Analytical Improvements for Assessing Dissolved Organic Carbon Concentrations and Dynamics in the Ocean

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ANALYTICAL IMPROVEMENTS FOR ASSESSING DISSOLVED ORGANIC CARBON CONCENTRATIONS AND DYNAMICS IN THE OCEAN

By

Meredith K. Jennings

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ANALYTICAL IMPROVEMENTS FOR ASSESSING
DISSOLVED ORGANIC CARBON CONCENTRATIONS AND
DYNAMICS IN THE OCEAN

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Marine dissolved organic carbon (DOC) represents a large and dynamic reservoir of reduced carbon ($662 \times 10^{15} \text{g C}$) comparable in size to the Earth’s reservoir of atmospheric CO$_2$. Our knowledge on the transformation and removal of DOC through various sinks and processes is often obscured by a lack of observable and quantifiable mechanisms. For example, self-assembling microgels, transitional in size between dissolved and particulate matter, have been suggested to play a key intermediary role in organic matter bioreactivity. Microgel formation is the first step in creating a particulate sink for dissolved organic matter, with up to 10% of DOC in the ocean ($70 \times 10^{15} \text{g C}$) predicted to exist in the gel phase. Although they are considered macrogels due to their larger size and greater stickiness than microgels, transparent exopolymer particles (TEP) also abiotically assemble from dissolved organic precursors (namely, polysaccharides). As microgels and TEP span the dissolved-to-particulate size continuum of organic matter, it is important to understand how polymer gel dynamics influence the total organic carbon (TOC) pool.

Analytical challenges limit our ability to directly quantify the organic carbon present in these gels, or even to measure DOC removal through gel formation. In regions of the ocean where DOC concentrations are as low 35 µM, the potential 10% thermodynamic...
yield of gel formation is close to the analytical uncertainty of DOC measurement (1-3 µM; CV ~3%). This uncertainty also makes pursuit of other studies on DOC dynamics, such as uptake and respiration, difficult. This dissertation uses an analytical chemistry approach to enhance understanding of sources and sinks of marine organic carbon in the ocean through field observations of dynamics in addition to improvements of DOC and gel methodologies.

In Chapter 2, the abundance of TEP was observed across a surface ocean gradient of TOC, from a phytoplankton bloom region in the western North Atlantic to oligotrophic waters in the Sargasso Sea, including a coastal region sampled near Cape Cod. Results from this chapter support the hypothesis that TEP aggregation with non-gel particulate organic carbon (POC) enhances ballasting, thereby mutually facilitating export and subsequent sedimentation of both TEP and POC, and increasing the efficiency of the biological pump. Chapter 3 investigated the fluorescence quenching assay previously developed by Ding et al. (2007) to estimate the carbon content of microgels in natural seawater samples, because results obtained by applying the method are often inconclusive. Sensitivity to pH was identified as the primary driver for the variability, suggesting that the method should be discontinued in its present form. To improve the precision of the DOC measurement, Chapter 4 described the development of a prototype flow-through TOC/DOC analyzer system that combines UV and wet chemical oxidation and is capable of sub-micromolar precision. While the prototype instrument shows superior analytical skill relative to the standard HTC technique, application is currently limited to freshwater analyses due to oxidation interference by chloride ion.
“The sea is everything. It covers seven tenths of the terrestrial globe. Its breath is pure and healthy. It is an immense desert, where man is never lonely, for he feels life stirring on all sides. The sea is only the embodiment of a supernatural and wonderful existence. It is nothing but love and emotion; it is the Living Infinite.”

— Jules Verne, *Twenty Thousand Leagues Under the Sea*
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CHAPTER 1. Introduction

1.1 Background

One of the least understood carbon transformations in the ocean is the interaction between particulate and dissolved organic matter (POM and DOM, respectively). At 662 Pg C, marine DOM is the biggest and most dynamic reservoir of reduced carbon in the ocean (Hansell et al. 2009). Depending on its composition and source material, DOC has varying biological and photochemical reactivity that determines its fate. Consequently, the lifetime of dissolved organic carbon (DOC) varies from hours (highly labile) to millennia (highly refractory). The majority of DOC produced daily supports microbial substrate demands and so is rapidly transformed back to CO₂ as a product of respiration. Semi-labile DOC has a mean lifetime of ~1.5 years and is presently quantified from the seasonal variability of DOC concentration in the euphotic zone (Hansell 2013). DOC that is not remineralized accumulates as recalcitrant material that is advected into the deep ocean, where it persists for thousands of years and has radiocarbon ages between 4000 and 6000 years. Our knowledge of the transformation and removal of this refractory DOC through various sinks and processes is obscured by a lack of observable and quantifiable mechanisms (Hansell 2013).

In order to bridge the size continuum (Fig. 1.1) between dissolved and particulate organic carbon (DOC and POC, respectively), the roles of polymer gels such as microgels and transparent exopolymer particles (TEP) are actively being examined (Baltar et al., 2016; Mari et al., 2017; Orellana et al., 2011; Passow, 2002a; Verdugo et al., 2004; Zetsche and Ploug, 2015). Microgels spontaneously and reversibly self-assemble as colloidal- to micrometer-sized networks of negatively charged polymers.
stabilized by Ca\textsuperscript{2+} ionic bonds (Chin et al. 1998). Hydrophobic interactions, such as miscelle formation, can also facilitate gel assembly (Orellana et al. 2011; Ding et al. 2008). Microgel formation is the first step in creating a particulate sink for DOM; it has been suggested that up to 10% of DOC, or 70 Pg C, in the ocean exists in the gel phase (Chin et al. 1998).

**Fig. 1.1.** a) Size spectrum of organic matter adapted from Verdugo (2012) and images of b) macrogels, c) TEP, and d) microgels.

TEP also abiotically form from dissolved carbohydrate precursors, which are commonly referred to as gels or gel-like particles. TEP are operationally defined as transparent particles that are stainable with Alcian Blue, a dye that preferentially binds to acidic polysaccharides (Alldredge et al. 1993; Passow and Alldredge 1995). As TEP are inherently sticky, they have the potential to form aggregates heavy enough to sink
through the water column (Azetsu-Scott & Passow 2004). However, TEP have known surface-active properties, enabling them to be scavenged by rising bubbles to concentrate at the air-sea interface where the sea surface microlayer (SML) is formed (Mopper et al. 1995; Wurl et al. 2009; Cunliffe et al. 2013). Once concentrated in the SML, organic material may be introduced to the atmosphere via bubble bursting as sea spray aerosols capable of cloud condensation and ice nucleation (Quinn et al. 2014; Wilson et al. 2015; Orellana et al. 2011).

1.2 A role for gels in marine carbon cycling

Mechanisms for TEP and microgels interactions in the ocean carbon cycle and food web are important to understand. In particular, it is not well known what exact roles each play in the microbial loop and to what extent they facilitate POM export out of the euphotic zone. The ocean carbon cycle can briefly be summarized into three major processes (1) the solubility pump, the flux of CO₂ between the atmosphere and the surface ocean through physical processes (ventilation and advection); (2) the biological pump, biologically driven processes (primary production and respiration) and sequestration of carbon away from the atmosphere for longer timescales in carbon reservoirs such as the deep ocean and sediments; and intermediately, (3) the microbial loop in which DOC is recycled by bacteria and protozoan grazers (Anderson and Ducklow, 2001). As rising CO₂ concentrations in the atmosphere cause the oceans to warm and become more acidic, the role for gels in the carbon cycle and organic carbon cycle, particularly, could become more significant.
Several studies have found enhanced organic carbon in the form of TEP under elevated CO$_2$ conditions by performing mesocosm and incubation experiments (Borchard and Engel, 2012; Engel et al., 2004; Engel and Händel, 2011; Passow, 2012; Riebesell et al., 2007). For example, Riebesell et al. (2007) found a four-fold increase in TEP concentration during a mesocosm experiment involving a natural plankton community in a fjord exposed to 1,050 µatm CO$_2$ (or 3 times the present atmospheric concentration). They suggested TEP was a sink for DOC in the upper mixed layer because DOC concentration was lower than expected when considering the expected difference between POC build up and DIC drawdown. Other studies similarly reported a significant increase in POC attributed to TEP transformed from DOC produced by *Emiliania huxleyi* growing under different CO$_2$ concentrations and nutrient limitations (Borchard and Engel, 2012; Engel, 2004).

Verdugo (2012) identified the need to better understand the effects of temperature and pH on microgel assembly, as these parameters will be impacted by future elevated CO$_2$ concentrations. As temperature increases from 5–30 °C, the hydrodynamic diameter, or size, of microgels decreases (Verdugo, 2012). Below a critical point of pH 4.5, the networks of microgels condense and undergo volume phase transition, decreasing the hydrodynamic diameter from 5 µm to 1 µm (Chin et al., 1998). Although pH < 4.5 is unrealistic in modern or future oceans, there is a scenario where organic pollutants could create acidic microenvironments with cumulative, broader effects. Baltar et al. (2016) reported an increase in the proportion of organic carbon present in self-assembled gels when pH was reduced 0.3 units, due to higher Ca$^{2+}$ concentrations upon dissolution of CaCO$_3$ at lower pH.
Chen et al. (2015) identified a synergism between ocean warming and acidification that hindered the self-assembly of microgels in seawater at pH 8 and 32 °C that was not present at 22 °C or even 30 °C. However, Ding et al. (2008) reported rapid assembly (<15 h) of hydrophobic Sagittula stellata polymers in Ca\textsuperscript{2+}-free media (pH unknown) at 30 °C compared to slower assembly at 20 °C (~100 h) and hindered assembly at 4 °C. This contrast in temperature dynamics between isolated hydrophobic polymers and natural seawater containing potentially both hydrophobic and Ca\textsuperscript{2+} crosslinked polymers implies that covalent bonds in Ca\textsuperscript{2+} crosslinking likely dominate assembly dynamics.

Only a small fraction of primary produced material persists below the euphotic zone as sinking refractory organic carbon, with POC declining exponentially due to respiration. Numerous studies have correlated TEP formation with increased POC formation and consequent particle export, i.e. TEP-aided aggregation and sinking (Alldredge et al., 1993; Borchard and Engel, 2012; Martin et al., 2011; Schartau et al., 2007). Martin et al. (2011) combined particle fluxes from sediment traps and water column isotopically labeled thorium (\textsuperscript{234}Th) measurements to examine particle export during a North Atlantic spring diatom bloom. Because \textsuperscript{234}Th has high affinity for acidic polysaccharides, TEP-rich material will have lower POC:234\textsuperscript{Th} ratios (Passow et al., 2006). Their data affirmed that diatom blooms cause large export events with efficient transfer of POC through the mesopelagic (transfer efficiency ranges from 43 ± 11% to 25 ± 6%). They hypothesized that this large export event was caused by nutrient limitation, which promoted TEP formation and increased particle export.

The microbial ecology of marine aggregates (or “marine snow”) and TEP is well studied with respect to the speciation of colonizing or grazing microbes (Artolozaga et
al., 1997; Kiørboe et al., 2003; Mari and Kiørboe, 1996; Mari and Rassoulzadegan, 2004; Yamada et al., 2013). Degradation of marine snow (larger than 0.5 mm) is caused by microbial activity as bacterial enzymes hydrolyze POM to DOM (Smith et al., 1992). Instead of being described as simple sinking particles, marine snow can be described as a mixture of biopolymers representing a downward flux of slowly degradable DOM (Smith et al., 1992). This DOM includes dissolved polymers freshly produced from the enzymatic hydrolysis of marine snow by bacteria. Because sinking aggregates could collect more particles from the surrounding environment to supply enzymes with new particles (Azam and Long, 2001; Kiørboe, 2001), refractory material gradually accumulates and extends the lifetime of marine snow (Smith et al., 1992).

1.3 Analytical challenges

Direct measurements of carbon content in gel particles have yet to be published. This absence exists because in order to quantify the carbon held in gels, the gels must first be isolated from other particles of the same size class (e.g., bacteria and detritus), then isolated for carbon analysis. Attempts to measure carbon content in microgels have proven to be semi-quantitative as the fluorescence-labeling assay developed by Ding et al. (2007) only measures the contribution of microgels that disperse with EDTA (i.e. Ca$^{2+}$ linked microgels, but not hydrophobically linked microgels) as indicated by the fluorescence of bound Ca$^{2+}$. The standard method for determining TEP concentrations is also semi-quantitative, as measurements are calibrated by the Alcian Blue absorption of gum xanthan standards for calibration (Passow and Alldredge, 1995). Therefore, in order to estimate the concentration of TEP in carbon units (TEP-C), one must use a conversion
factor, which widely varies based on the dominant phytoplankton species (Engel et al., 2004; Engel and Passow, 2001).

Because of the semi-quantitative nature of the methodologies, TEP and microgels are constrained by operational definitions that limit their ability to be compared. Both are regarded as pathways to export carbon residue in the water column by bridging the size continuum between DOC and POC, but whether microgels can further anneal and aggregate into TEP or if these are even exclusive groups of particles are still open questions (Verdugo, 2012). Comprehensive analyses of the formation dynamics and carbon content of both TEP and microgels are necessary to better understand TOC dynamics in the marine carbon cycle, which influences global climate.

It would be ideal to quantify the gel/DOC continuum by measuring the removal of DOC upon gel formation, except that ~10% assembly from 35 µM (DOC concentration in Pacific Deep Water) is ~3.5 µM, which is closely within reasonable instrumental error. Even though the analytical precision of TOC measurement has been greatly improved since the mid-1990s (Sharp et al. 2002), the community is still unable to answer many of the outstanding questions that require a finer analytical resolution in measuring DOC concentration. Today’s standard method for determination of TOC in seawater employs high temperature combustion (HTC) techniques. They typically use Shimadzu brand instrumentation, which involves combustion of TOC to CO₂ upon sample injection and detection of CO₂ by a non-dispersive infrared (NDIR) detector (Dickson et al. 2007). At best, the Shimadzu TOC analyzer has 1-2 µM C precision (Hansell 2005). It is good enough for a routine understanding of DOC dynamics in the ocean, but insufficient for
more specific studies involving DOC uptake and respiration, conservative mixing of water masses, photochemical oxidation, and photosynthesis.

1.4 Objectives and approach

This dissertation combines laboratory and field measurements to advance our understanding of processes that control the concentration and distribution of organic carbon in the ocean. The objectives are to address the following questions: (1) What is the distribution of gel particles (i.e. microgels and transparent exopolymer particles) in the ocean, and what do the spatial gradients tell us about TOC dynamics? (2) To what extent does gel formation play a role as a particulate sink of DOC? (3) Can the analytical precision of DOC measurement be improved and thus better address questions requiring higher resolution? By providing new observations of TEP and TOC dynamics in the ocean and offering insights for improving the measurements of microgels and DOC, this work will also help address analytical uncertainties in making practical and useful measurements.

In Chapter 2, TEP distribution in the coastal and open ocean is evaluated by identifying potential sources and sinks of TEP in the upper 200 m ocean along biogeochemical gradients (Jennings et al., 2017). The study area encompassed four hydrographically distinct regions in the western North Atlantic Ocean along chlorophyll, temperature, and salinity gradients. The area included a phytoplankton bloom region (located between the Scotian Shelf and the Gulf Stream), an oligotrophic region near Bermuda in the Sargasso Sea, an intermediate region in the Gulf Stream, and a station off the coast of Cape Cod. Through the distribution of TEP and TOC in the water column,
surface water, and sea surface microlayer across coastal, productive, and oligotrophic conditions, the extent of TEP as a particulate sink of TOC was evaluated as a function of spatial and biogeochemical gradients. Although TEP measurements are still a semi-quantitative assessment of acidic carbohydrates, the contribution of TEP to the regional carbon pool was estimated by employing TEP-carbon conversion factors. This study compares enrichments of TEP and TOC in the microlayer to POC, TOC, and TEP concentrations in surface waters across the regions of varying productivity. These results provide new evidence to test the hypothesis that the abundance of particles in the environment affects the sinking velocity, residence time, and fate of TEP and POC in the water column (Mari et al., 2017).

Chapter 3 aims to address the extent to which microgel formation acts as a particulate sink of DOC through experiments in natural seawater and culture. This will additionally serve to validate the fluorescence-quenching method established by Ding et al. (2007) for estimating the carbon content of microgels. Present methods that analyze the abundance, size, and carbon content of microgels were also evaluated. Analyses were performed in various samples (Milli-Q water, artificial seawater (ASW), natural seawater, and phytoplankton cultures) as a function of pH and organic carbon concentration. However, some of the preliminary results were found to contradict Ding et al. (2007), prompting the calculation of “negative” carbon concentrations. This contradiction is based on an apparent increase or decrease in fluorescence of the molecule used to indicate bound Ca$^{2+}$ in assembled microgels compared to the fluorescence when gels were disassembled. The need to elucidate the disagreement of CTC fluorescence shifts upon physical dispersal of assembled gels prompts an investigation of the experimental equilibrium variables.
To better address questions that require greater resolution of DOC concentration, Chapter 4 attempts to improve the analytical precision and accuracy of DOC measurements through optimization and application of a prototype TOC analyzer. First developed in the Mopper Lab at Old Dominion University, this work began by testing and optimizing this high resolution TOC prototype instrument and replicating it with a second instrument in the Hansell Lab at the University of Miami. The effects of various instrumental parameters on figures of merit, signal stability, and response times were evaluated and results were compared with those obtained from traditional HTC measurements to validate the accuracy of the prototype.

Similar to HTC, the prototype also quantifies TOC by measuring its oxidation product through CO\textsubscript{2} detection using a NDIR gas analyzer detector. Briefly, acidifying and sparging the sample with O\textsubscript{2} or N\textsubscript{2} gas prior to analysis removes the pre-existing inorganic carbon. TOC is converted to CO\textsubscript{2} through several oxidative radical reactions promoted by UV radiation, controlled and continuous addition of acidified persulfate reagent, and heat. The oxidized sample solution is continuously pumped through the system at a constant flow rate using high precision pumps. CO\textsubscript{2} is removed using a mass-flow controlled (MFC) gas extraction cell (or sparging chamber) and sent to the detector, modeling a previous high accuracy and precision method for analyzing DIC in seawater by Kaltin, Haraldsson, & Anderson (2005). By recycling persulfate reagent alone through the system until essentially zero CO\textsubscript{2} is detected, a near-zero carbon blank/baseline for the analysis may be achieved, which should enhance sensitivity and lower the detection limit.
A seawater application is contingent on a successful optimization of the chloride interference anticipated during persulfate oxidation of organic matter (Aiken 1992; Bauer et al. 1991; Osburn and St-Jean 2007). However, in a freshwater system, the prototype instrument could measure the in-situ rates of DOC production/removal in cultures or in-situ rates of photodegradation or flocculation of DOC. Overall, this dissertation aims to assess the carbon content of gel particles, advance understanding of their role in the carbon cycle as particulate controls of DOC concentration and potentially significant contributors to overall TOC dynamics, and improve the analytical methodology to accurately measure microgels and OC.
CHAPTER 2. Distribution of transparent exopolymer particles (TEP) across an organic carbon gradient in the western North Atlantic Ocean

2.1 Overview

In this study, the abundance of transparent exopolymer particles (TEP) was examined across a surface water gradient of organic carbon from a phytoplankton bloom region in the western North Atlantic to oligotrophic waters in the Sargasso Sea, including a coastal region sampled near Cape Cod. TEP are macrogels that reach up to millimeters in size and abiotically assemble from dissolved acidic polysaccharides secreted by phytoplankton and bacteria. Due to their great stickiness, TEP self-aggregate and also form aggregates with non-TEP particulate organic carbon (POC). Aggregation enhances ballasting, thereby mutually facilitating export and subsequent sedimentation of both TEP and POC, increasing the efficiency of the biological pump. Here, four distinct regions with varying chlorophyll $a$ concentrations, temperature, and salinity were sampled in the upper column, at the surface, and from the sea surface microlayer (SML). While TEP in seawater shows no correlation to chlorophyll $a$, nutrients, or total organic carbon (TOC) concentration, a strong correlation exists between TEP and TOC in the SML; and concentrations of both variables are inversely proportional to surface productivity as indicated by chlorophyll $a$ concentrations. As open ocean regions show greater enrichments of TEP and TOC in the SML compared with the coastal region, the role of the SML in organic carbon cycling is dependent on regional biogeochemistry and productivity. The lower abundance of particles in oligotrophic regions compared with bloom regions is hypothesized to limit TEP export by sinking, thus increasing the residence time of TEP in the upper water column and the SML.
2.2 Background

Transparent exopolymer particles (TEP) are surface active macrogels that play a role in the marine carbon cycle by spanning the size continuum between dissolved and particulate organic carbon (DOC and POC, respectively), in addition to supporting particle aggregation (Alldredge et al., 1993; Passow, 2002a; Verdugo et al., 2004). TEP are operationally defined as transparent particles stainable with Alcian Blue, a dye that preferentially binds to acidic polysaccharides by complexing with half-ester sulfate and carboxyl groups (Alldredge et al., 1993; Passow and Alldredge, 1995). TEP abiotically assemble from dissolved polysaccharides produced by phytoplankton and bacteria (Logan et al., 1995; Passow, 2002a; Thuy et al., 2015). Rising bubbles also promote the formation of TEP, which adhere to the rising bubbles due to their high surface-activity and neutral buoyancy. TEP further aggregate and concentrate at the air-sea interface where these rising bubbles ultimately collapse. Thus they contribute appreciably to the organics in the sea surface microlayer (SML) (Azetsu-Scott and Passow, 2004; Cunliffe et al., 2013; Mopper et al., 1995; Wurl et al., 2009).

TEP formation and distribution are controlled by a combination of regional physical and biological factors, including wind-induced turbulence, salinity, and production of dissolved polysaccharide precursors by phytoplankton and bacteria. Nutrient levels control phytoplankton composition, which is one of the underlying factors that mediate the partitioning of organic matter between dissolved and particulate phases (Carlson et al., 1998; Conan et al., 2007; Lomas and Bates, 2004; Thornton, 2014), and production of TEP and organic matter (Claquin et al., 2008; Corzo et al., 2000; Mari et al., 2005; Passow, 2002b). TEP-chlorophyll a (Chl-a) relationships exist during the development of
a bloom, reflecting the production of TEP by phytoplankton (Passow, 2002a). However, the relationship between Chl-\textit{a} and TEP during bloom development is species-specific, with the cellular production rate of TEP by phytoplankton affected by their growth phase. Therefore, no overall TEP – Chl-\textit{a} relationship would be expected along a spatial gradient where different phytoplankton taxa exist at different life stages. Nutrient limitation often increases TEP production, but also may impede bacterial consumption of TEP (Bar-Zeev and Rahav, 2015), allowing TEP to accumulate and aggregate as C-rich N-poor organic material in surface waters (Passow, 2002a).

Elevated concentrations and formation rates of gel particles have been observed in the SML (Orellana et al., 2011; Wurl et al., 2011a), indicating the importance of gels for carbon cycling at the air-sea interface. Low density, surface active material (such as TEP) is transferred to the SML after absorbing to rising bubbles created by breaking waves (Wurl et al., 2011b; Wurl and Holmes, 2008). Although these breaking waves temporarily disperse its components into underlying waters, the SML has a rapid reformation rate (Cunliffe et al., 2013). Through bubble bursting, biogenic material concentrated in the SML may also be introduced to the atmosphere as components of sea spray aerosols (SSA) capable of cloud condensation and ice nucleation (Bigg and Leck, 2008; DeMott et al., 2015; Orellana et al., 2011; Quinn et al., 2014; Wang et al., 2015; Wilson et al., 2015). Since TEP adhere to rising bubbles (Mopper et al., 1995) and are enriched in the SML, TEP could contribute to the organic enrichment of sea spray aerosols derived from film droplets (Aller et al., 2005).

TEP are often 1-2 orders of magnitude stickier than non-TEP particles, acting as a glue to promote aggregate formation of particles and subsequently enhance sedimentation
(Logan et al., 1995; Passow et al., 1994). If TEP are ballasted by particles with adequate density (such as large phytoplankton or mineral dust aggregates), TEP and the associated particles will be stripped from surface waters (Azetsu-Scott and Passow, 2004). As TEP and POC sink in the water column, they provide a source of carbon to the deep ocean as microbial hotspots. Although sinking mechanisms of marine aggregates have been investigated (Burd and Jackson, 2009; Iversen and Robert, 2015; Jokulsdottir and Archer, 2016; Prairie et al., 2015), there remains the need to understand the role of TEP in the biological carbon pump (Burd et al., 2016; Zetsche and Ploug, 2015).

More comprehensive analyses of TEP distributions that include SML measurements are required to better describe the role of TEP in the carbon cycle as a particulate sink for DOC and dynamic component of total organic carbon (TOC). Here, we assess TEP and TOC concentrations within the upper water column, the surface layer, and SML across biogeochemical gradients to evaluate the potential sources and impacts of TEP accumulation in the coastal and open ocean.

2.3 Methods

2.3.1 Study areas

Samples were collected during the Western Atlantic Climate Study II (WACS2) field campaign conducted May 20 to June 5, 2014 onboard the RV Knorr (Cruise KN219). The cruise stations encompassed four hydrographically distinct areas along Chl-a, sea surface temperature (SST) and sea surface salinity (SSS) gradients. These areas, designated as A) Slope Water, B) Gulf Stream, C) Sargasso Sea, and D) Coastal regions, ranged from a phytoplankton bloom located between the Scotian Shelf and the Gulf
Stream (Slope Water; 42.5°N, 61.5°W) to an oligotrophic region near Bermuda (Sargasso Sea; 33.5°N, 63°W), and including an intermediate region (Gulf Stream; 40°N, 62°W) and a station off the coast of Cape Cod (Coastal; 40.5°N, 70.5°W) (Fig. 2.1). The entire study area encompassed a spectrum of potential end-members from lower salinity coastal water to higher salinity open ocean seawater, from subpolar to subtropical temperature ranges, and eutrophic to oligotrophic status, which influence the biogeochemistry of carbon and nutrients. Further hydrographic complexities are present in the intermediate region as it captures the meandering of the Gulf Stream.

2.3.2 Sampling

Seawater samples were collected from the upper 200 m of the water column, from the surface layer, and from the SML. Water column samples were collected on 9 casts (2 in region A, 4 in region B, 2 in region C, and 1 in region D; Fig. 2.1) using clean 12 L Niskin bottles attached to a conductivity temperature depth (CTD) rosette (Sea-bird SBE-911plus). Water column sample collection was evenly distributed from the surface to 200 m for TOC and nutrient analyses (~ 5-10 depths per cast) and TEP analyses (~ 4-8 depths per cast). Surface water samples (n=12) were collected by lowering a clean 5L polypropylene bucket to approximately 1 m depth, recovering onto the deck, and immediately transferring to a 9-L polycarbonate carboy for subsequent sampling for TOC, TEP, and POC. SML samples (n=3) were obtained using the ‘Interface II’, a battery operated and remote controlled catamaran sampler containing a rotating drum coated in a thin layer of hydrophilic Teflon film (Knulst et al., 2003) that collected SML material for TOC and TEP analysis into an amber glass bottle. The actual thickness of the SML was not determined because modifications to the scraping assembly of the sampler
prevented accurate measurements of the volume of SML collected (not all of it is scraped into the bottles). The SML of the oligotrophic area was not sampled due to prohibitive wind and sea conditions. Surface water and SML samples were collected near the ship, as the sampling devices were required to be tethered. Samples for nutrient (n=36) and Chl-a (n=138) analyses were continuously collected from the ship’s underway sample line (intake at ~ 5m depth) such that collections were evenly distributed throughout the cruise track.

2.3.3 Sample Analysis

Temperature, salinity, and wind speed — Underway temperature and salinity were measured using the ship’s thermosalinograph (Sea-Bird SBE-45). The conductivity and temperature sensors packaged on the CTD rosette were used for upper water column samples (Sea-bird SBE-911plus). Mixed layer depth was estimated as the depth in the water column where the vertical change in potential density was >0.01 kg m$^{-3}$. Potential density was calculated from the temperature and salinity of continuous CTD profiles averaged to 1-m bins. Wind speed was measured from the ship’s meteorological instrumentation (Vaisala WXT520) and averaged to 1-minute intervals.

Chlorophyll a — Chl-a concentrations (Fig. 2.1) were taken from NASA MODIS AQUA 9-km Products 8 day composite (17 May – 24 May 2014) and visualized with Ocean Data View software (Schlitzer, 2015). Discrete fluorometric Chl-a samples were collected from the ship’s underway seawater line into well-rinse d 500 ml amber Nalgene bottles and gently vacuum filtered (<5 mm Hg) onto 25 mm GF/F filters (Whatman; 0.7 µm nominal pore size). Samples were extracted into 7 ml 100% methanol for 24 h in the
dark at -20°C and read on a Turner Designs 10AU fluorometer calibrated with a commercial Chlorophyll-a standard (Sigma) and checked against a solid standard daily.

**Fig. 2.1.** Hydrographic stations of WACS2 cruise, sampled during May and June 2014 in the western North Atlantic Ocean, Sargasso Sea, and off the coast of New England. Chlorophyll a concentrations (background color; mg m⁻³) during 17 May – 24 May 2014 were taken from NASA MODIS AQUA 9-km Products 8 day composite. Boxes A – C represent open ocean stations distinguished as Slope Water (A), Gulf Stream (B), and Sargasso Sea (C); Box D represents a shallow, coastal station on the southern New England shelf (referred to as Coastal). The symbols represent types of samples collected for TOC and TEP concentration, i.e. surface samples (grey circles), water column CTD samples (yellow squares, labeled by cast number), and surface microlayer samples (red triangles). Smaller brown circles represent locations where underway nutrients were sampled along the cruise track. The curved arrow details the approximate location of the Gulf Stream where a temperature difference of nearly 10°C was observed. Dotted and dashed lines represent the bathymetry of the continental shelf.
**Nutrients** – Samples were collected directly into HDPE bottles and stored frozen (for phosphate and combined nitrate and nitrite samples) or at 4°C (for silicic acid samples) until analysis post-cruise (no later than 3 months). Concentrations of combined nitrate and nitrite (NO$_3^−$+NO$_2^−$) were first reduced to NO using a heated, acidic vanadium (III) solution and determined in duplicate by chemiluminescent detection of NO (Braman and Hendrix, 1989) using KNO$_3$ as a standard. Analyses of phosphate (PO$_4^{3−}$) and silicic acid (SiO$_4^{4−}$) followed standard methods established by Grasshoff (1976) and Strickland and Parsons (1972), respectively. Both phosphate and silicic acid were determined colorimetrically following using a spectrophotometer (Shimadzu UV-1800) with KPO$_4$ and silicic acid certified material (www.osil.co.uk) as standards.

**Total organic carbon** – Water column, surface, and SML samples were collected unfiltered into acid washed 60 mL polycarbonate bottles and stored frozen until analysis post-cruise at the University of Miami. TOC was measured in triplicate injections by high temperature combustion using a Shimadzu TOC-L auto analyzer (CV = 1.5 to 2.5%) following methods from Dickson et al. (2007) and quality controlled using consensus reference materials (Hansell, 2005).

**Particulate organic carbon** – Surface seawater was subsampled (500 mL) directly in-line from a 9L polycarbonate carboy and filtered through pre-combusted (450°C) glass fiber filters (Whatman; 0.7 μm nominal pore size) using a polycarbonate filter holder (Pall; 47 mm diameter); the material remaining on the filter was retained for measurement of POC. Inorganic carbonates were removed from the samples by acidification with 1M HCl and the samples dried in a drying oven (60°C, 24 h) prior to analysis. Portions of the acidified filters were placed in 5x9 mm tin cups and combusted
at 900°C in the presence of O₂ and measured on a CE Elantech Flash EA 1112 elemental analyzer in duplicate analyses. Sample response areas were calibrated to a standard curve generated with an aspartic acid standard.

*Transparent exopolymer particles* – TEP was collected from the upper water column by subsampling directly from the Niskin bottle into 1 L HDPE bottles, from the surface water by subsampling directly from a 9 L polycarbonate carboy (following POC collection), and from the SML by subsampling from a secondary polycarbonate container into 60 mL polycarbonate bottles. Samples were either filtered immediately after collection or stored at 4°C prior to filtration (no later than 24 hours after collection). Samples were filtered in triplicate using 0.4 µm (and also 0.2 µm for SML samples, surface samples, and casts 1, 2, 9, 13, 16) pore size 25 mm polycarbonate filters (Whatman Nuclepore), stained with 0.5 mL Alcian Blue solution (pH= 2; 0.16% w/v Alcian Blue 8GX, Sigma) calibrated with Gum Xanthan (Sigma) by the Passow laboratory at the University of California Santa Barbara, and frozen onboard (Passow and Alldredge, 1995). The volume of water necessary for each TEP analysis varied from 10 mL collected from the SML to 1000 mL collected from the maximum depth sampled in the water column (e.g., 200 m), depending on the general concentration of particles in the sample. Ideally, enough seawater was filtered from each depth to measure TEP concentrations well above the detection limit, while avoiding seawater volumes that would clog the filter with extraneous non-TEP particles. Post-cruise, the Alcian Blue stain was extracted from sample filters in 80% H₂SO₄ and analyzed for UV absorption at 787 nm using a spectrophotometer (Shimadzu UV-1800). As the dye binds to acidic groups, this method is semi-quantitative when the chemical composition of TEP is
unknown. The concentration of TEP was determined in units of Gum Xanthan equivalents per volume sampled, or µg Xeq. L⁻¹.

**Fig. 2.2.** Surface water measurements of a) sea surface temperature (SST; °C), b) sea surface salinity (SSS), c) silicic acid (µmol SiO₄²⁻ L⁻¹), d) phosphate (µmol PO₄³⁻ L⁻¹), e) POC (µmol L⁻¹), f) fluorometric chlorophyll a (µg Chl-a L⁻¹), g) TOC (µmol L⁻¹), and h) TEP-C (µmol L⁻¹) larger than 0.4 µm collected from ~1m depth. Measurements are shown in color. Symbols represent samples collected from underway/surface bucket sampling (circles) and from surface CTD niskin bottles (squares). Background color of a) is satellite SST during 17 May – 24 May 2014 taken from NASA MODIS AQUA 9-km Products 8 day composite, while the background of b) – h) is bathymetry from the standard map resource in Ocean Data View software.
For the majority of our analyses, only the concentration of TEP collected using the 0.4 µm filters is reported in order to provide comparison to most literature values. When both 0.2 µm and 0.4 µm pore sizes were used (SML samples, surface samples, and samples from casts 1, 2, 9, 13, 16), the 0.2 µm filters retained on average 37% more TEP than the 0.4 µm filters (SD=16.7%), similar to the 42% higher retention differential reported by Sun et al. (2012). By sampling with both filter sizes, we assessed the size spectrum exhibited by TEP. However, there is likely a significant concentration of bacteria retained on 0.2 µm filters, which may vary by region, thus potentially interfering with this assessment.

2.3.4 Derived values

*TEP carbon unit conversion* – To simplify comparisons of TEP to TOC and POC, the concentration of TEP (collected on both 0.2 and 0.4 µm filters) was converted from units of µg Xeq. L⁻¹ to units of TEP carbon (µmol TEP-C L⁻¹). TEP-C conversion factors depend on the chemical composition of TEP and range from 0.51 to 0.88 µg TEP-C L⁻¹ per µg Xeq. L⁻¹ as determined experimentally from phytoplankton cultures (Engel, 2004; Engel and Passow, 2001). It is therefore important to consider our TEP-C estimates as semi-quantitative yet indicative of gradients (Filella, 2014). This study used a conversion factor value of 0.5 µg TEP-C L⁻¹ per µg Xeq. L⁻¹. It is expected that lower values are more appropriate under non-culture conditions and the colorimetric method for determining TEP concentration has been shown to overestimate carbon (Passow, 2002a). The following equation summarizes the unit conversion to TEP carbon (µmol TEP-C L⁻¹) from µg Xeq. L⁻¹:
\[ \mu \text{mol TEP-C L}^{-1} = \mu \text{g Xeq. L}^{-1} \times \frac{0.5 \mu \text{g TEP-C L}^{-1}}{\mu \text{g Xeq. L}^{-1}} \times \frac{\mu \text{mol TEP-C}}{12.0 \mu \text{g TEP-C}} \quad \text{Equation 2.1} \]

*Enrichment Factors* – Enrichment factors (EF) were calculated by dividing the TEP or TOC concentration in the SML by the concentration found in the underlying surface seawater (SSW), e.g.

\[ \text{EF} = \frac{[\text{TEP}]_{\text{SML}}}{[\text{TEP}]_{\text{SSW}}}. \quad \text{Equation 2.2} \]

### 2.4. Results

#### 2.4.1 Regional hydrography

Sea surface temperature (SST) and salinity (SSS) measurements from the ship’s underway seawater line help define warm Gulf Stream water and colder waters to the north marked by a sharp change in temperature (Fig. 2.2a,b). North of the Gulf Stream, the Slope Water region (Fig. 2.1, region A) had the coldest SST (~10.6 °C) and freshest SSS (~33.6) of the open ocean regions sampled along the cruise track. The cruise track captured meandering of the Gulf Stream (region B), where SST ranged from ~19.4 to ~22 °C and SSS ranged from ~35.9 to ~36.5. South of the Gulf Stream, the Sargasso Sea (region C) is identifiable by warm SST (~20.2 to ~23.4 °C) and high SSS (~36.4 to ~36.6). West of the Gulf Stream, the Coastal station (region D) was distinguished by relatively fresh (~33 to ~34.7) and cold (10 to 17°C) surface water.

A T-S diagram of the upper 200 m of the water column supports our classifications of the four regions: Slope Water, Gulf Stream, Sargasso Sea, and Coastal (Fig. 2.3). Sargasso Sea waters (casts 13 and 15, blue symbols) had a narrow range and elevated salinity and temperature (36.5 to 36.6; 18.7 to 21.5 °C). Gulf Stream waters (casts 1, 2,
and 10; yellow symbols) had high salinity (36.1 to 36.7) and temperature (15.8 to 21.9 °C), similar to the subtropical Sargasso Sea. Slope Water (casts 5 and 9, green symbols) was colder and fresher than subtropical waters, with a wide range of salinity (33.6 to 35.5) and a narrow range in temperature (9.8 to 13.5 °C). Cast 12 (purple triangles) is apparently a mixture between Slope Water and subtropical waters, demonstrating intermediate temperature and salinity ranges (13.9 to 21.7 °C; 35.6 to 36.4). Coastal water (cast 16, red symbols) was the freshest and coldest water sampled, with a relatively narrow range of both temperature and salinity (8.5 to 13.2 °C; 33.0 to 33.3).

Mixed layer depths (MLD) are given in Table 2.1. Slope Water had a shallower MLD (~16 to 18m) than the Gulf Stream and Sargasso Sea regions (~26 to 49 m). The Coastal MLD was shallowest at ~12 m (~60 m bottom depth).

Table 2.1. Sampling dates, locations and mixed layer depths (MLD) for water column samples (200 m vertical CTD casts).

<table>
<thead>
<tr>
<th>CTD cast no.</th>
<th>Region</th>
<th>Date</th>
<th>Lat. (°N)</th>
<th>Long. (°W)</th>
<th>MLD (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td><em>Slope Water</em>, A</td>
<td>2014-05-25</td>
<td>42.4850</td>
<td>61.552</td>
<td>16.4</td>
</tr>
<tr>
<td>9</td>
<td><em>Slope Water</em>, A</td>
<td>2014-05-27</td>
<td>42.1860</td>
<td>61.528</td>
<td>9.97</td>
</tr>
<tr>
<td>1&amp;2</td>
<td><em>Gulf Stream</em>, B</td>
<td>2014-05-22</td>
<td>40.4180</td>
<td>63.211</td>
<td>28.7</td>
</tr>
<tr>
<td>10</td>
<td><em>Gulf Stream</em>, B</td>
<td>2014-05-28</td>
<td>40.1620</td>
<td>60.970</td>
<td>36.1</td>
</tr>
<tr>
<td>12</td>
<td><em>Gulf Stream</em>, B</td>
<td>2014-05-30</td>
<td>39.6460</td>
<td>60.846</td>
<td>35.3</td>
</tr>
<tr>
<td>13</td>
<td><em>Sargasso Sea</em>, C</td>
<td>2014-06-01</td>
<td>33.2680</td>
<td>62.906</td>
<td>29.5</td>
</tr>
<tr>
<td>15</td>
<td><em>Sargasso Sea</em>, C</td>
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</tbody>
</table>

### 2.4.2 Biogeochemical parameters

*Nutrients* – Surface (5m) distributions of silicic acid and phosphate are shown in Fig. 2.2c,d; combined nitrite and nitrate is not shown because it was very low (< 0.9 µmol NO₃⁻ + NO₂⁻ L⁻¹) or undetectable (< 0.03 µmol NO₃⁻ + NO₂⁻ L⁻¹) in the open ocean. The Slope Water region contained elevated silicic acid and phosphate (up to 1.4 µmol SiO₄⁴⁻ L⁻¹ and 0.2 µmol PO₄³⁻ L⁻¹), with extremely low values of combined nitrite and nitrate (<
0.05 μmol NO$_3^-$ + NO$_2^-$ L$^{-1}$). Silicic acid and phosphate were detectable in both Gulf Stream and Sargasso Sea waters (0.45 to 2 μmol SiO$_4^{4-}$ L$^{-1}$ and 0.01 to 0.11μmol PO$_4^{3-}$ L$^{-1}$). All three nutrients were highest in the Coastal region, where silicic acid ranged from 0.7 to 4.1 μmol SiO$_4^{4-}$ L$^{-1}$, phosphate ranged from 0.08 to 0.37 μmol PO$_4^{3-}$ L$^{-1}$, and nitrate was as high as 0.9 μmol NO$_3^-$ + NO$_2^-$ L$^{-1}$.

**Fig. 2.3.** Temperature-Salinity (T-S) plot of the upper 200m of water column (in continuous 1m bins). In the background, isopycnals are depicted as contours of constant potential density ($\sigma_\theta$). Casts from each region are grouped by color: Slope Water (A, green symbols), Gulf Stream (B, yellow), Sargasso Sea (C, blue), an apparent mixture between Slope Water and subtropical waters (cast 12; purple), and Coastal (D, red).
Water column nutrient profiles for each cast (Fig. 2.4a) reflect surface distributions for silicic acid and phosphate (Fig. 2.2 c-d). Nutrient concentrations in the upper water column are lower in the Gulf Stream (yellow symbols) and Sargasso Sea casts (blue symbols) (0.02 to 0.18 µmol PO$_4$$^{3-}$ L$^{-1}$, 0.3 to 5.4 µmol SiO$_4$$^{4-}$ L$^{-1}$, and 0 to 9.2 µmol NO$_3^{-}$ + NO$_2^{-}$ L$^{-1}$) relative to the more nutrient-rich Slope Water (green symbols; 0.2 to 1.1 µmol PO$_4$$^{3-}$ L$^{-1}$, 0 to 8.3 µmol SiO$_4$$^{4-}$ L$^{-1}$, and 0 to 16.8 µmol NO$_3^{-}$ + NO$_2^{-}$ L$^{-1}$) and the Coastal (red symbols; 0.26 to 0.65 µmol PO$_4$$^{3-}$ L$^{-1}$, 1.2 to 8.8 µmol SiO$_4$$^{4-}$ L$^{-1}$, and 0 to 1.1 µmol NO$_3^{-}$ + NO$_2^{-}$ L$^{-1}$) casts. Nutrient concentrations of the Slope Water-subtropical mixture (cast 12; purple symbols; 0.01 to 0.6 µmol PO$_4$$^{3-}$ L$^{-1}$, 0.7 to 3.3 µmol SiO$_4$$^{4-}$ L$^{-1}$, and 0 to 8.7 µmol NO$_3^{-}$ + NO$_2^{-}$ L$^{-1}$) fall within the ranges exhibited by Sargasso Sea and Gulf Stream casts. Nutrient concentrations increased with potential density (Fig. 2.4b), and Gulf Stream casts generally had the highest nutrient concentrations on a given isopycnal. Sargasso Sea water and the Slope Water-subtropical mixture (cast 12) showed great similarity.

*Chl-a* – Satellite imaging (Fig. 2.1) confirmed by discrete fluorometric analyses (Fig. 2.2f) reveals a regional gradient of surface Chl-*a* spanning three orders of magnitude, with concentrations ranging as high as 4 µg Chl-*a* L$^{-1}$ within the bloom in the Slope Water region to as low as 0.04 µg Chl-*a* L$^{-1}$ in the Sargasso Sea. Intermediate Chl-*a* concentrations were detected in the Gulf Stream region (~0.5 µg Chl-*a* L$^{-1}$) and the Coastal region (~0.7 µg Chl-*a* L$^{-1}$).
**Fig. 2.4.** Profiles of silicic acid (µmol SiO$_4^{4-}$ L$^{-1}$), phosphate (µmol PO$_4^{3-}$ L$^{-1}$), combined nitrate and nitrite (µmol NO$_3^{-} +$ NO$_2^{-}$ L$^{-1}$) and TOC (µmol L$^{-1}$) concentration in upper 200 m shown with a) depth and b) increasing potential density (σ$_0$). Symbols follow the same legend presented in Figure 2.3 and are grouped by color: Slope Water (green symbols), Gulf Stream (yellow), Slope Water-Subtropical mixture (cast 12; purple), Sargasso Sea (blue), and Coastal (red). Combined nitrate and nitrite concentrations in the upper 50 m were below the detection limit in the Sargasso Sea and Gulf Stream regions.

TOC — Surface (upper 1 m) TOC concentrations are shown in Fig. 2.2 and Table 2.2. A TOC gradient was observed with latitude, with the highest values in Slope Water (83.5 ± 6 µmol L$^{-1}$), lowest values in the Sargasso Sea (64.8 ± 0 µmol L$^{-1}$), and intermediate values in the Gulf Stream and Coastal regions (72.0 ± 1 µmol L$^{-1}$; 79.5 µmol L$^{-1}$, respectively).

TOC decreased with depth and density (Fig. 2.4). Spatial gradients were observed in samples from the upper 25 m (from 86 µmol L$^{-1}$ in Slope Water to 65 µmol L$^{-1}$ in Sargasso Sea waters), while TOC approached a unified value of 55 µmol L$^{-1}$ deeper in the water column (~200m) in the open ocean casts. At constant σ$_0$, Gulf Stream waters had the lowest TOC, with Slope Water having the highest TOC.
TOC was elevated in the SML from each region, with values up to 4x greater than those observed in underlying waters (Fig. 2.5, Table 2.2). TOC concentrations in the SML of the Gulf Stream were highest (303 µmol L\(^{-1}\)), compared to the SML of Slope Water (208 µmol L\(^{-1}\)) or Coastal (94 µmol L\(^{-1}\)) regions.

**POC** — Surface (upper 1 m) POC is shown in Fig. 2.2. Similar to TOC, a gradient of POC is observed with latitude. Highest values were in Slope Water and Coastal regions (average 18.2 ± 5.5 µmol L\(^{-1}\); 24.4 µmol L\(^{-1}\) respectively), lowest values in the Sargasso Sea (8.25 µmol L\(^{-1}\)), and intermediate values in the Gulf Stream (9.3 ± 1 µmol L\(^{-1}\)).

Fig. 2.5. Concentrations of TOC (µmol L\(^{-1}\)) (solid bars) and TEP-C >0.4 µm (µmol L\(^{-1}\)) (vertical striped bars) and >0.2 µm (µmol L\(^{-1}\)) (diagonal striped bars) in the SML. Error bars represent the standard deviation of triplicate measurements.

### 2.4.3 TEP-C

The regional average of surface TEP-C concentrations (0.4 µm pore size) from individual stations were lowest in the Gulf Stream region (10 ± 3 µmol L\(^{-1}\)), nearly equivalent in Slope Water and Sargasso Sea regions (18 ± 3 µmol L\(^{-1}\) and 17.5 ± 4 µmol L\(^{-1}\), respectively), and highest in the Coastal region (33 ± 5 µmol L\(^{-1}\)) (Fig. 2.2f, Table 2.2). Concentrations were highest at the surface and decreased with depth (Fig 2.6). With
respect to depth, the Coastal region had higher TEP-C than any of the open ocean areas (Slope Water, Gulf Stream, Sargasso Sea) sampled. In the upper 25 m of the water column, the TEP concentrations agreed within repetitive casts per region in both Slope Water (9 ± 1 µmol L⁻¹ in cast 5; 9 ± 1 µmol L⁻¹ in cast 9) and Gulf Stream (9 ± 2 µmol L⁻¹ in cast 1 and 9 ± 1 µmol L⁻¹ in cast 10) regions, while the Sargasso Sea had more variability between casts (10 ± 4 µmol L⁻¹ in cast 13; 7 ± 0 µmol L⁻¹ in cast 15). Below the mixed layer (~10 m to 50 m) in the open ocean, TEP concentrations were generally lower in the Slope Water region, higher in the subtropical Gulf Stream and Sargasso Sea, and highest in cast 12 (Slope Water-Subtropical mixture). The inset in Fig. 2.6 shows the distribution of TEP with σθ to demonstrate that the Coastal and Slope Waters have greater TEP concentrations than Sargasso Sea and Gulf Stream waters at the same σθ.

Overall, TEP was 1-2 orders of magnitude greater in the SML than in the underlying upper water column (Table 2.2). TEP concentrations in the SML (Fig. 2.5, Table 2.2) were highest in the Gulf Stream region (405 ± 20 µmol L⁻¹ for 0.4 µm pore size and 527 ± 42 µmol L⁻¹ for 0.2 µm pore size) and lowest in the Coastal region (94 ± 9 µmol L⁻¹ for 0.4 µm and 223 ± 26 µmol L⁻¹ for 0.2 µm). Falling between the two extremes, TEP in the Slope Water region SML were 241 ± 17 µmol L⁻¹ for 0.4 µm and 405 ± 94 µg µmol L⁻¹ for 0.2 µm.

Because TEP was collected on both 0.2 and 0.4 µm pore size filters from the SML, the percentage of total TEP (larger than 0.2 µm) sized between 0.2 and 0.4 µm (i.e. smaller size fraction), or >0.4 µm (i.e. a larger size fraction) was estimated. The majority of TEP in the Gulf Stream SML (~80%) and the Slope Water SML (~60%) fell within the larger size fraction (> 0.4 µm). Conversely, the majority of TEP in the Coastal SML
(~60%) fell within the smaller size fraction (between 0.2 and 0.4 µm). Bacterial counts in the same SML samples used in this study were reported by Aller et al. (2017) and can help estimate how bacterial biomass might contribute to these differences. They showed similar bacterial abundance in the Gulf Stream and Slope Water regions (17 – 17.5 x10^5 cells mL⁻¹), with significantly lower abundance observed in the Coastal region (3 x10^5 cells mL⁻¹). Therefore, based on similar bacterial abundance, these estimated size differences in TEP concentration might be appropriately compared within the open ocean SML samples. Although TEP concentrations were lowest in the Coastal SML, where the majority of TEP size fell within the smaller fraction, such a low abundance of bacteria in this regional SML would not substantially interfere with TEP retention on the filters.

2.4.4 Organic carbon partitioning and enrichments

The surface waters of the Slope Water and Coastal regions had the highest percentage of TOC as POC (24 and 23%, respectively) compared to the Gulf Stream and Sargasso Sea surface waters that each had 13% of TOC as POC (Table 2.2). The elevated contributions of POC to TOC in the Slope Water and Coastal regions coincided with higher concentrations of Chl-a (Fig. 2.2f).

The contribution of TEP-C to TOC was calculated in the SML, surface waters, and at selected depth ranges of the water column (Table 2.3). TEP-C in the Gulf Stream SML contributed a greater fraction to the organic carbon pool (134%) compared to the SML sampled in the Slope Water and Coastal regions (116% and 100%, respectively). Clearly values over 100% are impossible and emphasize the semi-quantitative character of the measurements. However, such high contributions do highlight that most of the TOC in the SML of all water masses consisted of TEP. TEP contributions in surface water, in
contrast, were low, with TEP-C to TOC of only 14% in the Gulf Stream, 26% in the Sargasso Sea, and 22% and 42% in the Slope Water and Coastal regions, respectively. Overall, the contribution of TEP-C to TOC decreased with depth in the water column, ranging from 8% at 10-50 m to <3% at 100-200m in the open ocean areas. The Coastal station had a shallow bottom depth of ~60m, with TEP-C representing 16% of the TOC at its deepest depths.

**Fig. 2.6:** Water column profiles (upper 200m) of TEP-C >0.4 µm (µmol L\(^{-1}\)) with depth and increasing density, \(\sigma_0\) (inset). Symbols follow the same legend presented in Figure 2.3 and are grouped by color: Slope Water (green symbols), Gulf Stream (yellow), Slope Water-Subtropical mixture (cast 12; purple), Sargasso Sea (blue), and Coastal (red). Error bars represent standard deviation of three replicates.
Table 2.2. Meta-data and results from sea surface water (SSW, upper 1 m) and sea surface microlayer (SML) samples (total organic carbon (TOC), particulate organic carbon (POC), and transparent exopolymeric particle concentration measured in units of Gum Xanthan equivalents per volume sampled, or µg Xeq. L$^{-1}$ and estimated in units of carbon (TEP-C)) are presented for the four different regions. Derived values for percent (%) contribution of POC or TEP-C to TOC (% POC of TOC= POC/TOC $\times$100), enrichment factors (EF= SML/SSW$_{avg}$) of TEP-C and TOC in the SML, and the ratio of POC to TEP-C (POC/TEP-C) are listed. Only TEP >0.4 µm pore size is shown.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Date</th>
<th>Lat. (N)</th>
<th>Long. (W)</th>
<th>TOC (µmol L$^{-1}$)</th>
<th>POC (µmol L$^{-1}$)</th>
<th>TEP-C (µg Xeq. L$^{-1}$)</th>
<th>% POC of TOC</th>
<th>% TEP-C of TOC</th>
<th>EF$_{TEP}$</th>
<th>EF$_{TOC}$</th>
<th>POC/TEP-C</th>
</tr>
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<td></td>
<td></td>
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<td>20</td>
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<td>15 ± 2</td>
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<td>17</td>
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<td>71.4</td>
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<td>10 ± 1</td>
<td>12</td>
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<td>SSW (3)</td>
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<td>40.4010</td>
<td>62.2099</td>
<td>71.7</td>
<td>10.6 ± 0.3</td>
<td>161 ± 8</td>
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<td>15</td>
<td>10</td>
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<td>61.3501</td>
<td>72.9</td>
<td>91 ± 1.0</td>
<td>298 ± 124</td>
<td>13 ± 5</td>
<td>12.5</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>SSW$_{avg}$</td>
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<td>72 ± 0.8</td>
<td>9.4 ± 1.0</td>
<td>279 ± 111</td>
<td>10 ± 3</td>
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<td>8.25 ± 1.8</td>
<td>460 ± 173</td>
<td>20 ± 7</td>
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<td>30</td>
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<td>0.4</td>
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<td>83 ± 13</td>
<td>20 ± 7</td>
<td>13 ± 1</td>
<td>-</td>
<td>18</td>
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<td>-</td>
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<tr>
<td>SSW$_{avg}$</td>
<td></td>
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<td>64.8 ± 0.4</td>
<td>8.25 ± 1.8</td>
<td>372 ± 125</td>
<td>17 ± 4</td>
<td>13</td>
<td>24</td>
<td>-</td>
<td>0.4</td>
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<td><strong>Coastal, D</strong></td>
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<td>94 ± 9</td>
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<tr>
<td>SSW (12)</td>
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<td>70.526</td>
<td>79.5</td>
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<td>787 ± 119</td>
<td>33 ± 5</td>
<td>23</td>
<td>42</td>
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The highest enrichments were observed in the Gulf Stream region (34.2- and 4.2-fold for TEP and TOC, respectively) followed by lower enrichments in the Slope Water region (13.4 and 2.3, respectively; Table 2.2). The Coastal region SML had the lowest enrichments of TEP and TOC (2.8 and 1.2 fold, respectively; Table 2.2).

**Table 2.3.** Contribution (%) of TEP-C to TOC throughout sea surface microlayer (SML), sea surface water (SSW), and water column for the four different regions (A-D).

<table>
<thead>
<tr>
<th>Layer/Region</th>
<th>Slope Water (A)</th>
<th>Slope Water-subtropical mixture (B)</th>
<th>Gulfstream (subtropical) (B)</th>
<th>Sargasso Sea (C)</th>
<th>Coastal (D)</th>
</tr>
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<tbody>
<tr>
<td>SML</td>
<td>116</td>
<td>-</td>
<td>134</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>SSW (~1 m)</td>
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<td>-</td>
<td>14</td>
<td>26</td>
<td>42</td>
</tr>
<tr>
<td>0–10 m</td>
<td>12</td>
<td>19</td>
<td>9</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>10–50 m</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>16</td>
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<tr>
<td>50–100 m</td>
<td>2</td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>100–200 m</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>-</td>
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</tbody>
</table>

**2.5 Discussion**

New evidence is presented that TEP plays a significant role in organic carbon dynamics of the surface ocean, particularly in the SML where TEP-C represented nearly 100% of TOC (Tables 2.2 and 2.3). These TOC values agreed with previously reported measurements (Wanninkhof et al., 2013), and establish an organic carbon gradient extending from a phytoplankton bloom (higher TOC) to an oligotrophic region (lower TOC). By sampling four regions of varying productivity that encompassed a wide range of water mass characteristics, the relative proportions of TEP-C to TOC were examined. Also considered were which biogeochemical parameters were likely to control the spatial distribution of TEP concentration and enrichment. Although there was no correlation between the surface distribution of TEP and nutrients, chlorophyll $a$, nor TOC
concentration, there was a correlation between TEP and TOC in the SML with concentrations of both variables being inversely proportional to surface productivity as indicated by chlorophyll \( a \) concentrations.

A horizontal density gradient established by differences in temperature and salinity was used to classify each of the four regions (Slope Water, Gulf Stream, Sargasso Sea, and Coastal) and helped further characterize subtropical or subpolar influences. The Gulf Stream and Sargasso Sea regions are comparable based on similar subtropical temperature and salinity, nutrient depletion, and lower surface TOC and POC concentrations (Figs 2.1, 2.2, and 2.3). Since the SML in the Sargasso Sea was not sampled, the Gulf Stream was used as the oligotrophic reference to contrast the biologically productive Slope Water and Coastal regions that were both distinguished by colder and fresher waters containing higher TOC, POC, and nutrient concentrations. These differences in density likely also affected the settling behavior of aggregates (including TEP) as aggregate settling speeds are slowed down in waters with increased stratification (Prairie et al., 2013). Even across this diverse set of systems, a relationship between TEP and TOC is observed in the SML (Fig. 2.7a), and lack thereof in underlying surface waters (Fig. 2.7b), which further suggests that TEP is a substantial component of TOC in the SML.

2.5.1 TEP and TOC enrichments in the SML

The finding that TEP-C contributed to nearly all TOC in the SML, but only to a small fraction in the water below (Table 2.3), is consistent with the result that TEP and TOC were heavily enriched in the SML relative to underlying surface water throughout the study area. The Gulf Stream region had the highest enrichments of TEP and TOC,
followed by the Slope Water region, with the lowest enrichments found in the Coastal region SML (Table 2.2). A similar trend of higher enrichment in the SML of open ocean versus coastal regions was observed by previous studies (Carlson, 1983; Wurl and Holmes, 2008). Furthermore, there was an inverse trend with apparent productivity, as the Coastal region SML was the least concentrated and enriched in TEP and TOC (Fig. 2.5) and the subtropical Gulf Stream region had greater concentrations and enrichments than the more biologically productive Slope Water region. Wurl et al. (2011b) also reported greater enrichment of surfactants in the SML of oligotrophic waters versus more productive waters.

**Fig. 2.7.** Correlations of concentrations of TEP-C >0.4 µm (µmol L⁻¹) (solid symbols) and >0.2 µm (µmol L⁻¹) (striped symbols) with TOC (µmol L⁻¹) in a) SML and b) surface waters. Colors represent sampled areas: Slope Water (green), Gulf Stream (yellow), Sargasso Sea (blue), and Coastal (red).

2.5.2 Suggested mechanisms for TEP enrichment

The greater enrichment of TEP in oligotrophic conditions likely results from multiple interactive processes, for many of which we do not have direct measurements (bacterial abundance, SML thickness and density, phytoplankton composition, particle flux, or TEP
precursor concentrations) or the observed ranges were too small to be quantitatively useful. Wurl et al. (2011a) described a comprehensive conceptual model of TEP cycling in the ocean and a discussion of biogeochemical drivers of TEP production and fate. This model describes the primary source of TEP in surface waters as aggregates of dissolved polysaccharides that were either directly exuded from phytoplankton or biproducts of grazing or viral lysis. Once formed, TEP adsorb to bubbles and enrich the microlayer where they are exposed to UV radiation, which physically destroys the TEP and stimulates microbial consumption of TEP. TEP may be distributed throughout the water column and SML, while undergoing several cycles of lysis, grazing, and aggregation. Because TEP are neutrally buoyant, they are capable of both ascending and descending depending on environmental circumstances, the presence or absence of ballasting particles, or act of scavenging by rising bubbles. However, here the role of non-gel organic particle concentrations in providing ballast for TEP to escape this cycling in the surface ocean is specifically evaluated, as well as speculations on temperature, salinity, and wind as physical controls of the environment.

The concentration of non-gel organic particles present in surface waters was likely the primary driver affecting the concentration and vertical distribution of TEP by controlling TEP aggregation and residence time. A conceptual framework is presented to explain why TEP is driven to sink out of the SML and surface waters in more productive areas because there are more organic carbon particles to aggregate with TEP and provide ballast (Fig. 2.8). If there is a greater ratio of organic particles to TEP (such as the phytoplankton bloom in the Slope Water region), then TEP will not accumulate in the SML and may be heavy enough to sink out of the water column, thereby decreasing the
TEP residence time (Azetsu-Scott and Passow, 2004; Anja Engel et al., 2004; Mari et al., 2007). If there is a lower concentration of organic particles (such as the oligotrophic subtropical waters), TEP is less likely to sink and will accumulate in the SML and surface waters. The elevated TEP concentrations observed in the surface and upper 100m in the oligotrophic Sargasso Sea region (Fig. 2.6) could be explained by a longer residence time of TEP related to lower particle export. This scenario would also explain the higher contribution of larger TEP in the Gulf Stream SML, as a higher fraction of large TEP at higher TEP concentrations is consistent with the idea of self-aggregation of TEP that could be enhanced with longer residence times.

Though this study did not have the appropriate measurements to estimate TEP residence times (i.e. TEP production rates and sinking speeds or heterotrophic production rates), surface POC concentrations were used as a proxy for particle abundance. The presence of a phytoplankton bloom was identified in the Slope Water region from the elevated Chl-a, nutrient and POC concentrations (Fig. 2.1; Fig 2.2f), suggesting that there were probably more particles being exported from that region compared to oligotrophic regions such as the Sargasso Sea. The Slope Water region had the highest average ratio of POC concentration per TEP-C concentration (1.1; expressed as POC/TEP-C in Table 2.2) in surface waters, while the Gulf Stream region had a ratio of 0.9, and the Sargasso Sea had an even lower ratio of 0.4, indicating that the relative importance of POC with respect to TEP diminished along the TOC gradient (Table 2.2). The POC/TEP ratio was relatively low in the Coastal region (0.5), which did not follow the trend observed along the productivity gradient in the open ocean. This decoupling could signify that the residence time of TEP in a coastal system is less influenced by particles, although still
subject to high variability from localized processes (turbidity, resuspension of sediments, mineral input, and anthropogenic effects) that dictate DOM-POM exchanges (He et al., 2016).

**Fig. 2.8.** Conceptual scheme of TEP interactions in marine environments with contrasting productivity and POC concentrations (together indicated by green rectangles) to explain the observed distribution of TEP (blue globules) enrichments in the SML. TEP form heavy particulate aggregates and sink when there are higher concentrations of POC (e.g. bloom conditions), while TEP ascend and accumulate in the SML when POC concentrations are insufficient to ballast TEP (e.g. oligotrophic conditions).

Wind variation was not considered to be significant to this study because the sampling of surface water and SML was biased towards relatively low wind conditions, as all of the SML samples were collected in winds <5 m s\(^{-1}\) and surface water samples were collected in winds <10 m s\(^{-1}\) (Fig. 2.9). However, it is possible that the thickness of the Gulf Stream SML, distinguished by a SST ~10 °C warmer than the Slope Water and Coastal regions, was affected by temperature. As the viscosity of water decreases in warmer temperatures, the thickness of the SML should also decrease with increasing
temperature (Carlson, 1982; Falkowska, 1999). The greater enrichment of organic material in the Gulf Stream SML is likely partially affected by a decreased SML thickness that presumably helped concentrate organic matter in the SML. In addition, a higher degree of density stratification is more likely in the Gulf Stream SML where there was elevated SST, which would result in slower settling speeds of aggregates (Prairie et al., 2013), further supporting the hypothesis that TEP experiences longer residence time in the Gulf Stream SML.

Fig. 2.9. Wind speed (m s\(^{-1}\)) observed during cruise (small black circles). Overlaid symbols indicate wind speed during sampling routines of surface water (large grey circles), SML (triangles) and CTD casts (squares; labeled by cast number). Colors for SML and CTD casts represent sampled areas: Slope Water (green), Gulf Stream (yellow), Slope Water-Subtropical mixture (purple), Sargasso Sea (blue), and Coastal (red).

2.5.3 Contributions of TEP to global carbon cycle

The elevated contributions of TEP-C to TOC estimated in the SML (Table 2.3), in combination with the observed relationship between TEP and TOC in the SML (Fig 2.7a), suggest that TEP is an important organic constituent of the SML. In each of the
regions sampled, TEP-C in the SML represented more than 100% of the TOC (Table 2.3), implying that the standard range of conversion factors are not ideal for application to SML samples. There was a larger fraction of TOC estimated as TEP-C in the upper 200 m of the oligotrophic and coastal water columns compared to the Slope Water region. For Slope Water and Gulf Stream surface waters, the contribution of TEP-C to TOC (Table 2.3) is comparable to the contribution of POC to TOC (Table 2.2), which suggests that TEP is an important fraction of the POC sampled in those regions.

Similar to the observed TEP and TOC enrichments in the SML in this study, greater organic carbon enrichment was observed in nascent sea spray aerosols (SSA) formed in the Sargasso Sea compared to a region containing higher sea surface Chl-a and greater phytoplankton biomass (Quinn et al., 2014). These data show that the SML of oligotrophic systems have greater TEP and TOC enrichment, which is suggested to be the major source of the organic enrichment of SSA. If the processes governing organic enrichment in the SML can be connected to the generation of organically enriched SSA, then there could be an additional sink for surface-active organic matter to consider (Aller et al., 2017; Kieber et al., 2016; Long et al., 2011), and an expanded role for TEP in the global carbon cycle (Engel and Galgani, 2016), with greater emphasis on oligotrophic conditions.

### 2.6 Summary

Throughout the study area, TEP is heavily enriched in the SML relative to underlying surface water (Table 2.2), and TEP contributes significantly to the organic carbon content of the SML. An organic carbon gradient was observed in the surface waters, with higher
TOC, POC, Chl-α and TEP concentrations in more productive waters in the northern Slope Water region compared to the more oligotrophic Sargasso Sea or Gulf Stream to the south. A different TOC distribution was observed in overlying SML waters, where appreciably more TOC was concentrated in the less productive areas. The correlation between TEP and TOC in the SML suggests that TEP is a significant component of the organic carbon in this gelatinous interfacial layer. These data suggest that TEP accumulates in the SML in areas where “ballasting” particles heavy enough to carry TEP-particle aggregates downwards are absent (oligotrophic areas). As a consequence, a more pronounced SML, enriched with surface-active organic matter, would be expected in oligotrophic regions that are characterized by lower particle export and nutrients than in more productive regions. Future work should trace the production of TEP to its distribution into the SML and upper water column, while controlling the effect of particle abundance on TEP accumulation.
CHAPTER 3. An examination of the fluorescence-quenching assay for quantifying self-assembled gels

3.1 Overview

The fluorescence quenching assay introduced by Ding et al. (2007) to quantify the proportion of organic carbon present as marine microgels is, in its present description, not reproducible under varying seawater conditions. Sensitivity to pH was identified as the primary contributor to this lack of reproducibility for using chlortetracycline (CTC) as a fluorescent indicator of bound Ca\textsuperscript{2+} in marine microgels. Although the method description recognized that the pH of the sample must be consistent between fluorescence measurements (before and after EDTA addition), the method does not provide a solution for consolidating and managing the varying sensitivity of CTC to pH, natural gradients of in-situ pH in seawater, and pH dependent assembly dynamics of polymer gel equilibria.

3.2 Background

One of the least understood carbon transformations in the ocean is the interaction between particulate and dissolved organic matter (POM and DOM, respectively). Marine gels are colloidal- to micrometer-sized polymer networks stabilized by Ca\textsuperscript{2+} ionic bonds that spontaneously and reversibly assemble from DOM, thus spanning the size continuum between POM and DOM (Chin et al., 1998; Orellana and Leck, 2015; Verdugo, 2012; Verdugo et al., 2004). It has been suggested that up to 10% of DOM, or 70 Pg C, in the ocean exists in the gel phase (Chin et al., 1998; Verdugo, 2012). Once assembled, microgels act as hotspots for microbial degradation (Orellana and Verdugo, 2003; Verdugo, 2012; Yamada et al., 2016) and may provide an important source of microbial
substrate to the deep ocean. Understanding these dynamics is important to predict the fate of this massive pool of carbon (Verdugo et al., 2008). However, very few measurements of the proportion of organic matter present as microgels have actually been made in the ocean (Baltar et al., 2016; Ding et al., 2007; Orellana et al., 2011; Verdugo, 2007).

There are essentially three properties that define and enable the detection of marine microgels stabilized by Ca\(^{2+}\) ionic bonds (Chin et al., 1998). First, microgels undergo volume phase transition that rapidly and reversibly dehydrates and condenses the gels into smaller, tighter networks in response to changes in the surrounding environment, including pH (Chin et al., 1998; Tanaka et al., 1980), temperature (Chen et al., 2015), and concentration of polyanions such as DMS and DMSP (Orellana et al., 2011). This property distinguishes polymer microgels from other larger classifications of particles with a gel-like nature such as transparent exopolymer particles (TEP) (Orellana and Leck, 2015). Whether or not a relationship exists between microgels and TEP is still an open question.

Second, marine microgels are held together by Ca\(^{2+}\) ionic links, thus they can be stained with chlorotetracycline (CTC), a fluorescent probe indicating bound calcium (Rudolf et al., 2003). CTC interacts with bound divalent cations such as Ca\(^{2+}\) and Mg\(^{2+}\), forming fluorescent metal chelates. Alone in aqueous solutions (e.g. Milli-Q water), CTC does not show emission fluorescence bands. CTC has three titratable functional groups (pKa’s = 3.4, 7.7, and 9.3) that affect the conformation and fluorescent properties of the CTC-chelate complex (Mathew and Balaram, 1980). CTC fluorescence observed at 530 nm on excitation at 400 nm is assigned to the chromophores in ring A (pK\(_a\) = 3.5; Fig. 3.1), which bends back toward rings B and C to form a Ca\(^{2+}\) chelate at the specified
coordination sites. Above pH 7.5, a second emission band corresponding to the conjugated BCD ring system (pKₐ = 7.7) appears at 430 nm on excitation at 345 nm. In the presence of Ca²⁺, excitation energy is transferred intramolecularly from the BCD chromophore to the ring A chromophore (Mathew and Balaram, 1980).

Fig. 3.1. a) Chemical structure of CTC with identification of the three functional groups/fluorophores and associated pKa’s. b) Emission spectra (380 nm ex) of seawater with CTC alone (blue solid line) and 20 mM Ca²⁺ added (dotted line). The peak at 430 nm corresponds to the BCD ring system fluorophore and the peak at 530 nm corresponds to the ring A fluorophore.

Third, microgels disperse into free polymers (< 20 nm) when their Ca²⁺ ionic crosslinks are chelated by ethylaminediaminetetraacetic acid (EDTA) (Chin et al., 1998). Notably, EDTA has also been shown to disrupt TEP (Alldredge et al., 1993; Cisternas-Novoa et al., 2015; Mopper et al., 1995; Passow, 2002a). Consequently, EDTA treatment is often used to control the dispersed versus assembled fraction of marine polymers in
microgel detection analyses (Chin et al., 1998; Ding et al., 2009; Ding et al., 2007; Orellana et al., 2007; Orellana and Verdugo, 2003).

Microgel detection analyses have the potential to measure either size and/or abundance of gel material, each with limitations. For example, traditional dynamic laser scattering (DLS) instrumentation measures the hydrodynamic diameter (proportional to size) of gel particles with a 2 nm limit of detection, but does not measure particle concentration. However, newer instrumentation and software may be capable of doing both (Particle-metrix, Germany; Malvern, UK; Horiba, CA, USA). Ca$^{2+}$-crosslinked microgels can be visualized using an epifluorescence microscope with the appropriate optical filters by staining with CTC. A flow cytometer can count and sort individual CTC-fluorescent particles (with and without EDTA addition) to provide microgel concentrations in units of gel particles mL$^{-1}$ (Orellana et al., 2007, 2003), however the limit of detection is instrument dependent and varies from 250 nm to >1µm.

Direct measurements of carbon content in microgels have not been published. Chin et al. (1998) reported that 10% of DOC assembles into polymer gels, but the specific methods used to determine this yield were not described, except that the results agreed with both Guo and Santschi (1997) and Stordal et al. (1996). To estimate the carbon content of microgels, the Ding et al. (2007) method attempts to measure the fraction of organic matter present as a gel by observing changes in the fluorescence of assembled and dispersed polymers that stain with CTC. Because they observed an increase in CTC fluorescence when microgels were dispersed with EDTA, Ding et al. hypothesized that the assembled microgel networks quench CTC fluorescence.
Quenching refers to any process that results in the decrease in the fluorescence of a sample, including processes that result in the transfer of energy, excited state reactions, and the formation of non-fluorescent ground state species (Kwon and Carson, 1998). Dequenching, or the “relief of quenching”, occurs when the distance between fluorophores is increased (Kwon and Carson, 1998). Ding et al. (2007) explain their observed increase in fluorescence upon dispersion of gels with EDTA to be a result of CTC dequenching (Fig 3.2), as the self-absorbance, the physical shielding, or shadowing of the microgel network prevented excitation energy from reaching the CTC fluorophores and/or emission fluorescence from reaching the detector. They demonstrated that the difference between the quenching fluorescence of assembled gels and the dequenching fluorescence of EDTA dispersion was proportional to size (measured by DLS). They also showed that the calculated gel carbon concentrations derived from these fluorescence measurements correlated with the concentration of gels (particles mL⁻¹) determined by flow cytometry.

However, when the present study analyzed several seawater samples from various ocean regions according to the Ding et al. (2007) method, there was often uncertainty for how to interpret data when “negative” gel C concentrations resulted. These negative results occurred when a decrease in CTC fluorescence was observed upon dispersion of gels with EDTA, in contrast to the increase in fluorescence detailed in Ding et al. (2007). A compilation of new and published data (including Baltar et al., 2016) obtained using the assay are shown as a plot of proportion of OC present as a gel (%) versus pH of the analysis (Fig. 3.3; supporting data in Tables 3.1 and 3.2). At lower pH, the gel (%) is skewed positive due to higher CTC fluorescence when microgels are dispersed with
EDTA (Fig. 3.2). However, at higher analysis pH, gel (%) is skewed negative due to higher CTC fluorescence when microgels are assembled.

**Fig. 3.2.** Conceptual scheme of the quenching/dequenching hypothesis described by Ding et al. (2007). EDTA is added to disperse assembled gels into unhindered CTC-tagged polymers, resulting in an increase in fluorescence emission at 520 nm (380 nm ex), or dequenching.

This method of estimating gel C depends on a thorough understanding of the fluorescent properties and selective reactivity of CTC - yet the use of CTC in seawater DOM analyses is insufficiently described in the literature (Domozych et al., 1993). The need to elucidate the disagreement of CTC fluorescence shifts upon physical dispersal of assembled gels prompts an investigation of the experimental equilibrium variables. The assembly of marine polymer gels has been described as both spontaneous and reversible, so it is logical that the distribution of OC across the dissolved-gel continuum would be affected by pH and temperature, as equilibrium constants are dependent on these variables.
In response to the method described by Ding et al. (2007), this study examines two alternative hypotheses that could explain the processes controlling fluorescence quenching and dequenching: (i) pH is altering gel-DOM equilibria, and/or (ii) quenching dynamics were simply misinterpreted or poorly described in the original method (Fig 3.2). Furthermore, this study provides new evidence to explain these quenching dynamics and advance future methodology to measure marine polymer gels.

3.3 Methods

3.3.1 Fluorescence quenching assay

Following Ding et al. (2007), a spectrofluorometer (Molecular Devices, Spectra Max M2; Perkin-Elmer LS55) was used to quantify the change in fluorescence (ex 380 nm/ em 522 nm) of a sample stained with 100 µM chlortetracycline (CTC, pH 2.7, MP Biomedical) with addition of 10 mM Na₂EDTA (pH 8, filtered 0.2 µm daily). When EDTA was added to the stained sample, hydrogen ions were released, lowering the pH of the sample to ~4.5. Before making the second fluorescence measurement, the pH was titrated back to the original pH of the first measurement using 0.5M NaOH using an automatic titrator (Dosimat, Metrohm). The change in CTC fluorescence resulting from the dispersion of assembled gel material upon addition of EDTA was used to estimate the gel fraction of total material, i.e. the proportion of organic carbon (OC) present as a gel. CTC fluorescence before (F1) and after (F2) addition of EDTA was used to calculate the “quenching fraction” or QF:

\[ QF = \frac{(F2 - F1)}{F2} \]

(Equation 3.1)
Fig. 3.3. Compilation of individual results collected from using the Ding et al. (2007) method displayed as the proportion of OC as a gel (%) as a function of the analysis pH. Above 0%, fluorescence increases with EDTA dispersion (dequenching); below 0%, fluorescence decreases with EDTA dispersion (quenching). Supplementary details are provided in Tables 3.1 and 3.2.
By multiplying the observed QF by TOC concentration of the sample ([TOC]), the carbon concentration of gels (gel C) was estimated according to the following formula:

\[ [\text{gel C}] = \text{QF} \times [\text{TOC}] \]  

(Equation 3.2)

The assay was performed at room temperature (with the exception of the Ross Sea samples, which were performed on ice).

3.3.2 Experimental framework

*Culture dilutions with varying pH* – To examine equilibrium dynamics of gels and provide evidence for hypothesis (i), concentrated phytoplankton cultures were diluted in series (0, 25, 50, 100%) with ASW (Berges et al., 2001) to vary the concentration of DOC at different pH scenarios from pH 7.5 to pH 9. pH was adjusted using dilute NaOH and HCl and monitored using a pH electrode (Orion/Ross, Thermo Scientific). Properties of the cultures and ASW used in the dilution experiments are described in Table 3.2. Upon excitation at 380 nm, emission spectra (475 - 600 nm) were collected to observe changes in fluorescence upon addition of EDTA in each of the tested conditions. Gel C, DOC, and TOC concentrations were also measured or calculated to estimate equilibrium constants for each dilution series as a function of pH. Equilibrium constants (K\text{eq}) were estimated using the following equation:

\[ K_{\text{eq}} = \frac{[\text{products}]}{[\text{reactants}]} = \frac{[\text{gel C}]}{[\text{DOC}]} = \frac{(F_2 - F_1)}{F_2} \times \frac{[\text{TOC}][\text{DOC}]}{[\text{DOC}]} \]  

(Equation 3.3)

*Quenching with Ca\textsuperscript{2+} serial addition* – To investigate fundamentals of CTC fluorescence quenching at controlled pH (7.5, 7.7, 8, 8.2, 8.5) and provide evidence for hypothesis (ii), experiments were designed to assess the extent of self-quenching (i.e. if assembled gels were shielding and blocking excitation energy or emission energy from
reaching the detector). By adding 2M CaCl\(_2\) in serial addition (0 – 20 mM final concentration) to Puget Sound seawater (pre-filtered 4 days prior; [DOC]= ~95 µM C) with and without EDTA, the quenching process was examined using the Stern-Volmer relationship, represented by the equation:

\[
\frac{F_0}{F} = 1 + K_v [Q]
\]

(Equation 3.4)

Where \(F_0\) is the fluorescence without the quencher (no additional Ca\(^{2+}\)), \(K_v\) is the Stern-Volmer constant (or slope), and \([Q]\) is the concentration of the quencher (Ca\(^{2+}\) added to the sample). Samples were excited at both 345 and 380 nm to examine the two emission bands that correspond to ring A and the conjugated BCD ring system in the CTC molecule (Fig. 3.1).

**Comparison with other methods** – Selected samples were analyzed according to Ding et al. (2007) and microgels were additionally counted either by flow cytometry or analyzed for TEP concentrations. Flow cytometry allows the discrimination of single particles in a laminar flow based on forward scatter (FSC; approximating size), side scatter (granularity), and CTC fluorescence to define changes in distinct populations (Orellana et al., 2007), instead of assuming changes in populations based on bulk fluorescence measurements (Ding et al., 2007). Although a formal relationship between microgels and TEP has not yet been established, comparison with TEP concentrations could help guide understanding for the relative increase or decrease in fluorescence quenching (i.e. gel C).

### 3.3.3 Sample collection and storage

Samples were either analyzed in natural seawater (detailed in Table 3.1), ASW (Berges et al., 2001), or in laboratory diatom cultures grown in ASW (Berges et al., 2001;
Kester et al., 1967), with added nutrients (f/2; (Guillard and Ryther, 1962)) (detailed in Table 3.2). Samples were collected unfiltered in dark HDPE bottles, preserved with (0.1%) sodium azide (Alfa Aesar), and stored at 4°C until analysis. Unless otherwise noted, samples were analyzed unfiltered. Samples used in the quenching experiments with Ca\(^{2+}\) serial addition were pre-filtered using 0.2 µm pore size 25 mm syringe filter (Acrodisc) four days prior to analysis.

3.3.4 Determination of TOC and DOC concentrations

Samples were collected either unfiltered (for TOC) or filtered (for DOC) using either glass fiber filters precombusted at 450°C overnight (GF/F Whatman; nominal pore size 0.7 µm) or acid washed 0.4 µm pore size 25 mm polycarbonate filters (Whatman Nuclepore). Samples were either stored frozen in acid washed 60-mL polycarbonate bottles or at 4°C in precombusted glass vials with acid (2 µL per mL sample) and analyzed at the University of Miami. TOC/DOC was measured by high temperature combustion (HTC) using a Shimadzu TOC-L auto analyzer (CV = 1.5 to 2.5%) following methods from Dickson et al. (2007) and quality controlled using Hansell consensus reference materials (CRM) (Hansell, 2005).

3.3.5 Microgel detection by flow cytometry

An InFlux flow cytometer (Cytopeia, Inc.) was also used to sort and count microgels that stain with CTC and disperse with EDTA (Orellana et al., 2011, 2007; Orellana and Hansell, 2012). Particle size was calibrated using 1µm reference beads (Becton Dickenson). Data analysis was performed using single cell analysis software (FlowJo, LLC).
Table 3.1. Locations, depth ranges, cruise information, sampling dates, and pH ranges analyzed for seawater samples in Fig. 3.3.

<table>
<thead>
<tr>
<th>Location</th>
<th>Depths collected</th>
<th>Cruise identifiers</th>
<th>Date</th>
<th>pH range analyzed</th>
<th>[DOC] and/or [TOC] range (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic</td>
<td>Surface</td>
<td>RV <em>Knorr</em> (Cruise KN219)</td>
<td>May 20 - June 5, 2014</td>
<td>7.5</td>
<td>71-90 (TOC)</td>
</tr>
<tr>
<td>Pacific</td>
<td>Surface</td>
<td>RV <em>Melville</em> (Cruise MV1310)</td>
<td>August 5-24, 2013</td>
<td>7.5-8</td>
<td>63-73 (TOC)</td>
</tr>
<tr>
<td>Pacific</td>
<td>Surface – 4000m</td>
<td>CCGS <em>John P. Tully</em> (Cruise 2014-01)</td>
<td>February 11-25, 2014</td>
<td>7.5</td>
<td>38-63 (TOC)</td>
</tr>
<tr>
<td>Ross Sea, Antarctica</td>
<td>Surface – 800m</td>
<td>RVIB <em>Nathaniel B Palmer</em> (Cruise NBP-1302)</td>
<td>Feb-Mar 2013</td>
<td>7.7 – 8.5</td>
<td>44-57 (DOC); 45-101 (TOC)</td>
</tr>
<tr>
<td>Florida Coastal – University of Miami (RSMAS Pier)</td>
<td>Surface</td>
<td>n/a</td>
<td>November 2013</td>
<td>7.5 – 8.2</td>
<td>-</td>
</tr>
<tr>
<td>California Coastal – University of California Santa Barbara (UCSB) (Goletta Pier)</td>
<td>Surface</td>
<td>n/a</td>
<td>December 2016</td>
<td>7.5, 8</td>
<td>91 (DOC) 97 (TOC)</td>
</tr>
<tr>
<td>California Coastal – UCSB - Del Playa Beach</td>
<td>Surface</td>
<td>n/a</td>
<td>December 2016</td>
<td>7.5, 8.1</td>
<td>69 (DOC) 82 (TOC)</td>
</tr>
<tr>
<td>South Pacific subtropical front</td>
<td>Surface-500 m</td>
<td>Baltar et a. (2016)</td>
<td>March and April 2015</td>
<td>7.5 - 8</td>
<td>-</td>
</tr>
</tbody>
</table>

3.3.6 Determination of TEP concentrations

TEP concentrations were analyzed at the University of California Santa Barbara by the Passow Lab following Passow and Alldredge (1995) before samples were shipped (overnight, chilled) to the University of Washington for subsequent analyses. Sample pH was not adjusted prior to TEP analysis (i.e. the in situ pH was maintained). Samples were filtered in triplicate (200 mL per replicate for seawater samples; 10-20 mL per replicate for culture samples) using 0.4 µm pore size 25 mm polycarbonate filters (Whatman Nuclepore), stained with 0.5 mL Alcian Blue solution (pH = 2; 0.16% w/v Alcian Blue 8GX, Sigma) calibrated with Gum Xanthan (Sigma). TEP concentrations were maintained in units of Gum Xanthan Equivalents (µg Gum X eq L⁻¹) and were not
converted to TEP-C units because conversion factors are species-specific (Passow, 2002a).

**Table 3.2.** Description of cultures used for specific experiments in this study.

<table>
<thead>
<tr>
<th>Species (growth phase)</th>
<th>Date</th>
<th>Growth medium</th>
<th>Lab grown</th>
<th>Experiment</th>
<th>([\text{Ca}^{2+}]) in media (mM)</th>
<th>[TOC] in ASW for dilutions Fig 3.4 and 3.5 (µM)</th>
<th>[DOC] and/or [TOC] in culture (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thalassiosira pseudonana</em> (stationary)</td>
<td>03/2015</td>
<td>autoclaved ASW (Berges et al., 2001) with f/2 nutrients (Guillard and Ryther, 1962)</td>
<td>U Miami; 12hr light/dark cycles</td>
<td>Culture spectra and equilibria Fig. 3.4 and 3.5</td>
<td>9.14</td>
<td>17</td>
<td>213 (DOC) 287 (TOC)</td>
</tr>
<tr>
<td><em>Thalassiosira pseudonana</em> (stationary)</td>
<td>04/2015</td>
<td>autoclaved Blue Water media (BWM; Brand, 1990)</td>
<td>U Miami; 12hr light/dark cycles</td>
<td>Culture equilibria Fig. 3.5</td>
<td>~10</td>
<td>35</td>
<td>350 (DOC) 816 (TOC)</td>
</tr>
<tr>
<td><em>Chaetoceros graciles</em> (stationary)</td>
<td>08/2014</td>
<td>autoclaved BWM</td>
<td>U Miami; 12hr light/dark cycles</td>
<td>Culture equilibria Fig. 3.5</td>
<td>~10</td>
<td>89</td>
<td>775 (DOC) 1931 (TOC)</td>
</tr>
<tr>
<td><em>Thalassiosira pseudonana</em> (stationary)</td>
<td>04/2014</td>
<td>autoclaved ASW (Berges et al., 2001) with f/2 nutrients</td>
<td>Institute for Systems Biology (Seattle, WA)</td>
<td>Culture equilibria Fig. 3.5</td>
<td>9.14</td>
<td>60</td>
<td>630 (DOC)</td>
</tr>
<tr>
<td><em>Thalassiosira pseudonana</em> (Near end of exponential)</td>
<td>12/2016</td>
<td>ASW modified from (Kester et al., 1967) with f/2 nutrients</td>
<td>UCSB</td>
<td>Compare with TEP; Fig. 7</td>
<td>10.5</td>
<td>-</td>
<td>311 (DOC) 506 (TOC)</td>
</tr>
<tr>
<td><em>Thalassiosira weissflogii</em> (Near end of exponential)</td>
<td>12/2016</td>
<td>filtered (0.2µm) SW with f/2 nutrients</td>
<td>UCSB</td>
<td>Compare with TEP; Fig. 7</td>
<td>~10</td>
<td>-</td>
<td>196 (DOC) 631 (TOC)</td>
</tr>
<tr>
<td><em>Skeletonema grethae</em> (Near end of exponential)</td>
<td>12/2016</td>
<td>filtered (0.2µm) SW with f/2 nutrients</td>
<td>UCSB</td>
<td>Compare with TEP; Fig. 7</td>
<td>~10</td>
<td>-</td>
<td>214 (DOC) 467 (TOC)</td>
</tr>
</tbody>
</table>
3.4 Results

3.4.1 Effect of pH on CTC emission and gel equilibria

*Emission spectra* – CTC emission spectra (380 nm ex) of assembled and EDTA-dispersed gels in *T. pseudonana* culture dilutions (0%, 25%, 50% and 100% culture) at varying pH (7.5, 7.9, 8.2) are shown in Figure 3.4 and described in Table 3.2. The CTC fluorescence of assembled and EDTA-dispersed gels present in the cultures generally decreased with increasing pH, with the ranges of fluorescence exhibited by each dilution series also narrowing. The highest CTC emission was observed at pH 7.5, where the fluorescence maximum of the 530 emission band ranged from ~270 to ~380 raw fluorescence units (RFU) across the culture dilution series. At pH 7.9, the max fluorescence ranged from ~220 to ~310 RFU. The range of fluorescence narrowed to ~215 to ~230 at pH 8.2. Regardless of pH and EDTA treatment, 0% culture (or 100% ASW; [TOC] = 17 µM; Table 3.2) had the highest fluorescence.

*Microgel equilibria* – Calculated equilibrium constants (\(K_{eq}\)) as a function of pH (7.5 – 9) using various phytoplankton cultures are shown in Figure 3.5. The measured TOC and DOC used to calculate \(K_{eq}\) in Equation 3.3 are supplied in Table 3.2. With increasing pH, the proportion of fluorescence that increased due to EDTA-induced dispersion of polymers decreased, resulting in all negative values above pH 8.3.
Fig. 3.4. CTC emission spectra from 470-600 nm (380 ex) of assembled (solid lines) and EDTA-dispersed (dashed lines) polymers in dilutions of *T. pseudonana* (0%, black/grey; 25%, green; 50%, orange; 100%, blue) at different pH a) 7.5, b) 7.9, and c) 8.2 shown in raw fluorescence units (RFU) measured 03/2015.

![Emission spectra](image)

Fig. 3.5. Equilibrium constant ($K_{eq}$) as a function of pH calculated in various phytoplankton cultures described in Table 3.2 (*T. pseudonana* sampled in 03/2015, open diamonds; *T. pseudonana* sampled in 04/2014, crisscrosses; *C. graciles*, filled triangles; *T. pseudonana* sampled in 04/2015, filled circles) diluted at different concentrations (0%, 25%, 50%, 100%) with ASW.

![Equilibrium constant](image)
3.4.2 Ca\(^{2+}\) quenching as a function of pH

Applying the Stern-Volmer relationship to Ca\(^{2+}\) added in series addition to the same Puget Sound seawater ([DOC]= \(\sim\)95 µM) with and without EDTA revealed that Ca\(^{2+}\) was quenching fluorescence at 530 nm (380 ex) regardless of EDTA treatment. Stern-volmer constants (K\(_q\)) were determined from the slope of the regression between additional [Ca\(^{2+}\)] and the quenched fluorescence (Equation 3.3) and are summarized in Table 3.3 as a function of pH (7.5 – 8.5) and EDTA treatment. The more negative K\(_q\) values correspond to greater quenching with [Ca\(^{2+}\)]. There were two major trends observed. First, there was generally greater quenching in the EDTA treated samples compared to the samples without EDTA (with the exception of pH 8.5, where EDTA treatment resulted in less quenching). Second, as pH increased from 7.5 to 8.5, [Ca\(^{2+}\)] quenching decreased in samples treated with EDTA and increased in samples without EDTA.

**Table 3.3.** Stern-Volmer constant (K\(_0\)) with and without EDTA at various pH. The R\(^2\) for the regression of [Ca\(^{2+}\)] and quenching of fluorescence is given in parentheses.

<table>
<thead>
<tr>
<th>pH</th>
<th>K(_q) (R(^2)) with EDTA “dispersed gels”</th>
<th>K(_q) (R(^2)) without EDTA “assembled gels”</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>-7.43E-03 (0.9958)</td>
<td>-4.82E-03 (0.98)</td>
</tr>
<tr>
<td>7.7</td>
<td>-6.36E-03 (0.9849)</td>
<td>-4.76E-03 (0.99)</td>
</tr>
<tr>
<td>8.0</td>
<td>-6.73E-03 (0.9983)</td>
<td>-5.51E-03 (0.98)</td>
</tr>
<tr>
<td>8.2</td>
<td>-6.21E-03 (0.9723)</td>
<td>-5.34E-03 (0.99)</td>
</tr>
<tr>
<td>8.5</td>
<td>-4.85E-03 (0.9177)</td>
<td>-5.63E-03 (0.99)</td>
</tr>
</tbody>
</table>

3.4.3 Comparison with other methods

**Flow cytometry** – Shifts in the optical properties of CTC fluorescent populations in Ross Sea seawater (TRACERS stations 66, 80, 85, and 93) with EDTA were determined by flow cytometry and compared to gel C concentration determined following Ding et al. (2007) (Fig. 3.6). There was no relationship observed between gel C concentration (µM) and gel particle concentrations (mL\(^{-1}\)) determined using flow cytometry (Fig. 3.6a).
Regressions between mean 520 nm emission (405 nm ex) and mean FSC signals of samples analyzed by flow cytometry with EDTA (filled triangles; yellow regression line) and without EDTA (open circles; dotted regression line) treatments (Fig. 3.6b). These results showed that EDTA does not affect the relationship between average size and CTC emission of particle populations.

**Fig. 3.6.** Flow cytometry results from four profiles from the Ross Sea (stations 66, 80, 85, and 93). Each data point represents the mean result for a sample. a) Plot of gel C concentration (µM) determined using Ding et al. (2007) against gel particle concentrations (mL⁻¹) determined using flow cytometry. b) Regressions between mean 520 emission (405 ex) and mean FSC signals of samples analyzed by flow cytometry with EDTA (filled triangles; yellow regression line) and without EDTA (open circles; dotted regression line).

**TEP (in situ pH vs. controlled pH)** — The fluorescence quenching assay was performed at both a constant pH (7.5) and at the in situ pH of the samples, which varied from 8.0-8.9, and was compared to the TEP concentrations determined in the same samples. Due to photosynthesis, the pH and OC concentrations were higher in the culture samples (*T. pseudonana, T. weissflogii, S. grethae*) compared with the seawater samples (California Coastal: Goleta Pier and Del Playa Beach; Table 3.4). TEP concentrations
ranged from 733.7 (±64.4) µg Gum xan Eq L\(^{-1}\) in Goleta Pier seawater to 14,642.9 (±361.6) µg Gum xan Eq L\(^{-1}\) in *S. grethae* culture. These values negatively correlated with the proportion of OC present as a gel, which varied from -4.7% (±1.5) in Goleta Pier seawater to -19.8% (±3.0) in *S. grethae* culture when the fluorescence quenching analysis was performed at the in situ pH of the samples (pH 8 – 8.9; filled blue circles, Fig. 3.7a; Table 3.4). There was also a strong negative correlation observed between the proportion of OC present as a gel and pH (Fig. 3.7b), which resulted from a greater decrease in fluorescence at higher pH when gels were dispersed with EDTA.

When the fluorescence quenching analysis was performed at constant pH (7.5), the resulting proportion of OC present as a gel ranged from -1.1% (±1.6) to 2.3% (±0.1) and did not correlate with TEP concentrations (open diamonds, Fig. 3.7a). pH 7.5 was selected because it appeared to have the highest CTC fluorescence (and presumptively, the highest sensitivity) of the pH ranges (Fig 3.3; Fig 3.4).

**Table 3.4.** Results from fluorescence quenching assay QF (% average ± SD), at in situ and constant pH, compared to TEP (average ± SD), TOC, and DOC concentrations.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>TEP (µg Gum xan Eq L(^{-1}))</th>
<th>QF (%) pH 7.5</th>
<th>QF (%) in situ pH</th>
<th>in situ pH</th>
<th>DOC (µM)</th>
<th>TOC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goleta Pier</td>
<td>733.7 (±64.4)</td>
<td>0.3 (±0.2)</td>
<td>-4.7 (±1.5)</td>
<td>8.043</td>
<td>91.0</td>
<td>96.7</td>
</tr>
<tr>
<td>Del Playa B.</td>
<td>1136.7 (±32.3)</td>
<td>0.6 (±2.9)</td>
<td>-5.8 (±0.9)</td>
<td>8.138</td>
<td>69.0</td>
<td>81.7</td>
</tr>
<tr>
<td><em>T. pseudonana</em></td>
<td>4868.9 (±781.0)</td>
<td>0.3 (±2.0)</td>
<td>-7.5 (±0.7)</td>
<td>8.300</td>
<td>310.5</td>
<td>506.2</td>
</tr>
<tr>
<td><em>T. weissflogii</em></td>
<td>5921.0 (±444.7)</td>
<td>2.3 (±0.1)</td>
<td>-16.7 (±1.9)</td>
<td>8.609</td>
<td>195.9</td>
<td>630.6</td>
</tr>
<tr>
<td><em>S. grethae</em></td>
<td>14,642.9 (±361.6)</td>
<td>-1.1 (±1.6)</td>
<td>-19.8 (±3.0)</td>
<td>8.889</td>
<td>213.7</td>
<td>466.6</td>
</tr>
</tbody>
</table>
Fig. 3.7. From data given in Table 3.4, a) Comparison of results obtained following Ding et al. (2007) with TEP at a range of in situ pH (8-8.9; filled circles) and fixed pH (7.5; open diamonds). b) Relationship between the proportion of OC as a gel (%) and pH of the analysis for samples analyzed at the in situ pH.

3.5 Discussion

In relation to the method described by Ding et al. (2007), two alternative (and not necessarily independent) hypotheses were offered to explain the processes controlling fluorescence quenching and dequenching previously reported to correlate with the carbon content of microgels. The first hypothesis suggested that the trend displayed in Figure 3.3 was indicative of a pH dependence of the reversible reaction between DOC polymers (reactant) and assembled gels (product). Although the results demonstrated that $K_{eq}$ was primarily controlled by pH (Fig. 3.5), the resulting negative $K_{eq}$ values (due to calculated negative gel C) above pH 8.2 prohibits application to the Gibbs function for determining thermodynamic potential when a system reaches chemical equilibrium. This suggests that equilibrium conditions (i.e. reversibility) do not apply to marine gel assembly above pH 8.2, perhaps due to enhanced Ca$^{2+}$ crystallization of the gel matrix (Chin et al. 1998).
Note that these conditions would also affect the interpretation of quenching dynamics that dictate the gel C calculation (hypothesis ii).

3.5.1 Testing critical assumptions of the assay

Source of fluorescence quenching – Ding et al. (2007) hypothesized that the topology of the assembled gels drove the observed fluorescence quenching by shadowing excitation light from reaching other assembled gels, and/or blocking emission light from reaching the detector. They acknowledged that this constraint does not apply to flow cytometry because flow cytometry counts single gel particles in a laminar flow and thus has no shadowing effect. However, they but did not offer an explanation to the observed correlation. By reinvestigating the relationship between size and fluorescence of single particles in flow cytometry, in addition to testing the quenching effects of Ca\(^{2+}\), EDTA, and DOC concentration on the bulk fluorescence spectra, the sources of the fluorescence quenching and resulting implications can be better identified.

The Stern-Volmer relationships (Table 3.3) indicated that [Ca\(^{2+}\)] is quenching CTC emission fluorescence at 530 nm in Puget Sound seawater samples with and without EDTA, although greater quenching is observed with EDTA. At higher relative Ca\(^{2+}\) concentrations, the CTC molecule can form a complex with up to two Ca\(^{2+}\) through independent coordination by ring A and conjugated BCD ring system (Fig. 3.1a) (Mathew and Balaram, 1980). At low Ca\(^{2+}\) concentrations (potentially when EDTA is added), 1:1 metal complexes create favorable geometry to more efficiently transfer excitation energy from the conjugated BCD ring system to ring A (Mathew and Balaram, 1980). Therefore, the observed quenching with higher Ca\(^{2+}\) concentration is probably due to a reduction in energy transfer from the emission band at 430 nm (BCD ring system) to
the emission band at 530 nm (ring A). This hypothesis is supported by a lower ratio of fluorescence emission at band A (530 emission) to band BCD (430 emission) at 380 nm excitation (F_A/F_{BCD}) (Fig 3.1; Fig. 3.8a) at elevated [Ca^{2+}] (see color legend). However, when EDTA was added to chelate Ca^{2+}, the transfer of energy from the BCD band to the A band appeared to decrease, observed by lower emission at 530 nm (solid squares, Fig. 3.8a). In order for excitation energy to be transferred to the ring A chromophore (via the BCD chromophore), the CTC-Ca^{2+} chelate must bind through Ca^{2+} to the polymers (Mathew and Balaram, 1980). Thus, EDTA, a strong Ca^{2+} chelator, is likely interfering with this transfer of energy between fluorophores bands and is contributing to the fluorescence quenching.

**Fig. 3.8.** Puget Sound seawater quenching experiments with Ca^{2+} serial addition: a) Ratio of fluorescence emission at band A (530 emission) to band BCD (430 emission) (F_A/F_{BCD}) at 380 excitation as a function of pH. Each cluster at various pH (7.5, 7.7, 8, and 8.2) contains treatments with and without EDTA and additional Ca^{2+} treatments (0-20 mM). b) Proportion of OC as a gel (%) as a function of the ratio of fluorescence of the A and BCD emission bands (530 and 430 nm, respectively) excited at 380 nm with and without EDTA treatments (filled squares and open circles, respectively), with pH values ranging from 7.5 to 8.2.
However, at constant pH, higher DOC concentrations also appear to quench fluorescence of the assembled gel fraction (F1) (Fig 3.4). This effect likely contributes to the greater quenching observed in cultures at higher pH due to enhanced primary production and elevated DOC (Fig 3.3, Fig 3.5, Table 3.2). Although higher DOC concentration contributes to fluorescence quenching of CTC, when DOC held is constant and pH is varied (Fig 3.4 and 3.8), pH appears to be the primary driver.

*Selectivity of EDTA* – The second assumption of Ding et al. (2007) was that the background fluorescence does not change when EDTA is added, and thus the observed change in CTC emission upon addition of EDTA can be used to quantify the fraction of OC present assembled as a gel. In testing this assumption, it appears that the relationships between CTC fluorescence, Ca$^{2+}$ and EDTA are subtler than described in Ding et al. (2007). As demonstrated in Fig. 3.6b, EDTA does not affect the relationship between mean particle size and fluorescence, but it does appear to affect the ratio of the fluorescence in the two fluorophores of CTC, or $F_A/F_{BCD}$ (Fig 3.8). Again, this is probably related to EDTA removing Ca$^{2+}$ necessary to transfer excitation energy from the BCD ring system to ring A.

Another possibility is that EDTA is replacing CTC in its complex with Ca$^{2+}$ and consequently lowering the fluorescence of CTC (because CTC is no longer bound to Ca$^{2+}$). Multidendate ligands can rapidly replace other multidendate ligands in their complexes with metals (Carr et al., 1967). In a different study, EDTA changed the conformational structure and activity of an enzyme that required Ca$^{2+}$ complexation for catalysis (Shin et al., 2011). After reinoculating the Ca$^{2+}$ - deficient enzyme with CaCl$_2$, the original conformational structure and activity (before EDTA was added) was restored.
(Shin et al., 2011). The EDTA-induced conformational changes to the enzyme suggest that EDTA had chelated $\text{Ca}^{2+}$ that was bound to the enzyme, implying that EDTA is not exclusively chelating the free ionic $\text{Ca}^{2+}$ cross-links, but is also capable of chelating $\text{Ca}^{2+}$ bound to free DOC polyanions. If this is the case, then the assumption by Ding et al. (2007) that EDTA induces fluorescence dequenching by allowing CTC-stained free DOC polyanions to fluoresce is likely incorrect.

*Magnitude of CTC fluorescence in assembled microgels vs. polymers* – The third major assumption of Ding et al. (2007) is that CTC fluorescence emission should be higher when $\text{Ca}^{2+}$ is bound to free DOC polyanions compared to DOC polyanions in microgel networks. This assumption was tested when comparing the mean 520 nm emissions against FSC (size) in flow cytometry (Fig. 3.6b). The observed positive linear relationship between the mean size and 520 nm emission of particles negated Ding et al.’s (2007) assumption. The results comparing TEP concentrations to the proportion of OC present as a gel (%) (Fig 3.7a, at situ pH) also indicate that the calculation of QF (Equation 3.1) should be related to a decrease in fluorescence, instead of an increase. However, it is also possible that the assembled gels have too much steric hinderance for CTC to completely penetrate the interior of the gel network, and this could lead to the increase in CTC emission that was described by Ding et al. (2007) and observed by Baltar et al. (2016). If that is the case, then the degree of CTC penetration into assembled gels does not represent quenching and would contribute to the variability and lack of reproducibility of the method.
3.5.2 Implications for previous work

This study was not able to replicate the linear correlation between measurements of gel C and microgel concentration via flow cytometry that was reported by Ding et al. (2007) (Fig. 3.6a). Lack of replication could be related to the effect of colder analysis temperatures used for Ross Sea samples (See section 3.3.1) or improved detection limits for flow cytometers. Previously, the flow cytometry method used to analyze gel particle concentrations (Chin et al., 1998; Ding et al., 2007; Orellana et al., 2007; Orellana and Verdugo, 2003), relied on the guiding principle that EDTA dispersed microgel particles into polymers (< 20 nm) too small to be detected. As the flow cytometer used in this study had improved limits of detection (~0.1 µm), gel particles dispersed to polymer sizes (< 0.1 µm) are still lost to instrumental noise. Because the samples were not maintained at pH 8 as described in Orellana et al. (2007), the effect of EDTA in Fig. 3.6b is likely driven by the decrease in pH when EDTA is added.

Clearly, it is critical to understand how pH, DOC concentration, Ca\(^{2+}\) availability, and temperature affect the application of any microgel assay. pH was observed to affect the ratio of fluorescence between the two emission bands (F\(_A\)/F\(_{BCD}\)) at 380 nm excitation (Fig 3.8a), which consequently affected the proportion of quenching used to determine the proportion of OC present as a gel (QF) in analyses (Fig. 3.8b; R\(^2\)= 0.72 with EDTA, R\(^2\)= 0.5 without EDTA). When examining results obtained using Ding et al. (2007) to establish equilibria conditions (Figs. 3.4,3.5) and to compare against TEP concentrations (Fig 3.7) and single-particle analyses (Fig. 3.6a), the dependence on pH and DOC concentration is evident.
When Baltar et al. (2016) purposefully lowered the pH of samples collected along the Munida Time Series Transect off the coast of New Zealand to examine the effects of ocean acidification on the proportion of OC present as a gel (Ding et al., 2007), they reported greater gel proportions at lower pH (grey triangles; Fig. 3.3). They attributed this increase in gel proportion to the expected increase in free ionized [Ca$^{2+}$] at lower pH, which they hypothesized would enhance microgel crosslinking. Even so, from the evidence presented in the current study, if there were an increase in [Ca$^{2+}$], greater fluorescence quenching would occur.

3.5.3 Recommendations for future work

Experimental and environmental parameters such as pH, temperature, DOC concentration, and Ca$^{2+}$ concentration (and thus potentially alkalinity) should be carefully considered in future studies. For example in Fig. 3.7, there would be a positive linear correlation between gel C and TEP at the in situ pH (that also correlated with higher DOC and pH) if Equation 3.1 was modified to reflect the decrease in fluorescence expected with dispersion of smaller particles (Fig 3.6b). In this study, the comparison to TEP concentration was used to provide an upper limit to the proportion of OC determined as a gel, which seemed useful when the analysis at pH 7.5 showed little OC present as a gel (Fig. 3.7a). Although it is likely that pH is the primary driver of the variability (Fig. 3.7b; Fig 3.8) in the Ding et al. (2007) method, perhaps pH and DOC concentration also affect the determination of TEP concentration.

Future work should consider the potentially negative effects of high DOC concentrations and high pH to better understand how DOC quenches fluorescence and better characterize the pH where crystallization of the gel matrix might be occurring.
These experiments would be useful to determine the environmental conditions where the dissolved-to-particulate transition is reversible in polymer gel dynamics. Modifying the technique to buffer the samples would help prevent the negative side effects from lowered pH when EDTA is added to seawater, which may also enhance the stability of the measurements.

Fluorescence microscopy, flow cytometry, or DLS could be useful techniques to address the effect of pH on the microgel size. Future direction should also include consideration of how changing detection limits affect how microgels and polymers can be sorted. As detection limits of flow cytometry continue to decrease or new instrumentation proves capable of detecting concentration and size of nanopolymers, there will be increased opportunities to expand understanding of assembly dynamics of microgels that do not rely on detection of CTC.

3.6 Summary

Two processes have been identified to affect quenching of CTC fluorescence in seawater while following the Ding et al. (2007) method. First, the available \([\text{Ca}^{2+}]\) appears to affect the transfer efficiency of excitation energy (at 380 nm) between fluorophores corresponding to emission bands peaking at 430 nm and 530 nm. EDTA chelates the available \([\text{Ca}^{2+}]\) and likely also physically displaces CTC from any existing bonds with \(\text{Ca}^{2+}\), both of which lower the fluorescence of CTC in seawater, even after samples were carefully titrated back to the original pH. Second, pH plays an important role in the transfer efficiency of energy between the two emission bands, as the conjugated BCD ring system band is associated with a pKa \(\sim 7.7\). DOC concentration and
EDTA addition were also identified to play a role in quenching dynamics. In its current form, the fluorescence quenching method described by Ding et al. (2007) is not effective at determining the proportion of organic carbon present as a gel because of a misunderstanding of quenching dynamics. The current study provides new evidence to explain the quenching dynamics of CTC in seawater and enables future advances to methodology for measuring marine polymer gels.
CHAPTER 4. A dissolved organic carbon (DOC) analyzer capable of detecting sub-µM DOC differences in natural fresh waters: A proof of concept study

4.1 Overview

This study reports the development of a new type of organic carbon (OC) analyzer that combines flow through analysis and wet chemical oxidation (WCO). Total or dissolved organic carbon (TOC or DOC, respectively) is determined through detection of CO\(_2\) as the oxidation product using a vibration insensitive non-dispersive infrared (NDIR) gas analyzer detector. Aided by heat and UV, OC is oxidized by continuously pumping the liquid sample in-line with a carbon-free acidic persulfate reagent at a constant flow rate through the system. The CO\(_2\) produced from oxidation is sparged from solution at a precise flow rate and is continuously detected, producing a stable signal with enhanced high signal-to-noise ratio. A near-zero carbon blank/baseline for the analysis may be achieved by recycling persulfate reagent alone through the system until essentially zero CO\(_2\) is detected. Standard high temperature combustion (HTC) instrumentation is subject to high blank variability and micromolar precision (CV ~3%). Progress is shown for the optimization of this novel instrument, which, unlike HTC, could have potential applications for studies of DOC net production and consumption, by various mechanisms that typically require sub-micromolar resolution.

4.2 Background

DOC is an active and quantitatively significant component of the global carbon cycle (Dittmar and Stubbins, 2014). The recognition of DOC as an actively cycling, ecologically important factor in aquatic ecosystems was facilitated by advances in the
analytical precision of DOC measurement since the mid-1990s (Sharp et al., 2002). However, there are still many outstanding analytical questions that require higher precision DOC concentration measurements. Today’s standard methods for determination of DOC (or TOC, if unfiltered) employ HTC or wet oxidation techniques (Wangersky, 1993). Both methods measure CO₂ as the oxidation product of OC, after the initial dissolved inorganic carbon (DIC) has been sufficiently stripped from the sample through preliminary acidification and sparging. CO₂ determination is often performed using NDIR detectors (Dickson et al., 2007; Menzel and Vacarro, 1964; Peltzer et al., 1996; Sharp et al., 1993), but other techniques such as inductively coupled plasma atomic emission spectrometry (Maestre et al., 2003; Stefánsson et al., 2007) and sequential injection spectrophotometry with on-line UV photo-oxidation (Tue-Ngeun et al., 2005) are also used.

The aquatic science community has largely adopted HTC techniques because of their relative simplicity, high oxidation efficiency, and good accuracy and precision when properly blanked (Sharp et al., 2002, 1993; Wangersky, 1993). HTC techniques involve combustion of DOC to CO₂ upon direct aqueous sample injection into a high temperature column (680-900 °C), usually packed with a catalyst such as platinum, cobalt oxide, copper oxide, or silver oxide (Dickson et al., 2007). The precision of commercially available HTC systems is 1-2 µM C at best (Hansell, 2005), which is good enough for a routine understanding of DOC dynamics. However, this precision is insufficient for more specific studies that require higher resolution measurements on shorter timescales such as DOC production and consumption at environmentally relevant timescales (Allesson et al., 2016; Bertilsson and Tranvik, 2000; Hansell, 2013; Seekell et al., 2015; Tranvik et al.,
and modeling the conservative mixing of water masses (Bercovici and Hansell, 2016; Hansell and Carlson, 2013; Reinthaler et al., 2013; Romera-Castillo et al., 2016).

Wet oxidation methods include wet chemical oxidation (WCO), UV-assisted WCO (UV-WCO), and ultraviolet oxidation (UVO). In WCO, a strong chemical oxidant such as persulfate is added to the aqueous sample in either batch analyses (using a sealed glass ampule or reusable reaction chamber) (McKenna and Doering, 1995; Menzel and Vaccaro, 1964) or via flow injection analysis (FIA) (Cauwet, 1984; Collins et al., 1977). In UV-WCO, persulfate oxidation of organic matter is enhanced in the presence of UV via oxidation by reactive oxygen species (Benner and Hedges, 1993; Cauwet, 1984; Lee and Henrichs, 1993; Matzek and Carter, 2016; Rocha et al., 2015). Whereas, in UVO, only ultraviolet light is used to oxidize organic carbon (Armstrong et al., 1966; Mueller and Bandaranayake, 1983). Heating persulfate increases the kinetics of sulfate radical formation, thereby increasing its oxidative capacity as a reagent (Peyton, 1993). Pretreatment with heat reduces the organic carbon contamination in the persulfate reagent (McKenna and Doering, 1995; Osburn and St.-Jean, 2007). However, if the reaction temperature is too high, efficiency of OC remineralization decreases by releasing radicals too quickly (Peyton, 1993). Problems with batch analysis arise because the oxidation potential of the reagent decreases with reaction time (Wangersky, 1993). Employing a flow through system removes these problems because reagent is continuously added to the system.

Whether WCO or HTC, current techniques still give variable blanks and insufficient precision to allow for consistent determination of sub-micromolar changes between samples. Large variability and high system blanks result in lower precision and higher
detection limits, limiting the accuracy and precision of TOC measurement in low TOC waters. There are methods for aiding the blank evaluation of “zero carbon” water, whereby hydrogen peroxide/persulfate is added to the blank, or the blank is recycled online through the instrument (Benner and Strom, 1993).

To overcome the limitations of current instrumentation, this study designed a proof-of-concept UV-WCO DOC analyzer with a significantly reduced and highly stable system blank and higher precision than existing DOC analyzers. This instrument has the potential to resolve DOC dynamics (Craft et al., 2002; Findlay et al., 2003; Lapierre and del Giorgio, 2014; Read and Rose, 2013) to an unprecedented precision, allowing a more refined assessment of the role of aquatic carbon processing in global biogeochemical cycles and climate change. The development of this DOC/TOC analyzer can be applied in fields outside of aquatic sciences, especially where high precision, low detection limits are important, e.g., monitoring organics in process water (e.g., cooling water and condensate/boiler feed water in power plants), in high purity water generating systems, in drinking water, in sterilization water used in biotech processes, in semiconductor production and reclaim water, and in a variety of regulatory operations (Drewes and Horstmeyer, 2016; Gibert et al., 2013; Husted et al., 1996; Jjemba et al., 2014; Kamjunke et al., 2016; Kaplan et al., 2006). The stability and the low concentration of the blank of this prototype could also facilitate improved precision for the measurement of the carbon stable isotope value ($\delta^{13}$C) of DOC (Osburn and St-Jean, 2007). The ability to run the system in continuous mode, could also allow both DOC concentration and $\delta^{13}$C to be monitored in natural waters, managed waters, or during the course of experiments and treatment processes.
4.3 Methods

4.3.1 System design overview and principle

The analyzer described here was constructed at University of Miami (FL) in the Hansell laboratory and quantifies DOC by measuring its oxidation product through CO$_2$ detection using a NDIR gas analyzer. Acidifying and sparging the sample with high purity N$_2$ gas prior to analysis removes the pre-existing inorganic carbon. Fig. 4.1 is a schematic of the components of the instrument. Once DIC has been stripped from the original sample, DOC is converted to CO$_2$ through several oxidative radical reactions promoted by UV radiation, controlled and continuous addition of DOC-free acidified persulfate reagent, and heat. The oxidized sample solution is continuously pumped through the system at a constant flow rate using high precision pumps. CO$_2$ is removed using a mass-flow controlled gas extraction cell (or sparging chamber) and sent to the detector, which is an approach similar to that used for a high precision method for analyzing DIC in seawater (Kaltin et al., 2005). A near-zero carbon blank/baseline for the analysis is achieved by recycling the acidified persulfate reagent alone through the system until a stable signal is obtained after all trace OC is oxidized.

4.3.2 Materials and reagents

The persulfate reagent was prepared as 30% w/v Na$_2$S$_2$O$_8$ (Fisher Scientific) with 4% H$_3$PO$_4$ (Sigma Aldrich) in ultra-pure (Millipore) water. Organic carbon standards (0-150 µM C) were prepared from potassium hydrogen phthalate (KHP; Sigma Aldrich) in ultra-pure water. Standards, samples, and blanks (ultra-pure water) were acidified with 1 mL concentrated hydrochloric acid (Sigma Aldrich) per 1000 mL sample, though
acidification with phosphoric acid is also suitable. Sparging was done with ultra-high purity N₂ (Airgas) further purified with Drierite, Ascarite, and activated charcoal traps.

**Fig. 4.1.** Schematic of OC analyzer components. (1) Detector LI-6262, NDIR CO₂/H₂O gas analyzer (LI-COR, USA), (2.1) sample and (2.2) reagent dual-syringe high precision pumps (Pharmacia P-500), (3) mass-flow controller (Tylan), (4) N₂ gas tank, (5) UV lamp (pen-ray), (6) quartz coil, (7) oven from a gas chromatography system (Hewlett Packard, USA), (8) sparging chamber/extraction cell with glass frit, (9) peristaltic pump for liquid waste, (10) condenser, (11) copper halide trap, (12) switching valve for recycle/“clean up” mode or waste, (13) switching valve for samples S1 or S2, and (14) computer. Tubing connections are shown for gas lines (dashed). Liquid lines are shared by reagent recycle or “clean-up” mode (red dotted) and sample analysis mode (purple solid).

CO₂-impervious tubing (PEEK, thick-walled Teflon or 316 stainless steel) was used for all liquid and gas transfer lines. Liquid pumping was performed with two high precision, acid resistant syringe pumps (Model P-500, Amersham Pharmacia Biotech UK) designated for sample and reagent pumping. A low-pressure mercury UV lamp (Pen ray 3SC-9; 22.9 cm illuminated length; 254nm primary output; 5400 µW cm⁻² average intensity and ~50°C body temperature at a1.9 cm distance) was placed inside the clear fused quartz reaction coil (Quartz Scientific, Inc.; overall dimensions: 4 cm coil OD, 27
cm length; tube dimensions: 6 mm OD, 2 mm ID; 75-90% transmittance at 254 nm). Following the quartz reaction coil, Teflon tubing (3 mm OD, 2 mm ID, 6.7 m length), which has low permeability to liquids and gases such as CO₂, was placed inside a GC oven (Hewlett Packard 6890 Series), which was capable of maintaining precise and constant reaction temperatures. A high precision mass flow controller (50 SCCM; UFC-1100, UNIT Instruments, Inc.) connected to a four-channel readout (MKS Type 247C) regulated the gas flow to the sparging chamber. The CO₂ sparging chamber (Kaltin et al., 2005) that received liquid from the oven was fabricated in-house. A condenser recycling 5°C water was attached to the top of the sparging chamber to capture water vapor. The sparged CO₂ was quantified with a LI-COR model 6262 NDIR detector. In contrast to other NDIR detectors in common use, the LI-COR detector is insensitive to vibration and, thus, is more robust for use in the field. A copper trap was placed between the condenser and the detector to protect the detector from corrosion arising from halides and persulfate (Osburn and St-Jean, 2007). Lab-VIEW (National Instruments) was used to collect the detector signal. A peristaltic pump (Peri-star PERIPRO-4LS; World Precision Instruments) with silicon pump tubing (Cole-Palmer 07616-48) controlled the flow of liquid exiting the sparging chamber. Nafion tubing (not shown) may be added following the condenser in order to better remove water vapor if found to interfere with the detector.

A second system built in the Mopper laboratory (Old Dominion University, VA) equipped with a newer LI-COR model 7000 detector and minor modifications gave similar signal to noise and reaction efficiency results. For simplicity, only results from the system in the Hansell laboratory (University of Miami, FL) that was described above
are presented. The exception is that the halide interference experiments were performed on the system in the Mopper laboratory.

4.3.3 Instrumental modes

Reagent clean-up mode – Prior to placing the persulfate reagent solution into the instrument, it was heated to 80°C for one hour on a hotplate to remove organic contaminants (Osburn and St-Jean, 2007; Peyton, 1993). The reagent was further oxidized, sparged, and recycled online to remove all residual organic contaminants so that the reagent blank was essentially zero prior to mixing with the sample. The option to switch to reagent recycle (or clean-up mode) mode is illustrated by the dotted red path in Fig. 4.1, where the waste valve and sample pump would be switched off, so that the persulfate reagent recycles through the sample path, including the sparging chamber, and back to the reagent reservoir. The reagent was recycled through the system by a high precision pump at a flow rate of 8 mL min\(^{-1}\) and resultant DIC was sparged at a rate of 10 mL min\(^{-1}\) until the CO\(_2\) baseline stabilized at a low signal level.

Sample analysis mode – To remove pre-existing CO\(_2\) prior to analysis, all samples, KHP standards, and blanks were acidified with HCl, dropping the pH to < 2 to enable stripping of DIC, and sparged with high purity N\(_2\) gas (~35 mL min\(^{-1}\)), for at least 5 minutes before beginning to pump the sample into the system via the high precision pump. A second high precision syringe pump pushed acidified OC-free persulfate reagent into the sample stream at the prescribed, constant rate. The sample/persulfate mixture passed through quartz reaction tubing that was coiled around a low-pressure mercury UV-lamp to ensure complete mixing and oxidation and then through a constant temperature (90° C) GC oven to increase oxidation efficiency. Upon exiting the oven, the
sample/persulfate mixture went to the sparging chamber where DIC produced from OC oxidation was sparged from solution and carried in the gas stream through the in-line condenser and halogen trap to the NDIR CO₂ detector.

4.3.4 System optimization, precision and accuracy

Effects of changing various system parameters on system performance (i.e. response time and relative signal intensity) were investigated to optimize the instrument. Experiments were designed to test effects of reaction temperature, quartz reaction coil length (i.e. UV irradiation time), concentration of persulfate in the combined liquid flow, liquid flow rate and offline reagent pre-treatment heating on the oxidization of 50 µM C KHP standards. Reaction temperature was varied from 60 to 120°C. An additional reaction coil length (6 cm) was investigated and compared to the effect of removing UV from the system. The final concentration of persulfate in the sample/persulfate mixture was varied from 4 to 6%. The sample/persulfate mixture flow rate was varied from 8 to 15 mL min⁻¹, and the initial offline heat treatment to remove OC from the persulfate reagent was varied from 1 hour at 80°C to 3 hours at 100°C.

KHP standards were used to calibrate the instrument signal and confirm signal linearity. Precision was examined by calculating the signal-to-noise ratio (S/N) and coefficient of variation (CV) for each standard and sample. S/N was calculated by dividing the average signal by the standard deviation of the signal integrated over time (once oxidation is complete), and CV was calculated as the inverse of S/N times 100.

All calibration standards, blanks, and samples (without persulfate reagent) were also analyzed by HTC using a Shimadzu TOC-L equipped with an auto analyzer (ASI-L) following standard methods (Dickson et al., 2007) and quality controlled using consensus
reference materials (CRM) (Hansell, 2005). Bias was estimated as the percent difference between the HTC method (TOC-L) and prototype analyzer results for an unknown sample.

4.3.5 Freshwater sample collection

Two freshwater samples (salinity 0.2) were collected from Taylor Slough (-80.607 °E, 25.404 °N), the Florida Coastal Everglades Long Term Ecological Research Program site (FCE LTER Program) on February 11, 2016. Samples were collected whole and filtered through 47 mm glass fiber filters (GFF; Whatman; 0.7 µm nominal pore size), acidified, and stored at 4°C in dark bottles. The DOC concentration of the original samples were determined as 529 and 546 µM C by HTC. Prior to analysis on the prototype system, samples were gravimetrically diluted with ultra-pure water to provide a range of concentrations to test as sample unknowns.

4.3.6 Halide optimization experiments

Stock solutions of CoSO₄ (saturation above 1.5M) were added to the persulfate reagent (or in one experiment, directly to the sample) to obtain final concentrations of 0.05M, 0.3M, and 0.5M in the sample/reagent mixture. CoSO₄ was added after the persulfate reagent pre-cleaning step to minimize oxidation of Co(II) to Co(III). The final persulfate concentrations were 0.25M, 0.38M, 0.42M, or 1.3M Na₂S₂O₈. Experiments examined the efficiency of CoSO₄ to remove the chloride interference on the oxidation by persulfate by comparing analytical signals of 50 µM C standards prepared in MQ water with and without NaCl (pre-combusted overnight at 450 °C to remove OC contamination). Salinity was converted from chloride concentration and used to calculate the density of the standards prepared in NaCl. Experiments were performed in the
Mopper Lab at constant conditions of 11.5 mL min\(^{-1}\) reagent/sample mixture flow rate, 110 °C, 27 cm length quartz coil, LI-COR model 7000 NDIR detector.

4.4. Results

4.4.1 Obtaining a near-zero system blank

To determine the effectiveness of reagent clean-up mode (Fig. 4.1) in removing trace TOC over time, 2 L of persulfate reagent was recycled online for six hours per day for four consecutive days at 8 mL min\(^{-1}\) at 80 °C (Fig. 4.2). Within each day the baseline signal decreased over the six-hour recycling time, suggesting some rebound in DOC when recycling was paused. Over the course of the four days, the baseline decreased from day 1 to 2 to 3, with days 3 and 4 being near identical. Longer off-line treatment of persulfate reagent heating at higher temperature (100 °C) decreased the time required to reduce the baseline to one day. However, the baseline increased over consequent days and a lower slope for the standard calibration curve (slope = 7.75; \(R^2 = 0.99\)) relative to the average slope obtained from a lower temperature and shorter off-line treatment (mean slope = 10.2). Off-line heat treatment can also decompose persulfate (Kolthoff and Miller, 1951), potentially adversely affecting the oxidation efficiency of the reagent. Thus, a balance needs to be struck between reducing background reagent OC to near-zero and maintaining the oxidation efficiency of the reagent.

4.4.2 Sample analysis

After switching to sample (or standard) analysis mode, the \(CO_2\) signal increased dramatically compared to the blank signal (baseline) and reached a plateau (Fig. 4.3). The resulting signal of a 50 \(\mu M\) C standard took around 20 minutes to plateau, where the
standard deviation of the plateau signal was < 1 ppm CO₂ over a 5-minute period. Because the detector records data every 1-second, the analytical signal was determined by averaging hundreds to thousands of data points on the resultant plateau. The ability to integrate a signal plateau instead of integrating a single peak is one of the main reasons the method described here is able to attain a much higher precision than standard peak injection systems, such as the HTC Shimadzu TOC analyzer.

**Fig. 4.2.** Time-based signal of recycle, or “reagent clean-up,” mode of prototype system to remove OC contamination from persulfate reagent prior to use in sample analyses.

![Time-based signal of recycle, or “reagent clean-up,” mode of prototype system to remove OC contamination from persulfate reagent prior to use in sample analyses.](image1)

**Fig. 4.3.** Example of the signal difference between a blank and a 50 µM C standard on the prototype system. Once a signal plateau is reached, the sample/standard data are averaged over time and compared to the blank baseline.

![Example of the signal difference between a blank and a 50 µM C standard on the prototype system. Once a signal plateau is reached, the sample/standard data are averaged over time and compared to the blank baseline.](image2)
4.4.3 System optimization

There was a positive linear correlation between reaction temperature and the CO₂ signal generated by oxidation of 50 µM C (slope = 1.2 ppm CO₂ °C⁻¹; R²=0.99). However, temperatures >100°C resulted in boiling inside the liquid lines and reduced precision, therefore 90°C was selected as the optimum reaction temperature. Using tubing of 6.7 m length and 2 mm ID inside the oven at 90 °C, the approximate reaction time for 50 µM C to reach an oxidation signal plateau was 25 minutes. A final persulfate concentration of 6% in the sample/persulfate mixture was selected to maximize the oxidation kinetics. The 10 mL min⁻¹ combined flow rate of the sample/persulfate mixture was selected because at higher flow rates, the sensitivity decreased, while the analysis time trended higher for lower flow rates (Fig. 4.4)

Fig. 4.4. The flow rate of the sample/persulfate mixture (mL min⁻¹) was optimized by examining the resulting variations in a) time required to reach maximum oxidation (signal plateau) and b) signal intensity (ΔCO₂ abs x1000) on the oxidation of 50 µM C standards at 80°C

Different signals resulted from measuring 50 µM C standards with the prototype system when the length of the quartz reaction coils was changed or if the UV lamp was turned off. Relative to the blank signal, the longer 27 cm quartz reaction coil UV
oxidation generated a larger signal response when measuring the standard (120 ppm CO₂) compared to the shorter 6 cm quartz reaction coil (60 ppm CO₂). Removal of the UV component from the system resulted in a response of 17 ppm CO₂. Longer UV exposure time significantly improved the sensitivity of the prototype, thus the longer 27 cm coil was selected as the routine quartz reaction coil (Fig. 4.1).

4.4.4 System accuracy and oxidation efficiency with natural samples

Calibration experiments demonstrated that the prototype is linear within the ranges tested (0 – 150 µM C) and there is overall agreement between results obtained from the prototype and HTC (Fig. 4.5). Low concentration standards (0 – 10 µM C; Fig. 4.5 inset) were also calibrated on the prototype and the slope (10.2) fell within the range observed when calibrating with higher standard concentrations (8.20 – 11.2), whereas the HTC system overestimated these low concentrations (secondary y-axis; blue circles).

**Fig. 4.5.** Calibration of prototype system signal (CO₂ ppm) with TOC concentrations of standards determined externally on the TOC-L by HTC. *Inset:* Calibration of prototype system (black squares) and HTC (blue circles; secondary vertical axis) with a low concentration range of 0-10 µM C (determined gravimetrically).
Upon measuring DOC concentrations in diluted freshwater samples having concentrations ranging from 26.92 to 86.99 µM C, the prototype was on average 3.57% (SD= 3.5%) lower than results obtained from HTC on the same samples (Table 4.1). A paired t-test determined that the mean difference between prototype and HTC measurements was significant (-1.79 µM C, SD= 1.76 µM C); t(8)=3.05, p = .016. Fig. 4.6 compares the results obtained for sample determinations from both instruments.

**Table 4.1.** Comparison of sample determinations per method.

<table>
<thead>
<tr>
<th>Sample Analysis</th>
<th>prototype (µM C)</th>
<th>TOC-L (µM C)</th>
<th>Difference (Prototype – TOC-L) (µM C)</th>
<th>% difference (µM C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56.51</td>
<td>54.61</td>
<td>1.90</td>
<td>3.42</td>
</tr>
<tr>
<td>2</td>
<td>55.01</td>
<td>51.59</td>
<td>3.42</td>
<td>6.42</td>
</tr>
<tr>
<td>3</td>
<td>60.03</td>
<td>60.37</td>
<td>-0.34</td>
<td>0.56</td>
</tr>
<tr>
<td>4</td>
<td>60.64</td>
<td>56.58</td>
<td>4.06</td>
<td>6.93</td>
</tr>
<tr>
<td>5</td>
<td>29.11</td>
<td>26.92</td>
<td>2.19</td>
<td>7.83</td>
</tr>
<tr>
<td>6</td>
<td>61.88</td>
<td>62.04</td>
<td>-0.16</td>
<td>0.26</td>
</tr>
<tr>
<td>7</td>
<td>66.36</td>
<td>62.42</td>
<td>3.94</td>
<td>6.11</td>
</tr>
<tr>
<td>8</td>
<td>88.13</td>
<td>86.99</td>
<td>1.14</td>
<td>1.30</td>
</tr>
<tr>
<td>9</td>
<td>85.78</td>
<td>85.83</td>
<td>-0.05</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>average</strong></td>
<td><strong>1.79</strong></td>
<td><strong>3.65</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>s.d.</strong></td>
<td><strong>1.76</strong></td>
<td><strong>3.19</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**4.4.4 Figures of merit**

Figures of merit for each instrument are summarized in Table 4.2. When averaging individual analyses (standards and samples), the CV of the prototype was 0.42%, which was 3-fold lower than the HTC TOC-L system at 1.4% (Fig. 4.7). The average S/N ratio of the prototype was 370 (from 5 to 150 µM C), which was ~4 fold higher than the HTC analyses, averaging 84. The detection limit for the prototype system (3.6 µM C) was lower than for the HTC system (5.1 µM C), due to a lower signal blank average and deviation in the prototype (2.5 µM C, SD = 1.2) compared to HTC (5.4 µM C, SD= 1.8) (Table 4.2).
Fig. 4.6. Direct comparison of calibrated sample concentrations determined using the prototype instrument and HTC (TOC-L).

![Graph showing the comparison between prototype and TOC-L concentrations](image)

\[ y = 0.9616x + 4.1261 \]

\[ R^2 = 0.9914 \]

Fig. 4.7. Overall comparison coefficients of variation (CV%) for individual HTC TOC-L (open squares) and prototype (filled circles) results for analysis of standards and samples (from 5 – 150 µM C; does not include blanks).

![Scatter plot showing CV% vs. µM C](image)

The main rationale for the development of the prototype was to increase analytical precision enabling small differences between samples to be quantified. To examine if the prototype was capable of detecting ~200 nM differences, 45 µM C standards were analyzed and manually spiked with 0.5% of their volumes with 75 µM C stock solution. The spike addition of 75 µM C stock solution should yield an approximate increase of ~0.15 µM C, but the exact pre-spike volumes were not known because the spike was added during measurement in situ. Based on the known CV for each instrument (0.42%
prototype; 1.4% TOC-L; Table 4.2), the expected standard deviation for each instrument to analyze a 45 µM C solution is 0.19 µM C (prototype) and 0.63 µM C (HTC), although the HTC CV maybe further compromised by variations in the blank (average 5.6 µM C).

**Fig. 4.8.** In situ results for before (pink shading) and after (green shading) addition of a 0.05% volume spike of 75 µM C stock. Since the analysis was not paused for additional sparging following the manual spike, the peak highlighted in the dashed circle was a result of dissolved CO₂ contamination from the spike solution. This contamination signal was excluded from calculations. The undulations in the signals are likely due to slight sparging gas variations.

![Graph showing in situ results](image)

When testing the actual performance of the two instruments (Fig. 4.8), the prototype determined the respective average pre_spike and post_spike concentrations to be 45.31 µM C (SD = 0.02 µM C) and 45.53 µM C (SD = 0.01 µM C), a difference of 220 nM C, while the TOC-L measured values indicated a difference of 2.2 µM C. Therefore, the prototype appears capable of detecting ~200 nM differences at ~50 µM C, while the HTC analyzer is not. The undulations in the baseline signals of the prototype, Fig. 4.8, are likely due to slight variations in the liquid volume within the sparging chamber, which become noticeable at high detector sensitivities. Improvements in the sparging chamber
plumbing are being examined and these should enhance the system sensitivity, with the aim of detecting DOC (or TOC) differences of <100 nM.

**Table 4.2.** Comparison and description of the analytical figures of merit (CV, S/N, and detection limit) and description and values for mean blank signal, standard deviation of the blank signal, minimum distinguishable signal, and calibration sensitivity (in respective signal units) used to calculate the detection limits (µM C⁻¹) for the prototype and TOC-L HTC systems.

<table>
<thead>
<tr>
<th>Figure of merit or consideration (signal unit)</th>
<th>Definition</th>
<th>HTC TOC-L</th>
<th>prototype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal unit</td>
<td>area (no unit)</td>
<td>ppm CO₂</td>
<td></td>
</tr>
<tr>
<td>Coefficient of variation (CV) (unit less)</td>
<td>standard deviation divided by average value x 100%</td>
<td>1.4%</td>
<td>0.42%</td>
</tr>
<tr>
<td>Signal-to-noise ratio (S/N) (unit less)</td>
<td>average value divided by standard deviation of the measurement</td>
<td>84</td>
<td>370</td>
</tr>
<tr>
<td>Mean blank signal (S_bl) (respective signal unit)</td>
<td>0.62</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>Standard deviation of the blank signal (s_bl) (respective signal unit)</td>
<td>0.20</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Number of blanks averaged</td>
<td>74</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Minimum distinguishable signal (S_m) (respective signal unit)</td>
<td>average blank signal plus 3 times the standard deviation of the blank signal</td>
<td>1.2</td>
<td>61</td>
</tr>
<tr>
<td>Calibration sensitivity (respective signal unit µM C⁻¹)</td>
<td>Average slope (m)</td>
<td>0.114</td>
<td>10.2</td>
</tr>
<tr>
<td>Detection limit (C_m) (µM C)</td>
<td>Min. distinguishable signal minus the average blank signal, divided by slope</td>
<td>5.14</td>
<td>3.58</td>
</tr>
</tbody>
</table>

### 4.4.5 Halide optimization

The oxidation signal for 50 µM C standards decreased as salinity was increased from 0 to 35 using persulfate reagent (Fig. 4.9a), highlighting an interference of halides (mainly chloride) with the persulfate oxidation of OC. The chloride ion lowers the oxidative strength of the persulfate reagent by competing with DOC for persulfate, forming chlorinated intermediate compounds alongside CO₂ (Aiken, 1992; McKenna and Doering, 1995). Increasing the persulfate-to-chloride ratio (Conaway et al., 2015; Godec et al., 1992; McKenna and Doering, 1995; Osburn and St-Jean, 2007; Peltzer et al., 1996)
or addition of heavy metals (such as Co or Hg) capable of forming a stable complex with chloride (Williams et al., 1993) increases oxidation efficiencies. Other approaches include modification of the system into a reagent free system that utilizes a longer offline UV treatment.

Fig. 4.9. a) Analysis of standards (49.5-51.5 µM C) at various salinities (0, 10, 25, 35) using a reagent mixture of persulfate (0.25M) and CoSO₄ (0.05M). b) Percent recovery of standards (49.5-51.5 µM C) in 35 salinity relative to 0 salinity as a function of the molar ratio of CoSO₄ to chloride (0 to 1.4 [Co]/[Cl⁻]). CoSO₄ was added to the reagent (persulfate-to-chloride ratio ≈ 1) (filled squares), added directly to the sample (open triangle)(persulfate-to-chloride ratio of the reagent ≈ 1), or not added (open diamond) with a higher persulfate-to-chloride ratio of the reagent (≈ 9). The dashed line represents a linear regression (R²= 0.98) of all treatments excluding the highest concentration CoSO₄ added to the reagent.

Different concentrations of CoSO₄ and persulfate were tested to optimize the percent recovery in the presence of chloride (Fig 4.9b). Initially, CoSO₄ was first added to the
reagent (Fig. 4.9b, black squares) and then mixed with the sample (Fig. 4.1). Upon observing no increased recovery after nearly doubling the molar ratio of cobalt-to-chloride ([Co]/[Cl]) from 0.8 to 1.4, CoSO$_4$ was added directly to the sample (Fig. 4.9b, open triangle). This resulted in the highest percent recovery. Finally, when the molar ratio of persulfate-to-chloride (without CoSO$_4$) was dramatically enhanced from ~1 in previous experiments to ~9 (Fig. 4.9b, open diamond), the percent recovery of 50 µM C standard in the presence of chloride did not improve, even though previous studies recommended persulfate-to-chloride molar ratios of 8.75 (McKenna and Doering, 1995) or lower (Menzel and Vacarro, 1964; Strickland and Parsons, 1972).

4.5 Discussion

4.5.1 Advantages of prototype system

By integrating a signal plateau instead of integrating a single peak, there is greater potential to improve the S/N at low OC concentrations, which often limits the detection limit of most instruments that operate discrete injections (i.e. HTC). The prototype can be calibrated at low C concentrations with similar sensitivity as high C concentrations (Fig 4.5). The results from the prototype system demonstrated superior S/N and CV values for individual analyses of samples and standards compared to the lower precision HTC analyses (Fig. 4.7; Table 4.2), especially at low OC concentrations. The higher S/N for the prototype system is related to the longer integration time for the prototype and peak integration error of the HTC system.
4.5.2 Limitations of prototype system

At least one full day of recycling, or two circulations, is necessary to maximize a reduction in the baseline to remove OC contamination from the reagent (Fig 4.2). In similar acidic conditions, a longer off-line heat treatment could more rapidly decompose persulfate (Kolthoff and Miller, 1951), thus adversely affecting the oxidation efficiency of the reagent. A separate, offline reagent recycling system could be employed to prepare the reagent without causing instrument downtime. The recycling system could employ a simple peristaltic pump to recycle the reagent through the same oven, through a separate quartz coil and UV lamp, and returned to the reagent bottle for sparging. A system of valves could allow the recycled reagent to go through the sparging cell and be detected.

Although this experiment showed promise for overcoming the halide interference in the persulfate oxidation of OC (Fig 4.9), it would cost 63.2 g of CoSO₄ per 300 mL sample to obtain a final cobalt-to-chloride molar ratio of 1.4, which is simply not practical for daily use. The next step will be to investigate approaches to do the online oxidation at neutral to alkaline pH values, where chloride will less significantly interact with OH radicals (Collins et al., 1977; Liao et al., 2001).

The offset of 4.1 µM C in the y-intercept of the linear regression of prototype and HTC results is likely due to the high system blank that is observed in the TOC-L HTC system (Dickson et al., 2007). The slope of the linear regression was 0.96 (standard error = 0.034; LINEST function, MS Excel 2011), indicating that the oxidation efficiency of the prototype is slightly lower than HTC, but comparable.
4.6 Summary

This chapter presented results for the proof of concept and initial optimization of a prototype OC analyzer that were in very good agreement with the standard HTC techniques while being relatively simple and inexpensive to construct. The current prototype TOC system has a markedly higher S/N ratio than the traditional HTC system, and is therefore capable of differentiating much smaller difference in the TOC between samples. The current prototype can detect ~200 nM OC differences compared to 1-2 µM differences by the HTC analyzer. However, the oxidation efficiency of the prototype system in freshwater is less than 100% (~96%).

Future work will incorporate methods to increase the oxidation efficiency, such as increasing and intensifying sample exposure to UV radiation and heat (Beaupré et al., 2007). Increasing the off-line heat treatment for the reagent may reduce the time required for reagent clean-up mode, but excessive heat may affect the oxidation efficiency and sensitivity. The current operating times of the prototype require a minimum of 20 minutes for the oxidization signal for a 50 µM C sample to plateau. Longer time is required for greater concentrations, which is slightly longer than 15 to 20 minutes per sample for the HTC analyzer (for 3 to 4 injections). Thus, the flow rate may be adjusted, and the dead volume (e.g. the length of tubing) may be reduced to shorten the analyzing time. Future work will also include miniaturization and automation of the system to alleviate this issue.

Presently, a seawater application is contingent on a successful optimization of the chloride interference anticipated during persulfate oxidation of organic matter (Aiken, 1992; Bauer et al., 1991; Osburn and St-Jean, 2007). However, the OC analyzer may now
be applied in freshwater studies where high precision, low detection limits are important to distinguish DOC net production and consumption by various mechanisms, or even in fields outside of aquatic sciences interested in the removal of organic contaminants or water quality.
CHAPTER 5. Conclusions

Through field observations and analytical improvements to current methods, this dissertation enhanced the understanding of organic carbon dynamics in the ocean. The following questions posed in Chapter 1 focused the three studies in Chapters 2-4: (1) What is the distribution of gel particles (i.e. microgels and TEP) in the ocean, and what do the spatial gradients tell us about TOC dynamics? (2) To what extent does gel formation play a role as a particulate sink of DOC? (3) Can the analytical precision of DOC measurement be improved and thus better address questions requiring higher resolution? Overall, the results presented in this dissertation contributed useful evidence toward eventually answering these questions in full, while improving our understanding of the methodology necessary to address future questions and challenges.

(1) What is the distribution of gel particles (i.e. microgels and TEP) in the ocean, and what do the spatial gradients tell us about TOC dynamics? – Chapter 2 most directly addressed this question by examining the role for TEP to facilitate POM export out of the euphotic zone. As the ratio of POC to TEP decreased from the phytoplankton bloom in the Slope Water region into the oligotrophic Sargasso Sea region, the sea surface microlayer (SML) became more enriched in both TEP and TOC. This evidence supported the hypothesis that the abundance of non-TEP organic particles determines whether TEP will accumulate in the SML or be exported to greater depths. Because this study did not measure bacteria parameters or DOC concentration, nor perform any incubation, it cannot directly answer questions related to the microbial loop, TEP bioreactivity, or to what extent TEP is a particulate sink of DOC (question 2). However, it is likely that bacteria were also enhanced in the SML according to previous studies (Kurata et al., 2016;
Reinthaler et al., 2008; Stolle et al., 2010), thus future studies should try to incorporate these measurements. Understanding the implications for TEP and TOC concentrations in the SML is important because the large surface to volume ratio of TEP may enable TEP to physically cover the SML and reduce molecular diffusion rates of gasses at the air-sea interface (Engel and Galgani, 2016).

After Chapter 3 investigated the Ding et al. (2007) method used to estimate the proportion of OC present in microgels in response to decreased pH (Baltar et al., 2016), it is now unclear whether the results of Baltar et al. (2016) are significant without having any alternative, complimentary method for comparison. It is still likely that with ocean warming and acidification, the equilibrium size and concentration yield of microgels will be affected (Chen et al., 2015), which will further implicate TOC concentrations and dynamics. More studies are required to provide evidence for an ocean warming and acidification synergism (Chen et al., 2015), such that eventually these impacts can be modeled for the global ocean.

(2) To what extent does gel formation play a role as a particulate sink of DOC? – This dissertation was not able to sufficiently evaluate this question due to the analytical challenges presented in Chapters 3 and 4. Future work could further resolve this by addressing the calcification issue identified in Chapter 3. To better understand the effect of calcification of gels with higher pH or condensation of gels at lower pH, it might also be beneficial to combine multiple techniques such as flow cytometry and fluorescence microscopy to quantify and visualize the effects of crystallization on gel properties. This characterization is necessary to completely understand equilibrium dynamics of the
dissolved-to-particulate continuum, which should also include measurements of gel size using DLS, due to the instrument’s superior detection limits.

(3) Can the analytical precision of DOC measurement be improved and thus better address questions requiring higher resolution? – Chapter 4 described a proof of concept study of a prototype instrument with sub-μM precision capability superior to current standard methods. However, this improved precision will not yet benefit the marine carbon community, as the proof of concept instrument does not efficiently oxidize organic matter in seawater. Although experimented addition of CoSO₄ directly to the seawater sample seemed to greatly improve the recovery of oxidized organic carbon relative to the same concentration in fresh water, the required concentration of the environmentally toxic catalyst is unfeasible for daily use. Future work will be directed to improve oxidation efficiency in seawater through enhanced UV oxidation times and should also seek advanced chemical engineering applications that could remove chloride ion without scavenging DOC in the process.

Additional suggestions for future work – The findings presented in Chapter 3 suggest that the fluorescence quenching/dequenching observed in Ding et al. (2007) is primarily controlled by pH (among other likely factors such as DOC and Ca²⁺ concentrations), by controlling the energy transfer between fluorophores. The relationship between the associated fluorescence change with microgel dispersion and pH appears complicated, because often the results reflect what could be expected in nature (Baltar et al., 2016; Orellana et al., 2011). Whether these results were purely coincidental or indicative of a stronger relationship to an undetermined variable (i.e. temperature, DOC concentration, or alkalinity) is still not known.
The following recommendations are provided if the fluorescence quenching mechanism of CTC is to be used as a tool for investigating microgels. Instead of collecting the emission at a particular excitation/emission wavelength pair, the entire emission spectrum (350 nm to 700 nm) should be collected with 380 and 345 nm excitation. The assay should also be coupled with at least one additional method or technique, such as flow cytometry, dynamic laser scattering (DLS), or TEP and DOC concentration analysis, to have a more complete picture of gel assembly dynamics. There are also specific recommendations for controlling and knowing certain experimental parameters beyond DOC and TOC concentrations, such as temperature, pH, and Ca\textsuperscript{2+} concentration.

Furthermore, the relationship between microgels and TEP is still not known, because TEP are too large to be measured by DLS or flow cytometry. In Chapter 3, there was an observed relationship between TEP and microgels measured using the Ding et al. (2007) method, but given the uncertainty of this method in its current form, it is difficult to draw any conclusions. A preliminary experiment included in Appendix A shows microscope images of particles in Del Playa Beach seawater collected on 0.4 \(\mu\)m polycarbonate filters mounted on frosted glass slides. The filters were dual stained with Alcian Blue (pH ~2) and CTC (pH 8) for TEP and microgel visualizations, respectively. Visually, there are similar places where particles were positive for both gel indicators, and some places of particles or even whole particles that uniquely stained for either TEP or microgels. These results, though only qualitative, are promising and suggest that future work should continue to investigate this relationship by building a novel approach that potentially incorporates microscopy.
Ultimately, this dissertation improved understanding of the analytical uncertainty and tools required to measure and study the associated dynamics of marine DOC and polymer gel concentrations. Through field observations and an assessment of DOC and gel methodologies and dynamics, this work used an analytical chemistry approach to enhance understanding of sources and sinks of marine organic carbon in the ocean. Although the objectives primarily focused on analyzing carbon, this work prescribes that future work incorporate the biogeochemical cycles of oxygen and nutrients in order to completely understand the system and predict the impacts and fate of organic matter remineralization in the ocean.
Appendix A: Dual staining of particles for TEP and microgel visualization using fluorescence microscopy

Particles from Del Playa Beach raw seawater (described in Table 3.1) were dual stained with Alcian Blue and CTC on 0.4 µm polycarbonate filters (Whatman Nuclepore) mounted on frosted glass slides (Cytoclear; Sterlitech, USA). TEP was stained using 0.5 mL Alcian Blue solution (pH = 2; 0.16% w/v Alcian Blue 8GX, Sigma) and was visualized in bright field in a), c), and e). 10 mM CTC (pH 8) was used to stain microgels, which were visualized using acridine orange emission filter (blue light) in b), d), and f). Photographs were taken using 100x magnification (oil immersion); Scaling grid = 100 µm x 100 µm. Red circles are used to highlight particles that stained positively for both microgels and TEP

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The Florida Coastal Everglades (FCE) LTER Program. fcelter.fiu.edu (accessed 12.2.16).


