidly than the second step.

Both the 40% and 90% effluent samples exhibited a net production of DPA, but there was less production, or more consumption of DPA in the 90% samples. It is likely that in the 90% samples, DPA was being used as a reactant more quickly due to higher effluent concentration, resulting in a higher production of NH_4^+ and a smaller percent change in DPA. This explanation does not clarify the deviation observed for DON. It is possible that other reactions were occurring simultaneously, such as the formation of DON. For example, previous studies have noted the formation of humic acids through photooxidation of triglycerides, fatty acids and ammonia (NH_3) (Kieber *et al.* 1997). Although this mechanism uses NH_3 (conjugate base of NH_4^+) to form humics, it probably does not explain the increase in DON. It is possible that other forms of DIN, i.e. NO_2^- and NO_3^-, were being incorporated into DON in Experiment 2.

The pH for the different set of samples between 90% and 40% effluent was significantly different (p=0.04), although the difference was small. The average pH of samples at 90% and 40% effluent were 7.91 ± 0.06 and 8.01 ± 0.07, respectively. The study by Wang *et al.* (2000) noted that the second step of photoammonification (primary amines→ NH_4^+) is affected by pH changes; at lower pH this reaction occurs much faster. This could explain the faster rate of NH_4^+ release for the 90% effluent samples.

Another explanation for the observed differences in DON and NH_4^+ between effluent concentrations could be that other forms of DIN (e.g. NO_2^-, NO_3^-) were cleaved off more abundantly in the 40% effluent. Even though we did not see a significant relationship for NO_3^- in the combined effects treatment (Table 6), it exhibited significant changes in concentration in the light/salinity 30 treatments, and NO_2^- concentrations significantly changed at higher salinity (data not shown). The effluent molecules from the

samples made with 40% effluent may have experienced different salinity affects than molecules in the 90% effluent, which can be thought of in terms of effluent: salt ratios. The 40% samples had a lower effluent: salt ratio (i.e. more salt molecules per effluent molecule) than the 90% samples, meaning that these samples exhibited stronger salinity affects. These stronger salinity affects for the 40% effluent samples were observed for samples in the dark/salinity 30 treatment, which showed a significantly larger percent change (data not shown). In the dark/salinity 30 samples DON photolysis rates were - 0.027 ± 0.005 μmol N L^{-1} h^{-1} and were similar to samples exposed to light/salinity 30 - 0.022 ± 0.004 μmol N L^{-1} h^{-1}, which suggests that the decay rate of DON was mostly attributed to the salt affects rather than the sunlight affects. In summary, the amount of EON that is abiotically transformed to LMW-N in Experiment 2 is 3.3 ± 0.2 and 3.4 ± 0.4 μmol N L^{-1} for 40% and 90% effluent concentration, respectively.

v -Temperature Effects

For the temperature variable, significant changes were only observed for NO_3^- in Experiment 1. In pre and post UV disinfected effluent there was a 3% increase in concentration in the colder water bath at 18°C compared to the 30°C water bath, with an average change of 0.417 ± 0.376 μmol N L^{-1} (data not shown). For chlorine disinfection, there was a 0.3% decrease in concentration in the 30°C water bath, with an average change of 0.051 ± 0.009 μmol N L^{-1}. These changes are likely not significant from an environmental perspective, because of the small amount of NO_3^- that is released at the different temperatures. It should be noted that the temperature difference between water baths was about 12°C; perhaps this difference in temperatures was not high enough to see notable concentration changes.

vi - Changes in Nitrogen Concentrations due to Disinfection Method

Significant differences were seen between initial concentration of various nitrogen species for pre and post disinfected effluents in Experiment 1 (Figure 6). The disinfection process significantly ($p<0.05$) changed the concentration of NO_3^-, NH_4^+, DPA and DON in effluent. For most of the LMW-N, there was an increase in concentration after both of the two disinfection methods (UV and chlorine). This was expected, since these disinfection methods break apart large structural units into smaller molecules. UV disinfection can also cause photoammonification, cleaving off primary amines and NH_4^+ releasing them into the water column. Similar to the light treatment in Experiment 1, concentrations of NH_4^+ and NO_2^- nearly doubled, 0.066 and 1.370 μmol N L^{-1}, respectively, after UV disinfection. Dissolved primary amine concentrations decreased, by about 0.03 μmol N L^{-1}, and DON concentrations also decreased by about 2 μmol N L^{-1}, after UV disinfection. The decrease in DPA and DON, as explained before, was likely due to cleavage of NH_4^+ and NO_2^-.

After chlorine disinfection there was a ~0.05 μmol L^{-1} decrease in NO_2^- (Figure 6). This was expected because chlorine is a strong oxidizing agent, oxidizing NO_2^- to NO_3^-. Significant increases in NO_3^-, however, were not seen due to the small changes in NO_2^-. Chlorine also reacts with NH_4^+ and primary amines to form chloramines. The concentrations of NH_4^+ did not decrease after chlorination as expected, but decreases in DPA were observed. Perhaps the amount of NH_4^+ released from DON, after chlorination, was much greater than the amount of chlorine available to react with the free NH_4^+. Decreases in CDOM (a_{300}) and DON concentrations were much larger in effluent treated with chlorine disinfection than that treated with UV disinfection. This could mean that

organic matter in effluent is more sensitive to the oxidation reaction provided from chlorination than it is to electromagnetic radiation from UV disinfection

vii – Comparisons among the three Effluent Types after Exposure to Changes in Abiotic Conditions

Changes in LMW-N after exposure to changes abiotic conditions (exposure to sunlight and increasing salinity) for the different effluent types were compared in Experiment 1. The concentration differences between samples at 9 hours light/salinity 33 and samples at 0 hour dark/salinity 0 for each effluent type were calculated (Figure 7). These data suggests that the two disinfection methods resulted in EON that is less prone to abiotic LMW-N release and both methods appear to influence EON similarly. Dissolved organic nitrogen concentrations decreased by approximately -2.7 μmol N L^{-1}(10%), while UV and chlorine disinfected effluent decrease by about 0.7 μmol N L^{-1} (3%) and -1.3 μmol N L^{-1} (5%), respectively after abiotic effects (Figure 7). Pre-disinfected effluent also had greater changes in NH_4^+ than disinfected effluent. This trend is to be expected since it was previously noted that disinfection methods can significantly change the concentrations of LMW-N and DON (Figure 6). Therefore, there was less change in NH_4^+ and DON for disinfected effluent because the disinfection process had already broken down a portion of the EON. These findings suggest that disinfection methods could be used to break down EON prior to it being released into the environment. Unfortunately, having the disinfection method as the last process in WWTPs will cause all the LMW-N compounds released during disinfection to go directly into the environment. To alleviate this problem WWTPs could place the disinfection process before the biological nutrient removal (BNR) system, or could run the disinfected

effluent through the BNR process again to remove more DIN. The results of this study suggest WWTPs might benefit from longer disinfection periods to allow EON to break down even further.

viii - Environmental Impacts on EON Exposed to Abiotic Conditions

From this study (data from Experiment 1), we calculated that about 8% of the EON was transformed into LMW-N after it was exposed to variable abiotic conditions. This percent change is comparable to other studies that looked at photolysis of DON derived from coastal waters (i.e. Buffam and McGlathery 2003; Bushaw-Newton and Moran 1999). This percent transformation was for EON exposed to 9 hours of summer sunlight and salinity changes from 0 to 33. Other forms of labile DON that could not be measured were likely produced during these abiotic processes, thus the 8% transformation is an underestimation of how much EON was converted to labile N (assuming all EON maybe refractory).

Physical, chemical, and biological processes need to be considered when determining if a combination of abiotic conditions have an impact on water quality of the receiving waters, since these factors affect the transport and transformation of nutrients (Boynton and Kemp 2000; Lung 1995; Liu and Dagg 2003; Marti *et al.* 2004; Vandenberg *et al.* 2005). Another important factor to consider is the volume of effluent discharge and the total volume of the receiving waters (Swayne *et al.* 1980), which will affect the dilution of the effluent, and, as mentioned in the previous section, affect the photolysis rate of EON. A study that looked at effluent nutrient retention efficiency (defined as uptake over distance) for different streams in Catalonia, Spain, found that, generally pristine systems have shorter uptake lengths than those systems that are more

eutrophied (Marti *et al.* 2004). They also noted that nutrient loading was magnified for low stream discharge. In a low stream flow system EON will primarily reside at the head of the river where photochemical affects will predominantly contribute to DIN release. However, photochemical affects on EON will depend on the turbidity of the system since turbidity can significantly reduce the amount of light penetration in the water column and, therefore the amount available to react with EON. Conversely, if the system already has high nutrient concentrations with high river flow, the effluent will travel to the mouth of the river where EON will experience both photochemical (again depending on turbidity) and salinity release. Thus, abiotic conditions would only impact the mouth of the river.

Determining the effects of abiotic release of EON in the environment will be dependent on multiple parameters of a specific system, which may or may not significantly affect the water quality. Alone, abiotic conditions on EON will probably not have adverse affects on the water quality of the receiving waters, but the accumulation of nutrients from multiple point and non-point sources may add up and magnify the eutrophication problems along different sections of the system.

FIGURES

Figure 1. Proposed mechanism for the production of ammonium and primary amines during photolysis (Wang *et al.* 2000).

Figure 2. Chromophoric dissolved organic matter (CDOM) absorbance (300 nm) vs. dissolved organic nitrogen (DON) concentration – Experiment 1. Data points in graph are from pre-disinfected effluent at salinity 0. The filled symbols represent samples from the dark treatment, while the open symbols represent those from the light treatment.

Figure 3. Changes in nitrogen concentration between different salinity intervals – Experiment 1. Concentration changes of (**A**) nitrate (NO_3^-), (**B**) ammonium (NH_4^+), and (**C**) dissolved primary amines (DPA) between different salinity intervals in the dark. Bars represent average concentrations changes given in μmol N L^{-1} ± standard error for each effluent type: E_B (pre disinfection), E_{UV} (post UV disinfection) E_{Cl} (chlorine disinfection).

Figure 4. Changes in DON concentration and absorbance changes due to light exposure and increases in salinity – Experiment 1. Changes in (**A**) dissolved organic nitrogen (DON) concentrations and (**B**) chromophoric dissolved organic matter (CDOM) absorbance (300nm) between different salinity intervals in 9 hours of light. Bars represent average concentrations in μmol N L^{-1} or absorbance ($CDOM_{a300}$) changes ± standard error for each effluent type: E_B (pre disinfection), E_{UV} (post UV disinfection) E_{Cl} (chlorine disinfection).

Figure 5. Chromophoric dissolved organic matter (CDOM) absorbance and dissolved organic nitrogen (DON) vs. ammonium – Experiment 2. Ammonium concentration

(μmol N L^{-1}) plotted versus A) CDOM absorbance (300 nm) and B) DON concentration (μmol N L^{-1}). Data are from samples at 90% effluent with salinity 30.The filled symbols represent samples in the dark treatment; the open symbols are those from the light treatment.

Figure 6. Average initial concentrations of nitrogen species in each effluent - Experiment 1. Average initial concentrations (μmol N L^{-1} ± standard error) of (**A**) nitrite (NO_2^-), (**B**) nitrate (NO_3^-), (**C**) ammonium (NH_4^+), (**D**) dissolved primary amines (DPA), and (**E**) dissolved organic nitrogen (DON), and (**F**) chromophoric dissolved organic matter (CDOM) absorbance (300nm) for the three effluent types: E_B (pre disinfection), E_{UV} (post UV disinfection), and E_{Cl} (chlorine disinfection). * denotes significant ($p<0.05$) concentration differences between pre and post disinfection.

Figure 7. Average changes in nutrient concentrations due to light exposure and increases in salinity – Experiment 1. Average increase (left side of figure) concentration changes (μmol N L^{-1} ± standard error) of nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), dissolved primary amines (DPA) and decrease (right side of figure) concentration changes of dissolved organic nitrogen (DON) for samples that were exposed to 9 hours of light/ 30 salinity for each of the three effluent types: E_B (pre disinfection), E_{UV} (post UV disinfection), and E_{Cl} (chlorine disinfection).

Figure 1

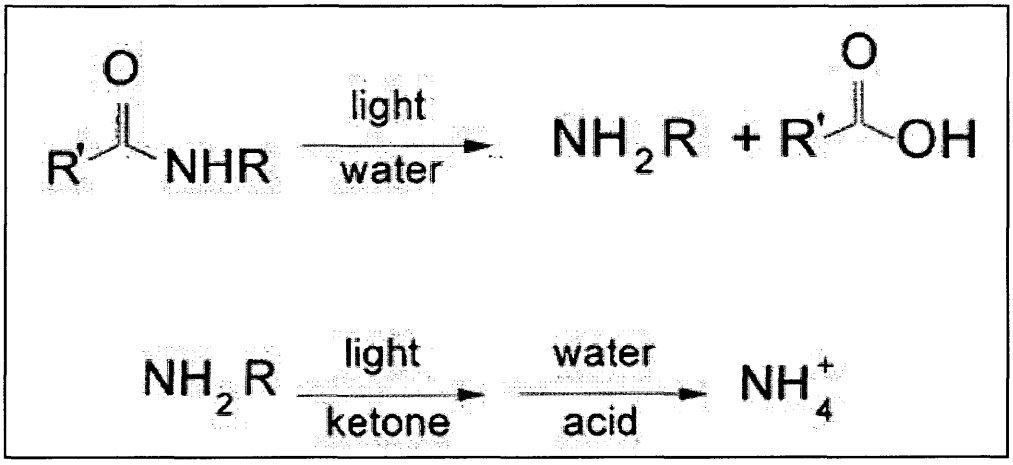

O
R'
NHR
light
water
NH2R + R'
O
OH
NH2R
light
ketone
water
acid
NH4+

Figure 2

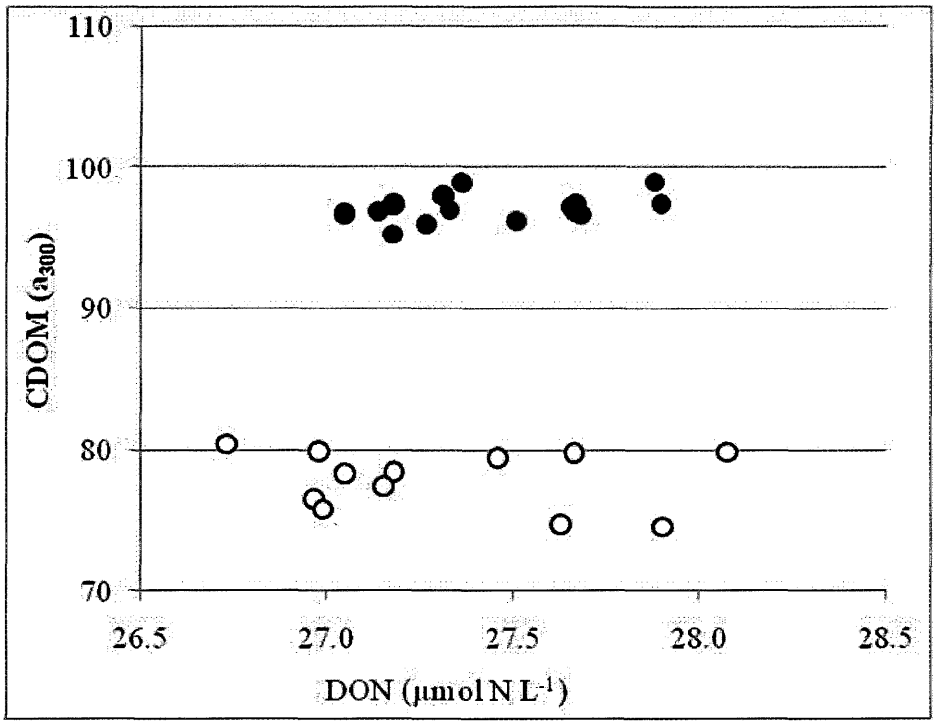


Figure 3

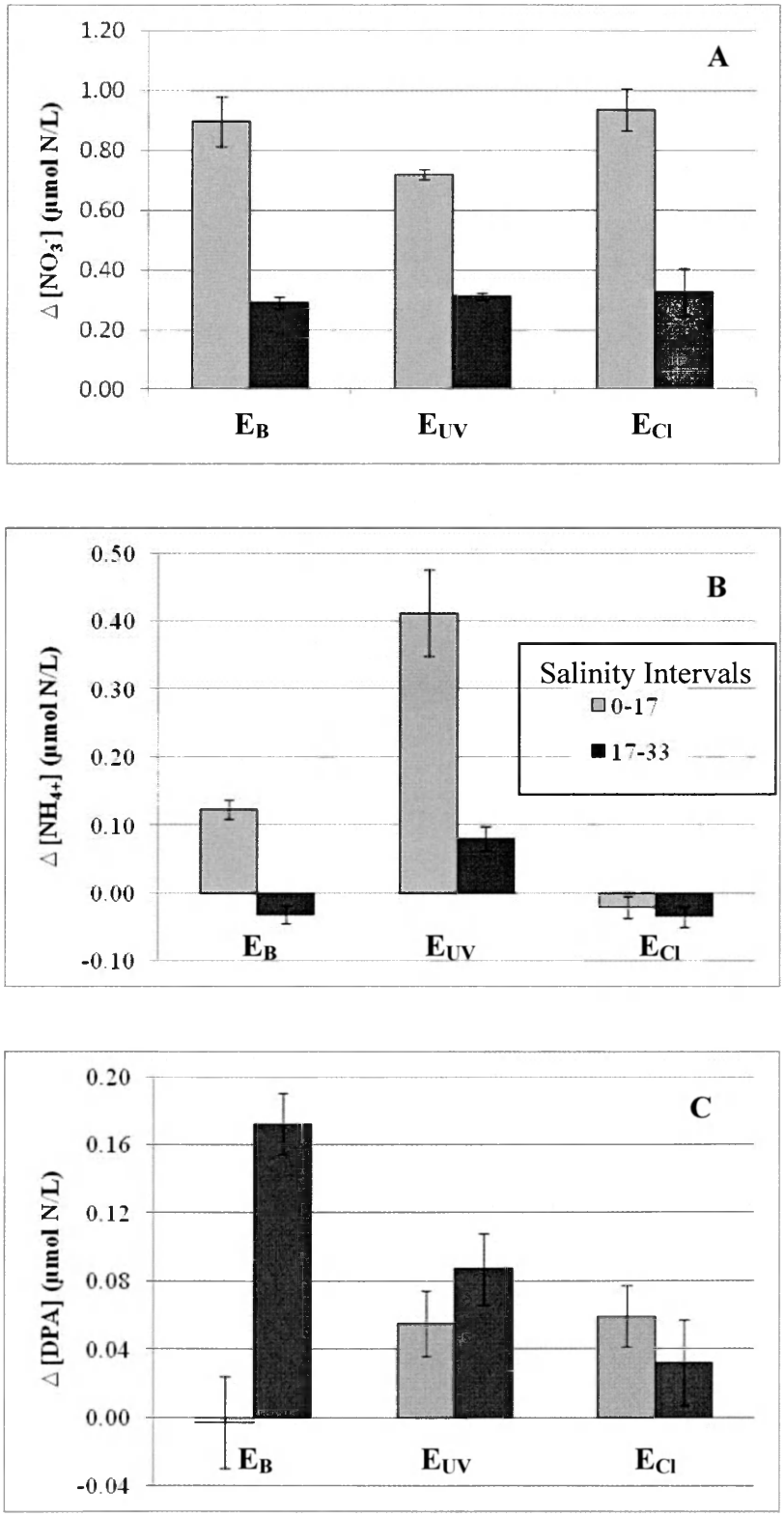

A
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Δ [NO3-] (µmol N/L)
EB
EUV
ECl
B
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
Δ [NH4+] (µmol N/L)
Salinity Intervals
0-17
17-33
EB
EUV
ECl
C
0.20
0.16
0.12
0.08
0.04
0.00
-0.04
Δ [DPA] (µmol N/L)
EB
EUV
ECl

Figure 4

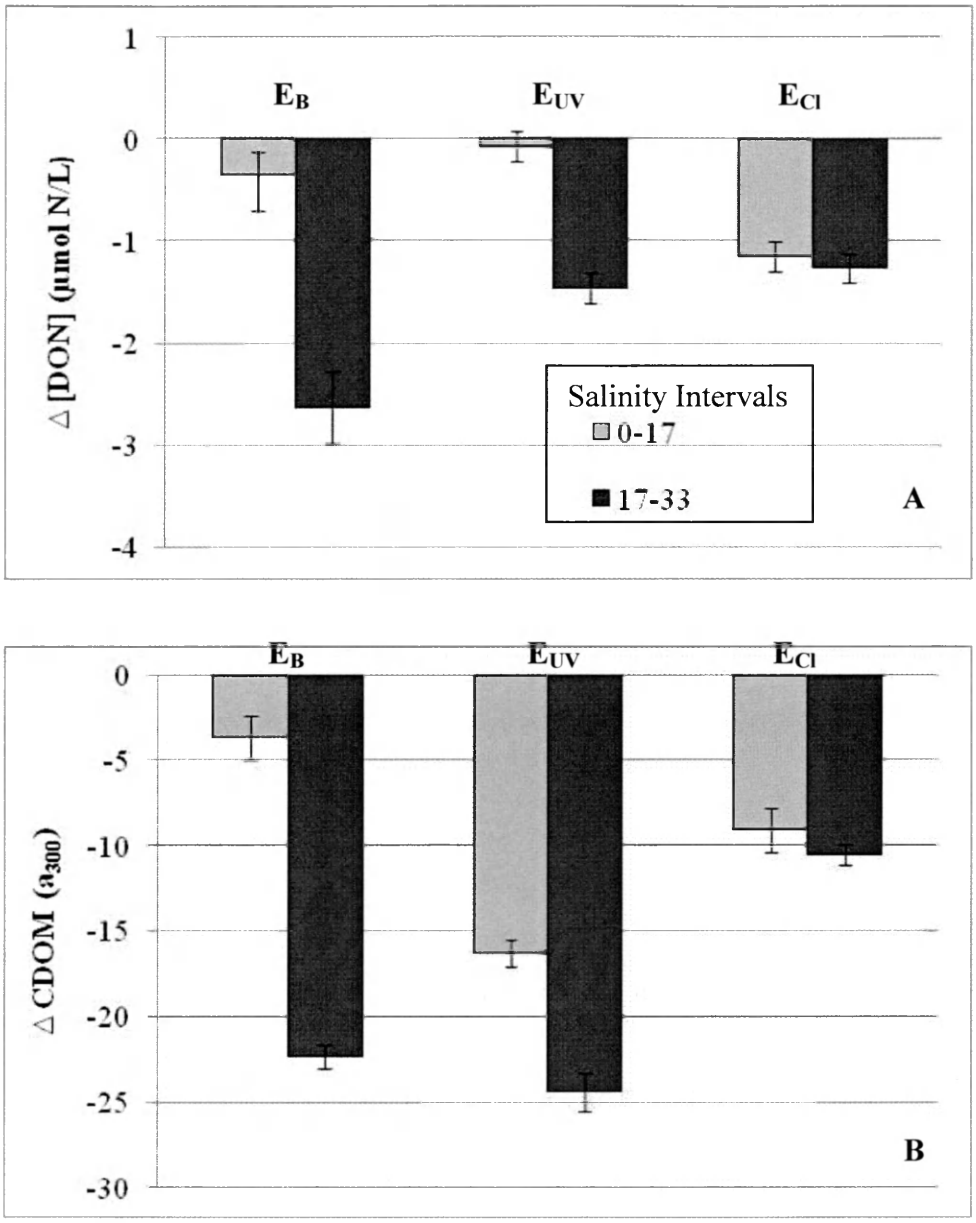

1
0
-1
-2
-3
-4
E_B
E_{UV}
E_{Cl}
Δ [DON] (μmol N/L)
Salinity Intervals
0-17
17-33
A
0
-5
-10
-15
-20
-25
-30
E_B
E_{UV}
E_{Cl}
Δ CDOM (a_{300})
B

Figure 5

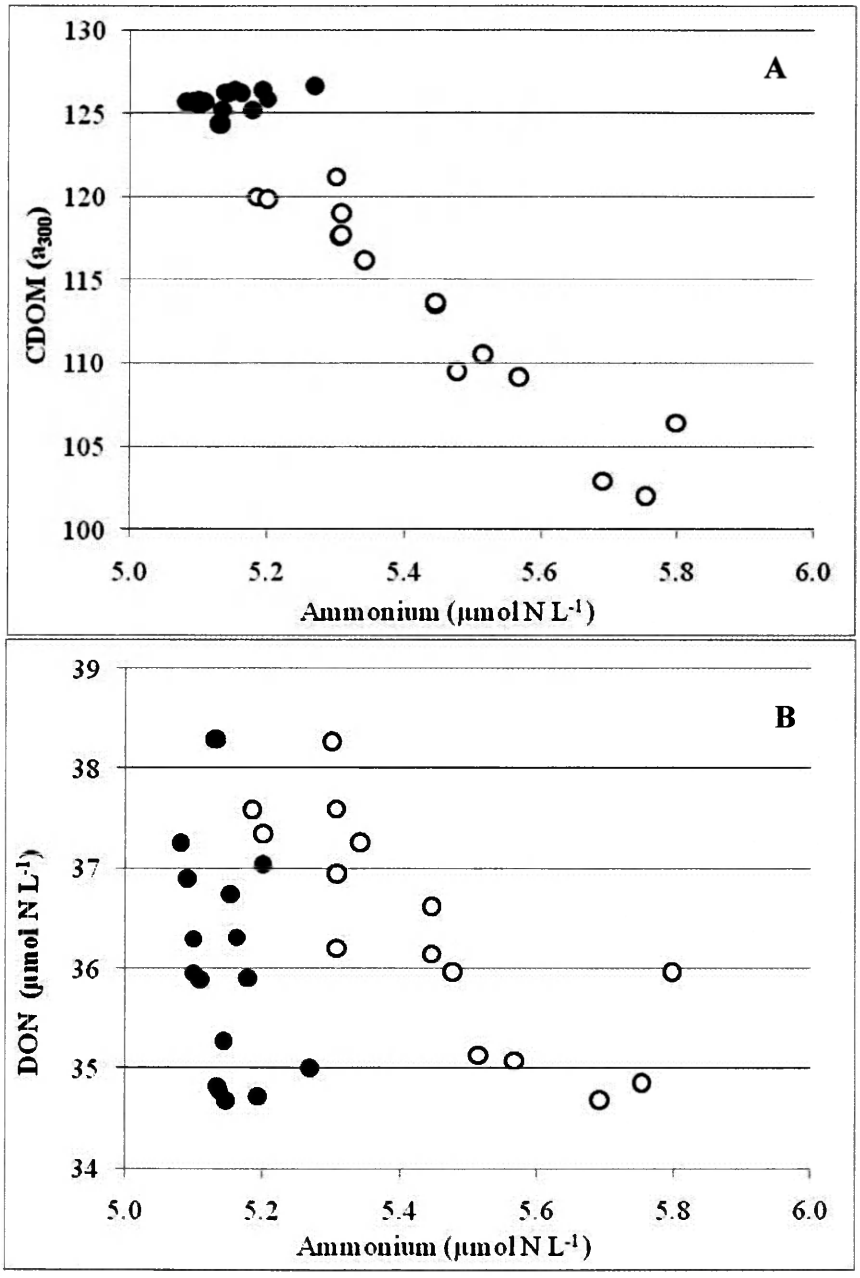

A
CDOM (a_{300})
130
125
120
115
110
105
100
5.0
5.2
5.4
5.6
5.8
6.0
Ammonium (µmol N L^{-1})
B
DON (µmol N L^{-1})
39
38
37
36
35
34
5.0
5.2
5.4
5.6
5.8
6.0
Ammonium (µmol N L^{-1})

Figure 6

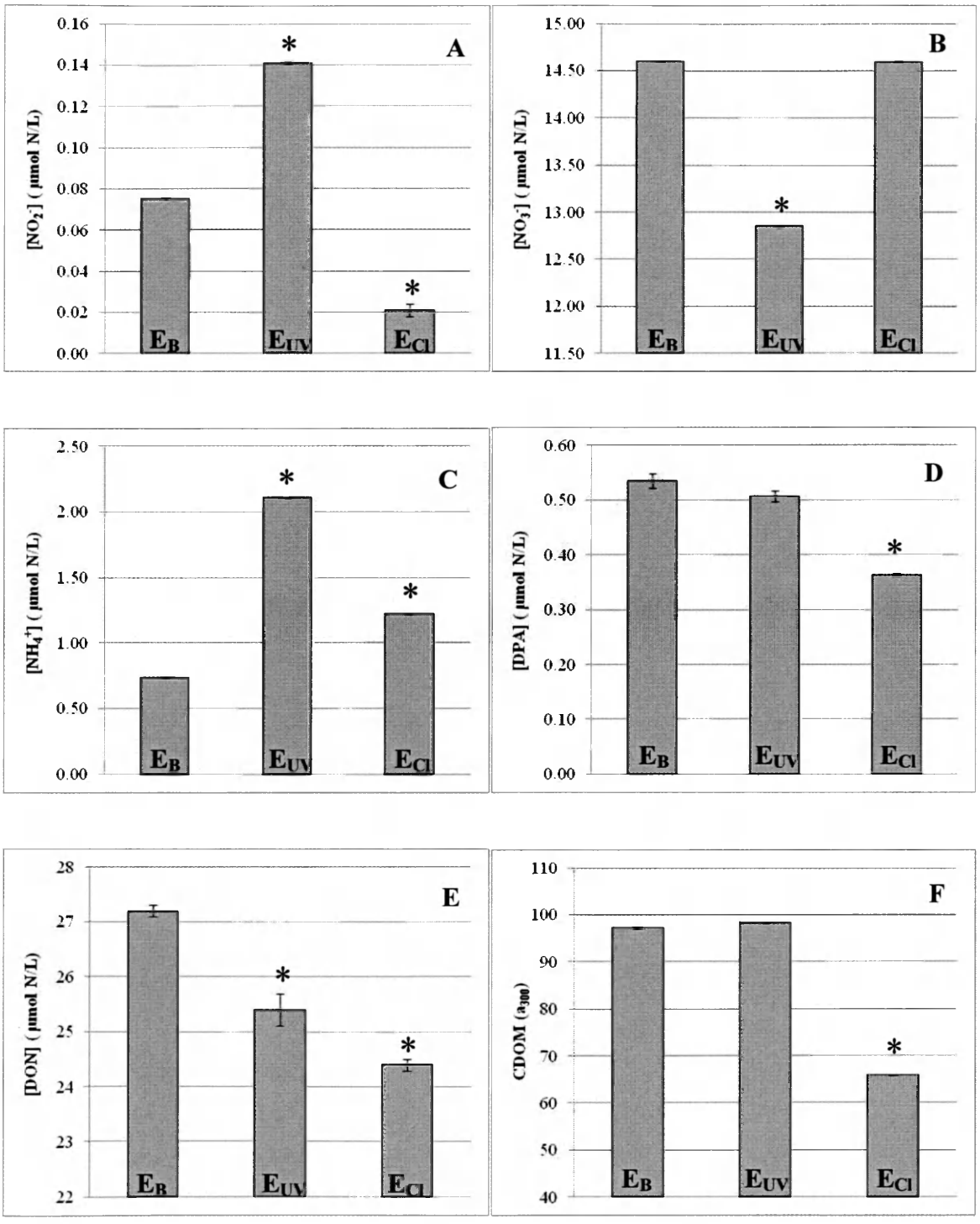
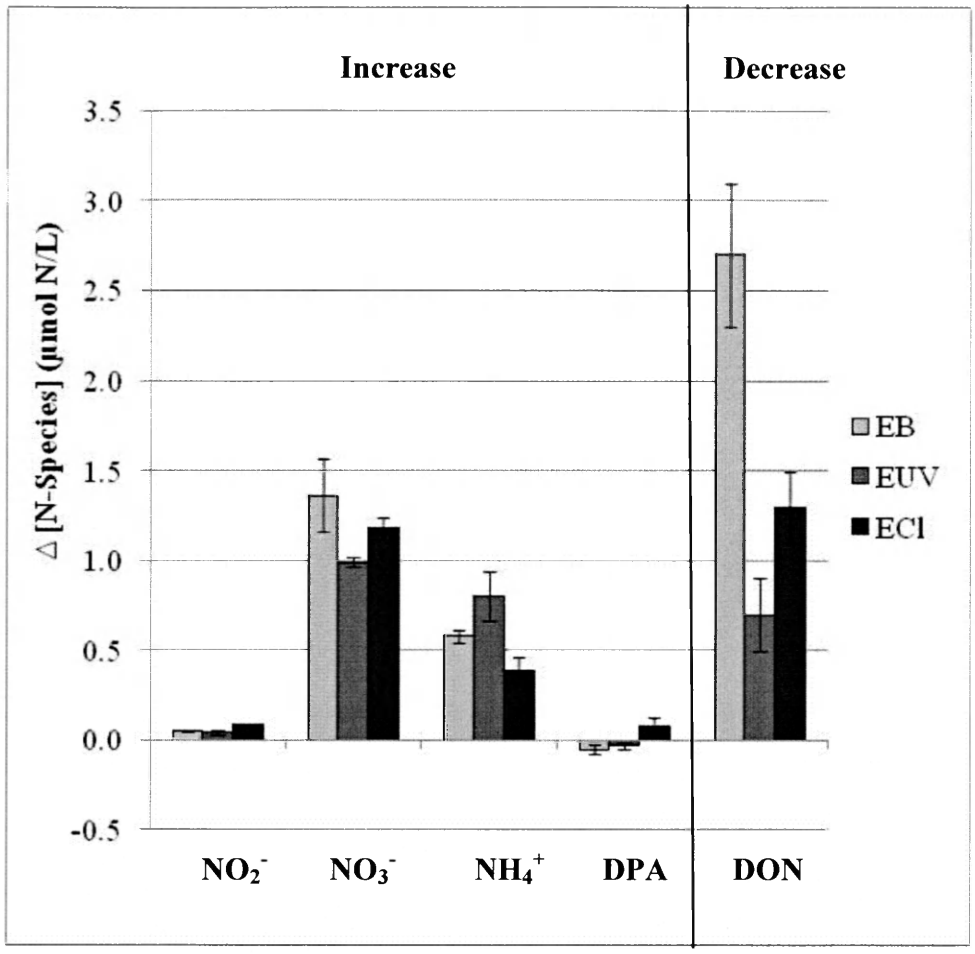

A
B
C
D
E
F
[NO2-] (µmol N/L)
[NO3-] (µmol N/L)
[NH4+] (µmol N/L)
[DPA] (µmol N/L)
[DON] (µmol N/L)
CDOM (a300)
EB
EUV
ECl

Figure 7


Increase
Decrease
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Δ [N-Species] (µmol N/L)
EB
EUV
ECl
NO2-
NO3-
NH4+
DPA
DON

TABLES

Table 1. Experimental design - Experiment 1. A five way nested factorial design was used to test the effect of five variables on the change in nutrient concentrations: three effluents (E_B (pre disinfection), E_{UV} (post UV disinfection) E_{Cl} (chlorine disinfection)), salinity (0, 17 and 33), sunlight (light) versus no sunlight exposure (dark), time duration (0, 4.5 and 9 hours) and temperature (18 and 30°C). Every possible combination of variables was created in triplicate.

E_B	E_{UV}	E_{Cl}	E_B	E_{UV}	E_{Cl}	E_B	E_{UV}	E_{Cl}	E_B	E_{UV}	E_{Cl}
(A) Sal 0	(B) Sal 17	(C) Sal 33	(A) Sal 0	(B) Sal 17	(C) Sal 33	(A) Sal 0	(B) Sal 17	(C) Sal 33	(A) Sal 0	(B) Sal 17	(C) Sal 33
Light			Dark			Light			Dark		
Time 0			Time 0			Time 0			Time 0		
Time 4.5 hr			Time 4.5 hr			Time 4.5 hr			Time 4.5 hr		
Time 9 hr			Time 9 hr			Time 9 hr			Time 9 hr		
Temperature ~18 °C						Temperature ~30 °C					

Table 2. Experimental design - Experiment 2. A four way nested factorial design was used to test four different variables on the change in nutrient concentrations: effluent concentration (40% and 90%), salinity (0 and 30), sunlight (light) versus no sunlight (dark) and time duration (0, 0.5, 1, 2, 4.5 and 9 hours). Every possible combination of variables was created in triplicate.

40% Effluent				90% Effluent			
Salinity 0	Salinity 30	Salinity 0	Salinity 30	Salinity 0	Salinity 30	Salinity 0	Salinity 30
Light		Dark		Light		Dark	
Time 0		Time 0		Time 0		Time 0	
Time 0.5 hr		Time 0.5 hr		Time 0.5 hr		Time 0.5 hr	
Time 1 hr		Time 1 hr		Time 1 hr		Time 1 hr	
Time 2 hr		Time 2 hr		Time 2 hr		Time 2 hr	
Time 4.5 hr		Time 4.5 hr		Time 4.5 hr		Time 4.5 hr	
Time 9 hr		Time 9 hr		Time 9 hr		Time 9 hr	

Table 3. Summary of Model Selection - Experiment 1. Various model equations for each response variable: nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), dissolved primary amines (DPA), and dissolved organic nitrogen (DON), and chromophoric dissolved organic matter (CDOM) absorbance (300 nm), with the corresponding Akaike's Information Criterion (AIC_c) values. The first line in each section is the equation with the variables that best explains the change of that nutrient species

Nutrient Species	Model	AIC_c
NO_2^-	**= *Light + Salinity + Effluent + Light * Time***	**-1986.7**
	= Light + Salinity + Effluent + Temperature + Light * Time	-1973.9
	= Light + Effluent + Salinity	-1847.7
	= Salinity + Light * Time	-1459.3
NO_3^-	**= *Salinity + Effluent +Temperature***	**-198.4**
	= Salinity + Effluent + Temp + Light * Time	-183.7
	= Salinity + Temperature + Light * Time	529.3
	= Effluent + Temp + Light * Time	565.0
NH_4^+	**= *Light + Salinity + Effluent + Light * Time***	**-378.7**
	= Salinity + Effluent + Temperature + Light * Time	- 366.5
	= Light + Effluent + Temperature + Light * Time	- 346.2
	= Salinity + Effluent	-3.4
DPA	**= *Light + Salinity + Effluent + Light * Time***	**-725.9**
	= Light + Salinity + Effluent + Temperature + Light * Time	-718.2
	= Light + Effluent + Temperature + Light * Time	-584.4
	= Light + Salinity +Light * Time	-519.7
DON	**= *Light + Salinity + Effluent + Light * Time***	**574.8**
	= Temperature +Effluent + Salinity	643.3
	= Temperature + Effluent + Light * Time	684.1
	= Light * Time + Salinity + Temperature	907.3
CDOM	**= *Light + Salinity + Effluent + Light * Time***	**1788.7**
	=Light + Effluent + Light * Time	2044.6
	= Light + Salinity + Effluent + Temperature	2184.7
	= Temperature +Effluent + Salinity	2337.2

Table 4. Changes in concentration due to light, salinity, and combined light and salinity - Experiment 1. Significant changes ($p< 0.05$) in concentrations of nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), dissolved primary amines (DPA) and dissolved organic nitrogen (DON) observed when effluent was exposed to **light** (UV in sunlight), increased **salinity** and **light and salinity combined**. NS denotes not significant. Values are in μmol N L^{-1} ± standard deviation, and the range of percent change from the starting concentration is reported in parenthesis. † denotes that averages were only taken from samples for pre and post UV disinfected effluent; chlorinated effluent was not included.

Nutrient Species	Light	Salinity	Light and Salinity combined
NO_2^-	0.036 ± 0.028 (42-345%)	0.032 ± 0.004 (22-167%)	0.059 ± 0.026 (19-662%)
NO_3^-	NS	1.005 ± 0.283 (4-9%)	1.178 ± 0.323 (4-9%)
NH_4^+	0.366 ± 0.177 (1-61%)	0.159 ± 0.073 (10-13%)†	0.692 ± 0.261 (14-93%)
DPA	-0.108 ± 0.071 (22-34%)	0.161 ± 0.0315 (20-57%)	-0.004 ± 0.067 (14-40%)
DON	-0.6 ± 0.8 (0-8%)	-0.4 ± 0.3 (0- 4%)	-1.6 ± 1.0 (1-13%)

Table 5. Photochemical rates – Experiment 2. Rate and rate constants with standard errors, half life and percent change for ammonium (NH_4^+), dissolved primary amines (DPA), dissolved organic nitrogen (DON), and chromophoric dissolved organic matter (CDOM) absorbance (300nm) for samples exposed in the light for 9 hours at 0 salinity. Half lives are only presented for nutrients that showed decay.

Nutrient Species	% Effluent	Rate or Rate Constant	Half Life (hours)	% Change
NH_4^+	40%	NS	--	--
	90%	-0.033 ± 0.007 μmol N $L^{-1}h^{-1}$	--	-8%
DPA	40%	NS	--	--
	90%	0.033 ± 0.005 μmol N $L^{-1}h^{-1}$	--	121%
DON	40%	0.011 ± 0.003 h^{-1}	58 ± 12	11%
	90%	NS	--	--
CDOM	40%	-0.017 ± 0.003 h^{-1}	41 ± 5	-15 %
	90%	-0.020 ± 0.002 h^{-1}	35 ± 4	-17%

Table 6. Combined light and salinity effects on rates – Experiment 2. Rate and rate constants, with standard errors, half life and percent change for ammonium (NH_4^+), dissolved primary amines (DPA), dissolved organic nitrogen (DON), and chromophoric dissolved organic matter (CDOM) absorbance (300nm) for samples exposed for 9 hours at light/30 salinity. Half lives are only shown for nutrients that exhibited decay.

Nutrient Species	% Effluent	Rate or Rate Constant	Half Life (hours)	% Change
NH_4^+	40%	0.026 ± 0.003 μmol N $L^{-1}h^{-1}$	--	12%
	90%	0.063 ± 0.005 μmol N $L^{-1}h^{-1}$	--	12%
DPA	40%	0.032 ± 0.004 μmol N $L^{-1}h^{-1}$	--	174%
	90%	0.024 ± 0.002 μmol N $L^{-1}h^{-1}$	--	54%
DON	40%	-0.024 ± 0.004 h^{-1}	31 ± 4	20%
	90%	-0.009 ± 0.002 h^{-1}	78 ± 11	8%
CDOM	40%	-0.015 ± 0.002 h^{-1}	49 ± 7	42%
	90%	-0.019 ± 0.002 h^{-1}	37 ± 3	17%

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CHAPTER 3: PROJECT SUMMARY

In order to improve the water quality of Chesapeake Bay, the Chesapeake Bay Total Maximum Daily Load (TMDL) has required a more stringent nutrient reduction that enters the Bay. Specific improvements address nutrient reduction in wastewater treatment plants (WWTPs) (CB TMDL2010). For WWTPs to reduce their N load it will require a large sum of tax dollars to renovate and modify these facilities. Since it was once believed that dissolved organic nitrogen (DON) was refractory, WWTPs thought it would be worthwhile to investigate if DON from effluent (i.e. effluent organic nitrogen (EON)) could be considered refractory and, thus, excluded from the N release budget. A number of studies have shown that up to 96% of EON can be taken up by microorganisms over the course of days in bioassay experiments (Urgun-Dimirtas *et al.* 2008; Sattayatewa *et al.* 2009; Bronk *et al.* 2010; Filippino *et al.* 2011). This study sought to expand on this earlier research by quantifying abiotic factors in more detail. This study addressed the question of how abiotic factors, particularly sunlight exposure, changes in salinity, and changes in temperature, affected the release of labile low molecular weight (LMW-N) from EON and determining the decay and production of various N species. Since there was already evidence that EON would have to be included in WWTP's new N release limit, the study also attempted to investigate ways in which WWTPs might be able to break down EON through abiotic methods.

In this study, photochemical and salinity changes were shown to significantly decrease EON concentrations (by approximately 5 to 10%) and increase concentrations of dissolved inorganic nitrogen (DIN), while concentrations of dissolved primary amines (DPA) increased with higher salinity (Chapter 2, Table 4). Changes in temperature were

not shown to significantly affect the change in concentration of most low molecular weight N (LMW-N) compounds, with the exception of nitrate (NO_3^-). In this study, approximately 8% of EON was transformed into LMW-N compounds through abiotic effects (in 9 hour sunlight exposure and changes in salinity from 0 to 33). Results from Experiment 2 indicate that higher concentrations of effluent exhibited faster photo production of ammonium (NH_4^+) and photobleaching of chromophoric dissolved organic matter (CDOM) (Chapter 2, Table 6). This trend was not true for DPA and DON, which had greater photolysis rates at lower effluent concentrations (40%) and can likely be attributed to the increased affect on salinity. A lower effluent: salt ratio could explain why DON had higher percent change and photolysis rates in the 40% effluent samples, since high salinity was also shown to cause more photobleaching of CDOM and decay of DON in Experiment 1 (Chapter 2, Figure 5).

Exposing EON to sunlight and increased salinity resulted in photolysis rates similar to those observed for terrestrially derived humic acids (i.e. Bushaw-Newton *et al.* 1999). These results suggest that EON has similar photo- reactivity to organic material known to be highly reactive in light. Yet, even with these high photo production rates, only a small percentage of EON was converted to LMW-N. This was surprising since it has been previously noted that EON is predominantly made up of amide functional groups (Dignac *et al.* 2000), which are believed to be a key player in photoammonification (Wang *et al.* 2000). A possible explanation for this small conversion of LMW-N could be attributed to the short duration of the experiment. In the environment EON will have longer exposure to light, however, turbidity of the receiving waters may reduce the amount of light that penetrates the water and available to react

with EON. Results from this study suggest that even if EON was refractory, EON would break down into known labile forms of N after abiotic exposure. Even though the release of LMW-N from EON through abiotic processes in the environment may not appear significant in a large estuary such as Chesapeake Bay, large accumulation of nutrients from multiple point and non-point sources could add up to a significant reduction in water quality and should be taken into consideration.

Disinfection methods were shown to increase the amount of LMW-N compounds and decrease EON concentration. Dissolved organic N concentrations decreased after UV and chlorine disinfected effluent by approximately 1.8 ± 0.2 μmol N L^{-1} (6%) and 2.8 ± 0.1 μmol N L^{-1} (10%), respectively (Chapter 2, Figure 6). It was also shown that disinfected effluents had a smaller concentration changes in DON (~3-5% concentration of DON change) after abiotic effects, than the effluent prior to disinfection, which changed by 10% (Chapter 2, Figure 7). These results suggest that disinfection methods have the ability to break down EON, which could be an effective way for WWTPs to reduce their EON concentrations. Although chlorination appears to be the better method for both disinfection and reduction of EON concentrations, chlorine has been shown to cause problems in the environment. Dissolved organic matter can react with chlorine to form disinfection by-products such as trihalomethanes, hatoacetic acids and halogenated organic compounds, which can be mutagenic (Wang *et al.* 2007; Fukui *et al.* 1990; US EPA F-99-062). In order to break down more EON, secondary effluent would need to be chlorinated for a longer period of time, which could potentially increase the mutagenicity of the solution. Therefore it would be advisable to use UV disinfection over chlorination.

Based on the growing body of results, including this study, WWTPs should not be able to exclude the organic N fraction of effluent from their new N release limit. As a result, WWTPs will need to develop new treatment technologies that can breakdown and remove the organic, as well as the inorganic fraction of effluent N. Disinfection methods already used by the facility may also help reduce the EON pool, by breaking it down into LMW-N compounds, which can then be removed through the BNR system. More research needs to be done in order to investigate the cost efficiency of breaking down EON through disinfection compared, or in addition, to other methods such as ammonification (Leslie Grady *et al.* 1999). The biggest problems WWTPs have when it comes to DON reduction, is that a large portion of EON comes from soluble microbial products within the BNR system (Sattayatewa *et al.* 2009). Thus moving the disinfection method in front of the BNR system may not drastically reduce the concentration of EON. Wastewater treatment plants will need to further investigate sources of EON and determining if disinfection methods, UV, oxidation or ozone, may be efficient methods for removing EON.

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Appendix 1

Experiment 1 - Nitrogen (N) mass balance for samples exposed to 9 hours of light/ 0 salinity for each effluent: E_B (pre disinfection), E_{UV} (post UV disinfection) and E_{Cl} (chlorine disinfection). Initial (T_0), final (T_f) and changes between final and initial (ΔT_f-T_0) concentrations in μmol N L^{-1} ± standard error are given for each N-species. Nitrogen species include total dissolved nitrogen (TDN), nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), and dissolved organic nitrogen (DON).

N-Species	E_B			E_{UV}			E_{Cl}		
	T_0	T_f	ΔT_f-T_0	T_0	T_f	ΔT_f-T_0	T_0	T_f	ΔT_f-T_0
TDN	42.59 ± 0.05	43.03 ± 0.22	0.44 ± 0.23	40.54 ± 0.11	40.52 ± 0.11	-0.02 ± 0.2	40.36 ± 0.06	39.3 ± 0.13	-1.06± 0.14
NO_2^-	0.08 ± 0.01	0.11 ± 0.01	0.03 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.01 ± 0.002	0.02 ± 0.01	0.09 ± 0.01	0.07 ± 0.01
NO_3^-	14.60 ± 0.01	14.46 ± 0.1	-0.14 ± 0.10	12.85 ± 0.01	12.90 ± 0.02	0.05 ± 0.02	14.60 ± 0.01	14.61 ± 0.01	0.01 ± 0.01
NH_4^+	0.74 ± 0.01	1.16 ± 0.01	0.42 ± 0.01	2.11 ± 0.01	2.42 ± 0.03	0.31 ± 0.03	1.22 ± 0.01	1.64 ± 0.03	0.42 ± 0.03
DON	27.2 ± 0.1	27.3 ± 0.2	0.1 ± 0.2	25.4 ± 0.1	25.1 ± 0.1	-0.39 ± 0.2	24.4 ± 0.01	22.9 ± 0.2	-1.5 ± 0.2

Appendix 2

Experiment 1 - Nitrogen (N) mass balance for samples exposed to the dark/ 30 salinity for each effluent: E_B (pre disinfection), E_{UV} (post UV disinfection) and E_{Cl} (chlorine disinfection). Initial (T_0), final (T_f) and change between final and initial (ΔT_f-T_0) concentrations in μmol N L^{-1} ± standard error are given for each N-species. Nitrogen species include total dissolved nitrogen (TDN), nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), and dissolved organic nitrogen (DON).

N-Species	E_B			E_{UV}			E_{Cl}		
	T_0	T_f	ΔT_f-T_0	T_0	T_f	ΔT_f-T_0	T_0	T_f	ΔT_f-T_0
TDN	42.59 ± 0.05	43.29 ± 0.17	0.70 ± 0.18	40.54 ± 0.11	41.19 ± 0.2	0.65 ± 0.24	40.36 ± 0.06	40.69 ± 0.08	0.33 ± 0.01
NO_2^-	0.08 ± 0.01	0.11 ± 0.01	0.03 ± 0.001	0.14 ± 0.01	0.17 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
NO_3^-	14.60 ± 0.01	15.29 ±0 .06	0.69 ± 0.06	12.85 ± 0.01	13.87 ± 0.01	1.02 ± 0.01	14.60 ± 0.01	15.91 ± 0.01	1.31 ± 0.01
NH_4^+	0.74 ± 0.01	0.83 ± 0.01	0.09 ± 0.01	2.11 ± 0.01	2.33 ± 0.01	0.22 ± 0.01	1.22 ± 0.01	1.09 ± 0.01	-0.13 ±0.01
DON	27.2 ± 0.1	27.1 ± 0.1	-0.1 ± 0.1	25.4 ± 0.1	25.1 ± 0.1	-0.3 ± 0.1	24.4 ± 0.1	23.7 ± 0.1	-0.7 ± 0.1

Appendix 3

Experiment 1 – Nitrogen (N) mass balance for samples exposed to 9 hours of light/ 30 salinity for each effluent: E_B (pre disinfection), E_{UV} (post UV disinfection) and E_{Cl} (chlorine disinfection). Initial (T_0), final (T_f) and change between final and initial (ΔT_f-T_0) concentrations in μmol N L^{-1} ± standard error are given for each N-species. Nitrogen species include total dissolved nitrogen (TDN), nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), and dissolved organic nitrogen (DON).

	E_B			E_{UV}			E_{Cl}		
N-Species	**T_0**	**T_f**	**ΔT_f-T_0**	**T_0**	**T_f**	**ΔT_f-T_0**	**T_0**	**T_f**	**ΔT_f-T_0**
TDN	42.59 ± 0.05	41.86 ± 0.5	-0.73 ± 0.52	40.54 ± 0.11	41.67 ± 0.08	1.13 ± 0.14	40.36 ± 0.06	40.59 ± 0.11	0.23 ± 0.13
NO_2^-	0.08 ± 0.01	0.13 ± 0.01	0.05 ± 0.01	0.14 ± 0.01	0.18 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.11 ± 0.01	0.09 ± 0.01
NO_3^-	14.60 ± 0.01	15.96 ± 0.20	1.36 ± 0.2	12.85 ± 0.01	13.84 ± 0.03	0.99 ± 0.03	14.60 ± 0.01	15.78 ± 0.06	1.18 ± 0.06
NH_4^+	0.74 ± 0.01	1.30 ± 0.04	0.56 ± 0.04	2.11 ± 0.01	2.91 ± 0.14	0.80 ± 0.14	1.22 ± 0.01	1.60 ± 0.08	0.38 ± 0.08
DON	27.2 ± 0.1	24.5 ± 0.4	-2.8 ± 0.4	25.4 ± 0.1	24.7 ± 0.14	-0.71 ± 0.17	24.4 ± 0.1	23.1 ± 0.1	-1.3 ± 0.13

Appendix 4.

Model information for photochemical rates - Experiment 2. For each nitrogen species specifying effluent concentration (denoted in parenthesis) data were fit to two equations, an exponential equation ($b^* e^{(m^*time)}$) and a linear equation (m*time + b), to determine whether the reaction proceeded as zero (exponential) or first (linear) order. R^2 values are given for the linear models only. Estimates for b (the intercept) and m (the slope or rate) are given for each model, along with Akaike's Information Criterion (AIC).

N Species	Model	R^2	b estimate	m estimate	AIC
NH_4^+ (90%)	$= b^* e^{(m^*time)}$	--	4.62	-0.007	-32.27
	= m*time + b	0.61	4.62	-0.033	-32.39
DPA (90%)	$= b^* e^{(m^*time)}$	--	0.23	0.088	-36.68
	= m*time + b	0.67	0.22	0.033	-37.76
DON (40%)	$= b^* e^{(m^*time)}$	--	14.53	0.012	37.15
	= m*time + b	0.51	14.53	0.190	37.52
CDOM (40%)	$= b^* e^{(m^*time)}$	--	52.00	-0.017	72.06
	= m*time + b	0.73	51.89	-0.822	73.06
CDOM (90%)	$= b^* e^{(m^*time)}$	--	117.91	-0.020	97.30
	= m*time + b	0.81	117.61	-2.149	98.93

Appendix 5.

Model information for combined light and salinity effects on rates - Experiment 2. For each nitrogen species specifying effluent concentration (denoted in parenthesis) data were fit to two equations, an exponential equation ($b^* e^{(m^*time)}$) and a linear equation (m*time + b) to determine whether the reaction proceeded as zero (exponential) or first (linear) order. R^2 values are given for the linear models only. Estimates for b (the intercept) and m (the slope or rate) are given for each model, along with Akaike's Information Criterion (AIC).

N-Species	Model	R^2	b estimate	m estimate	AIC
NH_4^+ (40%)	$= b^* e^{(m^*time)}$	--	2.38	0.010	-51.88
	= m*time + b	0.78	2.38	0.026	-51.96
NH_4^+ (90%)	$= b^* e^{(m^*time)}$	--	5.22	0.011	-42.07
	= m*time + b	0.91	5.21	0.063	-43.01
DPA (40%)	$= b^* e^{(m^*time)}$	--	0.18	0.111	-45.55
	= m*time + b	0.75	0.17	0.032	-42.26
DPA (90%)	$= b^* e^{(m^*time)}$	--	0.49	0.041	-69.06
	= m*time + b	0.88	0.49	0.025	-70.95
DON (40%)	$= b^* e^{(m^*time)}$	--	14.34	-0.024	32.89
	= m*time + b	0.74	14.28	-0.299	34.37
DON (90%)	$= b^* e^{(m^*time)}$	--	37.64	-0.009	42.59
	= m*time + b	0.69	37.61	-0.324	43.01
CDOM (40%)	$= b^* e^{(m^*time)}$	--	54.03	-0.015	66.25
	= m*time + b	0.71	53.94	-0.723	67.04
CDOM (90%)	$= b^* e^{(m^*time)}$	--	121.30	-0.019	83.93
	= m*time + b	0.90	121.07	-2.095	85.84

VITA

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Carolina was born in Tacoma Park, Maryland on April 18, 1985. She was raised overseas and graduate from George Mason High School in 2003. She attended college at the University of Mary Washington and obtained a B.S. in Chemistry in 2007. After graduation she worked at the Bermuda Institute of Ocean Sciences working in the Environmental Quality Department. In the Fall of 2008 she entered the masters program at the Virginia Institute of Marine Science and was advised by Dr. Deborah Bronk.