ASSESSING THE SEASONAL DISTRIBUTION, DYNAMICS, AND FLOCCULATION OF ORGANIC MATTER FROM THE LAND TO SEA ACROSS TWO BOREAL SYSTEMS

by

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ABSTRACT

Boreal zones contain globally significant organic matter (OM) reservoirs and are currently in a state of rapid change due to shifting temperatures and regional hydrology. Such ongoing change has the ability to alter the export of substantial amounts of OM from these C-rich systems to the coastal environment, which may have major consequences on coastal food web dynamics. In this thesis, I investigate the distribution, dynamics, and salt-induced flocculation of OM across two small adjacent boreal rivers and their immediate coastal zones over three consecutive seasons. Overall, biogeochemical diversity and variation in flocculation potential at local and weekly time scales emphasize the relevance and important inclusion of these underrepresented small coastal systems within regional and global elemental budgets, which will increase the efficacy of future biogeochemical flux predictions from the land to sea. In the second chapter of my thesis, observations of the distribution and dynamics of particulate and dissolved (D)OM highlight important linkages between OM dynamics and local and seasonal physico-chemical, biological, and hydrological processes that were potentially enhanced at a weekly scale during the summer-to-fall transition period. The findings of this observational study also revealed that over 95% of the freshwater organic carbon is in the dissolved fraction with characteristics of being terrestrially derived, aromatic, and C-rich. These characteristics, along with dissolved iron, were also selectively removed by salt-induced flocculation processes, as highlighted in the third chapter of my thesis. Overall, results from the experimental study emphasize the relevant contribution of flocculation as a process affecting the removal of OM from pelagic zones to the benthos, which can occur across a broad range of salinities (1-25 psu). The findings in my thesis also encourage the use of dissolved iron measurements and calculations for dissolved iron to dissolved organic carbon ratios in future biogeochemical studies as they were found to be especially informative for assessing the temporal stability of DOM as well as degree of flocculation across adjacent systems with differing catchment types. Changing environmental conditions will result in greater day-to-day variability in river hydrology and subsequently affect the fluxes, cycling, and fate of OM, and to better predict and understand the consequences of these changes, an increased representation of small near-shore coastal systems with recognition of its biogeochemical diversity in regional and global elemental budgets is required.

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LIST OF ABBREVIATIONS AND SYMBOLS

Arctic-GRO = Arctic Great Rivers Observatory

C = Carbon

°C = degrees Celsius

C:N = Carbon to nitrogen ratio

CBC = Come By Chance

CDOM = Chromophoric dissolved organic matter

CO_2 = Carbon dioxide

DOC = Dissolved organic carbon

DON = Dissolved organic nitrogen

DFe = Dissolved iron

DOM = Dissolved organic matter

ETM = Estuarine turbidity maxima

Fe = Iron

FDOM = Fluorescent dissolved organic matter

h = hour

HCl = Hydrochloric acid

HELCOM = Helsinki Commission

HMW-DOM = High molecular weight dissolved organic matter

HYDAT = Environment and Climate Change Canada Hydrometric Database

ICP-OES = Inductively coupled plasma-optical emission spectroscopy

km = kilometers

L = Liters

LMW-DOM = Low molecular weight dissolved organic matter

m = meters

mΩ = milli-Ohms

N = Nitrogen

°N = degrees North

NH_4^+ = Ammonium

NO_x^- = Nitrogen oxides

NMR = Nuclear Magnetic Resonance

NL = Newfoundland and Labrador

OM = Organic matter

P = Phosphorus

PARTNERS = Pan-Arctic River Transport of Nutrients, Organic Matter and Suspended Sediments

PC = Particulate carbon

PHR = Piper's Hole

PN = Particulate nitrogen

PM = Particulate matter

POM = Particulate organic matter

$SUVA_{254}$ = Specific UV absorbance at 254nm normalized to DOC

S_r = Spectral slope ratio

tDOM = terrestrially derived dissolved organic matter

TOC = Total organic carbon

tOM = terrestrially derived organic matter

$\mu mol\ L^{-1}$ = micro-mol per liter

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CHAPTER 1: OVERVIEW

1 Organic matter classifications in freshwater and marine ecosystems

Organic matter (OM) across fresh and marine ecosystems exists as many different chemical structures across a size continuum, stretching from nanometres and daltons to centimetres (Figure 1). These sizes give rise to the distinction of particulate (POM) and dissolved (D)OM pools, which are operationally defined as organic compounds that are either retained or passed through filters with pore sizes of 0.1 to 0.7 µm (Bianchi 2007, Repeta 2015). Generally, the POM pool consists of living biomass and dead cells such as phytoplankton, detritus and aggregates (Benner et al. 1997). On the other hand, DOM can be made up of biomolecules (E.g. proteins, nucleic acids, sugars), land-derived organic polymers (E.g. lignin, cellulose), bacteria and viruses, as well as anthropogenically-derived compounds (Hartnett 2018). The DOM pool can be further size fractionated via techniques such as ultrafiltration and size exclusion chromatography for insights into the complex heterogeneous compounds that make up this pool. For example, the use of ultrafiltration can physically separate hydrophilic high molecular weight DOM (HMW-DOM) from hydrophobic low molecular weight DOM (LMW-DOM) and dissolved inorganic species prevalent in these systems (Repeta 2015). This separation is particularly useful in saline systems, where elevated dissolved salt concentrations have been known to pose major challenges for chemical characterization (Bauer and Bianchi 2011, Repeta 2015, Bianchi and Canuel 2011). While all isolation techniques today exhibit bias and struggle to fully recover the entire DOM pool, these techniques have paved the way for studies to explore relationships between size classes of DOM and its reactivity and fate in aquatic systems (Amon and Benner 1996, Walker et al. 2016, Mannino and Harvey 2000, McCallister et al. 2006). Much of what we know about OM is not only dependent on size, but the combination of size distributions with radiocarbon ages, stable isotopes (Eg. $\delta^{13}C$, $\delta^{15}N$), molecular structural characteristics (E.g. NMR), optically-based measurements (Eg. Absorbance, Fluorescence), and compound specific analyses (Wagner et al. 2020, Bauer and Bianchi 2011, Canuel and Hardison 2016).

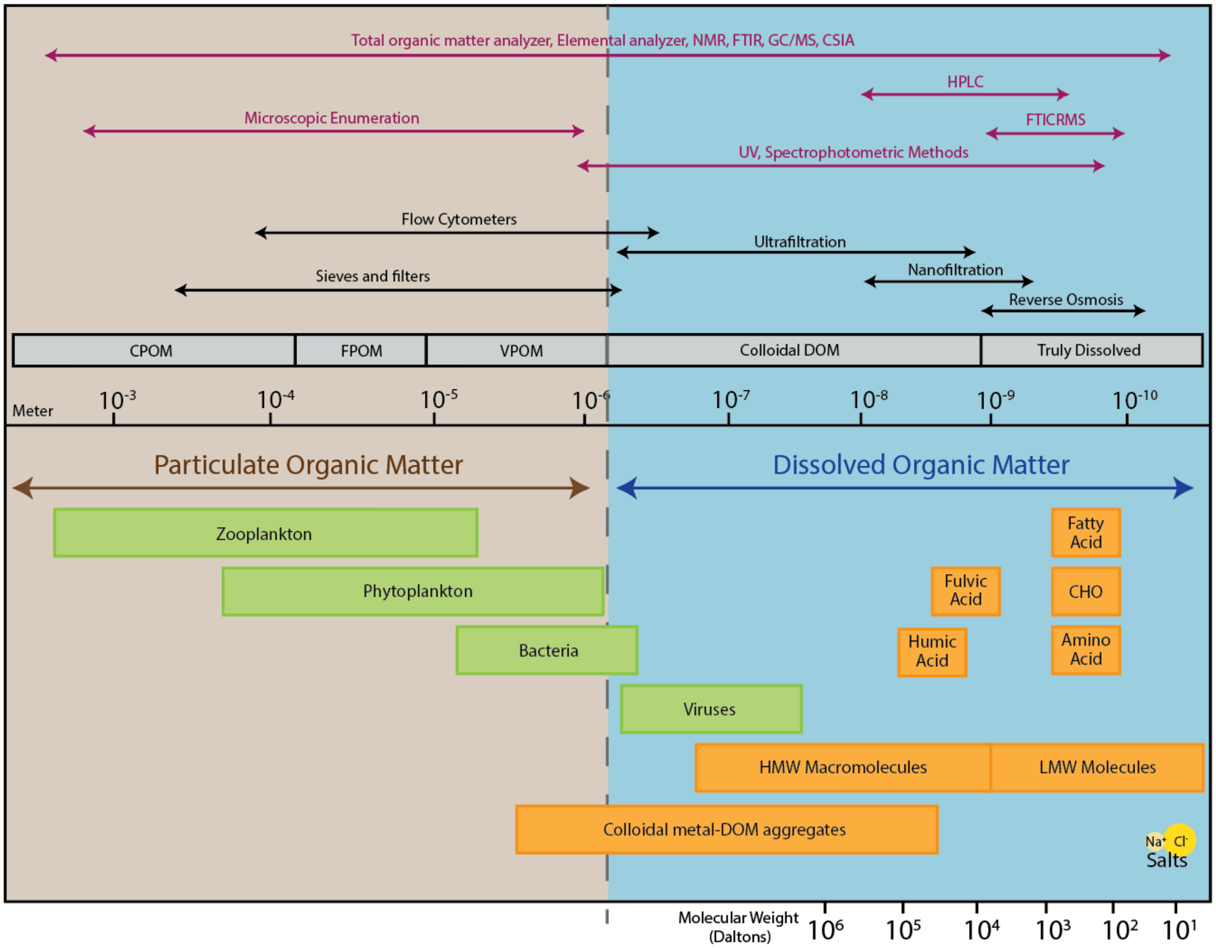

Figure 1.1. A summary of the chemical characterization (purple) and size separation techniques (black) used across the size continuum of organic matter in fresh and saline waters (modified from Hansell and Carlson, 2002 and He et al. 2016). POM often consists of living material (green boxes) whereas DOM is made out of a heterogeneous mixture of biomolecules, organic polymers, metals and ions (organge boxes).

Sources of OM along river-estuaries consists of materials that either 1) enter the aquatic system from terrestrial systems, referred to as allochthonous or terrestrially-derived material, or 2) is produced within the aquatic system via biological processes, referred to as autochthonous or algal-derived material (Bauer and Bianchi 2011, Bianchi 2007). Today, analytical advancements in areas spanning from elemental analyses (e.g. TOC) to the use of compound specific biomarkers across the aquatic continuum has led to a better understanding of the characteristics defining these two source categories (Bianchi and Canuel 2011). Broadly, terrestrially-derived DOM is known to be more aromatic, high in chromophoric (C)DOM and C-rich, often having high organic carbon to nitrogen ratios (C:N; 20-100) compared to algal-derived sources (C:N; 5-20; (Bianchi 2007)). Autochthonous sources of DOM are also generally more protein rich and consist of smaller monomeric compounds, which likely makes these sources a high quality substrate for microbial consumption (Donderski et al. 1998). In the case of POM, dominance of allochthonous OM can often shift abruptly to autochthonous sources below the estuarine turbidity maximum (Canuel and Hardison 2016, Savoye et al. 2012). An increase in light availability from the trapping of particles within the estuarine turbidity maximum, along with an increase in nutrient availability are some potential factors that contribute to this sudden shift (Bianchi 2007).

Since the early 1990s, our ability to analyse DOM has been limited to the availability and robustness of analytical techniques. Total (TOC) and dissolved organic carbon (DOC), which is typically the most popular metric for biogeochemical studies, were only developed around 25-30 years ago (Wangersky 1993). Since then, these methods along with many others continue to be re-evaluated and explored, particularly for salt-containing samples (Sharp et al. 2004, Laodong and Santschi 1997, Coble 1996, Osburn et al. 2016). In more recent years, the use of optical properties (e.g. absorbance and fluorescence) have gained popularity as such techniques are non-destructive and can be made using remote sensing. While these techniques are promising, their use in estuarine environments is often plagued by uncertainties surrounding conservative mixing and competing transformations that alter CDOM and FDOM components (Asmala et al. 2016). Because of the wide span in physicochemical gradients (e.g. salinity, pH), the analytical techniques used to measure OM parameters across fresh and saline systems is oftentimes different. As a result, it is difficult to compare OM along a land to sea continuum, especially in estuaries where these two systems meet and multiple sources contribute to the bulk OM pool. In addition, certain constituents of estuarine OM can often be in low concentrations and thus, the application of these tools to both phases (DOM and POM) can be made difficult by instrument detection limits. It is also important to note that while we are able to synthesize general patterns in OM distribution and dynamics across seasons and systems today, these conclusions are often derived from different techniques and associated analytical errors. As such, these complex coastal zones that vary greatly in OM composition, dynamics and distribution make the field of estuarine biogeochemistry relatively young and understudied compared to its fresh and marine counterparts.

2 Changing environmental conditions across northern mid-to-high latitudes

Northern mid-to-high latitudes (>45°N) encompass a broad range of landscapes and environmental gradients, ranging from permafrost-dominated Arctic tundra in the North to a mosaic of boreal forests and lakes in the South. Together, these regions account for up to 60% of the global soil carbon pool (Hobbie et al. 2000) and has since been recognized as a key region contributing to the global carbon cycle (Ciais et al. 2013). While carbon has been at the forefront of global biogeochemistry, in part due to our concerns with climate change and atmospheric CO_2, OM is a major ‘vehicle’ linking key elements with carbon in natural environments. In northern high latitudes, such accumulation of soil OM can be attributed to the regions characteristic extreme cold, water-logged conditions and short growing seasons (Hobbie et al. 2000). In the face of climate change, these large stores of soil OM are likely to undergo drastic changes as a result of accelerated warming (Ciais et al. 2013), earlier and longer growing seasons (Park et al. 2016) an increase in periodic land disturbances such as wildfires (Goetz et al. 2005) and abrupt winter thaw (Zhang et al. 2011), and increased regulation of river flow for hydroelectric power generation (Cherry et al. 2017). These changing natural conditions have the potential to affect vegetation cover and terrestrial productivity (Beck and Goetz 2011, Wang and Friedl 2019), which forces changes to soil OM (Loranty et al. 2016) and alterations to catchment hydrology (Walvoord and Striegl 2007). In coastal and marine systems, changing climatic conditions have led to a decrease in sea ice cover (Perovich 2011) and an increase in freshwater (Peterson et al. 2002, Ahmed et al. 2020), and export of terrestrial (t)DOM (McClelland et al. 2011), all of which, have cascading effects on microbial communities and energy transfer into higher trophic levels (Wikner and Andersson 2012, Rolff and Elmgren 2000, Langenheder et al. 2003, Sipler et al. 2017, Deininger and Frigstad 2019).

Over the last few decades, monitoring programs such as the Arctic Great Rivers Observatory (Arctic-GRO) and Pan-Arctic River Transport of Nutrients, Organic Matter and Suspended Sediments (PARTNERS) project have led to an increase in high-resolution biogeochemical studies in the Arctic. From such endeavours, large Arctic rivers have gained recognition in transporting disproportionately large quantities of freshwater and tDOM to the Arctic Ocean (Holmes et al. 2011, Retamal et al. 2007, Chupakov et al. 2020, Fichot et al. 2013). These studies have helped explain earlier reports of higher than average DOC concentrations in the Arctic Ocean relative to other ocean systems (Opsahl, Benner, and Amon 1999). In boreal regions, a large proportion of biogeochemical studies and environmental monitoring programs can be found based around the Baltic Sea, where the need for intergovernmental organizations are vital in protecting the marine environment from pollution (HELCOM). Similar to Arctic regions, the contribution of riverine OM to boreal coastal oceans is notable (Hoikkala et al. 2015, Frigstad et al. 2013) and have been reported as a potential source in sustaining annual productivity in the Baltic Sea (Wikner and Andersson 2012, Soares and Berggren 2019, Traving et al. 2017) and Norwegian Coast (Frigstad et al. 2020). While studies and programs today have made considerable leaps towards understanding biogeochemical cycles in these northern high latitudes, the region's vast expanse and characteristic extreme climatic conditions continue to make conducting high-resolution biogeochemical studies across space and time difficult. Furthermore, much of the attention across these regions has been directed towards large rivers and little towards the great many small rivers that are scattered across Arctic-boreal coastal zones. Consequently, these knowledge gaps hinder our ability to make informed estimations and predictions of spatial and seasonal patterns of OM concentration and composition across these regions.

3 Changing hydrology and riverine fluxes of organic matter to coastal ecosystems

Water connects OM from land to sea and in northern high latitudes, seasonal variations in precipitation and temperature exert strong controls on the accumulation, storage and transport of OM and nutrients to the coastal ocean. The most striking feature of the annual hydrograph across these regions is a dramatic increase in river discharge during the spring snowmelt period. This peak is more prominent in Arctic regions and tends to happen later in the year as a result of colder temperatures (Figure 2). Nevertheless, the spring snowmelt period acts as a key contributor to the substantial flux of water and OM to the oceans (Paerl et al. 1998, Stedmon et al. 2011, Davis and Benner 2005, Frigstad et al. 2020). For example, in the Gulf of Finland, DOC and DON concentrations both doubled in the spring, along with an approximate 6-fold increase in coloured (C)DOM (Ylöstalo et al. 2016). Higher POM concentrations have also been reported during this time in coastal waters (McGovern et al. 2020, Davis and Benner 2005). The drivers behind increases in POM concentrations are less straightforward than the delivery of DOM and freshwater via riverine discharge. Instead, such increases in POM are likely driven by the coupling of DOM and biological production as well as changes in community composition due to differences in salinity (Bittar et al. 2016). In addition, coupled increases in CDOM and freshwater to the coastal system during high flow periods can also result in vertical stratification and a decrease in light attenuation (Wikner and Andersson 2012, Frigstad et al. 2020), both of which play a vital role for coastal primary production (Figure 3B; 3C). While quantification of OM is an important aspect of biogeochemical cycles, understanding the characteristics of OM is also important as its chemical make-up ultimately determines its reactivity and fate in the environment.

Along with higher concentrations of OM being transported during the spring snowmelt period, DOM also tends to be more terrestrial, aromatic, and of higher molecular weight (Stedmon et al. 2011, Mutschlecner et al. 2018, Osterholz et al. 2016, Walker et al. 2013). Kaiser et al. (2017) also reported a general 3-4 fold increase in biochemicals such as carbohydrates,

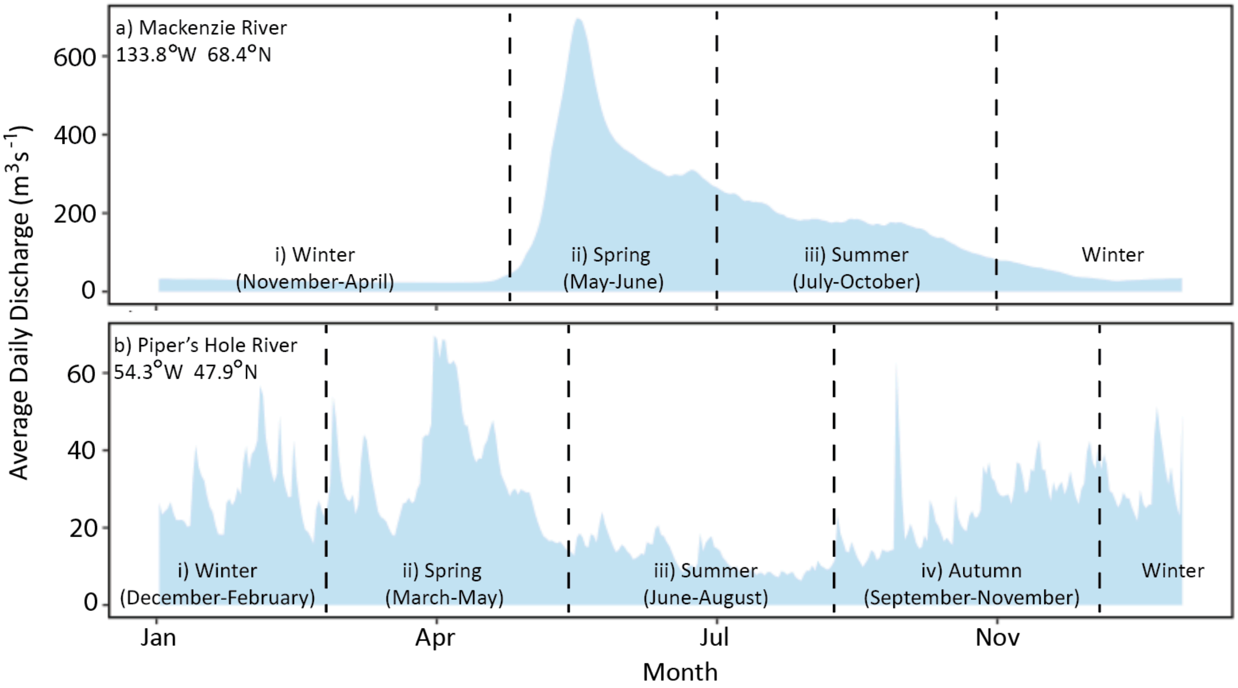

Figure 1.2. Seasonality as assessed by river discharge in the a) Arctic vs. b) boreal region. The spring snow melt period in the Arctic (a, ii) is more pronounced and can contribute up to 57% of the annual water discharge (Ahmed et al. 2020). High flow periods in boreal regions are more frequent, occurring not only during spring snowmelt but also in winter (b, i) as rain on snow events, and in fall (b, iv) as storm events. Data source: Environment and Climate Change Canada Hydrometric Database (HYDAT).

amino acids and plant phenols during this time, which are known to represent a notable portion of the bioavailable DOM pool. While these general trends have been linked to periods of high flow, many of these reported trends come from studies conducted in large rivers and may not hold true across all northern high-latitude rivers. For example, Johnston et al. (2018) noted similar general trends across the freshet in a mid-sized river in the Arctic but found a persistence of high DOC and aromatic indicators during winter baseflow conditions as well. These comparatively smaller systems may be more reflective of landscape characteristics and changes in water flow paths and residence times. As a result, the potential for misrepresentation of the fluxes, fate and reactivity of tDOM is evident in Arctic-boreal regions, particularly for individual river-estuaries, as regional models have been based on data from similar sets of river systems (e.g. large rivers in the Arctic). Additionally, typical features of boreal regions such as flat topography and the presence of wetlands and water bodies further regulate water flow and sources of OM to and within aquatic systems. By regulating water pathways, these features effectively provide areas for extensive transformations and cycling of OM, some of which include: greater production of authochthonous DOC and sedimentation (Winterdahl et al. 2014), an increased susceptibility of high molecular weight DOM to more extensive photodegradation (Stedmon et al. 2011, Mari et al. 2007) and a decrease in dissolved organic nitrogen (DON) bioavailability due to upstream biological uptake (Asmala et al. 2013). In a region where residence times are likely longer and OM is more likely to undergo transformations prior to reaching downstream coastal systems (Ledesma et al. 2017), episodic large discharge events such as the spring freshet can promote the flushing of reactive tDOM into coastal systems (Raymond et al. 2016).

The overall composition of OM is further influenced by inorganic nutrient concentrations and primary productivity in river-estuaries. The occurrence of a spring phytoplankton bloom, commonly attributed to the onset of higher water temperatures (Hjerne et al. 2019), is often a period where autochthonous sources dominate both POM and DOM pools (McGovern et al. 2020). Here, atmospheric controls such as warmer temperatures and wind patterns can shift the timing and magnitude of the bloom period (Hjerne et al. 2019), adding to the complexities of understanding OM sources and dynamics in these estuarine ecosystems. The release of marine DOC from autochthonous primary production of POM has also been reported to predominate over tDOC inputs in the Baltic Sea despite high tDOC concentrations found in rivers draining into the large basin (Hoikkala et al. 2015, Osburn and Stedmon 2011). It is important to note that these findings can vary significantly across spatial scales, which are ultimately decided upon by the scope of individual studies. In this case, conclusions made for the Baltic Sea reflect that of a large basin that likely expresses an average of all the various processes occurring in the many contributing river-estuaries. Large basins aside, these periods that are dominated by autochthonous OM can shift drastically in upper estuary, particularly with the spring freshet, towards a terrestrially dominated source of OM (McGovern et al. 2020). Clearly, there are many contributing sources of DOM to coastal waters and these differences can have a strong control on spatiotemporal POM and DOM dynamics. Because of these complexities, estuaries exhibit very high spatiotemporal variability that is likely to be system specific. As a result, our understanding of OM in estuaries, in particular high latitude estuaries, remain very much unconstrained.

4 Transformations of OM during Estuarine Mixing

Riverine OM was historically thought of as relatively stable and resistant to transformations along route to the ocean (Bianchi 2007, Hansell and Carlson 2015). Today, progress in the field of coastal biogeochemistry has identified estuaries and coastal systems as effective filters of OM, often trapping significant quantities of tOM from reaching the open ocean via transformational processes (He et al. 2016, Carstensen et al. 2020, Ward et al. 2017, Bianchi et al. 2018, Dagg et al. 2004). Simultaneous processes such as microbial production and respiration (Berggren et al. 2010, Andersson et al. 2018), photochemical degradation (Chupakova et al. 2018, Miller and Moran 1997), and flocculation and sedimentation (Sholkovitz 1976, Sholkovitz, Boyle, and Price 1978, Forsgren, Jansson, and Nilsson 1996) act as key removal mechanisms of tOM in coastal systems (Figure 3A). The relative role of these mechanisms are in part dependent on both OM composition and concentrations present within coastal zones. Today, our understanding of the relative contributions of these processes to bulk OM pools in the coastal zone remains unresolved, largely because it is difficult to tease apart the combination of multiple potential sources of OM along with these simultaneous processes.

Photochemical transformations play a vital role in altering OM composition and biogeochemical cycling across land to sea gradients. This process is driven by DOM absorption of sunlight and as such, primarily affects CDOM in the photic zone (Miller and Kieber, 2002). Generally, DOM can be transformed into additional lower molecular weight compounds and into biologically available photoproducts that can be nitrogen and phosphorus-rich (Moran and Zepp 1997, Miller and Moran 1997, Kieber, Li, and Seaton 1999, Kohler et al. 2002). These transformations can favour microbial uptake into biomass and production of CO_2 (Smith and

Benner 2005, Moran and Covert 2003) and accelerate the removal of tDOM in coastal waters (Figure 3; A3). Evidence also suggests that the destruction of biologically available DOM can also occur under prolonged irradiation (Reader and Miller 2014). The chemical composition of CDOM plays an important role in this process, with terrestrially derived and more humic-like CDOM having been reported to be more susceptible to photo-reactivity (Zhu, Yang, and Zhang 2017). Such photochemical transformations of DOM and CDOM are likely contributors of CO_2 export to the atmosphere (Miller and Moran, 1997; Figure 3; A2). While the susceptibility of tDOM to photochemical transformations has been relatively consistent, exceptions do exist. For example, Chupakova et al. (2018) found a high resistance of DOM and trace metals to photo-degradation in waters collected from the largest European Arctic estuary. Such disparities highlight the important role of CDOM composition in determining transformations along the land to sea continuum. Nevertheless, over the last few decades, studies addressing photochemical transformations have increased exponentially and have resulted in notable linkages to biological processes.

Biological and photochemical transformations aside, the mixing of fresh and saline waters often result in drastic and abrupt changes to water column physicochemical properties. Perhaps one of the most apparent changes is that of salinity and subsequently, an increase in the ionic strength of river water. Together, these changes can facilitate the aggregation of DOM by preventing the negatively charged DOM molecules from repelling one another with electrostatic forces (Gregory 2013, Asmala et al. 2014). This OM transformation is better known as flocculation. While this mechanism has been well recognized as a key contributor to OM removal within coastal margins, interacting factors such as OM size distribution, concentration and composition of OM and its association with metals (von Wachenfeldt and Tranvik 2008), the formation and maintenance of the estuarine turbidity maximum (Bianchi 2007) and changes to CDOM characteristics (Asmala et al. 2016) make understanding the degree and extent of flocculation and sedimentation difficult. For example, Gustafsson et al. (2000) found that flocculation processes that occur in the water column can be reversed before reaching the benthic layer and as such, may not lead to complete sedimentation. Overall, flocculation selectively transforms Fe and high molecular weight, aromatic OM into POM (Sholkovitz 1976, Sholkovitz et al. 1978, Asmala et al. 2014, Forsgren et al. 1996, Helms et al. 2013, Jilbert et al. 2018, Shulkin et al. 2018). This transformation could effectively intercept large amounts of tDOM being delivered by rivers through redirection into the benthos (Figure 3; A5-6), thereby contributing to the mismatch of high tDOM being delivered by rivers but a lack of it found in the ocean (Massicotte et al. 2017, Hedges et al. 1997). While flocculation could occur from changes in salinity alone, photo-flocculation (Helms et al. 2013, von Wachenfeldt et al. 2008) and biological flocculation (Lee, Hur, and Toorman 2017) have also been reported. These studies illustrate the complex interactions between physical, chemical and biological fields and as a result, the potential and consequences to C export of such transformations remain unclear.

5 Implications for coastal systems

Combined changes to environmental conditions and fluxes of riverine OM in northern high latitudes, along with transformations of OM across the land to sea continuum ultimately determine energy sources for food webs in the coastal ocean. In these systems, heterotrophic bacterioplankton are the primary consumers of DOM (Hansell and Carlson 2015). Reports of high bacterial production during high flow periods (Andersson et al. 2018, Figueroa et al. 2016) link tDOM inputs and basal production. In addition to an increase in nutrient delivery during these periods, the increased delivery of tDOM can further facilitate phytoplankton growth via bacterioplankton release of inorganic nutrients (Traving et al. 2017). While DOC is no doubt an important component for bacterial respiration (Rochelle-Newall et al. 2004), the complex mixture of macro (N and P) and micronutrients (Fe) making up DOM often act as limiting elements in coastal systems (Dittmar, Fitznar, and Kattner 2001, Holmes et al. 2011, Mutschlecner et al. 2018, Cai et al. 2008, Ylöstalo et al. 2016). In some systems, it has been suggested that river DOM is more of a source of nutrients to the estuary and thus, contributes to bacterial respiration (Soares and Berggren 2019). These limiting elements are often dynamic,

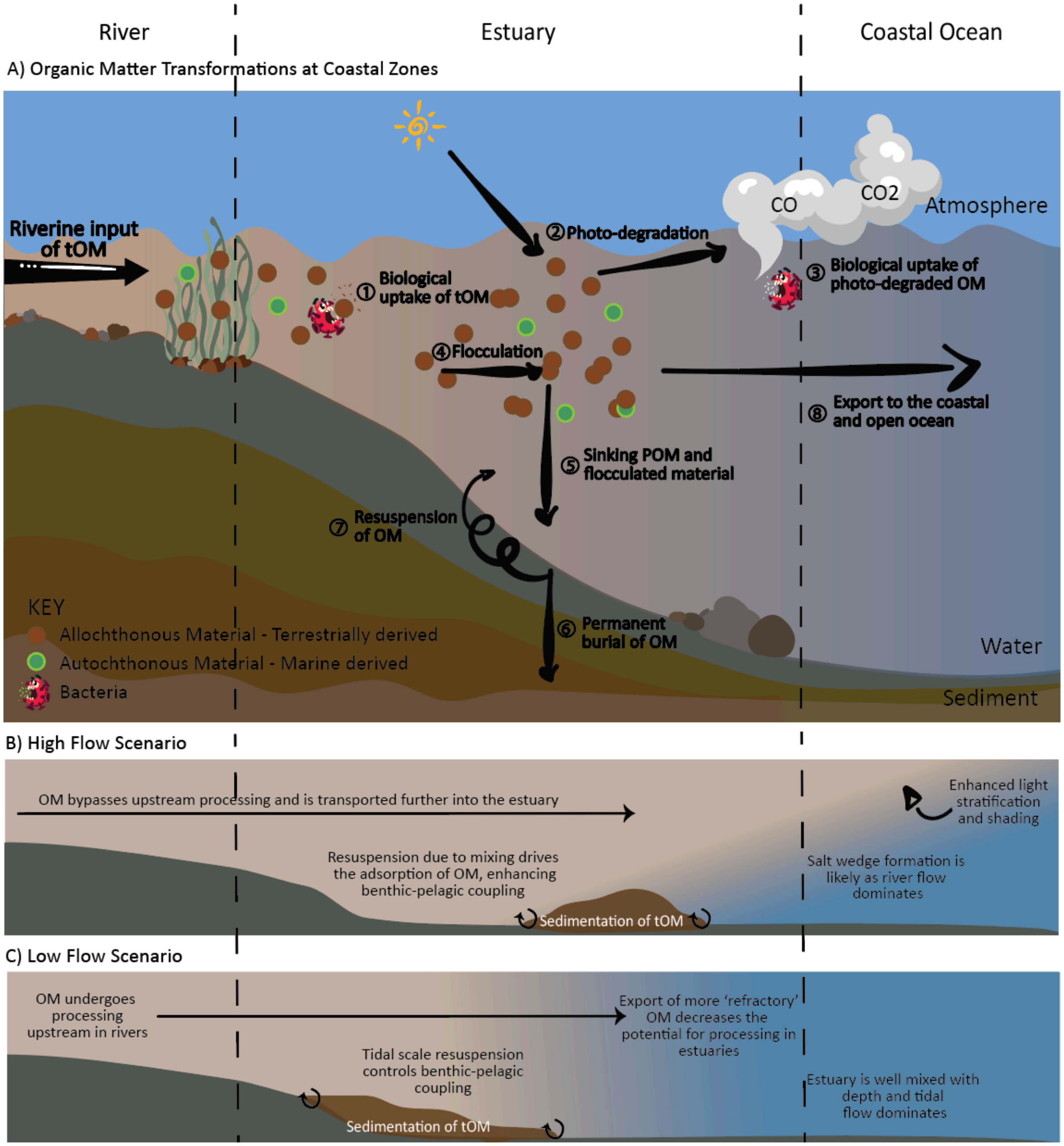

Figure 1.3. Interacting physical, chemical, and biological processes that transform organic matter (OM) along route to the ocean. OM can be transformed simultaneously via; biological uptake (Al; Berggren et al. 2010), photo-degradation and thus the release of inorganic nutrients and carbon gases (A2; Miller and Moran, 1997), biological uptake of photo-degraded material (A3; Moran et al. 2000), and flocculation (A4; Sholkovitz 1976). After transformation, OM can be exported into the benthos (A5-A7), to the coastal and open ocean (A8), and/or into the atmosphere as gases. The degree and extent of these processes can vary with hydrology, particularly in river-influenced coastal zones (B, C). The maximum turbidity zone and associated sedimentation can be present from low (right dashed line) to high salinities (right dashed line) within an estuary.

varying with space and time. For example, bacterial production in areas closer to the river mouth may be C-limited but, N and P-limited further towards the ocean (Figueroa et al. 2016, Hoikkala et al. 2009). Because estuaries can act as efficient coastal filters for tDOM (e.g. flocculation and sedimentation, upstream biological DOM consumption), bacterioplankton communities located further from the river mouth may need to be more reliant on phytoplankton-derived DOM and in doing so, may put them in direct competition with phytoplankton for inorganic nutrients (Thingstad et al. 2008). These local and regional heterogeneities highlight the importance of understanding processes within coastal zones such as flocculation and the impacts it may have on POM and DOM dynamics, along with how it may alter elemental ratios (C:N:P) that are key for biological productivity. Nevertheless, increasing river discharge and tDOM fluxes to the coastal ocean can shift basal resources and potentially lead to net heterotrophic systems (Wikner and Andersson 2012, Soares et al. 2018, Sipler et al. 2017a, Sipler et al. 2017b) and at the very least, shifts in microbial community composition (Sipler et al. 2017a, Traving et al. 2017).

In addition to nutrient and energy sources, basal production is highly dependent on light availability. While the increase in freshwater and tDOM may bring additional pulses of energy to the coastal ocean (Frigstad et al. 2020), the characteristic CDOM-rich rivers of Arctic-boreal regions can have negative impacts by limiting light availability. For example, a reduction in light needed for photosynthesis, linked to tDOM shading, had a negative effect on N utilization in Arctic microbial communities (Sipler, Baer, et al. 2017) and limited phytoplankton growth (Traving et al. 2017). In addition to light availability, temperature and salinity are also key factors in determining coastal productivity (Hoikkala, Aarnos, and Lignell 2009, Bittar et al. 2016). Because these interlinked and competing effects make predicting coastal ecosystem responses difficult, there remains a need to better understand the underlying delivery and reactivity of tOM at greater spatial and temporal resolutions.

6 Thesis Significance, Intent and Structure

Estuaries integrate multiple systems at vertical and horizontal scales across scientific fields (e.g. land-to-sea; atmosphere-to-benthos) and it is in these interlinkages that make estuarine biogeochemistry difficult to study. Since the first definition of an estuary in 1967, biogeochemical conclusions have remained broad, system specific, and OM pool (DOM or POM) specific. From field logistical challenges to the difficulties in analyses and interpretation, our understanding of these systems trail behind that of its neighbouring systems. For the coastal ocean, understanding ecosystem response requires detailed studies in OM and nutrient delivery by rivers, OM reactivity along transport and within controlled experimentation designed to explore underlying processes such as OM transformations and biological utilization. Ultimately, studies conducted in estuaries today are of vital importance in helping predict and understand global biogeochemical cycles, commercially important fisheries and ultimately, the livelihoods of billions of people that rely on these river and coastal systems for food security.

The overall intent of this thesis was to improve our understanding of the potential transformation, impact and fate of OM within high latitude river-influenced estuaries. To do this, I first studied how two neighbouring systems and their associated catchment characteristics and seasonal hydrology relate to riverine OM inputs by; 1) analysing the elemental (C, N, Fe) composition and reactivity of size fractionated OM distributions within contrasting rivers and their estuaries; and 2) assessed how they varied seasonally. I then conducted a series of salt-addition experiments alongside each of these observational samples to explore the potential for flocculation of the riverine DOM. This research resulted in two chapters; an observational study and an experimental study with the aims in answering the following two questions:

1. How does the size fractionated OM composition along river-estuarine gradients vary among: a) periods of high and low flow and b) neighbouring systems?
2. What is the relative potential for salt-induced flocculation and how does it relate to the observed variation in OM size fractions and their elemental composition?

Water samples for this thesis were collected from Piper's Hole and Come by Chance rivers and their respective estuaries in Placentia Bay, Newfoundland. The four established sampling sites were visited monthly from February to October 2019, with the exception of September where samples were taken weekly.

This thesis consists of 4 chapters, with the first and last chapters as the overall research overview and summary. Chapters two and three are individual research papers, each containing its own introduction and conclusion sections. These chapters address the two questions outlined above, starting with the observational chapter.

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CO-AUTHORSHIP STATEMENT

The initial idea for the project was proposed by Dr. Rachel Sipler and Dr. Susan Ziegler upon discussion of my research interests on aquatic biogeochemistry. Sampling campaigns were designed by myself, Dr. Rachel Sipler, Dr. Susan Ziegler and Sally Faulkner using historical climatological and hydrological data. Flocculation experiments were designed by myself, with input from my full supervisory committee.

The collection of field samples and initial sample processing was conducted by myself, Sally Faulkner, Colleen McBride, Maryam Beheshti Foroutani, Sean Boyd, and Alexandria Fudge, under the supervision of Dr. Rachel Sipler and Dr. Susan Ziegler. Flocculation experiments were carried out by myself with the help of Maryam Beheshti Foroutani, Alexandria Fudge, and Dr. Rachel Sipler.

Sample analysis was conducted by myself, Sean Boyd and Sally Faulkner. Specifically, Sally Faulkner conducted chlorophyll-a and inorganic nutrient analyses, and Sean Boyd conducted analyses on particulate matter samples. I conducted all other data analyses and interpretation, and wrote the initial manuscripts under guidance from Dr. Rachel Sipler and Dr. Susan Ziegler. Co-authors on each of the manuscripts then contributed to revisions prior to the papers being submitted for publication. I compiled these manuscripts into the presented thesis.

CHAPTER 2: A COMPARISON OF THE DISTRIBUTION AND DYNAMICS OF PARTICULATE AND DISSOLVED ORGANIC MATTER ACROSS TWO BOREAL COASTAL SYSTEMS

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ABSTRACT

Observed and predicted hydrological changes in C-rich northern mid-to-high latitudes has the ability to change the trajectory of organic matter (OM) export to ecologically, economically, and culturally important coastal systems. Yet, a large gap remains in our current understanding of the distribution and sources of particulate (PM) and dissolved organic matter (DOM) across salinity gradients, particularly for understudied remote small systems. In this study, we assessed the distribution and dynamics of these OM pools across two adjacent freshwater-to-saline systems in the boreal zone. Overall, the dissolved fraction dominated (95%) the total organic carbon pool at the freshwater sites, and was further characterized as C-rich, nutrient poor, and made up of larger, more aromatic DOM. Variation in DOM concentrations and dissolved Fe to DOC ratio highlighted the influence of catchment characteristics and were the best indicators for the temporal stability of the DOM pool. DOM concentrations, degree of aromaticity, and chromophoric DOM size were generally lower, more variable and coupled with PM at the saline sites relative to the freshwater sites. We found evidence that link these dynamics to system specific processes associated with variations in salinity and seasonal biological production. Combined, these results show that variation in the PM, DOM, and their potential coupling occurs locally and at weekly timescales, particularly during the summer-to-fall transition period. These findings highlight the important considerations of biogeochemical diversity in understanding the movement and fate of OM in near-shore coastal systems.

1 Introduction

Canada's boreal zone is one of the most carbon and water rich areas of the world (Brandt et al. 2013, Schuur et al. 2018). This volume of water acts as a vessel for organic matter (OM) and nutrients, intricately linking the carbon stored in boreal soils to biogeochemical cycles and food web dynamics in the coastal zone. For Atlantic Canada, these coastal ecosystems are vital in sustaining the region's essential fisheries, marine mammal populations, and seabird colonies (Bernier et al. 2018). Already, biogeochemical cycles across the boreal zone are threatened by changing hydrologic regimes (Wrona et al. 2016), with predictions of increasing winter flow that is coupled with earlier, more intermittent spring melts (Woo et al. 2008), along with changing intensity and onset of water fluxes during the summer to fall transition. Consequently, an increase in the export of terrestrial dissolved organic matter (tDOM) to the coastal ocean is expected (McClelland et al. 2011, Huntington et al. 2016) and is likely to have major consequences on coastal food web dynamics by changing microbial composition (Sipler et al. 2017a, Traving et al. 2017) and shifting estuarine systems towards net heterotrophy (Wikner and Andersson 2012, Sipler et al. 2017a, Soares et al. 2018).

In rivers, the composition and dynamics of particulate (PM) and dissolved organic matter (DOM) reflect catchment processes and characteristics such as hydrological flow paths, topography, and catchment size (Walvoord and Striegel, 2007, Harms et al. 2016, Mari et al. 2007). For example, smaller catchments have higher dissolved organic carbon (DOC) concentrations that are biologically and photochemically reactive in the short-term as a result of shorter water retention times and increased connectivity between the landscape and stream (Mattson et al. 2009, Franke et al. 2012). In addition to these determining factors, OM cannot be transported without a water flux, and as such, variations in seasonal hydrology like those aforementioned exert the greatest control on the source and flux of riverine OM (Raymond et al. 2016). Like DOM, PM can vary seasonally in relation to flow magnitude. For example, higher concentrations of soil-derived PM are mobilized during high flow periods via the drainage of surficial soils whereas summer baseflows result in low concentrations of PM that are more reflective of algal sources (Guo et al. 2015, Bright et al. 2020).

In estuaries, OM signatures not only reflect riverine material and its catchment influences, but also marine OM brought in from tidal action and OM produced from within the estuary itself. These different sources of OM have different biological and ecological consequences as they participate in local metabolism in different ways. Unlike tDOM, algal sources of DOM are more easily assimilated by microbial consumers as they are typically protein rich, enriched in nitrogen (C:N ~ 5-20; Bianchi 2007) and consist of smaller monomeric compounds (Donderski et al. 1998). Several studies, however, have found tDOM to be bioavailable for coastal plankton communities across Arctic and boreal regions (Autio et al. 2016, Kaiser et al. 2017, Sipler et al. 2017b). Likewise, while algal-derived PM is generally thought to be the highest quality food source in aquatic systems, studies suggest terrestrial-derived PM can be bioavailable and act as an important secondary food source for estuarine zooplankton (Harfmann et al. 2019, Berggren et al. 2015). These studies add to a growing body of evidence that highlight the important ecological consequences of different OM sources, one of which being an ecological shift towards net heterotrophy in response to increased fluxes of terrestrial-derived OM.

Along transit from headwaters to the ocean, exchanges between DOM and PM pools add to the list of possible OM sources within each pool. For example, exudation or breakdown of microbial cells and other higher trophic level organisms (a component of PM) can add to the DOM pool, or the aggregation of DOM can lead to the formation of PM (Thornton 2014, He et al. 2016). Our understanding of the sources and fate of OM along salinity gradients and its interlinkages across size fractions (dissolved and particulate phases) remains unclear, largely due to its inherently heterogeneous nature and the complex estuarine processes (e.g. photochemical degradation, flocculation, biological uptake) that are often in competition (Bauer and Bianchi 2011). In addition, our ability to analyse samples along physicochemical gradients accurately, consistently, and at low concentrations (Bauer and Bianchi 2011) pose further challenges in comparing across PM and DOM pools. Contributing to these challenges, simultaneous characterization of OM across size fractions along a river to estuarine continuum is rare, despite the dynamic interplay between the pools in such globally important ecosystems.

The objectives of this study were to: (1) examine the elemental and isotopic composition of size fractionated organic matter and its distribution within two arms of Placentia Bay, a boreal estuary in northeastern Canada; (2) explore the seasonal variation in OM concentration and composition across the salinity gradient; and (3) generate hypotheses on the potential effects of watershed characteristics and transformations on boreal OM dynamics.

2 Methods

2.1 Study sites and collection frequency

This study was conducted in two major arms of Placentia Bay, located on the southeastern coast of Newfoundland, Canada (Figure 1). The area is situated within the boreal shield ecozone (Vasseur and Catto 2008) and lies at the boundaries of major atmospheric circulation systems. Broadly, it is an area where cold air from the Arctic converges with warm air from subtropical and temperate regions (Drinkwater, 1996, Banfield and Jacobs, 1998), resulting in frequent fog cover and high precipitation (Catto et al. 1999, Cadigan 2009). The two arms defined by the river entering the coastal zone, Piper's Hole (PHR) and Come By Chance (CBC), are located within 30 kilometers of one another and as such, experience similar climatic conditions. Both arm systems have previously been geomorphologically classified by Catto et al. (1999) as salt marsh zones, supported by moderate sediment accumulation and low energy conditions. In terms of the catchment characteristics for each river discharging into these two arms, the PHR catchment area is considerably larger (787 km^2) relative to CBC (45.9 km^2). Lower relief (average percent slope = 5.46 $m\ m^{-1}$) at PHR contributes to a greater percent of the land being classified as water bodies (15.2%) relative to CBC (9.21%, average percent slope = 5.89 $m\ m^{-1}$).

A total of four study sites, two upstream freshwater and two downstream saline sites, were selected to represent a salinity gradient within each of the arms. The approximate distance between the fresh and saline sites of PHR was 12 kilometers and 14 kilometers for CBC.

Consistent with low energy conditions as described by Catto et al. (1999), the saline sites are also classified as micro tidal, experiencing a tidal range of up to two meters. Additionally, depth at the saline sites did not exceed 8 meters whereas at the freshwater sites, depth did not exceed 2 meters across the sampling period (associated data are submitted to the St. Lawrence Global Observatory-SLGO; awaiting DOI). At the freshwater sites, bottom type was observed to be predominantly boulder and bedrock at PHR and silt and boulder at CBC. Altogether, 34 discrete collections were made at these four sites from March to October 2019. The sites were sampled approximately every month during the study period and weekly during the summer-to-fall transition period spanning from mid-August to September (A1. Table 1; A1. Table 2).

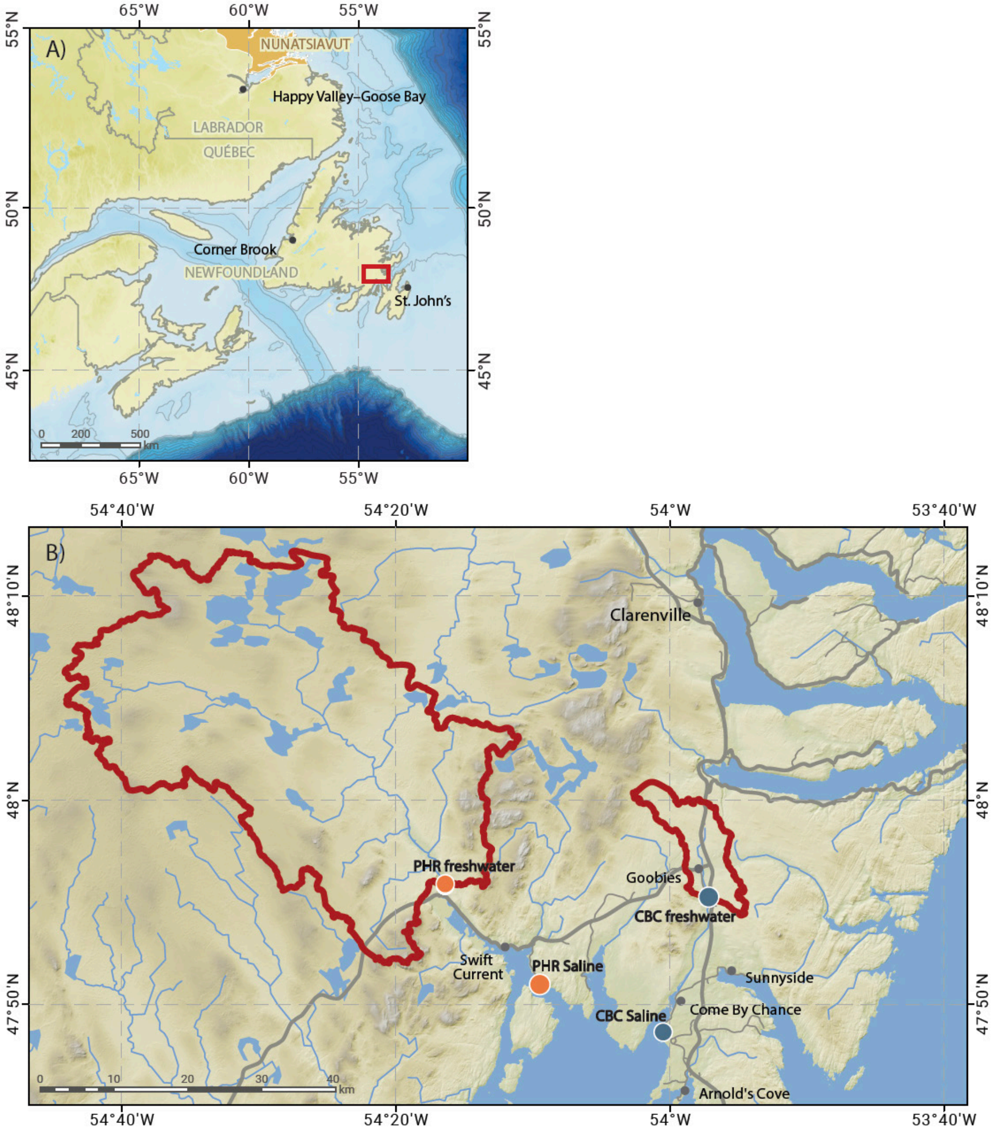


Figure 2.1. Placentia Bay, Newfoundland freshwater and saline study sites. Sites are located in easternmost Canada and in south-eastern Newfoundland (panel A). Red boundary lines in panel B indicate Piper's Hole and Come By Chance catchments, located on the left and right, respectively. Orange circles mark the sites within PHR, and blue circles mark the sites within CBC.

2.2 Sample collection

Field collection within each system occurred within an hour, while the two systems were visited within 1-3 days of the other to allow for timely sample processing. Surface samples were collected from each site using a conditioned and acid-washed (10% HCl) bucket and passed through a 200µm mesh into acid-washed (10% HCl) carboys. Following transport back to the laboratory at Memorial University of Newfoundland's Ocean Science Centre, samples underwent vacuum filtration through pre-combusted Advantec MFS GF-75 filters (0.3µm; 450°C for 4 h). Filters were then collected in pre-combusted (500°C for 4 h) foil, immediately frozen at -20°C and freeze-dried for 3-5 days prior to PM analyses. The filtrate was subsampled into several small volume (15-125 mL) acid-cleaned HDPE bottles or acid-cleaned and combusted glass (500°C for 4 h) and stored frozen until DOM analyses. Subsamples for coloured dissolved organic matter (CDOM) and fluorescent dissolved organic matter (FDOM) were stored in amber HDPE bottles at 4°C until analysis.

2.3 Sample Analysis

2.3.1 Dissolved organic matter (DOM)

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) samples were analysed at the CREAIT Aquatic Research Cluster at Memorial University of Newfoundland by high temperature combustion on a Shimadzu TOC-V_{CPH}/TN autoanalyzer. Samples were acidified manually using 6N HCl prior to analysis to remove inorganic fractions, and all methods were kept consistent for fresh and saline water samples. Low carbon and deep seawater reference material from the University of Miami were used for instrument checks and data quality assurances.

Dissolved inorganic nitrogen (DIN = NH_4^+ plus NO_x^-) concentrations were determined using a Lachat QuickChem 8500 autoanalyzer, and the detection limits for NH_4^+ and NO_x^- were 0.23 and 0.01 µmol N L^{-1}, respectively. Dissolved organic nitrogen (DON) was calculated by subtracting DIN from TDN, and errors were propagated throughout for standard deviation calculations. DOC:DON, a common source indicator for terrestrial vs. marine influenced DOM (Bianchi 2007, Canuel and Hardison, 2016), was then calculated as the molar ratio.

Dissolved iron (DFe) was determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES), with a detection limit of 0.036 µmol Fe L^{-1}. ICP-OES was conducted at the Geological Survey of Newfoundland and Labrador geochemical laboratory. Fe is an important micro- and macro-nutrient for primary producers in coastal waters. However, its availability in aquatic ecosystems is in part dependent on the movement, quantity, and quality of the DOC pool (Kritzberg et al. 2019). To investigate these relationships, the molar ratio of dissolved Fe to DOC (DFe:DOC) was calculated. All samples that were below the detection limit for DFe are reported as the detection limit value, and when observed, the detection limit value was used in the calculation of the DFe:DOC ratio, providing a maximum numerical value for DFe:DOC.

2.3.2 Chromophoric dissolved organic matter (CDOM)

Spectrophotometric analysis of CDOM was performed using Perkin Elmer Lambda 25 and Cary UVWin spectrophotometers with crossover of a subset of samples to confirm consistency between instruments. Analysis on all CDOM samples were conducted within a week of sample collection. Samples were analysed using a 1cm quartz cuvette, and where CDOM signals were low, a 5cm cuvette was used. Milli-Q water (18.2 mΩ) was used as a reference for all samples. Absorbance measurements were transformed to napierian absorption coefficients using Eq. 1, where A(λ) is the measured absorbance and l is the pathlength in meters. CDOM parameters; $SUVA_{254}$ and spectral slope ratio ($Sr = S_{275-296}/S_{350-400}$) were calculated according to Weishaar et al. (2003) and Helms et al. (2008) respectively. Additionally, we chose the absorbance at 350nm as a proxy for CDOM concentration, which has been positively correlated to DOC concentrations in estuaries (Osburn et al. 2016). $SUVA_{254}$ has been found to be correlated with DOM aromaticity (Weishaar et al. 2003) while S_r has been related to CDOM molecular weight (Helms et al. 2008).

$a(\lambda) = 2.303*A(\lambda)/l$ (**Eq.1**)

2.3.3 Particulate Matter

Whole 25mm 0.3µm GF75 (Advantec MFS) filters were analysed for particulate carbon (PC), particulate nitrogen (PN) and $\delta^{13}C$ values at the CREAIT Stable Isotope Laboratory at Memorial University of Newfoundland. These measurements were made with a Vario Isotope Cube (Elementar) elemental analyzer coupled via a ConFlo IV Interface to a Delta V Plus (Thermo Scientific) isotope ratio mass spectrometer. For PC and PN, daily correction factors were determined using acetanilide at the beginning of each run. For $\delta^{13}C$ analysis, measured isotopic values of two reference materials purchased from Indiana University (EDTA #2 with a $\delta^{13}C$ value of -40.38 ‰ (± 0.01 ‰), and caffeine (USGS62) with a $\delta^{13}C$ value of -14.79 ‰ (± 0.04 ‰)) were used to correct measured values of the samples. Protein B2155 (purchased from Elemental Microanalysis: 46.50 ± 0.78% C; 13.32 ± 0.40 % N, $\delta^{13}C$ = -27.03 ± 0.13 ‰) were also analysed multiple times throughout each run for quality checks. Stable C isotopic signatures are commonly used to distinguish between terrestrial and aquatic sources as they reflect differences in biosynthetic pathways (Bianchi 2007). Note that because we did not acidify filters prior analysis, these values may include traces of inorganic particulate matter.

Chlorophyll-a (Chl-a) measurements, which are used as a proxy for phytoplankton abundance, were determined from whole 25mm 0.3µm GF-75 (Advantec MFS) filters using a Turner 10-AU fluorometer. Pigments were extracted in 8mL of 90% acetone for 24 hours in a -20°C freezer. Filter blanks, treated in the same manner, were used as the reference blank for all samples. To estimate the proportion of phytoplankton derived carbon (Caperon et al. 1976), we then calculated the weight:weight ratio of PC to Chl-a (PC:Chl-a).

2.4 Statistical Analysis

In consideration of the sites being sampled two days apart and at irregular intervals, we bracketed the temporal component of the data into sampling events so that the 4 sites were represented in each sampling event. The exception to this is for the March sampling where only the Piper's Hole sites were sampled. A hierarchical two-way ANOVA was used to explore scales of spatial variability as well as the temporal effect among the samples. The samples were collected at sites within each river-estuary system and water body type (freshwater or saline); thus, sites are nested within these two categorical variables. The statistical model of this experimental design is as follows: $Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_{jk(l)} + \varepsilon_{ijk(l)}$ where Y_{ijkl} is the dependent variable, μ is the overall mean, α_i is the main fixed effect of sampling event where i = 1-9, β_j is the main fixed effect of river-estuary where j = 1-2 for either PHR or CBC, γ_k is the main fixed effect of water body type where k = 1-2 for either river-mouth or bay, $\delta_{jk(l)}$ is the term for the random effect of the 4 sites (l) nested within water body type (k) and river-estuary (j), and $\varepsilon_{ijk(l)}$ is the random error term. Visual inspection of the histogram of residuals, Q-Q plot, fitted vs. residuals plot, and Shapiro-Wilks were used to assess normality and homogeneity of variance assumptions. These assumptions were met by all untransformed DOM parameters except for $SUVA_{254}$ and S_r. For $SUVA_{254}$ and S_r, we conducted additional analysis on transformed data (log), however, assumptions of homogeneity were still violated, implying that there is strong evidence of the samples being non-identical in regard to these parameters. The proportion of variance explained by each of the terms in the statistical model is calculated by dividing the sum of squares of each term by the total sum of squares.

Correlation analyses were used to assess the relationships between the different OM parameters at each site during the study period. Analyses were conducted using the 'cor' function and plotted using the Hmisc package in R. For the correlation method, the non-parametric Kendall rank correlation was chosen to be inclusive of monotonic relationships that were not linear, and for its robustness regarding the smaller sample sizes in our study. All statistical analyses were carried out using R.4.1.0 (R Core Team, 2021).

3 Results

3.1 Hydrology and environmental conditions

Magnitudinal changes in river flow often determine biogeochemical fluxes from the land to sea. In both PHR and CBC, irregular non-seasonal and regular seasonal peaks were observed throughout the study period (Figure 2). The spring snowmelt period was not well defined in 2019 across both systems, as several large peaks were observed into July when snow and ice were no longer prevalent. Following summer low flow conditions, the reappearance of interspersed peaks along with a rise in base flow in September signified a change from summer to fall across the region. From these hydrologic patterns, we can identify four seasons, divided broadly as: winter (December-February), spring (March-May), summer (June-August), and fall (September-November).

Salinity is another factor that can affect the biogeochemistry of aquatic systems, especially in coastal areas. The freshwater sites represented true freshwater systems and remained outside the reaches of tidal influence throughout the study period. At the saline sites, salinity at CBC averaged at 32.4 psu ± 3.5 while PHR averaged at 28.3 psu ± 6 (Figure 2). At both sites, the highest salinities were recorded in late September. For PHR, salinity of the March sampling was notably lower than the rest of the year at 16 psu, which was likely a result of elevated river flow during this time.

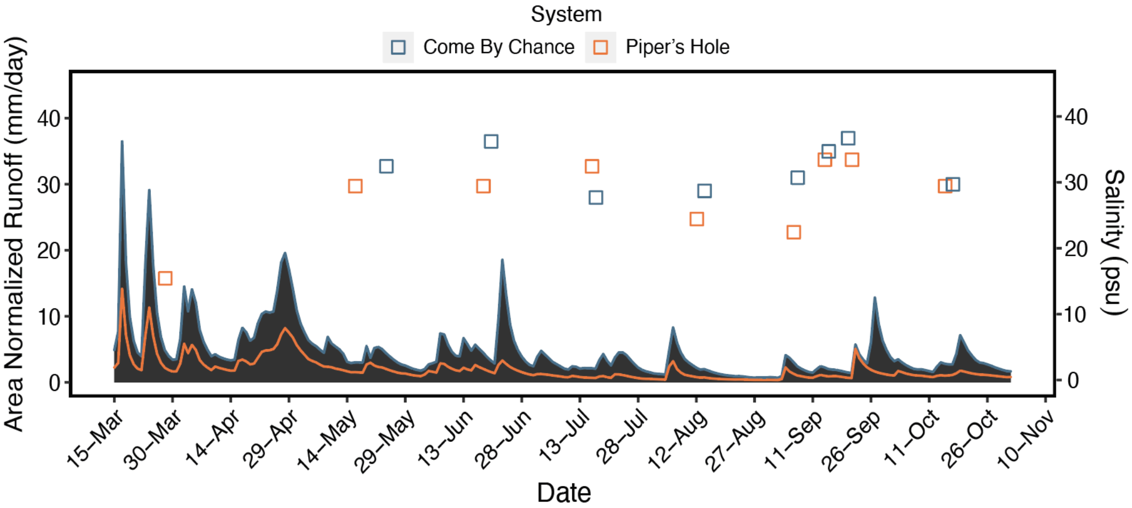


Figure 2.2. Environmental conditions for the Placentia Bay study sites. A time series of area normalized river runoff (solid line and area) for the freshwater sites and salinity (squares) for the

saline sites during the 2019 study period. Come By Chance is represented in blue and Piper's Hole is represented in orange. Streamflow data was extracted from the Environment and Climate Change Canada Hydrometric Database (Station numbers 02ZH002 and 02ZH001, respectively).

3.2 Distribution of DOM concentrations

A wide range in DOM concentrations were captured throughout the study period and across the four study sites. Overall, concentrations varied up to 8-fold in DOC, 2-fold in DON and 200-fold in DFe (Table 2.1; Figure 2.3a; 2.3b; 2.3d). A large portion of this variability was accounted for by water body type (Table 2.3), with concentrations being consistently lower at the saline sites. More specifically, freshwater DOC averaged 682.9 µmol C L^{-1} at PHR and 791.4 µmol C L^{-1} at CBC, whereas saline DOC concentrations averaged 260.7 and 153.8 µmol C L^{-1} at PHR and CBC, respectively. It is also important to note that across our study sites, DOC accounted for 88-96% of the total organic carbon pool smaller than 200µm (Table 2.2). DON was twice as high in the freshwater sites (PHR = 13.47 µmol N L^{-1}, CBC = 15.74 µmol N L^{-1}) than the saline sites (PHR = 9.04 µmol N L^{-1}, CBC = 8.52 µmol N L^{-1}). The most drastic differences between the sites were recorded for DFe, where the freshwater sites averaged 3.12 µmol Fe L^{-1} and 5.95 µmol Fe L^{-1} for PHR and CBC, respectively. At the saline sites, with the exception of PHR during the spring, DFe concentrations were below or close to detectable levels (0.036 µmol Fe L^{-1}) throughout the study period.

DOM concentrations (DOC, DON, DFe) simultaneously peaked in late summer for both freshwater sites and the PHR saline site (Figure 2.3). At these three sites, fluctuations throughout the year were also similar, and is supported by significant positive correlations ($p < 0.05$) between DOC, DON and DFe (Figure 2.5). Variability across the year is notable as a significant temporal effect (sample event) on DOC, DON and DFe was also observed among the samples (Table 2.3a). In comparison between the two systems, DOM concentrations at the freshwater and saline PHR sites mimicked one another, whereas concentrations at the saline CBC site remained more stable despite fluctuations at its freshwater endmember (Figure 2.3). It is interesting to note that while DOC and DFe remained stable throughout the year at the saline site of CBC, DON was more variable. These DON fluctuations were also observed for the PHR saline site.

Table 2.1. Dissolved organic matter characteristics for the four study sites. Numbers in parentheses reflect the range of values observed throughout the study period. Parameter abbreviations are as follows: DOC: dissolved organic carbon, DON: dissolved organic nitrogen, DOC:DON: dissolved organic carbon to nitrogen ratio, DFe: dissolved iron, DFe:DOC: dissolved iron to carbon ratio, $SUVA_{254}$: specific UV absorbance at 254nm normalized to DOC, $S_{(275-295/350-400)}$: spectral slope ratio.

Site	Salinity (psu)	DOC (µmol L-1)	DON (µmol L-1)	DOC:DON (Ratio)	DFe (µmol L-1)	DFe:DOC (Ratio)	SUVA254 (L mg-1 m-1)	S(275-295/350-400) (Ratio)
Piper's Hole Freshwater	0	682.9 (484.9 - 902.3)	13.47 (9.72 - 18.07)	47.03 (41.90 - 53.28)	3.12 (1.73 - 5.33)	0.0049 (0.0036 - 0.0062)	3.39 (3.14 -3.55)	0.80 (0.77 - 0.83)
Piper's Hole Saline	28.3 (16.0 - 34.0)	260.7 (117.2 - 473.4)	9.04 (6.13 - 12.41)	27.68 (17.74 - 41.60)	0.29 (0.04 - 0.97)	0.0010 (0.0002 - 0.0024)	2.29 (1.63 - 3.11)	1.06 (0.84 - 1.38)
Come By Chance Freshwater	0	791.4 (614.8 - 909.7)	15.74 (10.76 - 18.13)	50.46 (45.90 - 56.71)	5.95 (2.53 - 8.27)	0.0074 (0.0041 - 0.0096)	3.49 (3.08 - 4.02)	0.77 (0.73 - 0.79)
Come By Chance Saline	32.4 (28.0 - 37.0)	153.8 (111.8 - 299.2)	8.52 (6.31 - 11.15)	18.06 (12.33 - 26.82)	0.05 (0.04 - 0.11)	0.0003 (0.0003 - 0.0005)	1.69 (1.14 - 2.57)	1.41 (1.00 - 2.01)

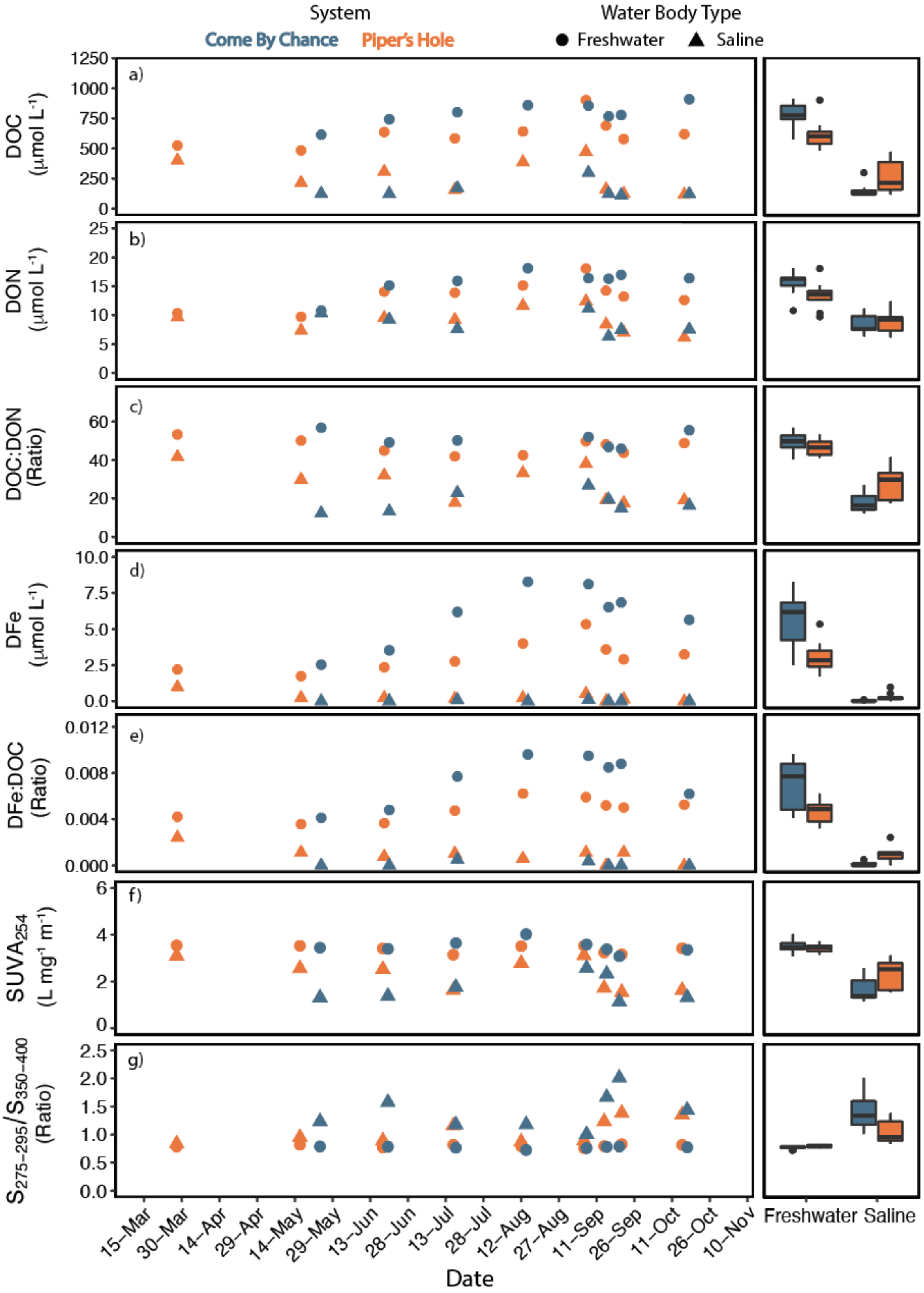


Figure 2.3. Sampling event (left panels) and total variation (right panels) in a) dissolved organic carbon (DOC), b) dissolved organic nitrogen (DON), c) dissolved organic carbon to nitrogen

ratio (DOC:DON), d) dissolved iron (DFe), e) dissolved iron to carbon ratio (DFe:DOC), f) $SUVA_{254}$, and g) spectral slope ratio ($S_{275-295/S350-400}$) across the Come By Chance (blue) and Piper's Hole (orange) freshwater (circles) and saline sites (triangles) in 2019.

3.3 Dynamics of the DOM pool

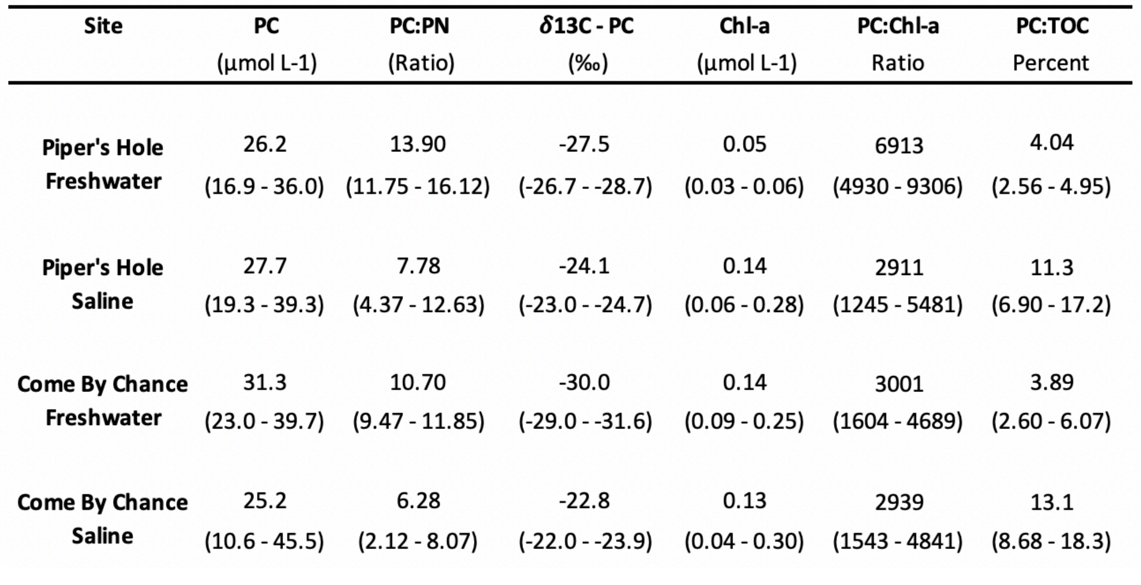
Overall, DOM compositional parameters (DOC:DON, $SUVA_{254}$ and S_r) were more stable at the freshwater sites than at the saline sites throughout the study period (Figure 2.3). DFe:DOC ratios of the bulk DOM pool were an exception to this as DFe concentrations at the saline sites were close to or below detectable levels and thus, not variable (Figure 2.3e). Moreover, DFe:DOC ratios were an order of magnitude higher at the freshwater sites (Table 2.1). While there is a strong positive correlation between DFe and DOC at these sites, DFe:DOC has a stronger correlation with DFe than DOC (Figure 2.5). Similar peaks in DFe:DOC, DFe and DOC in the late summer (Figure 2.3a; 2.3d; 2.3e) support a disproportionately greater change in DFe relative to DOC. A significant effect across all spatial scales and by sampling event was also observed for DFe:DOC (Table 2.3).

As expected, average DOC:DON ratios at the freshwater sites (PHR = 47.04, CBC = 50.46) were higher compared to the saline sites (PHR = 27.68, CBC = 18.06; Table 2.1). A lack of significant correlations between DOC:DON and DOC was observed for the freshwater sites, whereas significant but weak negative correlations were found between DOC:DON and DON (Figure 2.5a; 2.5c). For the saline sites, there were significant positive correlations between DOC:DON and DOC (Figure 2.5b; 2.5d). DOC:DON peaked in late September at the saline sites and do not exhibit clear patterns for the freshwater sites (Figure 2.3c).

The use of a_{350} as an indicator of CDOM and in developing robust CDOM-DOC relationships has proven to be an effective proxy across many estuarine studies (Osburn et al. 2016, Johnston et al. 2018). In our study, these relationships were evident across our study sites with a_{350} highly correlated to DOC ($R^2 = 0.966$, $n = 33$; A1. Figure 1). As a result, fluctuations in a_{350} were similar to that of DOC throughout the study period. These strong linear correlations suggest that general trends in CDOM can explain the bulk DOM pool with some certainty across our sample sites.

CDOM metrics, $SUVA_{254}$ and S_r, used to track relative changes in DOM aromaticity and CDOM molecular weight, both exhibited patterns associated with salinity. $SUVA_{254}$ values were higher and less variable at the freshwater sites, averaging at 3.39 and 3.49 for PHR and CBC, respectively (Table 2.1; Figure 2.3f). At the saline sites, values varied between 1.63 - 3.11 at PHR and 1.14 - 2.57 at CBC. S_r values are inversely correlated to $SUVA_{254}$ and as a result, S_r was higher and more variable at the saline sites, ranging from 0.84-1.38 at PHR and 1.00-2.01 at CBC. In comparison, S_r ranged from 0.77-0.83 and 0.73-0.79 at each, respective, freshwater site (Table 2.1; Figure 2.3g). The greatest fluctuations of $SUVA_{254}$ and S_r at these sites were recorded in late summer which also corresponds to similar fluctuations in DOM concentrations. Interestingly, $SUVA_{254}$ was not significantly correlated to any DOM concentration parameters at either freshwater site (Figure 2.5a; 2.5c). It was, however, positively correlated to both runoff and DOC:DON in the more wetland dominated PHR freshwater site (Figure 5a).

Table 2.2. Particulate matter characteristics for the four study sites. Numbers in parentheses reflect the range of values observed throughout the study period. Parameter abbreviations are as follows: PC: particulate carbon, PC:PN: particulate carbon to nitrogen ratio, δ^{13}C-PC: stable isotopic composition of the particulate carbon, Chl-a: chlorophyll-a, PC:Chl-a: particulate carbon to chlorophyll-a ratio, PC:TOC: percent of total organic carbon smaller than 200µm found in the particulate fraction.



Site	PC (µmol L-1)	PC:PN (Ratio)	δ13C - PC (‰)	Chl-a (µmol L-1)	PC:Chl-a Ratio	PC:TOC Percent
Piper's Hole Freshwater	26.2 (16.9 - 36.0)	13.90 (11.75 - 16.12)	-27.5 (-26.7 - -28.7)	0.05 (0.03 - 0.06)	6913 (4930 - 9306)	4.04 (2.56 - 4.95)
Piper's Hole Saline	27.7 (19.3 - 39.3)	7.78 (4.37 - 12.63)	-24.1 (-23.0 - -24.7)	0.14 (0.06 - 0.28)	2911 (1245 - 5481)	11.3 (6.90 - 17.2)
Come By Chance Freshwater	31.3 (23.0 - 39.7)	10.70 (9.47 - 11.85)	-30.0 (-29.0 - -31.6)	0.14 (0.09 - 0.25)	3001 (1604 - 4689)	3.89 (2.60 - 6.07)
Come By Chance Saline	25.2 (10.6 - 45.5)	6.28 (2.12 - 8.07)	-22.8 (-22.0 - -23.9)	0.13 (0.04 - 0.30)	2939 (1543 - 4841)	13.1 (8.68 - 18.3)

3.4 Distribution and dynamics of the PM pool

Across all sites throughout the study period, PC concentrations were similar, averaging at 27.6 µmol C L^{-1}, and were substantially lower than DOC concentrations at all sites (Table 2.2). Along with similar annual concentrations, there was a lack of significant temporal and spatial effect on PC concentrations (Table 2.3). Nevertheless, some patterns were observable across the study period. For example, during the spring and fall, PC concentrations were higher at both freshwater sites, whereas concentrations switched to being higher at the saline sites during the summer. For both the fresh and saline sites at PHR, PC peaked in late summer, which corresponded to a similar peak in the DOM parameters. However, for CBC, the highest concentrations occurred in May for the freshwater site and in September for its respective saline site (Figure 2.4a).

PM compositional parameters were more variable than DOM across the four sampling sites (Figure 2.4b; 2.4c). Average C:N of the PM pool were higher in the freshwater sites (PHR = 13.90, CBC = 10.70) than at the saline sites (PHR = 7.78, CBC = 6.28) (Table 2.2). Stable carbon isotopic signatures were more depleted for the freshwater sites (-27.5‰ for PHR and -30.0‰ for CBC) relative to the saline sites (-24.1‰ for PHR and -22.8‰ for CBC). Both PC:PN and δ^{13}C-PC did not vary significantly over the study period as confirmed by the lack of temporal effect among the samples (Table 2.3). However, a sudden drop in PC:PN at the CBC saline site was observed in September and corresponded to a decrease in PC, whereas a similar drop in PC:PN at the PHR saline site did not correspond to a decrease in PC. Moreover, despite fluctuations in PC:PN during this time, δ^{13}C-PC remained stable across the saline sites. In contrast to bulk PC concentrations, there was a significant effect across all scales of space (system, water body type and site within water body type) for these PM compositional parameters (Table 2.3a).

Understanding the contributions of biological production to the PC pool can be revealed through Chl-a analysis. The contributions of phytoplankton (as indicated by Chl-a concentrations) to PC were different among the four sites (Figure 2.4e; Figure 2.5). For the PHR freshwater site, Chl-a values were consistently lower and less variable (average 0.05 µg L^{-1}) than

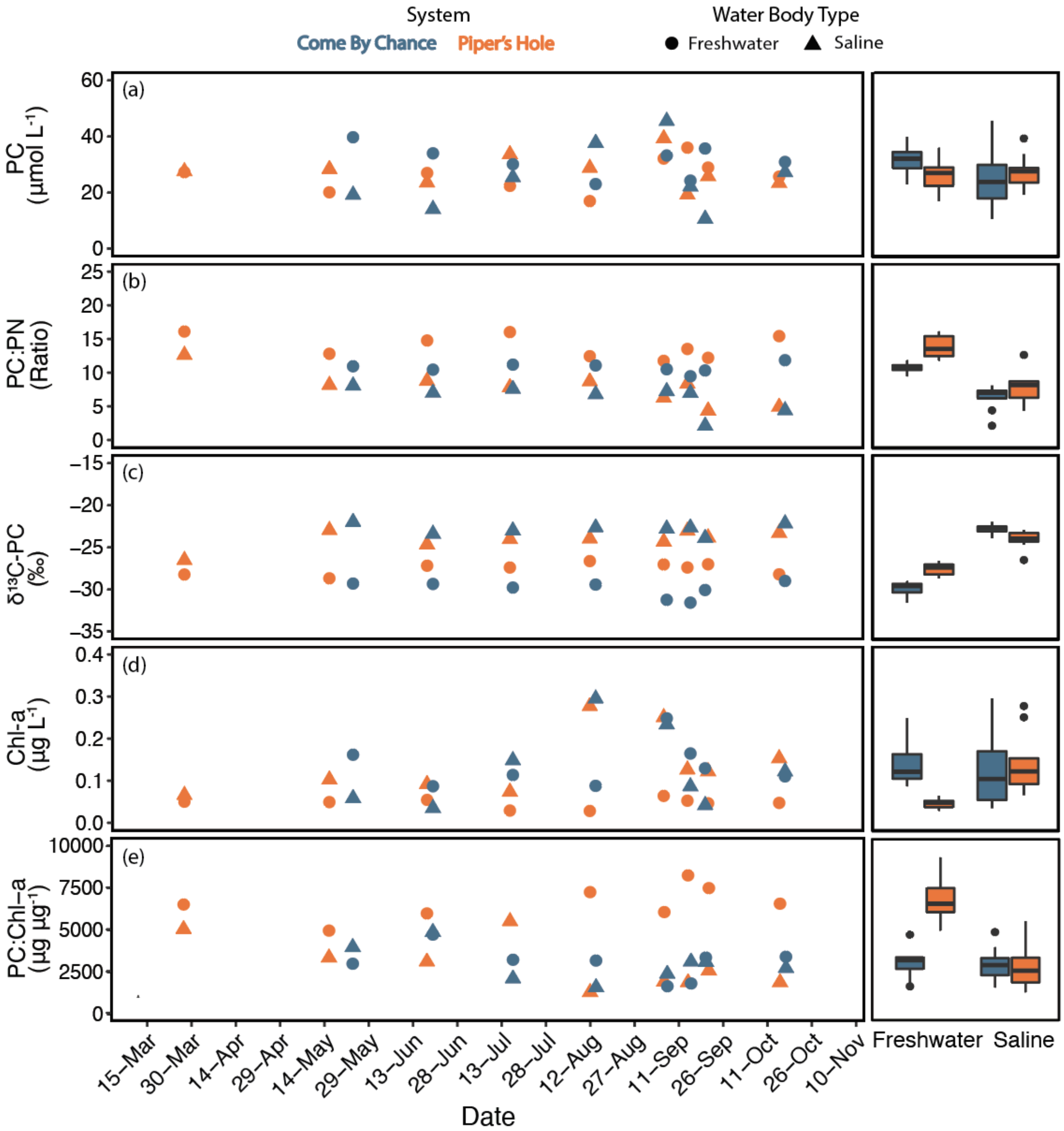
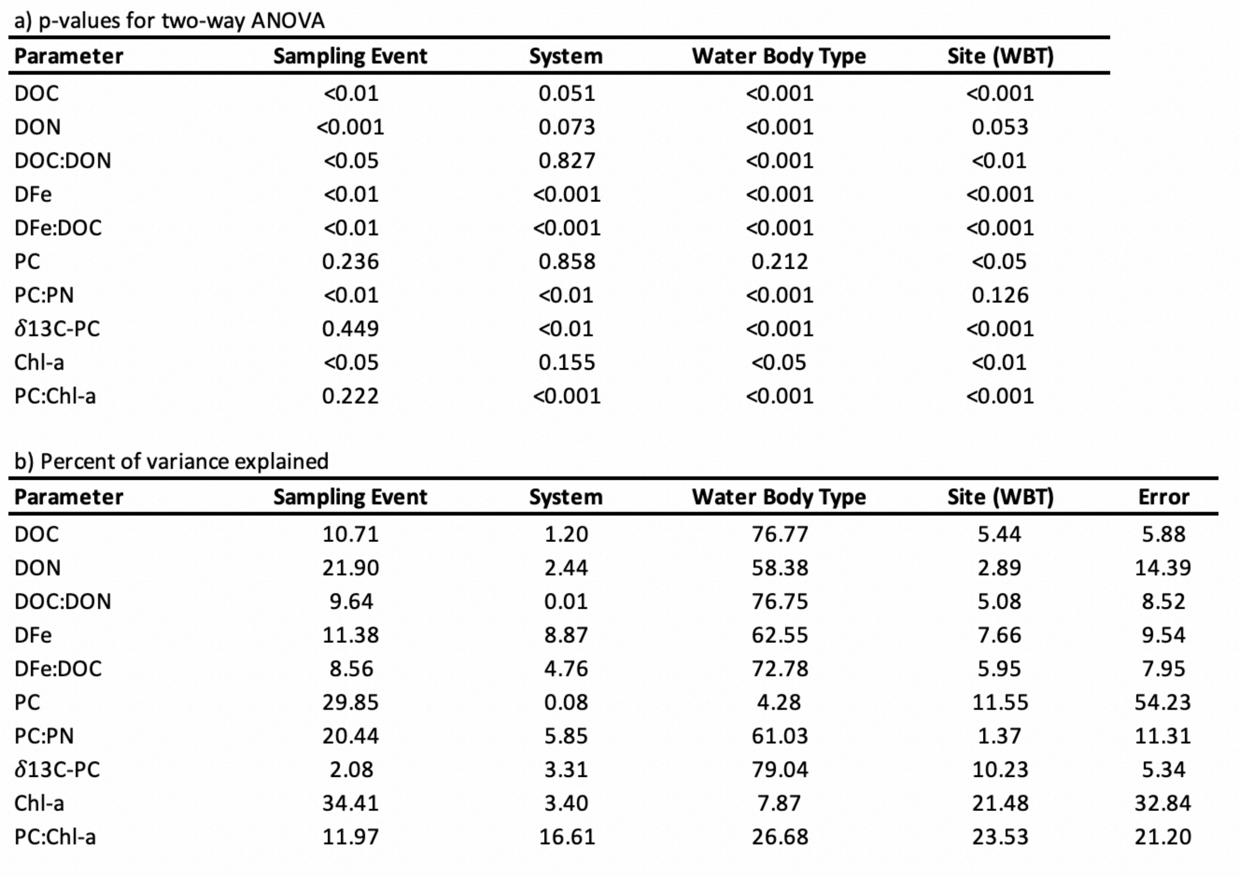


Figure 2.4. Sampling event (left panels) and total variation (right panels) in a) particulate carbon (PC), b) particulate carbon to nitrogen ratio (PC:PN), c) stable carbon isotope of the particulate carbon (δ^{13}C-PC), d) chlorophyll-a (Chl-a), and e) particulate carbon to chlorophyll-a ratio (PC:Chl-a) across the Come By Chance (blue) and Piper's Hole (orange) freshwater (circles) and saline sites (triangles) in 2019.

the other 3 sites (average 0.14 µg L^{-1}; Table 2.2). Notably, Chl-a increased and had similar peaks as PC for both saline sites and for the CBC freshwater site during the summer to fall period only (Figure 2.4d). Congruent with differences in PC, the PC:Chl-a ratio, which can be used to estimate the phytoplankton-derived carbon (Caperon et al. 1976), was consistently two-fold higher at the PHR freshwater site relative to the other three sites (Table 2.2). There was low variability in PC:Chl-a for both saline sites and the CBC freshwater site during the latter half of the sampling period (July onwards), corresponding to consistent δ^{13}C-PC values. Interestingly, despite consistent PC:Chl-a values throughout the study period (Figure 2.4e), there was not a significant correlation between PC and Chl-a at the CBC freshwater and PHR saline sites (Figure 2.5b; 2.5c).

Table 2.3. Results from the two-way hierarchical ANOVA showing a) significant effects and b) percent of explained variance for each parameter across temporal (sampling event: event 1-9) and spatial (system: PHR vs. CBC, water body type (WBT): river mouth vs. bay, site (WBT): sites nested within water body type) scales. Significant p-values are represented in three brackets: < 0.05, < 0.01, and < 0.001. DOC: dissolved organic carbon, DON: dissolved organic nitrogen, DFe: dissolved iron, PC: particulate carbon, δ^{13}C-PC: stable isotopic composition of the particulate carbon, Chl-a: chlorophyll-a. Molar ratios are expressed by x:y (e.g. DOC:DON).



a) p-values for two-way ANOVA

Parameter	Sampling Event	System	Water Body Type	Site (WBT)
DOC	<0.01	0.051	<0.001	<0.001
DON	<0.001	0.073	<0.001	0.053
DOC:DON	<0.05	0.827	<0.001	<0.01
DFe	<0.01	<0.001	<0.001	<0.001
DFe:DOC	<0.01	<0.001	<0.001	<0.001
PC	0.236	0.858	0.212	<0.05
PC:PN	<0.01	<0.01	<0.001	0.126
δ13C-PC	0.449	<0.01	<0.001	<0.001
Chl-a	<0.05	0.155	<0.05	<0.01
PC:Chl-a	0.222	<0.001	<0.001	<0.001

b) Percent of variance explained

Parameter	Sampling Event	System	Water Body Type	Site (WBT)	Error
DOC	10.71	1.20	76.77	5.44	5.88
DON	21.90	2.44	58.38	2.89	14.39
DOC:DON	9.64	0.01	76.75	5.08	8.52
DFe	11.38	8.87	62.55	7.66	9.54
DFe:DOC	8.56	4.76	72.78	5.95	7.95
PC	29.85	0.08	4.28	11.55	54.23
PC:PN	20.44	5.85	61.03	1.37	11.31
δ13C-PC	2.08	3.31	79.04	10.23	5.34
Chl-a	34.41	3.40	7.87	21.48	32.84
PC:Chl-a	11.97	16.61	26.68	23.53	21.20

3.5 Temporal and spatial scale effects on OM

The two-way hierarchical ANOVA approach revealed that overall, the OM parameters varied temporally and across scales of space, and that the effects varied across individual parameters (Table 2.3). Of the parameters exhibiting a sample event effect, the percent variance accounted for by this effect ranged from 8.56% to 34.4%. The effect of water body type (freshwater vs. saline) accounted for the greatest variation (percent variance explained > 50%; Table 2.3b) in DOM parameters among the samples. Variance explained by water body type encompassed a much larger range across the PM parameters, from 4.28% to 79.0%. Most notably, across all but two OM parameters (DON and PC:PN), there was variation at a site-specific scale. For the PM parameters, the effect of site explained 10-24% of the variance, whereas only 2-8% of the variance was explained for the DOM parameters.

3.6 Relationships among the OM parameters

Correlation strength between the parameters was highly variable, while the directionality of the correlations was similar across all sites. Within each size fraction (dissolved and particulate), there was a lack of correlation between OM concentration (DOC, DON, DFe, PC) and composition parameters at the PHR freshwater site (Figure 2.5a). On the other hand, apart from $SUVA_{254}$ and PC, OM concentration parameters correlated with composition parameters for the CBC freshwater site (Figure 2.5c). Freshwater DOM parameters were generally correlated with runoff.

The number of correlations between OM parameters were greater for the saline sites, and most were correlated with salinity (Figure 2.5b; 2.5d). At the PHR saline site, Chl-a did not correlate with any DOM or PM parameter, while δ^{13}C-PC was weakly correlated with PC and PN (Figure 2.5b). At the CBC saline site, Chl-a was correlated with all DOM and PM parameters (Figure 2.5d). Overall, the potential coupling of DOM and PM, as well as the relationships between OM concentration and composition, were most evident for the CBC saline site, as almost all the parameters were significantly correlated (Figure 2.5d).

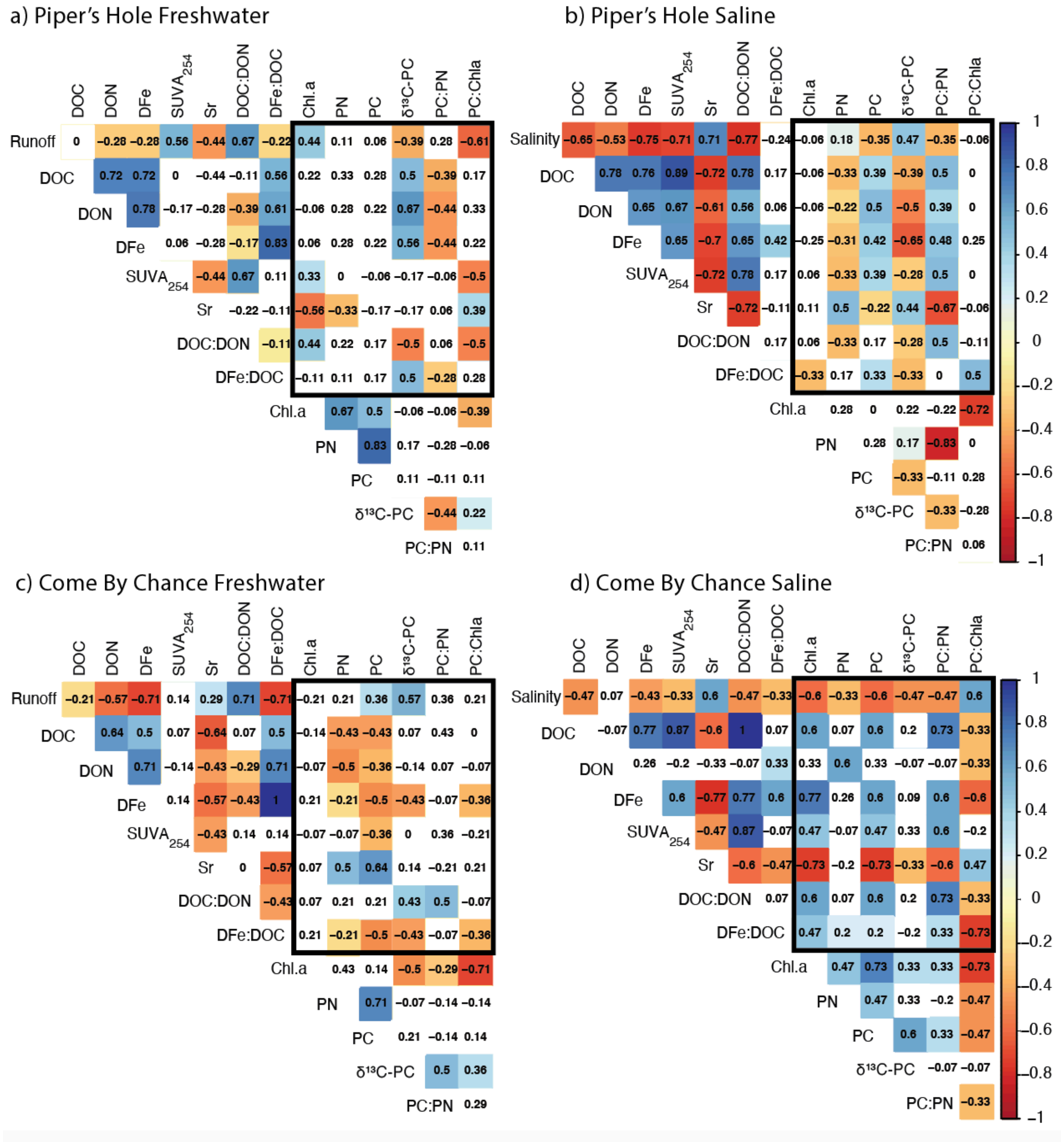


Figure 2.5. Correlogram for the a) Piper's Hole freshwater site, b) Piper's Hole saline site, c) Come By Chance freshwater site, and d) Come By Chance saline site. Significant positive correlations are represented in blue, and negative correlations are represented in red-orange. Correlations that are not significant ($p > 0.05$) are in white. Kendall's tau correlation coefficients (r) are included as numbers. DOM-PM correlations are highlighted within the black box.

4 Discussion

4.1 DOM characteristics suggests site specificity relevant to DOM fate

DOM analysis at our freshwater study sites uncovered DOM distinctive of C-rich boreal landscapes and a local effect on DOM concentration and DFe:DOC relevant to the fate of DOM in Placentia Bay. DOM composition parameters (DOC:DON, $SUVA_{254}$ and S_r) were similar and remained steady throughout the study period, while DOM concentration parameters (DOC, DON and DFe; Figure 2.3) varied simultaneously but to different extents across these sites. Correlation analysis validated these observations, particularly for $SUVA_{254}$, as it exhibited no correlation with DOC, DON or DFe in either freshwater site (Figure 2.5a; 2.5c; $p < 0.05$). A similar finding was reported in Jaffe et al. (2008) in which DOM relationships were only evident between composition parameters but not between DOC concentrations and the optical composition parameters (SUVA and Fluorescence Index).

Overall, high DOC:DON (>40), along with a small range in $SUVA_{254}$ (3-4 L mg^{-1} m^{-1}) and S_r (0.73-0.83) characterize our freshwater study sites as C-rich, aromatic, and primarily of high molecular weight (HMW)-CDOM that is typical of peatland and forest draining catchments in the boreal zone (Mattson et al. 2009, Asmala et al. 2016, Autio et al. 2016). While slightly higher DOC:DON and $SUVA_{254}$, as well as slightly lower S_r were observed at the smaller, upland catchment of CBC, differences in these bulk characteristics were negligible across the two freshwater sites (Figure 2.3f; 2.3g), suggesting that these bulk DOM measures likely do not fully capture DOM dynamics, particularly in systems that are C-rich. For example, little to no variation in DOM quality indicators such as SUVA seem to be a common occurrence in areas stemming from wetland and peatland draining ecosystems where high DOC concentrations have been observed (Schumacher et al. 2006, Jaffe et al. 2008, Asmala et al. 2016). The lack of variation in DOM composition may not hold true across all temporal scales, especially for hydrological events that reduce catchment residence times (Bukaveckas et al. 2020, Panton et al. 2020).

Disparities in DOM concentrations between the two freshwater sites in our study allude to potential processes occurring at a local scale. DOM concentrations (DOC, DON and DFe) gradually increased and simultaneously peaked in late summer at both freshwater sites (Figure 2.3a; 2.3b; 2.3d). Increase and persistence of DOC into the late summer, when river flow is low, has also been reported in regions where wetland, peatland and forest cover is prevalent (Laudon et al. 2014, Liu et al. 2014, Reader et al. 2014). While between-site differences were negligible for the DOM bulk characteristics, DOC, DON and DFe concentrations were relatively lower for the larger, lower relief catchment of PHR. The nearly two-fold difference in DFe concentrations (Figure 2.3d), which strongly influenced DFe:DOC ratios (Figure 2.5a; 2.5c), was the most striking difference between the two freshwater sites. Discerning relationships between Fe and catchment characteristics is challenging as the prevalence of Fe in aquatic systems is largely dependent on conditions (e.g. pH, OM concentration and composition) controlling Fe speciation and stability (Herzog et al. 2020). While close chemical associations between Fe and DOC are particularly evident in boreal waters (Kritzberg et al. 2014), temporal variation in DFe:DOC at the CBC freshwater site (Figure 3e) highlight differences in the degree of Fe and DOC coupling among systems, which may be linked to catchment characteristics. For example, the more stable DFe:DOC at the PHR freshwater site, contrasts with CBC, and is likely a result of the greater proportion of wetlands and water bodies in the PHR catchment. The presence of these catchment characteristics can increase upstream residence times and the likelihood for DOC and DFe removal processes such sedimentation (Winterdahl et al. 2014), biological uptake (Asmala et al. 2013) and photodegradation (Mari et al. 2007, Stedmon et al. 2011) to occur en route to our freshwater sites, which is also consistent with the lower DOM concentrations observed at PHR.

4.2 Evidence for potential system-specific DOM transformation in adjacent coastal systems

The transport of DOM from streams to the ocean is not only dependent on the flux of freshwater inflow but also on distinct estuarine morphology, mixing patterns and reactivity of the freshwater DOM (Bianchi, 2007, Ward et al. 2017). Typically, as water and DOM travel downstream into the coastal zone, DOM concentrations decrease (Goni et al. 2003, Voss et al. 2020) and transformations of its bulk characteristics often include a loss in aromaticity, a decrease in molecular size and a more enriched ^{13}C stable isotopic signature (Amon and Benner 1996, Goni et al. 2003, Asmala et al. 2016). Significant negative correlations between salinity and DOC, DON, DFe, SUVA$_{254}$ and DOC:DON, as well as a significant positive correlation to Sr are congruent with these previous observations. Unlike DOM at the freshwater sites, DOM concentrations and bulk characteristics seem to be more strongly linked at the saline sites. This linkage between bulk characteristics and salinity across all DOM parameters was more evident in PHR and weaker in the CBC saline site, suggesting that the role of salinity, and hence mixing, can exhibit site-specificity. However, mixing alone does not fully describe DOM characteristics at these sites. Many studies across Arctic and boreal regions have found significant contributions of photochemical degradation (Chupakova et al. 2018), microbial production and respiration (Berggren et al. 2010, Andersson et al. 2018), and flocculation and sedimentation (Sholkovitz 1976) in determining OM composition in coastal zones. Altogether, these transformations can contribute to non-conservative behaviour (non-linear decrease) of DOM across salinity gradients.

The relationships between DOM and salinity are often element-specific, despite the inherent coupling of C, N and Fe cycles. For example, the distribution of DON across salinity transects can be more variable relative to DOC (Tank et al. 2012, Voss et al. 2020). The 2-fold difference in DON, 8-fold difference in DOC, and 200-fold difference in DFe across the salinity gradient in our study systems suggest that these three pools undergo transformations of varying degrees. A higher likelihood for DON to turnover quickly via assimilation and release by biological microorganisms (phytoplankton, bacteria, and zooplankton; Sipler and Bronk, 2015), particularly when nitrate and ammonium are scarce (Zavarzin 2010), likely contributes to a more consistent distribution of N along the salinity gradient. For DOC, the clear decrease with salinity suggests that mixing and loss processes dominate over production processes. Substantial decreases in DFe allude to a dominance of loss processes such as particle formation (flocculation). These processes, as regulated by DOM composition and DFe:DOC, has implications for the transformation, loss, and production of C in boreal estuaries (Asmala et al.

2014, Shulkin et al. 2017).

4.3 Biological components contribute to site specificity in PM characteristics

Controls on PM concentration often include the flushing of decaying aquatic vegetation, suspension of surface sediments, seasonal algal production, and estuarine characteristics such as the turbidity maximum location (Canuel and Hardison 2016). In our study, these factors were difficult to determine given the lack of temporal and spatial effects on PC concentration (Figure 2.5a; Table 2.3a). However, compositional differences indicated PM changes between the fresh and saline sites, and that site-specificity was enhanced when the biological components of the PM were considered (Figure 2.5; Table 2.3a). The use of Chl-a and other biological components (e.g. lipids and amino acids) of the PM pool have been suggested to be more dynamic than bulk PM alone (Lee et al. 2004), and in congruence, the results in this study illustrate the value in making simultaneous measures to better understand contributing sources to PM.

Stable isotopic composition and C:N ratio of the PM differed among sites as described by their water body type (freshwater vs. saline; $p < 0.05$), accounting for 61 and 79% of the variance among the samples, respectively (Table 2.3b), and signifies differences in algal and terrestrially derived sources. Traditionally, the C:N ratio of marine phytoplankton is approximately 6.6 (Redfield 1934), while plant material is typically C-rich and depleted in N with C:N values ranging from 20-500 (Bianchi, 2007). More recently, variability from the Redfield C:N ratios have been observed in both marine (C:N = 11.4 - 6.1; Martiny et al. 2013) and freshwater systems (average seston C:N = 10.4; They et al. 2017). Therefore, the averaged PC:PN values of 13.9 and 10.7 for PHR and CBC freshwater sites suggests a combination of terrestrial and algal sources to freshwater PM in these systems. A greater algal influence was observed for the saline sites given that PC:PN averaged below 8 at both saline sites (A1. Table 2). Given the spatial separation of our sites and their position along the salinity gradient, these differences were expected. The δ^{13}C-PC values also reflected a more algal signature at the saline sites, as their more enriched ^{13}C signatures (-22 to -24.7 ‰) fell within range for marine bacteria (Savoye et al.

2003) and phytoplankton (Bianchi and Canuel 2011). The contribution of particulate inorganic carbon may also influence the enrichment of δ^{13}C-PC at the saline sites. Indeed, the contribution of estuarine and marine phytoplankton to total PM tends to increase with nutrient availability (Savoye et al. 2003, Dagg et al. 2004) and when sediments settle, and turbidity decreases at downstream coastal zones (Savoye et al. 2012, Canuel and Hardison 2016).

One major contributor to the distribution of PM in surface waters is primary production (eg. phytoplankton), and to date, the use of pigment analyses such as Chl-a has been one of the most common measures employed for understanding such distributions. Overall, all sites and sampling events were low in Chl-a (<0.3 µg L^{-1}) and phytoplankton derived PC (PC:Chl-a > 500 µg L^{-1}; A1. Table 2). In brackish systems of the European boreal, low phytoplankton biomass has been attributed to an indirect effect of high tDOM inputs, as these inputs favor heterotrophic biomass rather than phytoplankton (Sandberg et al. 2004, Thingstad et al. 2008). Given that DOM at our freshwater sites was characterized as C-rich and compositionally terrestrial in nature, it is likely that the chemical nature of the DOM observed, as well as its potential to attenuate light acted as contributing factors suppressing phytoplankton growth at our surface water sites. Despite low surface water Chl-a, differences in the dynamics and distribution of PM at our study sites were greater over the summer months or growing season, particularly for the saline sites. Further, interpretation of Chl-a in conjunction with ^{13}C signatures, PC:PN and hydro-geomorphological conditions highlighted local source contributions to the PM pool. For example, between the freshwater sites, comparatively lower PC:PN (10.7), three-fold higher Chl-a (0.14 µg L^{-1}), and a ^{13}C signature range between -29 to -31.6 ‰ at CBC alluded to a mixed contribution of freshwater phytoplankton (- 25 to -35 ‰; Remeikaitė-Nikienė et al. 2017), riparian vegetation and epilithic algae (Finlay and Kendall 2007, Rasmussen 2010) to PM. Observed hydro-geomorphological conditions such as shallow channel depth, a siltier channel bed and lower discharge also favored benthic production at this site (Tank et al. 2010).

4.4 Potential coupling of DOM and PM

Simultaneous changes to both the DOM and PM captured across our higher resolution sampling during the summer-to-fall transition period suggests that this period, in addition to spring-melt, may provide the best window for further studies regarding DOM-PM exchanges in this region. At the freshwater sites, a decoupling of DOM and PM is evident (Figure 2.5a; 2.5c) and can be attributed to the high terrestrial nature of the DOM and its dominance to the total OM pool (Davis and Benner, 2005). However, the potential coupling of these size fractionated pools seemed to be intensified at the saline sites (Figure 2.5b; 2.5d), where PC accounted for up to 18% of the total organic C pool (A1. Table 2). At these sites, PM (PC, ^{13}C stable isotope, and chl-a) and DOM (bulk elemental concentrations and optical signatures) characteristics simultaneously decreased following a peak in late summer, which likely reflects seasonal phytoplankton and microbial dynamics. For example, late summer production may increase freshly produced, bioavailable DOM, which would be reflected in CDOM proxies such as $SUVA_{254}$ (< 2.5 L mg^{-1} m^{-1}; Hansen et al. 2016). Given our analyses at the bulk level and our observational study design, we are limited in our interpretation of these potential couplings. Many other factors such as environmental nutrient dynamics (Soares et al. 2018, Bukaveckas et al. 2020), light availability, microbial and phytoplankton community composition (Thingstad et al. 2008, Hjerne et al. 2019), and temperature dependence (Pomeroy et al. 1991) could also contribute to observed dynamics in our systems. Without more targeted meso- and microcosm experiments, as well as higher resolution analyses, it is difficult to discern the specific processes contributing to and the directionality of PM-DOM exchanges.

5 Conclusion

Large gaps in biogeochemical observations across the land-to-sea continuum exist, especially for remote near-shore coastal regions (Ward et al. 2017). Our observations highlight a decoupling between DOM concentration and composition in C-rich boreal systems where most of the total organic carbon exists in the dissolved form. Despite DOM being compositionally stable, variation in DOM concentrations and DFe:DOC ratios were the best indicators for assessing the temporal stability of the DOM pool. We highlight the continued value of bulk DOM measurements and the importance of DFe in assessing the fate of OM in coastal boreal systems. Another key finding of our study is that variability in PM, DOM and their coupling is localized and potentially enhanced during windows of seasonal change such as the summer-to-fall transition period. Collectively, results here indicate the potential for poor predictions of the cycling, fluxes, and fate of OM when common OM dynamics are assumed among boreal systems, even within those in close proximity. Given that the boreal zone is undergoing rapid climate change and contains globally significant OM reservoirs, we encourage the recognition of the biogeochemical diversity among boreal systems, and their implication for carbon-climate feedbacks.

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7 Author Contributions

C.L.L.K, R.E.S, S.E.Z designed the study. All authors participated in the collection of field samples and initial sample processing. Inorganic nutrient and Chl-a data analysis was conducted by S.J.M.F, and S.B. conducted analysis on PM. C.L.L.K conducted all other data analysis, data interpretation and wrote the manuscript under guidance from R.E.S and S.E.Z. All authors contributed to manuscript revisions and approved of the final manuscript.

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CHAPTER 3: EVALUATING THE MECHANISMS INFLUENCING SALT-INDUCED FLOCCULATION OF DISSOLVED ORGANIC MATTER IN BOREAL COASTAL SYSTEMS

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ABSTRACT

Rivers and estuaries are important conduits and reactors for organic matter (OM). Despite the substantial export of terrestrial OM by rivers, only a small proportion of this material has been found in oceanic waters. One potentially important mechanism contributing to the removal of terrestrial dissolved (D)OM is flocculation; a process that transforms DOM into larger size fractions that can settle into sediment pools. Here we investigate the role of flocculation in adjacent boreal coastal systems over three consecutive seasons. Flocculation experiments, which include the salting of freshwater DOM to mid (12-15 psu) and high (25 psu) salinities, highlight the potentially important, and significant source of particulate carbon in boreal estuaries. Along with a consistent removal of dissolved organic carbon (DOC) and dissolved iron (DFe), the potential for flocculation of these constituents significantly increased with increasing salinity from 12 to 25 psu. Greater flocculation potentials were linked to higher initial elemental concentrations and DFe:DOC ratios, which were observed in the fall. Non-uniform changes in DOM across the ultraviolet and visible light regions of the absorption spectrum in response to flocculation at different salinities, seasons, and systems suggest potential DOM compositional drivers. These findings demonstrate the importance of quantitative and qualitative measures, and in particular, the predictive value of DFe:DOC ratios for understanding DOM susceptibility to flocculation and consequently, its relevance and contribution to regional and global carbon budgets.

1 Introduction

Occupying the interface between land and ocean, estuaries process a substantial amount of terrestrial and marine organic matter (OM) and play a vital role in determining the distribution and flux of OM (particulate and dissolved) to the coastal ocean as well as across benthic, pelagic and atmospheric bounds (Bianchi 2007, Canuel and Hardison 2018). Complex chemical, biological, and physical OM transformations can contribute to the removal, addition or reworking of OM in these transitional ecosystems (Carlson and Hansell 2015) and lead to non-linear changes in constituents along a salinity gradient (non-conservative mixing; Dagg et al. 2004, Osterholz et al. 2016, Asmala et al. 2014, Lasareva et al. 2019). Fundamental changes in salinity alone can facilitate the aggregation of OM (Sholkovitz 1976) and iron (Fe; Forsgren et al. 1996), which carries the potential to affect ecosystem functioning and estuarine structure. This transformation, known as flocculation, is widely thought to be a dominant process affecting the removal of OM from pelagic zones to the benthos during estuarine mixing (Jokinen et al. 2020, Mhashhash et al. 2017, Jilbert et al. 2018; Gebhart et al. 2005). Flocculation is also known for its contribution to estuarine turbidity maximums where intense biogeochemical cycling is known to occur (Wollast 2003). This transformation is hence critical for understanding biogeochemical cycling in estuaries as well as global and regional carbon budgeting.

Understanding the size distribution of OM is critical for elucidating its fate in aquatic systems and are consequently an essential component for the quantification of fluxes within carbon budgets. Most commonly, particulate (POM) and dissolved (D)OM represent two size classes that are operationally defined as organic compounds either retained or passed through filters with pore sizes of 0.1 to 0.7 μm (Bianchi 2007). One distinct difference between these size classes is the ability for particles greater than 0.2 μm to sink via gravitational settling (Blanchard et al. 2017). Organic carbon is often used as a quantitative measure of DOM and POM. These two pools can be further size fractionated or analyzed based on source material. For example, a subset of DOM, referred to as chromophoric or colored (C)DOM, can absorb light and thus be measured using optical methods. This pool largely contributes to the yellow or brown appearances of aquatic systems (Nelson and Coble, 2009), and has been linked to other dissolved constituents such as Fe (Poulin et al., 2014). While elemental iron is considered inorganic, a large majority of dissolved iron (DFe) is present as complexes with organic ligands or DOM (Muller, 2018). As such, DFe is often considered an important characteristic of DOM that can also be analyzed as part of CDOM. Although CDOM and DFe are encompassed within DOM, these components are often assessed via different analytical methods, and can exhibit distinct behaviours. As such, they are herein addressed as individual components to allow for exploration of the linkages between DOM (quantified as dissolved organic carbon (DOC)), DFe (concentrations) and CDOM (optical properties) as well as their individual susceptibility to flocculation.

Ecologically, the products and byproducts of flocculation can have multiple impacts on community structure and basal food webs. For example, newly formed flocculated material can positively favor secondary production in estuaries by providing a source of energy for heterotrophic bacteria (Yan et al. 2018), while changes in optical properties can reduce light availability for primary producers (Sipler et al. 2017). Flocculated materials are also thought to resemble snowflakes that are known to be attractive to microbes and predators (Kranck and Milligan 1980). Congregation of bacterial communities around flocs can enhance the production of exopolymers (e.g. polysaccharides, proteins, and lipids) and increase the size of the flocs, resulting in the growth of some species (e.g. rotifers) and alteration of oxygen conditions (Walczynska et al. 2018). Strong associations of metals with OM may also alter trace metal sequestration and exacerbate Fe limitation in oceanic systems (Laraseva et al. 2019, Jokinen et al. 2020).

Salt-induced flocculation of OM is typically explained by an increase in ionic strength as freshwater meets saline waters, thereby causing negatively charged OM compounds that would otherwise repel one another to destabilize and flocculate (Gregory 2013). However, complex interactions between estuarine hydrodynamics, floc formation and settling (Mhashhash et al. 2018, Manning et al. 2010), photo-induced exchanges (Helms et al. 2013, von Wachenfeldt et al.

2008), microbial degradation, and variability in DOM composition across different time scales pose difficulties in quantifying flocculation in natural systems. The selectivity of certain elements and characteristics that make up DOM has also resulted in mixed conclusions surrounding the relevance of flocculation in estuaries. In some cases, flocculation has been deemed insufficient to account for observed non-conservative behaviour in DOM (Guo et al. 2007, Del Castillo et al. 2000). Flocculation has also been reported to occur across a wide range of salinities, from 1 to 30 psu (Forsgren et al. 1996, Gustafsson et al. 2000, Sholkovitz 1976). While many uncertainties remain as to where, when, what, and how flocculation affects the distribution of OM in estuaries, there are also some common findings among flocculation studies to date. For example, flocculation has been reported to remove larger (high molecular weight CDOM), more aromatic DOM (Sholkovitz et al. 1978), that in some cases, may result in a decrease in average DOM molecular weight of up to 17% (Asmala et al. 2014).

The role of flocculation may be enhanced in boreal systems, as high DOC, Fe, and "browning" (CDOM) are common characteristics of freshwater DOM across this region (Mattson et al. 2005, Monteith et al. 2007). While OM concentration and composition varies with system and seasonal dynamics (Stedmon et al. 2011, Mutschlecner et al. 2018, Osterholz et al. 2016, Khoo et al. Chapter 2), overall, these commonly described boreal DOM characteristics are predicted to increase under rapid climate change across many systems in the boreal zone (Bjorneras et al. 2017, Kritzberg et al. 2020). Despite the wide acceptance of flocculation as a removal mechanism, the degree and extent of abiotic flocculation, its potential for temporal variation and its contribution to estuarine particulate matter (PM) remain questionable. In light of environmental stressors, gaining an understanding of the potential transformations of riverine DOM fluxes will be imperative to better predict the trajectories of change in these important coastal ecosystems.

In this study, we investigate the susceptibility of OM observed within two boreal river-estuarine systems to flocculation, across seasons. Through a series of flocculation experiments, our objectives were: (1) to determine the salinity and degree to which maximum potential for flocculation occurs throughout different seasons; (2) to explore the effects of salt addition relative to the addition of other water mass components in controlling flocculation and (3) to relate these findings to attributes including elemental quantification, optical properties, elemental ratios, and isotopic signatures of the OM observed in these systems. We hypothesize that flocculation is driven by these quantitative and qualitative properties (e.g. optical properties) of DOM and in the face of changing and variable OM fluxes from the land, it is likely to be an important transformation informing the fate of OM in estuaries.

2 Methods

2.1 Study sites and resolution

Our study was carried out within two systems located in the Canadian boreal zone on the island of Newfoundland: Come By Chance (CBC) and Piper's Hole (PHR). The two adjacent systems drain southward into Placentia Bay, a bay that supports essential fisheries such as Atlantic Cod (DFO 2016, Wells et al. 2019) and aquaculture (Maxwell and Filgueira 2020). Because of their proximity, they are subjected to similar climatic conditions with winter snow accumulation beginning in December and summer conditions beginning in June. Throughout the study period, the freshwater and saline sites remained ice free. These systems vary in catchment size, with PHR being considerably larger (787 km^2) relative to CBC (45.9 km^2). The PHR catchment also has a lower relief (average percent slope = 5.46 m m^{-1}) relative to CBC (9.21%, average percent slope = 5.89 m m^{-1}), resulting in a greater percent of water bodies in the PHR catchment (~15.2%, CBC ~ 9.21%).

Surface water sampling was conducted at fixed freshwater (CBC: 47°55'6" N, 53°56'55"W, PHR: 47°55'49"N, 54°16'23"W) and downstream saline sites within each system (CBC: 47°48'39"N, 54°00'30"W, PHR: 47°51'02"N, 54°09'29"W). The distance between the freshwater and saline sites was approximately 12 and 14 kilometers for PHR and CBC, respectively. These sites were sampled eight times throughout 2019, monthly from March to October with additional weekly sampling in September during the summer-to-fall transition period.

2.2 Cleaning Procedures

Plasticware was first brined in a salt bath (~80psu) for one week prior to copious rinsing with type 1 water (18.2 mΩ; Milli-Q) to reduce the potential for C leachate. All glassware and plasticware were then cleaned by soaking in 10% HCl for a minimum of 4 hours and rinsed 6 times with type 1 water. Glassware was then combusted at 500°C for 4 hours and glass fiber filters were combusted at 450°C for 4 hours before use.

2.3 *In-situ* Sampling

All samples were collected with a bucket and filtered through a 200µm mesh into 20L carboys at the field sites. Samples were then transported to Memorial University of Newfoundland's Ocean Sciences Centre for initial filtration. Upon arrival, samples underwent vacuum filtration at less than 7psi, through 0.3µm GF75 (Advantec MFS) filters for the removal of freshwater and estuarine bacteria (Palumbo et al. 1984), along with larger particles. Subsamples of the filtrate were then collected into small volume (15-125 mL) high density polyethylene (HDPE) bottles or glass vials and stored frozen until DOM analyses. Exceptions to these are subsamples for CDOM, which were collected in amber HDPE bottles and stored at 4°C until measurements were made. Subsamples collected at this point were used to represent the in-situ DOM pool. The remaining filtrate was stored in a 20L carboy at 4°C until the beginning of experiments (Figure 1), which were conducted within 10 hours of sample collection.

2.4 Salt Addition Flocculation Experiments

'Salting' of the river water DOM to mid (12psu) and high (25psu) salinities was conducted by adding artificial seawater solution prepared to 30 and 65psu, respectively. The artificial seawater solution was prepared by dissolving NaCl, $MgSO_4$, and $NaHCO_3$ in type 1 water. NaCl was baked at 500°C for 2h to reduce C and N contamination and salts were combined in a 321:71:1 weight ratio, respectively. One liter amber experimental bottles were first filled with 500mLs of the filtered water sample, followed by the addition of 300mL artificial seawater solution. All treatments were conducted in triplicate and each bottle was carefully inverted upon addition of the artificial seawater solution and incubation. In addition to the triplicate bottles, a separate bottle for each treatment was added to allow for measurements of salinity and pH (Figure 1). At the end of a one hour incubation at 4°C, each experimental bottle was filtered through pre-combusted 0.3μm GF75 (Advantec MFS; 450°C for 4 h) filters for the collection of both PM and DOM. Filters were gently folded and stored in pre-combusted (500°C for 4 h) foil and immediately frozen at -20°C. Prior to PM analyses, these filters were further freeze-dried for 3-5 days. The filtrate was then used to produce the same set of subsamples as for the *in-situ* DOM pool described above.

2.5 Mixed Water Flocculation Experiments

Parallel to the salt addition treatments described above, a 1:1 mixture of freshwater DOM to their respective saline water DOM counterparts was also conducted at each sampling event. For these treatments, 400mL of 0.3μm filtered freshwater was added to 400mL of 0.3μm saline water in 1L amber HDPE bottles. Sample collections, experimental procedures and subsampling were kept consistent with the methods described above.

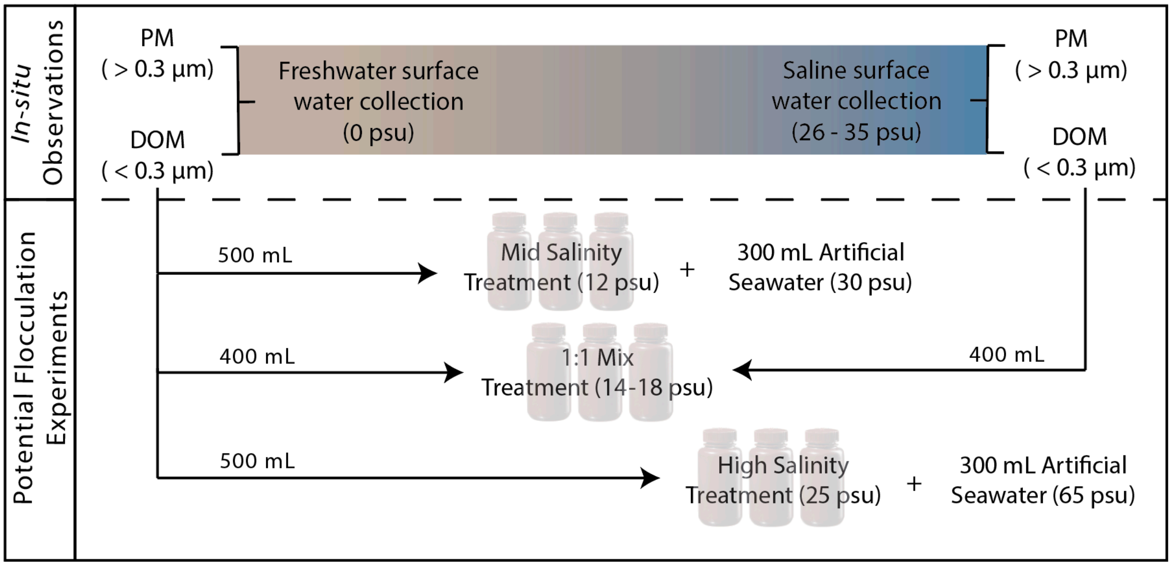


Figure 3.1. Visual representation of the flocculation experimental design. Each experimental treatment was conducted in triplicate across nine occasions on surface water collected from Piper's Hole and Come By Chance, Newfoundland. DOM: Dissolved organic matter; PM: Particulate matter; psu: practical salinity units

2.6 Analytical Methods

DOC and total dissolved nitrogen (TDN) concentrations were measured by high-temperature combustion using a Shimadzu TOC-V_{CPH}/TN autoanalyzer (Sharp et al. 2004, Laodong and Santschi 1997) after manual HCl acidification. Daily instrument checks were conducted using certified low carbon and deep seawater references from Miami University and analyses were carried out at Memorial University of Newfoundland's Ocean Sciences Centre CREAIT laboratory. Inorganic nutrient analyses (NH_4^+ and NO_x^-) were carried out on a Lachat QuickChem 8500 autoanalyzer, with detection limits of 0.23 and 0.01 µmol N L^{-1}, respectively. Dissolved organic nitrogen (DON) was then calculated by subtracting inorganic nitrogen (NH_4^+ and NO_x^-) from TDN. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) was used to measure DFe, with a detection limit of 0.036 µmol Fe L^{-1}. To investigate the removal of DFe in relation to DOC, the molar ratio of DFe:DOC was also calculated. In cases where DFe concentrations fell below the detection limit, the detection limit was used to represent DFe and the maximum possible DFe:DOC in these calculations.

Spectrophotometric analysis of CDOM was carried out over a spectral range of 200 to 800nm at 1nm intervals on Perkin Elmer and Cary UVWin spectrophotometers. A 1cm quartz cuvette was used for the majority of the samples and in instances where CDOM concentrations were low, a 5cm quartz cuvette was substituted. Type 1 water at 18.2 mΩ was used as the reference for all samples. Samples were warmed to room temperature prior to analysis. Absorbance measurements were then transformed to absorption coefficients by multiplying the absorbance of a specific wavelength (A) by 2.303 and dividing by the cuvette path length (L) in metres (Eq. 1). $SUVA_{254}$ and spectral slope ratio ($Sr = S_{275\text{-}296}/S_{350\text{-}400}$) were then calculated according to Weishaar et al. (2003) and Helms et al. (2008), respectively. These values were used as qualitative parameters, as they have been found to correlate with DOM aromaticity (Weishaar et al. 2003) and CDOM molecular weight (Helms et al. 2008).

$a(\lambda) = 2.303 * A(\lambda)/l$ (**Eq.1**)

Analyses of particulate carbon (PC) and stable C isotopic ($\delta^{13}C$) analyses were conducted at the CREAIT Stable Isotope Laboratory at Memorial University of Newfoundland. Whole 25mm 0.3μm GF75 (Advantec MFS) filters were analyzed with a Vario Isotope Cube (Elementar) elemental analyzer coupled via a ConFlo IV Interface to a Delta V Plus (Thermo Scientific) isotope ratio mass spectrometer. Reference materials for $\delta^{13}C$ analyses (EDTA #2 = -40.38 ± 0.01 ‰; USGS62 = -14.79 ± 0.04 ‰;) from Indiana University and protein B2155 (46.50 ± 0.78% C; 13.32 ± 0.40% N; $\delta^{13}C$ = -27.03 ± 0.13 ‰) from Elemental Microanalysis were used for daily correction factors and quality checks.

2.7 Flocculation Calculations and Statistical Analyses

To account for dilution effects, in-situ values were multiplied by the dilution factor of 0.625 for the salt addition experiments and averaged for the 1:1 mix experiments. These values are referred to as initial values henceforth. To compare across space, time, and variable initial values, the degree of C flocculation was then calculated as a percentage and referred to as potential C flocculation. For the salt addition treatments, percentages were calculated as:

Potential flocculation (%) : $[\ PM / DOM_F * DF\ ] * 100$ (**Eq.2**)

For the mixed water treatments, percent flocculation were calculated as:

Potential flocculation (%) : $[\ PM / ((DOM_F * 0.4) + (DOM_S * 0.4))\ ] * 100$ (**Eq.3**)

where PM is the concentration of flocculated C or the difference between the initial and post flocculation DFe concentrations in µmol L^{-1}, DOM_F is the initial DOC or DFe concentration of the freshwater in µmol L^{-1}, DOM_S is the initial DOC or DFe concentration of the saline water in µmol L^{-1} and DF is the dilution factor of 0.625.

To evaluate significant changes between initial and treatment values, we conducted paired two-tailed t-tests. Results from all experiments conducted throughout the study period were pooled by treatment type and system before t-test analyses. An analysis of variance (ANOVA) was further conducted to identify similarities and differences in the degree of flocculation across experiment types (mid, high and 1:1 mix). Lastly, linear regressions were also performed to explore the relationships between DOM quantitative (DOC, DFe) and qualitative (DOC:DON, $SUVA_{254}$, S_r and DFe:DOC) parameters with potential C and Fe flocculation. Linear regressions were performed on all flocculation data (salt addition and 1:1 mixed freshwater: saline treatments) collected from both systems except for the optical proxies ($SUVA_{254}$ and S_r) where only the salt addition data was used. The statistical model for these linear regression models are as follows: $Y = \mu + \alpha + \varepsilon$ where Y is the potential C or Fe flocculation, μ is the overall mean, α is the effect of DOM quantitative or qualitative parameters, and ε is the error. All statistical analyses were conducted using the basic functions of R.4.1.0 (R Core Team, 2021).

3 Results

3.1 Relevant in-situ conditions for potential flocculation

Overall, the dissolved fraction accounted for > 95% of the total organic carbon across both freshwater sites (Table 3.1), which is consistent with systems in the boreal zone (Mattson et al. 2005). Between these sites, DOM quantitative parameters (DOC, DFe) and qualitative parameters (DOC:DON, DFe:DOC, $SUVA_{254}$) were higher for CBC relative to PHR (Table 3.1; A2. Figure 1). While DOC and DFe concentrations were highest in late summer, all qualitative parameters except for DFe:DOC exhibited little seasonal variation. Differences in the freshwater PM pool were subtle, with average PC higher and δ^{13}C-PC lower at CBC (PC = 31.3 ± 5.6 µmol C L^{-1}; δ^{13}C-PC = -30.0 ± 0.9 ‰) relative to PHR (PC = 26.2 ± 5.9 µmol C L^{-1}; δ^{13}C-PC = -27.5 ± 0.7‰).

Table 3.1. Averaged in-situ organic matter characteristics for the four study sites including standard deviations. A full description showing the seasonal and spatial distribution can be found in Chapter 2. Data source: St. Lawrence Global Observatory (awaiting DOI)

Organic Matter Characteristics	**Come By Chance**		**Piper's Hole**	
	Freshwater	Saline	Freshwater	Saline
Salinity (psu)	0.0 ± 0.0	32.4 ± 3.5	0.0 ± 0.0	28.3 ± 6.0
pH (Dimensionless)	7.2 ± 0.0	7.7 ± 0.0	7.5 ± 0.0	7.7 ± 0.0
DOC (µmol L^{-1})	791 ± 90	154 ± 62	683 ± 120	261 ± 135
DOC:DON (Ratio)	50.5 ± 4.0	18.1 ± 5.3	47.0 ± 3.9	27.7 ± 9.3
DFe (µmol L^{-1})	5.95 ± 2.0	0.05 ± 0.03	3.12 ± 1.09	0.29 ± 0.29
DFe:DOC (Ratio)	0.0074 ± 0.002	0.0003 ± 0.0001	0.0049 ± 0.0009	0.0010 ± 0.0006
$S_{(275-295/350-400)}$ (Ratio)	0.77 ± 0.02	1.41 ± 0.33	0.80 ± 0.02	1.06 ± 0.22
$SUVA_{254}$ (L mg C^{-1} m^{-1})	3.5 ± 0.3	1.7 ± 0.6	3.4 ± 0.2	2.3 ± 0.7
PC (µmol L^{-1})	31.3 ± 5.6	25.2 ± 11.7	26.2 ± 5.9	27.2 ± 5.9
δ^{13}C-PC (‰)	-30.0 ± 0.9	-22.8 ± 0.6	-27.5 ± 0.7	-24.1 ± 1.1
DOC:TOC (Percent)	96.1 ± 1.1	86.9 ± 3.1	96.0 ± 0.8	88.7 ± 4.7

DOC was approximately 3 to 5-fold lower and more variable at the saline sites, ranging from 117.2 to 473.4 µmol C L^{-1} at PHR and from 111.8 to 299.2 µmol C L^{-1} at CBC (Table 3.1; A2. Figure 1). Similar to temporal variability at the freshwater sites, DOC concentrations at the saline sites were also highest in late summer. Unlike the freshwater sites, DFe concentrations did not exhibit any temporal variability, remaining consistently low and often fell below detectable levels (< 0.036 µmol Fe L^{-1}). Aromaticity and CDOM size also increased alongside increases in

DOC (A2. Figure 1). Average PC concentrations were similar to the freshwater sites averaging 27.2 and 25.2 µmol C L^{-1} for PHR and CBC, respectively while δ^{13}C-PC was consistently lower for PHR (-24.1 ± 1.1‰) relative to CBC (-22.8 ± 0.6‰; Figure 3.2). A more detailed evaluation of the distribution and dynamics of OM at these sites can be found in Khoo et al. Chapter 2.

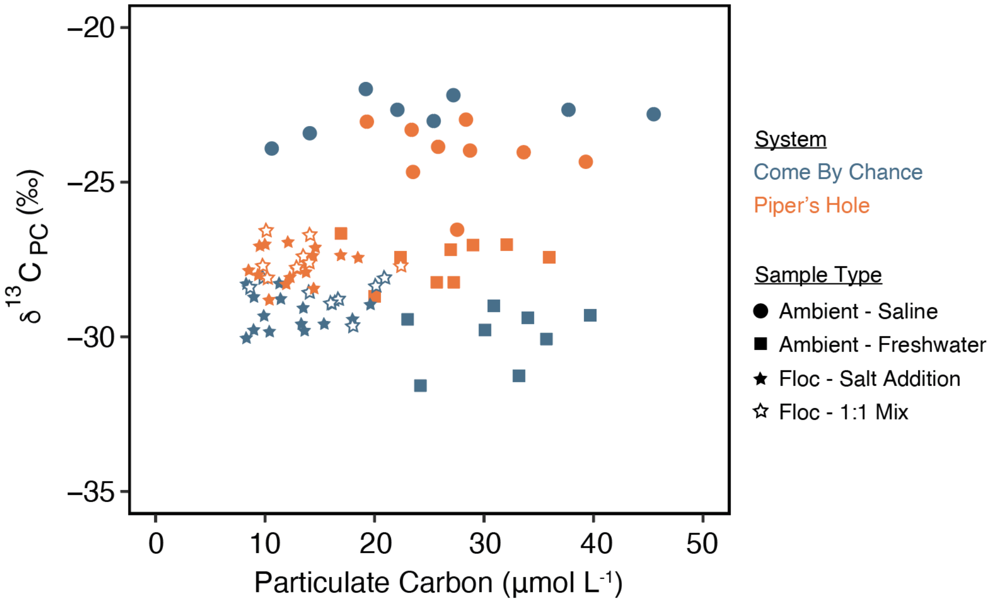


Figure 3.2. Particulate carbon and stable carbon isotopic composition of *in-situ* conditions prior to flocculation experiments (circle = saline sites; squares = freshwater sites) and flocculated particulate carbon (stars) conducted over the 2019 study period in Come By Chance (orange) and Piper's Hole (blue), Newfoundland.

3.2 Impact of flocculation on organic carbon pools

The potential for salt induced flocculation in each experimental treatment was quantified as the amount of PC captured on 0.3 µm filters at the end of the one hour incubation period. Flocculation of C was detectable across all flocculation experiments, ranging from 8 to 22 µmol C L^{-1} in PC (Figure 2). Carbon isotopic signatures (δ^{13}C -PC) of the flocculated C were consistent throughout the year and similar to in-situ δ^{13}C values found at the freshwater sites. Values were more ^{13}C enriched for the PHR experiments (-27.7‰) relative to CBC (-29.1‰; Figure 3.2).

Potential C flocculation was consistently greater for high salinity treatments relative to mid salinity treatments. For both systems, increasing the salinity from 12 to 25 psu resulted in additive flocculation by yielding an additional 30% increase in PC. With substantially higher DOC relative to PC, this increase translated into 0.8% and 1.0% of DOC removed for PHR and CBC, respectively (Figure 3.3, A2. Table 1). This brings the average potential C flocculation from 2.5% in the mid salinity treatments to 3.5% at high salinity.

Similar to the salt addition experiments, the 1:1 freshwater:saline mixed water treatments yielded an average potential flocculation of 3.2% across both systems. In terms of concentrations,

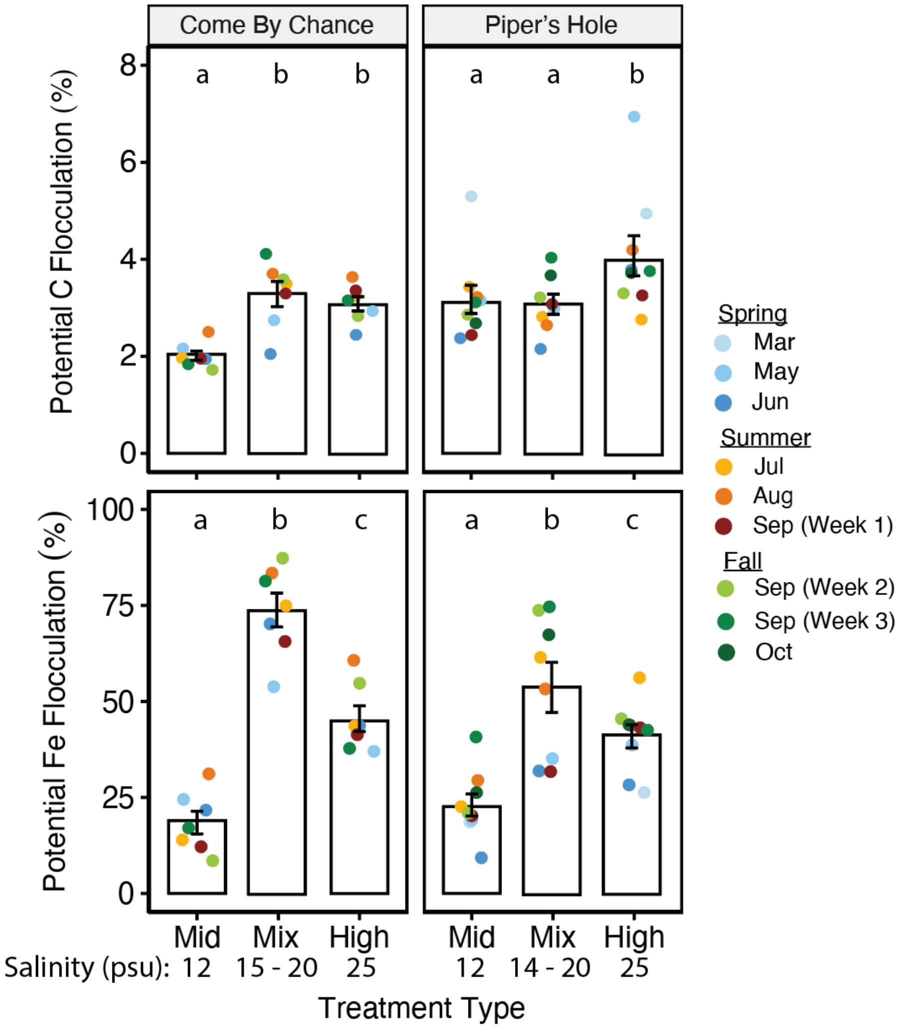


Figure 3.3. Potential carbon (top panels) and iron flocculation (bottom panels) representing the percent of dissolved organic carbon and iron being flocculated across mid salinity (12 psu), high salinity (25 psu) and 1:1 freshwater:saline mix (14-18 psu) treatments. Flocculation potentials for Come By Chance are displayed in the left panels and Piper's Hole is displayed in the right panels. Within each panel, groups that do not share the same letter are significantly different ($p < 0.05$).

this resulted in a gain of 13.4 and 15.5 µmol C L^{-1} in PC for PHR and CBC, respectively. Interestingly, mixed treatments (14-18 psu) were similar to the high salinity treatments (25 psu) for CBC but were similar to the mid salinity treatments (12 psu) for PHR (Figure 3.3, A2. Table 1). Seasonal variations were more notable in the 1:1 mix treatments, particularly for PHR where fall samples had greater C flocculation, followed by summer then spring.

3.3 Impact of flocculation on dissolved iron

Along with the measured C in the particulate phase, significant decreases in DFe were also observed (Table 2). DFe concentrations decreased on average by 19.7% at mid salinity and an additional 22.3% at high salinity across both PHR and CBC (Figure 3.3; A2. Table 1). Like potential C flocculation, the maximum potential for DFe flocculation occurred at high salinity, relative to low salinity. Potential DFe flocculation seemed to follow similar patterns to starting DFe concentrations, peaking in late summer and particularly for CBC where DFe concentrations were twice as high as PHR (Figure 3.5). This was confirmed by a significant positive linear relationship between DFe and potential Fe flocculation (Table 3.3).

Potential DFe flocculation was significantly ($p < 0.05$) greater in the 1:1 mix treatments, ranging from 32.1% to 75.0% loss in DFe for PHR and 54.1% to 87.6% loss for CBC (Figure 3.3; A2. Table 1). In comparison with the mid salinity treatments, the mixing of water mass DOM resulted in a 2- to 3-fold greater loss of DFe. The composition of DOM in fall resulted in greater C and Fe flocculation, relative to summer and spring. These variations were more evident for the 1:1 mix treatments and for PHR.

3.4 Impact of flocculation on optical characteristics

Flocculation has been linked to decreases in CDOM, particularly in the ultraviolet region of the absorbance spectra (Asmala et al. 2014). Consistent with this, a net loss in absorbance at shorter wavelengths (<350 nm) and a net gain at longer wavelengths was observed across all experiments (Figure 3.4). This resulted in a 3 to 6% increase in S_r due to salt addition (Table

3.2). Relative changes in absorbance across mid and high salinity salt addition, and mixed water treatments were grouped by season. Seasonal responses in absorbance to flocculation also differed by system, being more pronounced for CBC relative to PHR. For CBC, losses occurred across all wavelengths for experiments conducted in the fall and summer, with relative changes being greater for the fall, followed by summer then spring. While a smaller range in the relative change was recorded for PHR, it is clear that the PHR spring experiments yielded smaller relative losses at short wavelengths (<300 nm) and larger gains at high wavelengths compared to experiments in the summer and fall.

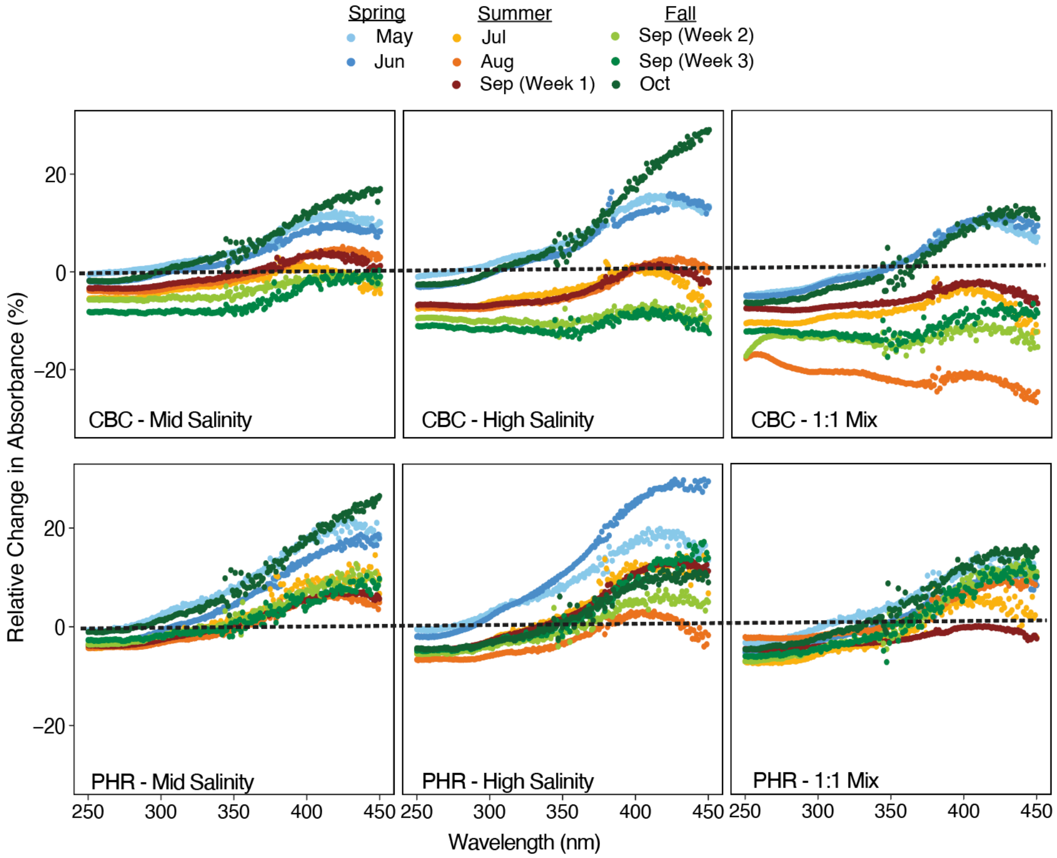
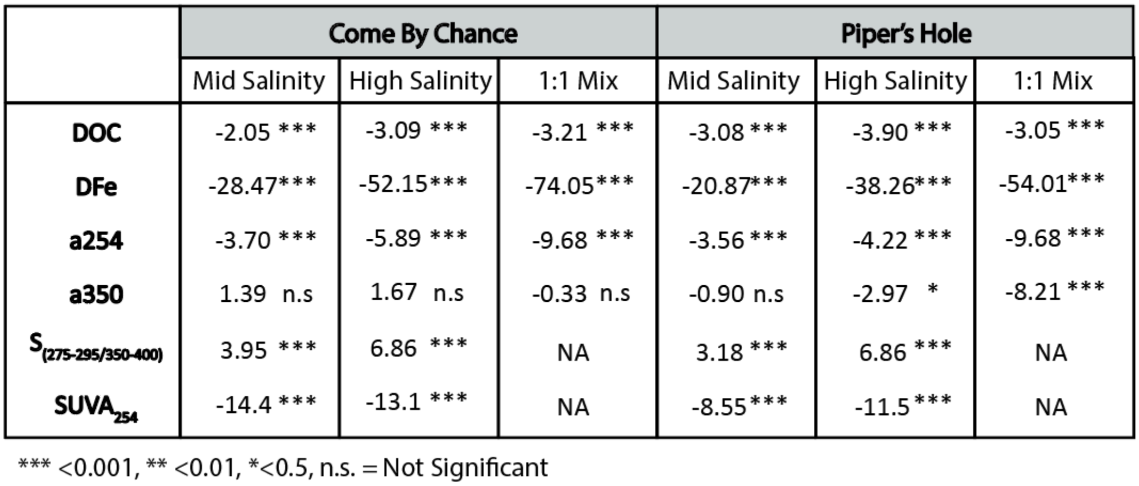


Figure 3.4. Relative changes in absorbance across 250 to 450 nm following flocculation. Values expressed as net change relative to initial water samples where the black dotted line indicates no net change in absorbance.

Typically, absorbance at 350 nm (a_{350}) is used to quantify CDOM and is often highly correlated to DOC concentrations (Osburn et al. 2016), including in these two systems (Khoo et al. Chapter 2). As changes to the absorbance spectra started to shift from loss to gain around 350 nm, changes to a_{350} were not significantly ($p > 0.05$) different from the initial values (Table 2). However, absorbance at 254 nm (a_{245}), which is more representative of the aromatic fraction of CDOM (Korshin et al. 2009), was statistically significantly different (Table 2; $p < 0.05$) and exhibited a relative decrease similar to the potential C flocculation at mid salinity (3.6%). At high salinity, decreases in a_{254} were generally higher, particularly for CBC (Table 2).

Table 3.2. Average potential for flocculation as represented by the percent change from initial dissolved organic matter values, as well as the p-value from t-tests assessing differences between the initial and treatment values. NA: not assessed. Asterisks denote level of significance.



	Come By Chance			Piper's Hole		
	Mid Salinity	High Salinity	1:1 Mix	Mid Salinity	High Salinity	1:1 Mix
DOC	-2.05 ***	-3.09 ***	-3.21 ***	-3.08 ***	-3.90 ***	-3.05 ***
DFe	-28.47***	-52.15***	-74.05***	-20.87***	-38.26***	-54.01***
a254	-3.70 ***	-5.89 ***	-9.68 ***	-3.56 ***	-4.22 ***	-9.68 ***
a350	1.39 n.s	1.67 n.s	-0.33 n.s	-0.90 n.s	-2.97 *	-8.21 ***
$S_{(275-295/350-400)}$	3.95 ***	6.86 ***	NA	3.18 ***	6.86 ***	NA
$SUVA_{254}$	-14.4 ***	-13.1 ***	NA	-8.55***	-11.5***	NA

*** <0.001, ** <0.01, *<0.5, n.s. = Not Significant

Significant ($p < 0.05$) decreases in $SUVA_{254}$ and increases in S_r in the dissolved phase indicate a change in aromaticity and CDOM size due to salt induced flocculation (Table 2). On average, $SUVA_{254}$ decreased -14.4% and -8.6% at mid salinity, and -13.1% and -11.5% at high salinity for CBC and PHR, respectively (Table 3.2). Large variations in the flocculation induced changes in $SUVA_{254}$ throughout the year were also observed. Increases in S_r, which indicate a decrease in CDOM size, were two-fold higher at high salinity (PHR = 6.9%, CBC = 6.9%) than at mid salinity (PHR = 3.2%, CBC = 4.0%; Table 3.2). Unlike potential DFe and C flocculation, decreases in S_r were greater in the latter half of the year despite initial values being similar across the study period.

Table 3.3. Relationships between the concentration (μmol L^{-1}) of potential dissolved iron and carbon flocculated with initial DOM characteristics showing regression slope, intercepts and p-values where significant relationships are present. DOC: Dissolved organic carbon, DFe: dissolved iron, DFe:DOC: dissolved iron to organic carbon ratio, DOC:DON: dissolved organic carbon to nitrogen ratio, $S_{(275\text{-}295/350\text{-}400)}$: Spectral slope ratio, and $SUVA_{254}$: specific UV absorbance at 254 nm normalized to DOC.

	Potential C Flocculation			**Potential Fe Flocculation**		
	p-value	Slope	Intercept	*p*-value	Slope	Intercept
DOC	< 0.001	+ 0.03	+ 2.07	< 0.01	+ 0.01	- 1.13
DFe	< 0.001	+ 2.09	+ 9.05	< 0.001	+ 0.60	- 0.10
DFe:DOC	< 0.001	+ 1.11	+ 8.32	< 0.001	+ 0.35	- 0.52
DOC:DON	0.13			0.53		
$S_{(275\text{-}295/350\text{-}400)}$	< 0.5	- 64. 5	+ 65.3	< 0.01	- 23.6	+19.9
$SUVA_{254}$	< 0.01	+ 7.58	-11.2	0.12		

3.5 Characteristics relating to flocculation

In assessing the predictive value of DOM parameters for flocculation, linear regression results showed significant relationships for potential C and Fe flocculation with *in-situ* DOC, DFe, DFe:DOC and S_r while DOC:DON and $SUVA_{254}$ failed to explain the degree of C and Fe flocculation (Table 3, Figure 5). Where significant relationships are present, potential flocculation increases with increasing values in the DOM parameters. Sr is an exception to this, as it is inversely correlated to CDOM size (lower values = larger CDOM size; Helms et al. 2008).

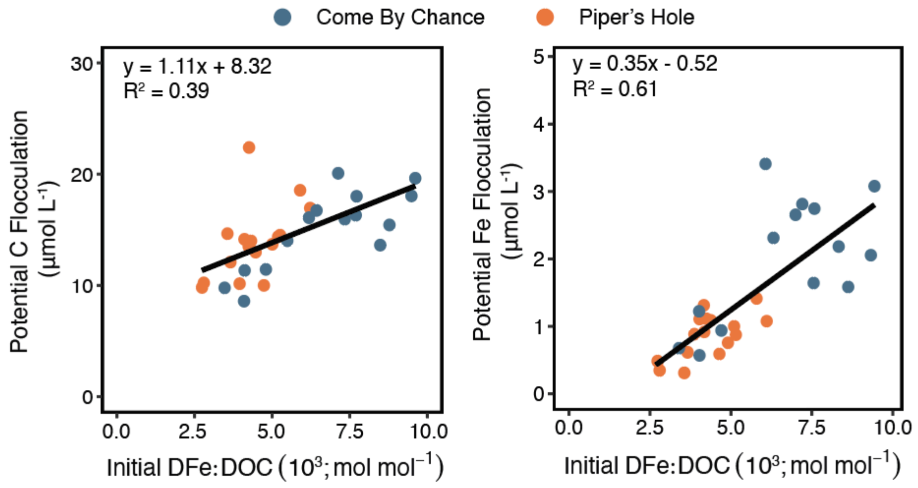


Figure 3.5. Relationships between potential carbon (left panel) and iron flocculation (right panel) with *in-situ* dissolved iron to organic carbon ratios (DFe:DOC), showing the use of DFe:DOC in its ability to predict flocculation potential. The data represented includes salt addition and 1:1 mixed freshwater:saline treatments. Black solid regression lines represent significant ($p < 0.05$) relationships, while blue dots represent data from Come By Chance, and orange dots represent data from Piper's Hole.

4 Discussion

This study demonstrates the relevance of flocculation in affecting the qualitative and quantitative properties of OM in coastal systems. Flocculation experiments revealed a potentially important source of PC in boreal estuaries while the selective transformation of DFe and optical properties highlighted a spatial and temporal susceptibility of DOM to flocculation. With increasing trajectories of DOC, CDOM ("browning") and Fe across the boreal zone (Kritzberg et al. 2020, Bjorneras at al. 2017), our results suggest a potential for an increase in PM derived from flocculation processes. However, understanding the controls on flocculation as outlined here (salinity; DFe:DOC; aromaticity) is vital in assessing and quantifying this possibility.

4.1 Flocculation as support for PM pools

With over 95% of in-situ total organic carbon in the dissolved fraction, salt-induced flocculation experiments demonstrate that a 3% transformation of DOC into the particulate fraction could amount to more than half of the *in-situ* PC in our study systems. Similarities in stable isotopic composition between flocculated and *in-situ* PC further suggest flocculation-derived contributions to PM. These results are likely conservative estimates of the potential flocculation in natural systems as flocculation is known to be enhanced in the presence of particles (Gebhardt et al. 2005, Forsgren et al. 1996, Skinnebach et al. 2019). The seemingly small proportion of DOC flocculation is on the same order of magnitude as those found for DOC-rich systems, varying in land use, across the boreal region (Sholkovitz 1976, Forsgren et al. 1996, Asmala et al. 2014).DOC-rich systems such as these have the potential to be a major source of floc-derived PM at the land-ocean interface. Our experiments also demonstrated a near complete removal of DFe (up to 87%) due to flocculation processes. With freshwater systems in the boreal region often reported as brown in appearance, owing to its characteristically high DOC and Fe content (Monteith et al. 2007, Weyhenmeyer et al. 2014), flocculation and sedimentation of these constituents may also be a potentially important pathway for regulating water color. This is especially important in a region undergoing freshwater "brownification", which has far-reaching consequences on ecosystem functioning (e.g. light availability effects on primary production) and ecosystem services (e.g. recreational value) across freshwater and estuarine environments (Kritzberg et al. 2020).

4.2 The role of salt on potential flocculation

Salinity exerts a dominant control on C and Fe flocculation, as raising the salinity from 12 to 25 psu yielded an additional 30% of DOC flocculation (Figure 3) and 50% of DFe flocculation. The salinity at which C flocculation has been suggested to occur hasranged anywhere from low (< 6psu; Asmala et al. 2014, Gustaffson et al., 2000) to mid (8 - 15psu; Eisma 1993, Mari et al. 2012; Dyu Vinh et al. 2018), and to high (> 25psu; Sholkovitz 1976). In contrast, near complete removal of DFe has been reported at low salinities (2-4 psu; Jilbert et al. 2018, Herzog et al. 2019). Our results suggest that there is a potential for both C and Fe to flocculate at downstream reaches of an estuary. This can affect estuarine structure and functioning such as the spatial occurrence of estuarine turbidity maximas (ETMs), which are known to enhance biogeochemical cycling and coupling of benthic, pelagic, and atmospheric zones (Wollast 2003). Flocculation at high salinities may also increase the potential for benthic OM sequestration as the stability (harder to break apart) of flocculated particles formed at higher salinities has been hypothesized to be greater than those formed at low salinities (Gregory 2013, Asmala et al. 2014, Mosley and Liss 2019).

4.3 Carbon source and DFe explain spatial and temporal variations in flocculation potential

Our results highlight the potential variability in flocculation across adjacent systems where differences in DOM composition can be observed and helps to explain the inconsistency among flocculation studies. As DOM is subjected to processing along transit from headwaters to the ocean, its transformation and fate in coastal environments is linked to source material, degree of upstream environmental processing, and time of year. Chemically, a multitude of factors are known to affect the potential for DOM to aggregate (e.g. flocculation), including ligand exchange, electrostatic interactions with metal oxides, and cation bridging (Philippe and Schaumann 2014). These mechanisms are complex and require understanding of DOM molecular structure, molecular weight, surface charge, and degree of hydrophobicity (Lasareva et al. 2019). Additionally, the physico-chemical environment in which molecules are present such as pH, ionic strength (salinity) and temperature are also known to affect the aggregation capabilities of DOM (Muller 2018). These factors act upon flocculation processes simultaneously, making it difficult to pinpoint the exact mechanism(s) that lead to DOM flocculation. For example, in systems where pH is greater than 7, such as those in our study, Fe content seems to be a more important precursor for flocculation (Kritzberg et al. 2014, Asmala et al. 2014).

A greater susceptibility for DFe and optical properties to flocculation in the fall, relative to summer and spring (Figure 4; Figure 5; Figure 6) highlights the importance in considering both the quantitative and qualitative parameters of DOM when evaluating the degree of DOM flocculation. For example, a more discernible seasonal pattern in the 1:1 mix treatments suggests that there are components (e.g. biochemical compounds) in saline waters, in addition to those in freshwater DOM, that enhance flocculation in the fall. Under current and projected changes to climate and regional hydrology, a redistribution in the timing and magnitude of water and DOM fluxes will affect the contribution and significance of transformative processes such as flocculation. For northern mid to high latitudes, increasing fluxes of DOC, Fe and CDOM (Huntington et al. 2016, Frigstad et al. 2013, Kritzberg et al. 2020, Bjorneras et al. 2017) has the potential to increase flocculation at an annual scale as higher flocculation potentials generally corresponded to higher initial DOM values (e.g. DOC, DFe, a_{254}). Seasonal variation in DOM composition and element speciation is also likely to affect the potential for flocculation. For example, enhanced infiltration during the fall relative to spring runoff may increase the mobilization of DOM (Bowering et al. 2021) but also the prevalence of Fe as Fe oxyhydroxides, which are thought to be more susceptible to flocculation (Herzog et al. 2017), may also enhance flocculation.

Comparisons between salt addition and 1:1 mix treatments highlights the potential for system-specific DOM susceptibility to C flocculation. While salinities in the 1:1 mix experiments (14-18 psu) were more comparable to mid salinity experiments (12 psu), C flocculation was only comparable across these two experiment types for the larger, lower relief system of PHR (Supplementary Table 1; Figure 3). For CBC, 1:1 mix treatments were more comparable to the high salinity treatments (25 psu; Figure 3). This suggests that there are components of the CBC freshwater DOM that may require greater exposure to salts, saline DOM or other environmental factors to reach its maximum potential for flocculation. One hypothesis for this is the presence of biochemical compounds influencing DOM composition. The presence of biopolymers resulting from marine primary production has been reported to positively influence the extent of flocculation (Furukawa et al. 2014, Skinnebach et al. 2019, Lee et al. 2017, 2019). In these studies, chain-like molecular structures of extracellular polymeric substances are said to intensify binding of particles. In the case of CBC, it is possible that flocculation was enhanced in the presence of biochemical compounds as a higher contribution of biological production was evident in the starting saline DOM (average chlorophyll-a: 0.05 µg L^{-1} (PHR); 0.13 µg L^{-1} (CBC), Average $\delta^{13}C$ -PC: -24.1‰ (PHR); -22.8‰ (CBC); Khoo et al. Chapter 2).

4.4 DFe:DOC and spectral slope ratios as predictors for flocculation

Flocculation of DOM is thought to be a highly discriminative process that selects for larger (high molecular weight), more aromatic DOM molecules that are more abundant in terrestrially-derived sources (Sholkovitz et al. 1978, Asmala et al. 2014, Forsgren et al. 1996, Shulkin et al. 2018). These properties are often ascertained through the use of carbon-to-nitrogen ratios (terrestrial > 20 vs. algal ~6.6; Bianchi 2007) and optical proxies for DOM composition such as aromaticity and CDOM size (Helms et al. 2003, Weishaar et al. 2003). Contrary to previous findings, we surprisingly found that neither DOC:DON and SUVA254 could significantly explain the degree of both C and Fe flocculation in our systems (Table 3). It is very likely that flocculation processes select for DOC and DFe compounds that are not reflected by these commonly employed measures. It is also important to note that initial DOC:DON ratios were relatively stable and high (average DOC:DON = 48.8), and coupled with a narrow range in SUVA_{254} (3.2 to 3.6 L mg C^{-1} m^{-1}), which could potentially contribute to its lack of predictability for flocculation with these parameters. While a more explicit predictability for flocculation may be extracted from studies conducted on DOM with a wider range in DOC:DON, SUVA_{254}, and S_r (e.g. SUVA_{254} = 3.5 to 5 L mg C^{-1} m^{-1}; Asmala et al. 2014), our results suggest that even in systems where these DOM characteristics are seemingly stable, there is still a potential to predict the degree of flocculation at regional scales via ranges in DFe:DOC.

Of the four DOM compositional parameters (DOC:DON, SUVA_{254}, S_r, and DFe:DOC) explored in this study, the DFe:DOC molar ratio and S_r were the only parameters that could significantly ($p < 0.05$) explain the degree of C and Fe flocculation (Figure 3.5, Table 3.3). A large portion is complexed with organic carbon in the colloidal size fraction (200 kDa < colloidal < 0.2 µm; Boye et al. 2010). These organic Fe complexes likely contribute to CDOM size, and more specifically, the reduction of both Fe and larger CDOM molecules during flocculation. In natural waters, a significant portion of Fe in surface waters is bound to organic ligands (Muller 2018), which keeps Fe in suspension. Such strong affinity of Fe to DOM is thought to increase resistance to flocculation (Herzog et al. 2020, Gustaffson et al. 2000), thereby enhancing their transport efficiency in coastal waters (Kritzberg et al. 2014). Our results support these findings as lower concentrations of DFe and DOC flocculate when DFe:DOC ratios are lower, which increases the potential export of DOC and DFe to downstream systems (Figure 3.5). In addition to the findings of Kritzberg et al. (2014), the possibility of increased DFe transport when there is less DFe per DOC is likely to hold true across seasonal cycles in adjacent boreal systems.

The association of DFe to DOC is particularly relevant for systems in the boreal as peatland and coniferous forests, which are common land characteristics within the region, are known for their higher DOC and DFe concentrations in comparison to other land cover types (Kortelainen et al. 2006, Moore 2003). Among peatland dominated systems, DFe:DOC ratios can be an order of magnitude lower for the boreal (1×10^{-2} to 1×10^{-3}; Kritzberg et al. 2020) relative to the tropics (1×10^{-1} to 1×10^{-2}; Zhang et al. 2020), and is driven primarily by characteristically high DOC concentrations found across boreal freshwaters. As such, the use of DFe:DOC as a predictor for flocculation should be further investigated in systems varying in land use, and in those with higher DFe:DOC ratios ($> 1 \times 10^{-2}$). Further investigation will not only benefit a greater understanding of flocculation on a global scale but also for boreal systems, which are undergoing an increase in DFe and DOC concentrations.

4.5 Response of optical properties to flocculation

Optical measurements of absorbance are a good indicator for water color, and have been linked to DOC (Osburn et al. 2016) and DFe concentrations (Poulin et al. 2014, Xiao et al. 2015). For example, Xiao et al. (2015) found Fe to be a dominant source of water color when Fe:C ratios fall below 0.22 in peatland dominated Finnish rivers. Expression of DOC and DFe typically occurs at specific wavelengths or regions across the absorbance spectra. DFe is known to absorb between 250 and 280 nm (Poulin et al. 2014), while absorption at 350 nm is highly correlated with DOC concentrations (Osburn et al. 2016). Absorbance at wavelengths shorter than 350 nm are also thought to represent aromatic molecules (Opsahl & Benner, 1997), which can be coupled to Fe(III) (Du et al., 2018). While our study shows that these regions thought to represent aromatic DOM and DFe generally decreased due to flocculation, it also revealed non-uniform responses across the absorbance spectra in relation to different salinities, seasons, and systems. For example, relative losses started to shift to gains in absorbance at wavelengths around 300 nm for experiments conducted in the spring, while shifts started around 350 nm for experiments conducted in the summer and fall for PHR. For most of the experiments conducted in the summer and fall for CBC, relative losses occurred across all wavelengths (250 - 450 nm), especially for the high salinity and 1:1 mix treatments. Asmala et al. (2014) also reported non-uniform changes in CDOM absorption due to flocculation in response to incremental salt additions of 0.5 psu, however, relative change in absorbance from losses to gains occurred around 400 nm. While spectral slope ratios were found to have some predictive value for C and Fe flocculation in this study, these variable responses across the absorbance spectra indicates that the control on CDOM flocculation is more complex than simple reductions in CDOM size and aromaticity. As such, the use of specific wavelengths and spectral slope ratios to elucidate transformations like flocculation within estuaries warrants further investigation.

5 Conclusion

Our findings highlight the relevant contribution of flocculation as a process removing OM from the water column to the benthos. Specifically, we show that even a small fraction (~3%) of DOC flocculation could amount to more than half of the PC found in boreal surface waters, and is likely to increase given current and predicted increases in riverine DOC, DFe and the overall browning effects of boreal freshwaters. We also highlight the potential for flocculation at downstream reaches of an estuary, in salinities greater than 20 psu, which may increase the spatial extent of benthic, pelagic, and atmospheric exchange through the existence of estuarine turbidity maximas. Higher elemental concentrations, DFe:DOC ratios and potentially enhanced biological sources of DOM contributed to a greater potential for flocculation in the fall, relative to summer and spring. This further highlights a temporal variation that will likely be affected by climate related changes in hydrology and seasonal fluxes of OM to coastal systems. We also call attention to the predictive value of DFe:DOC for understanding the degree of C and Fe flocculation among systems characterized as rich in aromatic and terrestrially-derived carbon sources. Further exploration of the relationship between DFe:DOC and flocculation is encouraged across the ubiquitous nature of DOM in coastal systems. Overall, our findings demonstrate the importance in considering both the quantitative and qualitative properties of

DOM in determining its susceptibility to transformation in coastal systems and consequently, its relevance and contribution to regional and global carbon budgets.

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7 Author Contributions

C.L.L.K, R.E.S, S.E.Z designed the study. All authors participated in the collection of field samples and initial sample processing. C.L.L.K conducted all experiments with helps from R.E.S., M.B.F., and A.F. S.B. conducted analysis on PM. C.L.L.K conducted all other data analysis, data interpretation and wrote the manuscript under guidance from R.E.S and S.E.Z. All authors contributed to manuscript revisions and approved of the final manuscript.

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CHAPTER 4: THESIS CONCLUSION

1 Thesis Summary

For decades, researchers have grappled with constraining the inputs, losses and transformations of carbon (organic and inorganic) from catchments to the ocean. Transitional environments, such as estuaries, are particularly difficult to constrain at local, regional, and global scales because of large spatial and temporal variation in carbon cycle processes. The findings in my thesis highlight the biogeochemical diversity and its influence on flocculation potentials among small (catchment area < 1,000 km^2) adjacent boreal coastal systems over three consecutive seasons. Overall, heterogeneity in the distribution, dynamics and fate (e.g. flocculation) of organic matter (OM) over narrow spatial and temporal scales (weekly) emphasizes the importance and relevance of these understudied small coastal systems to the efficacy of regional and global elemental budgets.

In-situ measurements of particulate and dissolved OM, chapter two of my thesis, revealed a dominance of OM in the dissolved fraction in boreal freshwaters (>95%), of which the majority of the molecules and compounds that make up the DOM pool are terrestrially derived, aromatic and C-rich. While common optical proxies for aromaticity ($SUVA_{254}$) and molecular weight ($S_{275-295/350-400}$) lacked spatial and temporal variation among the freshwater sites, dissolved elemental concentrations (DOC: Dissolved organic carbon, DON: Dissolved organic nitrogen, DFe: Dissolved iron) and their molar ratios (DOC:DON; DFe:DOC) exhibited seasonal variation with peaks in late summer. Measurements of dissolved iron and its ratio with DOC were found to be the best indicators for understanding the temporal stability of the DOM pool, and thus the fate of DOM among the adjacent systems. Correlation analyses also revealed a localized coupling of DOM with particulate matter (PM) pools, which were potentially enhanced at a weekly scale during the summer-to-fall transition period. This highlights the importance of biogeochemical diversity, even within small systems in close proximity, in understanding the fluxes, cycling, and transformations of OM in near-shore coastal systems.

Flocculation experiments, chapter three of my thesis, demonstrated a consistent removal of DOC and DFe, highlighting the relevance of flocculation as a potentially important, and significant source of particulate carbon in boreal systems. The potential for flocculation also increased with salinity, indicating a possibility for flocculation to occur at lower reaches of an estuary (salinities above 20 psu), which could extend the zones where estuarine turbidity maximas can be found and thus where intense biogeochemical cycling can occur. We further show flocculation to be a selective process with higher starting elemental concentrations and DFe:DOC yielding greater flocculation potentials while non-uniform changes to optical properties in response to flocculation at different salinities, seasons, and system were also evident. The use of DFe:DOC ratios were particularly useful in predicting the degree of flocculation across spatial and temporal scales, and likely hold predictive value at regional scales.

2 Applications for estuarine biogeochemistry

2.1 The utility of DFe:DOC ratios in estuarine biogeochemistry

For boreal regions where OM is predominantly in the dissolved fraction and terrestrial organic carbon is in abundance, assessments of OM including elemental quantification, optical properties, elemental ratios, and isotopic signatures, may act as a guide for method and analyte selection in future studies. As demonstrated in my thesis, measurements of DFe and calculations for DFe:DOC ratios will be especially informative for assessing the fluxes and fate of DOM across different catchment types, even among adjacent systems. Additionally, these measures have the potential to predict DOM susceptibility to flocculation when common DOM measures like $SUVA_{254}$ and DOC:DON exhibit narrow ranges (e.g. $SUVA_{254} = 3.4 \pm 0.2$; DOC:DON = 48 ± 7). Conducting simultaneous measures of OM using techniques from traditionally different fields (e.g. geochemistry: stable isotopes, biology: Chlorophyll-a) will also be beneficial for estuarine biogeochemistry given the complexities in accounting for multiple OM sources across estuarine zones. While recent analytical advancements and statistical tools create opportunities for a better glimpse at the tens of thousands of distinct molecular formulae that make up DOM (Derenne and

Tu, 2014, Mostovaya et al. 2017), the wide-spread applicability of such high resolution analytical techniques is limited by challenges in data interpretation, operational complexity and cost. When such techniques are unavailable, my thesis demonstrates that there is still much to gain from common OM measures.

2.2 Contribution of biogeochemical data from small coastal systems

The coupling of large OM reservoirs with rapid environmental change across northern mid-to-high latitudes introduces great uncertainties in the movement of elements across land-ocean and atmosphere-pelagic-benthos continuums. Despite an abundance of small and relatively untouched systems across the region, our current understanding of the trajectories of change are primarily based on biogeochemical data from large Arctic rivers and urbanized systems (Regnier et al. 2013, Ganju et al. 2016). Given the abundance of small systems, their cumulative impact can also be greater than those of larger systems. For example, Milliman and Syvitsky (1992) reported that 40 to 70% of the global sediment flux to the ocean comes from smaller rivers that are more abundant. The lack of representation of small systems within regional modelling efforts thus increases uncertainties and can potentially lead to poor predictions of the cycling, fluxes, and fate of OM. Uncovering and monitoring the dynamics of OM at a local scale and within smaller systems, such as those outlined in my thesis, helps to lay the foundation for identification of larger regional and global distributions.

2.4 Highlighting the relevance of the summer-to-fall transition period

The spring freshet, which occurs in systems affected by snow and ice, is typically considered to be one of the most important seasonal periods contributing to the mobilization of OM from the land to sea. For northern mid-to-high latitudes, much of the focus of the last decade has been on this distinct hydrologic period, however, the timing and magnitude of this large flux of water and OM is changing and becoming less distinct with climate change. Specifically, climate induced shifts in hydrology has resulted in earlier and more intermittent snowmelts, a reduction in the peak of the freshet, and an increased number and intensity of fall storms (Wrona et al. 2016, Woo et al. 2008). Collectively, these shifts highlight the need for a better understanding of OM fluxes beyond the spring freshet, and my thesis shows that the summer-to-fall transition period is an equally relevant period that should be studied more extensively moving forward.

3 Recommendations for future research

A major challenge facing estuarine biogeochemistry and organic matter research is the extensive and rapid alteration of these natural ecosystems and the climate feedbacks they support. Assessing and predicting the trajectories of such rapid change requires long-term monitoring of OM in rivers and estuaries, however, such datasets are rare. From those that do exist such as the Helsinki commission (HELCOM; formed for the Baltic Sea and surrounding areas), continued success in long term monitoring efforts have created possibilities for understanding not only the trends and drivers of OM exports and dynamics, but also the formation of strategic management programs crucial for maintaining healthy aquatic ecosystems. For the coastal systems of Piper's Hole and Come By Chance in Newfoundland, Canada, the data presented in this thesis acts as a baseline measure of OM dynamics and should be continued to better understand the impacts of OM on coastal ecosystem functioning and ultimately the livelihoods of people who rely on these systems for food security. OM monitoring should also be extended to include other coastal systems of Newfoundland and Labrador as well as Atlantic Canada to include representation of important climatic gradients (temperate to polar). Long term monitoring aside, efforts made today in understanding OM dynamics across this geographical area will provide key insights on the potential future dynamics of Arctic systems, which are undergoing borealization (Polyakov et al. 2020, Møller and Nielsen, 2019).

Rapid environmental change also increases the variability among and within systems, making it more difficult to understand and predict the distribution, cycling and fate of OM. This calls for a greater number of targeted studies around seasonal change where variability is greatest and at weekly resolutions to better allow understanding of the bounds of elemental fluxes across the land-ocean continuum. Already, local scale biogeochemical models that implement high resolution (daily) climatological, hydrological, and environmental datasets have seen success in disentangling the effects of complex simultaneous estuarine processes, and thus show promise in providing a more integrated assessment of fluxes across land-ocean and atmospheric-pelagic continuums (Regnier et al. 2013). Increasing the number of biogeochemical datasets across rivers and estuaries will also require further development and re-assessments of such modelling approaches to simulate processes at appropriate spatial and temporal scales. These scales should capture changes in anthropogenic land-use, climate related environmental change (e.g. hydrology, sea level changes etc.), and the breadth of variabilities in system size and classifications (e.g. salt wedge, fjords etc.). Modelling approaches should also integrate transformation processes such as flocculation.

Despite flocculation being widely referenced as an important transformation mechanism in estuaries, a predictive understanding of the concentration, capacity, and primary locations of flocculation will enable its use in refining coastal biogeochemical models. In assessing the current literature on flocculation, it also became apparent that there is a lack of understanding regarding the lifespan of floc-derived particles. For example, investigations tracking the pathways and alterations of floc-derived particles through food webs are needed to support its biological relevance in estuarine biogeochemical cycles. The byproducts from biological uptake of floc-derived particles and its impacts on current nutrient and OM cycling will also need future investigation. Finally, quantifying the potential for carbon sequestration via flocculation processes requires further understanding of the sinking rate, remineralization rates, and long term sediment storage of floc-derived particles.

Advancements in spectroscopic techniques and the identification of optical proxies such and $SUVA_{254}$ and spectral slope ratios have led to an increased use of optical properties in aquatic biogeochemistry. Understanding the optical properties of the DOM in pelagic zones of an estuary is particularly important given their control on light availability and DOM quality and their subsequent effect on basal productivity. Evaluating the effect of optical properties on coastal ecosystem functioning is in part challenged by a lack of understanding on how individual transformations alter DOM along transit from the land to ocean. Combining flocculation experiments with other transformative processes such as photochemistry will therefore benefit our understanding of the cumulative and individual impact of transformations on specific wavelengths, and thus common optical proxies such as $SUVA_{254}$ and spectral slope ratios. Future exploration of new techniques such as the calculation of the nominal oxidation states of the carbon (Wilson and Tfaily, 2018), a calculation of the energy provided by the oxidation of a compound and thus OM quality, may also supplement the use of optical properties as a measure for deciphering OM quality.

Estuarine biogeochemistry requires integration of ideas and techniques from traditionally different scientific fields. It is also relatively new compared to its freshwater and marine counterparts, and as a result, many of the techniques employed in estuaries and subsequent data interpretation are borrowed from these fields. Having consistent techniques and data across the land-ocean continuum is not always feasible due to interferences from salts and instrument detection limits, however, future observations should identify and prioritize the use of relevant OM measures that can be consistently employed across freshwater, estuarine, and marine environments. The findings in my thesis address these key gaps, paving the way forward for estuarine biogeochemistry.

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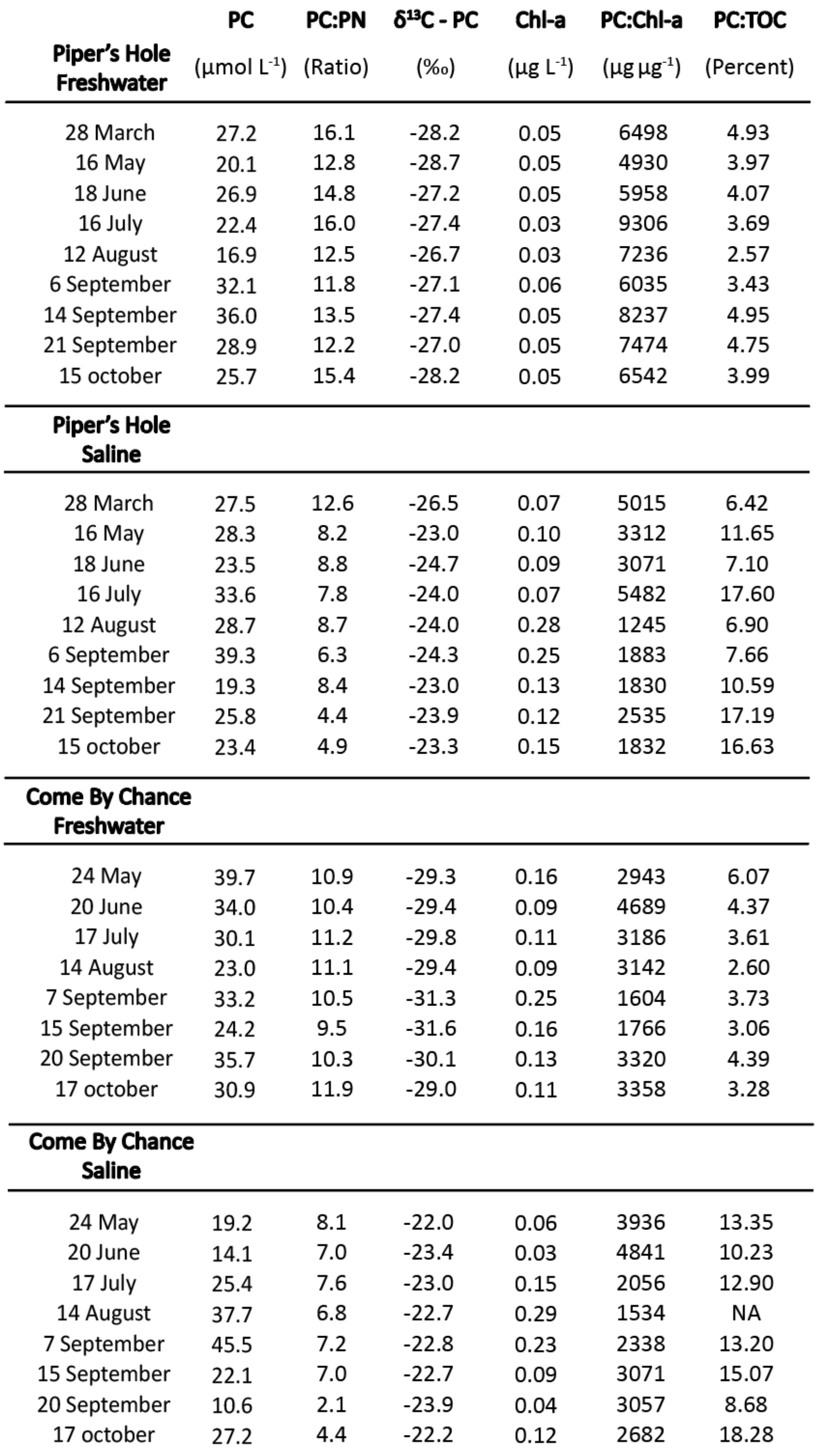
APPENDICES

Appendix 1: Chapter 2: A comparison of the distribution and dynamics of particulate and dissolved organic matter across two boreal river-influenced systems.

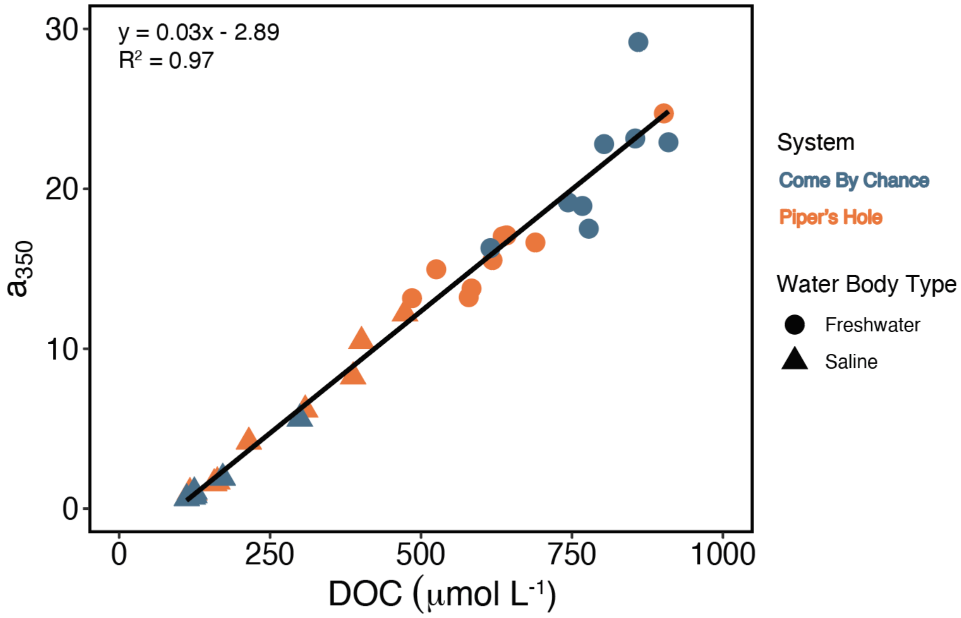
A1. Table 1. Dissolved organic matter parameters for the four study sites at each sampling event over the 2019 study period. DOC: dissolved organic carbon, DON: dissolved organic nitrogen, DOC:DON: dissolved carbon to nitrogen ratio, DFe: dissolved iron, DFe:DOC: dissolved iron to carbon ratio, $SUVA_{254}$: specific UV absorbance at 254 nm normalized to DOC, S_r: spectral slope ratio at 275-295nm over 350-400nm.

	Salinity	**DOC**	**DON**	**DOC:DON**	**DFe**	**DFe:DOC**	**SUVA$_{254}$**	**$S_{(275\text{-}295/350\text{-}400)}$**
Piper's Hole Freshwater	(psu)	(µmol L^{-1})	(µmol L^{-1})	(Ratio)	(µmol L^{-1})	(Ratio)	(L mg^{-1} m^{-1})	(Ratio)
28 March	0	525	10.3	53.3	2.20	0.0042	3.55	0.79
16 May	0	485	9.7	50.1	1.73	0.0036	3.53	0.82
18 June	0	636	14.1	45.0	2.35	0.0037	3.42	0.77
16 July	0	784	13.9	41.9	2.76	0.0047	3.14	0.82
12 August	0	642	15.1	42.4	3.99	0.0062	3.51	0.79
6 September	0	902	18.1	29.8	5.33	0.0059	3.53	0.76
14 September	0	690	14.3	48.2	3.58	0.0052	3.25	0.79
21 September	0	579	13.2	43.8	2.90	0.0050	3.16	0.83
15 october	0	618	12.6	48.8	3.25	0.0053	3.42	0.81
Piper's Hole Saline								
28 March	16	401	9.7	41.6	0.97	0.0024	3.10	0.84
16 May	30	215	7.3	29.9	0.23	0.0011	2.56	0.95
18 June	30	308	9.5	32.1	0.24	0.0008	2.53	0.89
16 July	33	157	9.2	17.9	0.16	0.0010	1.63	1.16
12 August	25	388	11.6	33.3	0.23	0.0006	2.79	0.87
6 September	23	473	12.4	38.1	0.53	0.0011	3.11	0.89
14 September	34	163	8.4	19.3	0.04	0.0002	1.72	1.23
21 September	34	124	7.0	17.7	0.14	0.0011	1.54	1.38
15 october	30	117	6.1	19.2	0.04	0.0003	1.62	1.35
Come By Chance Freshwater								
24 May	0	615	10.8	56.7	2.53	0.0041	3.45	0.79
20 June	0	744	15.1	49.2	3.52	0.0048	3.40	0.78
17 July	0	803	15.9	50.2	6.18	0.0077	3.64	0.77
14 August	0	860	18.1	47.4	8.27	0.0096	4.02	0.73
7 September	0	855	16.4	51.9	8.12	0.0095	3.59	0.76
15 September	0	767	16.3	46.8	6.51	0.0085	3.37	0.78
20 September	0	778	17.0	45.9	6.83	0.0088	3.08	0.79
17 october	0	910	16.4	55.5	5.63	0.0062	3.35	0.77
Come By Chance Saline								
24 May	33	124	10.4	12.3	0.04	0.0003	1.31	1.23
20 June	37	124	9.2	13.4	0.04	0.0003	1.38	1.58
17 July	28	172	7.6	23.0	0.09	0.0005	1.76	1.17
14 August	29	NA	NA	NA	0.04	NA	NA	1.18
7 September	31	299	11.1	26.8	0.11	0.0004	2.57	1.00
15 September	35	125	6.3	19.5	0.04	0.0003	2.33	1.67
20 September	37	112	7.5	15.0	0.04	0.0003	1.14	2.01
17 october	30	122	7.5	16.5	0.04	0.0003	1.32	1.44

A1. Table 2. Particulate matter parameters for the four study sites at each sampling event over the 2019 study period. PC: particulate carbon, PC:PN: particulate carbon to nitrogen ratio, δ^{13}C-PC: stable isotopic composition of the particulate carbon, Chl-a: chlorophyll-a, PC:Chl-a: particulate carbon to chlorophyll-a ratio, PC:TOC: percent of total organic carbon s maller than 200μm found in the particulate fraction.



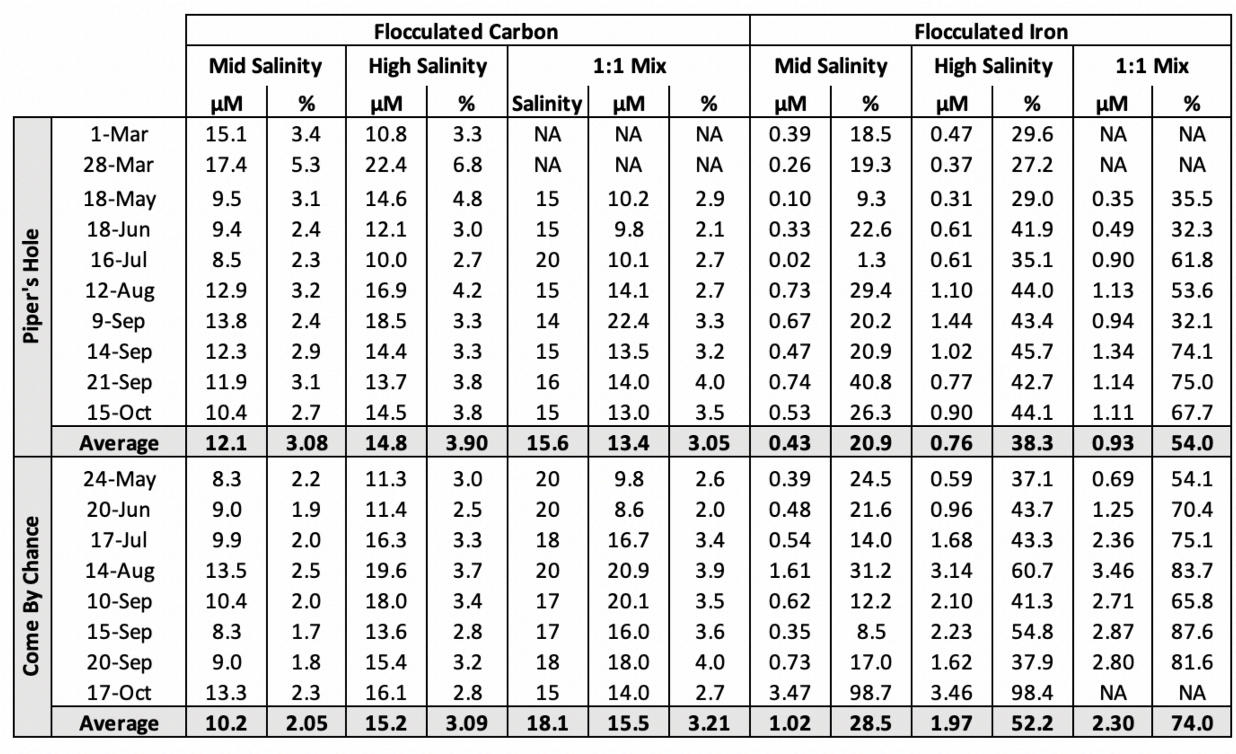
Piper's Hole Freshwater	**PC** (μmol L^{-1})	**PC:PN** (Ratio)	**δ^{13}C - PC** (‰)	**Chl-a** (μg L^{-1})	**PC:Chl-a** (μg μg^{-1})	**PC:TOC** (Percent)
28 March	27.2	16.1	-28.2	0.05	6498	4.93
16 May	20.1	12.8	-28.7	0.05	4930	3.97
18 June	26.9	14.8	-27.2	0.05	5958	4.07
16 July	22.4	16.0	-27.4	0.03	9306	3.69
12 August	16.9	12.5	-26.7	0.03	7236	2.57
6 September	32.1	11.8	-27.1	0.06	6035	3.43
14 September	36.0	13.5	-27.4	0.05	8237	4.95
21 September	28.9	12.2	-27.0	0.05	7474	4.75
15 october	25.7	15.4	-28.2	0.05	6542	3.99
Piper's Hole Saline						
28 March	27.5	12.6	-26.5	0.07	5015	6.42
16 May	28.3	8.2	-23.0	0.10	3312	11.65
18 June	23.5	8.8	-24.7	0.09	3071	7.10
16 July	33.6	7.8	-24.0	0.07	5482	17.60
12 August	28.7	8.7	-24.0	0.28	1245	6.90
6 September	39.3	6.3	-24.3	0.25	1883	7.66
14 September	19.3	8.4	-23.0	0.13	1830	10.59
21 September	25.8	4.4	-23.9	0.12	2535	17.19
15 october	23.4	4.9	-23.3	0.15	1832	16.63
Come By Chance Freshwater						
24 May	39.7	10.9	-29.3	0.16	2943	6.07
20 June	34.0	10.4	-29.4	0.09	4689	4.37
17 July	30.1	11.2	-29.8	0.11	3186	3.61
14 August	23.0	11.1	-29.4	0.09	3142	2.60
7 September	33.2	10.5	-31.3	0.25	1604	3.73
15 September	24.2	9.5	-31.6	0.16	1766	3.06
20 September	35.7	10.3	-30.1	0.13	3320	4.39
17 october	30.9	11.9	-29.0	0.11	3358	3.28
Come By Chance Saline						
24 May	19.2	8.1	-22.0	0.06	3936	13.35
20 June	14.1	7.0	-23.4	0.03	4841	10.23
17 July	25.4	7.6	-23.0	0.15	2056	12.90
14 August	37.7	6.8	-22.7	0.29	1534	NA
7 September	45.5	7.2	-22.8	0.23	2338	13.20
15 September	22.1	7.0	-22.7	0.09	3071	15.07
20 September	10.6	2.1	-23.9	0.04	3057	8.68
17 october	27.2	4.4	-22.2	0.12	2682	18.28



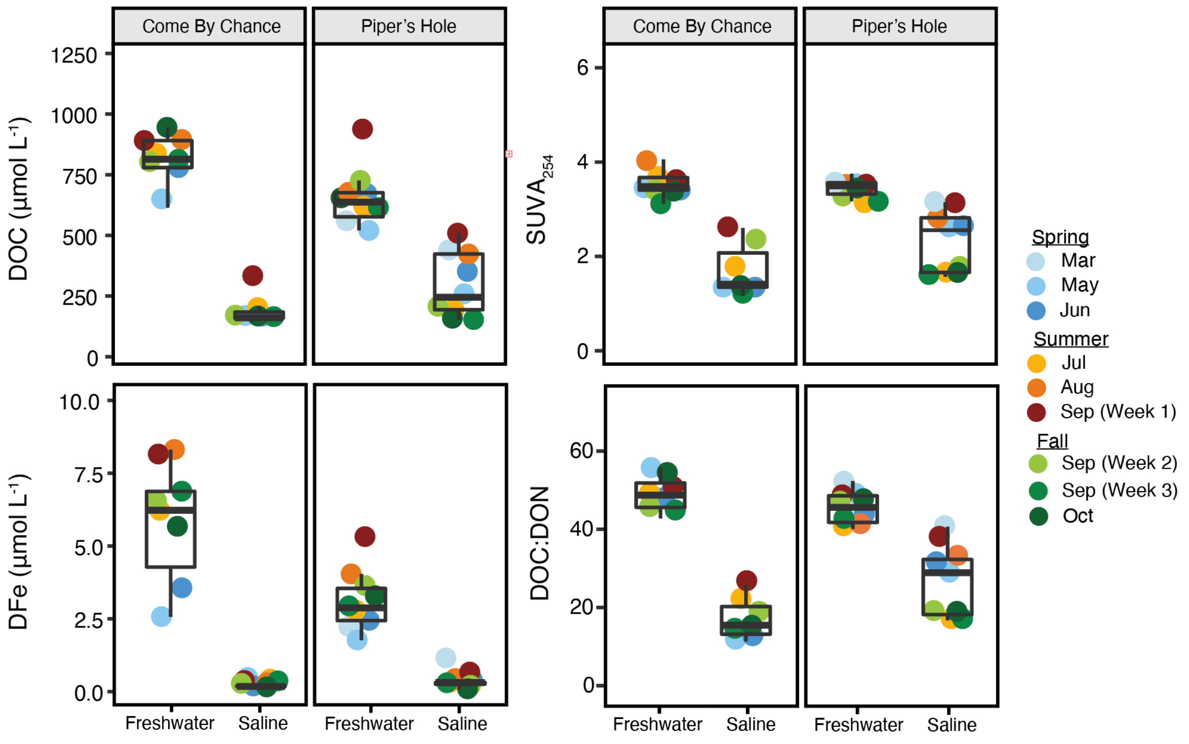
A1. Figure 1. Linear relationship between DOC and absorbance at 350nm showing the strength of the DOC-CDOM relationship in our study systems. Values for absorbance at 350nm are naperian absorption coefficients. Triangles represent data collected from saline sites and circles represent data from freshwater sites. Orange symbols represent Piper's Hole and blue symbols represent Come By Chance.

Appendix 2: Chapter 3: Evaluating the mechanisms influencing salt-induced flocculation of dissolved organic matter in boreal coastal systems

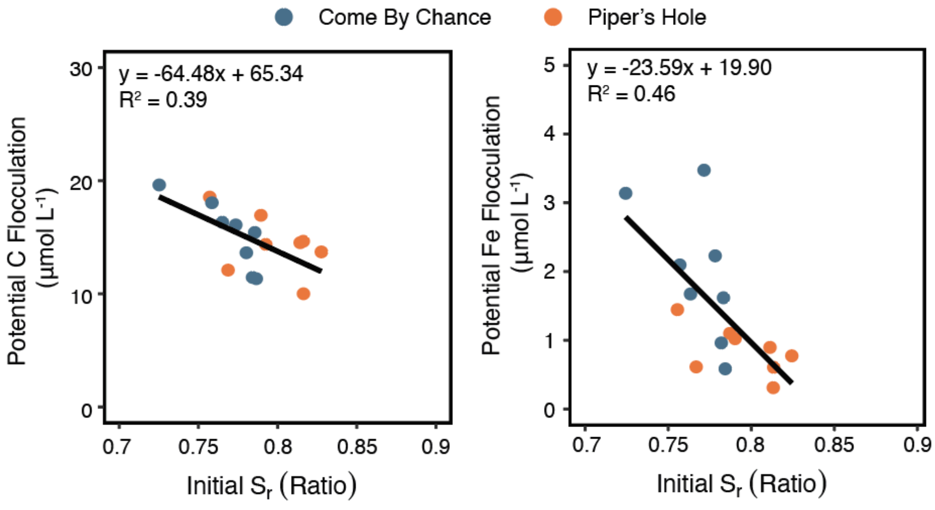
A2. Table 1. Concentration and percent flocculation of dissolved organic carbon and dissolved iron across the two study systems, Piper's Hole and Come By Chance. Flocculated iron is represented as the loss (change) in dissolved iron after the incubation period whereas flocculated carbon is represented as the gain in particulate carbon after the incubation period both expressed here as percent of initial dissolved iron and organic carbon, respectively.



		Flocculated Carbon							Flocculated Iron					
		Mid Salinity		High Salinity		1:1 Mix			Mid Salinity		High Salinity		1:1 Mix	
		µM	%	µM	%	Salinity	µM	%	µM	%	µM	%	µM	%
Piper's Hole	1-Mar	15.1	3.4	10.8	3.3	NA	NA	NA	0.39	18.5	0.47	29.6	NA	NA
	28-Mar	17.4	5.3	22.4	6.8	NA	NA	NA	0.26	19.3	0.37	27.2	NA	NA
	18-May	9.5	3.1	14.6	4.8	15	10.2	2.9	0.10	9.3	0.31	29.0	0.35	35.5
	18-Jun	9.4	2.4	12.1	3.0	15	9.8	2.1	0.33	22.6	0.61	41.9	0.49	32.3
	16-Jul	8.5	2.3	10.0	2.7	20	10.1	2.7	0.02	1.3	0.61	35.1	0.90	61.8
	12-Aug	12.9	3.2	16.9	4.2	15	14.1	2.7	0.73	29.4	1.10	44.0	1.13	53.6
	9-Sep	13.8	2.4	18.5	3.3	14	22.4	3.3	0.67	20.2	1.44	43.4	0.94	32.1
	14-Sep	12.3	2.9	14.4	3.3	15	13.5	3.2	0.47	20.9	1.02	45.7	1.34	74.1
	21-Sep	11.9	3.1	13.7	3.8	16	14.0	4.0	0.74	40.8	0.77	42.7	1.14	75.0
	15-Oct	10.4	2.7	14.5	3.8	15	13.0	3.5	0.53	26.3	0.90	44.1	1.11	67.7
	Average	**12.1**	**3.08**	**14.8**	**3.90**	**15.6**	**13.4**	**3.05**	**0.43**	**20.9**	**0.76**	**38.3**	**0.93**	**54.0**
Come By Chance	24-May	8.3	2.2	11.3	3.0	20	9.8	2.6	0.39	24.5	0.59	37.1	0.69	54.1
	20-Jun	9.0	1.9	11.4	2.5	20	8.6	2.0	0.48	21.6	0.96	43.7	1.25	70.4
	17-Jul	9.9	2.0	16.3	3.3	18	16.7	3.4	0.54	14.0	1.68	43.3	2.36	75.1
	14-Aug	13.5	2.5	19.6	3.7	20	20.9	3.9	1.61	31.2	3.14	60.7	3.46	83.7
	10-Sep	10.4	2.0	18.0	3.4	17	20.1	3.5	0.62	12.2	2.10	41.3	2.71	65.8
	15-Sep	8.3	1.7	13.6	2.8	17	16.0	3.6	0.35	8.5	2.23	54.8	2.87	87.6
	20-Sep	9.0	1.8	15.4	3.2	18	18.0	4.0	0.73	17.0	1.62	37.9	2.80	81.6
	17-Oct	13.3	2.3	16.1	2.8	15	14.0	2.7	3.47	98.7	3.46	98.4	NA	NA
	Average	**10.2**	**2.05**	**15.2**	**3.09**	**18.1**	**15.5**	**3.21**	**1.02**	**28.5**	**1.97**	**52.2**	**2.30**	**74.0**



A2. Figure 1. Spatial and annual variation in in-situ dissolved organic carbon (top left), dissolved iron (bottom left), $SUVA_{254}$ (top right), and DOC:DON ratio (bottom right) across the 2019 study period for Come By Chance and Piper's Hole. Data source: St. Lawrence Global Observatory (DOI)



A2. Figure 2. Relationships between potential carbon (left panel) and iron flocculation (right panel) with *in-situ* spectral slope ratios (Sr) for salt addition treatments. Black solid regression lines represent significant ($p < 0.05$) relationships, while blue dots represent data from Come By Chance and orange dots represent data from Piper's Hole.