CONSEQUENCES OF SALINIZATION ON CARBON AND NUTRIENT RELEASE FROM A RESTORED COASTAL FORESTED WETLAND

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Given that wetlands provide important ecosystem services, such as improving water quality and protecting inland regions from storms, it is necessary to understand how sea level rise and climate change will alter their structure and function. Wetlands play an important role in the global carbon cycle, thus much work has focused on how they will respond to climatic change. One aspect that has not received much attention is how increasing salinity, due to drought and sea level rise, alters the export of dissolved organic carbon and the process of flocculation. This study combined long-term field observations, laboratory assays, and a field experiment to examine the effects of salinity on the process of flocculation and the subsequent fate of particulate organic carbon (POC). I compiled long-term field data to determine control mechanisms on POC concentration. To assess the effects of salinity and floc reversibility, I conducted a lab assay. I also conducted a field saltwater addition with adapted sediment traps to determine if salt induced flocculation and changes in water clarity and nutrients. I then determined the bioavailability of the floc for heterotrophic microbial respiration. In the long-term field data, salinity did not enter the site and POC formation was variable and was weakly correlated to nitrate. In the lab assays, salinity induced POC formation and resulted in an increase in light penetration, and the floc did not re-dissolve after a simulated storm event. In the field experiment, salt addition induced flocculation and established a potential mechanism for

phosphorus (e.g. PO_4^{3-}) retention. DOC was converted to recalcitrant POC, reducing bioavailability for microbial respiration, which led to increased floc deposition. Salinity mobilized nitrogen (NH_4^+) and stimulated algal biomass production. My results suggest that saltwater intrusion could increase organic sediment accumulation in wetlands with high DOC loads and increase the potential for algal blooms in legacy impacted wetlands with low flows. My results suggest that increased salinity to freshwater wetlands could reduce carbon export and increase soil accretion rates, increasing ecosystem resilience to low salinity perturbations.

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TABLE OF CONTENTS

LIST OF TABLES ..... **vii**

LIST OF FIGURES ..... **viii**

CHAPTER 1: INTRODUCTION **1**

Wetlands and Vulnerability of Saltwater Intrusion 1

Organic C, Salt-Induced Flocculation, and Optical Parameters 3

Heterotrophic Respiration of Salt-Induced Flocs 9

Nutrient Dynamics in Response to Salinity and Subsequent Effect on Algal Biomass 10

Objectives, Purpose, Questions, and Hypotheses 12

CHAPTER 2: METHODS **14**

Site Description 14

Long-Term Sample Collection, Sample Processing, and Flow Data 15

Experimental Design, Flocculation, and Water Color for Lab Assays 16

Experimental Mesocosm Design 16

Sediment Trap Removal Timeline 19

Filter Analysis of POC, Chlorophyll *a*, Stable Isotopes and Heterotrophic Respiration 20

Water Analysis of PO_4, DOC/TDN, Water Color Parameters 22

Statistical Analysis 23

Long-term Data 23

Lab Assays 23

Field Experiment 24

CHAPTER 3: RESULTS 26

Long-Term Field POC for Inflow, Midpoint, and Outflow 26

Lab Assays of Salt-Induced Flocculation and Irreversibility 27

Mesocosm experiment 28

Organic Matter Dynamics 28

Heterotrophic Respiration 30

Nutrients and Algal Biomass.....31

CHAPTER 4: DISCUSSION 34

Long-Term Field Data 35

Lab Assays 36

Mesocosm Experiment 37

Organic Matter Dynamics 37

Heterotrophic Respiration 42

Nutrients and Algal Biomass.....44

Conclusions 47

TABLES & FIGURES 49

REFERENCES 74

LIST OF TABLES

CHAPTER 2: METHODS **51**

2.1 Recipe for artificial saltwater for a 1 L solution 51

CHAPTER 3: RESULTS **56**

3.1 Multiple linear regression fitted models for POC in TOWeR 56

3.2 Multiple linear regression parameter estimates for best model for POC concentrations in TOWeR 57

3.3 Means for Experimental Salt Addition 59

3.4 Heterotrophic respiration of floc for each treatment over stated time points to determine intercept, slope, and r^2 value 66

LIST OF FIGURES

CHAPTER 1: INTRODUCTION **49**

1.1 Conceptual diagram of hypotheses 49

CHAPTER 2: METHODS **50**

2.1 Map of Timberlake Observatory for Wetland Restoration (TOWeR) 50

2.2 Diagram of experimental salt addition to mesocosm chambers (n=15) 52

2.3 Timeline for sediment trap removal and replacements 53

CHAPTER 3: RESULTS **54**

3.1 Time series of POC and DOC dynamics for long-term field data at TOWeR. Linear relationship between DOC and POC and between chloride and POC at TOWeR (n=158) 54

3.2 Mean values for experimental lab assays (n=32).....58

3.3 Mean DOC depletion over time and without initial salinity exposure (n=15).....60

3.4 Mean POC formation over time and without initial salinity exposure (n=15) 61

3.5 Mean deposition rate of POC over time (n=15) 63

3.6 Mean specific UV absorbance values over time and without initial salinity exposure (n=15) 64

3.7 Mean stable isotope data over time and without initial salinity exposure (n=15) 65

3.8 Carbon dioxide flux of flocs during incubation (n=15) 67

3.9 Mean carbon dioxide flux of flocs, percent of floc respired, and predicted depletion (n=15) 68

3.10 Mean orthophosphate over time and without initial salinity exposure (n=15) 69

3.11 Mean total dissolved nitrogen over time and without initial salinity exposure (n=15).....70

3.12 Mean ammonium over time and without initial salinity exposure (n=15) 71

3.13 Mean absorbance values in the photosynthetically active radiation spectrum over time and without initial salinity exposure (n=15) 72

3.14 Mean chlorophyll *a* over time and without initial salinity exposure (n=15) 73

Chapter 1: Introduction

Wetlands and Vulnerability to Saltwater Intrusion

Coastal freshwater wetlands are important in the global carbon cycle because they are highly productive ecosystems and have low decomposition rates (Odum 1979; Houghton & Skole 1990). Wetlands are essential to conserve because they serve as storm surge barriers, improve water quality, assist in flood control, and store 20-30% of the terrestrial carbon (Mitsch & Gosselink 2007). The accumulation of organic matter is known as soil accretion, an important ecosystem function that aids in storm protection of inland regions. Ecosystem services of coastal wetlands have been valued at US$18,818 $ha^{-1}yr^{-1}$ (Barbier *et al.* 2011). Despite their economic value, the United States has lost over 50% of its wetlands since the 1780's due to land-use change and agriculture (Mitsch & Gosselink 2007). While the anthropogenic conversion of land is a major driver in wetland loss, structural additions (e.g. levees, impoundments) alter the natural hydrologic regime, increasing susceptibility to salinization, exacerbating coastal wetland loss (Ghose-Hajra *et al.* 2015; Lane *et al.* 2016). Historical agricultural practices such as the construction of irrigation and drainage networks provide routes of saltwater intrusion beyond naturally occurring rivers (Prat & Ibanez 1995).

Coastal wetland loss can be attributed to agricultural land-use increasing vulnerability to salinization (Antonellini *et al.* 2014; Benini *et al.* 2015; Islam *et al.* 2015). Along with anthropogenic drivers in coastal wetland loss, climate change models predict acceleration in sea level rise (Warrick & Oerlemans 1990; Arkema *et al.* 2013). Globally, sea levels have risen by 17-21 cm throughout the last century (IPCC 2014) and are currently rising at a rate of 2.8 to 3.1 mm yr^{-1} (Håkanson & Eklund 2010), and are expected to increase by 15.6 mm yr^{-1} by 2100 (Church *et al.* 2013). When these sea level rise rates are combined with increased frequency and

duration of storms, coastal wetlands will be greatly impacted (Michener *et al.* 1997). Rising sea levels facilitate exchange between the open ocean and the Albemarle Sound, increasing the salinity of hydrologically connected systems, making the coast of North Carolina a hot-spot for sea level rise compared to global averages (Sallenger *et al.* 2012).

Climate change models also predict increases in frequency and duration of droughts. When these effects are coupled with increased storm intensity, saltwater intrusion occurs through decreased freshwater export, with strong consequences for the southeastern US (Buesseler *et al.* 2007; Day *et al.* 2007; Ardón *et al.* 2013; Carter *et al.* 2014). Saltwater intrusion is the inward movement of high salinity surface water and groundwater into formerly freshwater wetlands (Ferguson & Gleeson 2012; Ardón *et al.* 2013; Herbert *et al.* 2015). As sea levels rise, coastal wetland regions migrate inland, but if there are human structures upland, coastal wetlands are "squeezed" (Brinson *et al.* 1995). Sea level rise will increase the frequency of flooding with high salinity waters being delivered to freshwater wetland regions (Ferguson & Gleeson 2012; Herbert *et al.* 2015). Increasing frequency of drought and sea level rise will increase saltwater intrusion events into former freshwater wetlands (Mulholland *et al.* 1997).

Wetlands are important carbon sinks, sequestering an average of 118 g C m^{-2} $year^{-1}$ (Mitsch *et al.* 2013). Carbon retention in coastal freshwater wetlands is negatively impacted due to the positive feedback loop between climate change and wetlands. For example, in northern peatlands, elevated CO_2 increases dissolved organic carbon (DOC) export and stimulates organic matter decomposition, releasing more carbon dioxide back into the atmosphere (Fenner *et al.* 2007). Atmospheric CO_2 increases temperature, melting ice sheets and accelerating the rate of sea level rise (Gregory & Oerlemans 1998). As coastal freshwater wetlands experience saltwater intrusion, the bulk density of inundated soils is reduced, leading to root loss and soil compaction,

increasing the susceptibility to peat collapse (Stagg & Mendelssohn 2010). Peat collapse also occurs through increased salinity, stimulating a rapid export of nutrients and DOC to downstream systems from wetlands (Chambers *et al.* 2014). Furthermore, when wetlands are exposed to high salinity, primary productivity of macrophytes is reduced, leading to a decrease in organic matter retention (Neubauer 2013). Organic matter retention is necessary for a wetland to function as a carbon sink and as wetland soils become anaerobic, methane production increases, converting the wetland to a carbon source (Schlesinger & Bernhardt 2013). However, in response to flooding or saltwater intrusion, methanogenesis decreases, which can reestablish the wetland as a carbon sink (Weston *et al.* 2006; Helton *et al.* 2014).

Organic C, Salt-Induced Flocculation, and Optical Parameters

Organic carbon in water is found primarily in two forms, dissolved organic carbon (DOC) and particulate organic carbon (POC). The distinction between DOC and POC is an operational definition, in that POC moves gravitationally to settle out in the sediments (Mulholland 1981). While opinions are varied on this matter and dependent upon the OC portion of interest, the common method to separate POC and DOC is to filter the water sample through a 0.45µm or 0.7µm filter, in which DOC passes through (Pike & Moran 1997; Moran *et al.* 1999). When examining POC formation, a 0.7µm filter is used because 0.45µm filters can capture up to 35% of the original DOC, skewing POC calculations (Abdel-Moati 1990). POC sinking and export is fairly uniform in low velocity water bodies, only decreasing in the presence of high algal concentrations (Lapoussière *et al.* 2009). POC has the ability to carry organic chemicals and assists in the export of hydrophobic pollutants (Ni *et al.* 2008; Luo *et al.* 2009).

Coastal freshwater wetlands typically have high concentrations of DOC, which links aquatic and terrestrial ecosystems by serving as the base of the microbial food web (Battin *et al.*

2008). Enhancing microbial activity, DOC is generally more bioavailable and abundant than POC (Hanson *et al.* 2003; Hoellein *et al.* 2013). POC, like other forms of carbon, is used by microbes and to assimilate OC, microbes release extracellular enzymes to break it down into smaller molecules (Battin *et al.* 2008). The quality of POC will determine the fate and its potential to be sequestered or assimilated (Osburn *et al.* 2012). As organic carbon is the main food source for heterotrophic microbes (Battin *et al.* 2008), understanding the bioavailability (i.e. ease of decomposition) of POC is important. Because DOC and POC have roles in regulating aquatic productivity and respiration (Cole 2013), understanding the effects of salinity on the relative concentrations of DOC and POC is important.

The fate and export of DOC and POC from coastal wetlands is influenced by flooding, drought, and salinity. The quantity of organic matter, as DOC, that is exported from wetlands to estuaries is dependent upon the frequency and intensity of rainfall (Raymond & Saiers 2010; Alvarez-Cobelas *et al.* 2012). Increases in rainfall stimulates flooding events which increases the export of DOC, and conversely, drought decreases DOC export (Clark *et al.* 2005; Evans *et al.* 2015). Flooding and drought periods alter the quality (e.g. aromatic and humic compounds) of carbon molecules (Wilson & Xenopoulos 2009; Mehring *et al.* 2013). Similar to DOC, POC is mobilized during storms and enters the water column from the sediments with increased opportunities for export (Dhillon & Inamdar 2014). While storm associated discharge events increase DOC concentrations, POC export concentrations peak earlier than DOC concentrations, suggesting increased mobility of POC and the primary export path is through surface water (Coynel *et al.* 2005; Jeong *et al.* 2012). As climate change models predict increased intensity of storms, it is important to understand the possible changes in POC.

POC quality varies depending on its source (e.g. allochthonous or autochthonous). Allochthonous POC is derived from terrestrial organic matter (e.g. riparian leaf litter) and is generally considered more recalcitrant (i.e. hard to degrade) because of the complex molecules (e.g. lignin and cellulose) (Carrington *et al.* 2012). Allochthonous POC is dominated by high molecular weight (HMW) humic substances which are aromatic, recalcitrant, and heterogeneous molecules (McKnight & Aiken 1998). While terrestrial OC inputs can fuel secondary production (Cole & Caraco 2001), bacterial respiration of autochthonous DOC is greater because it is more labile (i.e. easy to degrade) (Håkanson 2006). Autochthonous POC is organic matter that is produced within the system, derived from autotrophs, and labile due to the high protein content (Berg & McClaugherty 2014). Similarly, autochthonous OM is typically more bioavailable because it is less aromatic (Hotchkiss *et al.* 2014). In an autotrophic system, productivity surpasses respiration and autochthonous OM sustains the food web (Hoellein *et al.* 2013).

The source of carbon can be determined using stable isotopes. Carbon isotope values that are more depleted (i.e. negative) indicate a terrestrially derived origin (-26.5‰), and less depleted (-20.3‰) indicates marine or aquatic origin (Ogawa & Ogura 1997). Origin dependent isotopic signatures are present in POC, meaning that terrestrial origin is at -27‰, estuarine origin is at -30‰ and marine origin is at -17‰ (Middelburg & Nieuwenhuize 1998). The differences in the carbon signatures are attributed to the type of photosynthesis (e.g. C_3 and C_4) utilized by the autotroph (Ueno 2001). Typically, aquatic plants utilizing C_3 photosynthesis produce more labile, simple carbon exudates, comparatively, many terrestrial plants utilize C_4 photosynthesis and they produce more complex, heavier carbon exudates (Cloern *et al.* 2002; Van Dongen *et al.* 2002). Results derived from ecosystems with seasonal algal blooms need to be interpreted with caution because they are not always constant (Ogawa *et al.* 1994). Of the stable isotope

parameters, $\delta^{13}C$ is more reliable when determining POM origin because the $\delta^{15}N$ signature may be altered through fast-acting biochemical processes (Thornton & McManus 1994). As salinity increased in a hypereutrophic estuary, the $\delta^{13}C$ signature became less depleted and increased from -25.6‰ to -20‰, suggesting that increased salinity reduced the export of terrestrially derived carbon (Sato *et al.* 2006).

Salinity introduces increased ion concentrations which alters biogeochemical cycling processes by altering available electron acceptors and affecting the microbial community (Herbert *et al.* 2015). Salinity is derived from conductivity, which measures the concentration of ions in a solution and is used to relate chloride, conductivity, and salinity (Paine 2003). Some of the important conductive ions that conductivity measures are chlorides, sulfides, and carbonate ions, many of which are present in saltwater (Miller *et al.* 1988). An initial salinity effect on the biogeochemistry of an ecosystem is that it reduces movement of gases and ions in soil by decreasing the hydraulic conductivity and establishing anoxic conditions (Brady & Weil 2000). The onset of anoxia is accelerated because saltwater decreases gas solubility and oxygen permeability through the ion concentrations, creating a negative redox potential (Stumm & Morgan 1996). The negative redox and reduced oxygen availability forces microbes to respire anaerobically by utilizing other terminal electron acceptors (TEAs). As salinity increases available TEAs (e.g. sulfate), organic matter may be mineralized, increasing DOC production and export (Chambers *et al.* 2014). In anoxic conditions, TEAs are used in accordance with their energy yield (NO_3^-, Fe_3^+, SO_4^{2-}, and CO_2) (Mitsch & Gosselink 2007). Present at a high concentration in saltwater, sulfate is a preferred terminal electron acceptor and is used for sulfate reduction in anaerobic conditions (Weston *et al.* 2011). Sulfate reduction products (e.g. H_2S) can be toxic to vegetation, decreasing the productivity of macrophytes, resulting in a decrease of

primary productivity (Krauss *et al.* 2009). Furthermore, sulfate is rapidly utilized, increasing organic matter decomposition, and leading to soil subsidence and erosion (Wong *et al.* 2010).

Saltwater can alter the chemical and physical environment of primarily freshwater systems through the high concentration of monovalent (Na^+) and divalent (Ca^{2+}) cations. Exposure to divalent cations will release soil bound NH_4^+ into the water column (Giblin *et al.* 2010; Weston *et al.* 2010; Ardón *et al.* 2013). Due to the high divalent cation concentration (Ca^{2+} and Mg^{2+}) of saltwater, solubility of organic carbon decreases and stimulates flocculation, the aggregation of DOC to form POC (Sholkovitz 1976b; Mavi *et al.* 2012). Flocculation is driven primarily by physical (e.g. water velocity, turbulence), chemical (e.g. ion availability), or biological (e.g. bacteria) factors (Droppo *et al.* 1998). Flocculation is a heavily used process in wastewater treatment facilities that precipitates organic matter by aggregating dissolved compounds to form particulates (Marquardt 1984; Matilainen *et al.* 2010). Particulates are aggregated in the presence of salinity but if the ionic concentrations are reduced, the flocs can revert to the original DOC molecules (Hruška *et al.* 2009). DOC quantity and quality is reduced as flocculation occurs at low salinities (Asmala *et al.* 2014), through the aggregation of terrestrially derived HMW humic compounds (Kowalczuk *et al.* 2009).

While salinity stimulates flocculation, physical factors (e.g. shallow water depth, flooding, and high velocity) can resuspend POC that was deposited in the sediments (Koch *et al.* 2014b). When flocs are resuspended, flocculation can be reversed and converted back into DOC but the longer the carbon molecules are in the flocculent form, the more energy required to break apart the floc (Nan *et al.* 2015). Understanding the potential for reversibility is important as it has implications on carbon export and sedimentation. Previous work has investigated flocculation

through lab assays via field sample collection, and lab salinity adjustments (Eisma *et al.* 1991; Thill *et al.* 2001; Xia *et al.* 2004).

As salinity stimulates flocculation and sedimentation of DOC, there are several implications for water clarity and light penetration. Prior to salinization, high DOC concentrations support increased heterotrophic activity but DOC absorbs light in the photosynthetically active radiation (PAR) spectrum (400-700nm) in the water column, resulting in light attenuation and shading of autotrophic organisms (Karlsson *et al.* 2009). The access to light for primary producers is hindered by the high DOC load typical of wetlands because it absorbs most of the UV-B light (Arts *et al.* 2000). When salinity stimulates flocculation and deposition of POC, water clarity increases, enhancing photosynthetic activity of autotrophs but also increases the potential for photo-oxidation of benthic organic matter (Cory 2013). The bioavailability of benthic organic matter is reduced as light penetration increases due to photobleaching (Cory *et al.* 2007). Photodegradation of DOM occurs when DOM is oxidized to form dissolved inorganic carbon (DIC), but when temperature is elevated, DOM is converted to POM instead, supporting flocculation processes (Porcal *et al.* 2015).

The relative percent of aromatic carbon is the specific UV absorbance (SUVA) at 254nm and is correlated to carbon quality (Weishaar *et al.* 2003; Choi *et al.* 2008). When the percent of aromatic carbon is higher, the DOC likely originated from an allochthonous source and is more recalcitrant (Berggren *et al.* 2009). Recalcitrant and less bioavailable DOM is quantitatively measures through lower DOC:DON and the water color proxy, SUVA (Balcarczyk *et al.* 2009). As SUVA increases, bacterial production and bacterial growth efficiency decreases, establishing a link between recalcitrant, aromatic DOC and decreases in bioavailability (Berggren *et al.* 2009).

Heterotrophic Respiration of Salt-Induced Flocs

Microbial communities are impacted by salinity exposure and their response is mediated by the community structure (Allison & Martiny 2008). Microbial structure shifts when exposed to elevated salinity. The microbes may not survive the environmental stress but, as ecological niches become available, they are quickly filled by salt-tolerant microbes. Before a community shift, salinity hinders the ability of freshwater microbes to osmoregulate reducing respiration (Rietz & Haynes 2003). Respiration, the aerobic decomposition of OM, reduces DOC concentrations as well as through the flocculation process (Brouns *et al.* 2014).

Flocculation, the aggregation of DOC, incorporates humic substances to form POC in the presence of salinity (Ardón et al, unpublished data). Because salinity increases repulsive forces, extracellular carbohydrates that are produced by bacteria and plants may be mobilized and used in the flocculation process (Del Giorgio & Cole 1998; Von Wachenfeldt *et al.* 2009). Conversely, flocculation processes are inhibited by humic acids in agricultural soils because they reduce the bonding capacity in the presence of larger molecules (Furukawa *et al.* 2009; Furukawa *et al.* 2014). In humic-rich ecosystems, the microbial loop does not function at optimal efficiency because humic substances are a refractory energy source and less bioavailable compared to labile autochthonous sources (e.g. polysaccharides and amino acids) for food web structure (Farjalla *et al.* 2009).

The bioavailability of POC is important because saltwater intrusion stimulates the microbial mineralization of OM in anoxic conditions through the introduction of favorable TEAs, resulting in an overall loss of organic carbon and creating a risk of permanent inundation of coastal wetlands (Weston *et al.* 2011). Studies have identified bioavailability differences in DOC (Hotchkiss *et al.* 2014), but few studies have examined the differences in bioavailability of

salt-induced flocs. High salinity stimulates carbon mineralization in tidal freshwater sediments (Weston *et al.* 2006). While microbial respiration is positively correlated with flocculation in lakes (Von Wachenfeldt *et al.* 2009), if the organic matter is terrestrial in source (i.e. recalcitrant), a portion of the OC will be respired but a significant fraction will be exported in slow moving water systems like lakes (Hanson *et al.* 2004).

Salinity has been noted to increase microbial mineralization of carbon due to increased availability of TEAs but few studies address whether the increase in TEAs or floc bioavailability is the cause for increased carbon mineralization (Chambers *et al.* 2011; Weston *et al.* 2011; Neubauer 2013). Specifically, increases in carbon mineralization are driven through the microbial utilization of sulfate as the terminal electron acceptor because it is present at high concentrations in saltwater (Weston *et al.* 2011). It could be that flocculation makes carbon more available to microbes in the sediment surface, as observed in lakes (Von Wachenfeldt et al. 2009). It is unclear if the flocs produced through elevated salinity have a negative impact on microbial respiration coupled with a biological hindrance of salt stress on microbes.

Nutrient Dynamics in Response to Salinity and Subsequent Effect on Algal Biomass

Phosphorus is present in wetlands in organic and inorganic forms and the proportion of each is determined by vegetation and soil characteristics, as well as land use legacies (Reddy *et al.* 1996; Duff *et al.* 2009; Sharpley *et al.* 2013). Phosphorus retention is mediated by adsorption, sedimentation, and plant uptake (Reddy *et al.* 1999). P availability is mediated by microbial uptake activity, with much of orthophosphate being assimilated and immobilized rapidly (Walbridge 1991). Typically, inorganic phosphorus is bound to clays and organic matter by cation exchange with Fe^{3+}, Ca^{2+}, Al^{3+}, and Mg^{2+} (Richardson 1985). In anaerobic conditions (e.g. high salinity, inundation), the anaerobic reduction of Fe^{3+} to Fe^{2+} facilitates the release of Fe-

bound P from soils (Reddy *et al.* 1999; Ardón *et al.* 2010a). The release of inorganic P could have serious implications, especially in ecosystems with historical agricultural legacies (Pant & Reddy 2003). While Fe-bound P may be released from freshwater soils, if Fe^{2+} is oxidized, P may precipitate again (Jordan *et al.* 2008). Potentially reducing phosphorus export, salinity may precipitate orthophosphate with binding cations via flocculation (van Diggelen *et al.* 2014). Many studies have found differences in P movement when exposed to salinity (Herbert et al. 2015). Phosphorus is released from sediments in anoxic soils, as nitrogen is removed via denitrification during re-flooding, posing challenges for nutrient retention (Aldous *et al.* 2005).

Coastal freshwater wetlands can retain and transform nitrogen and phosphorus, reducing the potential for eutrophication and toxic algal blooms (Verhoeven *et al.* 2006). Downstream eutrophication potential increases as ammonium is ionically displaced and exported in the presence of saltwater (Weston *et al.* 2006; Ardón *et al.* 2013). In anaerobic conditions, denitrification occurs, compared to aerobic conditions when nitrogen mineralization and nitrification occurs (Davidson & Swank 1986). Denitrification is the process by which wetlands retain and remove nitrate. Inorganic nitrogen is toxic to freshwater fish and invertebrates at high concentrations, similar to concentrations present in restored wetlands with past agricultural use (e.g. ammonia is toxic at concentrations ranging from 0.08 – 0.65 NH_3-N mg/L) (Camargo *et al.* 2005).

As salinity increases ammonium and phosphorus concentrations in the water column through desorption processes, nitrification decreases, resulting in changes of limiting nutrient concentrations and increasing susceptibility to eutrophication. This increase in nutrient release depends on past and present land use and soil type (Brouns *et al.* 2014). Salinity typically hinders

freshwater algae, but algae may benefit from increased light penetrations due to flocculation of DOC (Davis *et al.* 2003; Nielsen *et al.* 2003; Davis *et al.* 2010).

In freshwater ecosystems experiencing periodic salinity perturbations and phosphorus mobilization from soils, algal growth may occur at an enhanced rate (Conley *et al.* 2009). As salinity exposure increases, phosphorus limits algal growth instead of salt stress (Marino *et al.* 2006). Many cyanobacterial bloom forming genera can tolerate low to moderate salinities (Moisander *et al.* 2002). Bloom formation is more likely to be disrupted by more intense storms and increased discharge. But as the discharge subsides, the nutrient load deposited into the system through runoff could promote blooms (Mitrovic *et al.* 2003). As salinity releases and mobilizes nutrients from the sediments, algal growth is likely to increase.

Objectives, Purpose, Questions, and Hypotheses

My overarching goal was to answer this question; **As a saltwater intrusion event occurs, what are the OC and nutrient consequences in the water column and in the sediments?**

Firstly, I established a baseline be examining the long term and current POC formation at TOWeR to determine if natural salt-induced flocculation is occurring. I hypothesized that upon exposure to increased conductivity, salt-induced flocculation will occur, resulting in a higher concentration of POC.

Secondly, I conducted an experiment to answer the question; **How does salinity affect the flocculation of DOC and light penetration through the water column? Is the flocculation process reversible?** I hypothesized that salinity would increase the flocculation of DOC, increase light penetration, and increase specific UV absorbance (SUVA). I hypothesized

that the addition of a highly turbulent event prior to filtration would reverse the flocculation process and result in a lower POC concentration.

Finally, I conducted an experimental salt addition to answer the following questions (Fig. 1.1); **1) How does the form and lability of organic carbon change in response to salinity in the water column and in the sediments?** I hypothesized that an increase in salinity would decrease DOC in the water column, increase the amount of POC entering the sediments, and the resulting floc would be characterized by allochthonous matter. I hypothesized that microbial respiration rates of salt-induced flocs would decrease as salinity increases due to recalcitrance of salt-induced flocs. **2) How do nutrient concentrations change in the water column in response to salinity?** I hypothesized that as salinity increases, the concentration of phosphorus (orthophosphate) in water would decrease, total dissolved nitrogen in the water column would increase, and ammonium in the water column would increase. **3) What are the consequences of salinity in the water column for autotrophs?** I hypothesized that an increase in salinity would decrease the absorbance of light in the PAR range and chlorophyll *a* concentrations would increase.

Chapter 2: Methods

Site Description

The Timberlake Observatory for Wetland Restoration (TOWeR) is located in Tyrrell County, Columbia, NC, USA (35°54′32.835″, 76°9′36.054″). The total area of TOWeR is 1704.2 ha and 440 ha of TOWeR has been restored from intensively farmed land to a wetland (Fig. 2.1). Historically, pocosin and cypress-tupelo wetlands dominated this region, but it was logged intensively beginning in the 1950s. After the area was extensively logged, the land was converted to agricultural fields in the 1970s and 1980s. Creating suitable farmland for soybeans and corn, the area was ditched and drained through canal construction. Upon initiation of restoration of TOWeR, ditches were filled and beginning in 2005, 750,000 saplings were planted throughout the site (Ardón *et al.* 2010b). When ecosystem monitoring began in 2007, the site has experienced saltwater intrusion, flooding, and droughts. Agricultural fields on the southern side of the mitigation bank input water with high concentrations of nitrogen annually. Water level overall has increased since the flooding in 2007. TOWeR experiences a bidirectional water flow in response to precipitation and wind direction with the general flow being from the south side to the north side of the site. Upon installation of a culvert, there is a single outflow which is hydrologically connected to the Little Alligator River. Being hydrologically connected to the Albemarle Sound (via Little Alligator River which flows into the Alligator River), TOWeR has experienced saltwater intrusion. During periods of decreased precipitation that occurred in 2007-2009, TOWeR experienced saltwater intrusion resulting in a salinity peak of 6 ppt (Ardón *et al.* 2013).

Long-Term Sample Collection, Sample Processing, and Flow Data

To determine dissolved organic carbon and nutrient concentrations, I collected filtered and unfiltered water samples weekly since December 2012 until September 2014 from the Inflow, Midpoint, and Outflow of TOWeR. The three sites had varied physical and chemical conditions due to distance from the agricultural site and depth of water column. I filtered the samples through GF/F Whatman 0.7 µm filters and kept at 4°C until analysis. DOC and total dissolved nitrogen were measured on a Shimadzu TOC-V total carbon analyzer with a TNM-l nitrogen module (Shimadzu Scientific Instruments, Columbia, Maryland, USA). Chemical concentrations of NH_4-N were measured using the phenate method on a Lachat QuickChem automated system and NO_3-N, Cl^-, and SO_4^{2-} were measured on a Dionex ICS-2000 ion chromatograph (Dionex Corporation, Sunnyvale, California, USA). Along with water sample collection, pH, dissolved oxygen, conductivity, and temperature were measured with a handheld YSI device (YSI Multiprobe 560, Yellow Springs, Ohio, USA).

Unfiltered samples were frozen (-2°C) until processing. Upon analysis, I thawed and filtered samples through pre-weighed pre-combusted Whatman GF/F 0.7µm filters. After initial filtration, I placed samples in a drying oven at 60ºC for 48 hours. Then, to obtain total dry floc mass, I weighed samples and placed filters in a muffle furnace at 500ºC for 3 hours to remove all organic matter from the filter. I calculated particulate organic carbon as ash free dry mass and half of the mass was assumed to be carbon (Mulholland 1981). Water depth and velocity were recorded every 15 minutes since February 2012 with a velocity meter (2150 Area Velocity Meter, Teledyne ISCO, Lincoln, Nebraska, USA) water velocity meters were downloaded monthly.

Experimental Design, Flocculation, and Water Color for Lab Assays

To determine baseline salt-induced flocculation, I collected unfiltered water was from the Outflow. I made artificial saltwater (Kester *et al.* 1967) (Table 2.1) and then added it to 60mL of the filtered sample to adjust the salinity to the desired treatment (Control, 4 ppt, 8 ppt, or 12 ppt). Samples were incubated for 48 hours. To determine if flocculation could be reversed through physical means, I used a Bench Mixer to simulate a turbulent event for 15 seconds immediately prior to filtration. I decided to select a 15s turbulent event because short periods of shear force have been shown to reverse the flocculation process (Hedborg & Lindström 1996; Hubbe 2000). I filtered samples immediately through a pre-weighed, combusted Whatman GF/F 0.7 filter.

To determine absorbance of light at varying levels of UV radiation (i.e. PAR = 400-700nm and SUVA parameter = 254nm), 600µL of each water sample for triplicate absorbance measurements was micropipetted onto a 96-well plate to be analyzed with the Microplate reader. Absorbance was measured from 200 to 700nm in two nm intervals. To gain measures of percent aromaticity of the carbon present and chromophoric dissolved organic matter (CDOM), I measured specific ultraviolet absorbance (SUVA) of each sample by dividing the UV absorbance at 254nm by the DOC concentration of the sample (Choi *et al.* 2008). To gain measures of light which may be utilized for photosynthesis, I measured the absorbance of UV radiation in the photosynthetically active radiation (PAR) spectrum. To acquire an overall measure of photosynthetic light-absorbing DOM, I averaged the absorbance of samples from 400–700nm.

Experimental Mesocosm Design

To collect POC that precipitates through the water column, I adapted sediment trap designs from marine and lake ecosystems to work in a shallow water system. Sediment traps

were constructed by securing 7cm diameter funnels inside a drilled opening on 125mL Nalgene bottles. To prevent the contents of the sediment trap from exchanging with the chamber, I used a silicon adhesive to create a water tight seal. To limit coarse particulate organic matter from entering the sediment trap, I covered the funnel opening with 1000µm mesh screen.

To conduct a field saltwater addition mesocosms, I acquired 18 rope-handled plastic containers (54.61cm by 41.91cm). For each chamber, I removed the base, created an opening to allow for free water exchange 5cm from the top of the chamber, and installed four sediment traps equidistant from one another 12cm from the bottom. The sediment traps were secured with zip ties and silicone. Mesocosms were installed at the Midpoint for four reason; 1) the flow was low which decreased the possibility of losing the chambers, 2) the likelihood of the chambers flooding over was low because the water depth was approximately 25 cm, 3) the soils were all relatively soft and chambers could be submerged 4-6 cm lowering the possibility of external water exchange with the mesocosms, and 4) the Midpoint data sonde location was close to chambers. I organized chambers in randomized block experimental design to control for possible external factors. Each chamber was assigned a number in ascending order from one through fifteen. In R, I applied the sample function to randomly select which chambers were assigned to the control group or one of the two treatments for each replicate individually. Chambers were installed on June 15th, 2015 and allowed to equilibrate with surroundings for 48 hours. Returning on June 17th, 2015, labeled sediment traps were installed in each chamber. I collected water samples from each chamber prior to the saltwater addition.

To measure flocculation in the field, I conducted an experimental salt addition to a chamber system at the Midpoint site in TOWeR (Fig. 2.2). Chambers were not sealed at the top to allow for air exchange. The three treatments for this experiment were a control with no

artificial saltwater addition and two artificial saltwater addition treatment levels (S = 4 and 8). These three treatments were replicated five times for a total of fifteen chambers. Each chamber was equipped with four sediment traps inside. Sediment traps were placed in four equidistant locations from each other (north, east, south, and west). The conical sediment traps were constructed as described by (Honjo 1992), with attention given to past sediment traps assessments and descriptions (Bloesch & Burns 1980; Gardner 1980; Blomqvist & Håkanson 1981; Butman *et al.* 1986). In low velocity systems like TOWeR, conical traps are recommended, in comparison to high velocity systems where they are known to under collect particles due to apparatus movement (Buesseler *et al.* 2007). The funnel was inserted into a 125mL Nalgene bottle and secured with silicon. Each sediment trap was installed in the chamber with a zip tie and submerged into the water. Funnels were labeled, indicating when the traps were to be removed.

To equilibrate the chambers for the salt addition experiment, I placed chambers approximately 6cm into the sediment with the holes unobstructed to allow chamber to calibrate for 48 hours with surrounding water. I placed the chambers 6cm into the sediments to minimize water exchange. To determine possible exchange, I measured salinity at the soil-water interface externally of the chambers at each time point with an YSI Multiparameter 550. Prior to saltwater addition, I collected an unfiltered water sample from each chamber and immediately filtered on a 0.7µm GF/F Whatman pre-weighed, pre-combusted filter and the holes in the chambers were plugged with rubber stoppers.

To create the artificial saltwater, I used Instant Ocean. Due to the high volume of salt required to adjust chambers to 4 and 8 ppt, I made the artificial saltwater to 140mS/cm. To add saltwater while minimizing disturbance of sediments within the chamber, I added 2.5L of

artificial saltwater to chambers with high (8 ppt) salinity treatment, 1.5L of artificial saltwater and 1L of deionized water to chambers with the low (4 ppt) salinity treatment, and 2.5L of deionized water to the control chambers. I measured salinity continuously with an YSI Multiparameter 550.

Sediment Trap Removal Timeline

To parse out the amount of flocculation based on length of time exposed to saltwater, sediment traps were removed and installed at different times. To control for immediate salt-induced flocculation, I removed the north side sediment trap after 30 minutes. Also, to understand the importance of the first 30 minutes of flocculation potential, one set of sediment traps was removed after 30 minutes, and a new sediment trap was installed (Fig. 2.3). To measure flocculation and the length of salinity exposure on POC formation, I removed sediment traps after 24 hours, 1 week, and 4 weeks. To determine how the potential for flocculation changes over time with exposure to salinity, I replaced the 30 minutes and 24 hours sediment traps with new traps that were collected with the 1 week sediment traps (Fig 2.3). Specifically, after 24 hours, a second set of sediment traps was removed and a new set was installed and after 1 week, three sets of sediment traps were removed. The final set of sediment traps was removed after 4 weeks.

Each time a sediment trap was retrieved, water pH, dissolved oxygen, salinity conductivity, redox potential and temperature were measured with a handheld multiprobe YSI device (YSI Multiprobe 560, Yellow Springs, Ohio, USA). Measurements were taken from the inside and the outside of the mesocosm to determine strength of the chamber seal in the soils. Water depth measurements were obtained at each sampling time point on the north, center, and

south side inside of the mesocosm. All sediment traps were capped and brought back to the lab for immediate filtration for POC and chlorophyll *a*.

Filter Analysis of POC, Chlorophyll a, Stable Isotopes and Heterotrophic Respiration

Sediment traps were thoroughly mixed and 30 mL was syringe filtered through a pre-weighed, pre-combusted GF/F Whatman 0.7µm filter. To gain measures of organic matter, I dried filters at 60ºC for 48 hours and ashed them at 500ºC for 3 hours, taking weights in between. I calculated POC formation as ash-free dry mass.

To obtain measures of algal biomass, I performed a digestion on the filters using acetone and chlorophyll *a* was measured on a fluorometer. I used one of the three filters (pre-weighed combusted Whatman GF/F 0.7µm filter) from each sediment trap for a chlorophyll *a* extraction experiment. Methods were acquired from Arar and Collins 1997, Standard Methods for the Examination of Water and Wastewater 2012, Strickland and Parsons 1968, and Parsons et al. 1984. I stored the filters in a –20ºC freezer until processing.

Due to differences in SUVA values in the presence of salinity, determining stable isotopes of isolated sediment traps will clarify potential flocculation preference of allochthonous carbon. To determine if the flocculation process is selective for allochthonous or autochthonous carbon due to variation in lability, I filtered samples through a third pre-weighed, combusted Whatman GF/F 0.7 µm filter. I divided filters in half and sent off one section for stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$. To determine effects of salinity on lability of carbon for heterotrophic microbes, stable isotope mass spectrometry (University of Georgia, Stable Isotope Ecology Laboratory) was used to determine the source of carbon. In effort to quantify source contribution to susceptibility to be flocculated, I calculated the stable carbon isotopic composition (Coffin *et*

al. 1994). I presented isotopic ratios for organic matter originating from the filters following this equation:

$$\delta X\ (‰) = (R_{sample} / R_{standard} - 1) \cdot 1000$$

I calculated X as the isotope of interest, either $\delta^{13}C$ or $\delta^{15}N$ by determining R_{sample}, the isotopic ratio of the sample as either $^{13}C/^{12}C$ or $^{15}N/^{14}N$, with the heavier isotope as the numerator. $R_{standard}$ is the isotopic ratio for the standard. Vienna PeeDee Belemnite was used for the carbon standard and ambient air was used for the nitrogen standard (Sharp 2005, McConnachie and Petticrew 2006). In aquatic systems, this technique is utilized because autochthonous carbon yields lower isotopic ratios than allochthonous carbon (Owen et al. 1999). Using the percent carbon and nitrogen, I determined the percent organic matter and the carbon to nitrogen ratio. Although stable nitrogen isotopic composition is used for distinction between terrestrial and marine origins, an artificial saltwater addition would not induce marine characteristics (Umezawa *et al.* 2002).

To examine the bioavailability of the salt-induced flocs, I measured heterotrophic aerobic microbial respiration rates. Overall, for each individual time treatment, vials were set up to determine heterotrophic respiration of the flocs by measuring carbon dioxide accumulation. I corrected carbon dioxide values for the amount of POC that was available for possible respiration. I cut the third filter from each sediment trap in half and placed into a 40mL glass scintillation vial with 20mL of previously frozen, filtered (0.7µm GF/F Whatman filter) TOWeR. In order to have an inoculum acclimated to the carbon in the flocs, I collected unfiltered water from the Midpoint on the same day that I started the experiment and filtered it through a 0.7µm GF/F Whatman filter. I added 2mL to each vial. To measure carbon bioavailability, I took gas

samples at T_0, T_1, T_3, and T_7, where T_x is the number of days of the carbon mineralization experiment. T_0 was measured prior to capping the samples. At each time point, I collected a 5mL gas sample and analyzed it using a gas chromatograph (Shimadzu Tech). I stored the capped samples at room temperature in the dark. I corrected the carbon dioxide production values for available headspace. Methods are adapted from Hotchkiss et al. 2014.

Water Analysis of PO_4, DOC/TDN, Water Color Parameters

To quantify changes in phosphorus due to salt-induced flocculation, I measured dissolved orthophosphate (PO_4 -P) with a SmartChem 200. I filtered sediment trap samples through a 0.7µm GF/F Whatman filter and analyzed filtered water samples within 36 hours of filtering or froze them for later analysis. I followed the protocol as established through methods described by SmartChem 200 Method, Standard Methods for the Examination of Water and Wastewater 2012, and EPA 1993. I measured DOC and total dissolved nitrogen (TDN) on a TOC-V with a TN module (Shimadzu Corporation). Because TDN values do not discriminate between NH_4^+ and NO_3^-, I measured NH_4^+ and reported it as NH_4^+-N with a SmartChem 200. I filtered and froze samples in accordance with phosphorus procedure. I did not exceed the holding time of 28 days for the samples for ammonium analysis.

To determine absorbance of light at varying levels of UV radiation (i.e. PAR = 400-700nm and SUVA parameter = 254nm), 600µL of each water sample for triplicate absorbance measurements was micropipetted onto a 96-well plate to be analyzed with the Microplate reader. I measured absorbance from 200 to 700nm in two nm intervals. To gain measures of percent aromaticity of the carbon present and chromophoric dissolved organic matter (CDOM), I measured the specific ultraviolet absorbance (SUVA) of each sample by using the UV absorbance at 254nm and then normalized by dividing it by the DOC concentration of the sample

(Choi *et al.* 2008). To gain measures of light which may be utilized for photosynthesis, I measured the absorbance of UV radiation in the photosynthetically active radiation (PAR) spectrum. To acquire an overall measure of photosynthetic light-absorbing DOM, I averaged the absorbance of samples from 400–700nm.

Statistical Analysis: Long-term Data

To determine the effects of increased conductivity on POC concentration within and between sites (e.g. Inflow, Midpoint, and Outflow) in the long-term field data in TOWeR, I used a linear regression analysis and model selection. To examine potential site specific effects on POC, I conducted an ANOVA by site. I used model selection methods to determine potential explanatory variables contributing to POC formation at different sites in TOWeR. I used the regsubset() and leaps() functions in the leaps package in RV3.2.2 to determine the r^2, Cp, and AIC scores associated with each model created. I did not include water level data in this analysis because it was strongly correlated with temperature (Ardón *et al.* 2016).

Statistical Analysis: Lab Assays

To examine the fixed effect of artificial saltwater on flocculation in lab assays, I compared POC concentrations among salinity treatments with an ANOVA. This statistical test was followed by a post hoc Tukey test of means to determine which treatments were significant. Specifically, I used an analysis of variance statistical test to determine the effect of an artificial saltwater addition on DOC, the percent of DOC converted to POC, the average absorbance in the photosynthetically active radiation range, and the specific UV absorbance as well. For the tests that were significant, a post hoc Tukey test of means was completed as well.

Statistical Analysis: Field Experiment

For the time treatments of 30 minutes, 24 hours, 1 week, and 4 weeks, I ran diagnostic tests to determine normality and linearity of values. For dependent variables with a Shapiro Wilks test score of 0.05 or higher, a log transformation was applied to data. For the percent variable with a Shapiro Wilks test score of 0.05 or higher, an arc sine transformation was applied. Overall, POC, specific UV absorbance, orthophosphate, total dissolved nitrogen, ammonium, and chlorophyll *a* data were log transformed. DOC, stable isotope, and absorbance in PAR spectrum were not log transformed. I used a repeated measures analysis of variance to compare POC between samples within each salinity treatment to the other treatment and control and to determine potential time dependence, followed by a post hoc Tukey test of means. I used a repeated measures ANOVA to examine the fixed effects of salinity and time on water color parameters, DOC, the percent of POC converted from original DOC, TDN, P, and chlorophyll *a*. To analyze the carbon dioxide produced through the heterotrophic respiration experiment, I normalized the carbon dioxide concentrations by the amount of POC available for consumption. To statistically analyze the respiration data, I calculated the respiration rate of each sample accounting for headspace and then used a repeated measures ANOVA, followed by a post hoc Tukey test of means.

For the time treatments of 1 week - 30 minutes, 1 week - 24 hours, and 1 week, I ran diagnostic tests to determine normality and linearity of values. For dependent variables with a Shapiro Wilks test score of 0.05 or higher, a log transformation was applied to data. For the percent variable with a Shapiro Wilks test score of 0.05 or higher, an arc sine transformation was applied. I used a two-way analysis of variance to determine the fixed effect of initial salinity exposure on the dependent variables. In these tests, statistical non-significance means that initial exposure to salinity is not important to eliciting the effect that occurs by 1 week of exposure.

Conversely, statistical significance means that initial exposure to salinity is crucial to the effect observed at 1 week. I used an ANOVA to compare POC between samples within each salinity to the other treatment and control, followed by a post hoc Tukey test of means. I used an ANOVA to examine salinity for water color parameters, DOC, the percent of POC converted from original DOC, TDN, P, and chlorophyll *a*. To analyze the carbon dioxide produced through the heterotrophic respiration experiment, I normalized the carbon dioxide concentrations by the amount of POC available for consumption. To statistically analyze the respiration data, I calculated the respiration rate of each sample accounting for headspace and then used a repeated measures ANOVA, followed by a post hoc Tukey test of means.

Chapter 3: Results

Long-Term Field POC for Inflow, Midpoint, and Outflow

During the two years of long-term field data (2012-2014), TOWeR did not experience a saltwater intrusion but variations in conductivity and chloride did increase POC concentrations (Fig. 3.1). Furthermore, there appears to be site specific effects on POC concentrations, with the Inflow having the highest POC of the four sites ($F_{3,182}$=5.128, p=0.00199, Fig. 3.1J). Average POC concentrations were higher at the Inflow (18.39 ± 5.37 mg/L), which corresponds to higher DOC values, than at the Midpoint (15.92 ± 4.96 mg/L) or at the Outflow (17.55 ± 5.85 mg/L). At the Outflow, there are two chloride measurements (79.18 & 116.62) which appear to be outliers altering the linear model. Correlated parameters (e.g. conductivity, sulfate) were not elevated.

POC appears to follow a seasonal pattern with higher values in the spring and summer and lower values in the winter. As POC is formed through DOC molecules suspended in the water column, higher original DOC concentrations resulted in higher POC formation (r^2 = 0.15, p=0.0051; Fig. 3.1B). POC values increased with higher chloride and conductivity, explaining 9 and 13%, respectively, of the variation at the Inflow (Fig. 3.1C). POC values at the Midpoint and Outflow were not correlated to chloride (Fig. 3.1F & I). It is likely that the conductivity values experienced over these two years were not strong enough to influence flocculation. Furthermore, if there was a saltwater intrusion event, it would have been unlikely surface water samples would have captured POC, as it moves gravitationally to settle out of the water column. Due to the strength of the correlation in the Outflow between conductivity and DOC concentrations, DOC was included as an explanatory variable in multiple linear regression model fitting analysis to predict POC concentration. Other significant explanatory variables were NO_3^-, Cl^-, and water

temperature. The best models were chosen through the lowest AIC value and these models did not provide strong explanations for the effects of salinity on flocculation. The best model for the Inflow showed that 29% of the variability in POC formation could be explained by NO_3^-, Cl^-, and water temperature (Table 3.1 & 3.2).

Lab Assays of Salt-Induced Flocculation and Irreversibility

The addition of artificial saltwater to water samples retrieved from TOWeR showed that increased salinity can decrease the amount of dissolved organic carbon (DOC) through salt-induced flocculation, the conversion of DOC to particulate organic carbon (POC) ($F_{3,28}$=42.81, p<0.0001; Fig. 3.2B). POC formation in the control group (4.204 ± 1.079 mg/L) was much lower than in any of the salinity treatments (4 ppt = 7.613 ± 2.169 mg/L and 8 ppt = 7.954 ± 1.165mg/L), with the highest salinity treatment having the most POC formation (12.272 ± 0.971; Fig. 3.2A). The formation of POC from the conversion of DOC was supported by the loss in DOC as salinity increases ($F_{3,28}$=353.8, p<0.0001; Fig. 3.2A). The amount of POC as percent of original DOC significantly increased with salinity ($F_{3,28}$=117.5, p<0.0001; Fig. 3.2C). The percent of DOC converted to POC ranged from the lowest salinity treatment (11.5%) to the highest salinity treatment (28.1%). Salt-induced flocculation removed DOC from the water column via sedimentation of POC, and resulted in significantly more light penetration ($F_{3,28}$=4.558, p=0.0101; Fig. 3.2D) and increased SUVA (measured at 254nm), which served as a proxy for relative percent of aromatic carbon in the water column ($F_{3,28}$=5.095, p=0.00612; Fig. 3.2E). When samples were subjected to rapid turbulence prior to filtration, POC formation did not decrease, which suggested that rapid physical factors did not reverse the flocculation process (t_{30}=0.03, p=0.9724; Fig. 3.2F). Due to the non-significance, the vortex effect was removed for the remaining statistical analyses.

Mesocosm experiment

Organic Matter Dynamics

DOC concentrations in the chambers did not decrease in the presence of salinity over time (Fig. 3.3A & 3.3B). While it was not significant (p=0.072), there were differences in samples without initial salinity exposure. In samples that lacked the first 24 hours of salinity exposure, DOC concentrations decreased from 29.75 mg/L in the control to 21.98 mg/L and 17.69 mg/L for the low and high salinity, respectively. POC formation increased, on average, 2-3 times in the presence of salinity over time. In the time series analysis, POC formation increased significantly in the presence of salinity, length of exposure, and interaction of salinity and time ($F_{2,44}$=9.301, p=0.0004, $F_{3,44}$=2.491, p=0.0725, and $F_{6,44}$ =2.720, p=0.0246, respectively; Fig. 3.4A). Furthermore, the deposition rate of floc increased significantly due to increased salinity, time, and length of salinity exposure ($F_{2,44}$=9.301, p=0.0004, $F_{3,44}$=99.51, p<0.0001, and $F_{6,44}$ =2.720, p=0.0246, respectively; Fig. 3.5A). When sediment traps were removed at 4 weeks, chambers were noticeably flooded and heavy rainfall had occurred twice between the 1 week and 4 weeks sampling time point (7.03 cm of rain on June 26, 2015 and 3.33 cm on July 3, 2015). POC formation decreased at the 4 weeks time point due to mesocosm inundation (Table 3.3). Further illustrating floc dissolution due to storm induced turbulence and dilution, Cohen's d effect size revealed a strong and positive effect of salinity on floc formation and then a negative but equally as strong effect on the amount of floc in the sediment traps after the storm (Fig. 3.4B).

When initial time periods were removed, POC formation did not increase in the presence of salinity within each time point ($F_{4,36}$=0.885, p=0.4828; Fig. 3.4C), but when compared among salt exposed time treatments, the sediment traps with full length of exposure to salinity were 2-3

times greater than those lacking the first 30 minutes or 24 hours ($F_{2,36}$=6.7, p=0.0033 and $F_{2,36}$=32.05, p<0.0001, salinity and time, respectively; Fig. 3.4C). Averaging over the salinity treatments, POC formation in mesocosms with 1 week salinity exposure was 11.87 mg, compared to 5.02 and 3.25 mg without the first 30 minutes and 24 hours of salinity exposure, respectively. Initial salinity exposure was crucial to the flocculation process. Without the first 30 minutes of salinity exposure, POC formation in the low and high salinity treatments increased by 27% compared to the control. Without the first 24 hours of initial salinity exposure, POC in the salinity treatments increase by 34%. Comparing the time series to the 1 week samples with full exposure, the salinity treatments had 55% more POC formation than the control.

SUVA did not respond to salinity or to the length of exposure ($F_{2,44}$=2.173, p=0.126, and $F_{3,44}$=1.762, p=0.168, salinity and time, respectively; Fig. 3.6A). Corresponding with mesocosm inundation, SUVA decreases between weeks 1 and 4. The observed decrease in SUVA was likely a response to the DOC rich water that inundated mesocosms. Without the initial exposure periods, salinity significantly decreased SUVA ($F_{2,36}$=3.33, p=0.0481; Fig. 3.6B). The decrease in SUVA without the first 24 hours of salinity exposure suggests that a large portion of highly aromatic carbon was used in the flocculation process.

The percent carbon increased significantly with length of exposure but not to the salinity treatment ($F_{2,44}$=2.233, p=0.1192 and $F_{3,44}$=3.962, p=0.0139, salinity and time, respectively; Fig. 3.7A). Similar to percent carbon, length of exposure significantly increased the percent nitrogen of the floc and salinity had an effect, though it was not significant at the 0.05 level ($F_{2,44}$=2.693, p=0.078 and $F_{3,44}$=4.432, p=0.0093, salinity and time, respectively; Fig. 3.7B). Over time, the carbon to nitrogen ratio significantly decreased but the decrease was not attributed to salinity ($F_{2,44}$=0.624, p=0.54 and $F_{3,44}$=6.016, p=0.0015, salinity and time, respectively; Fig. 3.7C).

Salinity and length of salinity exposure were nearly significant on the carbon isotopic signature, resulting in a slightly less depleted origin ($F_{2,44}$=2.539, p=0.09 and $F_{6,44}$=2.142, p=0.067, salinity and interaction, respectively; Fig. 3.7D). There was no time dependence on the carbon isotopic signature ($F_{3,44}$=1.715, p=0.177, Fig. 3.7D).

Heterotrophic Respiration

When heterotrophic microbes were exposed to flocs formed within the first 30 minutes, 24 hours, and 1 week, salinity significantly decreased the amount of carbon dioxide produced but overall respiration increased over the seven-day mineralization experiment (Table 3.4, Fig. 3.8). The interaction term between salinity and each sampling time point was non-significant. To determine the respiration, carbon dioxide measurements were corrected for the amount of floc available. Overall, the level of salinity at which the flocs were formed decreased the CO_2 flux rate ($F_{2,33}$=7.782, p=0.0.0017; Fig. 3.9A). Using the CO_2 flux rate and floc amount, the predicted time until carbon depletion was calculated. For flocs produced within the first 30 minutes of salinity exposure, the POC would sustain the microbial community for 4, 5, and 12 days for the control, 4 ppt, and 8 ppt, respectively (Fig. 3.9C). While the predicted days until depletion were relatively short amounts of time, the microbial inoculum was exposed to only a subset of the floc mass that precipitated in the sediment traps.

When data was scaled up to determine the carbon dioxide production as a percent of the total floc present, the level of salinity used to produce flocs significantly decreased the total percent of floc respired per day ($F_{2,33}$=10.901, p=0.0.0002; Fig. 3.9B). Salt-induced flocs produced within the first 30 minutes of salinity exposure had much lower respiration rates of 0.18 and 0.11 mg CO_2 m^{-2} day^{-1} mg $AFDM^{-1}$ floc for the 4 ppt and 8 ppt treatments, respectively, compared to the respiration rate of 0.29 mg CO_2 m^{-2} day^{-1} mg $AFDM^{-1}$ floc for the freshwater

produced flocs. Using the averages for respiration rates of flocs formed in 30 minutes of salinity exposure, the average aerobic respiration of the salt-induced flocs was reduced by 50% compared to the freshwater flocs (Fig. 3.9A). Averaging over flocs produced in the first week, respiration in the control was 0.17 mg CO_2 m^{-2} day^{-1} mg $AFDM^{-1}$ floc, the 4 ppt treatment was 0.11 mg CO_2 m^{-2} day^{-1} mg $AFDM^{-1}$ floc, and the 8 ppt treatment was 0.10 mg CO_2 m^{-2} day^{-1} mg $AFDM^{-1}$ floc. The respiration of freshwater produced floc was 63% greater than the salt-induced flocs at 1 week of salinity exposure.

Nutrients and Algal Biomass

Phosphorus concentrations increased with time, but there was no effect of salinity ($F_{3,44}$=4.298, p=0.0096 and $F_{2,44}$=0.054, p=0.947, time and salinity, respectively; Fig. 3.10A). In the first 30 minutes, PO_4 increased from 0.0199 to 0.0292 mg/L in the presence of salinity and from 0.0468 to 0.1069 mg/L in the first 24 hours. When the initial exposure periods were removed, results show that salinity exposure and the length of initial salinity exposure are important with elevated salinity and less exposure significantly reducing P in the water column ($F_{2,36}$=8.149, p=0.0012 and $F_{2,36}$=8.884, p=0.0007, salinity and time, respectively; Fig. 3.10B). While the length of initial exposure was not significant, differences were observed. When samples lacked the first 30 minutes of salinity exposure P decreased from 0.0639 to 0.0054 mg/L in the presence of salinity. When the first 24 hours of salinity exposure are removed, P decreased from 0.1018 to 0.0336 mg/L.

Total dissolved nitrogen concentrations increased significantly over time in the presence of elevated salinity ($F_{6,44}$=2.391, p=0.0437; Fig. 3.11A). In the time treatment of 4 weeks, the salinity treatments continued to show increased TDN values while the control chambers yielded decreasing TDN values. When the initial exposure periods were removed, results showed that

salinity and time had no effect ($F_{2,36}$=1.488, p=0.239 and $F_{2,36}$=0.597, p=0.556, salinity and time, respectively), meaning that the initial exposure to salinity has no effect on the total nitrogen concentration (Fig. 3.11B).

There were significant effects from salinity level and length of salinity exposure on ammonium concentrations ($F_{2,44}$=16.133, p<0.0001 and $F_{3,44}$=3.294, p=0.0291, salinity and time, respectively; Fig. 3.12). The interaction of elevated salinity level over time did not significantly increase ammonium ($F_{6,44}$=0.876, p=0.5201, salinity and time interaction; Fig. 3.12) and data showed a visible decrease between the 1 week and 4 weeks sampling time points, likely correlated to the observed increase in chlorophyll *a* or rainfall. Upon comparison of TDN and NH_4^+-N concentrations, it is likely that most, if not all, of the nitrogen is in the form of ammonium, and the variability was attributed to handling time altering nitrogen species or using different methods to determine nitrogen.

As nutrients were mobilized into the water column and DOC concentrations decreased, there was an effect on the absorbance of PAR. While neither salinity nor length of exposure affected the average absorbance in the photosynthetically active radiation spectrum over the course of 4 weeks ($F_{2,44}$=0.217, p=0.8053, $F_{3,44}$=1.368, p=0.2649, salinity and time, respectively; Fig. 3.13A), the elevated salinity over time interaction significantly decreased the average absorbance ($F_{6,44}$=2.6, p=0.0303). The significant interaction suggests that the combined effect increases water clarity, creating an opportunity for photosynthesis. The initial periods of saltwater exposure had no effect on the average absorbance of PAR ($F_{2,36}$=1.851, p=0.1718 and $F_{2,36}$=1.096, p=0.3450 salinity and time, respectively; Fig. 3.13B), meaning that initial periods of salinity exposure are not important to increasing light penetration but that over time, water clarity increases.

Chlorophyll *a* concentrations were significantly increased in response to salinity and over time ($F_{2,44}$=9.48, p=0.0004; $F_{3,44}$=3.834, p=0.0159, salinity and time, respectively; Fig. 3.14A) but the interaction between salinity and length exposed was not significant. While there is not much change from 30 minutes to 24 hours to 1 week, the chlorophyll *a* concentrations increase greatly at the 4 weeks time point. While this suggests that salinity has a delayed effect, ultimately increasing chlorophyll *a* productivity, the increase in algal biomass was observed after the storm events. Furthermore, chlorophyll *a* concentrations observed in the field experiment were much higher than background concentrations obtained from the Midpoint (10µg/L) but the increase in response to salinity was observed over a naturally occurring salinity gradient. When initial exposure to salinity was removed, results shows that there was an effect of time but not salinity on the concentration of chlorophyll *a* ($F_{2,36}$=4.76, p=0.0146; Fig. 3.14B).

Chapter 4: Discussion

My results show that flocculation increased with salinity and exposure time, until storm events inundated mesocosms, stimulating turbulence which led to POC resuspension. Initial exposure to salinity was critical to the flocculation process. While it has been suggested that the formation of POC may increase the bioavailability of dissolved organic matter to heterotrophs (Mulholland 1981), in my experiment, aerobic microbial decomposition of the salt-induced flocs was reduced by 50%. The decrease in respiration suggests that the process of flocculation converts DOC into recalcitrant POC, reducing the overall bioavailability of the organic carbon. The percent of original DOC converted to POC increased with salinity over time, suggesting that DOC is used in the flocculation process. This increase in POC production could increase organic C sediment accumulation, which could lead to increases in surface elevation. While it is known that climate change reduces organic matter retention at the ecosystem scale, my results suggest that salinity perturbations may aid in soil accretion (e.g. via carbon sequestration) to increase the elevation, potentially reducing the effects of sea level rise (Loomis & Craft 2010; Schile *et al.* 2014). My results suggest that salt-induced flocculation likely reduces dissolved phosphorus. Salinization did not affect algal biomass but, over time, increased algal biomass from 130 µg/L in control to 211 µg/L in salinized treatments. These reported values were well above the regulated water quality standard for chlorophyll *a* in NC (40µg/L). While salinization may reduce subsidence rates through increased sediment deposition, it negatively affected water quality (Brouns *et al.* 2014). These results show that salinization of restored wetlands may significantly reduce the export of carbon to estuarine systems through flocculation and subsequent deposition, and this process reduces the bioavailability of flocculent matter.

Long Term Data

In the long-term water samples, I found that seasonal variations in conductivity and chloride increased POC concentrations in the forested wetland (Inflow) but did not increase POC concentrations in the restored wetland sites (Midpoint and Outflow). Though, the salinity observed in this dataset was much lower than those in my experiments. At all three sites, NO_3^- was included in the best model as a control determining POC concentration (Table 3.2). While nitrate was included in the model, it had positive and negative controls on POC concentrations, suggesting that there is not a direct link. POC concentrations were variable overall and posed challenges for determining controls on flocculation in the field as obtained through water samples because flocs move gravitationally to settle out in the sediments. According to the model selection procedure, at the Inflow and Outflow, NO_3^- showed a negative correlation with POC concentration during periods of low temperature and pre-growing season fertilizer additions (Table 3.2 & Fig. 3.1A, G). The Inflow and Outflow had elevated POC concentration compared to the Midpoint (Fig. 3.1J). The negative relationship between NO_3^- and POC concentration at the Inflow could have been caused by nutrient runoff from the adjacent farm fueling algal and macrophyte production, which led to increased decomposition. At the Midpoint, NO_3^- was correlated to an increase in the amount of POC (Table 3.1). This model only included NO_3^- and did not explain much of the variability (r^2=0.04) in POC concentration (Table 3.2). While DOC was included in the model as a negative control on POC concentration at the Outflow, the relationship was not significant (Fig. 3.1H). Also, it is possible that fertilizer inputs (e.g. NO_3^-) stimulated microbial mineralization of DOC, limiting the potential for flocculation. Further supporting increases in POC concentration during warmer periods, temperature increased POC concentration (Table 3.2).

As the linear relationship and model predicted, POC concentrations were positively correlated with DOC at the Inflow and negatively correlated with DOC at the Midpoint and Outflow (Table 3.2 & Fig 3.1B, E, H). I did not observe a relationship between POC concentration and increased chloride. However, this conclusion was to be expected as POC was measured from surface water samples, not sediment traps, where POC would have been captured, if present. I observed increases in response to higher temperatures (i.e. during the growing season and aquatic plant productivity). Increases in POC concentration can occur naturally in light-intensive conditions and during the growing season when plant exudates are elevated (Dzierzbicka-Głowacka *et al.* 2011). During the years of 2012-2014, TOWeR did not experience a saltwater intrusion event (e.g. salinity exceeding the brackish limit of 0.5 ppt) as previously described (Ardón *et al.* 2016). Overall, these results suggest potential controls on flocculation and show that nutrients are involved in the flocculation process, be it through direct controls stimulating or hindering microbial decomposition or through increasing plant productivity and subsequent DOC release.

Laboratory Assays

In the laboratory assays, I did not observe reversibility of POC due to turbulence (Fig 3.2F). Increased turbulence and water velocity attributed to storm events have the potential to reverse the flocculation process through OM resuspension (Grant *et al.* 1997; Bonnin *et al.* 2006). I may have not seen an effect due to the short length of simulated turbulence (15s). However, it is likely that during heavy rainfall, the dilution with freshwater plays a role to reduce the ionic concentration of the water, promoting resuspension of OM. There was clear evidence that salinity stimulated flocculation through the conversion of DOC molecules into larger flocculent particles (Fig 3.2A, B). The percent of original DOC converted to POC ranged from

11-28% (Fig 3.2C), which was higher than previously reported percentages (4-20%) (Mulholland 1981). Specific UV absorbance increased with salinity (Fig. 3.2E), suggesting that aromatic carbon remains in suspension in the water column during the flocculation process. Light penetration of water samples increased greatly when flocs were removed via filtration methods (Fig. 3.2D). As POC formation and light penetration increased with salinity, there is potential for flocculation to increase light penetration through the water column, increasing primary productivity of aquatic macrophytes.

Mesocosm Experiment

Organic Matter Dynamics

In the field mesocosm experiment, artificial saltwater addition of 8 ppt increased POC formation, converting 3X more DOC to POC than the control within 30 minutes and the first 24 hours (Table 3.3). Salt-induced flocculation continued to increase for 1 week, converting 2.5X more DOC to POC than the control. My results show that studies examining salt-induced flocculation in a field setting that sampled along a pre-existing salinity gradient, likely failed to capture a significant portion of POC formation and underreported values. The initial salinity exposure is important as past studies have not been able to fully elucidate the effects of salinity on flocculation in the field because they were conducted in a lab or over a naturally occurring salinity gradient (Thill *et al.* 2001; Verney *et al.* 2009). While this research examined flocculation in salinity levels up to 8 ppt, based on the literature it is likely that DOC would continue to flocculate in low salinities (i.e. up to 20 ppt) and serve as the dominant form of organic matter transformation (Ertel *et al.* 1991).

Contrary to my initial hypotheses, POC formation decreased greatly at 4 weeks exposure (Fig. 3.4A). Upon arrival to the site at 4 weeks, mesocosms were noticeably inundated, as evidenced in the water level data (Table 3.3). Two storm events occurred, which stimulated turbulence and diluted salinity to 1-2 ppt depending on initial salinity concentration. As POC concentrations decreased with increased salinity and dilution, my results support arguments for resuspension and floc reversibility (Teodoru *et al.* 2013). Furthermore, if storm events (e.g. turbulence and dilution) led to POC resuspension, it was more prominent in the salt treatments than in the control, which continued to increase (Fig. 3.4A). Because floc dissolution was observed only in the salinized treatments, it is possible that salt-induced flocculation may form a cyclic pattern and not remain sequestered in the sediments. This dissolution pattern was also driven by a freshwater dilution, which decreased the salinity concentration. It is unlikely that precipitation events would deliver enough freshwater to an ecosystem experiencing saltwater intrusion. However, as shown through the lab assays, increased water velocity alone was not effective in the reversibility of flocculent matter, but a dilution with freshwater was necessary for successful resuspension. In the mesocosms treated with elevated salinity, my results show that the DOC concentrations were higher in the salinity chambers than the control chambers at 4 weeks (Table 3.3). My results suggest that there is potential for resuspension of deposited flocs with increased velocity and turbulence, typically attributed to a storm event (Van Raaphorst *et al.* 1998; Jago *et al.* 2007). While the lab assay determining the potential for reversibility of flocculation was unsuccessful, flocculation was reversed by a storm event in the field. It was likely that the turbulent event administered in the lab assay was not strong or long enough to reverse flocculation (Nan *et al.* 2015).

Although clear evidence was observed for salt-induced flocculation, DOC concentrations varied little throughout the field experiment (Fig. 3.3A). Previous reports show that salinity exposure in estuaries decreases DOC concentration from an initial concentration of 3.82 mg/L to 1.47 mg/L (He *et al.* 2010). This 2.5X decrease was similar to the decrease in DOC concentration that I observed in the lab assays. The small decrease in DOC concentration observed in the field experiment could be attributed to resuspension of POC, in situ DOM transformation, and algal release of exudates into the water column (Pakulski *et al.* 2000; Fukushima *et al.* 2001; Mulholland 2003). Supporting my results, published research in estuaries with salinities ranging from 1.5 – 9.5 ppt, the maximum flocculation occurred in low salinity conditions (1.5 – 5.8 ppt) with DOC concentration as the primary control in flocculation processes (Biati & Karbassi 2010). While flocculation was mediated by salinity, there may also have been controls from the constituents of suspended sediments and the concentrations of organic matter in the water column that determined the extent of flocculation (Verney *et al.* 2009).

Field saltwater addition transformed up to 68% of DOC into POC over 1 week exposure, which was higher than previously reported (Sholkovitz 1976a; Mulholland 1981), and was attributed to the DOC concentrations here that ranged from 20–30 mg/L (Table 3.3) compared to 4–20 mg/L in the published studies. Further supporting turbulence-induced resuspension of flocs, POC decreased after 1 week of salinity exposure correlating with the storm events. Initial salinity exposure was crucial to converting the maximum amount of original DOC to POC (Fig. 3.4C). Since the Midpoint in TOWeR is considered a low flow system, it is unlikely that resuspension would occur without a storm event. Even with the introduction of a storm, the rainfall needs to be large enough to effectively dilute the salinity to freshwater conditions, which is unlikely to

occur in the event of a saltwater intrusion event. My results suggest that the likelihood of floc being converted back to DOC without a storm event is small, which leads to an increase in sedimentation of POC and, thus, contributes to carbon sequestration (Von Wachenfeldt & Tranvik 2008). As sedimentation of POC occurs, microbial access to carbon is reduced, decreasing degradation (Kim & Kim 2010). Further increasing carbon sequestration potential, elevated salinity induces light-mediated flocculation as well as salt-induced flocculation (Sobek *et al.* 2007).

Coastal wetlands receiving terrestrial DOM input are less sensitive to photobleaching and photoremineralization due to the high relative percent of aromatic carbon (Minor *et al.* 2006). My results suggest that the DOC remaining suspended in the water column after flocculation was not highly aromatic and, thus, was not characterized as recalcitrant (Weishaar *et al.* 2003). Approaching 4 weeks, there was a rapid decrease in SUVA values (Fig. 3.6A), correlating with inundated mesocosm and increased DOC concentration in the salinity treatments when compared to the control. The initial exposure to salinity flocculated aromatic carbon, as shown through the significant decrease of SUVA in water samples without the initial periods of salinity exposure (Fig. 3.6B). Without the initial period of salinity exposure, SUVA values decreased, which meant that the relative percent of aromatic carbon decreased, suggesting that initial flocculation aggregates aromatic DOC preferentially. Due to the minimal differences with and without the initial 30 minutes of salinity exposure, flocculation likely could not convert enough labile DOC to POC to impact the relative percent aromaticity of DOC. Conversely, in the first 24 hours of salinity exposure, DOC was converted to POC without source discrimination (e.g. labile and recalcitrant carbon), leading to an overall reduction in SUVA values. These results support my

hypothesis that recalcitrant carbon is flocculated first and the process of flocculation converts a significant portion of DOC within the first 24 hours of salinity exposure.

Salinity did not alter the $\delta^{13}C$ signature (Fig. 3.7D). As salinity increased, I observed a slightly less depleted carbon signature indicating autochthonous or aquatic origin (McCallister *et al.* 2006) and then a movement towards more depleted, allochthonous carbon. The $\delta^{13}C$ lower limit signatures of aquatic macrophytes and algae are at -26‰ and -27‰, respectively, and the signal of terrestrial plant material is at -30‰ (Michener & Lajtha 2007). The only observable change due to salinity was from floc formed in 24 hours of salinity exposure and floc formed in 1 week where the $\delta^{13}C$ signature from a more depleted and terrestrial carbon source (-29.4‰) to a slightly more autochthonous source (-28.75‰) (Ray *et al.* 2015). While I hypothesized that the flocs would be comprised of more allochthonous POC, due to the degradation resistance, my results support that the salt-induced flocculation aggregates autochthonous carbon as well. Caution should be used when interpreting these results because the average for the salinity treatments was more depleted than the established autochthonous carbon signature.

While the $\delta^{13}C$ signal shifted slightly, my carbon isotope signatures were within the range of freshwater POM, which has a documented signal from -28.2‰ to -30‰ (Raymond & Bauer 2001; McCallister *et al.* 2004). In previous studies, the microbial degradation of POC was preferential for high-energy carbohydrates (e.g. polysaccharides), and resulted in a more depleted $\delta^{13}C$ signature and more lignin-rich POC remained in the floc (Benner *et al.* 1987). While coastal ecosystems receive mostly terrestrial inputs throughout the year, during summer, autochthonous carbon sources were more depleted due to autotrophic production and remineralization (Cifuentes *et al.* 1988), which may have affected my results because I conducted my field experiment during June and July of 2015.

Heterotrophic Respiration

While a recent review found that salinization inhibits the microbial community and respiration of organic carbon (Rath & Rousk 2015), little attention has been paid to the effect of salinity on POC formation and the subsequent bioavailability. To my knowledge, research has focused on the microbial degradation of DOC rather than salt-induced flocculation (Köster *et al.* 2005; Herlemann *et al.* 2014; Koch *et al.* 2014a). Limited research has been conducted on floc bioavailability but proxies have been developed such as the correlation between phosphorus concentration and autochthonous carbon quality to predict aerobic respiration in a Florida wetland (Pisani *et al.* 2015).

When exposed to flocs formed in the initial flocculation time window (e.g. first 30 minutes and 24 hours), respiration was lower in salt treatments than in the control (Table 3.4), suggesting that the process of salt-induced flocculation reduces the bioavailability of POC for heterotrophic microbial respiration. Aerobic respiration of salt-induced floc was lower than the control because of increased POC formation that may have stabilized OM, reducing microbial access to carbon. As a recent meta-analysis found, POC formation of organic matter protects the soil carbon pool from microbial degradation because it is physiochemically protected (Castellano *et al.* 2015). Freshwater produced flocs are more accessible for microbial degradation (Tremblay & Gagné 2009). My results showed that a salt-induced floc had a more depleted carbon isotope signature, suggesting that microbial access to the floc is reduced, decreasing respiration. In another study with low DOC concentrations, flocculation increased aerobic respiration (Muschenheim *et al.* 1989).

When exposed to flocs formed over 1 week of salinity exposure, microbial respiration decreased even with significantly more POC formed (Fig. 3.9) compared to flocs formed within

the first 30 minutes and 24 hours. These results suggest that the labile portion of OC that was flocculated was respired in situ prior to the 1 week collection time point. The portion of POC that is labile is more sensitive to microbial degradation in elevated salinity, resulting in sequestration of the more recalcitrant, humic fraction of POC (Setia *et al.* 2011). Supporting my results, a previous study noted that terrestrially produced DOC is not bioavailable for microbial use because salinity reduces the lability of humic compounds, converting any autochthonous carbon sources into recalcitrant flocs (Søndergaard *et al.* 2003).

As flocs remained in the elevated salinity, the percent of total floc respired was reduced over time, suggesting that salinity increases recalcitrance of POC over time, and reduces the floc bioavailability. The percent of total floc respired impacts the carbon turnover rate, with shorter turnover leading to higher OM mineralization and longer turnover leading to soil accretion. Respiration of salt-induced flocs decreased compared to freshwater flocs and the predicted time until depletion ranged from 4-12 days, supplementing literature that POC turnover is approximately 10 days in streams (Richardson *et al.* 2013). Consideration must be taken with these results because more flocculent matter was produced that could sustain the community but the carbon was still less accessible. In another study, increasing salinity to 8 ppt reduces microbial respiration by as much as 70% over a 27 day incubation period, but at lower salinities, the microbial communities adjusted and respiration was only reduced by 10% (Asghar *et al.* 2012).

My results further supplement literature investigating effects of salinity on microbial respiration and associated osmoregulation by showing that salinity produced less bioavailable flocs (i.e. recalcitrant food sources), which led to decreases in carbon dioxide production (Glatzel *et al.* 2004; Asghar *et al.* 2012). In wetlands with terrestrially derived DOC, increased salinity

promotes flocculation and the salt-induced flocs are deposited in the sediments, where microbial respiration is reduced. Potentially promoting respiration, when a freshwater wetland with legacy nutrients experiences a saltwater inundation, nutrients are released from soils, enhancing algal growth. Algal exudates can stimulate a priming effect, and result in higher respiration. Autochthonous (e.g. algal) leachates that stimulate microbial decomposition and respiration of terrestrially derived OC is known as the priming effect (Hotchkiss *et al.* 2014).

Nutrients and Algal Biomass

In soils with land use legacies (e.g. high nutrient concentrations adsorbed onto soil OM), salinization results in an increase in phosphate and ammonium concentrations (Brouns *et al.* 2014). My results support literature that salinity increases nitrogen and total phosphorus via soil desorption followed by an increase in chlorophyll *a*, a proxy for algal biomass (Håkanson & Eklund 2010). Similarly, in soils with legacy fertilizer, salinity led to a phosphate release (Baldwin *et al.* 2006). However, resorption of P can happen by adsorption onto Fe-DOM complexes which were bound to larger aggregates, resulting in floc formation (Maranger & Pullin 2003). My results support my hypothesis that phosphorus was desorbed from Fe in the sediments at the bottom of the chambers in the presence of salinity and that initial flocculation processes may have used phosphorus in the aggregation process, depositing the nutrient back into the sediments. My results established a potential mechanism for phosphate removal and nutrient retention (Fig. 3.10B). When soils with legacy fertilizers in tidal freshwater floodplain forests were inundated at low salinities (2-5 ppt), PO_4-P was rapidly desorbed which increased the P sorption potential when compared to non-sodic soils (Jun *et al.* 2013). In another study, the maximum removal of nutrients in estuaries via flocculation occurred in low salinity conditions with DOC, N, and P being used up in flocculation processes (Gregory 1989; Lisitsyn 1995). In

my study, phosphorus remained constant in the control treatment, suggesting that salinity is responsible for the nutrient release.

Salinity increased total dissolved nitrogen and ammonium, supporting that saltwater intrusion events into wetlands with fertilizer legacies are susceptible to algal blooms (Fig. 3.11A & 3.12). As saltwater inundates soils with fertilizer legacies, previous work in this TOWeR site has shown that ammonium was released from soils through multivalent cation exchange and increased overall nitrogen export (Ardón *et al.* 2013). After the storm events diluted mesocosms, ammonium remained elevated and mobilized in the water column, suggesting that there may be a delayed effect of inundation and ammonium was not able to adsorb back onto the soils. Ammonium concentrations were higher than TDN in 25 of the 60 samples, this should not happen. The differences between ammonium and TDN concentrations were likely due to different methods used to determine the concentrations, holding time of samples, and the preservation methods applied to the samples. If water velocity was low (e.g. observed at the Midpoint in TOWeR), ammonium was used preferentially over nitrate for phytoplankton growth (Dortch 1990). NH_4^+-N concentrations observed in elevated salinity conditions were within optimal growth ranges for many unicellular algae (Collos & Harrison 2014). As salinity concentrations decreased due to rainfall diluting the mesocosms, freshwater algae had a reduced osmotic stress and were able to assimilate the free ammonium, promoting algal blooms.

As salinity induced flocculation and subsequent deposition of POC, I observed increases in water clarity (Fig. 3.13A). Supporting my observations, published work shows that as salinity increases in coastal ecosystems, suspended particulate matter decreases, which leads to an increase in light penetration, as measured through Secchi disk depth (Håkanson 2006). In another study, increased salinity led to flocculation of high molecular weight DOM, and with low

molecular weight DOM compounds being suspended in the water column (Helms *et al.* 2008). These remaining low molecular weight DOM compounds absorbed less light, resulting in more light penetration of the water column. Other studies have found that salt-induced flocculation decreases absorbance of PAR and increases light availability, promoting photosynthesis (Schedlbauer *et al.* 2010). While my results for water color parameters were nonsignificant, I observed differences in water color. In the field experiment, absorbance in the PAR range did not increase with salinity, supporting that initial flocculation did not convert enough DOC to POC to increase water clarity and light penetration. These results suggest that salt-induced flocculation may not promote photosynthesis for benthic autotrophs.

Previous work has shown that in freshwater ecosystems, due to low salinity tolerance, chlorophyll *a* concentrations rapidly decreased (28.21 µg/L to 4.17 µg/L) until an equilibrium was reached when exposed to salt concentrations greater than 4 ppt (Nielsen *et al.* 2003; He *et al.* 2010). In the mesocosm experiment, chlorophyll *a* did not change initially, but then increased significantly at 4 weeks (Fig. 3.14A). There are several potential explanations for the significant increase in algal biomass. The addition of the mesocosms into the Midpoint provide a stable substrate to promote algal growth, potentially creating an experimental artifact. Furthermore, samples taken from the Midpoint revealed that chlorophyll *a* concentrations were much lower than observed here (10 µg/L). Because the background chlorophyll *a* concentrations observed at the Midpoint were much lower than what was observed during the field experiment, it was likely that the chlorophyll *a* concentrations were not representative. However, the increase in chlorophyll *a* in response to salinity was representative to observations made in a naturally occurring salinity gradient. Similarly, chlorophyll *a* concentrations in other forested wetlands range from 1-31 µg/L (Lane *et al.* 2003). Another study conducted during late summer when

phytoplankton concentrations are expected to be elevated found that chlorophyll *a* ranged from 12.6 – 54.9 µg/L (Sánchez-Carrillo & Álvarez-Cobelas 2001), which was much lower than averages observed here (211 µg/L). Spanning several trophic states, wetland lakes exhibit chlorophyll *a* concentrations ranging from 3.2 – 41.2 µg/L (Bayley & Prather 2003), suggesting that the observed increases here were likely due to an experimental artifact rather than a true response to salinity.

Alternatively, if the chlorophyll *a* concentrations observed were representative of saltwater intrusion events, my results suggest that salt induced nutrient desorption from soils and subsequent deposition of OM and flocs into the sediments, led to decreased turbidity and increased water clarity, promoting photosynthesis (Lane *et al.* 2007). Since macrophytes are negatively impacted by salinity, elevated chlorophyll *a* concentrations at 4 weeks were likely due to the time required to adjust and equilibrate with the salt water (Sim *et al.* 2006; Cant *et al.* 2010). Prior to the sampling point at 4 weeks, mesocosms were inundated, and salt concentrations decreased (Table 3.3), creating the opportunity for algae to utilize the excess nutrients. OM availability controls floc composition (Neto *et al.* 2006) and in the presence of algal exudates, a positive feedback loop may occur that enhances POC formation and sedimentation processes due to phytoplankton exudates creating bridges between forms of organic carbon that stimulates flocculation (Von Wachenfeldt & Tranvik 2008).

Conclusions

In conclusion, I found consistent results that salinity is altering carbon and nutrient concentrations in this restored wetland. Despite not documenting high salinity, the field data showed that POC did increase in one of the sites with higher salinity. The laboratory assays showed the potential for salt-induced flocculation leading to a decrease in DOC and an increase

in POC. My field experiment confirmed that salinity leads to rapid flocculation of DOC to POC. Furthermore, I showed that this salt-induced floc is of lower bioavailability than floc produced under freshwater conditions. My chamber experiment also confirmed field and microcosm observations of increased ammonium concentrations due to added salinity. Furthermore, my field experiment suggests that this nutrient release coupled with storm events, can lead to increases in algal biomass. I also found evidence suggesting that flocculation can also lead to the retention of P through flocculation, though this merits further research. Overall, my results suggest that we need a more mechanistic understanding of the interactions among storms, salinity, nutrients, and carbon to better forecast the trajectory of coastal wetlands under future scenarios of altered storms and sea level rise.

TABLES & FIGURES

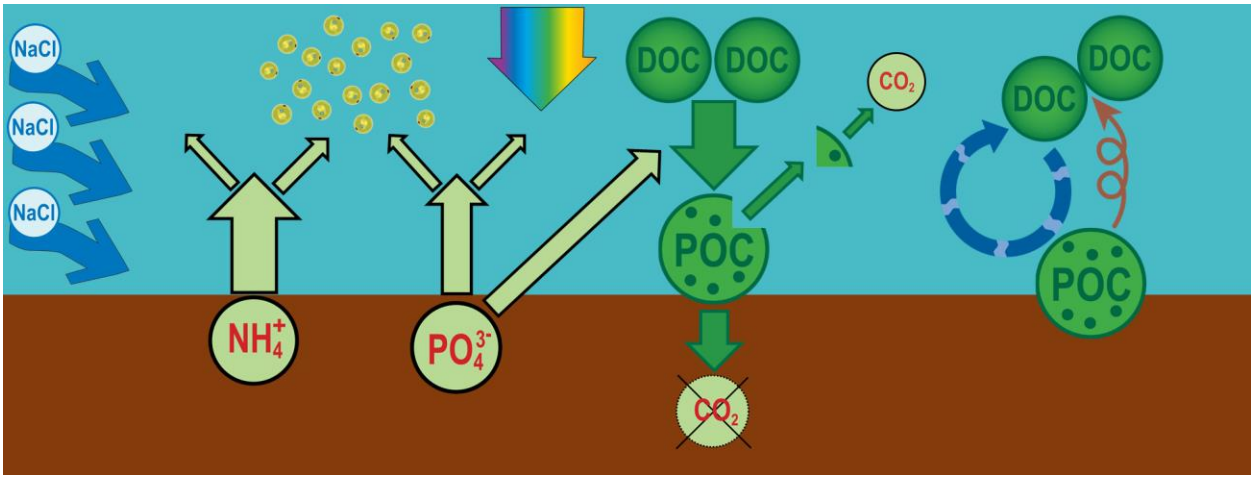


Figure 1.1. A conceptual diagram illustrating hypotheses. As saltwater intrusion occurs, ammonium and phosphate are desorbed from the sediments and mobilized into the water column. In the water column algae may use mobilized nutrients to promote algal growth. Phosphate may be used in the flocculation process, which converts DOC into POC, decreasing turbidity and increasing light penetration. POC moves gravitationally to settle in the sediments, where a portion of the floc is labile and is respired but the majority of the floc is sequestered due to recalcitrance. Storm events increase turbulence and dilution of saltwater, resuspending POC back into the water column.

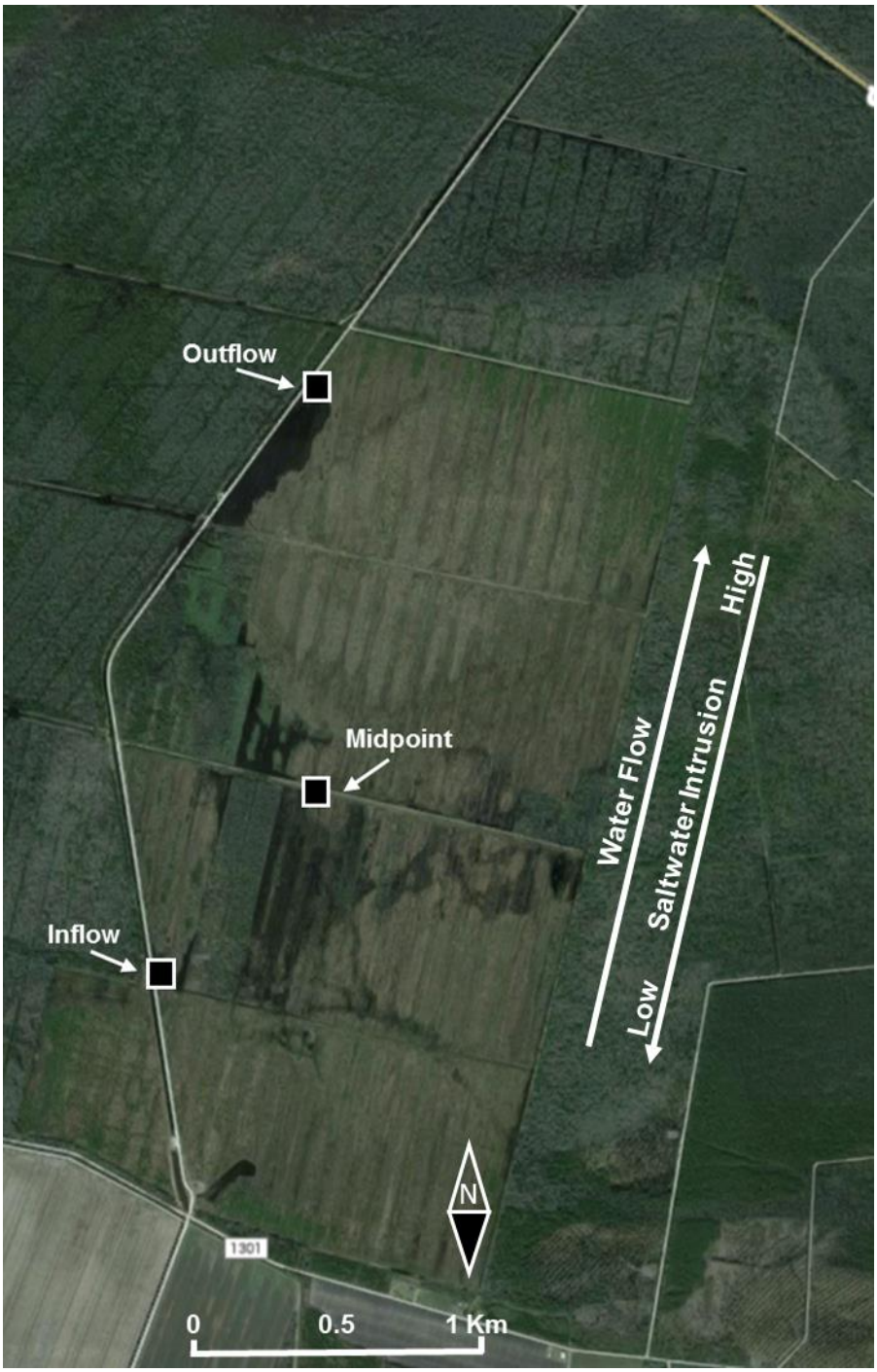


Figure 2.1. Map of Timberlake Observatory for Wetland Restoration (TOWeR).

Table 2.1. Recipe for artificial saltwater for a 1 L solution.

Substance	Unit of Measurement	Artificial Seawater
NaCl	g	23.944
K2SO4	g	4.011
KCl	g	0.679
NaHCO3	g	0.198
KBr	g	0.010
H3BO3	g	0.028
NaF	g	0.003
MgCl2-6H2O***	mL	57.053
CaCl2-2H2O***	mL	11.208
SrCl2-6H2O***	mL	0.912

*** Recipe to make

Substance	Deionized Water	Amount (g)
MgCl2-6H2O***	250	50.830
CaCl2-2H2O***	100	14.700
SrCl2-6H2O***	25	0.667

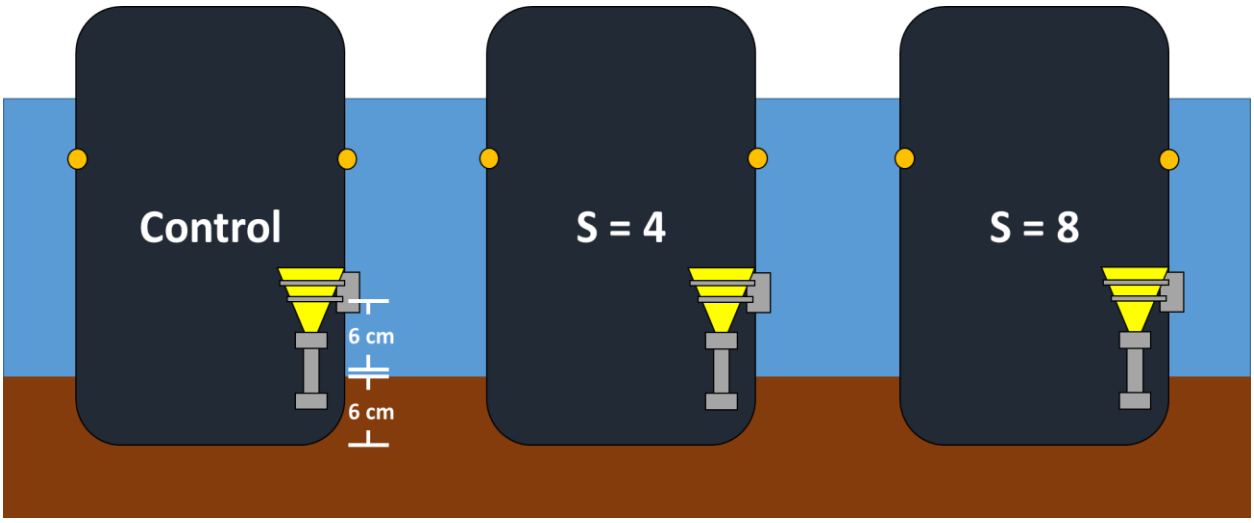


Figure 2.2. Diagram of experimental salt addition to mesocosm chambers (n=15). Each chamber is equipped with four sediment traps at the North, East, South, and West points in the mesocosm. Salinity is measured as parts per thousand and denoted as S.

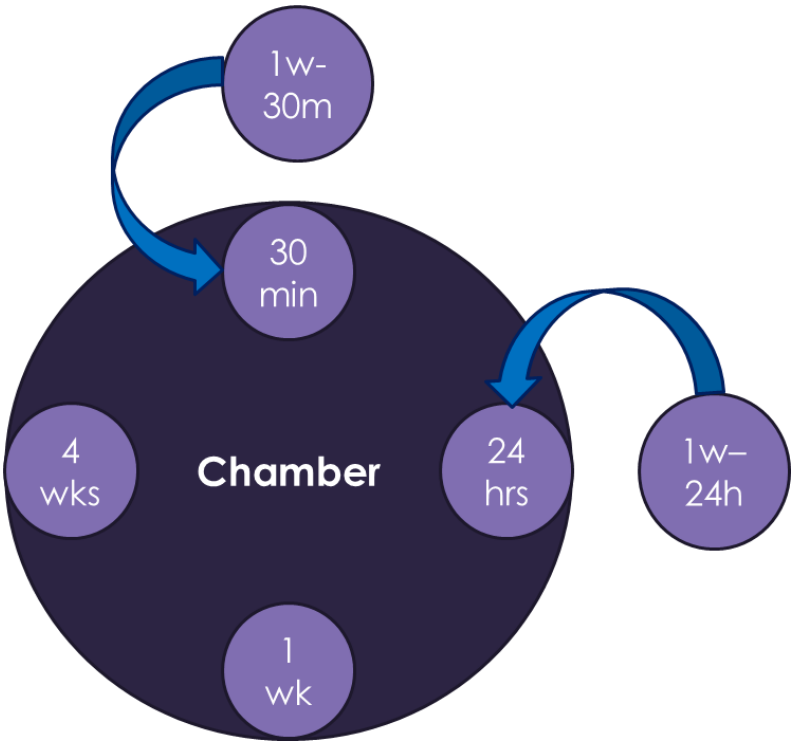
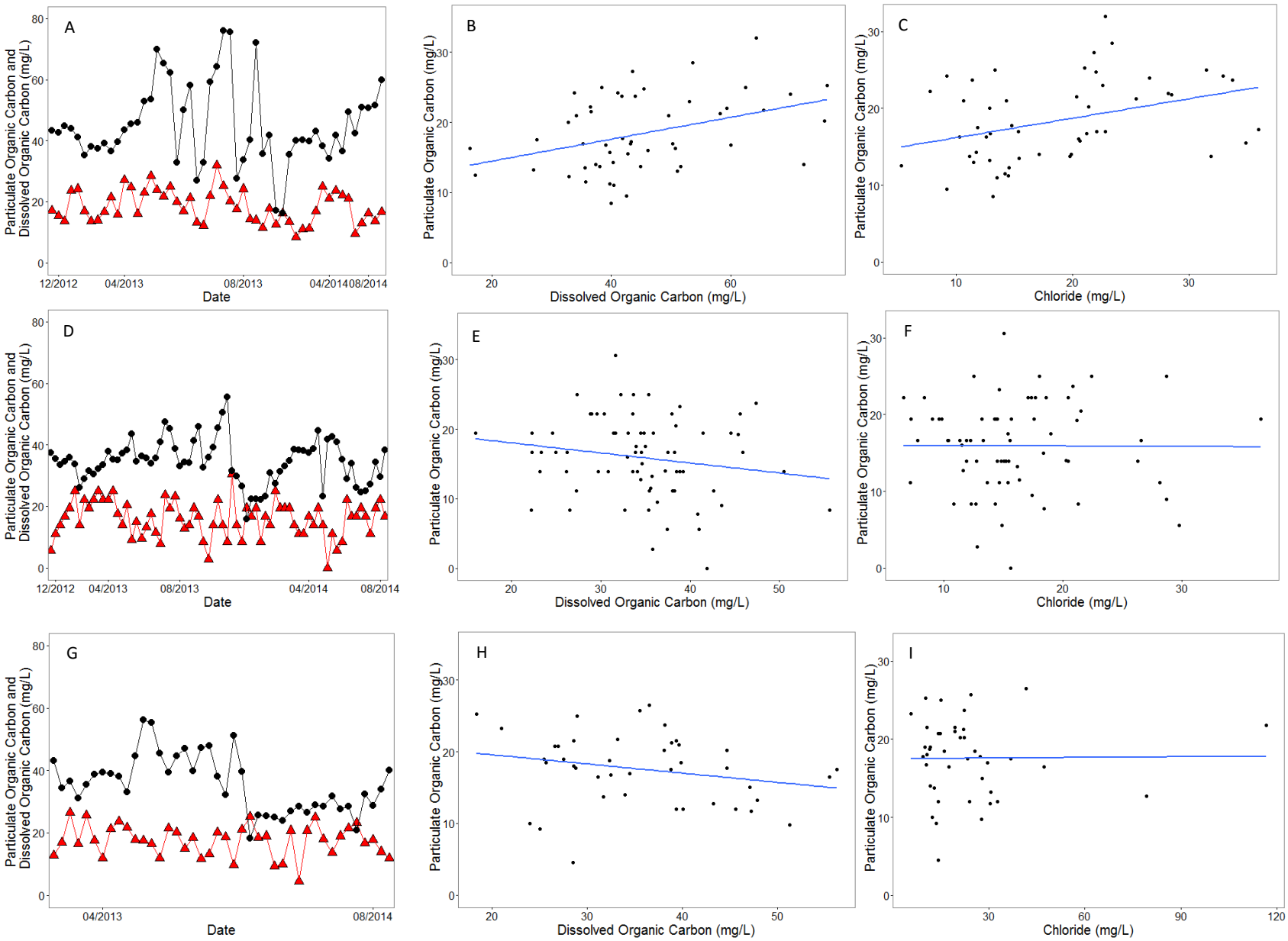


Figure 2.3. Timeline for sediment trap removal and replacements. Sediment traps were removed from the North position first and rotated removal around chamber in a clockwise fashion.


A
Particulate Organic Carbon and Dissolved Organic Carbon (mg/L)
Date
12/2012
04/2013
08/2013
04/2014
08/2014
B
Particulate Organic Carbon (mg/L)
Dissolved Organic Carbon (mg/L)
C
Particulate Organic Carbon (mg/L)
Chloride (mg/L)
D
Particulate Organic Carbon and Dissolved Organic Carbon (mg/L)
Date
12/2012
04/2013
08/2013
04/2014
08/2014
E
Particulate Organic Carbon (mg/L)
Dissolved Organic Carbon (mg/L)
F
Particulate Organic Carbon (mg/L)
Chloride (mg/L)
G
Particulate Organic Carbon and Dissolved Organic Carbon (mg/L)
Date
04/2013
08/2014
H
Particulate Organic Carbon (mg/L)
Dissolved Organic Carbon (mg/L)
I
Particulate Organic Carbon (mg/L)
Chloride (mg/L)

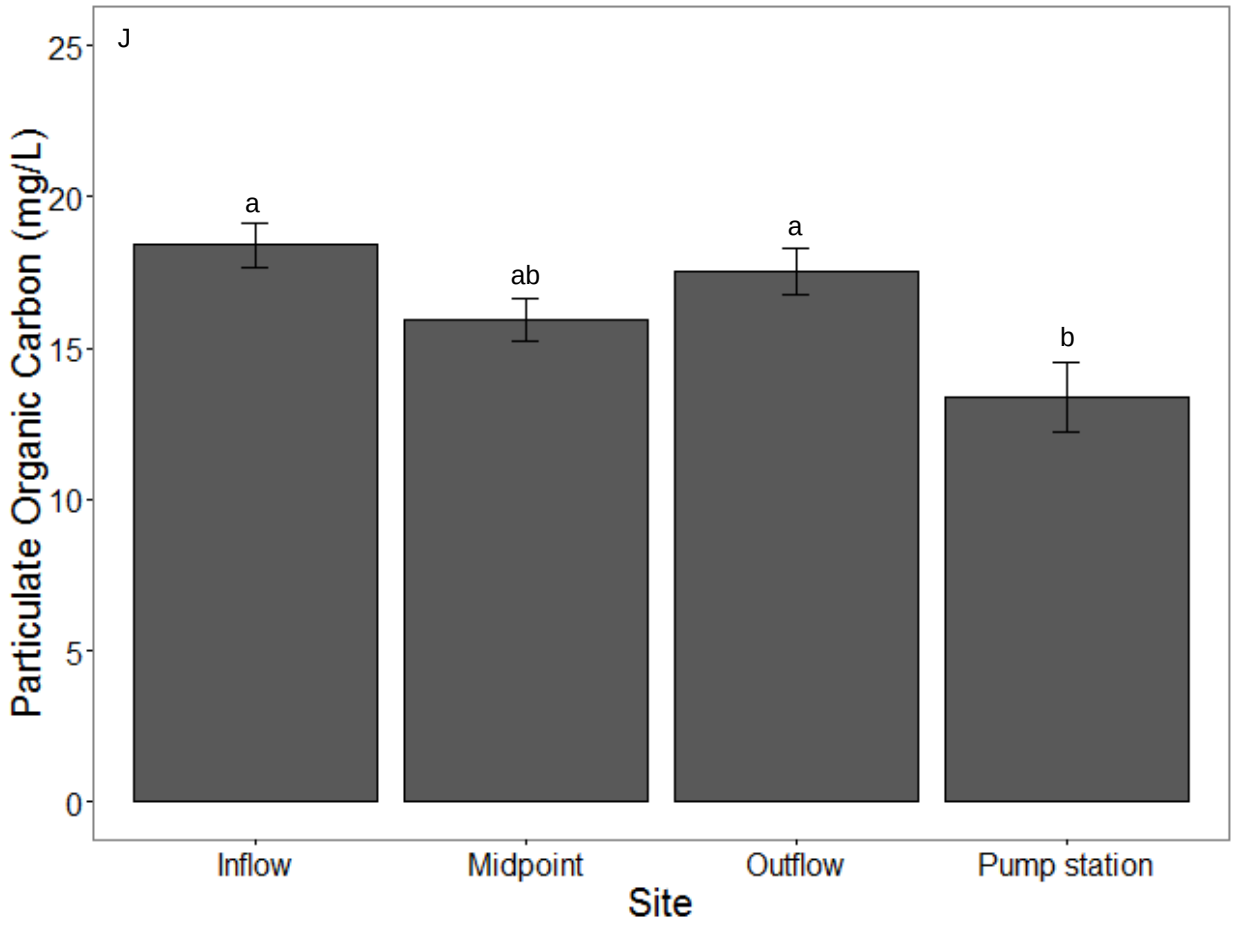


Figure 3.1. A) Time series of POC (▲) and DOC (●) dynamics for long-term field data at the Inflow. B) Linear relationship between DOC and POC at the Inflow (p=0.0051, r^2=0.15, n=49). C) Linear relationship between chloride and POC concentration at the Inflow (p=0.0086, r^2=0.13, n=49). D) Time series of POC (▲) and DOC (●) dynamics for long-term field data at the Midpoint. E) Linear relationship between DOC and POC at the Midpoint (p=0.1415, r^2=0.03, n=69). F) Linear relationship between chloride and POC concentration at the Midpoint (p=0.96, r^2=0.00, n=69). G) Time series of POC (▲) and DOC (●) dynamics for long-term field data at the Outflow. H) Linear relationship between DOC and POC at the Outflow (p=0.1329, r^2=0.05, n=49). I) Linear relationship between chloride and POC concentration at the Outflow (p=0.9451, r^2=0.00, n=40). J) POC formation by site with Tukey post-hoc test scores noted with error bars showing ± 1 standard error.

Table 3.1. Multiple linear regression fitted models for POC at the Inflow, Midpoint, and Outflow in TOWeR for the potential number of model coefficients (K), including the intercept of the linear model. Reported statistics include adjusted r_2 (r^2adj), Akaike's Information Criterion (AIC), the difference between each potential model and the best model AIC (Δ_i), and the residual sum of squares (RSS). The best model derived through the lowest AIC value is in bold and results are reported in Table 3.

Model	K	r^2adj	Cp	AIC	Δ_i	RSS
Inflow						
POC						
DOC	2	0.13	9.97	166.19	8.66	1226.6
Cl^-, Temp	3	0.25	3.17	159.63	2.10	1037.1
Cl^-, Temp, $NO3^-$	**4**	**0.29**	**1.45**	**157.53**	**0.00**	**957.0**
DOC, Cl^-, Temp, $NO3^-$	5	0.29	3.04	159.06	1.53	948.2
DOC, Cl^-, Temp, $NO3^-$, SRP	6	0.27	5.00	161.01	3.48	947.3
DOC, Cl^-, Temp, $NO3^-$, SRP, pH	7	0.25	7.00	163.01	5.48	947.3
Midpoint						
POC						
$NO3^-$	**2**	**0.04**	**-0.94**	**249.67**	**0.00**	**2259.3**
$NO3^-$, DOC	3	0.04	0.12	250.65	0.98	2227.0
$NO3^-$, DOC, pH	4	0.04	1.37	251.82	2.16	2201.3
$NO3^-$, DOC, pH, Cl^-	5	0.03	3.04	253.46	3.79	2189.9
$NO3^-$, DOC, pH, Cl^-, SRP	6	0.02	5.00	255.42	5.75	2188.7
$NO3^-$, DOC, pH, Cl^-, SRP, Temp	7	0.00	7.00	257.42	7.75	2188.7
Outflow						
POC						
DOC	2	0.03	2.75	135.23	1.25	955.4
DOC, $NO3^-$	**3**	**0.08**	**1.71**	**133.98**	**0.00**	**884.2**
DOC, $NO3^-$, Temp	4	0.08	2.88	135.04	1.06	864.7
DOC, $NO3^-$, Temp, Cl^-	5	0.07	4.11	136.15	2.18	864.7
DOC, $NO3^-$, Temp, Cl^-, pH	6	0.05	6.00	138.03	4.05	844.1

Table 3.2. Multiple linear regression parameter estimates for best model for POC concentrations at the Inflow, Midpoint, and Outflow. Significant estimates ($p<0.05$) are denoted with *.

Model	Inflow	Midpoint	Outflow
		POC	
R^2_{adj}	0.29	0.04	0.08
Intercept	8.01*	14.59*	25.05*
$NO3^-$	-4.37*	33.45*	-51.76
Cl^-	0.28*		
Temp	0.33*		
DOC			-0.16*

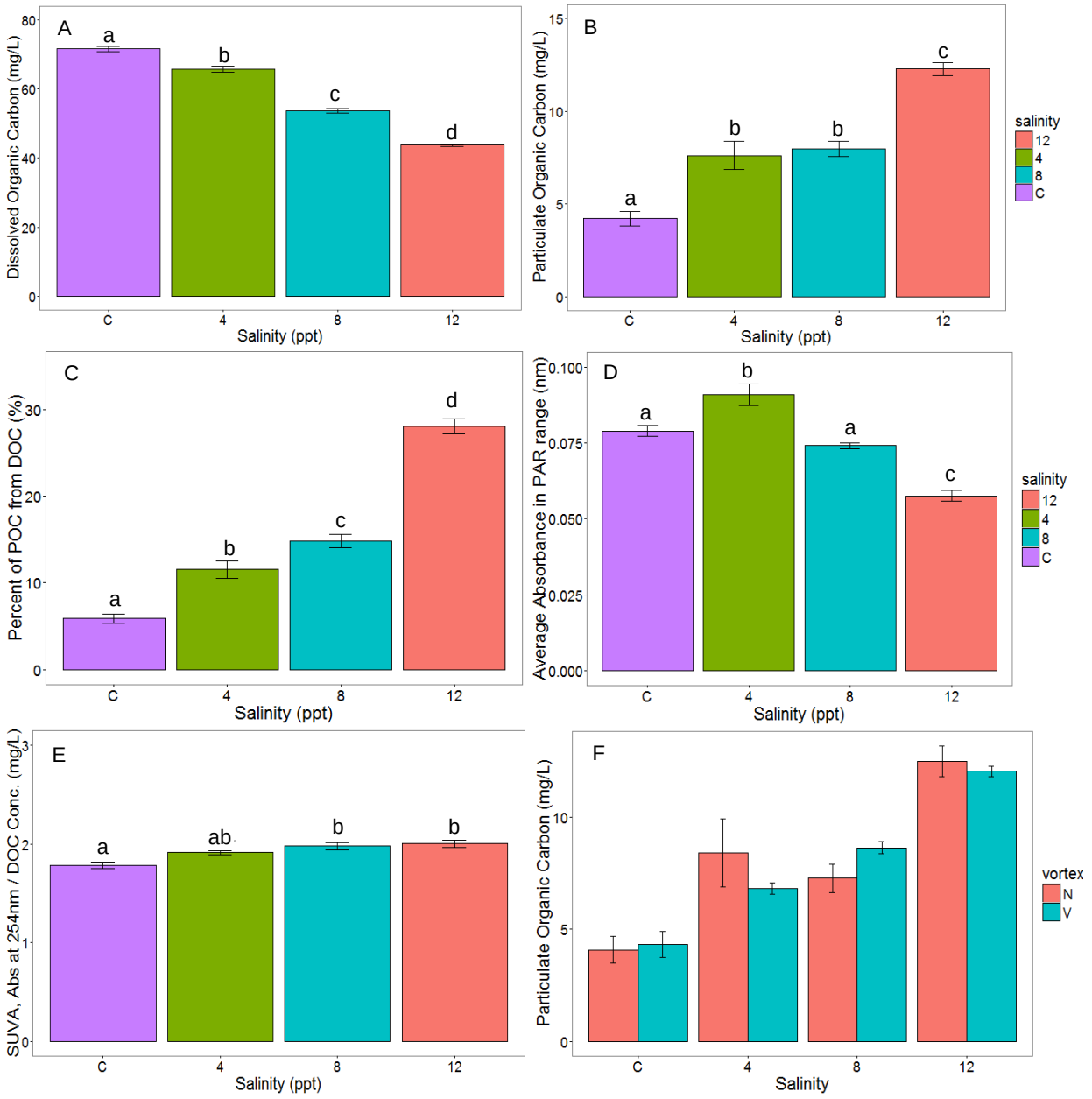


Figure 3.2. Mean values for experimental lab assays for dissolved organic carbon (A), salt-induced flocculation (B), percent of original DOC converted to POC (C), average absorbance in the PAR spectrum (D), specific UV absorbance at 254nm (E), and the effects of turbulence on salt-induced flocculation (F) in response to salinity. The colors in A-E represent the different salinity treatments (Control, 4 ppt, 8 ppt, 12ppt) (n=32, error bars = ± 1 standard error). The colors in F represent the vortexed/turbulent treatment and the non-vortex/control treatment. Treatments with the same letter were not significantly different from one another.

Table 3.3. Means for Experimental Salt Addition

Time In Chamber	Treatment	Salinity (ppt)	Conductivity (mS/cm)	pH	Water Level (cm)	POC (mg)	DOC (mg/L)	SUVA (254nm/DOC)	PAR (nm)	Chla (mug/L)	PO4 (mg/L)	TDN (mg/L)
30 minute	Control	0.06	0.12	5.71	27.03	2.24	28.79	2.20	0.049	74.85	0.020	1.58
30 minute	4	6.37	11.07	5.44	29.33	3.62	28.62	1.50	0.026	112.78	0.024	1.65
30 minute	8	8.85	15.43	5.20	29.58	6.34	26.29	1.70	0.033	129.42	0.029	1.56
24 hours	Control	0.03	0.06	4.92	27.03	3.29	28.28	2.54	0.059	98.19	0.047	1.92
24 hours	4	4.79	8.64	5.36	29.33	8.34	30.84	2.36	0.061	132.32	0.252	1.88
24 hours	8	7.43	12.94	5.17	29.58	11.17	30.50	2.32	0.044	146.59	0.107	2.19
1 Week -24 hours	Control	0.03	0.08	5.32	21.95	2.16	21.98	3.32	0.052	117.36	0.064	2.66
1 Week -24 hours	4	4.87	8.79	4.98	23.45	3.18	29.75	0.67	0.012	119.02	0.008	2.19
1 Week -24 hours	8	7.79	13.59	5.07	23.43	3.31	17.69	0.94	0.014	108.89	0.005	2.69
1 Week - 30 minutes	Control	0.03	0.08	5.32	21.95	3.60	21.57	2.48	0.036	155.16	0.102	2.99
1 Week - 30 minutes	4	4.87	8.79	4.98	23.45	5.70	28.25	1.74	0.036	174.51	0.028	2.22
1 Week - 30 minutes	8	7.79	13.59	5.07	23.43	4.34	25.77	2.52	0.040	126.91	0.034	2.63
1 Week	Control	0.03	0.08	5.32	21.95	5.38	27.81	1.76	0.031	156.01	0.098	3.55
1 Week	4	4.87	8.79	4.98	23.45	12.60	26.71	1.99	0.028	140.70	0.088	2.56
1 Week	8	7.79	13.59	5.07	23.43	11.14	22.02	2.54	0.036	191.78	0.135	2.79
4 Weeks	Control	0.02	0.04	5.34	33.65	6.79	21.69	1.11	0.012	179.18	0.091	1.60
4 Weeks	4	0.78	1.54	5.60	36.15	4.81	23.89	1.02	0.022	533.20	0.131	3.40
4 Weeks	8	2.06	3.86	5.61	35.85	4.58	24.91	1.30	0.031	618.87	0.054	3.33

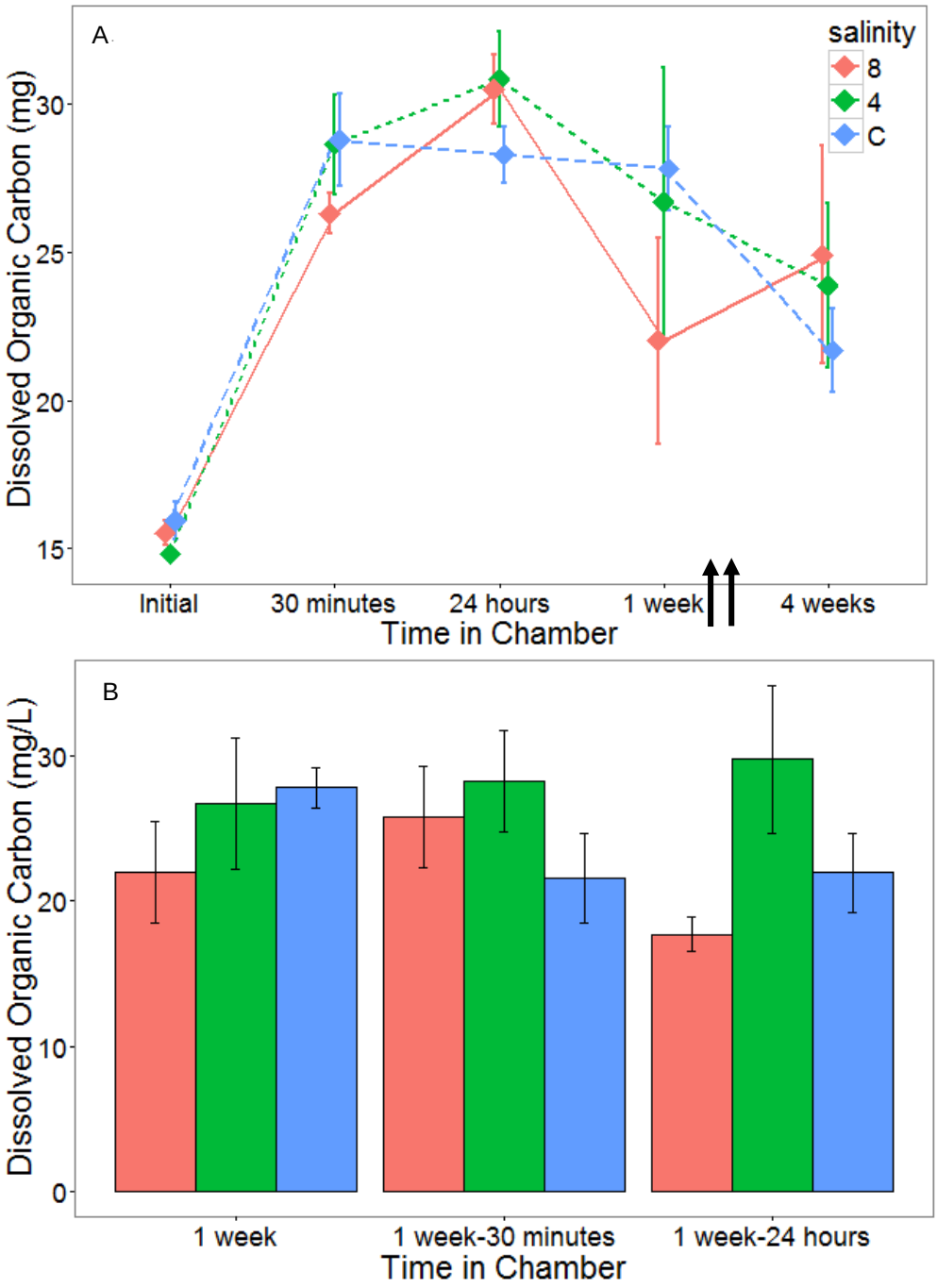
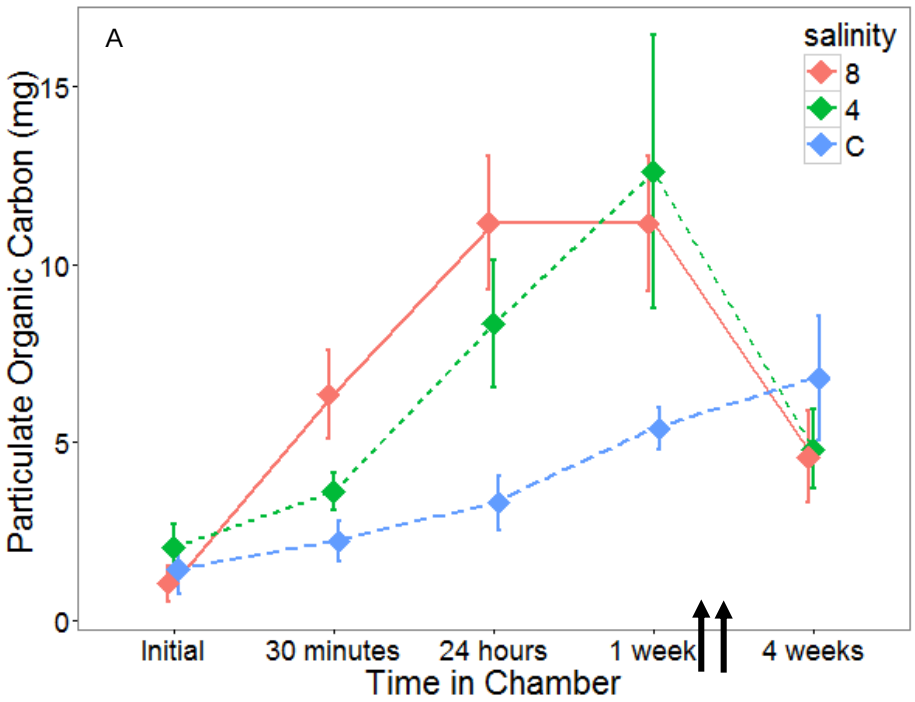
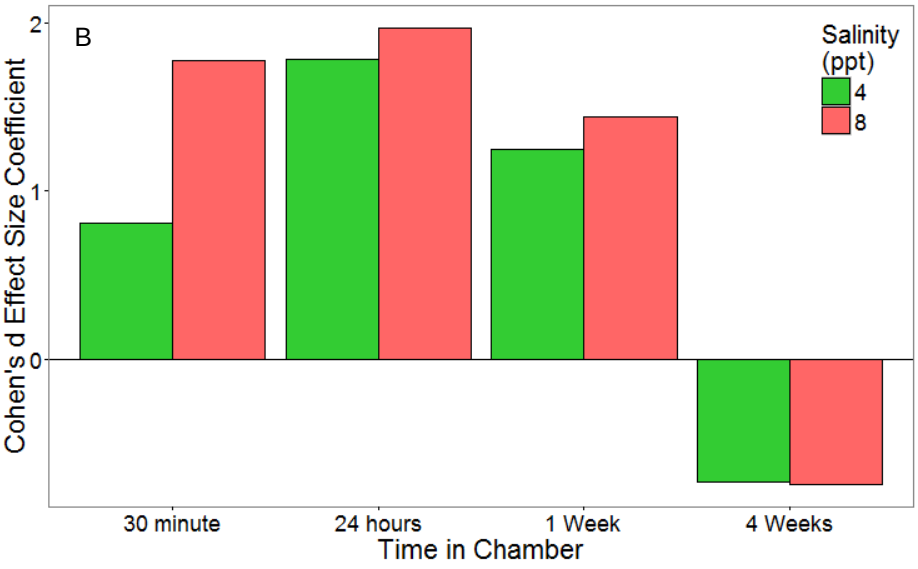


Figure 3.3. Mean DOC depletion due to increased salinity over time (A) and compared based on length of salinity exposure (B). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error). The arrows indicate two rainfall events in which chambers flooded.


A
salinity
8
4
C
Particulate Organic Carbon (mg)
0
5
10
15
Initial
30 minutes
24 hours
1 week
4 weeks
Time in Chamber


B
Salinity (ppt)
4
8
Cohen's d Effect Size Coefficient
0
1
2
30 minute
24 hours
1 Week
4 Weeks
Time in Chamber

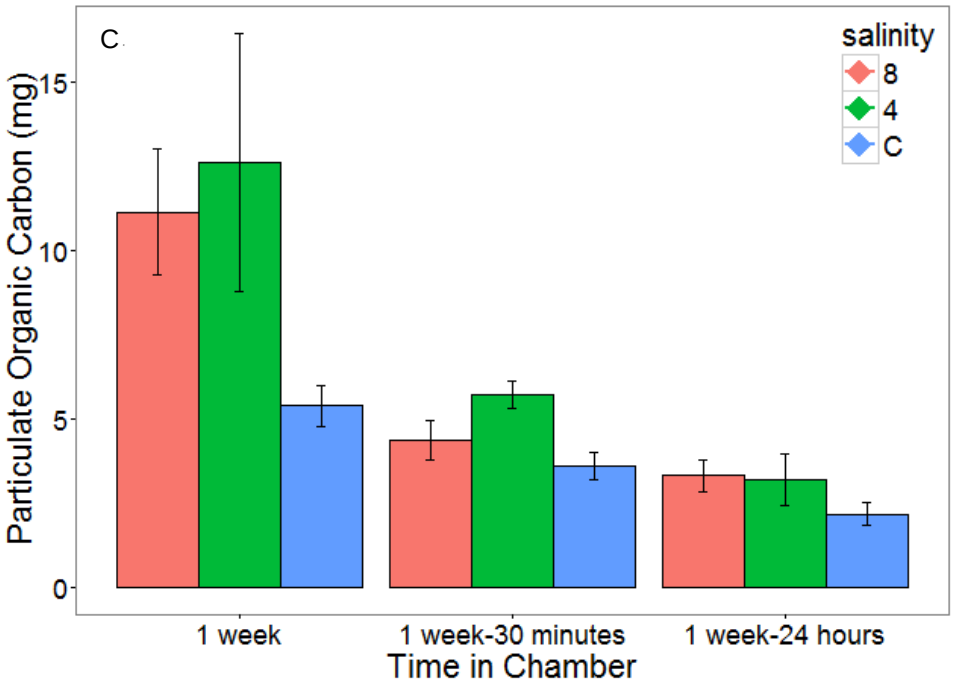


Figure 3.4. Mean POC accumulation over time (A), Cohen's d effect size of POC formation compared to the control in response to salinity (B), and mean POC accumulation compared based on length of salinity exposure (C). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error). The arrows indicate two rainfall events in which chambers flooded.

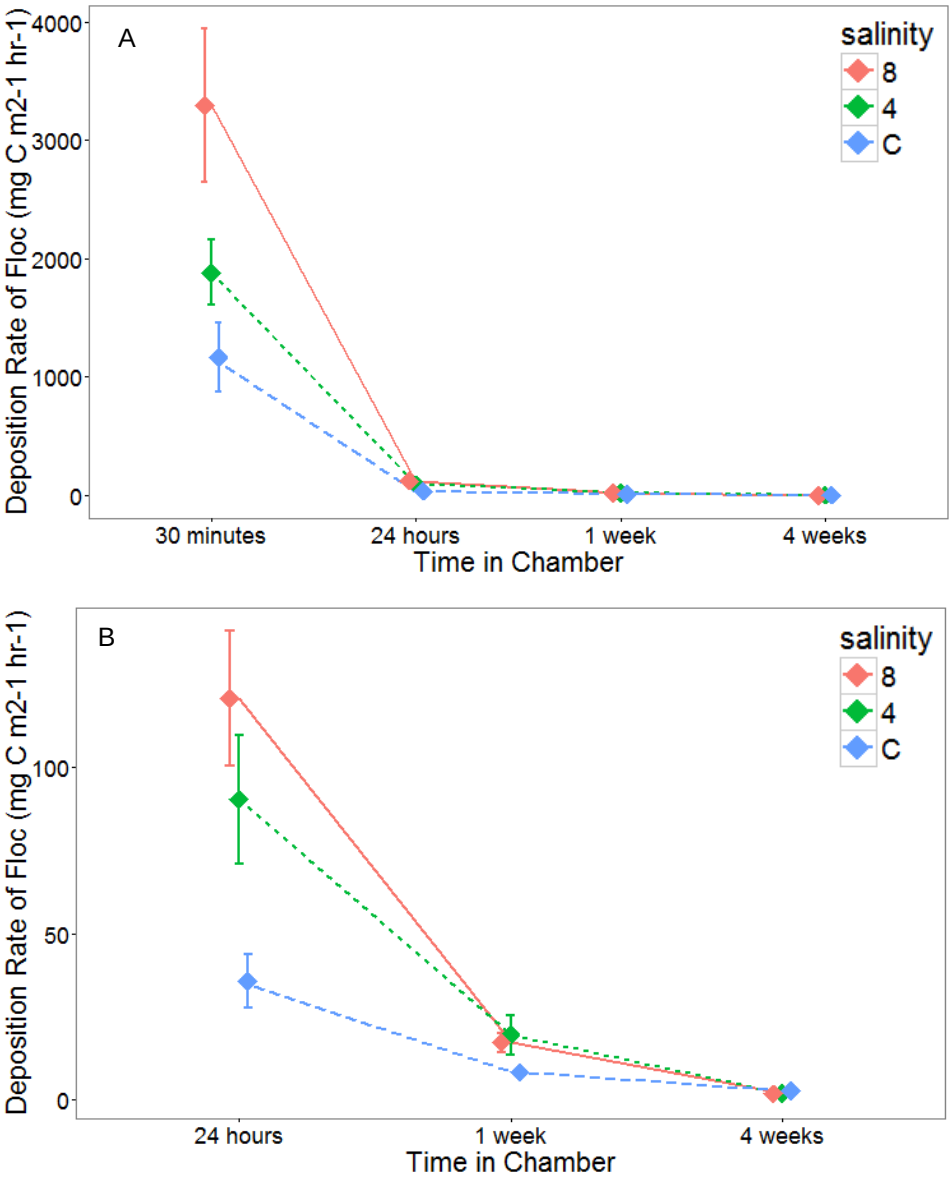


Figure 3.5. Mean deposition rate of floc over time including the first 30 minutes of salinity exposure (A) and the deposition rate without the first 30 minutes of salinity exposure (B). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error).

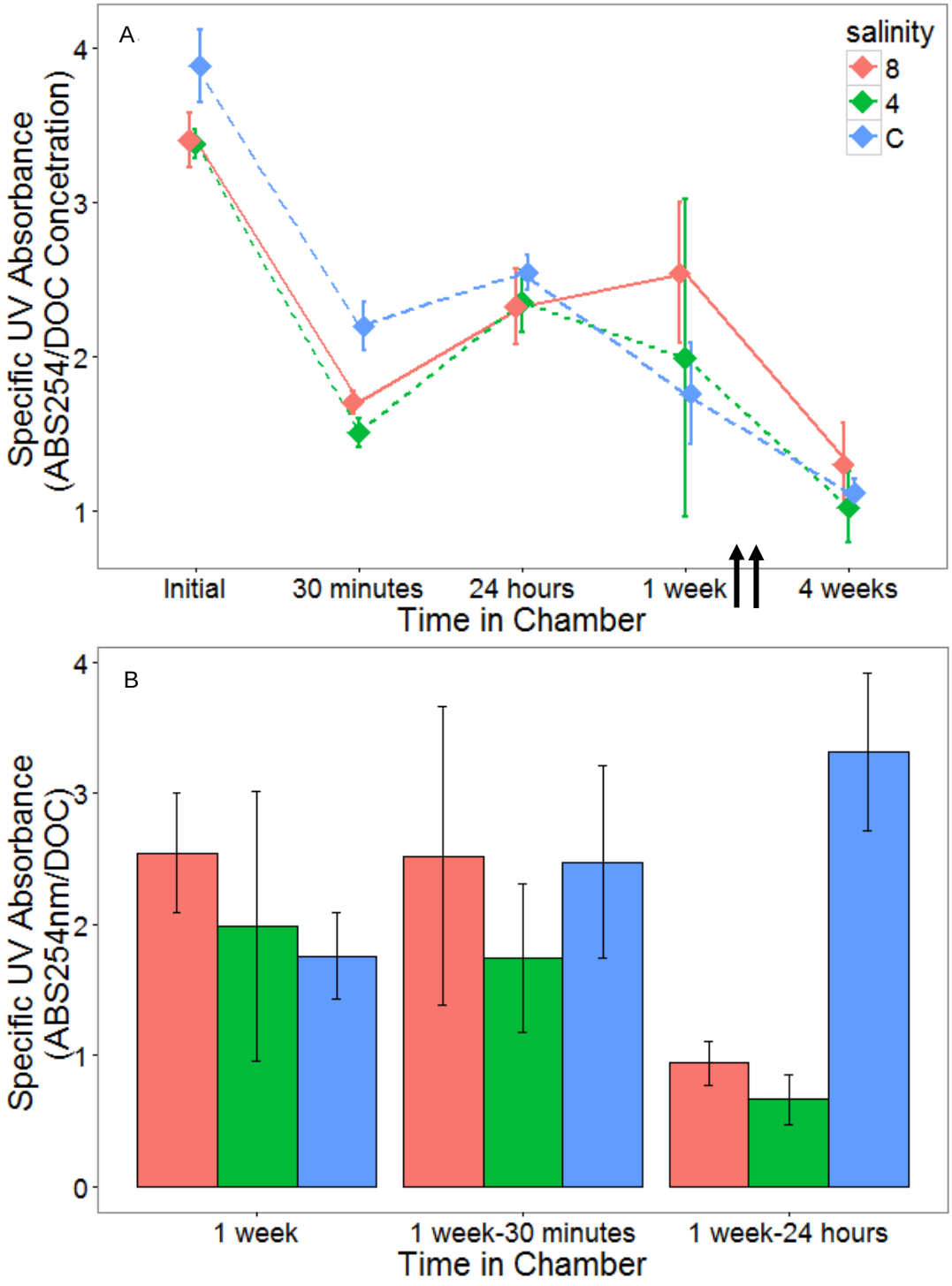


Figure 3.6. Mean salinity effects on relative percent aromaticity of DOC in the water column measured as specific UV absorbance values at 254nm corrected for DOC (mg/L) over time (A) and compared based on length of salinity exposure (B). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error). The arrows indicate two rainfall events in which chambers flooded.

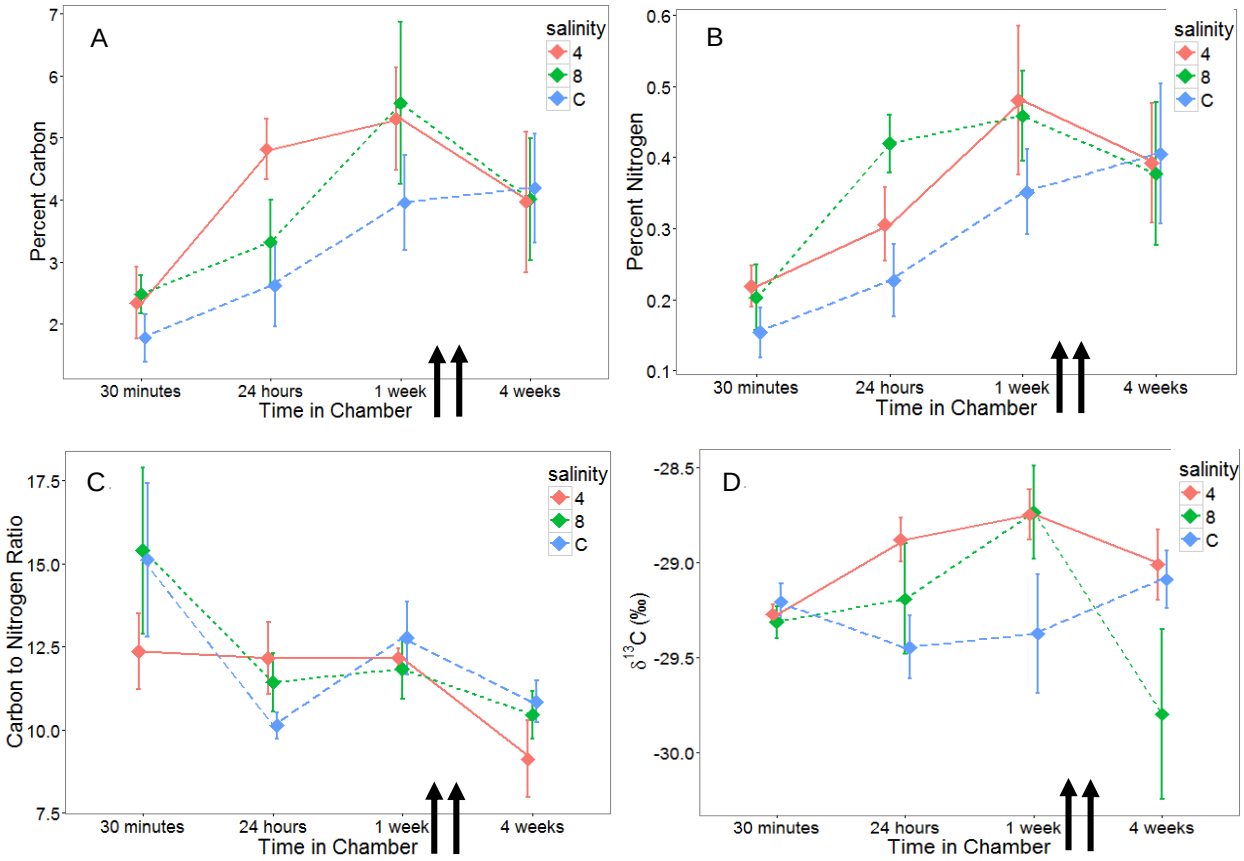


Figure 3.7. Mean percent carbon (A), percent nitrogen (B), carbon to nitrogen ratio (C), and carbon stable isotope (D) data from floc created through 30 minutes, 24 hours, 1 week, and 4 weeks exposure to salinity. The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error). The arrows indicate two rainfall events in which chambers flooded.

Table 3.4. Heterotrophic respiration of floc for each treatment over stated time points to determine intercept, slope, and r^2 value. Significant r^2 values are in bold.

Time	Treatment	n	Intercept	Slope	r^2
30 minutes	Control	5	0.49	0.10	**0.32**
30 minutes	4 ppt	5	0.26	0.06	**0.54**
30 minutes	8 ppt	5	0.16	0.04	**0.22**
24 hours	Control	5	0.33	0.10	**0.26**
24 hours	4 ppt	5	0.11	0.05	**0.42**
24 hours	8 ppt	5	0.07	0.04	**0.79**
1 week	Control	5	0.15	0.06	**0.64**
1 week	4 ppt	5	0.07	0.04	**0.51**
1 week	8 ppt	5	0.06	0.04	**0.62**

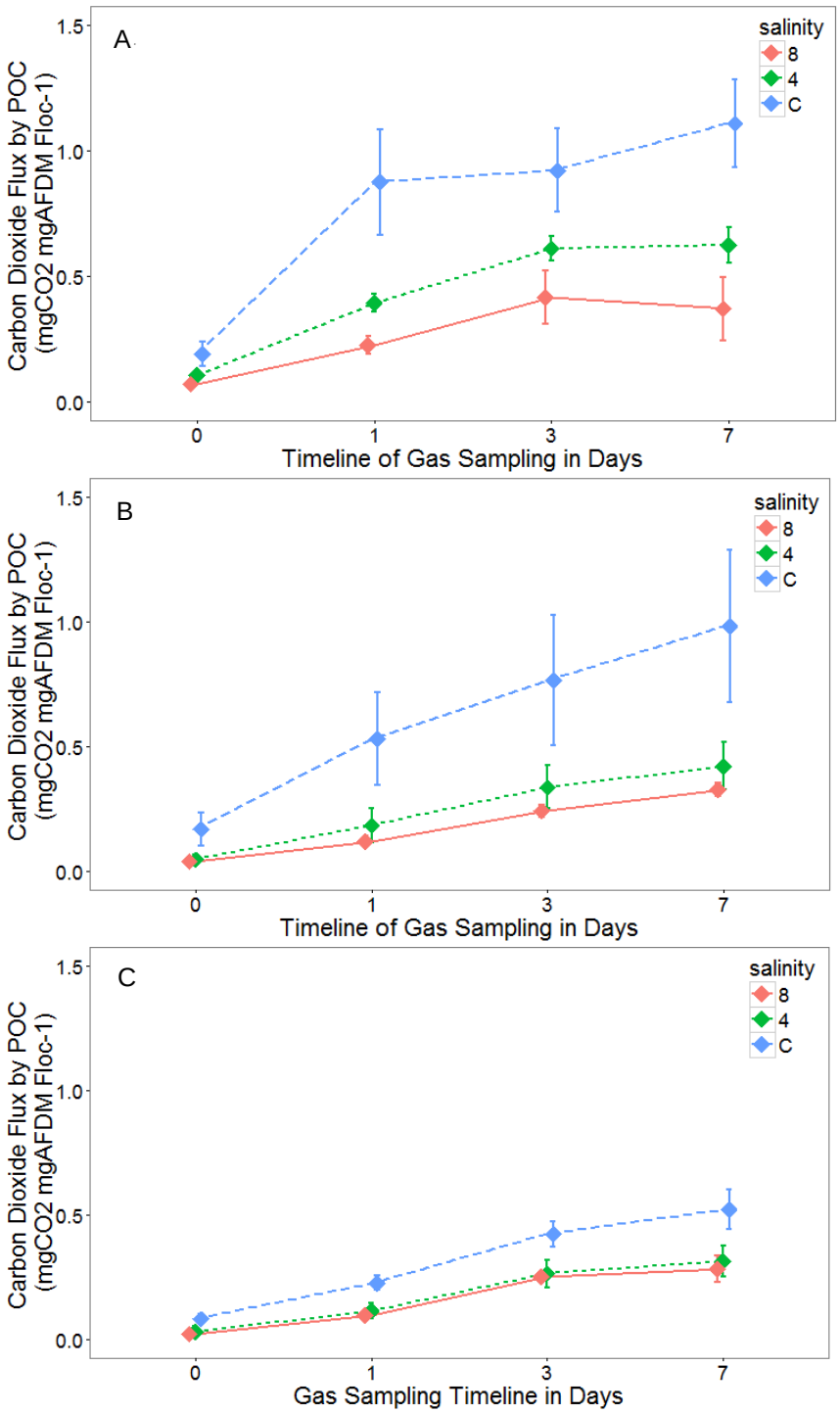


Figure 3.8. Carbon dioxide fluxes corrected for POC formation $mgCO_2$ mgAFDM $floc^{-1}$ over the 7-day incubation for 30 minutes (A), 24 hours (B), and 1 week (C). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error).

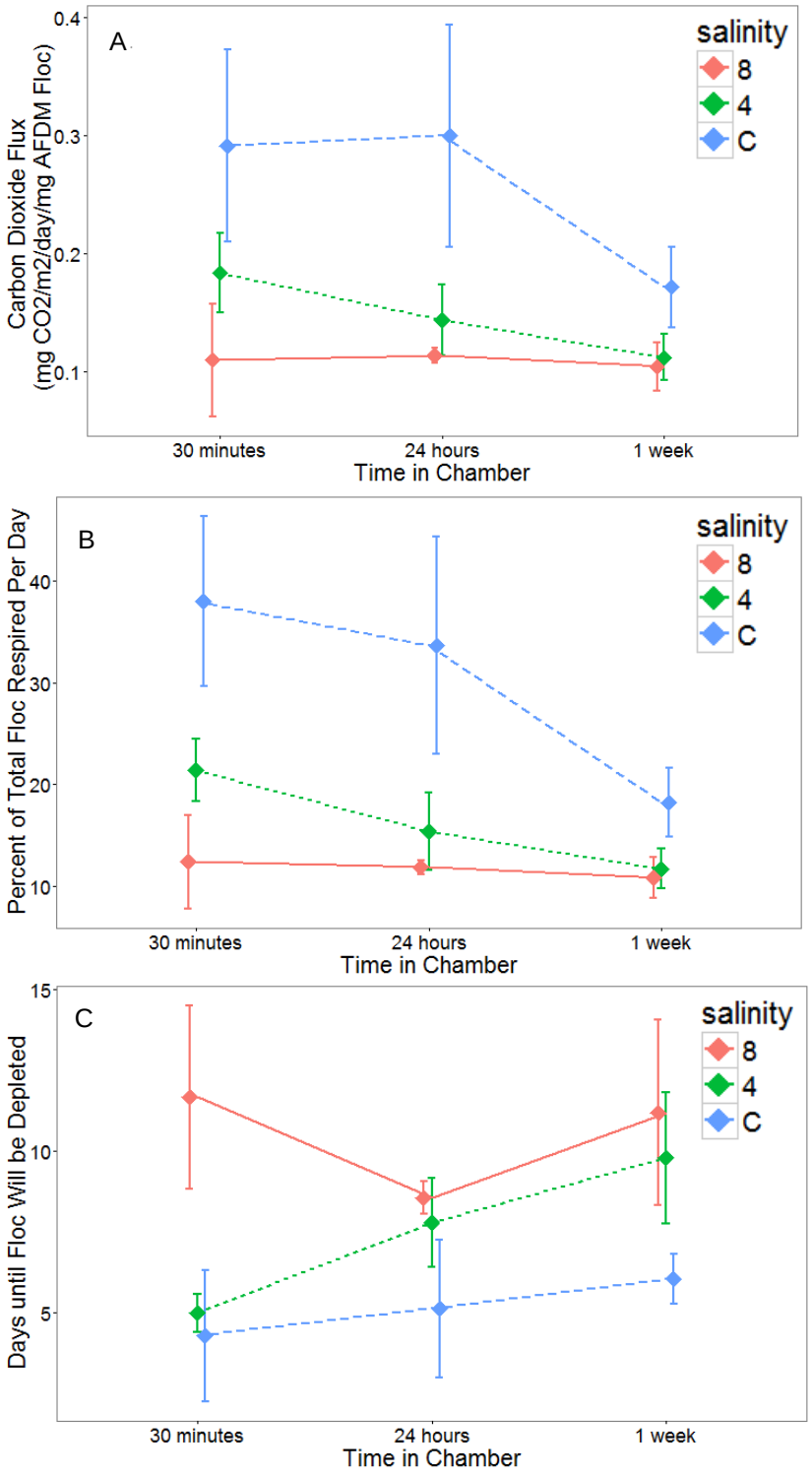


Figure 3.9. Mean heterotrophic respiration rates of floc formed at different salinities as $mgCO_2$ m^{-2} day^{-1} mgAFDM $floc^{-1}$ over time (A), the percent of total floc formed at each time point that was respired per day (B), and the predicted time until flocs will be fully depleted and microbial community will become carbon limited (C). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error).

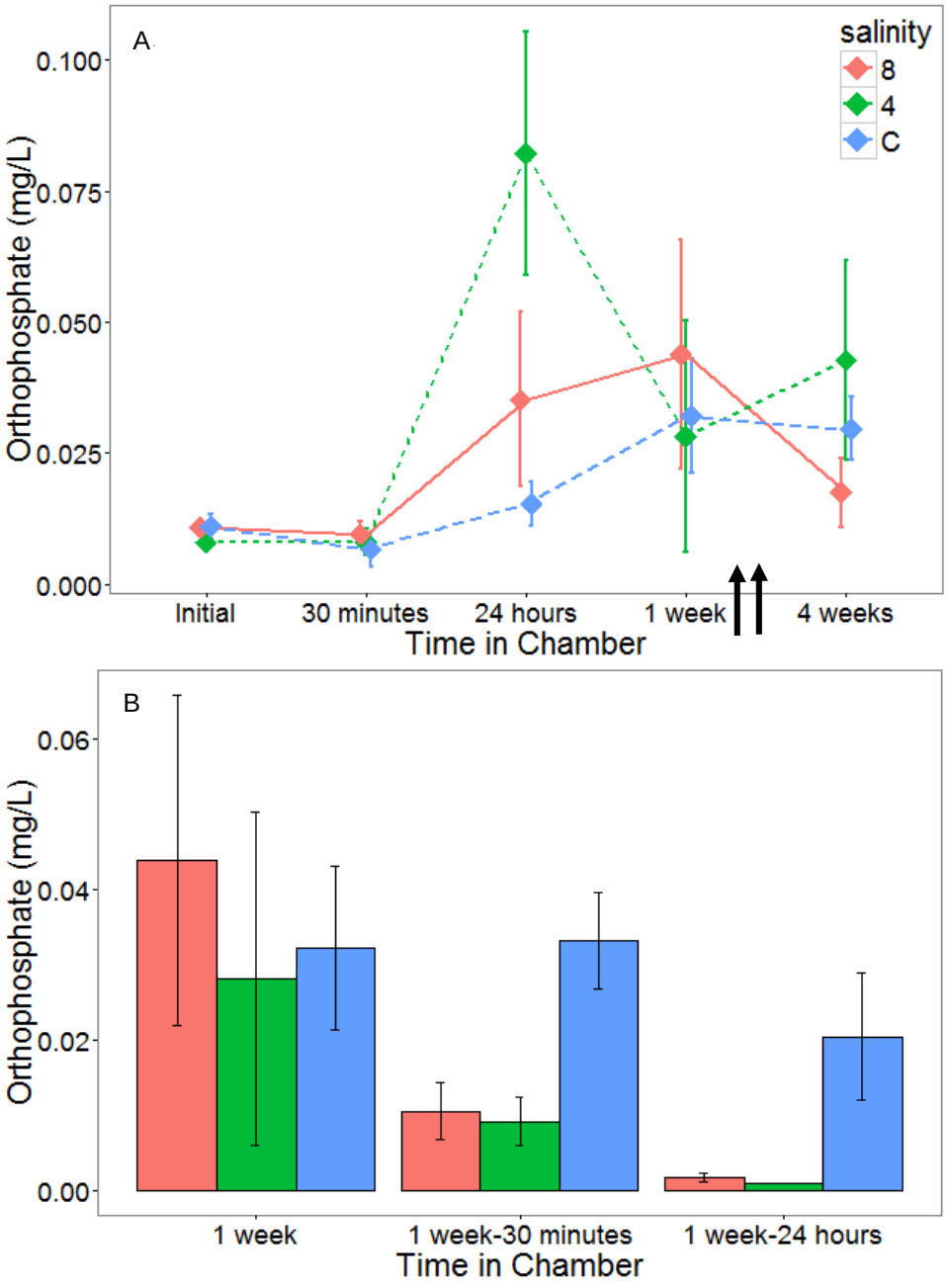


Figure 3.10. Mean phosphorus mobilization in the water column due to increased salinity measured as orthophosphate over time (A) and compared based on length of salinity exposure (B). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error). The arrows indicate two rainfall events in which chambers flooded.

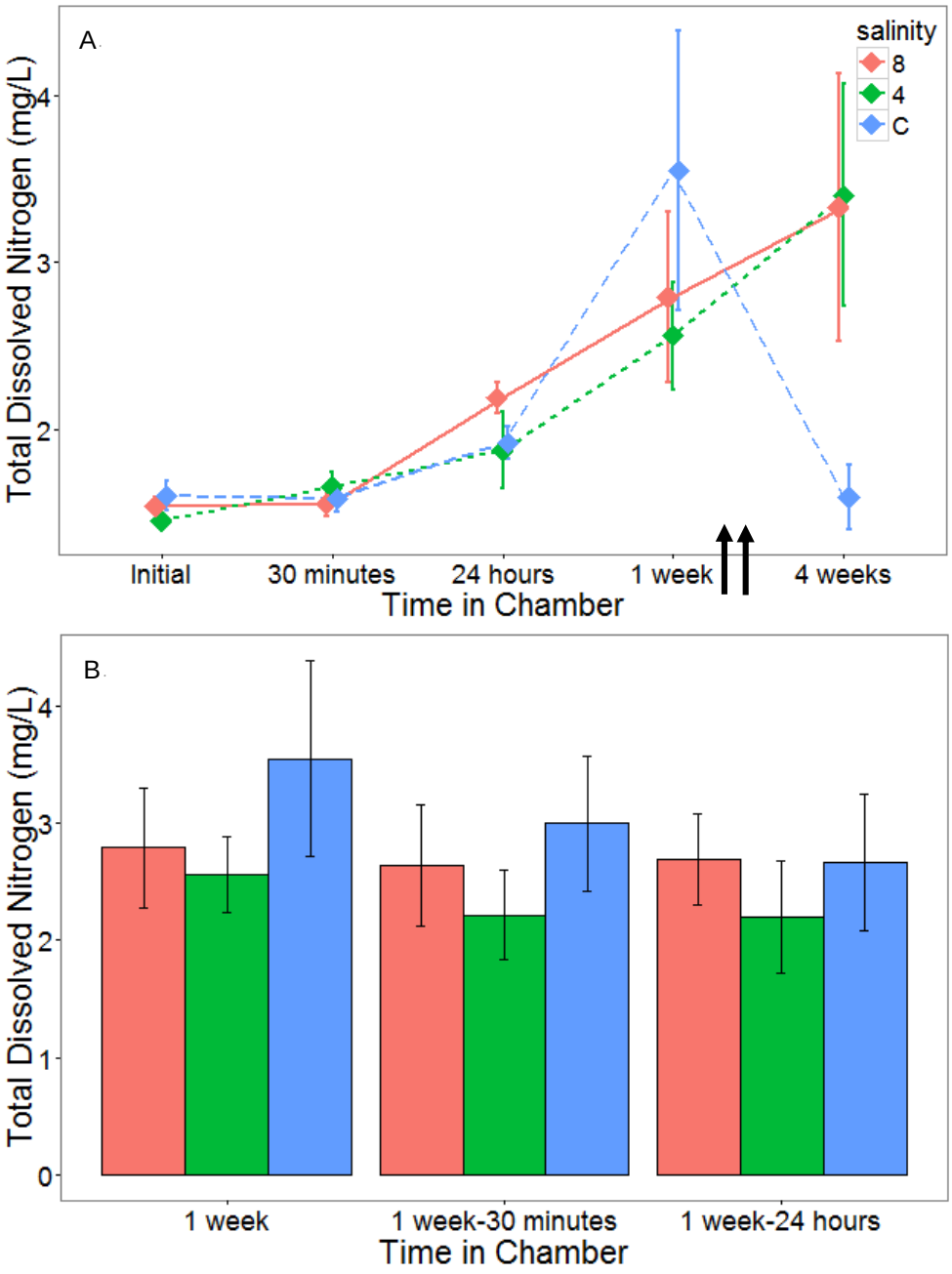


Figure 3.11. Salinity induced total dissolved nitrogen release over time (A) and compared based on length of salinity exposure (B). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error). The arrows indicate two rainfall events in which chambers flooded.

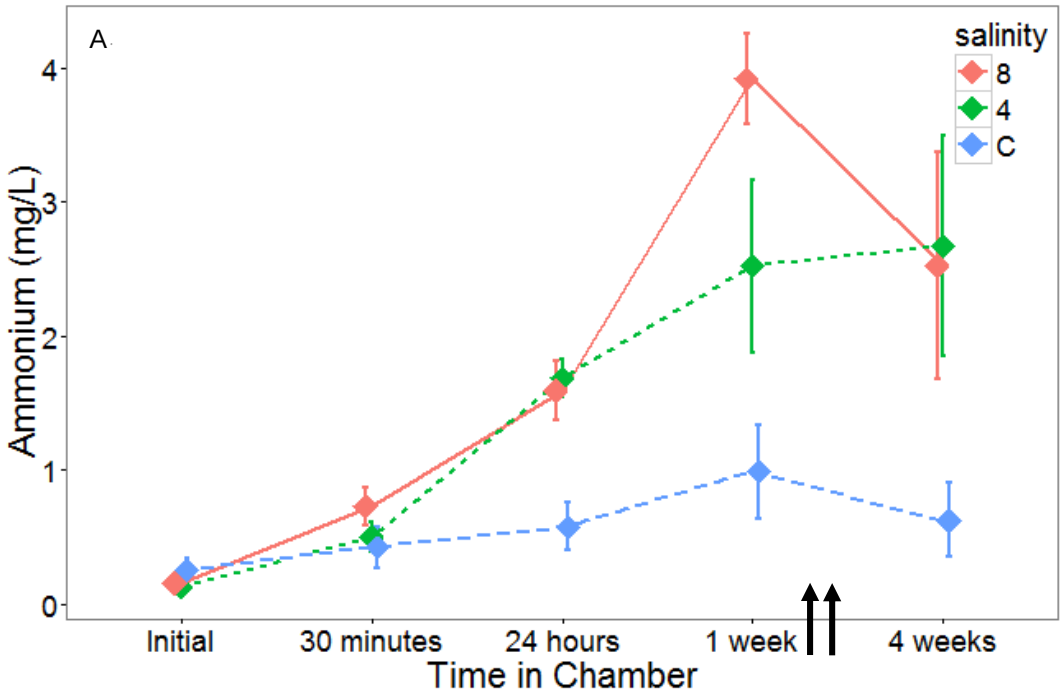


Figure 3.12. Effects of salinity on mean ammonium concentrations over time (A). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error). The arrows indicate two rainfall events in which chambers flooded.

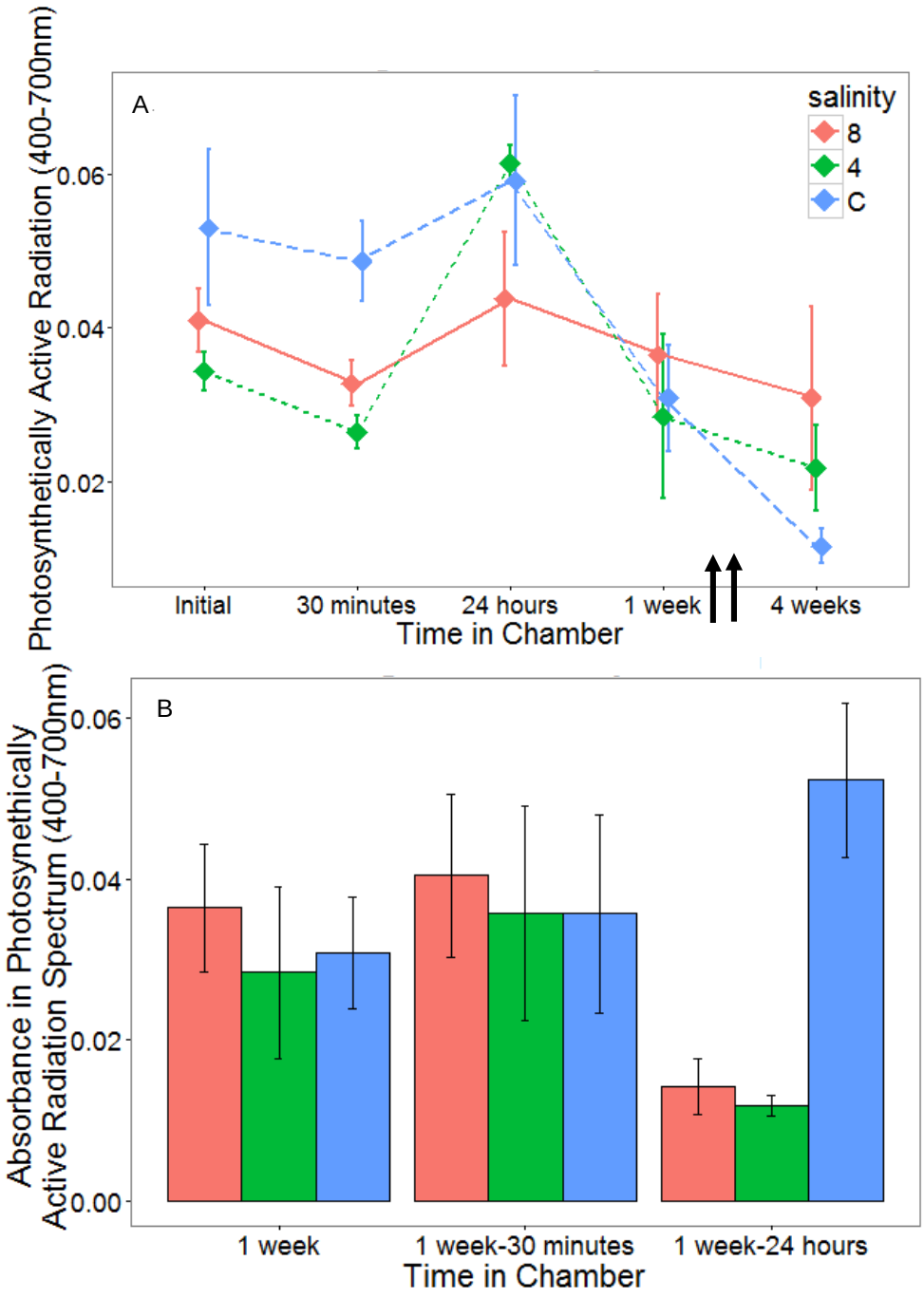


Figure 3.13. Salinity effects on the mean DOC absorbance of light in the photosynthetically active radiation spectrum over time (A) and compared based on length of salinity exposure (B). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error). The arrows indicate two rainfall events in which chambers flooded.

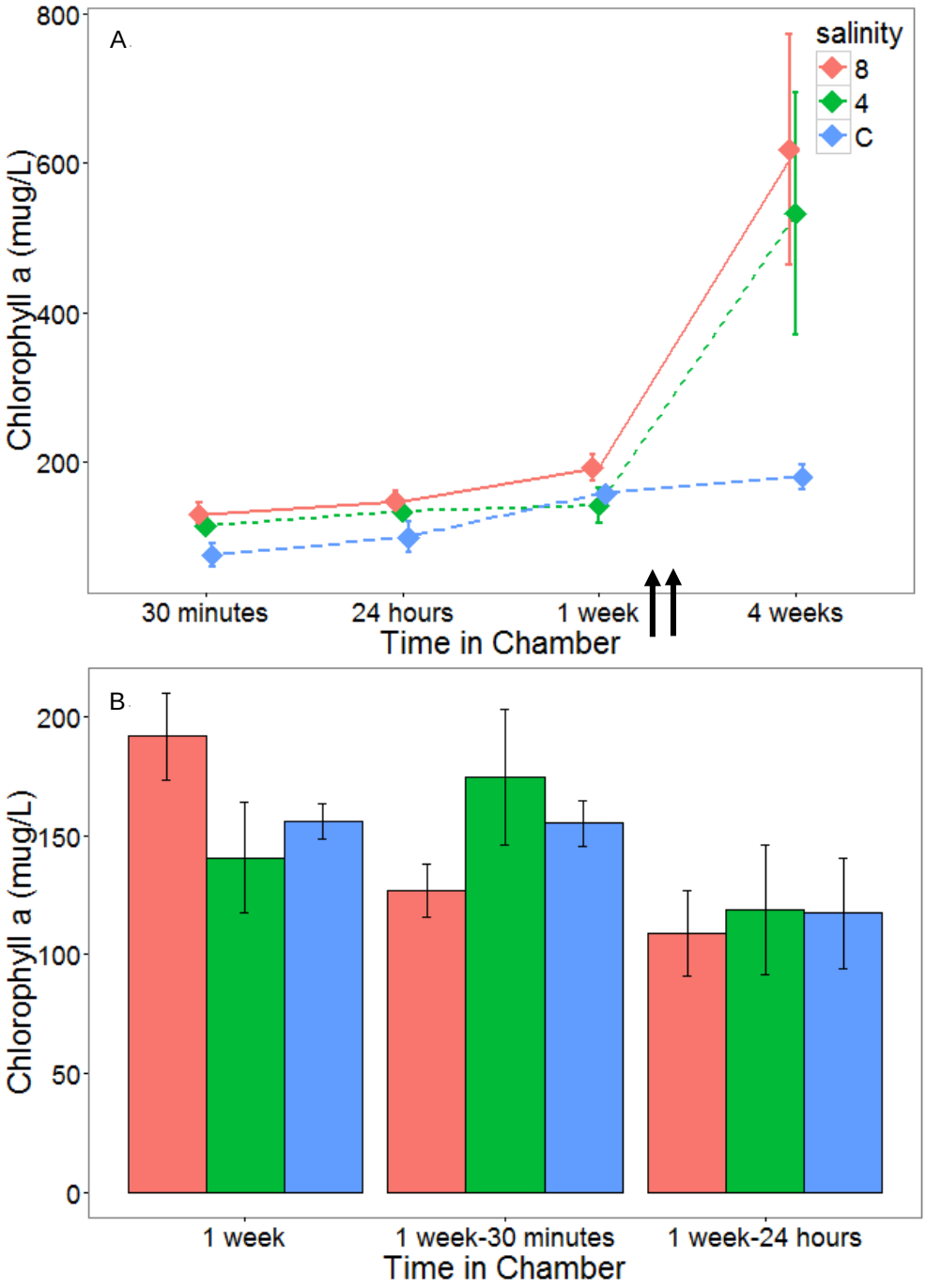


Figure 3.14. Mean chlorophyll *a* concentrations over time (A) and compared based on length of salinity exposure (B). The colors represent the different salinity treatments (Control, 4 ppt, 8 ppt) (n=15, error bars = ± 1 standard error). The arrows indicate two rainfall events in which chambers flooded.

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