Biogeochemistry of Redox-Sensitive Elements in the Subterranean Estuary

Alison E. O'Connor

College of William and Mary - Virginia Institute of Marine Science, alisoneoconnor@gmail.com

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Biogeochemistry of Redox Sensitive Elements in the Subterranean Estuary

A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements of the Degree of
Doctor of Philosophy

by
Alison E. O'Connor
January, 2017
This dissertation is submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Alison O’Connor

Approved by the Committee, December 2016

Aaron J. Beck, Ph.D.
Committee Chair/Advisor

Elizabeth Canuel, Ph.D.
Committee Chair/Advisor

Bongkeun Song, Ph.D.

Steven Kuehl, Ph.D.

Karen Johannesson, Ph.D.
Tulane University
New Orleans, Louisiana
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Dissertation Abstract

Submarine groundwater discharge (SGD) is any flow of water along the continental margins from the seabed into the coastal ocean, and it represents an important source of nutrients and trace metals to the coastal ocean. The chemical composition of SGD is strongly influenced by biogeochemical reactions that take place within the subterranean estuary (STE), the subsurface mixing zone of fresh and saline waters. Understanding the reactions that take place within the shallow STE is critical to evaluating the composition of SGD, and therefore SGD-driven chemical fluxes.

In this dissertation, I seek to determine the biogeochemical processes controlling the behavior of the redox-sensitive metals (RSMs) Mo, U, V, and Cr in a shallow subterranean estuary in Gloucester Point, VA (USA). These RSMs tend to form soluble oxyanions under oxidizing conditions but react to form more insoluble or particle-reactive (i.e., more likely to adsorb to sediments) species under reducing conditions.

In this STE, advection of water through the STE and the apparent respiration of organic matter drives the formation of a “classic” redox sequence typically observed in diffusion-dominated fine-grained sediments, with sequential zones with depth of high nitrate, dissolved Fe, and sulfide. While the general redox structure and RSM distributions in the STE remained consistent over time, concentrations and mixing behavior varied over the study period. Concentrations of DOC, humic carbon, and sulfide were higher in the summer, whereas Fe and Mn concentrations were higher in winter. This contrasting behavior may be due to sulfate and metal reducing bacteria responding differently to seasonally variable factors (such as temperature or substrate availability).

Mo and U were supplied to the STE by surface water, and both showed non-conservative removal. Removal of Mo was correlated with sulfide concentrations, but unlike sulfide concentrations, did not show seasonal differences. This was likely due to sulfide concentrations consistently in excess of the 11 µM threshold required to quantitatively react with and remove dissolved Mo. However, U showed greater removal in the summer, possibly driven by greater activity of U-reducing microbes. Dissolved V concentrations co-varied with DOC (with both greater in summer), indicating that V is likely complexed with dissolved organic matter. In contrast, Cr was correlated with both humic carbon and dissolved Fe in different parts of the STE. Over half of total dissolved DOC and Fe occurred in the colloidal phase, demonstrating the importance of colloidal transport in the STE. The relative proportion of RSMs in the colloidal phase increased in the order Mo<U<V<Cr, with up to 75% of Cr existing in the colloidal size fractions, suggesting the importance of colloidal transport for RSMs. Incubation experiments conducted under aerobic and anaerobic conditions showed that RSM concentration change on the order of hundreds of nM can take place in hours to weeks, within water residence times in the shallow STE. Furthermore, removal and mobilization rates between redox zones with distinct microbial populations.

The mechanistic approach used this work demonstrate how spatial and temporal variability of dissolved concentrations in the STE depend on redox zonation and microbe-mediated reactions. Findings from this work provide a basis for evaluating how changing environmental conditions may alter RSM fluxes.
AUTHOR’S NOTE

The chapters of this dissertation were written as manuscripts for publication in scientific journals. Citations for individual chapters at the time of writing are listed below:

Chapter 1


Chapter 2 & Chapter 3, as companion papers

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Chapter 4

O’Connor, A.E., and Beck, A.J. *In prep.* Colloid formation and Transport of Redox Sensitive Metals in a Shallow Subterranean Estuary. For submission to *Biogeochemistry.*

Chapter 5

Biogeochemistry of Redox Sensitive Elements in the Subterranean Estuary
INTRODUCTION

The Subterranean Estuary

Submarine groundwater discharge (SGD) is any flow of water along continental margins from the seabed into the coastal ocean (Burnett et al., 2003), and it can represent an important chemical flux to the coastal ocean. Radium isotope distributions across oceans indicate the global role of SGD as a source and sink for chemical species (Moore et al., 1996; Moore et al., 2008). SGD has been shown to be a source of nutrients (D’Elia et al., 1981; Slomp and Van Cappellen, 2004; Paytan et al., 2006), inorganic carbon (Cai et al., 2003), and trace metals (Spinelli et al., 2002; Windom et al. 2006; Bone et al., 2006; Beck et al., 2009) to coastal waters. Submarine groundwater discharge also plays an important role in the global budgets of elements such as Nd (Johannesson and Burdige, 2007), Ba (Shaw et al., 1998), and isotope ratios such as $^{87}\text{Sr}/^{86}\text{Sr}$ (Beck et al., 2013), potentially affecting their use as paleoproxies.

The chemical composition of SGD is strongly influenced by biogeochemical reactions that take place within the subterranean estuary (STE), the subsurface mixing zone of fresh and saline waters (Moore et al., 1999; Charette and Sholkovitz, 2006; Beck et al., 2007). Subterranean estuaries typically contain two mixing zones. One is a deep salt wedge, where denser, saline water underlies outflowing fresher water (Destouni and Prieto, 2003; Smith, 2004; Wilson, 2005; Fig. 1). The second is a near-shore zone where mixing is driven primarily by tidal oscillations and wave set-up (Li et al., 1999; Mango et al., 2004; Robinson et al., 2007; Fig. 1). The shallow mixing zone is associated with high SGD volume flux (e.g., Taniguchi et al., 2002; Burnett et al., 2006). Biogeochemical
reactions in the shallow STE can substantially modify the composition of the porewater and SGD (Beck et al., 2010; Riedel et al., 2011; McAllister et al., 2015) despite the relatively short residence time compared to the deeper STE (Robinson et al., 2007). Understanding the reactions that take place within the shallow STE is critical to evaluating the composition of SGD, and therefore SGD-driven chemical fluxes.

Like their surface namesake, STEs are characterized by mixing of freshwater and saline water with distinct chemical compositions, creating salinity and chemical concentration gradients and driving reactions that modify water composition (Moore, 1999). Reactions in the STE include changes in saturation state leading to precipitation or dissolution of minerals such as calcite and dolomite (Hanshaw et al., 1971; Badiozamani, 1973). Intrusion of saline water into coastal sediments also drives desorption of elements like radium and barium, which can be used as tracers of seawater recirculating in coastal sediments (Moore, 1996).

Subterranean estuaries are commonly characterized by steep redox gradients generated by the mixing of anoxic groundwater and oxic seawater. Redox reactions within the STE include the formation of iron and manganese oxides along the freshwater/saltwater interface (Charette and Sholkovitz, 2002; Windom et al., 2006), Fe and Mn reduction, and sulfide production (Snyder et al., 2004; Roy et al., 2010). Redox gradients play an important role in controlling the geochemical behavior of redox-sensitive metals (RSMs) such as V, Cr, Mo, and U. These RSMs tend to form soluble oxyanions under oxidizing conditions but react to form more insoluble or particle-reactive (i.e., more likely to adsorb to sediments) species under reducing conditions.
Due to their redox behavior, RSMs are widely used as marine paleoproxies to examine past oceanic oxygen levels, productivity, and circulation (e.g., Adelson et al., 2001; Nameroff et al., 2004; Rimmer et al., 2004; Tribovillard et al., 2006; Algeo et al., 2012). The use of elements like Mo and U as paleoindicators is influenced by material budgets controlling their oceanic concentrations and isotopic composition (Klinkhammer and Palmer, 1991; Siebert et al., 2003; Archer and Vance, 2008). As a result, submarine groundwater discharge may be an important component of RSM budgets in the coastal and global ocean.

In fresh groundwater systems and low-oxygen fine-grained sediments along continental margins, distributions of RSMs are influenced by reduction potential, sorption to Fe and Mn oxides, presence of sulfide, and complexation with organic matter (Brumsack and Gieskes, 1983; Goldberg and Forster, 1998; Morford and Emerson, 1999; Morford et al., 2005; Wright and Belitz, 2010; Scholz et al., 2011; Pourret et al., 2012). Metal oxides, sulfides, and organic matter are all cycled intensively in the STE, and likely influence the geochemical behavior of RSMs in coastal groundwater.

Previous work has shown that reactions within the STE can be important for determining the SGD-driven fluxes of RSMs. Molybdenum shows nonconservative removal from porewater in some locations (Windom and Niencheski., 2003), but is mobilized in the salinity transition zone in others (Beck et al., 2010). Dissolved U tends to exhibit non-conservative removal in the subterranean estuary (Windom and Niencheski, 2003; Duncan and Shaw, 2003; Charette and Sholkovitz, 2006; Santos et al., 2012).
2011), apparently due to reduction and partitioning into the sediment phase (Charette et al., 2005). At one site (Great South Bay, NY), dissolved V showed lowest concentrations in the salinity transition zone, indicating removal (Beck et al., 2010). To our knowledge, no data have been reported for dissolved Cr in the STE. Despite these observations of RSM distributions in the STE, detailed information about the mechanisms controlling RSM behavior in the STE is lacking.

**Redox Sensitive Metal Background**

Under oxidizing conditions, molybdenum is stable as the molybdate ion, MoO$_4^{2-}$ (Bruland and Lohan, 2006). Molybdate is typically present in the dissolved phase, but sorbs to Mn oxyhydroxides (Shimmield and Price, 1986). Under sulfidic conditions, dissolved Mo reacts to form thiomolybdates, which are particle reactive and readily removed from solution (Helz et al., 1996; Erikson and Helz, 2000):

$$\text{MoO}_4^{2-} \rightarrow \text{MoO}_x\text{S}_{4-x}^{2-} \rightarrow \text{MoS}_4^{2-}$$

Above a threshold concentration of approximately 11 µM sulfide, the reaction shifts sharply toward the tetrathiomolybdate product (Erikson and Helz, 2000). However, long-term Mo sequestration in sediments occurs primarily by incorporation into pyrites after reduction to Mo(III) or Mo(IV) (Bostick et al., 2003; Dahl et al., 2013). Because thiomolybdate species contain Mo(VI) rather than reduced Mo, they are not expected to be incorporated into pyrites. Molybdenum is typically reduced by ligand-induced reactions involving polysulfides such as S$_8$, S$_5^{2-}$, and S$_4^{2-}$ (Vorlicek et al., 2004).

Molybdenum typically exhibits conservative mixing in surface estuaries because
the relatively inert molybdate ion dominates in oxic estuarine waters (van der Sloot et al., 1985; Dalai et al., 2005; Audry et al., 2007; Strady et al., 2009; Rahaman et al., 2010). However, non-conservative addition of Mo can occur when Mn oxides are reductively dissolved in low oxygen bottom waters, releasing sorbed Mo (Dalai et al., 2005). Addition of Mo can also occur when increasing ionic strength drives Mo release from a high-Mo particulate source, such as pyrite (M. Beck et al., 2012). Molybdenum exhibits non-conservative removal in estuaries bordered by reducing environments that provide a sink, such as mangrove swamps (Rahaman et al., 2010). Molybdenum is not typically associated with the colloidal phase in estuaries (Pokrovsky and Schott, 2002; Vasyukova et al., 2010).

Uranium in natural waters can exist in three oxidation states: U(IV), U(V), and U(VI). In ocean and surface estuary waters, the most common dissolved form is oxidized U(VI). Depending on pH and solution composition, U(VI) can exist as the free uranyl ion (UO$_2^{2+}$, which sorbs to particles (Klinkhammer and Palmer, 1991)) or form unreactive complexes with carbonate (the most prevalent species in seawater) or phosphate (Markich, 2002).

Uranium behaves largely conservatively in the saline portions of estuaries, but is frequently removed in low salinity (<3) zones concurrent with the flocculation of Fe and Mn oxides and humic material (Maeda and Windom, 1982; Sarin and Church, 1994; M. Beck et al., 2012). Uranium can also be removed from estuaries in fringing reducing environments, such as salt marshes (Windom et al., 2000) and mangrove swamps (Rahaman et al., 2010). The majority of U entering the estuary from freshwater sources is
associated with the colloidal phase (~90% in the size fraction between 3 kD and 0.2 µm), whereas only a small amount (<5%) of U is colloidal at higher salinities (Andersson et al., 2001). Colloidal U is removed in the salinity transition zone via association with sinking Fe oxide and organic rich colloids.

Vanadium naturally occurs in oxidized V(V), and reduced species V(IV) and V(III). Oxidized vanadium (V(V)) is present in aquatic environments as vanadate (H$_4$VO$_4$/HVO$_4^{2-}$; Wanty and Goldhaber, 1992). Vanadate is generally soluble under oxic conditions, but also sorbs to iron oxide mineral surfaces (Wehrli and Stumm, 1989; Wright and Belitz, 2010; Pourret et al., 2012). Under reducing and low pH conditions, V can be reduced to V(IV) as the particle-reactive species VO$^{2+}$ or VO(OH)$^+$ (Wanty and Goldhaber 1992). Reduced V species are readily removed from the dissolved phase by binding strongly to solid phase organic and reactive metal oxyhydroxide solid phases (Wehrli and Stumm, 1989).

Vanadium exhibits variable behavior in surface estuaries. In some estuaries, V is correlated with phosphate and is non-conservatively removed at mid salinities through biological uptake (Shiller and Boyle, 1987; Shiller and Boyle, 1991). In others, V is reduced and removed from the dissolved phase in low salinity, low oxygen areas (e.g. van der Sloot, 1985), or desorbed from the solid phase at higher salinity (e.g. Strady et al., 2009). Although reduced V species are typically more particle-reactive, oxidized vanadate can also be strongly associated with the colloidal phase, in particular colloidal iron oxides (Pokrovsky and Schott, 2002; Vasyukova et al., 2010).

The major environmental Cr species are oxidized Cr (VI) and reduced Cr(III).
Under oxic conditions, Cr(VI) exists as the chromate ion (HCrO$_4^-$/CrO$_4^{2-}$), which can sorb to reactive metal oxide surfaces (Rai et al., 1989). However, Cr(VI) is highly oxidizing and readily reduced to Cr(III) by organic matter (James and Bartlett, 1983), ferrous iron (Rai et al., 1989), and sulfide (Smillie et al., 1981). Reduced Cr(III), on the other hand, is slowly oxidized only by dissolved oxygen and Mn oxides, with a rate constant for oxidation by Mn oxides of ~0.3 nmol/day (Eary and Rai, 1987).

Chromium(III) is particle-reactive and readily forms sparingly soluble hydroxide minerals, and thus is typically found in low concentrations in the dissolved phase (Richard and Bourg, 1991). However, high concentrations of organic ligands (such as fulvic acids) may complex Cr(III) and increase its effective solubility (Rai et al., 1989).

The behavior of Cr in a given estuary depends on Cr speciation. Riverine Cr was removed within the low-salinity estuarine turbidity maximum of the St. Lawrence River and Columbia River estuaries (Cranston and Murray, 1980; Campbell and Yeats, 1984). However, Cr in the Mississippi River estuary plume behaved conservatively in mixing experiments (Shiller and Boyle, 1991). Experiments showed that removal of dissolved Cr by flocculation was almost entirely due to removal of Cr(III) (as opposed to the less particle reactive Cr(VI); Cranston and Murray, 1980). Therefore, the distribution of Cr between Cr(III) and Cr(VI) in rivers affects its mixing behavior.

**Summary of the Dissertation**

In this work, I seek to determine the biogeochemical behavior of the RSMs Mo, U, V, and Cr in a shallow subterranean estuary. The following chapters (to be submitted
individually for publication) intend to answer the research questions 1) Does the STE act as a source or sink for RSMs? And 2) How does the redox structure of the STE affect the fate and transport of RSMs?

Chapter 1 (O’Connor et al., 2015) describes the general redox structure and RSM distributions in the Gloucester Point, VA STE and used equilibrium speciation modeling to draw conclusions about biogeochemical processes influencing RSM behavior. Chapters 2 and 3 use sediment and porewater data collected over a two-year time series to describe seasonal changes in the concentrations of organic carbon and redox-active metals Fe and Mn (Chapter 2) as well as RSMs (Chapter 3), and discuss potential biogeochemical drivers of this variability. Chapter 4 describes colloid formation in the STE, distributions of RSMs associated with the colloidal phase, and implications of these associations for transport of RSMs. Chapter 5 uses laboratory incubation experiments and microbial population analysis to constrains rates of RSM addition/removal in each STE redox zone under anaerobic and aerobic conditions.

Together, this work presents an uncommonly in-depth look at how STE redox processes affect the fate and transport of trace metals in permeable coastal sediments. The mechanistic approach used here advances understanding of the geochemical processes that control SGD fluxes of trace metals to estuaries and oceans.
Figures

Figure 1. Schematic of typical salinity structures in the shallow and deep STEs (based on Robinson et al., 2007).
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CHAPTER 1

**REDOX GRADIENTS AND REDOX SENSITIVE METAL DISTRIBUTIONS IN A SHALLOW STE**

Abstract

Submarine groundwater discharge (SGD) is an important component of chemical fluxes in coastal ocean. The composition of SGD is influenced by biogeochemical reactions that take place within the subterranean estuary (STE), the subsurface mixing zone of fresh and saline groundwaters. The STE is characterized by redox gradients that affect the speciation and mobility of redox-sensitive elements (RSEs). We examined the distributions and behavior of the RSEs Mo, U, V, and Cr within the larger redox framework of a shallow STE and evaluated the source-sink function of the STE for these elements. We found that the advection of water through the STE and the apparent respiration of organic matter drives the formation of a "classic" redox sequence typically observed in diffusion-dominated fine-grained sediments. High concentrations of dissolved organic matter (up to 2.9 mM) lead to extensive sulfide production (up to 1.8 mM) within 3 m of the surface. Both Mo and U are quantitatively removed as oxic surface waters mix into ferruginous and sulfidic zones. Molybdenum removal appears to occur where sulfide concentrations exceed ~11 µM, a previously reported threshold for quantitative formation of highly particle-reactive thiomolybdate species. Uranium removal apparently occurs via reduction and formation of insoluble phases. It is not clear how readily sequestered metals may be returned to solution, but SGD may be an important sink in the marine budget for both Mo and U. In contrast, both V and Cr show
non-conservative addition across the salinity mixing gradient. Increases in pH appear to promote dissolution of V from minerals within the shallow aquifer, and mobilization may also be associated with dissolved organic matter. Chromium enrichment is associated with increased dissolved organic matter and is likely due to the formation of soluble Cr-organic complexes. Fluxes of these elements were constrained using SGD volume fluxes, determined using radium isotopes as well as direct discharge measurements by Lee-type seepage meters, and concentrations in directly-sampled seepage (Mo: -0.21 to -7.7 µmol m$^{-2}$ day$^{-1}$; U: -0.02 to -0.6 µmol m$^{-2}$ day$^{-1}$; V: 0.05 to 2.0 µmol m$^{-2}$ day$^{-1}$; Cr: 0.12 to 4.4 µmol m$^{-2}$ day$^{-1}$).

**Introduction**

Submarine groundwater discharge (SGD) is any flow of water along continental margins from the seabed into the coastal ocean (Burnett et al., 2003), and represents an important chemical flux in the coastal ocean. For example, SGD has been shown to be a source of nutrients (D'Elia et al., 1981, Slomp and Van Cappellen, 2004) and metals (Windom et al. 2006; Bone et al., 2007) to coastal waters. Submarine groundwater discharge also plays an important role in the global budget of elements such as Nd (Johannesson and Burdige, 2007), Ba (Shaw et al., 1998), and $^{87}$Sr/$^{86}$Sr (Beck et al., 2013), potentially affecting their use as paleoproxies.

The chemical composition of SGD is strongly influenced by biogeochemical reactions that take place within the subterranean estuary (STE), the subsurface mixing zone of fresh and saline groundwaters (Moore, 1999; Charette and Sholkovitz, 2006;
Beck et al., 2007). Subterranean estuaries are characterized by steep redox gradients known to affect the speciation and mobility of redox sensitive elements (RSEs) in fine-grained sediments (Brumsack and Gieskes, 1983; Goldberg and Forster, 1998; Morford et al., 1999; Morford et al., 2005; Scholz et al., 2011), and STEs are also likely sites of intense RSE cycling. For example, previous studies have shown extensive formation of iron and manganese oxides along the freshwater/saltwater interface (Charette and Sholkovitz 2002; Windom et al. 2006), and DOC can fuel Fe and Mn reduction as well as sulfide production in the STE (Snyder et al., 2004; Roy et al., 2010).

Redox gradients play an important role in controlling the geochemical behavior of RSEs such as V, Cr, Mo, and U, though little is known about their behavior in the STE. These RSEs tend to form soluble oxyanions under oxidizing conditions but become more insoluble or particle-reactive (i.e., more likely to adsorb to sediments) under reducing conditions (Bruland and Lohan, 2006). Due to this behavior, these RSEs are widely used as paleoproxies to examine past oceanic oxygen levels, productivity, and circulation (e.g., Adelson et al., 2001; Lyons et al., 2003; Nameroff et al., 2004; Riboulleau et al., 2003; Rimmer et al., 2004; Rimmer, 2004; Morford et al., 2005; Tribouillard et al., 2006; Algeo et al., 2012; Arnold et al., 2012). The use of elements like Mo and U as paleoindicators is influenced by material budgets controlling their oceanic concentrations and isotopic composition (Klinkhammer and Palmer, 1991; Siebert et al., 2003; Archer and Vance, 2008). Submarine groundwater discharge is a poorly-characterized but likely important component of RSE budgets in the coastal and global ocean.

Previous work shows that reactions within the STE are important for determining
the SGD-driven fluxes of redox-sensitive trace metals. Molybdenum shows nonconservative removal from porewater in some STEs (Windom and Niencheski, 2003), but is mobilized in the salinity transition zone in others (Beck et al., 2010). Dissolved U tends to exhibit non-conservative removal in the subterranean estuary (Windom and Niencheski, 2003; Duncan and Shaw, 2003; Charette et al., 2005; Santos et al., 2011), apparently due to reduction and partitioning into the sediment phase (Charette et al., 2005). At one site (Great South Bay, NY), dissolved V showed lowest concentrations in the salinity transition zone, indicating removal (Beck et al., 2010). To our knowledge, no data have been reported for dissolved Cr in the STE.

In fresh groundwater systems and low-oxygen fine-grained sediments along continental margins, the distribution of RSEs is influenced by reduction potential, sorption to Fe and Mn oxides, presence of sulfide, and complexation with organic matter (Brumsack and Gieskes, 1983; Goldberg and Forster, 1998; Morford et al., 1999; Morford et al., 2005; Dotro et al., 2011; Wright and Belitz, 2010; Scholz et al., 2011; Pourret et al., 2012). Metal oxides, sulfides, and organic matter are all cycled intensively in the STE, and are likely important to the geochemical behavior of RSEs in coastal groundwater.

Detailed information about the mechanisms that control the behavior of these RSEs in the STE is lacking. Based on the redox geochemistry of these elements and previous observations in other STEs, we initially hypothesized that Mo, U, V, and Cr would be removed in the reducing portions of the subterranean estuary. Therefore, the current study examined the behavior of the RSEs Mo, U, V, and Cr within the larger
redox framework of a shallow STE, and evaluated the source-sink function of the STE for these elements.

**Methods**

*Study Site*

The study site is located in Gloucester Point, VA, USA (37.248884 N, 76.505324 W) on the York River estuary (a sub-estuary of the Chesapeake Bay; Fig. 1a). The York River estuary is microtidal, with a range of ~0.8 m at the study site. The site is a small (20-30 m wide) beach comprising fine to coarse sands, with a slope of approximately 0.2. The beach is flanked to the east by a maritime forest and upland marsh, and to the north by beachfront residences. An intermittent series of granite rock breakwaters lines the beach ~40 m from the dune line (Fig. 1b). Surface water salinity at the site varies seasonally between 15 and 25, with higher salinity in summer and lower salinity in winter (Luek and Beck, 2014).

*Conductivity Transects and Seepage Meters*

The location and structure of the STE was characterized using a bulk conductivity probe (Stieglitz et al., 2000; Stieglitz et al., 2008). Assuming reasonably homogenous sediment composition, observed variations in bulk conductivity reflect changes in porewater salinity. Sediment bulk conductivity was calibrated in the laboratory against water of known conductivity, and checked against surface water conductivity at the field site before deployment. A subsurface conductivity transect was measured in August
2013. The transect began at the mid-tide line and extended 50 m offshore, with depth profiles taken every 5 m. The depth of each profile was determined by the depth of probe refusal.

Seepage measurements and samples were obtained using Lee-type seepage meters (Lee, 1977) made from steel or polypropylene drums. Seepage meters were emplaced at least 3 days prior to sampling to allow for equilibration and headspace flushing. Seepage was collected in acid-washed soft polyethylene bags, and salinity was measured using a refractometer. Seepage rates and seepage metal concentrations were measured in the summer of 2012, and the seepage rate measurements repeated in summer of 2013, using four seepage meters deployed along a shore-normal transect starting immediately below the low tide line (Fig. 2).

**Groundwater Collection and Analysis**

Groundwater samples were collected monthly between July 2012 and September 2013 from a depth profile at the mid-tide line. Sampling occurred during the week of the spring tide during the ebb phase of the tidal cycle. From July 2012 to January 2013, samples were collected from 4–6 depths ranging from 25 to 200 cm using a retract-a-tip stainless steel sampler (Charette and Allen, 2006). From February to June 2013, samples were collected from 1 cm-long well-points (installed at 65 cm, 90 cm, 125 cm, 170 cm, 215 cm, and 325 cm depths) screened with stainless steel mesh (AMS, Inc.; e.g. Gonneea et al., 2013) and attached to 3 mm diameter No-Ox® PTFE tubing (Supelco/Sigma-Aldrich). From July to September 2013, additional shallow porewater samples were
collected from an all-plastic multi-level sampler with 10 cm depth increments to 70 cm (Beck, 2007). Groundwater was pumped from depth using a portable peristaltic pump and filtered through a 0.45 μM Millipore polypropylene capsule filter into sampling containers. Due to progressive changes in the sampling plan over the course of the project, not all geochemical parameters were measured at all time points. Specific periods during which different constituents were measured are noted below. Ancillary water quality parameters (salinity, pH, oxidation-reduction potential) were measured at the time of collection with a hand-held YSI556 multi-probe. Oxidation-reduction potential was converted to Eh by adding 200 mV (YSI Environmental, 2005).

Samples for inorganic nitrogen species and major ion analyses were collected in acid-washed LDPE bottles from February to August 2013. Dissolved NH$_4^+$ and NO$_x$ were measured by the manual hypochlorite and spongy cadmium methods, respectively, and analyzed using a Shimadzu UV-1601 spectrophotometer (detection limits of 0.6 μM and 0.1 μM respectively, with field sample replicate precision better than 10%; Grasshoff et al., 1999). Major ions were determined using a Dionex ICS2000 with suppressed conductivity detection ion chromatograph, with a CS12A column for cation determination and AS18 column for anion determination. Samples for trace metal analysis were collected in acid-washed LDPE bottles from October 2012 to February 2013. Samples for trace metal analysis were acidified immediately after collection, and analyzed by dilution and direct injection on a Thermo Element 2 inductively-coupled plasma mass spectrometer (detection limits less than 0.1 nM and field sample replicate precision better than 10% for all elements; accuracy validated by analysis of trace metal
Standard Reference Materials 1643d and SLRS-4). Samples for Fe speciation analysis were collected from June to September 2013. Samples were immediately placed in acid-washed polypropylene vials pre-charged with 200 µL 0.01 M Ferrozine solution, and Fe speciation measured the same day on a Shimadzu UV-1601 spectrophotometer using the Ferrozine method (detection limit of 0.2 µM; field sample replication better than 5%; Stookey, 1970; updated by Viollier et al., 2000). Samples for dissolved organic carbon (DOC) analysis were collected in acid-washed polycarbonate bottles from February to August 2013 and were analyzed using a Shimadzu TOC-V interfaced to a TNM-1 using methods modified from Sharp et al., (1993) for high-temperature combustion analysis (detection limit of 2.4 µM, field sample replicate precision better than 5%). Samples for humic acid analysis were collected in acid-washed LDPE bottles, and were analyzed according to Grasshoff et al. (1999) using a Shimadzu RF-1501 spectrofluorophotometer (detection limit of 24 µM, field sample replication better than 10%). Calibration was done using humic acid salt standard (Aldrich). Samples for sulfide analyses were taken from July to September 2013. Samples were collected in acid-washed polypropylene vials pre-charged with 1 mL 0.25 M zinc sulfide to fix volatile sulfide, and analyzed using either the Fonselius (detection limit 0.1 µM, field sample replication better than 10%) or Cline (field sample replication better than 20%) method, depending on the concentration (Grasshoff et al. 1999).

Because samples were collected over a number of months, seasonal effects on distribution may be relevant for interpreting geochemical distributions. The primary variables that could cause monthly or seasonal differences are 1) changes in hydraulic
gradient between groundwater and river level, 2) differences in wave and tidal forcings that drive mixing in the STE, and 3) changes in temperature. Previous work at this site has shown that the hydraulic gradient is greater in the winter than in the summer (Luek and Beck, 2014). Greater saline intrusion in the summer due to the lower hydraulic gradient could lead to greater oxygen intrusion into the sediments and a shifting of the redox zones. Likewise, changes in mixing due to waves and tides could change the supply of oxygen and other reactants to the STE, though previous work shows that these forcings tend to change on tidal, rather than seasonal, time scales (e.g. Santos et al., 2009). Because our sampling events all took place during the ebb phase of a spring tide, this variability should be minimized. Lower temperatures in the winter could slow the biogeochemical reactions that generate the geochemical zones observed, and therefore affect both reaction and transport of RSEs. The maximum concentrations of some constituents (such as Fe) did vary from month-to-month, but the depths of geochemical zone interfaces remained remarkably constant over time. Therefore, temporal variability appears to be of secondary importance in this system, and the observed patterns provide a framework for understanding the STE redox environment and associated redox cycling.

Geochemical Modeling

Geochemical modeling of dissolved species composition and mineral saturation indices was performed using PHREEQC (USGS) and the Lawrence Livermore National Laboratory database, edited to include molybdenum sulfide species (Erickson and Helz, 2000). Input parameters included pH, Eh, major ions (Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Cl$^-$, SO$_4^{2-}$, all
of which were conservative with respect to salinity), NO$_3^-$, NH$_4^+$, Fe(II), Fe(III), Mn, Mo, U, V, and Cr. Values and concentrations for these parameters were averaged over 25 – 50 cm depth intervals representing the observed redox zones within the STE (Table 1). Although geochemical trends were constant with depth, temporal variation in concentration should be noted when interpreting the speciation and mineral saturation modeling results (Supplementary Table 1).

Results

Conductivity Transects and Seepage Measurements

Porewater salinity at this site ranged from less than 2.9 to 18.2 (Fig. 2; ~5 to 26 mS cm$^{-1}$). Shallow porewater around the low tide line had low salinities, forming a freshwater discharge tube feature (Robinson et al., 2007). A subsurface freshwater plume also extended to around 30 m off-shore of the mid-tide line, with low salinity water evident 10s of centimeters below the sediment/water interface.

Discharge rates varied from 5.5 cm day$^{-1}$ at the shoreline to 2.8 cm day$^{-1}$ at 32 m off shore (Fig. 2; Supplementary Table 2. Salinity in the seepage meter water ranged from 14 close to shore to 20 farthest from shore, and varied inversely with discharge rate. Highest SGD rates at this site were therefore associated with the meteoric groundwater plume, but marine groundwater circulating through the STE comprised a major fraction of the seepage.
Porewater Depth Profiles

Vertical distributions of salinity and Eh showed that shallow porewater (down to 50 cm) was the most saline and most oxidizing. Due to issues with contamination, dissolved oxygen concentrations (not shown) were qualitative, but showed a sharp decrease was consistently observed within the upper 30 cm of the profile. The Eh decreased with depth through the salinity transition zone (STZ; 50-100 cm), with the most reducing conditions in the deep low-salinity zone (Table 1, Fig. 3). The lowest salinity measured at this site was 2.3, consistent with recharge occurring in a zone heavily impacted by sea salt aerosols (e.g., Rapaglia and Bokuniewicz, 2009; Beck et al., 2013). Porewater pH did not vary uniformly with salinity. The pH of the surface water averaged 8.0 and decreased rapidly to an average of 7.0 immediately below the sediment-water interface. The pH then gradually increased to ~7.5 at 70 cm, and then decreased to 6.8 at 250 cm. There was a consistent increase in pH at 325 cm to an average of 7.4.

Sequential peaks of nitrate, Fe/Mn, and sulfide were observed with depth (Fig. 4, Table 1). Nitrate was consistently low in the surface water (<1 µM), reached a maximum of ~76 µM around 12 cm depth, and decreased to less than the detection limit (<0.1 µM) below ~70 cm depth. Ammonium was generally below the detection limit (0.5 µM) in the upper 50 cm, and showed a sharp increase to more than 100 µM beneath the observed nitrate peak. Dissolved ammonium concentrations remained elevated down to 215 cm depth, but declined slightly to less than 70 µM at the deepest sampled point (325 cm). Dissolved Mn was low in surface water and shallow porewater, and showed a broad peak from ~50 cm to ~200 cm depth with maximum concentrations of 3 µM (with the
exception of two high concentration samples of up to 20 µM found around 30 cm depth). Maximum dissolved Fe concentrations (up to 30 µM) were observed between 50 and 60 cm depth. Iron concentrations were generally less than 10 µM throughout the rest of the depth profile. Sulfide was undetectable in the surface water and upper porewater, low between 50 and 90 cm, and sharply increased to maximum concentrations exceeding 1.5 mM below 100 cm.

DOC was relatively low in the surface water (~320 µM) and increased rapidly in the shallow porewater (Fig. 5, Table 1). DOC exhibited a broad maximum between 50 and 100 cm (reaching concentrations up to 2.9 mM), and showed a non-conservative mixing distribution with respect to salinity (Fig. 6). The vertical distribution of dissolved humic material matched that of DOC, with maximum concentrations of ~890 µM at 75 cm depth (Fig. 5). Humic carbon made up 43 ± 18% of total DOC.

Both Mo and U showed sharp declines within the upper 100 cm from maximum concentrations in the surface water to around 1 nM and 0.1 nM, respectively (Fig. 5, Table 1). In contrast, V and Cr both showed mid-depth maxima (Fig. 5, Table 1). Vanadium reached a maximum of 150 µM between 50 and 75 cm. Chromium showed a broad peak between 50 and 100 cm, reaching a maximum of nearly 60 nM (with the exception of a single shallow sample that reached 91 nM). All four RSEs showed non-conservative mixing behavior with respect to salinity (Fig. 6). Molybdenum and U showed low concentrations between salinities 5 and 15 (below the conservative mixing line), while V and Cr showed maxima at mid-range salinities (above the conservative mixing line).
Geochemical Modeling

The chemical speciation of dissolved RSEs was based on average values within different depth zones (Table 1), and calculated using the PHREEQC geochemical software package. The dominant dissolved Mo species was molybdate (MoO$_4^{2-}$) above the ferruginous/sulfidic transition, and sharply transitioned to primarily tetrathiolmolybdate (MoS$_4^{2-}$) at the interface between the ferruginous and sulfidic zones. Uranium was predominantly soluble U(VI) above the sulfidic zone, but was present as reduced U(IV) at and below the ferruginous/sulfidic interface. Oxidized V(V) was the predominant species where dissolved V was at a maximum, at and above the ferruginous/sulfidic interface. Reduced forms of V began to dominate below the ferruginous/sulfidic interface. However, at the deepest point (where pH increases), V(V) was the primary species. Chromium was entirely in the reduced Cr(III) oxidation state throughout the depth profile (Fig. 7). The polysulfide species S$_4^{2-}$ and S$_5^{2-}$ were positively correlated with total sulfide throughout the STE, and represented approximately 0.5 and 18% of reduced sulfur compounds, respectively.

Mineral solubility and equilibrium saturation states were also calculated using PHREEQC. Iron sulfide species (mackinawite and pyrite) were calculated to become saturated in and below the ferruginous zone (Fig. 8). Uraninite was slightly supersaturated just below the sulfidic zone (saturation index = 0.16) but was undersaturated in the deep, fresher zone where dissolved U concentrations were low (Fig. 8).
Discussion

**Seepage Measurements and Conductivity Transects**

Seepage measurements and conductivity transects (Fig. 2) showed that the fresh groundwater plume and shallow STE at the Gloucester Point beach had strong cross-shore variability in discharge rate and salinity. The shallow, high conductivity porewater at the mid-tide region and down-gradient zone of freshwater discharge reflect circulation patterns similar to those observed in other shallow STE environments (e.g., Mango et al., 2004; Robinson et al., 2007). This nearshore zone represents an area of high discharge (e.g., Taniguchi et al., 2002; Burnett et al., 2006) and intense, rapid mixing due to tidal and other forces (e.g. Li et al., 1999; Robinson et al., 2007). The mid-tide, shallow STE feature represents an important location for understanding geochemical transformations and determining elemental fluxes. Therefore, porewater samples for further chemical analysis were collected at this location and reflect processes occurring within high flow, rapid-turnover portion of the shallow STE.

Circulation within the shallow STE is complex, but can be simply conceptualized as saline water infiltrating, circulating, and discharging through the saline cell (i.e. above the STZ). Simple residence time calculations can then be made for waters in this zone. One way is to estimate the water transit distance as twice the depth of the STZ center, the minimum distance traveled to create the shallow saline cell. In the Gloucester Point shallow STE, the STZ is 75 cm deep, giving a total transit distance of 150 cm, and flow rate (measured as the seepage rate) is around 5.5 cm d$^{-1}$ (Fig. 2). This gives a minimum residence time for porewater in the shallow STE of ~27 days. The residence time of the
shallow STE can also be estimated by approximating the shallow STE as a tidal wedge, as it is recharged by tidal pumping. The following equation can be used to calculate the residence time of the water (Colbert and Hammond, 2008):

\[ t = \frac{2 \cdot A}{S_b \cdot (n/q)} \quad (1) \]

where \( t \) is the residence time, \( A \) is the tidal amplitude (0.8 m), \( S_b \) is the beach slope (0.2), \( n \) is the sediment porosity (0.3), and \( q \) is the linear flow rate (5.5 cm/day). Using this method, the residence time of water in the Gloucester Point shallow STE is around 46 days. These estimates are comparable to shallow STE residence times (5 – 20 days) modeled by Robinson et al. (2007).

Chemical reactions in the upper saline zone extensively modify the composition of recirculating water and determine SGD-driven fluxes of nutrients and DOC (Santos et al., 2009). The current work suggests that the redox gradients in the STE and the SGD-driven fluxes of RSEs are likewise controlled by reactions in the shallow mixing zone, despite the relatively short residence time of the upper saline plume (compared to the deeper STE; Robinson et al., 2007).

**Redox Framework**

As oxygen-rich surface water mixes into the STE, apparent microbial respiration of organic matter consumes oxygen and produces sequential peaks in nitrate, dissolved Fe, and sulfide (Fig. 4). There is a sharp drop in pH from the surface waters to shallow porewaters (Fig. 3), indicative of oxic respiration (Appelo and Postma, 2005). The subsurface peak in nitrate coincides with an ammonium minimum and low pH,
suggesting that the source of nitrate is oxic nitrification. The depletion of the nitrate peak with depth is consistent with consumption of nitrate as an electron acceptor in anaerobic respiration (e.g., Kroeger and Charette, 2008).

Immediately below the nitrate peak, the dissolved Fe maximum corresponds with an increase in pH, characteristic of iron oxide reduction (Appelo and Postma, 2005). The Fe and Mn porewater concentrations start to increase at approximately the same depth (~50 cm), and the Mn peak is broader and extends deeper than Fe peak. Removal of Fe and Mn at depth appears to be controlled by sulfide and alkalinity, respectively, which leads to a more well-defined peak for Fe than Mn. The sharp interface between dissolved Fe and dissolved sulfide suggests that presence of sulfide (starting around 90 cm) controls the dissolved Fe distribution via formation of solid-phase iron sulfides. The gradual decrease in dissolved Mn below 250 cm is likely due to formation of solid Mn-carbonate species. In the deeper sulfidic zone (below 250 cm), the pH increases as a result of alkalinity production during sulfate reduction (Appelo and Postma, 2005).

Chemical zonation driven by advective flow has been previously reported in terrestrial and coastal groundwater (e.g. Champ et al., 1979; Charette and Sholkovitz, 2002; Riedel et al., 2011). Salt transport in the shallow STE appears to be a controlled by an advection-dispersion process that mimics diffusive transport (Webster et al., 1996; Rapaglia and Bokuniewicz, 2009). In the Gloucester Point STE, this dispersive mixing creates the appearance of a "classic" redox sequence in the shallow STE reminiscent of geochemical zonation in fine-grained diffusion-dominated sediments (Froelich et al., 1979; Berner, 1981). Organic matter oxidation in the shallow STE appears to drive
reduction of various electron acceptors to create sequential oxic, nitrogenous, ferruginous, and sulfidic zones (Canfield and Thamdrup, 2009).

The development of this redox sequence within the STE may not be universal, and appears to depend on the availability of organic material to drive the succession of redox reactions. Some STEs are characterized by low DOC and are Fe-dominated (e.g., Table 2). In such systems, high dissolved Fe concentrations present in the fresh portion of the STE mix with low DOC/high oxygen water in the salinity transition zone, and are oxidized to form an oxidized "iron curtain" (Charette and Sholkovitz, 2006). Sulfide production in these areas appears to be negligible (Charette and Sholkovitz, 2002; Beck et al., 2007). In other STE locations, levels of organic matter and sulfate are sufficiently high that sulfide production occurs (Snyder et al., 2004; Roy et al., 2010; this study). This contrast was demonstrated clearly by characterization of SGD on opposite sides of the Okatee River estuary (South Carolina). Groundwater on opposite sides of the river contained different levels of dissolved organic matter and dissolved oxygen. This resulted in higher levels of reduced metabolites (such as dissolved Fe and sulfide) in the groundwater with high organic matter and low dissolved oxygen on one side of the river, and lower concentrations of reduced metabolites in the groundwater with low organic matter and oxygen on the opposite side (Porubsky et al., 2014). Moving toward a redox framework in examining STEs is important for understanding and predicting chemical fluxes via SGD.
Redox Sensitive Elements

Despite the short residence time of porewater in the shallow STE, reactions that occur in the near-surface zone (upper 1 – 3 m) substantially change the concentrations of metabolic products such as NO$_x$, NH$_4^+$, and sulfide (see also Roy et al., 2010; Sawyer et al., 2014). These same reactions also affect the distributions and cycling of RSEs in shallow porewater. The RSEs examined here fall into two general distributions: Mo and U show non-conservative removal from the porewater, while V and Cr exhibit non-conservative addition (Fig. 6). The removal and enrichment of these elements can be seen in both the salinity mixing diagrams, as well as calculations of enrichment or depletion relative to salinity. In contrast to the geochemistry of surface estuaries, where salinity gradient-driven flocculation often controls the cycling of metals (e.g. Sholkovitz et al. 1978; Bewers and Yeats, 1978), the cycling of RSEs in the subterranean estuary seems to be driven less by salinity gradients and more by redox gradients. These redox gradients are generated by the mixing of oxic and anoxic waters, as well as different concentrations of electron donors and acceptors. Such redox gradients also occur in coastal aquifers with negligible salinity change, and may instead depend primarily on sediment permeability, porewater advection, and reactant transport (M. Beck et al., 2008a, 2008b; Riedel et al., 2011; Lee et al., 2014).

Molybdenum. Under sulfidic conditions, dissolved Mo (as molybdate, MoO$_4^{2-}$) reacts to form thiomolybdates, which are particle reactive and readily removed from solution (Helz et al., 1996; Erikson and Helz, 2000):

\[ \text{MoO}_4^{2-} \rightarrow \text{MoO}_3\text{S}_4^{2-} \rightarrow \text{MoS}_4^{2-} \]
Above a threshold concentration of approximately 11 µM sulfide, the reaction shifts sharply toward the tetrathiomolybdate product (Erikson and Helz, 2000). Dissolved sulfide concentrations reached 11 µM at 50 cm depth in the Gloucester Point STE (Fig. 4), the depth at which Mo sharply declined (Fig. 5). This suggests that Mo removal occurred via thiomolybdate formation and subsequent adsorption to sediment surfaces. Because the removal of Mo is sulfide-dependent, the production of sulfide in a given STE is expected to control the behavior of Mo.

The long-term preservation of sedimentary Mo in the STE remains to be clearly elucidated. Long-term Mo sequestration in sediments is primarily via incorporation into pyrites after reduction to Mo(III) or Mo(IV) (Huerta-Diaz and Morse, 1992; Müller, 2002; Dellwig et al., 2002; Chaillou et al., 2002; Bostick et al., 2003). Molybdenum is typically reduced via ligand-induced reactions involving polysulfides such as S₈, S₅²⁻, and S₄²⁻ (Müller et al., 1978; Vorlicek et al., 2004), which speciation modeling predict in the Gloucester Point STE (Fig. 7). This suggests that while Mo may be initially removed from the porewater as thiomolybdate species, there is a potential for reduction and long-term storage in pyrite formed (Fig. 8) in the Gloucester Point STE.

Uranium. Removal of dissolved U in aqueous systems typically takes place when oxidized U(VI) is reduced to U(IV), which tends to sorb to sediments or form the mineral uraninite (UO₂) (Klinkhammer and Palmer, 1991). Reduction and removal of U in the Gloucester Point STE is supported by speciation calculations showing that U was stable as U(VI) within the upper 60 cm, but switched to predominantly U(IV) between 60 and 90 cm (Fig. 7). Uraninite became supersaturated at this depth (though was slightly
undersaturated below this depth due to low U concentrations in the fresher zone), indicating the potential for uraninite formation (Fig. 8). Most of the U removal was from seawater circulated through the reducing sediments, consistent with previous studies (e.g. Charette and Sholkovitz, 2006; Riedel et al., 2011). Where natural or anthropogenic U enrichment of coastal groundwater occurs (e.g. Kaplan et al., 1994; Swarzenski and Baskaran, 2007), removal in the reducing STE represents a potential control on export to the coastal ocean.

It is unclear if U reduction in the STE occurs via a microbial or an abiotic mechanism. Reduction of U(VI) to U(IV) can occur through biotic processes (directly reduced by iron- and sulfur-reducing microbes) or abiotic processes (by redox reactions with other chemical species) (Lovley et al. 1991; Lovley et al. 1993; Fredrickson et al., 2000; Behrends and Van Cappellen, 2005; Jeon et al., 2005; Hua et al., 2006). Both iron and sulfur reduction have been observed in various STEs, indicating that the microbial populations may be capable of U reduction. However, U may be abiotically reduced by Fe(II)-bearing minerals or Fe(II) adsorbed to reactive Fe(III) surfaces (Fredrickson et al., 2000; Behrends and Van Cappellen, 2005; Jeon et al., 2005), which may occur concurrently with iron-reducing microbes.

Uranium removal is observed in STEs which do not exhibit concentrations of DOC and/or sulfide as high as the Gloucester Point STE, but do show some degree of iron and/or sulfate reduction (Windom and Nienchski, 2003; Charette and Sholkovitz, 2006; Santos et al., 2011). This suggests that uranium reduction in the STE occurs by iron- or sulfur-reducing microbes, rather than by a particular abiotic chemical process.
The removal of U in various STEs suggests that they may act as a net sink for U in coastal waters (as proposed by Charette and Sholkovitz, 2006). However, the geology and lithology of certain SGD environments results in net uranium flux to surface waters. Karst groundwater can have high carbonate concentrations unrelated to salinity due to equilibration with carbonate minerals, which leads to the formation of highly soluble and stable uranium carbonate complexes and elevated uranium concentrations through the salinity mixing zone (Gonneea et al., 2014). Submarine groundwater discharge can also be a source for uranium in environments rich in uranium-containing phosphatic deposits (Swarzenski and Baskaran, 2007). A deeper understanding of the interactions of geology, redox environment, and microbial communities in global STEs and permeable sediments will be necessary to properly evaluate the role of porewater advection in the marine U budget.

The STE may not be a long-term sink for U in the York River estuary. The permanence of the STE as a U (or Mo) sink likely depends on the maintenance of reducing conditions in the shallow subsurface. The effects of short-term mixing of oxidizing surface water into the sediments driven by storms or large waves are unclear, and such events may oxidize and mobilize reduced species (e.g. Robinson et al., 2014).

*Vanadium.* Oxidized vanadium is present in the environment as vanadate ($\text{H}_2\text{VO}_4^-$/$\text{HVO}_4^{2-}$; Wanty and Goldhaber, 1992) which is generally soluble in oxic groundwater but does sorb to iron oxide mineral surfaces (Wehrli and Stumm, 1989; Wright and Belitz, 2010; Pourret et al, 2012). Reduced V is present as vanadyl cations ($\text{VO}^{2+}$, $\text{VO(OH)}^+$) which bind strongly to organic and oxyhydroxide solid phases (Wehrli and
Stumm, 1989).

The distribution of V in the Gloucester Point STE is consistent with known V geochemistry. Vanadium concentrations were lowest where reduced species were expected to dominate (in the more reducing portions of the STE), and highest where the soluble vanadate ion was the dominant species (within and above the salinity transition zone; Fig. 7). The mid-depth V maximum and nonconservative addition of V in the salinity transition zone therefore appears to be a result of oxidation and mobilization of V from the mineral phase (Fig. 5). Similar V mobilization from vanadium-rich minerals is observed in aquifers with high oxygen and circumneutral to slightly basic pH (Wright and Belitz, 2010; Pourret et al., 2012). Because V mobilization in the current study was observed in suboxic redox zones, V mobilization in this STE may be due to the mid-depth pH maximum. Alternatively, sediment-associated V may also be mobilized by DOC. The maxima of V and DOC concentrations coincide, and they are weakly but significantly correlated ($R^2 = 0.18, p = 0.03$). This indicates that there may be some influence of DOC on mobilization of V from the sediments.

Heavy mineral sands comprising V-rich minerals such as ilmenite are abundant in the Chesapeake Bay region (Firek et al., 1977; Darby, 1984; Carpenter and Carpenter, 1991). Only 0.03% of the acid-leachable sedimentary V (194 nmol cm$^{-3}$, unpubl.) in a given volume of sediment would need to be dissolved to produce observed porewater concentrations. Assuming steady-state conditions over the estimated residence time of the water (~35 days, discussed above), the sediments contain sufficient V to support these porewater concentrations for over 200 years, and could therefore be the source of V in the
While mobilization from aquifer sediments appears to be important for the distribution of V in this STE, V distribution may be controlled by different processes in other locations. In Waquoit Bay, phosphate and arsenate (which have chemical structures similar to vanadate) are removed via sorption to Fe oxides formed at the freshwater/saltwater interface (Charette and Sholkovitz, 2002; Bone et al., 2007). In an STE in Great South Bay, NY (one of the few STE locations where porewater V has been reported), V concentrations showed a minimum in the salinity transition zone where Fe oxides are formed (Beck et al., 2010). In contrast, V was enriched along the groundwater flow path of an intertidal creek bank on the German Wadden Sea, and co-varied with products of microbial organic matter degradation such as ammonium and DIC (Riedel et al., 2011). Additionally, there was little evidence in the current study for Fe oxide control of V in the high-DOC/partially sulfidic Gloucester Point STE. Vanadium speciation and transport STEs are evidently influenced by the redox framework of the STE, and the presence of absence of significant iron oxides.

Although the overall distribution of V in the STE indicated nonconservative addition, it should be noted that the samples collected closest to the sediment-water interface (25 cm depth) contained lower V concentrations than expected by conservative mixing (Fig. 6). It may be that there are processes close to the sediment-water interface which lead to the removal of V, which would ultimately affect the flux of V via SGD. Ongoing high-resolution sampling of shallow porewater will attempt to clarify the behavior of V in the shallow subsurface.
Chromium. Chromium exhibited nonconservative addition across the salinity gradient, with a broad concentration maximum that did not align with any of the established redox zones (Fig. 6). The acid leachable sediment Cr concentrations (147 nmol cm\(^{-3}\)) are sufficient to sustain these porewater concentrations for 500 years (as discussed above for V), and could therefore be the source of V in the STE. Geochemical modeling indicated that reduced Cr(III) dominated at all depths in the STE (Fig. 7). This was consistent with previously demonstrated Cr(VI) reduction by Fe(II) (Eary and Rai, 1989), organic matter (Bartlett and Kimble, 1976), and hydrogen sulfide (Smillie et al., 1981), all of which are abundant in the STE. The oxidation of Cr(III) is kinetically slow and occurs primarily by reaction with reactive manganese oxides or oxygen as oxidizing agents (Schroeder and Lee, 1975; Bartlett and James, 1979; Eary and Rai, 1987), so Cr(III) oxidation within the suboxic STE would not be expected.

At circumneutral pH, Cr(III) is particle reactive and forms low-solubility minerals (Rai et al., 1989), so it is surprising to find high levels of Cr in the porewater. Cr(III) may be kept in solution by complexation with small organic molecules such as fulvic compounds, particularly when the concentration of organic matter is in excess of Cr concentrations (James and Bartlett, 1983). Measured humic carbon concentrations in the STE are several orders of magnitude higher than Cr concentrations (Table 1), and these ligands may be responsible for maintaining Cr in solution. The distribution of dissolved Cr closely followed that of DOC and humic material, and DOC and Cr were significantly correlated (R\(^2\)=0.35, p=0.006). Therefore, the mobility of insoluble Cr(III) in the reducing STE was apparently enhanced by association with organic ligands, making SGD
a potential and unexpected source of Cr to surface waters.

**Redox-Sensitive Element Fluxes**

Estimates of SGD-driven RSE fluxes (\(F_c\)) were made according to Equation 2 (Table 3). Surface water concentrations (\(C_s\)) are given in Table 1. Porewater endmember RSE concentrations (\(C_p\)) were estimated using 16 samples collected from seepage meters (Table 3). One drawback of seepage meters is that they may isolate the sediment-water interface and alter the redox conditions within the seepage meter (e.g. Rapaglia, 2005), which may have implications for determining the fluxes of redox-sensitive species. However, the concentrations of the RSEs measured in the seepage meters match those measured in shallow porewater (Figure 6), indicating that in this case, redox artefacts were small. The seepage meter concentrations were therefore considered representative of the seepage composition \(C_p\) for flux calculations.

The volume flux of SGD (\(F_{SGD}\)) was determined both by seepage flux measurements and in previous work using a Ra isotope tracer method (Luek and Beck, 2014). The seepage meter volume flux (55 ± 9 L m\(^{-2}\) day\(^{-1}\)) was within the range determined for the whole York River using different Ra isotopes (5 - 181 L m\(^{-2}\) day\(^{-1}\)).

\[
F_c = (C_p - C_s) \cdot F_{SGD}
\]  

Molybdenum and U were removed at average rates of -3.4 and -0.26 µmol m\(^{-2}\) day\(^{-1}\), respectively. Previously reported Mo fluxes in fine-grained sediments overlain by oxic waters were on the order of -0.06 to -0.56 µmol m\(^{-2}\) day\(^{-1}\) (Morford et al., 2009), an order of magnitude less than Mo fluxes calculated for permeable sediments estimated in
the current study (-0.21 to -7.7 µmol m$^{-2}$ day$^{-1}$). Uranium fluxes in fine-grained sediments overlain by oxic waters were on the order of -0.02 to -0.07 µmol m$^{-2}$ day$^{-1}$ (Morford et al., 2009), generally less than U fluxes calculated for permeable sediments estimated in the current study (-0.02 to -0.60 µmol m$^{-2}$ day$^{-1}$). The U removal rate estimated in the current study was similar to those reported for other STE environments in South Carolina (0.08 – 0.16 µmol m$^{-2}$ day$^{-1}$; Duncan and Shaw, 2003), Waquoit Bay, MA (0.4 µmol m$^{-2}$ day$^{-1}$; Charette and Sholkovitz, 2006), and along the Florida coast (1 µmol m$^{-2}$ day$^{-1}$; Santos et al., 2011). This comparison suggests that permeable sediments may play a disproportionately large role in Mo and U removal from predominantly oxic marine waters, because relatively rapid advection of Mo- and U-rich surface waters into reducing sediments facilitates extensive removal of Mo.

V and Cr show average fluxes of 0.87 and 2.0 µmol m$^{-2}$ day$^{-1}$, respectively. Efflux of V from fine-grained sediments to the water column has also been reported for V (0.042 to 0.12 µmol m$^{-2}$ day$^{-1}$; Santos-Echeandia et al., 2009) and Cr (0.0095 µmol m$^{-2}$ day$^{-1}$; Rigaud et al., 2013) in oxic basins. The flux of V from sediments in anoxic basins (1.01 - 8.22 µmol m$^{-2}$ day$^{-1}$) is higher than in oxic basins, likely due to the deposition of V-rich particles from the water column and release of V during early diagenesis (Emerson and Huested, 1991). Relatively fast advection of water through permeable coastal sediments, as well as chemical transformations within the sediments, evidently lead to fluxes of V and Cr that are substantially greater than those observed in fine grained environments overlain by oxic water.
Conclusions

The Gloucester Point shallow STE is formed by mixing of saline, oxic water with fresher, reducing groundwater in the upper few meters of permeable beach sands. The resulting vertical distribution of redox-active constituents is similar to the "classic" diagenetic sequence commonly observed in fine-grained sediments, with oxic respiration, nitrification/denitrification, metal oxide reduction, and sulfate reduction occurring sequentially with depth.

Mixing processes in the shallow STE and the generation of reduced metabolic products (particularly sulfide) affect the cycling of RSEs and cause the STE to act as a sink for some elements, but a source for others. The STE is a sink for both Mo and U. Both sulfidic and non-sulfidic STEs have been observed to act as U sinks, and the reduction of U in a variety of chemical environments may suggest microbial U reduction by iron- and/or sulfur-reducing bacteria. In contrast, the STE is a sink for Mo only when sulfide concentrations exceed approximately 11 µM, driving the formation of particle-reactive thiomolybdates. The STE at Gloucester Point is a source for both V and Cr. Vanadium is mobilized from sediments under neutral- to high-pH conditions, so geochemical reactions (e.g. Fe reduction) within the STE that lead to a mid-depth pH maximum appear to drive V mobilization. Reduced Cr, which is typically insoluble and immobile, appears to be mobilized from the solid phase and kept in solution by complexation with organic ligands. The quantitative importance of SGD to the York River Estuary (and to the Chesapeake Bay region) RSE budget will require future work to determine other RSE sources and cycling in surface waters.
This and previous work show that RSE geochemistry in permeable sediments is closely tied to redox and pH conditions (Charette and Sholkovitz, 2006; M. Beck et al., 2008b; Beck et al., 2010; Santos et al., 2011), while salinity gradients in the STE appear more incidental. For example, the distributions of Mo, U, V, and Cr found in the Gloucester Point STE are similar to those observed in a permeable tidal flat system in the German Wadden Sea (e.g. removal of Mo and U in reducing sediments, mobility of Cr associated with DOC) which has no salinity gradient, but comparable levels of DOC and sulfide production (M. Beck et al., 2008a, b). Endmember composition is a more significant control on STE chemistry than salinity gradients.

Extensive efforts have been previously made to constrain SGD volumetric fluxes based on coastal typology (Bokuniewicz et al., 2003; Burnett et al., 2006). Because different STEs (and even different locations throughout an STE) are characterized by different redox chemistries (e.g. DOC concentrations, whether sulfide is produced), evaluating and quantifying the importance of SGD and the STE for RSE transport and global marine budgets requires working toward a coastal groundwater typological framework that encompasses organic matter supply and redox chemistry.
Table 1. Ancillary parameters and concentrations of porewater constituents (average ± standard deviation). Averages and standard deviations represent all samples taken from the indicated depth interval over all sampling events (individual measurements can be found in the electronic supplement). "NA" indicates that there are no measurements available for the indicated depth range.
Table 2. Comparison of DOC, Fe, and sulfide concentrations found in different STE/coastal groundwater environments as either a range or average ± standard deviation, as reported (NR = not reported). The presence of sulfide (which controls the cycling of many reactive trace elements) is generally associated with higher (on the order of 1 mM or greater) levels of organic matter.

<table>
<thead>
<tr>
<th>Location</th>
<th>DOC  (µM)</th>
<th>Fe  (µM)</th>
<th>Sulfide (µM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wequyoct Bay, MA</td>
<td>10 – 703</td>
<td>1 – 175</td>
<td>NR</td>
<td>Chareille and Stokkowitz, 2006</td>
</tr>
<tr>
<td>West Neck Bay, NY</td>
<td>21 – 118</td>
<td>NR</td>
<td>NR</td>
<td>Beck et al., 2007</td>
</tr>
<tr>
<td>Indian River Lagoon, FL</td>
<td>1 – 700</td>
<td>0 – 200</td>
<td>0 – 600</td>
<td>Roy et al., 2010</td>
</tr>
<tr>
<td>FL Gulf Coast</td>
<td>150 – 500</td>
<td>6 – 20</td>
<td>NR</td>
<td>Santos et al., 2011</td>
</tr>
<tr>
<td>Sapelo Island hammock, GA</td>
<td>250 – 1080</td>
<td>0 – 50</td>
<td>0 – 40</td>
<td>Snyder et al., 2004</td>
</tr>
<tr>
<td>Okatie R. estuary, SC, West Bank</td>
<td>613 ± 27</td>
<td>60 ± 15</td>
<td>34.4 ± 19</td>
<td>Poldyky et al., 2014</td>
</tr>
<tr>
<td>Okatie R. estuary, SC, East Bank</td>
<td>1360 ± 63</td>
<td>19.2 ± 5.2</td>
<td>2210 ± 300</td>
<td>Poldyky et al., 2014</td>
</tr>
<tr>
<td>Gloucester Point STE</td>
<td>300 – 3000</td>
<td>0 – 30</td>
<td>0 – 1800</td>
<td>This paper</td>
</tr>
</tbody>
</table>
Table 3. Redox-sensitive element fluxes calculated using SGD flux estimates made using Ra (Luek and Beck, 2014), as well as the volume fluxes in the seepage meters (this study).
Figures

Figure 1. (a) The Gloucester Point site on the York River estuary (which drains to the Chesapeake Bay). (b) The study site is on VIMS beach, down-gradient of a marsh. Samples were taken at the mid-tide line in the location indicated by the star.
Figure 2. Subsurface conductivity transect conducted perpendicular to the shoreline starting at the mid-tide sampler (location indicated by star; depths sampled given in electronic supplement). Arrows indicate the placement of seepage meters, along with the measured salinity of the discharge (color and number at base of arrow) and the average area-normalized discharge rate (number at top of arrow) measured from seepage meters over two summers.
Figure 3. Measurements of salinity, Eh, and pH from all sampling events. The dashed box indicates the salinity transition zone. The shading indicates different redox zones (described in Fig. 4).
Figure 4. Depth profiles of redox active porewater constituents NO$_x$, NH$_4$, Mn, Fe, and sulfide. Shaded areas indicate the approximate redox zones within the STE: darkest grey (top) is oxic, medium grey (middle) is nitrogenous, lightest grey (bottom) is ferruginous, and no shading is sulfidic. The dashed box indicates the salinity transition zone (Fig. 3).
Figure 5. Depth profiles of porewater DOC, humic material, Mo, U, V, and Cr. The dashed box indicates the salinity transition zone (Fig. 3), and the shaded regions indicate redox intervals are described in Fig. 4. The shaded points indicate surface concentrations.
Figure 6. Left: Mixing diagrams for porewater DOC, Mo, U, V, and Cr. Empty circles indicate subsurface porewater; black circles indicate concentrations in seepage meters; grey circles indicate surface water concentrations. The dashed lines indicate conservative mixing of the most fresh and most saline samples. Right: Depth profiles of concentration anomalies as the percent difference between observed concentration, and expected concentration given conservative mixing between surface water and the freshest sampled point.
Figure 7. Calculated speciation of Mo, U, V, Cr, and reduced sulfur species. The dashed line indicates the average depth profile of the total element (values used in the model). The dashed box indicates the salinity transition zone (Fig. 3), and shaded regions indicate redox intervals as described in Fig. 4.
Figure 8. Calculated saturation states for iron sulfide minerals and uraninite. Shaded regions indicate redox intervals as described in Fig. 4.
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CHAPTER 2

SEASONALITY OF MAJOR REDOX CONSTITUENTS IN A SHALLOW SUBTERRANEAN ESTUARY

Abstract

The subterranean estuary (STE), the subsurface mixing zone of outflowing fresh groundwater and infiltrating seawater, is an area of extensive geochemical reactions that determine the composition of groundwater that flows into coastal environments. This study monitored the porewater composition of a shallow STE in Gloucester Point, VA (USA) over two years to determine seasonal variations in dissolved organic carbon (DOC) and reduced metabolites (including sulfide, Fe, and Mn). An additional aim of this study was to investigate the relative importance of salinity gradients (which have great geochemical influence in surface estuaries) versus redox gradients on STE geochemistry. Freshwater endmember redox potential and composition varied with depth — a shallow freshwater endmember was oxidizing and high in DOC, whereas a deep freshwater endmember was reducing, lower in DOC, and high in sulfide. The findings from this work demonstrated that Fe, Mn, and sulfide varied along a redox gradient (rather than a salinity gradient), and prompted the use of three-endmember mixing to quantify nonconservative addition/removal in the STE. In addition to salinity, humic carbon was used as a quasi-conservative tracer to quantify mixing according to a three-endmember model. The vertical distributions of DOC and reduced metabolites remained approximately constant over time, but concentrations varied with season. Dissolved organic carbon concentrations were greatest in the summer, and shallow meteoric
groundwater supplied the majority of DOC to the STE, except in summer when there was evidence for shallow non-conservative addition of DOC. Dissolved Fe and Mn were highest in a subsurface plume through the middle of the STE (100-140 cm) which was characterized by both higher concentrations and greater non-conservative addition in the winter. In contrast, sulfide was higher in summer at depths within the Fe and Mn plume (100 to 140 cm). We attribute the contrasting seasonal behavior of Fe and Mn and sulfide to microbial competition for organic matter and electron acceptors in the transition zone between dissimilatory metal reduction and sulfate reduction which led to sulfate/sulfur reducing bacteria (SRB) being more active in summer, and metal reducers being more active in winter. Throughout the STE, seasonal temperature variations influenced the redox environment and determined the geochemical cycling of DOC, Fe, Mn, and sulfur.

**Introduction**

Permeable sediments, previously assumed to be biogeochemically unimportant due to their low organic content and short porewater residence times, are in reality "hot spots" of biogeochemical activity (Boudreau et al., 2001). Permeable sediments in the coastal zone (such as sandy beaches) are subject to rapid porewater advection, and a growing body of evidence supports the conclusion that intertidal beaches are important areas of biogeochemical transformations (Charbonnier et al., 2013; Santos et al., 2008; Seidel et al., 2015). The subsurface mixing of outflowing fresh groundwater and infiltrating seawater creates a zone of extensive geochemical reactions called the subterranean estuary (STE) (Moore, 1999). The flow of water in intertidal beaches is
particularly driven by tidal forcing and wave run-up (Mango et al., 2004; Robinson et al., 2007; Gibbes et al., 2007), which typically results in recharge near the high tide line and discharge around the low tide line, creating a shallow saline circulation cell that spans the intertidal zone.

Submarine groundwater discharge (SGD), water that flows from sediments into the coastal ocean, is an important source of nutrients and trace metals to the sea (Slomp and Van Cappellen, 2004; Spinelli et al., 2002; Beck et al., 2009). The composition of SGD, and therefore chemical delivery to the coastal ocean, is determined by reactions that occur within the STE. The term subterranean estuary implies that changing salinity is the driving force behind reactions in this mixing zone (as is the case for surface estuaries). However, many of the STE reactions that modify the composition of SGD are driven not by the salinity gradient, but by the redox gradient (Charette and Sholkovitz 2002; Roy et al, 2010; McAllister et al., 2015). The advection of oxygenated water into the beach face and mixing with outflowing anoxic meteoric groundwater creates a redox interface. Reactions within the shallow STE have been shown to affect the cycling of reduced metabolites such as Fe and sulfide (Santos et al., 2011; McAllister et al., 2015; O’Connor et al., 2015; Seidel et al., 2015).

Our current understanding of biogeochemical processes within the STE is largely based on short-term sampling events (i.e., tidal cycle scale or shorter). Although SGD volume flux is affected by short-term variables such as tide (Santos et al., 2009a), processes and reactions in the STE occurring over longer timescales (e.g., seasonal, annual, interannual) are less well understood. Seasonal variations in surface and
groundwater height can drive variability in SGD flux, the STE salinity interface, and cause changes in Ra and Ba concentrations (Gonneea et al., 2013a, b; Wilson et al., 2015). In surface estuaries, seasonal variability in freshwater inputs and temperature affect trace element cycling (Eaton, 1979; Baird and Ulanowicz, 1989; Sholkovitz et al., 1992). Microbially-mediated processes, such as organic matter respiration and concomitant generation of redox gradients, are particularly temperature sensitive, and likely influence processes occurring in the STE over seasonal timescales (Apple et al., 2006). The STE is likely more susceptible to redox changes than surface estuaries due to isolation from the atmosphere and strong redox gradients that influence biogeochemical cycling (Santos et al., 2011; McAllister et al., 2015; O'Connor et al., 2015; Seidel et al., 2015).

Studies in coastal groundwater systems also indicate that seasonal variations in physical and chemical factors can affect geochemical reactions. One comparison of STE nutrient chemistry found higher production of dissolved organic carbon (DOC) and N species in the summer than winter (Santos et al., 2009b), and another found variable DOC composition and Fe concentrations throughout a year (Seidel et al., 2015). The extent of redox processes coupled to organic matter breakdown (e.g., denitrification and Mn reduction) during river surface water infiltration into groundwater were greater during periods of warmer temperatures (Jacobs et al., 1988). Changes in concentrations of organic matter, reduced metabolites, and redox-sensitive trace metals in a tidal flat were also attributed to seasonal variation in temperature and substrate availability (Beck et al., 2008a, b).
The current study builds on previous work at the Gloucester Point site documenting seasonality in the physical and chemical processes in the STE. To assess seasonal effects on redox processes, monthly measurements of salinity, temperature Eh, DOC and humic carbon, and reduced metabolites (dissolved Fe, Mn, and sulfide) were collected in a shallow STE over a two-year time series. Specifically, we tested the hypothesis that concentrations of reduced metabolites, as indicators of microbial respiration, would be greater during warmer summer months when rates of microbial processes are highest. An additional aim of this study was to investigate the relative influence of salinity gradients, which exert one of the strongest controls on chemical cycling in surface estuaries (e.g., Sholkovitz et al., 1978; Sanudo-Wilhelmy et al., 1996) versus redox gradients on STE geochemistry.

Methods

Study Site

The study site is located on the York River Estuary (YRE) in Gloucester Point, Virginia, USA (37.248884, -76.505324) (Fig. 1a). The YRE is a microtidal (0.7 to 0.85 m tidal range), relatively uncontaminated tributary of the Chesapeake Bay (Moore and Reay, 2009). Salinities of YRE surface water at this study site typically range from 14 in the winter to 25 in the summer (Luek and Beck, 2014). The Gloucester Point beach is a narrow (20-30 m wide) sandy beach with a gradual slope (0.2). It is bordered to the east by an upland marsh. Granite breakwaters line the beach ~40 m from the dune line to prevent erosion and stabilize the beach morphology (Fig. 1b).
Previous work at the study site used a Ra budget to estimate seasonally variable SGD volume fluxes to the York River which ranged between 93 and 178 L m\(^{-2}\) d\(^{-1}\) (Luek and Beck, 2014). Groundwater hydraulic head at the study site is inversely correlated with tidal height (Beck et al., 2015), and seasonal fluctuations in groundwater and sea level control saline intrusion and groundwater discharge (Luek and Beck, 2014). Porewater sampling at a mid-tide location (described further below) showed no tidal variation of redox-sensitive parameters (dissolved Fe and NH\(_4^+\)) at 50 and 100 cm depths (Beck et al., 2016). This suggests that oxygen intrusion during the recharge/discharge cycle was minor and had little effect on redox cycling over tidal cycles. 

Previous sampling of redox-active constituents at the same mid-tide location showed that advection of water through the sandy sediments drove the formation of a "classic" redox sequence typical of diffusion-dominated fine-grained sediments (O'Connor et al., 2015). Sediments were oxic down to ~70 cm depth (relative to the high tide sediment surface level), high in NO\(_x\) between 70 and 100 cm, high in dissolved Fe and Mn starting at 100 cm, and sulfidic beginning at ~140 cm depth. Although seasonal variations in solute concentrations are discussed here, the vertical distributions remained constant over time, resulting in four major redox zones — oxic, nitrogenous, ferruginous, and sulfidic. Further information regarding oxygen and nitrogen can be found elsewhere (O'Connor et al., 2015), and is not discussed further here: oxygen data were unreliable due to air contamination during sampling, and the complexity of the nitrogen cycle in the STE is beyond the scope of the current work.
Groundwater Collection and Analysis

Porewater samples were collected from locations along the Gloucester Point beach (Fig. 1c). Samples were collected from the mid-tide (MT) site, located at the center of the shallow saline recirculation cell, each month (May 2013 to February 2015) during the week of spring tide at the ebb phase of the tidal cycle. Tidal phase was controlled for during sampling in order to remove effects due to tidal variation, and to characterize the shallow STE during the period of greatest discharge. Previous work at the Gloucester Point site and other locations have focused on tidal effects on the shallow STE (Beck et al., 2016; Santos et al., 2009a), and the goal of the current study was to focus instead on seasonal variability. Samples were also collected from depth profiles along a shore-normal transect (Fig. 1c) during April 2014, July 2014, October 2014, and February 2015 to represent spring, summer, fall, and winter, respectively. The depth profiles were located at the average water levels during high tide (HT), intertidal (IT, between HT and MT), and low tide (LT). To remain consistent between profiles, all depths are reported relative the sediment surface at the high tide profile rather than sediment surface at the location of collection.

Porewater samples were collected using permanently installed wells comprising a 1 cm-long stainless steel screened point (AMS, Inc.; e.g. Gonneea et al., 2013) attached to 3 mm diameter No-Ox® PTFE tubing (Supelco/Sigma-Aldrich), or from an all-plastic multi-level sampler (Beck et al., 2010). Groundwater was pumped from depth using a portable peristaltic pump and filtered through a 0.45 μm Millipore polypropylene capsule.
filter into sampling containers. Salinity, temperature, and redox potential (Eh) were measured at the time of collection with a hand-held YSI556 multi-probe.

Samples for DOC analysis were collected in acid-washed polycarbonate bottles and frozen. Samples were analyzed on a Shimadzu TOC-V using methods modified from Sharp et al., (1993) for high-temperature combustion analysis (detection limit of 2.4 μM, field sample replicate <5%). Samples for humic carbon analysis were collected in acid-washed HDPE vials and frozen prior to analysis. Humic carbon was determined using a Shimadzu RF-1501 spectrofluorophotometer (excitation: 320 nm; emission: 420 nm; method described in Grasshoff et al., 1999), calibrated using a humic acid salt standard (Aldrich). A comparison with humic carbon measurements made using Amberlite XAD-2 resin (Harvey et al., 1983) indicated that fluorescent detection tended to underestimate concentrations of humic carbon from this site by 10% to 50%. Because humic carbon in this study was analyzed using fluorescence (a process more favorable in inflexible molecules with extensive conjugated bonds), the method likely detected primarily larger, more aromatic humic material.

Samples for dissolved Fe and Mn analysis were collected in acid-washed LDPE bottles, acidified to pH<2 immediately after collection, and analyzed by dilution and direct injection on a Thermo Element 2 inductively-coupled plasma mass spectrometer at the University of Southern Mississippi’s Center for Trace Analysis. The detection limit was 7 nM for Fe, and 1 nM for Mn. Samples for sulfide analysis were collected in acid-washed polypropylene vials pre-charged with 1 mL 0.25 M zinc acetate to fix volatile sulfide, and analyzed using either the Fonselius method (detection limit 0.1 μM, field
sample replication <10%) or, for samples with >120 μM sulfide, the Cline (field sample replication ≥20%) method (Grasshoff et al., 1999).

Salinity Mixing

To evaluate chemical addition or removal along the seasonal STE transects, two mixing lines were inferred on salinity-concentration plots: one between the saline surface endmember and the shallow fresh endmember, and one between the surface and deep fresh endmember. Points that fall within the triangle created by the two mixing lines were interpreted as resulting from mixing among the three endmembers, whereas those that fall outside the mixing lines indicate non-conservative addition or removal.

To quantify the addition or removal of DOC, Fe, and Mn at the MT profile over the two year time series, we calculated the difference between the observed concentration and the concentration that would be expected given conservative mixing. This difference, referred to here as the concentration anomaly, was calculated using the following equation:

\[ C_{\text{anomaly}} = \frac{C_{\text{observed}} - C_{\text{expected}}}{C_{\text{expected}}} \]

For each sample, three anomaly estimates were calculated based on: 1) mixing entirely between the surface water and the shallow freshwater endmember, 2) mixing entirely between the surface water and deep freshwater endmember, and 3) mixing between all three endmembers. The first two estimates were made with two endmember mixing equations using salinity as a conservative tracer. These anomalies represent the
limits of freshwater endmember mixing with surface water.

Quantifying mixing between three endmembers requires the use of a second conservative tracer (in addition to salinity); here, we used humic carbon as a quasi-conservative tracer. Whereas organic constituents are not generally considered ideal conservative tracers, humic carbon is relatively refractory (MacCarthy, 2001) and is present at different concentrations in the three endmembers (Table 1). In addition, concentrations were consistently within the range of the observed endmembers. The use of humic carbon as a quasi-conservative tracer in this study is intended to better constrain the anomaly calculations and to demonstrate the use of three-endmember mixing in characterizing the subterranean estuary.

Sediments

A 4-meter long vibracore was collected adjacent to the mid-tide (MT) sampling location in May 2013. The core was sectioned into 5 cm intervals and dried in a 90°C oven for 48 hours. Sample mass was measured before and after drying to determine sediment porosity. Sediment metal concentrations were normalized to volume of sediment, determined using porosity and an average mineral density of 2.65 g/cm³ (the density of quartz; Vanoni, 1964).

Sequential chemical leaches were performed to isolate different sedimentary metal fractions (Tessier et al., 1979). Dried and weighed sediments (1 g) were shaken for 180 minutes in 5 mL of 1 M hydroxylamine hydrochloride in 25% acetic acid, followed by centrifugation at 3000g for 10 minutes. Four milliliters of supernatant were removed
to a clean tube and diluted with 0.5 M HNO₃ (Aristar Plus, trace metal grade). This fraction represented the reducible fraction, comprising primarily amorphous Fe and Mn oxides (Tessier et al., 1979). The remaining sediment was shaken in 5 mL aqua regia (1:3 HNO₃:HCl) for 13 hours. Samples were centrifuged and the supernatant removed and diluted with MilliQ water. This fraction represents more recalcitrant sedimentary phases, but does not dissolve pyrite (Tessier et al., 1979; Huerta-Diaz and Morse, 1990). Metal concentrations in these fractions were analyzed as described for groundwater samples above. Average surface area in sediment samples were measured using a Micrometrics FlowPrep 060 and Gemini V surface area and pore size analyzer (according to manufacturer-provided instructions).

**Statistical Analyses**

All statistical analyses were conducted in RStudio (R Core Team). Prior to statistical analyses, non-normal data (salinity; dissolved Fe, Mn, sulfide, DOC) were natural log transformed to achieve greater normality (checked using q-q plots). Seasonal peak sub-surface concentrations at the MT profile (summer and winter, 2013 and 2014) were first analyzed using repeated measures ANOVA, and post-hoc comparisons were made using paired t-tests with a Bonferroni p-value adjustment. The paired test was used to account for samples being collected from the same location each month.

**Results**

*Temperature, Salinity, and Eh*

Temperature showed strong variability with season throughout the shallow STE,
as far as 4 m below the sediment surface (Fig. 2a). Surface water temperatures ranged from $9.5 \pm 5^\circ\text{C}$ in winter to $26 \pm 3^\circ\text{C}$ in summer (Fig. 3a). Temperatures at depth were somewhat less variable, ranging from $12 \pm 2^\circ\text{C}$ in winter to $21 \pm 1^\circ\text{C}$ in summer (Fig. 3a). Four seasons can be differentiated by their temperature profiles ($p<0.05$): Summer 2013 (May to October 2013; $23 \pm 1^\circ\text{C}$), Winter 2013 (November 2013 to April 2014; $11 \pm 1^\circ\text{C}$), Summer 2014 (May to October 2014; $24 \pm 1^\circ\text{C}$), and Winter 2014 (November 2014 to February 2015; $10 \pm 2^\circ\text{C}$) (Fig. 3a). These designations were corroborated by the seasonal transect temperatures: April 2014 ($14 \pm 1^\circ\text{C}$) and February 2015 ($6 \pm 2^\circ\text{C}$) were colder than July ($25 \pm 2^\circ\text{C}$) and October 2014 ($22 \pm 1^\circ\text{C}$) (Fig. 2a). Based on the temperature profiles, "summer" will be used hereafter to refer to May through October, and "winter" to refer to November through April.

The shallow saline plume extended several meters up the beach face, ending between HT and MT (Fig. 2b). Salinity remained high (>10) to a depth of 90 to 120 cm throughout the STE (Fig. 2b). Below ~150 cm, there was a zone of intermediate salinity (0.3 to 10) that extended from the HT to the MT profile. Deeper than 250 cm, salinity was consistently <5 (Fig. 2b).

The salinity distribution at HT showed two salinity minima: one shallow (90 cm or 130 cm) and one deep (410 cm) (Fig. 2b). These endmembers were not perfectly fresh (i.e., salinity=0), with salinities of $0.6 \pm 0.5$ (shallow) and $2.3 \pm 0.4$ (deep), but were the lowest salinity attainable due to physical restrictions of the study site (i.e., the presence of a boardwalk and dense plant cover farther upgradient). The locations of these endmembers indicated the up-gradient edges of the shallow circulation cell, and both are
freshwater sources to the STE and contribute to the overall mixing structure. Therefore, these HT samples represent "freshwater" endmembers in all mixing diagrams and concentration anomaly calculations, and are described as freshwater endmembers hereafter.

At MT (Fig. 3b), the salinity decreased from 18 ± 3 (summer) and 15 ± 4 (winter) at 62 cm to a local minimum of 1.7 ± 0.2 (summer) and 3 ± 3 (winter) at 130 cm depth (Fig 3b). This was followed by an increase in salinity to 220 cm depth (5.1 ± 0.4 in summer, 4 ± 2 in winter), and another decrease to a deeper salinity minimum at 375 cm depth (4 ± 1 in the summer, 3 ± 1 in the winter). The two salinity minima in the MT profile reflect the contributions from the two freshwater endmembers.

Salinity was lower in April 2014 than in other months, in contrast to the otherwise consistent salinity distributions (Supplementary Data). Monthly rainfall and tidal height were considered as potential reasons for the lower salinities, but particularly high rainfall or low tides did not coincide with fresher porewaters in April.

Redox potential was highest (most oxidizing) in surface water (179 ± 29 mV) and shallow porewater (169 ± 51 mV above 100 cm depth) and decreased to approximately zero (-27 ± 99 mV) between 100 and 200 cm depth. Porewater was most reducing below 200 cm depth, where Eh was consistently negative (-131 ± 34 mV) (Fig. 2c, 3c).

Dissolved Organic Carbon and Humic Carbon

Concentrations of DOC in the transect samples were highest in July. The highest concentrations of DOC throughout the STE were between 100 cm and 200 cm depth,
extending from the HT profile at the high DOC shallow fresh endmember (Fig. 2d, Table 1), through the deep IT samples, to the MT profile (Fig. 2d). A slight mid-depth maximum was evident at the LT profile as well, though it was generally far less pronounced than in the rest of the profiles. At the LT profile, in contrast to the rest of the seasonal transect profiles, DOC was highest in February (Fig. 2d). The shallow freshwater endmember DOC concentrations (1405 ± 244 μM) were higher than both the deep freshwater endmember (349 ± 328 μM) and the surface water concentrations (367 ± 112 μM) (Table 1).

Dissolved organic carbon showed a mid-depth peak in the MT profile time series from ~80 cm to ~210 cm depth. Results from the repeated measures ANOVA showed that only season (p=0.05) and year (p=0.01) had a significant effect on DOC concentrations within the maximum (approximately 100 to 140 cm depth) (Table 2). Concentrations of DOC within the maximum tended to be higher during summer (1301 ± 180 μM) than in winter (1013 ± 208 μM), and the highest concentrations occurred during Summer 2013 (1561 ± 475 μM; significantly higher than every other season, p<0.05) (Fig. 4a).

In the seasonal transect mixing diagrams for each month, several samples (primarily from the IT and MT profiles) fell above the conservative mixing triangle indicating non-conservative DOC addition. In February and April, several samples (primarily from the MT and LT profiles) also fell below the mixing triangle, indicating DOC removal (Fig. 5a). Concentration anomalies at the MT profile showed slight nonconservative DOC addition between 80 and 140 cm depth in all seasons in both two-
endmember mixing scenario extremes and the three-endmember mixing calculation (Fig. 6a).

The humic carbon maximum extended from the HT to the LT profile, with the highest concentrations between 80 to 210 cm depth (Fig. 2e). Generally, humic carbon concentrations between HT and MT were highest in July and October. In contrast, humic carbon was highest at the LT profile in February (Fig. 2e). The proportion of humic carbon in DOC was generally between 30% and 70% (Appendix), and tended to increase with depth in the shallow STE (Fig. 3f).

Like DOC, humic carbon was highest between ~80 cm to ~210 cm depth in the MT profiles. Results from the repeated measures ANOVA showed that only season (p=0.03) had a significant effect on humic carbon concentrations within the maximum (approximately 100 to 140 cm depth) (Table 2). The highest mean seasonal concentrations within the peak ranged from 461 μM (winter 2013) to 909 μM (summer 2014) (p=0.01) (Fig. 3e). Concentrations of humic carbon within the MT maximum tended to be higher in the summers (473 ± 226 μM) than the winters (384 ± 154 μM), although this difference was not statistically significant (Fig. 4b).

Humic carbon concentrations, as well at the humic carbon to total DOC ratio (hum:DOC), varied among the three identified endmembers (Table 1). Concentrations of humic carbon were highest in the shallow freshwater endmember (497 ± 225 μM), lowest in the surface water (82 ± 37 μM), and intermediate in the deep freshwater endmember (107 ± 25 μM). However, hum:DOC followed a different pattern; hum:DOC was highest in the deep freshwater endmember (0.5 ± 0.2), and similar in the shallow freshwater
endmember (0.3 ± 0.1) and surface water (0.3 ± 0.1).

Fe, Mn, and Sulfide

Concentrations of Fe in the STE were highest between 100 cm and 200 cm depth (Fig. 2f). The mid-depth Fe maximum extended from the HT profile, along the deep transect samples to the MT profile. Dissolved Fe concentrations in the seasonal transects tended to increase in concentration from the HT profile maximum to the IT profile maxima, and then to decline again by the MT profile (Fig. 2f). Dissolved Fe was consistently low at LT, with concentrations below 4 μM at the shallowest profile depth and less than 1 μM at all deeper points (Fig. 2f). The shallow freshwater endmember Fe concentrations (68 ± 54 μM) were higher than both the deep freshwater endmember (1.7 ± 3 μM) and the surface water (0.09 ± 0.09 μM) concentrations (Table 2).

As previously observed, the vertical distribution of Fe was constant over time at MT (O’Connor et al., 2015). The Fe maximum at the MT time series was consistently observed between 110 cm and 140 cm depth. Results from the repeated measures ANOVA (using samples from 100-140 cm depth) showed that, statistically, neither year nor season had an effect on Fe concentrations within the MT maximum (Table 2). Although the ANOVA results were not statistically significant, maximum concentrations reached as high as 38 ± 23 μM during Winter 2014, and as low as 10 ± 3 μM during Summer 2014 (p=0.03; Fig. 3f). Although the summer and winter means were not significantly different, the highest overall concentrations of Fe within the MT profile maximum occurred during winter (Fig. 4c).
In all months when transect samples were collected, concentrations of Fe in the deep IT samples were higher than in either of the freshwater endmembers. Concentrations of Fe and enrichment relative to concentrations expected for conservative mixing were greatest in February, and at the IT locations (Fig. 5b). Within the MT maximum, Fe anomalies calculated using two endmember mixing fell at or around zero in all seasons, indicating that the concentration anomalies were not distinguishable from conservative mixing. Both shallow and deep freshwater endmember anomaly estimates were generally <0 in shallow (<90 cm) and deep (>300 cm) sediments, indicating non-conservative removal (Fig. 6b). The anomalies calculated using three endmember mixing followed the same trends as those calculated using the two freshwater endmembers individually, and commonly fell between those calculated using two endmember mixing. These anomalies showed that MT maximum Fe concentrations were enriched above conservative mixing in the winter, depleted below conservative mixing in the summer, and in all seasons depleted below conservative mixing both shallow (<90 cm) and deep (>150 cm) in the profile where Fe concentrations were lowest (Fig. 6b).

The highest Mn concentrations in the STE were observed between 100 cm and 300 cm depth (Fig. 2g). The mid-depth Mn maximum extended from the HT profile to the LT profile. In general, Mn concentrations were highest at the HT and IT profiles (Fig. 2g). Dissolved Mn tended to increase in concentration down-gradient along the deep IT profile samples (Fig. 3h). The shallow freshwater endmember Mn concentrations (0.58 ± 0.36 μM) were higher than both the deep freshwater endmember (0.07 ± 0.03 μM) and the surface water (0.17 ± 0.14 μM) concentrations, except in April 2014 (Table 2).
The MT time series Mn maximum was observed between 90 cm and 265 cm depth, and ANOVA results showed that concentrations within the maximum (100 to 140 cm) varied between seasons (p=0.08), and that there was an interaction between year and season (p=0.08) (Table 2). Within the MT maximum, median concentrations were lower in 2014 than 2013 (p=0.02). Concentrations of Mn tended to be higher in winter compared with summer, although this difference was only significant between Winter 2013 and Summer 2014 (Fig. 4d).

In all transects, concentrations of Mn at intermediate salinities (including all deep IT samples) commonly fell above the conservative mixing triangle (Fig. 5c). Both dissolved Mn concentrations and excess dissolved Mn were greatest in February (Fig. 5c). Mn concentration anomalies calculated using three endmember mixing fell between those calculated using the most extreme two endmember mixing cases (i.e., mixing of surface water completely with either the shallow or deep freshwater endmember) (Fig. 6c). Within the MT Mn maximum, concentration anomalies were greater than zero for both the two- and the three-endmember estimations, indicating non-conservative Mn addition. This addition was greater in winter than summer in both years (Fig. 6c). Concentration anomalies calculated using both two- and-three endmember mixing estimates were <0 in shallow (<90 cm) sediments, indicating non-conservative Mn removal (Fig. 6c).

Dissolved Fe and Mn concentrations in the shallow freshwater endmember were lower in April (i.e., more similar to the deep freshwater endmembers) resulting in a narrower conservative mixing triangle (Fig 5b, c). Increased input of fresh groundwater
into the STE potentially caused the low salinities, leading to decreased shallow groundwater residence times and therefore less accumulation of reduced metabolites.

Dissolved sulfide was greatest in the deep portions of the STE (Fig. 2h). At the MT profile, concentrations increased from below detection limit in the shallow sediments (<100 cm) to between 1 and 1.5 mM at 265 cm (Fig. 3i). A similar trend was observed at the HT profile, where sulfide was below detection to 130 cm and increased to 1 - 1.5 mM by 330 cm depth (Fig. 2h). No sulfide was detectable in the IT samples, whereas sulfide was detectable at all depth in the LT profile, ranging from ~250 μM at 125 cm to 1 - 1.5 mM at 190 cm (Fig. 2h).

In the MT time series, there was no observable or statistical seasonal difference between sulfide depth profiles (Fig. 2h). Likewise, maximum sulfide concentrations in the seasonal transects did not show a clear seasonal trend (Fig. 3i).

Unlike DOC, Fe, and Mn, sulfide did not reach a maximum in the 100 to 140 cm depth range at MT; however, sulfide began to be detectable and co-occurred with dissolved Fe within this zone (Fig. 4c, e). In this 100 to 140 cm depth range, sulfide tended to be greater in the summers than in the winters (Fig. 4e). There was no statistically significant seasonal difference in mean concentrations (with the possible exception of Summer 2013 and Winter 2014, p=0.1), but the upper range of sulfide concentrations was higher during both summers (upper quartiles of 121 μM and 45 μM in summers compared to 18 μM and 10 μM in winters).
Sedimentary Metals

Reducible Fe and Mn were lowest in the shallowest sediments (<100 cm), and reached a maximum of ~15 mmol/cm$^3$ and ~0.46 mmol/cm$^3$, respectively, with the peak between 100 and 150 cm (Fig. 7a, b) — the general depth range in which Fe and Mn exhibited maximum porewater concentrations. Both reducible Fe and Mn decreased below 150 cm depth, then decreased in concentration at 275 cm before reaching another maximum (to 7.8 and 0.14 mmol/cm$^3$, respectively) at 342 cm depth, well within the sulfidic/reducing zone of the profile. The aqua regia leach concentrations followed a similar trend, but concentrations were around an order of magnitude higher overall.

Discussion

Salinity & Eh

The salinity structure of this shallow STE deviated slightly from what is typically expected and modeled (Robinson et al., 2007). Rather than a simple shallow high-salinity circulation cell underlain by fresh water, we observed a mid-depth zone of intermediate salinity water that diminished as it flowed down gradient (Fig. 2b). In contrast, porewater Eh uniformly decreased with depth throughout the STE, indicating more reducing conditions with depth (Figs. 2c, 3c). Difference in the distribution of salinity and Eh contributed to a significant but weak positive correlation between salinity and Eh throughout the shallow STE (R$^2$=0.15, p=1x10$^{-5}$). The mechanism causing the mid-depth salinity maximum is unclear. It may be due to along-shore advection of saline water (as observed by Zhang et al., 2016), perhaps driven by the shoreline curvature at
this site (Fig. 1b). Alternatively, some variability in sediment porosity may create preferential flow paths for the intrusion of saline water into the beach face (Michael et al., 2016).

Despite any salinity variation, previous work showed that biogeochemical zonation in this STE followed the typical sequence predicted by free energy yield (O'Connor et al., 2015). The mid-depth higher salinity water was evidently isolated from the atmosphere and modified to have a consistent Eh with the surrounding water, and to fall within the observed redox succession of the system. Slomp and Van Cappellen (2004) proposed four general classifications for deep STEs based on the redox potentials of fresh (oxic or anoxic) and saline (oxic or anoxic) water inputs. Our findings demonstrate that, in this shallow STE, characterizing redox condition of STE inputs is more complicated: freshwater here had redox potentials that varied from oxidizing to reducing (Fig. 2c).

**Multiple Endmember Mixing in the STE**

Salinity mixing diagrams are commonly used to evaluate the addition or removal of a chemical constituent along an estuarine mixing gradient (Loder and Reichard, 1981). Conservative behavior during mixing of fresh and saline waters results in intermediate chemical concentrations that fall on a linear trend line with salinity between those in the two endmembers. Addition or removal of solutes in the STE can be estimated using an "effective zero-salinity endmember" concentration, which is extrapolated from the salinity-concentration relationship in samples from either surface water (Charette and
Sholkovitz, 2006) or shallow porewater (Santos et al., 2011). The effective fresh endmember can then be compared to the range of observed fresh endmember concentrations to determine degree of addition or removal in the shallow STE. While effective as a basic mechanism for evaluating chemical behavior in some STEs, this method is inaccurate where more than two endmembers exist.

The salinity and geochemical distributions observed in this study serve to highlight an important point about endmember assumptions in the STE. Groundwater chemistry can be vertically heterogeneous on meter or even centimeter scales (e.g., Appelo and Postma, 2005; Charette and Sholkovitz, 2006; Beck et al., 2010; O'Connor et al., 2015), Observations from this and other STE sites have shown vertical variation in concentrations of solutes such as Fe and DOC in the up-gradient freshwater source (e.g., Charette and Sholkovitz, 2006; Santos et al., 2011; this paper). Characterizing the boundary conditions of the STE is therefore important for determining water sources and mixing.

Three-endmember mixing has not generally been considered in STE studies, but where appropriate, represents a more realistic framework for determining nonconservative mixing of dissolved constituents in the subterranean estuary. Previous work in a deep STE demonstrated three endmember mixing in a deep STE, and used the $^{87}\text{Sr}/^{86}\text{Sr}$ isotope ratio to quantify three-endmember mixing (Jorgensen et al., 2008). Our study demonstrates how a shallow STE can receive water from two upgradient low-salinity sources, representing distinct chemical endmembers, providing clear evidence for three-endmember mixing (Table 1; Fig. 2), and showed the effectiveness of a three-
endmember approach in constraining addition or removal of dissolved chemical constituents (Fig. 6). The concentration anomalies calculated using humic carbon fell between the extreme two-endmember mixing scenarios (mixing between the surface water and either the shallow fresh endmember or the deep fresh endmember), narrowing down the wide range of estimates (Fig. 6). Using the three endmember approach therefore allowed for more accurate characterization of how the biogeochemistry of the STE changed seasonally.

The calculated contributions from each of the endmembers were not always a positive fraction (Supplementary Information), indicating either imperfect conservative behavior of one of the tracers, or a flaw in assigning endmember concentrations. Specifically, there will be some degree of temporal decoupling between the mixing of the observed endmembers and the STE samples, exacerbated by changing endmember concentrations. This characterization of endmembers and mixing patterns across seasons highlights the importance of endmember variability in interpreting mixing patterns as well as chemical fluxes.

Seasonal Variation in the STE

Temperature. Seasonal temperature variation at MT was ~15°C in the upper 100 cm and ~9°C at 375 cm depth (Fig. 3a). According to the Q_{10} rule (Lloyd and Taylor, 1994), rates for chemical reactions are expected to approximately double for every 10°C increase in temperature. As a result, microbial respiration rates typically increase with temperature. These temperature changes therefore have the potential to generate a two-
fold variation in microbial respiration rates throughout the shallow STE.

Dissolved Organic Carbon and Humic Carbon. Dissolved organic carbon concentrations tended to be higher in summer compared with winter throughout the STE, as within both the DOC maximum (Fig. 2d, 3d, 4a) and the shallow freshwater endmember (Table 2). Furthermore, greater non-conservative addition of DOC was observed in summer (Fig. 5a, 6a). These same trends were observed for humic carbon, as the two co-varied throughout the STE (Fig. 2e, 3e).

The mid-depth DOC maximum, which showed the greatest seasonal concentration variation, likely resulted from input of shallow fresh groundwater with high DOC concentrations (Fig. 2d). The shallow freshwater endmember and mid-depth DOC plume were characterized by both high concentrations of both DOC and humic carbon, as well as a high humic:DOC ratio (Fig. 2d-e, 3d-f). This endmember was very similar in DOC concentration, humic carbon concentration, and hum:DOC to pooled water in a small marsh located a few dozen meters up-gradient of the beach (Table 1). Seasonal variation in DOC concentrations occurred within the mid-depth DOC plume, and coincided with variations in humic carbon (Fig. 2d, e; 3d, e; 4a, b). The higher DOC and humic carbon during summer in the MT time series and seasonal transects (Fig. 2d, e; 3d, e) is then likely partially due to increased organic matter supply from the marsh. This could be caused by greater plant growth and microbial degradation of detritus under warmer temperatures.

The highest DOC concentrations occurred in the IT and MT profiles rather than farther up-gradient (Fig. 2d), indicating non-conservative inputs in these samples (Figs.
5a and 6a). The non-conservative addition of DOC in the shallow porewater may be from a surface estuarine source, such as benthic algal production or straining of algae through the intertidal sands (Santos et al., 2008, Roy et al., 2010). The York River Estuary is more biologically productive during spring and summer (Sin et al., 1999), which could lead to an increased supply of organic material to the shallow sediments followed by breakdown of this particulate carbon, generating the greater non-conservative DOC addition observed in summer.

Previous studies have also found higher summer concentrations of DOC in the STE, derived from either increased inputs from surface water (Santos et al., 2009b) or release from deep sediments by higher summer respiration (M. Beck et al., 2008). In this STE, higher summer DOC concentrations were driven by increases in the shallow freshwater endmember concentration and by greater input from surface water, both of which were likely controlled by warmer temperatures. In all of these cases, increased biological activity led to increases in DOC.

*Fe, Mn, and Sulfide.* Overall seasonal trends in this STE were higher concentrations and enrichment of Fe and Mn during the winter, and higher concentrations of DOC and sulfide (in the intermediate depth range) in the summer. Higher summer DOC concentrations were explained by seasonally variable freshwater organic matter source (i.e., organic matter production and breakdown in the marsh), and increased surface estuary organic matter supply to and breakdown in shallow STE sediments. However, the inverse trends observed in reduced metabolite concentrations did not support the hypothesis that all microbial respiration processes (and resulting reduced
metabolite concentrations) in the STE increase with temperature. Observed patterns of dissolved Fe, Mn, and sulfide can be combined with existing knowledge of microbial behavior to understand the seasonal cycling of these elements in the shallow STE.

Despite the fact that Fe concentrations in the shallow fresh endmember (at the upgradient edge of the Fe plume) were highest in summer (Table 1), concentrations of Fe in both the transects (Fig. 2f) and MT time series (Fig. 3g, 4c) were higher in winter than summer. Additionally, non-conservative addition of Fe to the STE was greatest during February, when Fe concentrations were highest (Fig. 5b). Therefore, high Fe within the mid-depth maximum during winter resulted from non-conservative addition of Fe from reductive dissolution within the STE (Fig. 5b, 6b), and not an increase in the fresh endmember.

Higher concentrations of dissolved Mn in the winter paralleled the seasonal dissolved Fe trends (Fig. 2g 3h, 4d). Non-conservative addition of Mn was observed in all seasons, and was greatest in winter in both the transects and MT time series (Fig. 5c, 6c). Similar to Fe, the high concentrations of Mn in the STE were therefore, like Fe, due to non-conservative addition by reductive dissolution.

Between 100 and 140 cm (the depth zone in which Fe and sulfide overlap) in the MT time series, sulfide tended to be higher in the summers than in the winters (Fig. 4e), the opposite behavior of Fe and Mn. Sulfide is a major control on Fe cycling in the environment. Sulfide and Fe(II) readily react to form solid-phase Fe sulfide minerals such as mackinawite and pyrite (Rickard et al., 1995), both shown previously to be saturated below 125 cm at the MT profile (O'Connor et al., 2015). Contrasting seasonal trends in
Fe and sulfide concentrations (Fig. 4c, e) were consistent with their reactivity with each other.

Unlike Mn, the addition of Fe at IT was evidently attenuated during the summer as water moved down-gradient. Although both Fe and Mn were higher and showed greater non-conservative addition in winter, their distributions differed moving down-gradient through the STE. Dissolved Fe was only enriched at MT in winter, whereas Mn enrichment at MT occurred in all seasons (Fig. 6b, c). Additionally, Fe anomalies were highest in deep IT samples, but Mn anomalies were high in both IT, MT, and even LT samples (Fig. 5b, c).

Solid-phase Fe and Mn oxides are reduced by similar microbes (Lovley, 2000), accounting for their analogous distributions within the STE. However, Mn does not readily react to form solid-phase sulfide compounds (Vairavamurthy and Schoonen, 1995). Sediment Fe and Mn maxima (in both the reducing and aqua regia leaches) coincided with the dissolved peaks for these constituents (Fig. 3g, h; Fig. 7), suggesting that some oxidation of both Fe and Mn occurs at MT. However, oxidation of Mn is less thermodynamically favorable than oxidation of Fe (Canfield and Thamdrup, 2009).

Removal of Fe along the STE, by either oxidation or reaction with sulfide, was greater in the summer, resulting in lower MT summer anomalies compared to Mn. Concurrently high Mn and Fe and enrichment at the IT wells indicated that the higher winter concentrations resulted from an increased input by dissimilatory metal reduction, rather than simply less removal. This suggested that dissimilatory metal reduction as a form of anaerobic respiration was more important in the winter than sulfate.
Despite having sedimentary metal data for only summer, field observations may allow us to infer how oxidation of Fe and Mn at MT may affect interpretation of seasonal trends. A red-colored sand layer was consistently observed at ~75 cm depth in hand-dug pits on the beach face, and was clearest during the late summer. This pattern suggested that the Fe oxide (and, perhaps, the concurrent Mn oxide) layer built up in the summer, and was dissolved by dissimilatory metal reduction in the winter. In contrast to other STE locations (i.e., Spiteri et al., 2006), the pH gradient within the zone of interest spans only one pH unit, from 6.5 to 7.5 (O'Connor et al., 2015), and is unlikely to drive a dramatic increase in Fe oxidation. Although speculative, higher activity of metal oxidizing microbes during summer could explain the greater oxidation of metals (McAllister et al., 2015). The decline in metal oxides during winter is consistent with greater dissimilatory metal reduction at this time of year.

Seasonal Drivers. Because both temperature and DOC increased in the summers, it is important to consider both variables as a potential control on the redox framework of the STE. Dissolved organic matter can fuel microbial respiration, and in a Florida STE, higher N cycling rates were linked to greater marine DOC supply (Santos et al., 2009b). In an STE in Taiwan, DOC contained a large amount of fluorescent humic carbon, and was relatively unreactive in incubation experiments (Yang et al., 2014). In the current study, DOC from the mid-depth maximum tended to have a high proportion of refractory humic carbon (similar to Yang et al., 2014), and therefore temperature may have had a greater effect than organic carbon supply on microbial metabolism and seasonal redox
fluctuations. However, estuarine organic carbon input to the STE in the summers (which led to positive DOC anomalies) may have been more labile, and may have affected microbial activity as well.

Observations from this study are consistent with observations from a salt marsh showing a collapse in the Fe reducing microbial community during the summer (Koretsky et al., 2003). The collapse was attributed to an increase in the rate of microbial sulfate reduction in summer driven by higher temperature and greater concentrations of organic matter. The population of Fe reducers was inhibited and reduced in number by the higher concentrations of sulfide in summer. When the activity of the SRB slowed in the winter, the Fe reducing bacterial population recovered, and more dissolved iron was generated.

The following framework, based on these previous observations (Koretsky et al., 2003), is therefore proposed to explain the seasonal variations in redox chemistry within this STE. In the transition zone between dissimilatory metal reduction and sulfur reduction, the microbial populations mediating these reactions compete for organic matter and electron acceptors. Increases in SRB activity during the summer (better able than metal reducers to take metabolic advantage of warmer temperatures and more organic matter) leads to higher concentrations of sulfide, inhibiting metal reducing microbes, leading to lower concentrations of both Fe and Mn. In winter, the activity of SRB slows, allowing the population and/or activity of metal reducers to increase, leading to higher concentrations of Fe and Mn.

This work expands our understanding of redox dynamics in the STE to include
seasonal controls. The majority of prior work on redox active constituents in the STE has occurred during only one season, most frequently the spring and summer (e.g., Beck et al., 2007; Charette and Sholkovitz 2006, Roy et al 2010, McAllister et al., 2015). However, seasonal sampling has demonstrated seasonal variability in Ba and Ra STE cycling and fluxes (Gonneea et al., 2013b). Increased temporal coverage at the Gloucester Point site reveals, for example, that the STE itself is a source of dissolved Fe in winter; without this information, the dissolved Fe flux via SGD would likely be underestimated.

It has been widely concluded that SGD is a source of Fe to coastal waters because of inputs from Fe-rich meteoric groundwater (Charette and Sholkovitz, 2002; Windom et al., 2006; Roy et al., 2010). The input of Fe to the ocean is limited in this scenario by oxidation of iron as meteoric groundwater mixes with oxic, high pH infiltrating seawater (Roy et al., 2010; Spiteri et al., 2006, McAllister et al., 2015). This work demonstrates that Fe may be remobilized seasonally within the shallow STE itself and may be a periodic source of dissolved Fe. In addition to oxidation, reactions with sulfide within the STE can inhibit Fe transport to the ocean (this study; Roy et al., 2010), suggesting that the Fe flux to the coastal ocean is controlled by both production and removal mechanisms within the STE.

In addition to ramifications for Fe and Mn fluxes to the coastal ocean, the formation and dissolution of reactive Fe and Mn oxides affect the transport of other chemical constituents to the coastal ocean such as Ba, Mo, V (Charette and Sholkovitz, 2006; Beck et al., 2007). Therefore, seasonal variation in STE redox chemistry likely affects the speciation and transport of many trace elements.
Conclusions

For many redox-sensitive chemical constituents, the term "subterranean estuary" may be somewhat biogeochemically misleading. Both surface and subterranean estuaries are defined by mixing of fresh and saline waters, and serve as links between terrestrial and marine systems. However, major geochemical transformations in most surface estuaries are driven by salinity changes (e.g. flocculation and Fe removal), phytoplankton, and oxic processes. In contrast, the biogeochemistry of STEs is largely dominated by redox reactions, not salinity gradients.

Although salinity may be useful in tracing addition or removal of elements, endmember mixing and transport in STEs can be complex and make chemical behavior difficult to evaluate. The current work used a three-endmember mixing approach, but further development of these mixing models for the STE (perhaps moving toward a redox-based, rather than a salinity-based, framework) will be needed to accurately quantify addition and removal.

Although the depth distributions of DOC and major redox active constituents in the STE remained remarkably consistent over time, their concentrations showed clear seasonal trends. These seasonal variations affected how DOC, Fe, and Mn were transported to coastal waters, and demonstrated the complexity of STE redox response to variations in temperature. The seasonal changes in redox cycling likely have ramifications for the solubility and transport of other redox-active species (including some trace metals).
# Tables

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* Mar, Apr  
** May, Jun, Jul  
† Aug, Sep, Oct  
‡ Nov, Dec, Jan, Feb

Table 1. Salinities and dissolved constituents of the three endmembers considered in this paper: a shallow freshwater endmember, a deep freshwater endmember, and surface water. Noted are the months in which each endmember was used for calculating seasonal MT concentration anomalies. April was used in fewer months due to the unusual salinity range compared to all other sampled months over the two years (discussed in text). NA indicates a sample not collected or measured.
Table 2. P values from repeated measures ANOVA (described in Methods). Values <0.1 were considered statistically significant (rather than 0.05, as these results are from field observations rather than a controlled experiment) and are shown in bold.

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<td>Sulfide</td>
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Figures

Figure 1. (a) The study site is located on the western side of the Gloucester Point peninsula on the York River estuary (Virginia, USA). (b) The sample site is located by the VIMS boat basin. Up-gradient of the sampling site is a marsh, and granite breakwaters (part of a larger York River shoreline restoration project) line the shore. (c) Permanent sampling wells were installed at the points shown (specific depths found in Supplementary Information). Depth profiles were located at the spring high tide (HT), mid tide (MT), and just below low tide (LT) water levels. Shorter depth profiles were installed in a transect in the intertidal zone (IT, including T1, T2, and T3) between the HT and MT profiles. Dotted lines indicate example salinity contours.
Figure 2. Concentrations of dissolved constituents from STE transects in July (summer) and February (winter), created using Ocean Data View. Well locations are described in the caption to Fig. 1. Depths are measured relative to the high tide sediment surface.
Figure 3. Dissolved constituent concentrations from the MT profile over the two-year time series. The months making up each summer and winter are described in the text. At each depth, points represent the mean concentration, and the shaded areas represent one standard deviation the mean, for all the months sampled in each season. The number of months averaged in each profile is shown in (a), with any exceptions shown in italics in the appropriate profile.
Figure 4. Concentrations of dissolved constituents for the MT profile between 100 and 140 cm over the two-year sampling period. The thick lines indicate the median concentration. The top and bottom edges of the boxes indicate the first and third quartiles. The whiskers extend to 1.5 times the interquartile range of the box, and any data outside this range are shown as hollow circles. The stars indicate which seasons are different from one another at the given p value (p values over 0.1 not indicated). The p values were determined using paired t tests, with the alpha level corrected for multiple comparisons using the Bonferroni adjustment.
Figure 5. Salinity mixing diagrams of dissolved Fe and Mn. Points are indicated by shape and color as originating from the HT profile, IT profiles, MT profile, LT profile, or surface water. Filled in points are from depths at which sulfide concentrations exceed 10 µM. For visual clarity over a wide concentration range, Fe and Mn concentrations are shown on a log scale. Conservative mixing lines are drawn between the surface water and both the deep and shallow freshwater endmembers (Table 1).
Figure 6. Concentration anomalies (calculated as described in the text) of DOC, Fe, and Mn at the MT profile. The two sets of points shown in solid circles (dark and light) are the anomalies calculated using either the deep or shallow freshwater endmembers, respectively. The shaded region between these two estimates represents the uncertainly range of the anomaly at a given depth. The stars indicate anomalies calculated using three end-member mixing. The points shown are the median anomaly values for each season.
Figure 7. Sediment concentrations of (a) Fe and (b) Mn dissolved by reducing (open circles) and aqua regia (filled circles) leaches (described in Methods). The sediment core was collected adjacent to the MT profile. The dotted line represents the sediment surface at the sampling location; depths are presented relative to the HT sediment surface.
References


sediments (Sapelo Island, GA, USA). Biogeochemistry 64, 179–203.


Abstract

The subterranean estuary (STE), where outflowing fresh groundwater mixes with infiltrating seawater, is a 'hotspot' of biogeochemical processes. Reactions occurring within the STE determine the composition of water that flows into coastal environments. This study monitored the porewater composition of a shallow STE in Gloucester Point, VA, USA over two years to determine seasonal variation in the distribution and concentrations of redox sensitive metals (RSMs) including Mo, U, V, and Cr. Molybdenum and uranium were both supplied to the STE by surface water, but differed in their removal mechanisms and seasonal behavior. Molybdenum showed non-conservative removal by reaction with sulfide in all seasons. Sulfide concentrations at this site were consistently higher than required for quantitative reaction with Mo (11 µM sulfide), evidently leading to quantitative removal at the same depth regardless of season. In contrast, U appeared to depend directly on microbial activity for removal, and showed more extensive removal at shallower depths in summer. Both V and Cr were elevated in meteoric groundwater (2.5 - 297 nM and 12.6 - 42.1 nM, respectively), with higher endmember concentrations in summer. Both V and Cr also showed non-conservative addition within the STE relative to conservative mixing among the observed endmembers. The mobility of V and Cr in the STE, and therefore their supply to the coastal ocean, was controlled by the availability of dissolved organic matter and Fe,
potentially complexing RSMs as colloids. However, complexation by different organic matter pools led to seasonal variations in V (possibly complexed by polar fulvic compounds, with higher summer porewater concentrations), but greater interannual variability of Cr (perhaps complexed by humic material, with higher concentrations in 2013 than 2014, regardless of season). These results emphasize the importance of seasonal considerations when evaluating the role of the STE in global biogeochemical cycles. Although the broader redox structure of the shallow STE was stable over the two-year time series, its role as a source or sink for these RSM (as shown by varying porewater concentrations and non-conservative behavior) changed with time, and these results indicate that seasonal changes in chemical cycling may therefore be reflected in the SGD-driven chemical flux to the ocean.

Introduction

Submarine groundwater discharge (SGD), water that flows from sediments into the coastal ocean, is an important source of nutrients and trace metals to the sea (Burnett et al., 2003; Slomp and Van Cappellen, 2004; Spinelli et al., 2002; Beck et al., 2009). The chemical composition of SGD is determined by reactions that occur within the subterranean estuary (STE), the mixing zone of fresh and saline water within permeable sediments (Moore et al., 1999; Charette and Sholkovitz, 2006; Santos et al., 2009). Recent work has shown that the circulation of water through sandy beaches drives substantial biogeochemical redox reactions: intrusion of oxygenated surface water into the beach face and mixing with outflowing anoxic meteoric groundwater creates a redox
interface. Redox reactions within the shallow STE have been shown to affect the cycling of reduced metabolites (such as Fe and sulfide) as well as redox-active trace metals (O'Connor et al., 2015; Santos et al., 2011; McAllister et al., 2015).

Redox sensitive metals (RSMs) are characterized by different environmental behavior depending on their redox state. The trace metals Mo, U, V, and Cr form soluble oxyanions under oxidizing conditions, but their reduced species are particle reactive (Bruland and Lohan, 2006). Due to their redox-active behavior, these RSMs are widely used as marine paleoproxies to examine past oceanic oxygen levels, productivity, and circulation (e.g., Nameroff et al., 2004; Rimmer et al., 2004; Tribovillard et al., 2006; Algeo et al., 2012). The use of elements like Mo and U as paleoindicators is influenced by material budgets controlling their oceanic concentrations and isotopic composition (Klinkhammer and Palmer, 1991; Siebert et al., 2003; Archer and Vance, 2008).

The STE may play an important role in controlling RSM budgets in coastal and global oceans; however, the reactions that control RSM concentrations in SGD remain poorly studied. Redox gradients control the porewater concentrations of these elements in both fine-grained sediments (Brumsack and Gieskes, 1983; Morford and Emerson, 1999; Morford et al., 2005; Scholz et al., 2011), and, although less well studied, in permeable sediments. Uranium removal in the STE has been observed in many locations (Windom and Niencheski, 2003; Duncan and Shaw, 2003; Charette and Sholkovitz, 2006; Santos et al., 2011, Riedel et al., 2011). Both non-conservative removal (Windom and Niencheski, 2003; Riedel et al., 2011) and addition (Beck et al., 2010) of Mo have been observed in different locations. Although data on V in the STE are limited, removal has been
observed in the salinity transition zone (Beck et al., 2010).

In this work, we seek to answer the questions 'What biogeochemical processes control RSM distribution in the shallow STE' and 'How do these processes change on seasonal and inter-annual time scales?' Previous work at this study site characterized the general distributions of reduced metabolic compounds and RSMs in the STE. Both Mo and U were supplied to the STE from the surface water, and V and Cr had a mid-depth maximum at a sampling profile bisecting the shallow STE (O'Connor et al., 2015). Redox processes controlling organic matter, Fe, and sulfide cycling varied seasonally, resulting in increased freshwater DOC inputs in the summer, higher sulfide production in the summer, and greater dissimilatory Fe reduction in the winter (O'Connor, Ch. 2).

Distinct behaviors for Mo, U, V, and Cr have been observed previously in coastal ocean porewaters (Brumsack and Gieskes, 1983; Morford et al., 2005; Scholz et al., 2011) as well as at this STE (O'Connor et al., 2015). These elements differ in their responses to geochemical variables such as reduction/oxidation at distinct Eh/pH, sorption to reactive metal oxides, complexation with organic matter or sulfide, and the activity of certain microbial species (Helz et al., 1996; Barnes and Cochran, 1993; Lovley et al., 1993; Wanty and Goldhaber, 1992; Rai et al., 1989). Therefore, seasonal variation in environmental conditions within the STE suggests that RSMs are likely to exhibit seasonally variable behavior within this zone as well.
Methods

Study Site

The study site is located on the York River Estuary (YRE) in Gloucester Point, Virginia, USA (37.248884 N, -76.505324 E) (Fig. 1a). The YRE is a microtidal (0.7 to 0.85 m tidal range), relatively pristine tributary of the Chesapeake Bay (Moore and Reay, 2009). Salinities of YRE surface water at this study site typically range from 14 in the winter to 25 in the summer (Luek and Beck, 2014). Benthic microalgae are a major contributor to organic matter and nutrient cycling in YRE shallow porewater at this location (Salisbury, 2011). The Gloucester Point beach is a narrow (20-30 m wide) sandy beach with a gradual slope (0.2). It is bordered to the east by an upland marsh. Granite breakwaters line the beach ~40 m from the dune line to prevent erosion and stabilize the beach morphology (Fig. 1b).

Previous work at the study site used a Ra budget to estimate seasonally variable SGD volume fluxes to the York River between 93 and 178 L m$^{-2}$ d$^{-1}$ (Luek and Beck, 2014). Groundwater hydraulic head at the study site is inversely correlated with tidal height (Beck et al., 2016), and seasonal fluctuations in groundwater and sea level control saline water intrusion and groundwater discharge (Luek and Beck, 2014). Previous sampling of redox-active constituents at the same mid-tide location showed that advection of water through the sandy sediments drives the formation of a "classic" redox sequence typical of diffusion-dominated fine-grained sediments (O'Connor et al., 2015). Sediments at the mid-tide line (MT) are oxic down to ~70 cm depth, high in NO$_x$ between 70 and 100 cm, high in dissolved Fe and Mn starting at 100 cm, and sulfidic
beginning at ~140 cm depth (depths relative to the high tide sediment surface level) (O’Connor et al., 2015). The vertical distributions of DOC and reduced metabolites remained consistent over time, but concentrations varied with season (O’Connor, Ch. 2). Dissolved organic carbon concentrations were greatest in the summer, and shallow meteoric groundwater supplied the majority of DOC to the STE. Dissolved Fe and Mn were highest in a plume through the middle of the STE (100-140 cm) which was characterized by both higher concentrations and greater nonconservative addition in the winter. In contrast, sulfide was higher in summer at depths within the Fe and Mn plume (100 to 140 cm).

Groundwater Collection and Analysis

Sample collection and analyses have been previously described (O’Connor, Ch. 2), and will be discussed briefly here. Porewater samples were collected using permanently installed wells comprising a 1 cm-long stainless steel screened point (AMS, Inc.; e.g. Gonneea et al., 2013) attached to 3 mm diameter No-Ox® PTFE tubing (Supelco/Sigma-Aldrich), or from an all-plastic multi-level sampler (Beck, 2007). Groundwater was pumped from depth using a portable peristaltic pump and filtered through an in-line 0.45 μm Millipore polypropylene capsule filter into sampling containers. Salinity, temperature, and Eh were measured in a flow-through unit at the time of collection with a hand-held YSI556 multiprobe.

Samples were collected from the mid-tide (MT) profile, located at the center of the shallow saline recirculation cell (Fig. 1c), each month (March 2013 to February 2015).
during the week of spring tide at the ebb phase of the tidal cycle. Porewater samples were also collected from a shore-normal transect (Fig. 1c) during four months – April 2014, July 2014, October 2014, and February 2015 to represent spring, summer, fall, and winter, respectively. The depth profiles were located at the locations of average water levels during high tide (HT), intertidal (IT, between HT and MT), and low tide (LT) water levels. To remain consistent between profiles, all depths were calculated relative the sediment surface at the high tide profile (rather than sediment surface at the location of collection).

Samples for trace metal analysis were collected in acid-washed LDPE bottles, acidified immediately after collection, and analyzed by dilution and direct injection on a Thermo Element 2 inductively-coupled plasma mass spectrometer by the University of Southern Mississippi's Center for Trace Analysis. Samples for organic-metal complex measurements were collected during the February seasonal transect sampling in 1 L acid-washed LDPE bottles and immediately frozen. These samples were later thawed and extracted through a Bond Elut C_{18} column (Agilent, 500 mg bed mass). Columns were acid-washed in dilute trace metal grade HCl and rinsed thoroughly. Before use, columns were conditioned with 100 mL of methanol followed by 500 mL of MilliQ water. Approximately 250 mL of sample was loaded through the column (confirmed by sample mass difference), followed by a 10 mL MilliQ water rinse (Lemaire et al., 2006). The column was dried by pulling air through for thirty minutes, and then elution was done with 10 mL of HPLC-grade methanol. Concentrated ultrapure HNO_{3} (100 uL) was added to samples before they were allowed to dry under a HEPA-filtered laminar flow hood.
Samples were reconstituted with 1 N ultrapure HNO$_3$ for analysis as described above. This method isolates 20-60% of DOC, primarily hydrophobic species (Lemaire et al., 2006). The amount of DOC recovered in the extractions here is not reported because efforts to prevent metal contamination were prioritized over preventing organic carbon contamination (e.g., collecting eluent in LDPE bottles). The metals extracted along with this organic phase will be referred to here as associated with hydrophobic dissolved organic matter associated (hDOM).

As previously described (O’Connor, Ch. 2), this STE is characterized by three endmember mixing between saline, estuarine surface water and two up-gradient freshwater sources (one shallow and one deep). To evaluate chemical addition or removal along the seasonal STE transects, two mixing lines were inferred on salinity-concentration plots: one between the surface endmember and the shallow fresh endmember, and one between the surface and deep fresh endmember. Points that fall within the triangle created by the two mixing lines were interpreted as resulting from mixing between the three endmembers, while those falling outside result from non-conservative addition or removal.

To quantify the addition or removal of an element at the MT profile over the 2-year time series, we calculated the concentration anomaly relative to conservative mixing. The concentration anomaly is the difference between the measured concentration and the expected concentration given conservative mixing between the endmembers, calculated as:
For each sample, three anomaly estimates were calculated based on: 1) mixing entirely between the surface water and the shallow freshwater endmember, 2) mixing entirely between the surface water and deep freshwater endmember, and 3) mixing between all three endmembers (Fig. 6). The first two estimates were made with two endmember mixing equations using salinity as a conservative tracer, and represent the limits of freshwater endmember mixing with the surface water. The third was made using humic carbon as a quasi-conservative tracer (described in O’Connor, Ch. 2), and its effectiveness as a conservative tracer with respect to these elements in discussed here.

**Sediment Collection and Analyses**

A 4-meter long vibracore was collected adjacent to the mid-tide sampling location in May 2013, and processed as described previously (O’Connor, Ch. 2). Sequential leaches were performed to isolate different metal fractions (Tessier et al., 1979). Dried and weighed sediments (~1 g) were shaken for 180 minutes in 5 mL of 1 M hydroxylamine hydrochloride in 25% acetic acid, centrifuged at 3000g for 10 minutes. Four milliliters of supernatant were removed to a new, clean tube and diluted in 0.5 M HNO₃ (Aristar Plus, trace metal grade). This represented the fraction liberated upon reductive dissolution of Fe and Mn (hydr)oxides (Tessier et al., 1979), and will be referred to here as the 'metal oxide fraction'. The remaining sediment was shaken in 5 mL aqua regia (1:3 HNO₃:HCl) for 13 hours. Samples were centrifuged, and the supernatant
removed and diluted with MilliQ water. Metal concentrations in these fractions were analyzed as described for groundwater samples above. The first leach recovered metals bound to Fe and Mn oxide phases. This fraction represents more recalcitrant sedimentary phases (but does not dissolve pyrite), and will be referred to as the 'reclacitrant fraction' (Tessier et al., 1979; Huerta-Diaz and Morse, 1990). Metal concentrations in both fractions were analyzed as described for groundwater samples above.

Statistics

Multiple linear regression models were used to quantify the response of the RSEs (Mo, U, V, and Cr) to changes in biogeochemically relevant predictor variables (all analyses were performed using existing functions in R; R Core Team). The goal of these regressions was to quantify the effect of major porewater constituents on the distribution of RSMs in order to provide insight into how these elements respond to geochemical changes in the STE. These relationships also provide insight regarding what major constituents could be measured to predict RSM behavior in other coastal groundwaters.

Two sets of regressions were done to understand the responses of redox-sensitive trace metals to major chemical distributions in the STE across both time and space. The first, using data measured monthly at the MT location, explored changes over different seasons during the two-year time series (referred to here as 'time effects'). The second set was used to assess how RSM responses to predictor variables differ spatially in the STE, as measured along a shore-normal transect (referred to here as 'space effects'). All
response variables (i.e. trace metal concentrations), as well as salinity, dissolved Fe, DOC, humic C, and sulfide concentrations were natural log transformed to achieve data normality. Partial $R^2$ values for each predictor variable were calculated using the R package *relaimpo* (Grömping, 2006).

Time effects were modeled using the MT profile data from each season (summer 2013, winter 2013, summer 2014, and winter 2014) to determine if the response to predictor variables differed seasonally. The depth ranges used for Mo and U were 62 cm to 175 cm (inclusive), and 62 cm to 265 (inclusive), respectively. Within these depth ranges, Mo and U decrease from their maximum concentrations to values consistently below the detection limit (below which any correlation analysis becomes meaningless). Because V and Cr vary throughout the depth profile, all porewater depths were included in these regressions.

The space effect regressions were done on the HT, MT, and LT profile data separately (using all four months of data in each) to determine if RSM response to predictor variables differed spatially throughout the STE. Similar to the MT profile regressions, only depths where Mo and U concentrations were above the detection limit were used (Mo: 330 cm and shallower at the HT profile; 175 cm and shallower at the MT profile; U: 256 cm and shallower at the MT profile). Concentrations of Mo and U were consistently above the detection limit deeper at the LT profile, so all depths were included.

Models using various permutations of geochemically relevant predictor variables were considered. Autocorrelations were found between sulfide and Eh, and DOC and
humic C. As a result, these variables were never considered in the same model. Final models were selected using the Akaike information criterion (Supplemental Information).

For Mo and U, the predictor variables tested were salinity, dissolved Fe, Eh/sulfide, and temperature. Temperature was only included in the space effect model because the time effects model, already split up by season, accounted for temperature variability in its design. Dissolved Fe was included because Mo(VI) can sorb to iron oxides, and some Fe reducing bacteria are capable of reducing U. Sulfide was included because of its reactivity with Mo, and because several sulfur-reducing microbial species are capable of reducing U. Although U(VI) can be reduced by Fe(II) (Behrends and Van Capellen, 2005) and sulfide (Hua et al., 2006), the rates of these reactions are comparable to those of enzymatic reduction only under certain conditions (e.g., the ready availability of certain reactive metal hydroxide surfaces; dominance of uranium hydroxide species). Therefore, geochemical associations of U with Fe and S here are generally considered to be proxy relationships for the microbes that generate those reduced metabolites, although abiotic reduction may also play a role.

For V and Cr, the predictor variables tested were salinity, DOC/humic C, dissolved Fe, and temperature (space effect model only). Reduced V and Cr species can be complexed by DOC (Wehrli and Stumm, 1989; Remoundaki et al., 2007), and co-precipitate with iron (hydr)oxides (Rai et al., 1989).

**Results**

*Molybdenum*
Molybdenum was highest in the surface water (63 ± 8 nM) and shallow STE porewater, with the 25 nM contour falling between 50 cm and 150 cm depth, and the 10 nM contour between 100 cm and 200 cm depth. At MT, concentrations decreased steadily from surface water concentrations (62 ± 11 nM in summer; 57 ± 9 nM in winter), to 24 ± 12 nM at 115 cm depth across all seasons. Below 115 cm depth, Mo declined sharply to less than the detection limit of 1 nM (Fig. 3a). At LT, Mo was consistently lower than at other locations (3 ± 5 nM) but tended to be highest in the shallowest sampled depth (Fig. 2a). Concentrations of Mo associated with hDOM were highest at depths between 50 and 100 cm throughout the STE (Fig. 4). The percent of Mo associated with hDOM increased with depth in the STE, from 2.7% in the surface to 54% ± 30 below 100 cm depth (Fig. 4).

The shallow freshwater endmember Mo concentration (5.9 ± 2.1 nM) tended to be higher than the deep freshwater endmember (0.84 ± 1.5 nM), but both were much lower than surface water concentrations (66.7 ± 7.5 nM) (Table 1).

In the seasonal transects, sulfidic samples showed evidence of non-conservative removal (Fig. 5a). Most non-sulfidic (shallow) samples fell within the conservative mixing area or slightly below, but several IT samples showed non-conservative excess in July, October, and February. Molybdenum anomalies calculated at the MT profile were near zero in surface porewater, and the concentration anomaly dropped to below zero at 130 to 140 cm. The anomalies calculated from three-endmember mixing typically fell between those calculated from mixing between the surface water and either the shallow or deep freshwater end-member.
The time effects linear model selected (based on AIC) for Mo was:

\[ \log(\text{Mo}) \sim \log(\text{salinity}) + \log(\text{Fe}) + \log(\text{sulfide}) \]

The models explained between 61% and 77% of the variability in Mo concentrations (Table 2). Molybdenum was correlated negatively with sulfide across all seasons (Fig. 7a). In seasons where the correlation with sulfide explained a lower percent of Mo variability (summer 2013 and winter 2014), the correlation with salinity was stronger and more significant. Dissolved Fe was a significant predictor of Mo in only one season (summer 2014).

The space effects linear model selected for Mo was:

\[ \log(\text{Mo}) \sim \log(\text{salinity}) + \text{Eh} + \log(\text{Fe}) \]

The models explained between 64% and 74% of the variability in Mo concentrations (Table 3). Salinity was most important for Mo distribution at LT, whereas Fe and Eh were most significant at HT and MT (Fig. 8a). The positive correlation with Eh indicated that Mo concentrations decrease as conditions become more reducing (i.e. more negative Eh).

**Uranium**

The shallow freshwater endmember U concentration (1.1 ± 0.8 nM) tended to be higher than the deep freshwater endmember (0.24 ± 0.2 nM), but both were much lower than surface water concentrations (7.5 ± 0.5 nM) (Table 1).

Dissolved U was highest in the surface water and shallowest parts of the STE. The 1 nM contour fell between 100 and 150 cm in April and July, and between 150 cm
and 350 cm in October and February. Summer U concentrations at MT remained relatively constant down to 90 cm (5 ± 3 nM), then decreased to <1 nM by 130 cm. Winter U concentrations at the MT profile remained relatively constant down to 100 cm (6 ± 4 nM), then decreased to <1 nM by 130 cm depth (Fig. 3b). At LT, U was consistently low (0.6 ± 0.6 nM), but was highest at the shallowest (125 cm, 0.9 ± 0.2 nM) and deepest (300 cm, 1.6 ± 0.5 nM) sampled depths (Fig. 2b). Concentrations of U were highest between 200 and 300 cm depth (Fig. 4). The percent of U associated with hDOM was low in the surface (2.0%) and reached a maximum in the 201-300 cm depth range (15% ± 12) (Fig. 4).

In the seasonal transects, nearly all samples above salinity ~3 showed non-conservative removal (in all months except April) (Fig. 5b). Greater non-conservative behavior was observed in July than in other months. Uranium anomalies calculated at the MT were generally at or below zero from 62 to 115 cm, except in winter 2014, when anomalies were between zero and one. Nonconservative removal occurred at all depths in summer 2013; at 90 cm and deeper in summer 2014; and at 140 cm and deeper in both winters. The anomalies calculated from three-endmember mixing typically fell between those calculated from mixing between the surface water and either the shallow or deep freshwater end-member.

The time effects linear model selected for U was:

\[ \log(U) \sim \log(\text{salinity}) + \log(\text{Fe}) + \log(\text{sulfide}) \]

The most significant predictors of U concentrations over the two year sampling period were sulfide (negative correlation) and Fe (positive correlation), and the effect of sulfide
was greater than the effect of Fe (Fig. 7b, Table 2). In winter 2014, the effect of Fe was not significant, but unlike other seasons, the effect of salinity was statistically significant.

The space effects linear model selected for U was:

\[
\log(U) \sim \log(\text{salinity}) + \text{Eh} + \log(\text{Fe})
\]

At HT and MT, Eh and Fe were directly correlated with U. At MT, salinity also was a significant predictor of U concentrations (Fig. 8b, Table 3). Salinity was the strongest predictor of U concentrations at the MT and LT locations. Dissolved Fe was also a predictor of U at LT, but had a negative correlation.

**Vanadium**

Dissolved V showed a mid-depth concentration maximum throughout the STE (Fig. 2c). The maximum was generally between 50 cm and 250 cm from HT to MT, and 150 cm to 250 cm at LT. Dissolved V concentrations were highest from HT to MT, and were never below the detection limit (2 nM) at any location (Fig. 2c). Within the MT profile maximum (from 100 to 140 cm), V concentrations ranged from a low of 147 ± 24 nM in winter 2014 to a high of 256 ± 54 nM in summer 2014 (Fig. 3c), with generally higher concentrations in summer than in winter (Fig. 2c). Concentrations of V associated with hDOM were typically 1 to 2 nM at all depths (Fig. 4). The fraction of V associated with hDOM was low throughout the surface water and STE (<5%; Fig. 4).

On average, vanadium was lowest in the deep freshwater endmember and highest in the shallow freshwater endmember. The shallow freshwater endmember concentration was variable, increasing by a factor of six between April (2.48 nM) and July (296.9 nM).
(Table 1). The deep freshwater endmember varied between a low of 8.8 nM in February to a high of 30.7 nM in July (Table 1).

In salinity mixing diagrams for the seasonal transects, V generally showed non-conservative addition, although patterns varied by month (Fig. 5c). In July (with the highest shallow freshwater endmember concentration), most samples showed conservative behavior. In April and February (with lower shallow freshwater endmember concentrations), several sulfidic and non-sulfidic samples showed non-conservative addition. The calculated V anomalies at MT were greatest between 100 and 175 cm in all seasons, coinciding with the mid-depth V concentration maximum. The concentration anomalies within the peak were higher in winter than in summer (Fig. 6c). The anomalies within the V concentration maximum from three-endmember mixing typically were lower than those calculated from mixing between the surface water and the shallow or deep freshwater end-member.

The time effects linear model selected for V was:
\[ \log(V) \sim \log(\text{salinity}) + \text{Eh} + \log(\text{Fe}) + \log(\text{DOC}) \]
Dissolved organic carbon was a strong predictor of V in all seasons (Fig. 7c, Table 2). Dissolved Fe was also a significant predictor in winter 2013, summer 2014, and winter 2014, and tended to explain less of the variation in dissolved V than DOC (except for winter 2014). Eh was significantly and positively correlated with V in summer, although it accounted for a low percent of variation in V concentration.

The space effects linear model selected for V was:
\[ \log(V) \sim \log(\text{salinity}) + \log(\text{Fe}) + \log(\text{DOC}) + \text{Eh} + \text{temp.} \]
Both dissolved organic carbon and temperature were significant predictors of V at all STE locations (Fig. 8c, Table 3). Vanadium was only significantly correlated with Fe at MT, and was negatively correlated with Eh at the HT profile.

**Chromium**

Chromium was lowest in the surface water (3 ± 3 nM) and highest in the shallow freshwater endmember (19.4 to 235.6 nM) (Table 1).

Similar to V, Cr showed a mid-depth concentration maximum throughout the shallow STE (Fig. 2d). Maximum Cr concentrations were observed from 90 to 130 cm depth at HT, from 100 cm to 200 cm depth at MT, and 125 to 150 cm at LT. Concentrations of Cr were typically highest at HT, with concentrations commonly >100 nM, and up to 235 nM (Fig. 2d). In the MT time series, Cr concentrations within the maximum ranged from 28 ± 3 nM in winter 2014 to a high of 53 ± 31 nM in winter 2013 (Fig. 3d). Like V, Cr concentrations were always above the detection limit (1 nM, Fig. 2d). Concentrations of Cr within the MT maximum did not show any consistent trends with season, but were higher during 2013 (Fig. 3d). Concentrations of Cr associated with hDOM were highest in the same depth range as the dissolved Cr maximum, between 100 and 200 cm depth (Fig. 4). The percent of Cr associated with hDOM increased with depth in the STE from the surface water (2.8%) to the 301 to 410 cm depth interval (81% ± 26; Fig. 4).

In salinity mixing diagrams for the seasonal transects, Cr concentrations generally exhibited conservative mixing behavior. In October and February, when shallow
freshwater endmember Cr concentrations were low, several points showed non-conservative addition (Fig. 5d). Additionally, positive Cr concentration anomalies (indicating addition) were observed at the MT profile, and tended to be higher in winter than summer (Fig. 6d). Calculated Cr anomalies using three-endmember mixing low relative to those calculated from mixing between the surface water and the shallow or deep freshwater end-member.

The time effects linear model selected for Cr was:

$$\log(Cr) \sim \log(\text{salinity}) + \text{Eh} + \log(\text{Fe}) + \log(\text{humic C})$$

Humic C was consistently a significant predictor of Cr concentrations in the STE (Fig. 7d; Table 2). Dissolved Fe was a significant predictor of Cr in all seasons excluding winter 2013, but was weaker than humic C. Chromium was inversely correlated with salinity in summer 2014 and winter 2013. Eh was a significant predictor of Cr in all seasons except summer 2013.

The space effects linear model selected for Cr was:

$$\log(Cr) \sim \log(\text{salinity}) + \log(\text{Fe}) + \log(\text{DOC}) + \text{Eh} + \text{temp}.$$ 

The only predictor variable significant across all the profiles was Fe (Fig. 8d; Table 3). DOC was a significant predictor at HT, and salinity was inversely correlated with Cr at MT.

**Sediment Constituents**

Metal oxide-associated Mo content in sediments was low (<1.5 µmol/cm³) in the shallowest sediments, increased to ~4 µmol/cm³ between 70 and 150 cm, then increased
sharply to reach as high as 7.8 µmol/cm³ at depth (Fig. 9). Metal oxide-associated U was low (~0.1 µmol/cm³) down to 45 cm depth, increased to 1 to 2 µmol/cm³ between 70 cm and 250 cm depth, then increased sharply to 5 µmol/cm³ at the base of the profile. Recalcitrant Mo and U profiles showed similar distributions to the metal oxide fractions. Sediment U contents were lower than Mo in both leaches (Fig. 9), consistent with porewater concentrations.

Metal oxide-associated V and Cr content were highest between 60-90 cm and 190 to 300 cm depths (Fig. 9). Metal oxide-associated V reached as high as 86 µmol/cm³, and reducible Cr reaches as high as 11 µmol/cm³. Recalcitrant V and Cr showed similar distributions as the metal oxide fractions, but with a sharper peak at 70 cm depth and concentrations much more similar to one another (Fig. 9).

Discussion

Molybdenum

High Mo concentrations in the surface water and shallow STE (compared to freshwater endmembers) showed Mo was supplied to the STE by surface water (Fig. 2a, 3a). Once in STE porewaters, Mo was non-conservatively removed during advection into sulfidic portions of the STE (Fig. 5a, 6a). Mo in sulfidic samples was always below concentrations expected for conservative mixing (Fig. 5a), and calculated anomalies showed that Mo was consistently removed from porewaters between 100 and 140 cm depth at MT (Fig. 6a), where dissolved sulfide was first present (O'Connor, Ch. 2). These results are consistent with non-conservative Mo removal with sulfide.
Laboratory and field studies suggest that oxidized Mo (as molybdate) quantitatively reacts to form particle reactive thiomolybdate (MoS\(_4^{2-}\)) at a sulfide threshold of ~10 µM (Helz, 1996; Erickson and Helz, 2000). The observed Mo removal at depths where sulfide is present at concentrations higher than 10 µM is consistent with this model: dissolved Mo and sulfide react to form dissolved thiomolybdate species, which is highly particle-reactive and removed via sorption to sediments.

However, detectable Mo was occasionally detectable at highly sulfidic depths in the STE (Fig. 2a). The occurrence of hDOM-associated Mo deep in the STE (up to 5 nM) suggested that the mechanism causing these low but detectable Mo concentrations may have been the complexation of particle-reactive Mo sulfide compounds by organic matter, making them more soluble.

Several shallow samples showed non-conservative addition of Mo. Molybdate can sorb to Mn oxides under oxic conditions (Shimmield and Price, 1986), so shallow addition of Mo could be due to release of Mo with the reductive dissolution of metal oxides observed in this STE (as described in O'Connor, Ch. 2).

The time and space regression models supported the conclusion that Mo was primarily supplied to the STE from high-salinity surface water, was removed from porewater under sulfidic/reducing conditions, and that high concentrations of Fe (generated by reductive dissolution of metal oxides; O'Connor, Ch. 2) were associated with higher Mo. Salinity was only positively correlated with Mo in two of four seasons and explained a large proportion of Mo variability in these times, consistent with Mo being supplied to the STE from high-salinity surface waters. The significant negative
correlation between Mo and sulfide throughout the two-year time series suggests the importance of Mo removal by sulfide in every season (Fig. 7a). Dissolved Fe was a predictor of Mo in summer 2014, suggesting the release of Mo from reductive dissolution of Fe oxides may have been the cause of the shallow Mo addition in July 2014. Additionally, the correlation between Mo with Eh and Fe at HT and MT provides additional support for Mo removal under sulfidic/reducing conditions and some mobilization of Mo along with dissolved Fe occurs within the intertidal saline circulation cell.

Lower Mo concentrations and stronger correlation of Mo with salinity at LT (Fig. 8a) indicated that the majority of Mo removal in these sediments happens shallow enough (sulfide concentrations were in excess of 250 µM at every sampled depth; O'Connor, Ch. 2) that increasingly reducing conditions at depth did not drive further changes in Mo. The continued presence of Mo, even under reducing conditions, could have been due to Mo associated with porewater-associated colloidal material.

Sulfide concentrations varied seasonally in this STE (O'Connor, Ch. 2), with higher concentrations during summer in the depth range where Mo was removed (100 to 140 cm; Fig. 3a, 6a). Although this suggests Mo removal may also be greater in summer, mixing diagrams (Fig. 5a) and anomaly calculations (Fig. 6a) showed no seasonal effect on Mo removal. This may be because sulfide reached sufficiently high concentrations to drive Mo removal (>10 µM) by 140 cm depth in all seasons (O'Connor, Ch. 2). Seasonal removal of Mo may be different in STEs where sulfide concentrations are lower or vary around the 11 µM threshold.
Sediment Mo contents support the conclusion that it is removed from porewater to form sulfide compounds. Both metal oxide-associated and recalcitrant Mo increased in the 100-140 cm depth range (Fig. 9) where sulfide and Mo co-occurred and dissolved Mo was removed (Fig. 6a). If Mo were simply being supplied by the surface water and quantitatively removed within a narrow depth range, a single sedimentary peak in Mo would be expected. However, sediment Mo contents continued to increase with depth (Fig. 9).

The higher sediment Mo content deep in the sediment may be due to a difference in sequestration permanence. The mixing of saline and oxic surface water into the STE depends on the hydraulic gradient between groundwater and surface water (Gonneea et al., 2013; Luek and Beck, 2014), which may be disturbed by short-term events such as storm surges or long-term events such as sea level rise. Periodic intrusion of oxic water into the STE may cause the oxidation and dissolution of solid-phase sulfides and associated Mo at shallower depths (140 cm to 200 cm). This suggests that the permanence of the shallow STE as a Mo sink is variable with depth, and that periodic oxygen intrusion into the STE may have an effect on trace metal budgets.

Uranium

Uranium, like Mo, was supplied to the shallow STE by surface water and showed non-conservative removal with depth. Uranium concentrations were highest in surface waters and decreased with depth in the STE (Figs. 2b, 3b). Non-conservative removal was evident in both the salinity mixing diagrams and apparent U anomalies (Fig. 5b, 6b).
Additionally, non-conservative removal was greater in summer, demonstrated by concentrations falling farther below the conservative mixing range (Fig. 5b) and apparent U anomalies indicating shallower removal depths in the summer than the winter (Fig. 6b). Uranium removal in the shallow STE was therefore more extensive during the warmer, summer months.

Several microbial species, including many iron reducing bacteria (FRB) and sulfate/sulfur reducing bacteria (SRB), (e.g. *Geobacter metallireducens* strain GS-15, *Alteromonas putrefaciens*, *Shewanella putrefaciens*, *Desulfovibrio desulfuricans*, *Desulfovibrio vulgaris*), are capable of reducing U to its particle reactive species (Lovley et al., 1991; Lovley and Phillips, 1992, Lovley et al., 1993; Fredrickson et al., 2000). High concentrations of dissolved Fe and sulfide were present in all seasons at this location, suggesting activity of both FRB and SRB (O'Connor, Ch. 2). Seasonal shifts in concentrations from high sulfide (summer) to high Fe (winter) indicated seasonal variations in microbial activity (i.e., metal reduction versus sulfate/sulfur reduction; O'Connor, Ch. 2). Therefore, greater U removal in summer suggested that the activity of SRB may have been more important for U reduction in this STE.

Over the two year MT time series, U was consistently correlated more strongly with sulfide (inversely) than Fe (directly), and the direction of the correlation between U and sulfide was consistent with U removal (Fig. 7b). The greater U removal observed in summer was then likely due primarily to SRB microbes, and greater rates of SRB metabolism in summer enhanced U removal.

Similar to Mo, salinity exerted a stronger control on U concentrations at LT than
in other locations in this STE (Fig. 8b). Despite the strongly reducing conditions observed within the LT profile and consistently low U concentrations, U concentrations were always above the detection limit. As with Mo, this may suggest U association with colloidal material.

Although both metal oxide-associated and recalcitrant U increased steadily with sediment depth (Fig. 9), there was no U maximum indicating a clear depth of removal (as observed by Charette et al., 2005). Additionally, the increase in sediment U content was not as dramatic as the increase of Mo, despite similar contents in shallow (<100 cm) sediments. This may indicate that U removed to the sediments is more susceptible to remobilization during temporary increases in porewater redox potential.

In the regression analyses, Eh proved the better predictor of Mo and U concentrations than sulfide throughout the STE. In the case of Mo, this may be because sulfide concentrations varied over three orders of magnitude along the transect, and often exceeded concentrations required for Mo removal (O'Connor, Ch. 2). Uranium cycling appeared to be related to both Fe and sulfur reducing processes, and using Eh as a predictor combines these parameters.

Vanadium

Dissolved V concentrations in the STE were highest in a mid-depth maximum, evidently originating from shallow fresh groundwater (Fig. 2c). Vanadium concentrations within the maximum (Fig. 3c) and throughout the STE (Fig. 8c) were higher in summer/during warmer temperatures. These higher summer concentrations appear partly
due to higher concentrations of V in the shallow freshwater endmember, the source of V during summer (Table 1).

In inland groundwaters, V tends to be highest in aquifers composed of mafic and ultramafic rocks (Luengo-Oroz et al., 2014; Aiuppa et al., 2003; Wright and Belitz, 2010). Sediments in the Chesapeake Bay region are rich in mafic and ultramafic minerals such as ilmenite (Darby, 1984; Carpenter and Carpenter, 1991), epidote (Firek et al., 1977), and staurolite (Firek et al., 1977; Carpenter and Carpenter, 1991). The lithology of the Gloucester Point region therefore likely partially responsible for the high groundwater V concentrations observed in this study.

Dissolved V tends to be highest in groundwaters that are oxidizing and slightly basic (Wright and Belitz, 2010; Pourret et al., 2012), unlike the reducing conditions throughout large portions of this STE. Through the MT profile two-year time series and the entire STE, V was consistently correlated with DOC, suggesting that complexation by organic matter kept particle reactive reduced V species soluble (Pourret et al., 2012; Telfeyan et al., 2015). Higher summer DOC concentrations (O'Connor, Ch. 2) could have driven higher summer V concentrations. However, only a small percent of V measured in the hDOM pool (Fig. 4c), suggesting that V may be complexed by more hydrophilic organic matter, such as fulvic acids (Stolpe et al., 2013; Telfeyan et al., 2015).

In addition to the seasonal meteoric V supply, non-conservative addition of V occurred within the STE itself. Although higher V concentrations occurred in summer, greater addition occurred during winter in both years (shown in seasonal transects and MT anomalies; Figs. 5c, 6c). Reduced V can sorb to hydrous metal oxides (Wehrli and
Stumm, 1989), and V was correlated with dissolved Fe in both winters (Fig. 7c) and only at MT (Fig. 8c). This is consistent with V release during the dissolution of Fe oxides, which appears to occur just up-gradient of the MT profile (O'Connor, Ch. 2).

Although using humic carbon as a pseudo-conservative tracer in three end-member mixing analysis has been successful in quantifying anomalies for Fe, Mn (O'Connor, Ch. 2), Mo, and U (this study), dissolved V anomalies calculated using three end-members appeared to underestimate the V anomaly when V concentrations were high. This could have been due to the observed association of V with organic material.

Chromium

Similar to V, Cr concentrations were highest in a mid-depth maximum originating from shallow fresh groundwater (shown by high Cr concentrations at HT (Fig. 2d) and in the shallow freshwater endmember (Table 1). Like V, Cr is enriched in minerals associated with mafic and ultramafic rock (Robles-Camacho & Armienta, 2000; Oze et al., 2007), which are common in the Chesapeake Bay and Virginia coastal plain region (Firek et al., 1977; Darby, 1984; Carpenter and Carpenter, 1991). Chromium is readily reduced by Fe(II) (Eary and Rai, 1989), organic matter (Bartlett and Kimble, 1976), and sulfide (Smillie et al., 1981), all of which are abundant in the Gloucester Point STE. Additionally, previous equilibrium calculations showed that Cr would be reduced and therefore highly particle reactive throughout the STE (O'Connor et al., 2015). The high concentrations of Cr are likely driven by association of Cr with organic matter and colloidal metal (hydroxide) material, which increases its apparent solubility in these
porewaters.

High concentrations of Cr in the STE were associated with organic matter. Compared with V, Cr was more strongly associated with the hDOC pool (Fig. 4d) and with humic carbon (Fig. 8d). This suggests that Cr was likely complexed by humic material specifically, which is functionally defined by a nonpolar character (Harvey et al., 1983), rather than with total DOC. This complexation would keep Cr in solution even in the reducing environment of the STE (James and Bartlett, 1983).

Dissolved Cr also co-varied with Fe in most seasons and in all profiles of this transect, with this association strength increasing moving seaward (Fig. 9d). In the presence of Fe, Cr(III) is likely to precipitate as the highly insoluble (Fe,Cr)(OH)₃ (Rai et al., 1989). Correlation of Cr with DOC and Fe through the STE suggests that Cr may be associated with both organic and Fe-based colloids, with Fe colloids becoming more important moving seaward. Iron oxide colloids are mobilized at oxic/anoxic interfaces (Hassellov and von der Kammer, 2008; Pokrovsky and Schott, 2002). The occurrence of sharp redox interfaces through the center of the saline circulation cell, including reductive dissolution of Fe oxides (O'Connor et al., 2015), may result in greater mobilization of Fe-based colloids in this zone. This mobilization of Fe colloids may result in the shift from DOC- to Fe-mediated Cr transport.

Non-conservative addition of Cr within the STE itself occurred in addition to the meteoric groundwater inputs (Fig. 5d, Fig. 6d), and as with V, this addition tended to be greater in winter. As Cr species can sorb to reactive metal (hydr)oxide surfaces, this addition may also be due to release upon reductive dissolution of Fe oxides, which is
greater in winter (O'Connor, Ch. 2).

As with V, dissolved Cr anomalies calculated using three end-members appeared lower than the two-endmember estimates when Cr concentrations were high. This suggests that although humic carbon may be a useful semi-conservative tracer for certain porewater constituents, it should be used with caution when interpreting non-conservative mixing behavior of elements that are readily complexed by organic matter.

Because Cr and V concentrations in the shallow freshwater endmember were seasonally variable, there was likely temporal decoupling between changes in the freshwater endmember and effects on the rest of the STE porewater. The observation of changing endmember concentrations affirms the need for increased coverage of STE geochemistry over longer time scales to more accurately define STE boundary conditions. Additionally, these findings indicate that characterizing groundwater flow paths and transit times will further improve geochemical mixing analyses.

Unlike the other redox-active metals in this study, Cr concentrations did not show a clear seasonal trend despite seasonally-dependent non-conservative addition. There was, however, a strong interannual component to Cr concentrations in the STE, indicating that interannual changes in meteoric groundwater Cr concentrations likely drove this variability (although freshwater end-members were not sampled during 2013). An explanation may be found in interannual variation of DOC, which had higher concentrations at the MT maximum in 2013 compared to 2014 (O'Connor, Ch. 2). High concentrations of DOC may have enhanced Cr solubility, driving higher porewater Cr concentrations. However, interannual variability was not observed for V, possibly due to
the different pools of organic matter complexing these elements.

**Conclusions**

Despite having broadly similar chemical properties, Mo, U, V, and Cr behave differently in the STE due to differences in source, mixing within the STE, and distinct biogeochemical reactivities. These differences also cause seasonal variation in concentrations of these elements in coastal groundwater at this study site.

Dissolved Mo and U had higher concentrations in the surface water than freshwater endmembers, and showed non-conservative removal when oxic surface waters are mixed into the reducing portions of the shallow STE. Molybdenum was evidently removed by reaction with sulfide, but sulfide concentrations at this site were sufficiently high (i.e., in great excess of the 11 µM required to quantitatively react with Mo) that seasonal trends in removal were not evident. In contrast, U removal appeared linked to SRB activity, and showed greater removal during summer (when temperatures are warmer and microbial metabolic rates are higher).

Both V and Cr were primarily supplied to the STE by meteoric groundwater and also showed non-conservative addition within the STE. Minerals common to sediments in the region are enriched in V and Cr, and were the most likely source of these elements to local groundwater. The mobility of V and Cr within the STE, and therefore their supply to the coastal ocean, was controlled by association/complexation by porewater organic matter and Fe-rich colloids. However, complexation by different organic matter pools may have led to seasonal variability of V (fulvic-associated), but greater interannual
variability of Cr (humic-associated).

Results from this study emphasize the importance of seasonality on biogeochemical cycling in the STE. Although the broader redox structure of the shallow STE was stable over this two-year study, the magnitude of its source or sink function changed in predictable ways — for example, greater U removal in summer — that could be applied to create more accurate regional or global SGD flux estimates. Additionally, more easily-measured major redox species (such as organic carbon and Fe) can help predict the behavior of redox-active trace metals, and may therefore be useful for improving estimates of global RSM fluxes.
Table 1. Salinities and RSM concentrations used to estimate mixing and concentration anomalies. Noted which month's endmember was used in the anomaly calculations.

<table>
<thead>
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<th>Surface</th>
<th>Freshwater - shallow</th>
<th>Freshwater - deep</th>
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<tr>
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<td>Salinity</td>
<td>Mo (nM)</td>
<td>U (nM)</td>
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<td>6.47</td>
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<tr>
<td>July**</td>
<td>18.3</td>
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* Mar, Apr
** May, Jun, Jul
† Aug, Sep, Oct
‡ Nov, Dec, Jan, Feb
Table 2. Results from multiple linear regressions of RSMs in the seasonal transects. Comparisons were done using paired t tests with a Bonferroni correction for multiple comparisons in R over the noted depth interval (if no depth interval is noted, all sampled depths were used). P values less than 0.05 are shown in bold.
Table 3. Results from multiple linear regressions on Mo, U, V, and Cr at the MT profile throughout the observed seasons. Comparisons were done using paired t tests with a Bonferroni correction for multiple comparisons in R over the noted depth interval (if no depth interval is noted, all sampled depths were used). P values less than 0.05 are shown in bold.
Figure 1. (a) The study site is located on the western side of the Gloucester Point peninsula on the York River estuary (Virginia, USA). (b) The sample site is located by the VIMS boat basin. Upgradient of the sampling site is a marsh, and granite breakwaters (part of a larger York River shoreline restoration project) line the shore. (c) Permanent sampling wells were installed at the points shown (specific depths found in Supplementary Information). Depth profiles were located at the spring high tide (HT), mid tide (MT), and just below low tide (LT) water levels. Shorter depth profiles were installed in a transect in the intertidal zone (IT, including T1, T2, and T3) between the HT and MT profiles. Dotted lines indicate example salinity contours.
Figure 2. Concentrations of dissolved RSMs (a) Mo, (b) U, (c) V, and (d) Cr from the STE transect. Well locations are described in Fig. 1. Depths are measured relative to the high tide sediment surface (0 cm). Plots and contours were created in Ocean Data View.
Figure 3. Dissolved RSM (a) Mo, (b) U, (c) V, and (d) Cr concentrations from the MT profile over the two-year time series. Summer is defined as May to October, and winter is defined as November to April. At each depth, points represent the mean, and shaded areas represent one standard deviation, of all the months sampled in each season. Boxplots (right) showing concentrations of (c) V and (d) Cr at MT between 100 and 140 cm are included for comparison with DOC, Fe, and Mn concentrations (O’Connor, Ch. 2), which also show maxima in this depth range. The thick lines indicate the median.
concentration. The top and bottom edges of the boxes indicate the first and third quartiles. The whiskers extend to the farthest point 1.5 times the interquartile range of the box. The stars indicate which seasons are different from one another at the given p value (p values over 0.1 not indicated). The p values were determined using paired t tests, with the alpha level corrected for multiple comparisons using the Bonferroni adjustment.
Figure 4. Percent of total dissolved metal associated with hydrophobic organic matter (as determined by extraction on a C$_{18}$ column) in depth increments throughout the STE.
Figure 5. Salinity mixing diagrams of dissolved (a) Mo, (b) U, (c) V, and (d) Cr. Points are indicated by shape and color as originating from the HT profile, IT profiles, MT profile, LT profile, or surface water. Filled in points are from depths at which sulfide concentrations exceed 11 uM. Conservative mixing lines are drawn between the saline surface water endmember and both the deep and shallow freshwater endmembers (Table 1).
Figure 6. Concentration anomalies (calculated as described in the text) of (a) Mo, (b) U, (c) V, and (d) Cr at the MT profile. The two sets of points are the anomalies.
calculated assuming conservative mixing between the saline surface endmember and either the deep or shallow freshwater endmembers, and the shaded region between these two estimates represents range of potential anomalies at that depth. The stars indicate anomalies calculated using three end-member mixing. The points shown are the median anomaly values for each season.
Figure 7. Linear model results from the MT profile time series (see Results for regression equations). Transparent bars indicate a p value greater than 0.05.
Positive/negative signs at the bottom of the bars indicate the direction of the correlation.
Figure 8. Linear model results from the HT, MT, and LT locations (see Results for regression equations). Transparent bars indicate a p value greater than 0.05. Positive/negative signs at the base of the bars indicate the direction of the correlation.
Figure 9. Sediment concentrations of metals dissolved by reducing (Metal Ox.) and aqua regia (Recal.) leaches (described in Methods). The sediment core was taken adjacent to the MT profile. The dotted line represents the sediment surface; depths are relative to the HT sediment surface.
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McAllister S. M., Barnett J. M., Heiss J. W., Findlay A. J., Macdonald D. J., Dow C. L.,


CHAPTER 4

COLLOID FORMATION AND TRANSPORT OF REDOX SENSITIVE METALS IN A SHALLOW SUBTERRANEAN ESTUARY

Abstract

In surface estuaries, the transport of organic matter, iron, and trace metals to the sea is modified by the flocculation and removal of colloids, functionally defined as particles from 1 nm to 1 µm in diameter. Like surface estuaries and rivers, subterranean estuaries (STEs) exert considerable control on the composition of groundwater reaching the coastal ocean. However, little is known about colloid behavior or the importance of the colloidal phase for trace metal transport in the STE. The current study investigated how surface charge and composition of colloidal particles in an STE changed along the salinity gradient, and how these properties affected the transport of the redox sensitive metals (RSMs) Mo, U, V, and Cr. Colloidal surface charges (as determined by electrophoretic mobility) were similar to those found in surface estuaries, ranging between \(-0.1\) and \(-2.2 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}\). The abundance of colloidal particles, as determined by the concentrations of DOC, Fe, and Mn in the truly dissolved (<3 kD), small colloidal (3-30 kD), and large colloidal (30 kD to 0.45 µm) size fractions, generally followed the distribution and salinity mixing behavior of the total dissolved concentrations. In contrast to surface estuaries, the proportion of colloidal DOC, Fe, and Mn were directly correlated to salinity. Principal Component Analysis (PCA) was used to clarify controls in the two colloidal size fractions, and indicated that the percent DOC in both colloidal size fractions was directly related to salinity. In contrast, an inverse
relationship with pH was the main control on the proportion of small colloidal Fe and Mn in the small colloidal size fraction. The geochemistry of each RSM determined its partitioning into the colloidal pools. The fraction of Mo, U, and V in the small colloidal phase was inversely related to salinity, similar to surface estuaries, but PCA of environmental variables (salinity, Eh, electrophoretic mobility) and colloidal composition (DOC, Fe, and Mn) suggested that different controls were important for the partitioning of each RSM. In the small colloidal size fraction, percent Mo was directly proportional to Eh; the proportions of U and Cr were directly related to small colloidal concentrations of Fe and Mn; and the fraction of V covaried with small colloidal DOC concentrations, indicating distinct controls. In contrast, electrophoretic mobility was the major control on partitioning of all RSMs into the large colloidal phase, indicating that higher surface charge alone was sufficient to bind these elements. This work showed that up to 70% of trace metals are associated with the colloidal phase in the STE, and further suggests that other particle reactive elements may have similar enhanced mobility in the STE.

Introduction

In the environment, small particles (typically 1 nm to 1 μm in diameter) often comprising aluminosilicate, iron (hydr)oxide, and organic matter components are referred to as colloids (Buffle et al., 1998). Colloidal transport affects the fate of metals in both surface estuaries and groundwaters. In estuaries, salinity-driven particle flocculation leads to the removal of metals such as Fe, Al, and Cu (Sholkovitz et al., 1978; Sanudo-Wilhelmy et al., 1996). In groundwater, the presence of colloids can enhance the
transport of particle-reactive metals (Sanudo-Wilhelmy et al., 2002; De Jonge et al., 2004). Despite the geochemical importance of colloids in both surface estuaries and groundwater, there is a lack of information about colloids in subterranean estuaries (STEs). The STE, the coastal subsurface zone where infiltrating seawater and outflowing groundwater mix, is biogeochemically active and regulates the flux of chemicals to the ocean via submarine groundwater discharge (SGD). Influenced by both the land and the sea, the STE incorporates properties of both surface estuarine (salinity gradients) and groundwater (presence of a stationary solid phase) environments. However, the formation of colloids and their importance for trace metal transport in the STE are poorly understood.

Chemical transport in sediment porewater is determined by advection, dispersion, and the sorption tendency of a given chemical. Particle-reactive constituents tend to sorb to aquifer solids more strongly and exhibit slowed transport. However, colloids can enhance the transport of particle reactive chemical constituents because they are typically similar in composition to aquifer solids, but are transported as water flows between aquifer solids (McCarthy and Zachara, 1989). For this reason, groundwater colloids have been called a 'third phase' of aquifer systems (McCarthy and Zachara, 1989).

Colloid formation in groundwater is typically driven by four major mechanisms: coagulation of smaller molecules or particles, erosion of soils, mobilization from sediment grains by changes in pH and/or ionic strength, and dissolution of phases (such as metal oxides) that cement grains together (Ryan and Gschwend 1994). The STE is characterized by many of these processes, demonstrating the potential for colloidal
formation and a role for colloid-associated transport. Both salinity and pH vary throughout the mixing zone, and these interfaces can move through time (Gonneea et al., 2013). The redox interface created by mixing of anoxic groundwater and oxic surface water drives cycling of authigenic mineral phases (Charette and Sholkovitz, 2006; Roy et al., 2010; O’Connor, Ch. 2).

Previous work in coastal groundwater has found that colloidal transport is important for certain elements. Rare earth elements (REEs) in coastal groundwater were found predominantly in the 4 kD to 0.22 μm colloidal phase, and increasing salinity drove removal of light REEs from the colloidal load (Duncan and Shaw, 2003). Additionally, increasing groundwater salinity can drive partitioning of REEs into the truly dissolved phase (Johannesson et al., 2017). Colloidal transport of some metals (Fe, Mn, Cr) was recently measured in the shallow, oxic portion of coastal groundwater and was found to account for between 10 and 45% of total dissolved metal species (Kim and Kim, 2015).

Redox sensitive metals (RSMs) exhibit different environmental behavior depending on their redox state. The trace metals Mo, U, V, Cr form soluble oxyanions under oxidizing conditions, but their reduced species are particle reactive (Bruland and Lohan, 2006). The STE can act as either a source (Beck et al., 2010; O’Connor, Ch. 3) and a sink (Windom and Niencheski, Charette and Sholkovitz, 2006; Santos et al., 2011) for these elements. However, the influence of colloids on RSMs throughout the STE has not been examined.

The current study examined colloidal composition and distribution in the STE,
and the portioning of RSMs into the colloidal phase. This work seeks to answer the questions 1) what are the properties of colloids formed in the STE and 2) how important are these colloids for the transport of RSMs?

Methods

Study Site

This study was conducted in an STE in Gloucester Point, VA, USA (37.248884 N, -76.505324 E) (Fig. 1a). The STE is adjacent to the York River estuary, a microtidal (0.7 to 0.85 m tidal range) tributary to the Chesapeake Bay (Moore and Reay, 2009). The Gloucester Point beach is a 20-30 m wide sandy beach with a gradual slope (0.2). An upland marsh borders the beach to the east. Previous work at the study site used a Ra budget to determin the flux of SGD to the York River (93 to 178 L m$^{-2}$ d$^{-1}$) (Luek and Beck, 2014). At the study location, hydraulic head is inversely correlated with both distance from shoreline and tidal height (Beck et al., 2015). The relative rates of river discharge and aquifer recharge lead to lower hydraulic gradients from the groundwater to the estuary from approximately June to November (Luek and Beck, 2014).

The porewater redox conditions in the STE have been previously characterized over different time scales. Redox-sensitive parameters (dissolved Fe and NH$_4^+$) at 50 and 100 cm below the sediment surface do not vary with the tidal cycle, suggesting that oxygen intrusion during the recharge/discharge cycle does not affect redox cycling on this time scale (Beck et al., 2015). There are sequential zones of Fe and sulfide production with depth throughout the STE, with higher concentrations of Fe in the
winters and sulfide in the summers. Elevated concentrations of dissolved V and Cr were associated with a zone of high dissolved organic carbon concentrations, suggesting the potential for transport of metals associated with organic colloids (O'Connor et al, 2015; O'Connor, Ch. 3).

Sample Collection and Analyses

Porewater samples were collected from depth profiles along a shore-normal transect that bisected the shallow recirculation cell (Fig. 1b). Samples were collected in July 2014, October 2014, and February 2015 (further described in O'Connor, Ch. 2). Although seasonal variation in RSM concentrations have been observed at this site (O'Connor, Ch. 3), spatial variability in the data and number of colloidal samples collected in each month proved to be too few to discern meaningful seasonal trends. Here, the compiled data are used, and the conclusions drawn represent findings integrated throughout the year.

We collected porewater samples using permanently installed wells comprising a 1 cm-long stainless steel screened point (AMS, Inc.; e.g. Gonneea et al., 2013) attached to 3 mm diameter No-Ox® PTFE tubing (Supelco/Sigma-Aldrich), or from an all-plastic multi-level sampler (Beck, 2007). Groundwater was pumped from depth using a portable peristaltic pump and filtered through a 0.45 μm Millipore polypropylene capsule filter into sampling containers.

To preserve redox conditions, samples for colloidal analysis were collected into gas-impermeable Tedlar bags (deBruyn and Rasmussen, 1999) which had been acid-
washed (3x with 1 N HCl), rinsed with MilliQ water (3x), and purged with Ar (3x) before being sealed. To partition the sample for analyses, water was pumped from the Tedlar bag directly into an Ar-filled glovebag for sub-sampling. Electrophoretic mobility, a proxy for particle surface charge, was measured using a Zetasizer Nano ZS90 (Malvern).

To separate the colloidal fractions, the 0.45 μm-filtered sample was ultrafiltered in parallel at 30 kD and 3 kD using acid-washed, MilliQ-rinsed Amicon Ultracel filters (Millipore). Masses of the filtrate and retentate were measured after centrifugation at 4000g for 20 minutes. Ultrafiltrates were acidified to pH <2 and frozen until analyzed for DOC, Fe, Mn and RSMs as described previously (O'Connor, Ch. 2, Ch. 3). Retentate subsamples collected during February were acidified and analyzed for metals to determine recoveries (70-100%). Due to the small volume of retentate (<0.5 mL), recovery of DOC could not be directly measured. In this study, three size fractions will be discussed based on the filtration cut-offs: truly dissolved (<3 kD), small colloidal (3 to 30 kD), and large colloidal (30 kD to 0.45 μm).

According to the methods described in Sanudo-Wilhelmy et al. (2002), the percent colloidal concentration of each constituent was calculated by difference, rather than by using concentrations in the retentate. Although some material is commonly lost to the membrane filter, calculating the percent colloidal material by difference (amount of material in the ultrafiltrate subtracted from the total dissolved concentration) yields similar results to flushing the membrane filter with acid. Therefore, we calculated the percent colloidal material based on the amount in the ultrafiltrate. Blanks for each filter size during each month were analyzed in triplicate.
Statistical Analyses

Principal component analysis (PCA) was used to explore multivariate relationships within the two colloidal size fractions. First, PCA was used to determine how major environmental variables (salinity, pH, and Eh) related to the partitioning of DOC, Fe, and Mn into each colloidal size pool. Then, PCA was used to understand how properties affecting colloidal properties (salinity, electrophoretic mobility, DOC, Fe, and Mn) and RSM geochemistry (Eh) determined the fractionation of RSMs into the colloidal phase. Although pH can also affect RSM speciation, its inclusion in the PCA reduced the proportion of variability explained by the dominant principal components, and it was therefore excluded from the analysis. In all cases, three principal components were required to explain more than 50% of the variability in the data.

Results

Electrophoretic Mobility

Electrophoretic mobility varied between -2.2 and -0.1 x 10^{-8} m^2 V^{-1} s^{-1} (Fig. 2a), and was significantly correlated with salinity (p=0.006, R^2=0.13), with the more strongly negative electrophoretic mobilities associated with fresher water. Variability in electrophoretic mobility was high at salinities between 3 and 5 (-0.19 to -1.9 x 10^{-8} m^2 V^{-1} s^{-1}). The distribution of electrophoretic mobility with salinity was similar to trends previously observed in this system due to three endmember mixing (O'Connor, Ch. 2; Ch. 3). Electrophoretic mobility was not significantly correlated with either Eh (Fig. 2b) or pH (Fig. 2c).
**DOC, Fe, and Mn**

The behavior of total dissolved DOC, Fe, and Mn in this STE have been previously described (O'Connor, Ch. 2). These components (along with aluminosilicates) make up the bulk structure of colloidal particles, and affect colloidal surface properties such as charge and response to pH changes (Buffle et al., 1998). Those results are briefly summarized here, followed by a description of the mixing behavior of DOC, Fe, and Mn in the three size fractions.

Both total dissolved DOC and Fe were highest in plumes occurring between 100 and 200 cm depth, whereas the Mn peak extended deeper into the STE. Primarily conservative behavior of DOC with salinity was observed, but total dissolved Fe and Mn showed non-conservative addition within the STE. Three distinct endmembers, with different concentrations of DOC, Fe, and Mn, were identified as sources of water to the STE: shallow meteoric groundwater, deep meteoric groundwater, and surface estuary water (O'Connor, Ch. 2; Fig. 3).

The mixing of each of the three size fractions with respect to salinity was similar to the mixing of total DOC, Fe, and Mn (Fig. 3). In all three size fractions, DOC, Fe, and Mn concentrations were highest in shallow, low salinity samples and lower in deep low salinity and high salinity samples (Fig. 3). The major trends in colloid concentrations along the salinity gradient did not differ from those observed in the total dissolved fraction (Fig. 3).

The proportions of DOC, Fe, and Mn in the different size fractions were affected
by salinity changes. The fraction of truly dissolved DOC decreased with increasing salinity ($R^2=0.16$), whereas the fraction of DOC in the large colloid size class increased with salinity ($R^2=0.12$; Fig. 4a). Overall, a greater percentage of DOC was in the combined colloidal size fractions (76 ± 20%) than in the truly dissolved fraction (24 ± 20%).

Similar to DOC (and in contrast to typical surface estuary behavior), the fraction of truly dissolved Fe decreased with increasing salinity ($R^2=0.05$) and the fraction of Fe in large colloids increased with increasing salinity ($R^2=0.08$; Fig. 4b). In fresher water (<5 salinity), the percent of truly dissolved Fe (56 ± 46%) was comparable to the combined colloidal phases (46 ± 32%), whereas at high salinities (>15) the percent of truly dissolved Fe (33 ± 27%) was lower than in the combined colloidal phases (70 ± 24%).

The same trends were observed for Mn as Fe, with percent in the truly dissolved fraction decreasing ($R^2=0.04$) and the percent in the large colloidal fraction increasing ($R^2=0.05$; Fig. 4c) with increasing salinity. The fraction of truly dissolved Mn (85 ± 12%) is much higher than total colloidal Mn (15 ± 12%) at low salinities (<5), and the fractions of truly dissolved (47 ± 39%) and total colloidal (55 ± 39%) Mn are similar at high salinities (>15).

In the small colloid pool, PCA indicated that partitioning of DOC was directly related to salinity (PC 3; Fig. 5a, Table 1) and Fe and Mn partitioning was inversely related to pH (PC 2, Fig. 5). The fraction of DOC in the large colloids covaried with salinity (PC 3, Fig. 6). In contrast, there was no clear environmental drivers of Fe and Mn
partitioning, although the fractions of Fe and Mn in the colloidal phase were inversely related to the fraction of DOC in the colloidal phase (PC 1, Fig. 5).

**Redox Sensitive Metals**

Behavior of the total dissolved RSMs discussed here (Mo, U, V, and Cr) has been previously described (O'Connor, Ch. 3). To briefly summarize: Molybdenum and uranium were both supplied to the STE by estuary surface water, but differed in their removal mechanisms. Molybdenum was removed by reaction with sulfide, whereas U removal was microbially-mediated. Both V and Cr were supplied to the STE by inland groundwater and showed non-conservative addition within the STE. The mobility of V and Cr through the STE, and therefore their supply to the coastal ocean, was related to porewater DOC and Fe — possibly due to enhanced solubility by complexation with these constituents.

Overall, the percent of each RSMs in the truly dissolved phase decreased in the order Mo (89 ± 20%), U (83 ± 17%), V (73 ± 20%), and Cr (66 ± 23%) (Fig. 6). Correspondingly, the reverse is true for percent of each RSM in the colloidal phase, and the percent distribution for each RSM between the two colloidal size fractions was roughly equivalent.

In the truly dissolved size fraction, both Mo and U showed trends similar to the total dissolved mixing patterns, primarily showing non-conservative removal between salinities 10 to 15 (Fig. 7a, b). Similarly, Mo and U in the colloidal size fractions tended to show removal relative to conservative mixing of the observed endmembers (Fig. 7a, b). The percent of truly dissolved Mo and U decreased as salinity decreased ($R^2=0.25$ and
$R^2=0.22$, respectively; Fig. 8a, b). The percent of Mo and U in the small colloidal phase increased as salinity decreased ($R^2=0.35$ and $R^2=0.18$, respectively), while the percent in the large colloidal phase did not change (Fig. 8a, b).

Both V and Cr showed trends with salinity in the truly dissolved size fraction similar to the total dissolved concentrations, both indicating non-conservative addition primarily between salinities 3 and 10 (Fig. 7c, d). In the colloidal fractions, V and Cr show similar, although less pronounced, non-conservative addition (Fig. 7c, d). The percent of truly dissolved V increased with salinity ($R^2=0.11$), but the percent of V in the small colloidal fraction decreased as salinity increased ($R^2=0.22$; Fig. 8c). Cr showed no significant changes with salinity in any size fraction (Fig. 8d), although the median percent truly dissolved Cr was lowest (70%) at low salinities (<5).

Principal component analysis of the small colloidal phase suggested that the three major groups of variables could be identified. Small colloid concentrations of Fe and DOC grouped with colloidal fractions of U, V, and Cr (dimension 1; Fig. 9, Table 1); Eh and the fraction of small colloidal Mo were directly related (dimension 2; Fig. 9, Table 1); and electrophoretic mobility varied with dimension 3 (Fig. 9, Table 1). The placement of the constituents along the three axes showed that colloidal DOC and the percent of colloidal V grouped together, and that colloidal Fe and the percent colloidal U and Cr grouped together (Fig. 9, Table 1).

Electrophoretic mobility was a major control of the fraction of each RSM in the large colloidal pool (dimension 1, Fig. 9). Large colloidal DOC plotted close to fraction V along PCs one and two, indicating a positive correlation.
Discussion

Electrophoretic Mobility

The colloidal electrophoretic mobilities observed in this STE were consistent with those in the surface estuary (Fig. 2). Observations from a number of estuaries have found that colloidal electrophoretic mobilities typically fall between $-0.5$ and $-2 \times 10^{-8}$ m$^2$ V$^{-1}$ s$^{-1}$, and become more negative with decreasing salinity (i.e., have a stronger negative surface charge; Hunter and Liss, 1979).

The mechanism posited for the decrease in electrophoretic mobility in surface estuaries is that increasing salinity (which means increasing concentrations of divalent cations such as Ca$^{2+}$ and Mg$^{2+}$) masks the colloidal surface charge (for which electrophoretic mobility serves as a proxy). This leads to both lower electrophoretic mobility and flocculation due to lower electrostatic repulsion between particles. The rate of electrophoretic mobility decrease and flocculation with salinity is relatively consistent across estuaries, despite markedly different laboratory rates observed for different colloidal mineral compositions (Hahn and Stumm, 1970). This has been attributed to the surface charge of natural colloids being dominated by metal oxides and adsorbed organic matter (Gibbs, 1977; Hunter and Liss, 1979). The similarity between surface estuary and STE colloidal surface charges indicate similar geochemical surface properties.

One major difference between surface estuary and STE colloidal surface charges
is the manner in which electrophoretic mobility changes with salinity. In surface estuaries, electrophoretic mobilities at salinities greater than ~5 fell consistently between -0.75 and -1.25 x 10^{-8} m^2 V^{-1} s^{-1}, whereas electrophoretic mobilities at salinities less than ~3 fell between -1.0 and -2.0 x 10^{-8} m^2 V^{-1} s^{-1} (Hunter and Liss, 1979). In contrast, the trend with salinity in this STE showed greater variability across the salinity range, and particularly at low salinities (with electrophoretic mobilities ranging from -0.25 to -2.0 x 10^{-8} m^2 V^{-1} s^{-1}). Differences in colloidal response to salinity can be attributed to different compositions of the inorganic and organic phases (Gibbs, 1977, 1983). Compared to surface estuaries, shallow STEs contain sharp redox and chemical gradients (e.g., Roy et al., 2010, McAllister et al., 2015, Seidel et al., 2015, O'Connor, Ch. 2). For example, the STE studied here exhibited three distinct organic matter endmembers, characterized by different proportions of humic material (O'Connor, Ch. 2), and colloidal DOC, Fe, and Mn concentrations (Fig. 3), even at a given salinity. Variability in colloidal composition driven by these gradients in this STE could drive the more variable trend of electrophoretic mobility with salinity.

**DOC, Fe, and Mn**

Overall, concentrations of DOC, Fe, and Mn in the three size fractions mirrored the distribution of the total dissolved concentrations (Fig. 3), suggesting that the dominant controls on colloidal concentration (as measured by these constituents) were the same processes that determine total concentrations (O'Connor, Ch. 2). These results present a major difference from the behavior of colloidal Fe and DOC in surface
estuaries, where high molecular weight organic matter (corresponding to the large colloidal size fraction in this study) was removed with increases in salinity (Sholkovitz, 1976; Boyle et al., 1977; Sholkovitz et al., 1978).

In other surface estuary and groundwater locations, distinct pools of colloidal materials characterized by either high DOC or high Fe were observed (Vasyukova et al. 2010, Pedrot et al., 2008; Pourret et al. 2012). The ratio of Fe and Mn to DOC in both colloidal size fractions showed no variation across salinity or redox gradients in the current study (data not shown), suggesting that bulk colloidal composition was similar throughout the STE. This uniformity may be due to the relative excess of DOC relative to total dissolved Fe creating mixed organo-metallic colloidal particles (Pokrovsky et al., 2005).

The distribution of these constituents across each size range, in conjunction with the salinity mixing data, can be used to determine the geochemical factors driving partitioning of DOC, Fe, and Mn into the colloidal phases. First, the effect of salinity on colloidal partitioning is discussed and compared to surface estuaries. Results from PCA are then discussed to give a more complete, multivariate picture of colloidal processes within the STE.

In most samples, over half of the total dissolved DOC and Fe was in the combined colloidal pools (Fig. 4), suggesting that organic and Fe (hydr)oxide colloids are abundant in the STE. A greater proportion of Mn was in the truly dissolved phase, and colloidal Mn had much lower concentrations than DOC or Fe, indicating that Mn is likely a less important colloidal component.
The increasing proportion of large colloid-associated DOC with salinity was likely not due to increasing proportions of humic material (typically high molecular weight) in the DOC pool, as humic carbon concentrations and the ratio of humic to total DOC were both lower at high salinities (O'Connor, Ch. 2). Additionally, the coupled increasing percent of DOC, Fe, and Mn in the large colloids and decreasing proportion of truly dissolved DOC, Fe, and Mn with increasing salinity was likely not due simply to removal of truly dissolved constituents or addition of large colloidal species. Although both removal of truly dissolved or addition of large colloidal DOC, Fe, and Mn could cause the observed changes in proportion, either is unlikely due to consistent mixing behavior (ruling out addition/removal processes). Additionally, either of these processes alone would also affect the proportion of small colloidal concentrations, which was unchanged with salinity.

The changing proportions of DOC, Fe, and Mn in the truly dissolved and large colloidal size fractions could be due to a combination of factors including: 1) movement of DOC/Fe/Mn from the truly dissolved to the small colloidal and large colloidal pools; and 2) addition of large colloidal DOC/Fe/Mn to the total dissolved pool in greater amounts than addition to the truly dissolved and small colloidal pools. Increasing salinity causes aggregation of colloidal particles in surface estuaries (Sholkovitz et al., 1978; Gibbs, 1983), but in the STE, these larger particles have no means of settling out of solution (supported by our mixing results indicating no distinct non-conservative colloidal removal). Additionally, salinity fluctuations, such as those observed over seasonal and tidal cycles in the shallow portions of this STE (Luek et al., 2014; Beck et
al., 2015), promote desorption of colloidal material from aquifer solids (Kretzschmar and Shafer, 2005).

The proportion of colloidal DOC, Fe, and Mn explained by salinity alone was highest for DOC (12%), but low overall (between 4% and 12%; Fig. 4). The PCA results showed relationships between environmental variables and the partitioning of DOC, Fe, and Mn between the three size fractions helped clarify these controls.

The proportion of DOC in both small and large colloidal phases was directly related to salinity, suggesting that salinity is the dominant control on organic colloid partitioning in the STE. The inverse relationship between pH and the fraction of Fe and Mn in small colloids, and lack of a clear relationship between Fe and Mn and any environmental variables in the large colloids indicated more complex controls on the partitioning of these metals among the size fractions (Fig. 5; Table 1).

The inverse relationship Fe and Mn in small colloids with pH is consistent with colloid formation in other environments. The oxidation of Fe(II) has been show to drive the formation of Fe(III) colloids in other aquifers (Pokrovsky and Schott, 2002; Hassellov and von der Kammer, 2008) in other aquifers, and Fe oxidation in the STE is driven by pH gradients (rather than simply Eh gradients; Spiteri et al., 2006). The formation of large reactive metal oxide colloids may also be related to the pH or redox gradient, but in a more complex manner than is apparent here. For example, PCA highlights only linear correlations between variables, whereas large colloid formation could be related to a factor like the rate of change of pH or Eh.
Redox Sensitive Metals

Similar to surface estuaries, which contain little to no colloidal Mo (<5%; Pokrovsky and Schott, 2002; Vasyukova et al., 2010), the proportion of Mo in colloids was generally low relative to the other elements studied. Colloidal transport in the STE was most important for V and Cr, RSMs which had high proportions in colloids in other groundwater and surface estuary environments (Pokrovsky and Schott, 2002; Pokrovsky et al., 2005; Vasyukova et al. 2010). Additionally, both V and Cr were supplied to the STE by lower salinity groundwater (O’Connor, Ch. 3) suggesting that the source of the RSM to the STE affects the importance of colloidal phase transport. This could be due to the speciation of these elements as they enter the STE — possibly as reduced, particle reactive species already associated with colloids, as opposed to the oxidized species of Mo and U, which exist in solution in estuarine surface waters.

The proportions of Mo, U, and V in the small colloidal fraction were inversely related with salinity. Although colloid flocculation and removal with increasing salinity can drive removal of these elements in surface estuaries (Cranston and Murray, 1980; Andersson et al., 2001; Pokrovsky and Schott, 2002; Strady et al., 2009; Beck et al., 2012), this is unlikely here because there was no evidence of this process in DOC and Fe distributions. Alternatively, changing concentrations with salinity could also cause changes in the proportion of colloidal RSMs. However, concentrations of truly dissolved and small colloidal Mo, U, and V showed different trends with salinity, whereas their proportional responses were similar. This indicated that the increase in the truly dissolved and decrease in the small colloidal proportions of these elements was instead likely due to
some changing property of the RSMs (e.g., speciation) or of the colloids themselves with changing salinity.

Results from the PCA helped clarify the environmental controls on RSM partitioning into the colloidal fractions. Although the percent of Mo, U, and V in the small colloidal phase was inversely correlated with salinity when examined alone, their relationships with colloid abundance and environmental variables showed that these elements are bound to different components of the small colloidal pool. In the small colloidal phase, percent Mo was directly correlated with Eh, indicating that a greater proportion of Mo was found in small colloids under oxidizing conditions, in contrast to the expectation that Mo would be more likely to sorb to colloids as a particle-reactive reduced species. The grouping together of the percent U and Cr with Fe suggested that the presence of reactive Fe surfaces in small colloids may be important for sorbing U and Cr, whereas the relationship between fraction of V with DOC suggested that small colloidal V is complexed by organic matter. Therefore, the composition of the colloidal pool in the STE determines which metals will be most effectively transported on colloids.

The behavior of RSMs in large colloids differed from those in the small colloids by showing more similar environmental controls across elements. All colloidal RSM fractions were inversely proportional to electrophoretic mobility and salinity, indicating that large colloidal surface charge strongly affected metal sorption. Although changes in the proportion of large colloidal RSMs with electrophoretic mobility could have been driven by processes external to the total dissolved pool, these RSMs showed different trends with salinity, suggesting that an external processes driving the same changes in
concentration is unlikely. In contrast to small colloids, the sorption of RSMs to these large colloids is evidently more dependent on non-specific electrostatic binding rather than association/complexation by a particular colloidal component. Additionally, the locations of DOC and percent V on dimensions one and two suggested that DOC complexation helped drive V partitioning in the large colloidal phase as well as in the small colloidal phase.

Previous work found that dissolved Cr was much more strongly associated with hydrophobic organic matter than V (O’Connor, Ch. 3). This study shows similar percentages of colloidal V and Cr and that colloidal V is more strongly associated with DOC than is Cr. Given these results along with those from previous work (O’Connor, Ch. 3), it seems likely that V and Cr may be complexed by different organic matter and colloidal pools in this STE: colloidal V tended to be associated with a less hydrophobic DOC pool (possibly fulvic acids, which are known to readily associate with colloidal phases; Buffle et al., 1998), but Cr was associated with mixed Fe (hydr)oxide and hydrophobic organic carbon colloids.

**Conclusions**

Colloids proved to be an important 'third phase' in this subterranean estuary. Colloidal surface charge responded to salinity similarly to surface estuaries, and was more strongly negative in lower-salinity waters. However, typical estuarine colloidal removal by flocculation did not occur in the STE — instead, the proportion of DOC, Mn, and Fe in the colloidal phase increased along the entire salinity gradient, likely due to a
combination of colloid flocculation (without the sedimentation and removal observed in surface estuaries) and mobilization of new colloids from aquifer solids.

This work demonstrated the importance of colloids for transport of RSMs in the STE, particularly those from a groundwater origin. Colloidal properties such as surface charge, and amount of colloidal DOC and Fe affected the partitioning of Mo, U, V, and Cr into the colloidal phase. One important implication of these findings is that otherwise particle-reactive metals (such as Pb or Hg) may be mobile in the STE, and therefore SGD could be a source of such elements to coastal waters.
Table 1. Correlation coefficients for the relationship between constituents and the three dominant principal components (plotted as dimensions 1, 2, and 3 in following figures, and described the same here). The upper portions of the table are from the PCA used to determine controls on the percent DOC, Fe, and Mn (here, DOC.f, Fe.f, and Mn.f) in the colloids, and the lower portions are from the PCA used to determine controls on the percent RSMs (Mo.f, U.f, V.f, and Cr.f) in the colloidal phases. Results shown in light text show correlations between the environmental/predictor variables alone.
Figures

Figure 1. (a) The study site (identified with a white box) is located on the western side of the Gloucester Point peninsula on the York River estuary (Virginia, USA). (b) Permanent sampling wells were installed at the points shown (specific depths found in Supplementary Information) to capture the geochemical gradients in this STE (O’Connor, Ch. 2; O’Connor, Ch. 3).
Figure 2. The relationships between electrophoretic mobility and (a) salinity, (b) Eh, and (c) pH. The depths of each sampling point are indicated by color to give a sense of spatial variability in these relationships. Grey lines indicate linear fits of the data using least squares regressions, and significant relationships, when they exist, are provided in the upper right corner of the panel.
Figure 3. Concentrations of (a) DOC, (b) Fe, and (c) Mn in each size fraction (as well as the total dissolved concentrations; see also O'Connor, Ch. 2) plotted against salinity to show mixing patterns along the gradient.
Figure 4. Percent (of total dissolved concentrations) of (a) DOC, (b) Fe, and (c) Mn in each size fraction in the porewater (open circles) and surface water (shown in X’s). Least squares linear regression were done only on the porewater data, and $R^2$ values are shown only for those with statistical significance (p<0.05).
Figure 5. Results from the principal components analyses used to determine controls on the partitioning of DOC, Fe, and Mn into the colloidal size fractions. Locations of each variable along the first three principal component dimensions for the small and large colloidal fractions are shown (see text for details). Color shading indicates distance along dimension 2 (running perpendicular to the plane of the page). Dotted circles show constituents that grouped together along a particular axis (Table 1).
Figure 6. Boxplots showing the percent of each RSM in three size fractions throughout the STE. The thick bar represents the median; the outer edges of the boxes represent the 25th and 75th percentiles of the data; the whiskers extend to the highest data point within 1.5 times the range of the box; and outliers are plotted as points.
Figure 7. Concentrations of (a) Mo, (b) U, (c) V, and (d) Cr in each size fraction (as well as total dissolved concentrations; see also O'Connor, Ch. 3) plotted against salinity to show mixing patterns along the transect.
Figure 8. Percent (of total dissolved concentrations) of (a) Mo, (b) U, (c) V, and (d) Cr in each size fraction in the porewater (open circles) and surface water (shown in X's). Least squares linear regression were done only on the porewater data, and results are shown only for those with statistical significance (p<0.05).
Figure 9. Results from the principal components analyses used to determine controls on the partitioning of RSMs into the colloidal size fractions. Locations of each variable along the first three principal component dimensions for the small and large colloidal fractions are shown (see text for details). Color shading indicates distance along dimension 2 (running perpendicular to the plane of the page). Dotted circles show constituents that grouped together along a particular axis (Table 1).
References


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CHAPTER 5

RATES OF REDOX SENSITIVE METAL SEQUESTRATION AND MOBILIZATION IN THE SUBTERRANEAN ESTUARY

Abstract

Submarine groundwater discharge (SGD), the mixture of fresh groundwater and recirculated seawater that flows from coastal sediments, is an important source of nutrients and trace metals to the ocean. Biogeochemical redox reactions within the subterranean estuary (STE), the subsurface region where fresh groundwater and infiltrating seawater mix, determine the composition of SGD. In this study, rates of redox-sensitive metal (RSM) removal and mobilization under anaerobic and aerobic conditions (respectively) were determined using laboratory experiments. Sediments from different redox zones (oxic, nitrogenous, ferruginous, and sulfidic) in the Gloucester Point, VA STE were mixed with artificial porewater under anaerobic or aerobic conditions, and changes in porewater concentrations of Mo, U, V, and Cr were measured over time. This study found that, for most STE sediments, RSMs were removed from the porewater under anaerobic conditions in two steps: one faster step, evidently from non-specific sorption to sediments; and one slower step, likely from reduction followed by immobilization. Additionally, reaction rates were influenced by the original redox conditions of the sediments and their microbial community. Molybdenum removal rates were greatest in sulfidic sediments, U removal rates were greatest in ferruginous sediments, and V removal rates were highest in oxic and nitrogenous sediments. Rates of Cr removal were too rapid to be measured in this experiment. Under aerobic conditions,
RSMs were primarily mobilized into the porewater. As with removal under anaerobic conditions, mobilization took place in two steps. Mobilization rates for all RSMs were fastest in sulfidic sediments, indicating that under oxidizing conditions, sulfidic STE sediments could present a significant source of RSMs.

Introduction

Submarine groundwater discharge (SGD) is an important source of nutrients and trace metals to the coastal ocean (Slomp and Van Cappellen, 2004; Spinelli et al., 2002; Bone et al., 2006; Beck et al., 2009). Biogeochemical reactions in the subterranean estuary (STE), created by the subsurface mixing of outflowing fresh groundwater and infiltrating seawater, determine the composition of SGD (Charette and Sholkovitz 2002; Roy et al., 2010; McAllister et al., 2015; O'Connor et al., 2015).

Redox reactions within the STE create biogeochemical zones (Spiteri et al., 2008; O'Connor et al., 2015; McAllister et al., 2015), which affect the cycling of trace elements such as redox sensitive metals (RSMs) (Windom and Niencheski, 2003; Riedel et al., 2011; Beck et al., 2010; O'Connor, Ch. 3). Some RSMs, such as Mo, U, V, and Cr, form soluble oxyanion species under oxidizing conditions, but their reduced species are highly particle reactive (Bruland and Lohan, 2006). Due to their redox-active behavior, these RSMs are widely used as marine paleoproxies to examine past oceanic oxygen levels, productivity, and circulation (e.g., Nameroff et al., 2004; Rimmer et al., 2004; Tribovillard et al., 2006; Algeo et al., 2012).

The hydraulic gradient between groundwater and sea level determines the position
of the salinity and redox interface of the STE, and can change at seasonal and annual time scales (Gonneea et al., 2013a, b). The movement of this interface has been shown to affect fluxes of elements such as Ra and Ba in SGD (Gonneea et al., 2013a, b). As redox conditions within the STE change, RSM speciation and mobility may change as well. Specifically, RSMs would be expected to be reduced to their particle-reactive species and removed to the sediments under reducing conditions. Although equilibrium speciation under new redox conditions can be readily calculated, water advection in permeable sediments is several orders of magnitude more rapid than in diffusion-dominated environments (Huettel et al., 2003), and kinetic constraints on biogeochemical reactions may limit the extent of geochemical reactions counter to equilibrium predictions. Therefore, reaction rates will determine how RSM distributions in the STE respond to changing redox conditions.

Respiration of organic matter in the STE generates distinct redox zones, resulting in sediments with different organic matter, metal oxides, and RSM contents (Charette et al., 2005; O’Connor, Ch. 2, Ch. 3). Additionally, microbial community structure varies throughout the STE, driving different nitrogen, Fe cycling, and sulfide cycling (Santoro, 2009; McAllister et al., 2015) reactions. As a result, sediments with different chemical compositions and microbial communities will likely have unique responses to changing salinity and redox conditions.

In this work, we build on previous studies of redox zonation and RSM cycling in an STE to determine rates of RSM removal under anoxic conditions and mobilization under oxic conditions, and put these results in the context of typical residence times (i.e.,
days to weeks) for the shallow STE (Robinson et al., 2007). Additionally, we explore the role of microbial community composition in determining reaction rates by analyzing the sediment microbial population of distinct STE redox zones.

**Methods**

*Study Location*

The study site is located on the York River Estuary (YRE) in Gloucester Point, Virginia, USA (37.248884 N, -76.505324 E) (Fig. 1a). The YRE is a relatively pristine microtidal (0.7 to 0.85 m tidal range) tributary of the Chesapeake Bay (Moore and Reay, 2009). Surface water salinity at this study site typically ranges from 14 in the winter to 25 in the summer (Luek and Beck, 2014). The Gloucester Point beach is a narrow (20-30 m wide) sandy beach with a gradual slope (0.2).

All samples were collected from a profile bisecting the center of the shallow STE circulation cell (Fig. 1b). The biogeochemical environment of this profile has been described previously (O'Connor et al., 2015; O'Connor et al., Ch. 2, O'Connor et al., Ch. 3) and will be summarized briefly here. In previous work, all depths were normalized instead to the sediment surface at the high tide location. Here, depths are normalized to depth below the mid-tide sediment surface to better describe the extracted core and in-situ conditions. For comparison, the depths presented here are 50 less than those previously reported.

Advection of water through the sandy sediments of this study site contributed to the formation of a "classic" redox sequence. At the mid-tide line, conditions were oxic
down to ~20 cm below the mid-tide sediment surface, concentrations of NO₃ peak between 20 and and 40 cm, dissolved Fe and Mn increase reach a maximum between 40 and 90 cm, and sediments become sulfidic at ~90 cm depth. Sediment contents of reducible Fe and Mn were highest from 50 to 100 cm. Although maximum concentrations of the solutes varied seasonally, the vertical distributions remained constant over time (O'Connor, Ch. 2), resulting in four major redox zones — oxic, nitrogenous, ferruginous, and sulfidic. Sediments collected for this study were partitioned according to these previously observed zones.

Porewater Mo and U concentrations were highest in shallow, saline water, indicating a surface estuarine source (O’Connor, Ch. 3). Dissolved V and Cr were highest at intermediate depths (100 to 150 cm depth and 100 to 250 cm depth, respectively), consistent with a terrestrial groundwater source. Sediment distributions of Mo and U were similar to each other, with reducing (hydroxylamine hydrochloride) and aqua regia leach concentrations increasing at ~120 cm depth. Sediment contents of V and Cr were similar to one another, and highest between 100 and 200 cm depth.

Sediment Collection and Analyses

A 1-meter long vibracore of sediments for incubation experiments and microbial analysis was collected in April 2015. The core was subsectioned under Ar according to the previously mentioned geochemical redox zones — oxic, nitrogenous, ferruginous, and sulfidic — and each horizon was homogenized. Subsamples for microbial analysis from each of these zones were stored at -80 °C until analysis.
Dried sediments were analyzed for porosity, surface area (described previously; O'Connor, Ch. 2), organic content, and reducible Fe contents. Sediment organic matter content was determined by loss on ignition at 550 °C for 12 hours (Dean, 1974). Reducible Fe was determined using methods described previously (O'Connor, Ch 2), using hydroxylamine hydrochloride leach (Tessier et al., 1979). Concentrations of these constituents are normalized to amount per volume of sediment, using surface area, porosity, and a sediment density of 2.65 g/cm³ (the density of quartz; Vanoni, 1964).

Experimental Set-up

The RSMs studied here tend to be particle reactive when reduced and soluble when oxidized. Therefore, two incubation experiments were performed: one to determine the removal rate of RSMs in each STE redox zone under anaerobic conditions, and one to determine the mobilization rate of RSMs under aerobic conditions in each sediment redox zone. These incubation experiments used sediments collected from the STE to introduce the naturally occurring minerals and microbial consortia to each sample. Microwave-sterilized (Keller et al., 1988) artificial seawater was used for consistency across treatments. Differences in RSM removal or mobilization rates were therefore due only to differences between sediments and microbial population. These experiments were carried out over 500 to 600 hours (20 to 25 days), which covers the range of shallow STE residence times (Robinson et al., 2007).

The intent of these incubations was not to mimic in situ redox conditions, but to demonstrate the potential of these sediments for RSM mobilization or sequestration under
changing aerobic or anaerobic conditions, which could occur due to environmental changes, such as storm surges, groundwater level lowering due to overdraw, or an increase in organic carbon loading to the STE due to a warmer, wetter summer. Treatments are referred to as oxic, nitrogenous, ferruginous, or sulfidic to indicate the redox zone from which the sediments originated, not necessarily the geochemical conditions during the experiment.

A schematic of the experimental setup is shown in Fig. 2 to help illustrate the following description. Immediately after collection, the core was split under Ar and sediments were divided according to pre-determined redox zones and homogenized. On the benchtop, approximately 90 g of sediment from each redox zone was rapidly added in triplicate to acid-washed 125 mL borosilicate glass serum vials (for anaerobic incubations, with one replicate sacrificed for oxygen measurement) and in duplicate to 500 mL polycarbonate flasks (for aerobic incubations). Approximately 105 mL of Ar-sparged artificial seawater at salinity 10 was added to the anaerobic incubation samples (which were then sealed with air-tight stoppers), and approximately 105 mL air-sparged artificial seawater at salinity 20 was added to the aerobic incubation samples.

A single salinity was used across samples in each treatment to ensure that any differences in reaction rate were due to differences between sediment redox zones. The salinities of water added to the experimental samples were selected based on ambient environmental conditions. The median salinity in the STE was ~10, and porewater at this salinity was typically anaerobic, so salinity 10 was used in the anaerobic experimental treatment. The STE would be exposed to aerobic conditions by intrusion of high-oxygen
surface water, which would also lead to higher salinities. Surface salinity in the York River Estuary is generally between 16 and 22. Therefore, salinity 20 was chosen for the aerobic experiment.

Anaerobic incubation samples were immediately sealed with rubber stoppers, and the headspace was sparged with Ar to remove remaining oxygen. These samples were shaken for a four-day pre-incubation period to remove residual oxygen, after which a 1 mL spike of concentrated trace metal solution was added. The initial RSM concentrations in the incubation vials were calculated to be roughly ten times those in YRE surface water — 730 nM Mo, 80 nM U, 430 nM V, and 24 nM Cr. These concentrations were chosen to be similar to those found naturally, but large enough to detect changes within the range of experimental error. After the trace metal spike, anaerobic conditions were confirmed in a test sample (using a Clark-type amperometric microsensor; Gieseke and de Beer, 2004), leaving duplicate samples for the duration of the experiment.

At each time point, a 6 mL aliquot was collected from each incubation sample using a needle attached to a syringe inserted through the rubber stopper. The syringe was then fitted with a 0.2 μM Nuclepore membrane filter, and the sample filtered into the appropriate collection containers. Of this sample, 2 mL were filtered into an acid-washed vial and acidified for trace metal analysis as described above. The remaining 4 mL were filtered into two polypropylene tubes (~2 mL each) containing 0.25 mL 0.25 M zinc acetate to fix dissolved sulfide. Sulfide concentrations were analyzed spectrophotometrically using the Fonselius method adapted for lower sample volumes (Grasshoff et al., 1999). The time zero sample collection was completed within 30
minutes of the trace metal spike.

Aerobic incubation samples were sampled for time zero (within 30 minutes of combining sediment and artificial porewater), covered in parafilm with needlepoint holes to allow for air exchange, and placed on a shaking table until the next time point. At each time point, a 2 mL aliquot of water from each sample was filtered (0.2 µM Nuclepore membrane filters) into an acid-washed vial. Samples were acidified to pH <2 with ultrapure HNO₃, and analyzed by dilution and direct injection on a Thermo Element 2 inductively-coupled plasma mass spectrometer at the University of Southern Mississippi’s Center for Trace Analysis.

Rate constants were determined by fitting time series data to integrated rate laws for zeroth, first, and second order reactions. Integrated rate laws are linear relationships of time vs. concentration which allow easy determination of rate constants. Generalized reaction equations and corresponding integrated rate laws for zeroth order, first order, and second order reactions are:

Zeroth Order:  \[ \text{A} \rightarrow \text{C} \quad \text{rate} = k \quad [\text{A}]_t = -kt + [\text{A}]_0 \]

First Order:  \[ \text{A} \rightarrow \text{C} \quad \text{rate} = k[\text{A}] \quad \ln[\text{A}]_t = -kt + \ln[\text{A}]_0 \]

Second Order:  \[ \text{A} + \text{B} \rightarrow \text{C} \quad \text{rate} = k[\text{A}][\text{B}] \quad 1/[\text{A}]_t = kt + 1/[\text{A}]_0 \]

where \([\text{A}]_t\) is the concentration at a given time during the reaction, \([\text{A}]_0\) is the initial concentration, \(t\) is the length of time elapsed, and \(k\) is the rate constant. These same equations, with the sign of \(k\) reversed, also apply to the generation of product C. When
[A], ln[A], or 1/[A]t (for the appropriate reaction order) are plotted against time, the magnitude of the slope indicates the value of k (Atkins and Jones, 2008).

Incubation data were fitted to each of these rate laws, and the equation that yielded the best linear fit was used to calculate k and to determine the reaction order. When there was no detectable change in concentration (i.e., none of these rate laws yielded a significant (p < 0.1 slope), k equaled zero.

If the full time series data fit poorly to these equations, this indicated that a single reaction mechanism failed to explain the changes in concentration. In these cases, two reaction rates were calculated to describe addition or removal as two distinct steps, and to explore these separate geochemical mechanisms.

**Microbial Analyses**

Bacterial species were identified in each sediment zone in order to understand how the microbial community contributed to geochemical outcomes. In addition to species identification, rRNA was extracted and identified in the oxic and sulfidic sediments to assess the relative activity of microbial populations in the two redox extremes. Ribosomes, the enzyme responsible for protein synthesis, are made of rRNA. Greater relative abundance of rRNA therefore suggests greater metabolic activity of particular microbial class or species (Jones and Lennon, 2010).

From each redox zone used in the incubation experiment, sediment samples were frozen at -80 °C immediately following collection, and later thawed and extracted using the RNA PowerSoil Total RNA Isolation and DNA Elution Accessory kits according to
the manufacturer's protocols (MoBio Laboratories). Product from the RNA isolation was converted to cDNA using the TURBO DNA Free Kit (Life technologies) and iScript cDNA Synthesis kit according to the provided instructions. Sequences were amplified using the Taq DNA Polymerase Kit (Invitrogen) with the primers 515F and 685R. Samples were sequenced using the Ion Torrent PGM with the Ion Torrent 400 bp sequencing kit protocol (Life Technologies). Sequence identification was performed using mothur SOP (Kozich et al., 2013) and the Silva database (v. 119; Quast et al., 2013; Table 1).

The distributions of eight selected bacterial classes, which make up a large proportion of the total population and provide insight into redox processes in each sediment zone, are discussed here. These classes were the alpha, delta, and gamma proteobacteria; chloroplast and cyanobacteria, which are autotrophs that require light to grow; deferribacteres, which use Fe in anaerobic metabolic processes (Alauzet and Jumas-Bilak, 2014); anaerolineae, which are primarily anaerobes with some facultative aerobic metabolic capacity (Yamada et al., 2006); and clostridia, which are obligate anaerobes (Yutin and Galperin, 2013). Additionally, sulfur/sulfate reducing bacteria (SRB) in the families Desulfobacteraceae, Desulfarculaceae, and Desulfuromonadaceae were compared between redox zones.

**Results**

**Sediment Characteristics**

Sediment organic (0.4 ± 0.3%) and reducible iron contents (2 ± 1 μmol m⁻²) were
variable throughout the core. Sediment organic content was highest in sulfidic sediments (0.54 ± 0.02%) and lowest in ferruginous sediments (0.14 ± 0.01%) (Fig. 3a). Sediment reducible Fe was lowest in sulfidic sediments (1.3 ± 0.4 μmol m$^{-2}$), and similar in all others (3.0 ± 0.7, 2.5 ± 1.1, and 2.5 ± 0.3 μmol m$^{-2}$ in oxic, nitrogenous, and ferruginous sediments respectively) (Fig. 3b).

**Anaerobic Treatments**

In the anaerobic treatments, dissolved Fe and sulfide (two indicators of anaerobic respiration) increased in the oxic and sulfidic samples, and only Fe increased in the nitrogenous samples (Fig. 4, Table 2). In all cases, rate constants were zeroth order. The rate constants for Fe and sulfide production were highest in the sulfidic samples and lowest (zero) in ferruginous samples (Fig. 5). Rate constants for Fe were higher than those for sulfide (Fig. 5, Table 2).

In many samples, RSM concentrations initially declined rapidly (between 0 and 51 hours), followed by a slower decrease through the rest of the incubation (Fig. 6). These two rates are referred to here as $k_{1R}$ and $k_{2R}$, respectively (R for 'removal'; shown schematically in Fig. 6). In the following, the magnitude of k will be described according to its absolute value — that is, a larger magnitude of k (whether positive or negative) implies a faster reaction, and the sign indicates the reaction direction.

The initial rate of change ($k_{1R}$) appeared to be zeroth order (when significant) (Table 3). However, because this initial change happened very rapidly (within the first 51 hours), only a linear fit against concentration was possible. Because only the zeroth order...
reaction equation (i.e., a straight line) could be fit two points, making the initial reaction order unreliable, the $k_{1R}$ rate constants are can be compared to one another in order to elucidate relative reaction rates. In oxic, nitrogenous, and ferruginous samples, V removal had the greatest constant, whereas in sulfidic samples, Mo had the greatest rate constant (Fig. 7a, Table 3). For V, $k_{1R}$ was highest in oxic samples (-89 nmol day$^{-1}$) and lowest (with zero initial change) in sulfidic samples; for U, $k_{1R}$ was similar in oxic, nitrogenous, and ferruginous samples (-14, -9, and -10 nmol day$^{-1}$, respectively) and lowest in sulfidic samples (-5 nmol day$^{-1}$); and $k_{1R}$ for Mo was greatest in sulfidic samples (-54 nmol day$^{-1}$) compared to oxic (-36 nmol day$^{-1}$), nitrogenous (-19 nmol day$^{-1}$), and ferruginous (-36 nmol day$^{-1}$) samples (Fig. 7a, Table 3). Chromium showed no change in any sediment redox zones early into (~50 hours) the experiment (Table 3).

Most RSM reactions that occurred after the first 51 hours of the anaerobic treatments ($k_{2R}$) were second order (Table 3), with the exception of zeroth order increases of V and Cr in sulfidic samples (Fig. 7b, c, Table 3). The $k_{2R}$ for RSM removal was greatest overall for U (Fig. 7b). The $k_{2R}$ removal rate was greatest in sulfidic samples for Mo (-1.6 ± 0.5 × 10$^{-5}$ nmol$^{-1}$ day$^{-1}$); in ferruginous samples for U (-1.79 ± 0.03 × 10$^{-1}$ nmol$^{-1}$ day$^{-1}$); and in nitrogenous samples for V (-4.1 ± 1 × 10$^{-3}$ nmol$^{-1}$ day$^{-1}$) (Fig. 7b).

Exceptions to these trends were V in oxic samples and sulfidic samples, and Cr in sulfidic samples, all of which showed some increase over the experiment (positive $k_{2R}$, Fig. 7c); Mo in ferruginous samples, which showed no significant decrease after 51 hours (no $k_{2R}$); and Cr in oxic, nitrogenous, and ferruginous samples, which showed no
significant change over time (Fig. 6).

**Aerobic Treatments**

In the aerobic treatments, mobilization of RSMs commonly occurred in two rate steps (similar to the anaerobic treatment), referred to here as $k_{1M}$ and $k_{2M}$ ('M' for mobilization; Table 4). The $k_{1M}$ represented a rapid concentration change early in the experiment, whereas $k_{2m}$ represented typically slower reactions occurring later in the experiment.

The concentration trends in some samples indicated mobilization that took place in the brief interval between the addition of metal spike and the collection of time zero samples (less than 20 minutes). For example, time zero concentrations of V were much higher in sulfidic samples (82.5 ± 0.7 nM) than in other sediment samples (8.7 ± 3.6 nM), suggesting that rapid mobilization occurred in the short time frame between experiment preparation and sample collection (Fig. 8). Similarly, varying time zero Cr concentrations (Fig. 8) suggested that some Cr may have been mobilized into solution prior to the initial sample collection. The rate constants for this type of mobilization are not constrained, and therefore cannot be determined.

Initial rates of mobilization ($k_{1M}$) were commonly zeroth order (Table 4). As was the case with rapid initial removal in the anaerobic treatments, this mobilization occurred too rapidly to accurately characterize the reaction order, and is here described as zeroth order for consistency. The $k_{1M}$ for Mo, U, and V indicated rapid mobilization from all sediment samples (Fig. 9a), but the $k_{1M}$ for V showed removal from sulfidic samples
The $k_{1M}$ rate constant for Mo and U was highest in sulfidic samples, but it was highest in nitrogenous samples for V (although $k_{1M}$ could not be quantified for V in sulfidic samples) (Fig. 9a).

The second mobilization rate constants ($k_{2M}$) were zeroth order for Mo, U, and V (with the exception of Mo in sulfidic samples) (Fig. 9b). For V and U, $k_{2M}$ was greatest in sulfidic samples (Fig. 9b). In contrast, Mo showed first order removal in sulfidic samples, resulting in a positive $k_{2M}$ (Table 4).

Dissolved Fe only changed significantly in sulfidic samples, in which Fe increased after ~64 hours (Fig. 8, Table 4). Similarly, U and V in sulfidic samples showed zeroth order increases after ~64 hours ($k_{2M}$), whereas Mo in sulfidic samples decreased in this time frame (Fig. 8).

Addition of Cr in sulfidic samples (the only redox condition under which Cr showed a significant change in concentration) occurred in a single step and was a first order reaction (Fig. 8, Table 4). Also in sulfidic samples, V showed $k_{1M}$ first order removal, and Mo showed $k_{2M}$ first order removal (Fig. 8, Table 4).

**Microbial Population**

The microbial populations in the four redox zones were distinct from one another (as determined by principle components analysis: Fig. 10a). In all redox zones, alpha, delta, and gamma proteobacteria were the greatest overall contributors to the bacterial community, accounting for between 12% and 35% of the total population (Table 5; Fig. 10b). In oxic sediments, gammaproteobacteria were most abundant, whereas in sulfidic
sediments deltaproteobacteria were most abundant. Anaerolineae were the next most abundant class after the proteobacteria in all sediments, but contributed most to the sulfidic sediment population. The proportion of photosynthetic bacteria was highest in oxic sediments. Clostridia were highest in sulfidic sediments, followed by ferruginous sediments.

The ratio of rRNA to DNA (Fig. 10) showed that proteobacteria were relatively more active in sulfidic compared to oxic sediments. Additionally, the primarily anaerobic classes Deferrribacteres, Anaerolineae, and Clostridia were more active in sulfidic sediments. The rRNA to DNA ratio of photosynthetic bacteria was very high in both oxic and sulfidic sediments, which may be an artefact of samples being partitioned under exposure to light.

SRB genera were most abundant in oxic and sulfidic sediments (Fig. 11), comprising from 1.5% to 2% of total bacteria. However, rRNA to DNA indicated much greater SRB activity in sulfidic sediments.

Discussion

Anaerobic Treatments

Sediments from different redox zones in the Gloucester Point STE had distinct microbial populations (Fig. 10a), which reflected their unique in situ geochemical environments. Although oxic sediments (from 0 to 15 cm depth) contained greater abundance (Fig. 10b) of photosynthetic bacterial classes, sulfidic sediments showed higher activities (Fig. 10c) of the primarily anaerobic class Anaerolineae, the obligate
anaerobic class Clostridia, and deltaproteobacteria (which contains a large number of iron-reducing microbes (Weber et al., 2006) and SRB (Munn, 2011)). Deferribacteres, which contain many Fe cycling bacteria, were most abundant in ferruginous and sulfidic sediment, indicating the potential of these sediment zones for carrying out metals cycling. These differences demonstrate the microbial diversity within the shallow STE (spanning, in this case, approximately the upper meter of sediment).

Upon exposure to reducing conditions, sediments from different redox zones responded with distinct anaerobic metabolic processes, shown by differences in the generation of dissolved Fe and sulfide (Fig. 4). Under anaerobic conditions, both oxic and sulfidic sediment zones have the capacity to rapidly begin Fe and sulfate/sulfur reduction processes, generating reduced metabolites.

The production of dissolved Fe and sulfide in sulfidic sediment samples may have been driven by the higher concentrations of sediment organic matter (Fig. 3). Oxic sediments generated Fe and sulfide, whereas production of dissolved Fe and sulfide was slow or nonexistent in nitrogenous and ferruginous sediments (Fig. 4). These sediments contained similar levels of organic matter, suggesting that there was another reason for this metabolic difference.

One reason for Fe and sulfide production in oxic zone sediments may be the available in situ microbial community, which will influence the initial geochemical response to changing environmental conditions. SRB were more abundant in oxic sediments than in nitrogenous and ferruginous sediments (Fig. 11a), indicating that oxic zone sediments have a natural microbial consortium with the ability to become
metabolically active during anaerobic conditions, leading to the generation of reduced metabolites in the oxic zone sediment samples.

Another possible reason for greater anaerobic activity in oxic zone sediments may be the quality of organic matter available. Oxic sediments, which are shallow and closest to the sediment-water interface, may have more labile organic matter. The presence and activity of photosynthesizing microbes in the shallow oxic sediments (Fig. 10), which require light to carry out metabolic processes, suggested that fresh organic matter was supplied to these sediments either by photosynthetic microbial production at the sediment surface, or from the surface estuary (via pumping and straining through the sediments; Santos et al., 2009). Fresh, labile organic matter could provide energy for microbes responsible for the reduction of Fe and sulfur in the oxic samples.

In general, porewater RSMs were removed to the sediments under anaerobic conditions. However, the rates of removal differed between sediment zones and removal was not always the dominant process, indicating an influence of sediment characteristics, such as chemical composition or microbial population. The removal of RSMs under anaerobic conditions typically occurred in two reactions, indicated by the different time scales and reaction orders of $k_{1R}$ and $k_{2R}$.

The variation of $k_{1R}$ between RSMs and sediment zones suggested removal to the sediments by adsorption. Rates of sorption are typically faster than redox reactions (Appelo and Postma, 2005), and even the typically-soluble oxidized RSM species can sorb to particles to some extent. Specifically, oxidized V and U can sorb to Fe oxides (Wehrli and Stumm, 1989; Davis et al., 2004), and molybdate can sorb to iron sulfide.
particles (Helz et al., 2004).

Our results indicate that on short time scales (hours to days), oxic, nitrogenous, and ferruginous sediments at this STE (all of which contained relatively high levels of reducible Fe) can sorb Mo, U, and V. Additionally, the sorption of V species was several times more rapid that U, demonstrating that its removal after the onset of reducing conditions STE will be faster than Mo and U removal.

In these experiments, Mo showed the greatest $k_{1R}$ in sulfidic samples (Fig. 7a), indicating that Mo will be rapidly removed under sulfidic conditions within the STE. Removal occurred before high concentrations of sulfide had been reached in the experimental samples. Additionally, rapid Mo removal occurred in all of the sediments even though dissolved sulfide never reached the threshold for quantitative reaction with Mo (~11 μM; Erickson and Helz, 2000) (Fig. 4). These findings indicate that Mo removal in the STE under reducing conditions may occur faster and more extensively than what might be predicted from reactions only with dissolved sulfide.

In contrast, the $k_{1R}$ for V was zero in sulfidic samples (Fig. 7a). The initial time point concentration in sulfidic samples was ~200 nM in oxic, nitrogenous, and ferruginous sediment samples, but 40 nM in sulfidic sediment samples despite being initially spiked with the same solution. This suggests that a lack of a detectable $k_{1R}$ in sulfidic samples was likely due to removal so rapid that it was not captured in the zero time point, which took place within thirty minutes of experimental sample preparation. Under anaerobic environmental conditions, V is therefore likely to be removed very rapidly to sulfidic zone sediments.
Chromium showed no initial removal in any of the sediment samples (Fig. 6). However, the trace metal spike should have resulted in initial concentrations of ~24 nM Cr, whereas all incubation treatments had initial Cr of <8 nM. This suggests that Cr removal occurred, but on a very rapid (<30 minutes) time scale (similar to V in sulfidic sediment samples). This removal could have been through sorption to sediment surfaces, as chromate tends to adsorb to metal oxide minerals (Rai et al., 1989). However, chromate was also by far the strongest oxidizing agent of the RSMs studied here (standard reduction potential (E°) of -0.41 V; Appelo and Postma, 2005) and may have been rapidly reduced prior to sorption. In either case, the very rapid removal suggests that Cr will be quickly removed in the STE under any reducing conditions on short time scales.

The second rate constants (k2R) for Mo, U, and V occurred on longer time scales than k1R, indicating a different removal mechanism. The k2R rate constants varied across several orders of magnitude (Fig. 7b), indicating widely different response times and reduction mechanisms.

As with k1R, the k2R rate of Mo removal was greatest in sulfidic sediment samples, despite the fact that dissolved sulfide was also present in oxic sediment samples in similar concentrations (Fig. 4). This indicated that dissolved sulfide concentrations were not the only determinant of Mo removal, despite previous work showing that Mo removal to particles is particularly sensitive to dissolved sulfide (Helz et al., 1996; Erickson and Helz, 2000). The presence of dissolved sulfide alone did not determine Mo removal rates in these experiments, which showed that some Mo removal in the STE may
occur under anaerobic conditions with low dissolved sulfide. The high removal rate constants for Mo in sulfidic sediments may have been enhanced by sorption on particulate iron sulfides (Helz et al., 2004) formed in the sulfidic-zone sediments. This suggests that present porewater conditions as well as sediment composition influence Mo removal. These results can inform future estimations of the rate and extent of Mo removal in other STE environments.

The $k_2R$ for U was greatest in the ferruginous sediments (Fig. 7b), the same zone where U is removal is observed in situ (O'Connor, Ch. 3). Because OM and Fe contents in the ferruginous sediments were not measurably different from those of the oxic and nitrogenous zones (Fig. 3), the microbial consortium is likely responsible for the rapid rate of U removal in the Fe cycling zone. These results suggest that after the onset of anaerobic conditions, sediments with U-reducing microbes will remain the most efficient at U removal over the following weeks.

Biologically mediated reactions typically follow Michaelis-Menten enzyme kinetics (Dowd and Riggs, 1965). The relationship between U removal and U concentration was similar to that expected from Michaelis-Menten kinetics (Fig. 12). This was in contrast to when the same plots are made for Mo and V, in which there was no relationship (not shown).

Previous work has demonstrated that certain microbes (such as *G. metallireducens* strain GS-15, *S. putrefaciens, D. desulfuricans, D. vulgaris*) are able directly reduce and remove U from porewater (Lovley et al., 1991; Lovley and Phillips, 1992, Lovley et al., 1993; Fredrickson et al., 2000). *Desulfovibrio* species were found
only in ferruginous sediments, which may contribute to the greater U removal rate constants in this redox zone. Additionally, U removal in fine-grained sediments also often coincides with depths of Fe reduction (Crusius and Thomson, 2000; Klinkhammer and Palmer, 1991; Morford et al., 2005). However, none of the species demonstrated to carry out U reduction in pure culture were particularly abundant in samples from this study. Because experiments cannot determine the ability of the ‘uncultured majority’ of microbes to reduce U, other microbes may also be responsible for U reduction in the STE sediments studied here, as well as in other iron reducing zones.

Over the time scale for $k_{2R}$, V removal was only evident in nitrogenous and ferruginous sediment samples (Fig. 7b). Environmental reduction and removal of V can occur through abiotic or biologically catalyzed reactions. For example, abiotic chemical reduction of vanadate can be driven by humic substances (Wilson and Weber, 1979; Lu et al., 1998) or by dissolved sulfide (Wanty and Goldhaber, 1992). A number of microbial species are capable of respiring vanadate, including G. metallireducens (Ortiz-Bernad, 2004); autohydrogenotrophic bacteria, such as Rhodocyclus and Clostridium genus (Xu et al., 2015); P. isachenkovii (Antipov et al., 2000); S. oneidensis (Carpentier et al., 2003); and A. ferrooxidans and thiooxidans (Bredberg et al., 2004).

Because there was no production of dissolved sulfide (Fig. 4) and relatively little organic matter (Fig. 3) in the nitrogenous and ferruginous samples, it seems unlikely that these would drive abiotic V reduction. However, in situ porewater in these sediment zones contained high concentrations of humic material (O'Connor, Ch. 2). It is possible that residual humic material remained in these samples and was capable of reducing V to
a particle reactive species (Lu et al., 1998).

As with U, specific cultured species of bacteria known to reduce V were in low abundance. However, many of the known V reducers (S. Oneidensis, P. Isachenkovii, A. Ferrooxidans, and thiooxidans) are in the gammaproteobacteria, which were abundant in oxic, nitrogenous, and ferruginous sediments (Fig. 10a), and were active in oxic sediments (Fig. 10b). This suggests that there may be uncultured Gammaproteobacteria species in this class capable of reducing V in the STE. Although $k_{2R}$ removal of V was not observed in oxic sediments, this may have been due to mobilization processes eclipsing reductive removal or a lack of V reducing microbes.

The $k_{2R}$ indicated second-order mobilization of V in oxic and sulfidic sediments, rather than removal (Fig. 6). In oxic sediments, the increase occurred after an initial concentration decrease ($k_{1R}$ of -89 nmol day$^{-1}$) consistent with sorption to sediments (as previously discussed). Iron oxide reduction was evident in oxic sediment samples from dissolved Fe increases. Vanadium was likely to have been adsorbed to these metal oxides (Wehrli and Stumm, 1989), and released upon reductive dissolution of the metal oxides.

Both V and Cr in sulfidic samples showed second-order concentration increases in $k_{2R}$ (Fig. 6, 7b), which could have been due to two mechanisms. The first explanation is that V and Cr (either oxidized or reduced species) were associated with metal oxides in the sediment phase and released when these minerals were reductively dissolved. These sediments were from depth where porewater contained high concentrations of sulfide (O'Connor, Ch. 2), and the original sediments were dark in color, indicating sulfidic conditions unlikely to support the presence of metal oxide species. It is possible that iron
sulfide colloidal material was mobilized during sample agitation, as can occur when water is too rapidly pumped through permeable sediments during sample collection (Creasey and Flegal, 1999). Dissolved V and Cr were highly correlated with Fe (V: $R^2=0.98$, Cr: $R^2=0.93$) and, to a lesser degree, sulfide (V: $R^2=0.21$, Cr: $R^2=0.41$) in these samples, suggesting that V and Cr may have been associated with sedimentary Fe sulfides or other Fe minerals in the STE. Although the anaerobic mobilization shown in this experiment was likely an artefact of the experiment, it indicates that V and Cr may be associated with and sequestrated in sedimentary iron sulfides.

*Aerobic Treatments*

The primary RSM behavior observed in the aerobic treatments was mobilization from the sediments. Mobilization can occur by displacement from surface binding sites, or by oxidation of RSMs to a more soluble species. Typically, a larger $k_1M$ suggests rapid electrostatic desorption of RSMs from the sediments rather than oxidation, and a smaller $k_2M$ would indicate a different mobilization mechanism, such as release due to oxidation and dissolution of sediment-associated organic matter, sulfides, and RSMs.

An increase in dissolved Fe concentrations was observed in nitrogenous and sulfidic samples (Fig. 8) despite the fact that Fe is expected to exist as particulate iron oxide minerals under aerobic/oxidizing conditions and circumneutral pH (Takeno, 2005). Mobilization of Fe from sulfidic samples was likely due to oxidation of iron sulfide minerals. The reason for Fe mobilization in nitrogenous samples is unclear, but did not appear to affect RSM mobilization (compared to oxic and ferruginous samples).
Similar trends for $k_{1M}$ of Mo, U, and V (Fig. 9a) indicated a similar mechanism of mobilization, which, along with the rapid reaction rate, suggests desorption of electrostatically bound species. Results from this experiment demonstrated that sediments in the STE contained a pool of these elements that can be mobilized during short (i.e., hours to days) oxidizing/saline intrusion events.

In sulfidic samples, Mo, U, and V concentrations declined after the initial increase (Fig. 8). These results suggested that even if these RSMs are mobilized from the sediment surface in the short term, they could subsequently undergo reactions returning them to the solid phase. Dissolved sulfide, possibly supplied from the sediments as they equilibrate with more saline water, is capable of reacting with all of these elements and could cause such an effect (Helz et al., 1996; Hua et al., 2006; Wanty and Goldhaber, 1992).

Even though removal was observed over the course of the experiment, the $k_{2M}$ for U and V in all sediment samples (and for Mo in all but sulfidic sediment samples) showed continued second-order mobilization over longer (i.e., weeks) exposure to aerobic conditions (Fig. 8, 9b). This release may be due to either: release from distinct binding sites (i.e., which take longer to reach an equilibrium with surrounding conditions) from those responsible for $k_{1M}$ mobilization; or aerobic conditions driving oxidation of sediment-bound RSMs, changing their adsorption coefficient and facilitating release. In either case, these sediments may be expected to continue releasing RSMs to the porewater upon longer exposure to aerobic, saline conditions.

In oxic, nitrogenous, and ferruginous sediments, the rates of mobilization were highest for V, followed by Mo, then U (Fig. 9a, b). Upon changing redox conditions, this
release will therefore affect Mo, U, and V delivery to the coastal ocean. Although Mo and U are relatively abundant in coastal waters, a mobilization of V (identified as an emerging contaminant by the EPA; Federal Register, 2009) on the order of hundreds of nM warrants further investigation into potential impacts.

In contrast to the other RSMs, Cr was removed (negative $k_{1M}$) in all but sulfidic sediment samples. As was proposed for Mo in sulfidic samples, it is possible that oxidized chromate was stabilized by sorption to sediments, mobilized on exposure to high ionic strength, and then reduced once free in the ‘porewater’. Chromate is the most oxidizing of all the RSMs studied here, and could have been reduced and removed by any organic matter (Bartlett and James, 1983), ferrous Fe (Rai et al., 1989), and sulfide (Smillie et al., 1981) found in these sediments. This suggests that under an intrusion of oxic/saline water on the time scales here, Cr mobilized from non-sulfidic STE sediments may be sequestered again. The ultimate effect on Cr flux will depend on the rate of porewater flow through the STE, which could deliver Cr to the surface water before it is reduced and removed to the sediments.

The behavior of Cr in sulfidic samples differed the other RSMs, being mobilized at a single rate to concentrations an order of magnitude higher than observed at the field site (O'Connor, Ch. 3). This occurred despite the potential for subsequent removal of Cr after initial mobilization, and the removal of the other RSMs in sulfidic sediments after initial mobilization (Fig. 8).

The high concentrations of U, V, and Cr generated from sulfidic samples (Fig. 8) suggested that these sediments hold a large reserve of RSMs which may be mobilized
under increasingly saline and/or oxic hydrologic conditions. This is likely because, in addition to mobilization from RSMs from mineral surfaces, iron sulfide minerals may be oxidized and dissolved (supported by the increase in dissolved Fe in sulfidic sediment samples), releasing an additional load of incorporated RSMs. However, this increase can be only in the short term for highly sulfide reactive elements like Mo (Fig. 8). The ultimate impact on RSM fluxes from the STE will depend on both the geochemical reactions shown in this study, as well as hydrologic conditions controlling flow rates and the movement of porewater constituents through the redox zones.

Conclusions

This work provided initial constraints on the rates of RSM sequestration and mobilization under changing redox conditions. Results from this experiment show that under anaerobic conditions, RSMs will primarily be removed from the porewater over days to weeks. However, elements that sorb to Fe oxides, such as vanadium, can then be re-mobilized into the porewater on dissolution of these metal oxides. The rate constants of removal varied between sediment redox zones, indicating that STE response to changing redox conditions is not uniform. Under saline, aerobic conditions (which might occur during intrusion of oxidizing surface water), RSMs were mobilized from all sediment redox horizons. Sulfidic STE sediments contained a particularly large pool of RSMs, which were released under saline, oxidizing conditions.

The results from this work indicate that dramatic changes in porewater RSM concentrations can occur over time scales relevant to shallow STE water residence times.
In situ, the influence of changing redox conditions on RSM mobility will also depend on the spatial extent of the change (i.e., which existing redox zones are affected) and the residence time of water in each of these zones. For example, an anaerobic event may drive V removal to the sediments in the short term, but over longer time scales cause reductive dissolution of metal oxides and release of associated V. The rate constants determined in this work can help estimate the effect of changing redox conditions on porewater RSM concentrations throughout the STE.

Under conditions of prolonged anoxia or oxygen exposure, microbial populations can shift and sediment characteristics change. However, on shorter time scales, these results can help predict the response of RSMs in the subterranean estuary.
Table 1. Number of 16S rDNA and rRNA sequences filtered during different steps in bioinformatic analysis.

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<th>Nucleic Acid</th>
<th>Redox Zone</th>
<th>Trimmed</th>
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<th>Total</th>
<th>Bacteria</th>
<th>Percent</th>
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Table 2. Rate constants for production of Fe and sulfide during anaerobic incubation experiments. A rate constant of zero indicates no significant concentration change over the course of the incubation in those samples.
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<tr>
<th>Redox</th>
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<th>k2_R</th>
<th>U</th>
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<td>-1.7 ± 0.9 - 10⁻⁵ nmol⁻¹ day⁻¹</td>
<td>2⁰</td>
<td>-1.9 ± 0.5 - 10⁻³ nmol⁻¹ day⁻¹</td>
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<td>2⁰</td>
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<td>2⁰</td>
<td>-5 nmol day⁻¹</td>
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Table 3. Rate constants and reaction orders of RSM concentration changes during the anaerobic incubation experiments. A positive rate constant indicates an increase in concentration, and a negative rate constant indicates a decrease. The two rates constants (k1_R and k2_R) are used to describe reactions where there were two clear rate steps, typically a rapid change in concentration (k1_R) followed by a slower change lasting the rest of the experimental time frame(k2_R). A rate constant of zero (and corresponding reaction order of NA) indicates that there was no detectable change during that time interval (indicated in Fig. 1). A null value (NA) for both rate constant and reaction order indicates that there was no detectable concentration change over the entire time frame of the anaerobic incubation experiment.
Table 4. Rate constants and reaction orders of RSM concentration changes during the aerobic incubation experiments. A positive rate constant indicates an increase in concentration, and a negative rate constant indicates a decrease. The two rates constants (k1R and k2R) are used to describe reactions where there were two clear rate steps, typically a rapid change in concentration (k1R) followed by a slower change lasting the rest of the experimental time frame (k2R). A rate constant of zero (and corresponding reaction order of NA) indicates that there was no detectable change during that time interval (indicated in Fig. 1). A null value (NA) for both rate constant and reaction order indicates that there was no detectable concentration change over the entire time frame of the aerobic incubation experiment.
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<th>Nitrogenous</th>
<th>Ferruginous</th>
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Table 5. Relative percent of present and active major bacterial classes and SRB families.
Figures

Figure 1. (a) Location of the study site along the York River in Gloucester Point, VA, USA. (b) A schematic of the salinity distributions and coring location in the STE, as previously described in detail (O'Connor, Ch. 2).
Figure 2. A schematic illustrating the experimental set-up. A sediment core from the center of the STE was collected and partitioned according to previously observed redox conditions (O’Connor et al., 2015; O’Connor, Ch. 2): oxic sediments from the sediment surface to 15 cm; nitrogenous sediments from 20 to 30 cm; ferruginous from 30 to 40 cm; and sulfidic from 65 cm to 90 cm. For comparison with sediment depths relative to the high tide sediment surface (used in previous work O’Connor, Ch. 2, Ch. 3), add 50 to these values. Some of each homogenized sediment group was used in the anaerobic (removal) and aerobic (mobilization) incubations experiments.
Figure 3. The weight percent organic matter and reducible Fe in each sediment zone used in the experiments.
Figure 4. Concentrations of Fe and sulfide in each sediment sample over the course of the anaerobic incubation experiment. Error bars indicate the standard deviation of experimental replicates (n=2). Lines for least squares linear regression are only shown for those samples which had a statistically significant (p<0.05) slope.
Figure 5. Rate constants for Fe and sulfide in each sediment sample in the anaerobic incubation experiment. Error bars indicate the error in the regression used to calculate k.
Figure 6. Concentrations of RSMs in each sediment sample over the course of the anaerobic incubation experiment. Error bars indicate the standard deviation of experimental replicates (n=2). Lines on the figures are not regressions, but are intended to indicate $k_{1R}$ and $k_{2R}$ (as indicated in the legend), and where slopes for an integrated rate law were statistically significant (p<0.05).
Figure 7. Rate constants determined for the change in RSM concentrations in each sediment type of the anaerobic incubation. Negative values indicate removal, and positive values indicate addition.
Figure 8. Concentrations of RSMs in each sediment sample over the course of the aerobic incubation experiment. Error bars indicate the standard deviation of experimental replicates (n=2). Lines on the figures are not regressions, but are intended to indicate $k_{1R}$ and $k_{2R}$ (as indicated in the legend), and where slopes for an integrated rate law were statistically significant ($p<0.05$).
Figure 9. Rate constants determined for the change in RSM concentrations in each sediment type of the aerobic incubation. Error bars indicate the error in the slope used to determine k.
Figure 10. (a) Ordination plot of the sediment microbial populations (as determined by DNA) in the redox horizons. Relative (b) abundances and (c) activities of bacterial classes based on measurements of 16S rDNA and rRNA.
Figure 11. Relative (a) abundances and (b) activities of selected SRB genera in the studied redox zones based on measurements of 16S rDNA and rRNA.
Figure 12. Rate of U removal between each time point (from the anaerobic incubation experiment) plotted against the U concentration at that time point (as per the Michaelis-Menten equation). In the nitrogenous and ferruginous sediment samples, the general trend was generally consistent with Michaelis-Menten kinetics.
References


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anaerobic microbial iron oxidation and reduction. 4, 752–764.


CONCLUSIONS

The composition of groundwater discharging into the coastal ocean is determined by reactions that take place within the STE, and predicting SGD-driven chemical fluxes requires understanding how STE biogeochemistry affects chemical concentrations. This body of work represents a novel, mechanistic view of STE redox chemistry and RSM biogeochemistry.

Initial work at the study location found that the redox framework of this STE followed a 'classic' redox sequence, with sequential zones of high oxygen, nitrate, dissolved Fe, and sulfide occurring with increasing depth below the sediment surface. Both Mo and U were removed from the porewater as saline, oxic surface waters mixed into the ferruginous and sulfidic zones. In contrast, V and Cr showed non-conservative addition across the salinity gradient. High V and Cr concentrations occurred in reducing zones of the STE despite thermodynamic predictions that they would exist as reduced, particle-reactive species, suggesting that a soluble ligand was maintaining the solubility of these elements.

Geochemical heterogeneity and non-linear flow patterns led to three-endmember mixing behavior in the STE. To address this, we used a three-endmember mixing approach to characterize distribution of chemical constituents in the STE. The three endmembers were the surface water and two freshwater endmember (shallow and deep), representing spatial boundaries of the STE. The three-endmember mixing calculations used humic carbon as a semi-conservative tracer. Three-endmember mixing presents a
new and, where applicable, more accurate framework for determining non-conservative mixing of dissolved constituents in the subterranean estuary, and future efforts to develop an easily-measured conservative tracer (perhaps a ratio of stable isotopes influenced by exposure aquifer sediments, such as $^{87}$Sr/$^{86}$Sr) would greatly enhance our understanding of shallow STE geochemical processes.

Although the general redox structure and RSM distributions in the STE remained consistent over time, concentrations and mixing behavior varied over this two-year time series. I initially hypothesized that warmer temperatures in summer would increase microbial metabolic rates, leading to higher concentrations of reduced metabolites. However, concentrations of DOC, humic carbon, and sulfide were higher in the summer, whereas Fe and Mn concentrations were higher in the winter. One conclusion from this study is that the activity of SRB and metal reducing bacteria respond differently to seasonally variable factors (such as temperature or substrate availability).

Concentrations of some, but not all, RSMs also showed seasonal variability in the STE, highlighting the importance of considering the individual biogeochemical behavior of these similar elements in predicting their environmental responses. Non-conservative removal of Mo was correlated with sulfide concentrations, but unlike sulfide concentrations, did not show seasonal differences. This was likely due to sulfide concentrations (up to 1.5 mM) consistently in excess of the 11 µM threshold required to quantitatively react with and remove dissolved Mo. However, U showed greater removal in the summer, possibly driven by greater activity of U-reducing microbes. Dissolved V concentrations co-varied with DOC (with both being greater in summer), indicating that...
V is likely complexed with dissolved organic matter. In contrast, Cr was correlated with both humic carbon and dissolved Fe in different parts of the STE, and showed interannual rather than seasonal variability.

We further explored complexed dissolved RSMs by measuring partitioning among small (3 kD to 30 kD) and large (30 kD - 0.45 µm) colloidal size fractions. Over half of the total DOC and dissolved Fe occurred in the colloidal phase, demonstrating the importance of colloidal transport in the STE. The relative proportion of RSMs in the colloidal phase increased in the order Mo<U<V<Cr, with up to 75% of Cr existing in the colloidal size fractions. Association of RSMs with large colloids was greater at lower salinities/higher surface charges (as determined by electrophoretic mobility). In the small colloidal size fraction, percent Mo was proportional to Eh, in contrast to expectations that reduced Mo would be the more particle reactive species; percent U and Cr were directly correlated to colloidal concentrations of Fe and Mn, suggesting association with reactive metal (hydr)oxides; and percent V was correlated to colloidal DOC, indicating complexation with organic matter. These results demonstrated that association with colloids plays a role in RSM transport in the STE. This study also showed that RSMs associate with different colloidal components, revealing some of the complexities associated with predicting the distribution and fate of RSMs in the STE.

This work, as well as previous studies, have shown non-conservative behavior of RSMs in the STE, and that this behavior depends on redox conditions; however, rates of addition or removal had not been investigated previously. Water advection in permeable sediments is several order of magnitude more rapid than in diffusion-dominated
environments, and kinetic constraints on biogeochemical reactions may limit the extent of geochemical reactions counter to equilibrium predictions. Our incubation experiments showed that RSM concentration changes on the order of hundreds of nM can take place in hours to weeks. As shallow STE residence times range from days to weeks, these results suggest that reaction rates likely affect porewater composition beyond what might be expected from equilibrium conditions. Additionally, reaction rates differed between sediment redox zones, indicating that the response of the STE to changing redox conditions will be heterogeneous.

Results from this work highlight the spatial and temporal variability of STE biogeochemical reactions, and offer geochemical explanations of RSM distributions and behavior. This approach also allows for more effective predictions of RSM behavior in other study locations with different redox conditions. For example, molybdenum has previously shown different behavior across STEs in different locations, which may now be understood in terms of sulfide production in these environments. Rising sea level is expected to drive greater intrusion of saline water into permeable coastal sediments, causing a landward shift of the salinity interface (Gonneea et al., 2013). The results from this work suggest that as more coastal surface water circulates through permeable sediments, the processes controlling RSM concentrations will change. If STE advection rates are slow enough to allow consumption of dissolved oxygen, rising sea level may contribute to increased removal of Mo and U as this water recirculates in the sediments, oxygen is drawn down, and reducing conditions occur. However, if increased surface water intrusion results in rapid advection and introduction of oxic water into previously
reduced sediments, resulting SGD will likely deliver a greater load of V and Cr, mobilized from the sediments, to the coastal ocean. These effects may be regional, as advection rates through the STE depend on both sediment permeability and coastal topography (Robinson et al., 2007; Seidel et al., 2015).

Concentrations of organic matter, reduced metabolites, and RSMs in the STE vary throughout the year, and this work demonstrates that data derived from a single time point may be insufficient to accurately determine SGD-driven chemical fluxes. The response of the STE as a whole to changing environmental conditions, such as seasonal temperature changes or oxygen fluctuations, depends on the in situ redox environments and sediment characteristics. Findings from this work demonstrate both how current geochemical conditions influence RSM behavior, and provide a basis for evaluating how changing conditions may alter RSM fluxes.
References


VITA
Alison E. O'Connor