Title: Soluble Mn(III)-L complexes are abundant in oxygenated waters and stabilized by humic ligands

Authors: Véronique E. Oldham^{1*}, Alfonso Mucci², Bradley M. Tebo³, George W. Luther III¹

Affiliations:

¹School of Marine Science and Policy, University of Delaware, Lewes, DE, 19958

²GEOTOP and Department of Earth and Planetary Sciences, McGill University, Montreal, QC, H3A 0E8

³Division of Environmental and Biomolecular Systems, Institute of Environmental Health, Oregon Health & Science University, Portland, OR 97239

*Correspondence to:

Véronique E. Oldham

School of Marine Science and Policy

University of Delaware

700 Pilottown Road

Lewes, DE 19958, USA

Phone (302) 645-4208

voldham@udel.edu

Abstract:

Dissolved Mn (dMn_T) is thought to be dominated by metastable Mn(II) in the presence of oxygen, as the stable form is insoluble Mn(IV). We show, for the first time, that Mn(III) is also stable as a soluble species in the oxygenated water column, when stabilized by organic ligands as Mn(III)-L complexes. We measured Mn(III)-L complexes in the oxygenated waters of a coastal fjord and a hemipelagic system where they make up to 86 % of the dMn_T . Although Mn(III) forms similar complexes to Fe(III), unlike most of the analogous Fe(III)-L complexes, the Mn(III)-L complexes are not colloidal, as they pass through both 0.20 μm and 0.02 μm filters. Depending on the kinetic stability of the Mn(III) complexes and the microbial community of a given system, these Mn(III)-L complexes are capable of donating or accepting electrons and may therefore serve as both reductants or oxidants, can be biologically available, and can thus participate in a multitude of redox reactions and biogeochemical processes. Furthermore, sample acidification experiments revealed that Mn(III) binding to humic ligands is responsible for up to 100 % of this complexation, which can influence the formation of other metal complexes including Fe(III) and thus impact nutrient availability and uptake. Hence, humic ligands may play a greater role in dissolved Mn transport from coastal areas to the ocean than previously thought.

1.0 Introduction:

Manganese (Mn) is ubiquitous in the global ocean, where it is an essential trace nutrient in the electron transfer processes of several metallo-enzymes - notably in photosystem II for photosynthetic organisms. In its solid oxidized form, it serves as an electron acceptor in the bacterial decomposition of sedimentary organic matter and acts as an important scavenger for

many trace elements and radionuclides. The chemical speciation of Mn in marine systems governs its diverse functions and is ultimately controlled by the redox conditions of the environment and the microbial community. The favorable oxidation state of Mn in oxic waters is Mn(IV), and thus Mn in oxygenated waters is primarily bound to oxygen as solid Mn(III/IV)-oxides (MnO_x). However, soluble Mn(II) is metastable in oxygenated waters because the oxidation of $\text{Mn}(\text{H}_2\text{O})_6^{2+}$ to MnO_x by O_2 in an one electron transfer is thermodynamically unfavorable and the two electron transfer is kinetically slow (Luther 2010), unless facilitated by microbes, surface catalysis or superoxide-promotion (Stumm and Morgan, 1996).

In the last decade, soluble manganese (dMn_T) speciation has been re-evaluated to include soluble Mn(III) bound to ligands (Mn(III)-L complexes) in low oxygen environments. Mn(III)-L complexes have been measured in the suboxic porewaters of the St. Lawrence Estuary (Madison et al., 2013), the suboxic waters of the Black Sea (Yakushev et al., 2007, 2009; Trouwborst et al., 2006) and the Baltic Sea (Dellwig et al., 2012) as well as the anoxic bottom waters of the Chesapeake Bay (Trouwborst et al., 2006; Oldham et al., 2015). Because Mn(III)-L complexes can donate or accept electrons, they can potentially have a profound impact on the redox chemistry of a given environment. In addition to their reactivity, Mn(III)-L complexes can affect the uptake of other metals due to metal-ligand competition reactions (Luther et al., 2015). For example, the siderophore pyoverdine from the Mn(II)-oxidizing *Pseudomonas putida* GB-1 has a higher affinity for Mn(III) than for Fe(III) (Parker et al., 2004). Likewise, kinetic experimentation, using known laboratory ligands (desferrioxamine-B, pyrophosphate, to name two) and suboxic porewater samples from the St. Lawrence Estuary also indicate that Mn(III) may bind more strongly than Fe(III) to the same ligands (Luther et al., 2015). These findings are

important given that many microorganisms use Fe(III)-binding ligands for Fe uptake. However, the impact of this ligand competition is poorly described because the presence of Mn(III)-L has not yet been documented in oxygenated marine environments as dMn_T concentrations in these environments are typically lower than the detection limits of current speciation methods (50 nM, Madison et al., 2011). Consequently, thus far, dMn_T speciation in oxygenated systems has been thought to be dominated by Mn(II) (e.g. Landing and Bruland, 1986).

Using a modification of the UV-Vis method of Madison et al. (2011), we show for the first time that, like Fe(III)-L complexes which account for up to 99.9% of dFe_T (e.g. Gledhill and van den Berg, 1994) in oxygenated marine waters, Mn(III)-L complexes are present in the oxygenated waters of both a coastal fjord and in the hemipelagic waters of the St. Lawrence Estuary, the largest enclosed estuary in the world. These Mn(III)-L complexes account for most of the dMn_T , but, unlike most Fe(III)-L complexes (Schlosser et al., 2013), are not colloidal (operational size class between 20 to 200 nm). Finally, our findings indicate that binding to humic ligands is responsible for up to 100 % of the Mn(III)-L in these waters.

2.0 Materials and Methods:

2.1 Water column sample processing

Water column samples were collected in September 2014 from two sites in the St. Lawrence Estuary (Figure 1; Station 23 [~ 350 m depth]: $48^{\circ}42.29'N$, $68^{\circ}38.83'W$; and Station SAG-30 [~ 260 m depth]: $48^{\circ}21.72'N$, $70^{\circ}23.71'W$) into 12 x 12 L Niskin PVC bottles mounted on the CTD-rosette. Contamination was avoided by sampling through a trace metal clean tube into clean 50 mL polypropylene centrifuge tubes (Falcon), which were overflowed three times before

filling. Water samples were immediately filtered through 0.20 μm syringe filters (Nylon, Whatman), in a fume hood aboard the R/V Coriolis II, into new 50 mL centrifuge tubes, and were kept in the dark until analysis. Aliquots were frozen at -20 $^{\circ}\text{C}$ in 50 mL centrifuge tubes for future analyses.

To obtain true bottom-water samples, undisturbed sediment multi-cores were recovered at Station 23 using a Bowers & Connelly Multicorer and 10-cm diameter clear plastic barrels. The overlying water at the sediment-water interface was withdrawn from the barrels with a trace metal clean polypropylene syringe, filtered and handled as above. The water analyzed is termed overlying sediment-core water and its composition, relative to waters above it, provides insights on the direction and magnitude of the flux of chemical species across the sediment-water interface.

2.2 Dissolved Mn speciation

All samples were analyzed for soluble Mn speciation within two hours of collection following the addition of a soluble porphyrin ligand [α , β , γ , δ -tetrakis(4-carboxyphenyl)porphine (T(4-CP)P)] , according to the method described by Madison et al. (2011). The method allows for the subsequent spectrophotometric determination of both Mn(II) and Mn(III)-L_{weak} (Mn(III)-L_{weak} can be outcompeted by the added porphyrin ligand, and has $\log K_{\text{cond}} < 13.2$; Oldham et al., 2015; Luther et al., 2015) using the differential kinetics of the reaction between Mn and the added porphyrin which is initially complexed to Cd(II). The Mn(II) displaces the Cd(II) and reacts rapidly with the porphyrin, forming a Mn(III)-porphyrin complex as Mn(II) oxidizes in the presence of oxygen, whereas the Mn(III)-L_{weak} undergoes a ligand substitution reaction with the

porphyrin to form the Mn(III)-porphyrin complex ($\log K_{\text{cond}} < 13.2$, Oldham et al., 2015; Luther et al., 2015). The complex yields a sharp Soret band at 468 nm ($\epsilon = 94,600 \text{ mol}^{-1} \text{ cm}^{-1}$), which can be measured by UV-Vis spectrophotometry. To measure Mn(III)-L_{strong} (cannot be outcompeted by the added porphyrin ligand, $\log K_{\text{cond}} > 13.2$; Oldham et al., 2015), a reducing agent (hydroxylamine, 100-fold excess of expected dMn_T, does not dissociate the Mn-porphyrin complex at this concentration due to abundant O₂) is added to the filtered sample, converting all Mn(III)-L to Mn(II), and the sample is reanalyzed for dMn_T. The difference between the reduced (dMn_T) and non-reduced (dMn(II) + dMn(III)-L_{weak}) sample gives the concentration of Mn(III)-L_{strong}. Laboratory-synthesized colloidal MnO₂ does not react with the porphyrin (Madison et al., 2011). The detection limit for this method is 50 nM, which is sufficiently low for porewater analysis, but not low enough for water column samples.

We modified the method of Madison et al. (2011) for water column samples to achieve a detection limit of 0.3 nM (3 times the standard deviation of a blank) by using a 100-cm liquid waveguide capillary cell and the addition of a heating step as well as a strong reducing agent for Mn speciation. All chemicals were A.C.S. reagent grade, and all solutions were prepared with deionized water (18.2 mΩ). As the absorbance is proportional to the cell pathlength, for low levels of Mn, the reagents described by Madison et al. (2011) were diluted 100-fold to fit into the analytical detection window. The [T(4-CP)P] stock solution was $2.00 \times 10^{-4} \text{ M}$ (final sample concentration of $2.33 \times 10^{-7} \text{ M}$), the borate buffer was made up to pH 8.0-8.2 using trace metal clean NaOH (final sample buffer concentration $4.16 \times 10^{-4} \text{ M}$), and the CdCl₂ stock solution was prepared to $1.20 \times 10^{-4} \text{ M}$ (final sample concentration $2.40 \times 10^{-7} \text{ M}$). All samples were analyzed

in triplicate following 12 to 30-fold dilution into the reagents to yield a total Mn concentration in the range of 10-100 nM.

The rate of reaction is proportional to the concentration of reactants, and at the low levels of the modified method, the reaction is slow. Hence, a heating step, prior to analysis, is employed to accelerate the reaction kinetics. Because the Cd(II) in the porphyrin reacts with chloride in seawater, seawater samples are diluted to ensure reaction of the porphyrin with Mn(II); thus, the practical detection limit of the method is 3.0 nM. Recovery tests with MnCl₂ standards were performed, and the most successful recovery in a reasonable time period was achieved by heating at 90 °C for 1 hour (98-106 % recovery, Table 1). A NASS-6 seawater standard was tested for recovery using this heating step, and a triplicate measurement was within 1 standard deviation of the known value of the standard [dMn_T] (Table 1). For Mn speciation, strong Mn(III)-L complexes are reduced by an excess of a strong reducing agent, hydroxylamine (at least 100-fold expected dMn_T concentration). Samples are analyzed first without hydroxylamine for dissolved Mn(II) and then with the addition of hydroxylamine for dMn_T (Mn(II) + Mn(III)-L). This method does not allow for the independent kinetic assessment of weak Mn(III)-L complexes and can only determine the concentration of Mn(II) and strong Mn(III)-L complexes. Hence, the Mn(II) fraction reported in Table 2 likely includes weak Mn(III)-L complexes that react during the heating step.

The liquid waveguide capillary cell (World Precision Instruments) was coupled to a UV-Vis spectrophotometer (Ocean Optics). In this spectrophotometric set up, a mini deuterium halogen light source (DT-Mini-2-GS) was coupled to a USB2000+ fiber optic spectrometer controlled

with SpectraSuite software. The Mn(III)-T(4-CP)P complex was measured at its absorbance maximum (468 nm) against a reagent and sample blank. For small peaks, peak height was determined using an algorithm adapted to resolve small peaks with the application of linear fit tangent baseline subtraction (ECDSOFT, which can be found at: <https://mojoblak.irb.hr/index.php/s/dxB24S8m5H6fsZW?path=%2FProMCC>).

2.3 MnO_x measurements

Known volumes of sample (265 mL) were filtered through 47 mm diameter 0.20 μ m Whatman Nucleopore track-etched polycarbonate filters. Using the method of Altmann (1972), filters were amended with 2 mL of 0.0032 % leucoberbelin blue dye (LBB, 65 % diluted, Sigma-Aldrich). The dye color, formed on oxidation by particulate manganese or MnO_x , was measured in a 100-cm liquid waveguide capillary cell at 620 nm using the Ocean Optics spectrophotometric set-up described above. The LBB (410.5 g mol^{-1}) stock solution is prepared by dissolution in Milli-Q water to 4 % w/w and the addition of 40 μ L of 10 M sodium hydroxide (NaOH) per 10 mL of solution. Working solutions are made by diluting the stock into 1% acetic acid (made up in Milli-Q water), to 0.04 % w/w. A calibration curve was generated using potassium permanganate ($KMnO_4$). We expect that to produce an equivalent absorbance, 2.5 times more Mn(IV) is required than Mn(VII), since Mn(VII) oxidizes 5 LBB molecules, compared to 2 LBB molecules for Mn(IV). In a 100-cm pathlength cell, the limit of detection is 0.05 nM.

3.0 Results:

At the hemipelagic site, Station 23 (Figure 2A), the water column is characterized by the presence of three water masses: (1) a 25 – 50 m thick surface layer of low salinity (27 – 32) that flows seaward, (2) an intermediate layer of cold water (-1° to 2°C with intermediate salinity; 32)

that extends to about 150 m depth and flows landward from the Gulf, and (3) an underlying deep layer with warmer (4° to 6°C) and saltier (34 – 34.6) water (Figure 2B) that flows landward and originates in the northwestern Atlantic Ocean. Concentrations of dissolved O_2 (dO_2) decreased from the surface ($339\ \mu\text{M}$) to the bottom ($55\ \mu\text{M}$) (Figure 2B) whereas dMn_T increased almost monotonically with depth, reaching the highest concentrations in the deepest sample recovered by the CTD-rosette ($289\ \text{nM}$ ~5-10 m above bottom) and in the overlying sediment-core water ($2.93\ \mu\text{M}$) (Figure 2A). The dMn_T speciation (Figure 2A) is dominated by Mn(III)-L between 150-300 m (52 – 86 % of dMn_T) whereas the % Mn(III)-L was lower in surface (17.6 %) and bottom waters (2.0 %). In the two deepest samples, dMn_T was dominated by Mn(II), indicating that dMn_T escapes from the sediment to the overlying waters as Mn(II).

The water column in the Saguenay Fjord (SAG-30) is fully oxygenated ($228\text{-}302\ \mu\text{M dO}_2$). The practical salinity (S_P) of the 3 to 10-m surface-water wedge was 14.6 – 24.8 at SAG-30 at the time of sampling, whereas more saline waters ($S_\text{P} = 28.2 – 30.8$) originating from the St. Lawrence Estuary are found below. Evidence of Mn(II) oxidation to Mn(III)-L (see eqs. 1 – 4 below) and to MnO_x in the water column is also observed here (Figure 2C). Like Station 23, the highest dMn_T concentrations were measured in the bottom waters. Mn(III)-L accounted for 9.5 – 70 % of dMn_T in the water column (Table 2), but the speciation profile was shaped differently than at Station 23. At SAG-30, the highest fractions of Mn(III)-L (65-70 %) are observed in the bottom and surface waters. Mn(II) accounted for 100% of the dMn_T speciation in the overlying sediment-core water, its concentration ($2.28\ \mu\text{M}$) being higher than in the bottom waters at Station 23.

Previous studies may have neglected a fraction of the dMn_T in natural waters since nearly all dissolved metal studies employ an acidification step for sample storage which would precipitate humic substances. To test this hypothesis, three frozen, filtered samples were thawed and amended with concentrated HCl (Optima) to $pH < 1.5$ to precipitate Mn(III)-humic complexes. After 1 hour, the acidified sample was centrifuged, to remove the humic precipitate, and the Mn speciation of the centrifuged supernatant was subsequently determined (Table 3). The % Mn(III)-humic is calculated as the difference between the original shipboard dMn_T and shore-based analysis after freezing, thawing and acidification (removal of precipitated humic material). The % Mn(III)-humic agreed with the original shipboard % Mn(III)-L, indicating that the acidification step removed all of the Mn(III)-L and that the Mn(III)-L was in the form of humic complexes. In the bottom waters of the Saguenay Fjord, most of the Mn(III)-L complexes were also lost upon acidification, with ~75 % of the Mn(III)-L (53.4 % of dMn_T) lost in the 250-m sample (Table 3).

We additionally performed analysis on frozen samples and compared them to original shipboard measurements, to show that freezing filtered samples is suitable for Mn speciation preservation. Three frozen, filtered water column samples from Station 23 and two samples from SAG-30 were thawed in October 2015, filtered through 0.02 μm filters (Anatop), and the dMn speciation analyses repeated. The 0.02 μm filtered samples had the same concentrations and speciation at both sites as the original 0.20 μm filtered samples analyzed shipboard (Table 4), even at basal depths where particulate MnO_x concentrations were high. Thus, we conclude that the measured Mn(III) is not colloidal and that freezing filtered samples is suitable for storage of soluble Mn samples destined for total and speciation analyses.

4.0 Discussion:

Mn(III)-L complexes were detected at both Station 23 in the Lower St. Lawrence Estuary (LSLE) and in the Saguenay Fjord, indicating for the first time that Mn(III)-L complexes are stable in oxic waters (Figure 2, Table 2). The concentration of dO₂, the bacterial community and the abundance as well as the nature of Mn(III)-binding ligands at each site are responsible for the formation and stabilization of Mn(III)-L, via the following potential oxidative (eqs. 1-4) and reductive (eqs. 5-6) pathways (unbalanced):

The high dMn_T in bottom waters was predominantly in the form of Mn(II) at both sites, indicating that dMn escapes mostly in the form of Mn(II) from the sediments to the overlying water. Madison et al., (2013) showed that the sediment porewaters in the St. Lawrence Estuary are rich in Mn(III)-L complexes, which are concentrated at the oxic-suboxic interface, and that oxidation of Mn(II) by O₂ accounts for up to 100 % of the Mn(III) inventory. We propose that Mn(II) diffusing up from the sediment anoxic zone is responsible for the Mn(III)-L production in the suboxic porewaters, and that these complexes may be recycled to Mn(II) upon burial or

oxidized to MnO_x in the oxic zone. Upward diffusion of porewater Mn(II) and/or advection of
 Mn(II) -bearing particles by bioturbation (Richard et al., 2013) likely supply Mn(II) to the
 overlying water column from the sediments. Associated with the flux of Mn(II) to the bottom
 waters at Station 23, we also observed a maximum $[\text{MnO}_x]$ (1070 nM) in bottom waters (340 m,
 Figure 2A), indicating the oxidation of Mn(II) to MnO_x , is the dominant prominent sink of the
 soluble Mn. The large fraction of dMn_T (66.3 %) in the form of Mn(III)-L at 300 m may result
 from the oxidation of Mn(II) escaping from the sediment (eqs. 1 – 4) or the reductive dissolution
 of sinking or advected authigenic Mn oxides (eqs. 5 and 6), but likely represents a residual
 species from the dominant oxidation of Mn(II) to MnO_x in bottom waters. The abiotic oxidation
 of Mn(II) by O_2 is slow, but can be accelerated by adsorption onto mineral surfaces (eq.1; Davies
 and Morgan, 1989), through bacterial mediation (eq.2; Tebo et al., 1997), and/or via *in situ*
 ligand-promoted oxidation by an oxidizing ligand such as photo-altered dissolved organic matter
 or a ligand in the presence of oxygen, such has been shown for the formation of Mn(III) with
 desferrioxamine-B (eq.3; Duckworth and Sposito, 2005; Parker et al., 2007). In the St. Lawrence
 Estuary, particle concentrations are greater in surface waters ($2.5 - 2.7 \text{ mg L}^{-1}$) than in bottom
 waters ($1.2 - 1.5 \text{ mg L}^{-1}$; Bourgoïn and Tremblay, 2010), and we predict that surface-catalyzed
 oxidation (eq.1) is not the dominant oxidizing mechanism at this site, particularly as our highest
 MnO_x concentrations were measured in bottom water samples. Bacterial oxidation rates of
 Mn(II) are ~5 times faster than surface-catalyzed oxidation rates in most seawater systems (Tebo
 et al., 2004), thus bacterial oxidation may dominate MnO_x formation at this site. As there is no
 light penetration at the depth of our deep samples, these measurements could also be a
 manifestation of dark production of reactive oxygen species, such as O_2^- , and their reaction with
 Mn(II) (eq.4; Hansard et al., 2011; Learman et al., 2011).

At station SAG-30, the abundance of Mn(II), coupled with the appearance of Mn(III)-L within 5 m above the sediment-water interface (our deepest Niskin bottle sample) is also interpreted as evidence of the oxidation of Mn(II) fluxing out of the sediment. Furthermore, the much lower MnO_x concentrations (5.9 – 21.6 nM) in the water column at SAG-30 than at Station 23 (Figure 2) may reflect the greater abundance of terrestrially-derived ligands, inhibiting the complete oxidation of Mn(II) to MnO_x or reductively dissolving MnO_x to produce Mn(III)-L complexes (eqs. 5 and 6) as shown in Oldham et al. (2015). It is also possible that the lower [MnO_x] in the Saguenay Fjord could be attributed to the greater particulate load of the Saguenay Fjord relative to Station 23 (Bourgoin and Tremblay, 2010) rendering MnO_x removal by scavenging more likely.

Given its dimensions and tributary inputs, the dissolved and particulate load in the Saguenay Fjord carries a greater terrestrial signature than the Lower St. Lawrence Estuary (LSLE). As in most estuaries, the humic substances originate from terrestrial runoff and humification of marine organic matter in the water column and sediments (Beck et al., 1974). Humic substances make up to 62 - 100% of the sedimentary organic matter at our study site in the Saguenay Fjord (Tremblay and Gagné, 2007), and these are mostly terrigenous. Shiller et al., (2006) indicated that the presence of photofresh, riverine allochthonous dissolved organic matter promotes stronger metal complexation than photodegraded material found downstream, consistent with our surface water speciation data from SAG-30 relative to surface water at Station 23. Tremblay and Gagné (2007) report dissolved organic matter concentrations ([DOM]) in the water column at each of our study sites, and find that DOM concentrations are nearly three times larger in the surface waters of the Saguenay Fjord (~13.0 mg L⁻¹) than the surface waters

of Station 23 ($\sim 2.6 \text{ mg L}^{-1}$). This is consistent with our observation of high % Mn(III)-L complexation in the surface waters of the Saguenay Fjord compared to Station 23. Accordingly, we propose that Mn(III)-binding terrigenous ligands enter the Saguenay Fjord from surface runoff and nearby wetlands, and ultimately accumulate in the sediments.

In Figure 3, we compare our dMn_T speciation results from 2014 at Station 23 to the extractable- dMn_T results (measured by sample acidification and subsequent ammonium pyrrolidine dithiocarbamate (APDC) extraction) on samples collected in May of 1974 by Yeats et al (1979) at their Station 63, within 0.5 km from our Station 23. The salinities of our 2014 samples are within ± 1.0 of the salinities reported by Yeats et al (1979) for similar depth intervals, and thus we feel the water masses are comparable. The 2014 profile for Mn(II) follows the shape of the extractable- dMn_T obtained in 1974, with higher dMn_T measured at all depths in 2014. The accumulation of dMn_T in the water column since 1974 may have resulted from decreased dO_2 (Gilbert et al., 2005), but the 2014 dMn_T is significantly higher at all depths (irrespective of dO_2), except in the bottom waters where 2014 dMn_T is almost entirely Mn(II). We attribute the difference to the presence of Mn(III)-L complexes, and propose that the extractable fraction of dMn_T analyzed in 1974 (Yeats et al., 1979) is missing most of the Mn(III)-L. We postulate that the acidification step ($\text{pH} < 2$), employed in the APDC extraction applied by Yeats et al (1979), resulted in the precipitation of humic Mn(III)-L complexes. Furthermore, Yeats et al (1979) explicitly state that the APDC-extractable Mn is defined by the availability to the chelating agent and stability at storage pH. The loss of Mn(III)-L upon acidification is supported by our finding that our $\text{dMn}_{T(\text{acidified})}$ corresponds to Mn(II) in the 2014 samples as well as the shape of the 1974 acidified extractable Mn profile (Yeats et al., 1979).

Several research groups have shown that Fe(III)-humic complexation is also important in coastal environments (Laglera and van den Berg, 2009; Abualhaija et al., 2015; Krachler et al., 2015). Mn(III) binds to the same ligands as dissolved Fe(III) and their thermodynamic stability constants are greater or similar to Fe(III) complexes (Kostka et al., 1995; Klewicki and Morgan, 1998; Parker et al., 2004; Duckworth and Sposito, 2007; Luther et al., 2015). Because many microorganisms use complexed Fe(III) to facilitate Fe uptake (Goyne and Carpenter, 1974; Trick, 1989), the presence of excess Mn(III) could affect Fe(III) bioavailability by competing for the same ligands, especially where Fe(III) may be a limiting nutrient for some microorganisms. Furthermore, because dMn_T is generally not limiting in surface waters compared to dFe_T (Morel and Price 2003), we anticipate that humic binding and competition for these ligands has been underestimated.

Aliquots of 5 samples (Table 4) were filtered shipboard, frozen and stored for future analyses. Figure 4 shows the linear correlation between samples analyzed shipboard (x-axis) and samples analyzed after preservation (y-axis, filtered, frozen and thawed 12 months later). Thawed samples (closed circles) were compared to thawed and further filtered samples (0.02 μm , open squares). Given the near 1:1 slope in relation to shipboard measurements, the excellent R^2 values, and the overlap between thawed 0.20 μm -filtered and thawed 0.02 μm -filtered samples, we conclude that freezing filtered samples is suitable for sample preservation. Moreover, the Mn(III)-L complexes we measured at this site are not operationally colloidal (size class between 20 to 200 nm), unlike Fe(III)-L species for which up to 90 % are found in the colloidal fraction (Schlosser et al., 2013). One possible interpretation of these results is that Mn(III)-L species

are more mobile and transported farther than their Fe(III)-L counterparts. Mn redox reactivity in a given system will vary pending the stability of the complex, which depends on many factors including ligand concentration, photochemical effects and microbial mediation. Mn(III)-L complexes formed at sites like the St. Lawrence Estuary are likely kinetically (meta)stable, and their long range transport may be sustained by high fluxes of Mn from the coast. Because Mn(III) can donate or accept electrons, upon transport from coastal to oceanic environments, these Mn(III)-L complexes may eventually act as important redox active species in the cycling of other elements.

5.0 Conclusions:

Our findings have broad implications for understanding the cycling of Mn in the global ocean. We demonstrate that Mn(III)-L complexes are present in oxygenated waters, and are thus likely ubiquitous in the marine environment. The presence of Mn(III)-L in the marine environment can have a profound impact on the mobility and uptake of other nutrients (Kostka et al., 1995). In the Lower St. Lawrence Estuary (LSLE), the fate (oxidation and complexation to Mn(III)-L or oxidation to MnO_x) of Mn(II) fluxing out of the sediments is critical in determining the transport and residence time of dMn_T in the water column (Mucci et al., 2003). The rate of Mn(II) oxidation is a function of the flux of dMn_T out of the sediments, the nature of the ligand(s) in the water column, the abundance of particles in the water column, and the dO_2 . Mn(II) escapes from the sediments to the overlying waters at both study sites, and the appearance of Mn oxides and Mn(III)-L in bottom waters is clear evidence of Mn(II) oxidation in the water column (Sundby et al., 1981; Richard et al., 2013). In the deep waters at Station 23, the Mn(II) is oxidized to MnO_x , whereas, in the Saguenay Fjord, $[\text{Mn(III)-L}] \gg [\text{MnO}_x]$. We attribute this difference to the

presence of stronger terrestrially-derived ligands that prevent the complete oxidation of Mn(II) to MnO_x in the Saguenay Fjord, and/or higher reductive dissolution rates of authigenic MnO_x. At Station 23, the initial fate of Mn(II) fluxing from sediments is its oxidation to MnO_x in bottom waters. Nevertheless, we still observe Mn(III)-L in the water column indicating that reduction of MnO_x and stabilization of Mn(III)-L may be an important formation pathway of Mn(III). It is also possible that Mn(III) is a residual product of the two-step oxidation of Mn(II) to Mn(IV), and stabilized as Mn(III)-L by ambient ligands. Our findings suggest that the development of hypoxic bottom waters in the LSLE may have led to increased dMn_T at Station 23, and that this additional dMn_T is stabilized as Mn(III)-L complexes in the presence of strong organic ligands (with logK_{cond} >13.2). Mn speciation studies need to include the measurement of Mn(III)-L complexes, which cannot be accomplished upon acidification for sample storage, as acidification precipitates Mn(III)-L humic complexes, a previously overlooked class of Mn species. Finally, our data highlight the importance of humic ligands to the coastal ocean, and provide evidence of a neglected potential pathway for the transport of dissolved metal-organic complexes to the global ocean.

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Figures:

Figure 1 Map of the two study sites in the St. Lawrence Estuary.

Figure 2 Water column Mn speciation data from Station 23 (2A) and SAG-30 (2C), with dMnT, Mn(II), and % Mn(III) determined by their difference, and MnOx measured by leucoberbelin blue (LBB) on solids collected onto a 0.20 μ m filter. Physical parameters are given for Station 23 (2B) and for SAG-30 (2D) with salinity and temperature measured using the CTD-rosette system, pH measured spectrophotometrically or potentiometrically (NBS scale) on discrete samples and dO₂ determined on discrete samples by Winkler titration. Standard deviations are given for Mn speciation measurements, and where not visible, are smaller than the symbols.

Figure 3 A comparison of dMnT from 1974 at Station 63 (Yeats et al., 1979) and the data from this study at Station 23. The Yeats et al. (1979) water samples were collected, filtered, acidified, and analyzed following an ammonium pyrrolidine dithiocarbamate (APDC) extraction. Our samples were analyzed immediately after filtration using the porphyrin addition method.

Figure 4 Linear correlation of total dissolved Mn from samples analyzed shipboard (x-axis) and samples analyzed 12 months after sampling, stored by filtering and freezing. Closed circles represent samples which were thawed and reanalyzed, open squares represent samples that were thawed, and filtered again through 0.02 μ m filters. Standard deviations of triplicate measurements are shown as error bars.

Tables:

Table 1 Recovery tests of dMn_T measured using a 100-cm cell coupled to a UV/Vis spectrophotometer following a heating step. Standard deviations for recovery were $\pm 5\%$ based on a standard calibration curve and the standard deviation for the NASS-6 seawater standard was based on triplicate analyses.

Treatment	dMnT(known) (nM)	dMnT(measured) (nM)	% Recovery
60 minutes in 60 °C bath	5	0.6 ± 0.03	<i>12.3</i>
	10	2.3 ± 0.17	<i>23.4</i>
	25	12.6 ± 0.63	<i>50.3</i>
30 minutes in 80 °C bath	5	1.7 ± 0.09	<i>34.7</i>
	10	5.0 ± 0.25	<i>50.0</i>
	25	19.8 ± 0.10	<i>79.4</i>
15 minutes in 90 °C bath	5	2.1 ± 0.1	<i>42.6</i>
	10	8.6 ± 0.43	<i>86.4</i>
	25	17.3 ± 0.9	<i>69.4</i>
60 minutes in 90 °C bath	5	5.2 ± 0.25	<i>104.9</i>
	10	9.8 ± 0.50	<i>98.0</i>
	25	26.6 ± 1.3	<i>106.4</i>
NASS-6 Seawater Standard	6.1 ± 0.6	5.8 ± 0.7	<i>95.1</i>

529 **Table 2** Water column dMn_T speciation (nM), dO₂ from recalibrated CTD probe (μM; Seabird
530 SBE-43), and salinity by CTD probe (Seabird SBE-911). % Mn(III) represents the difference
531 between measured dMn_T and Mn(II).

Station	Depth (m)	Mn (II)			dMn _T			% Mn(III)-L	MnO _x			dO ₂	Salinity	Temperature (°C)	pH
23	3	66.7	±	1.9	80.9	±	3.4	17.6	4.2	±	0.0	339	27.78	7.89	8.15
	20	69.1	±	2.5	86.0	±	0.6	19.6	18.1	±	0.2	277	29.96	4.15	8.00
	50	51.8	±	2.7	108.1	±	27.3	52.1	10.7	±	0.0	304	31.86	0.80	8.04
	80	45.2	±	0.6	125.8	±	15.8	64.1	9.2	±	0.2	299	32.25	0.32	8.02
	100	47.0	±	3.5	102.8	±	4.8	54.3	24.2	±	0.0	235	32.92	1.55	7.92
	150	35.2	±	5.5	253.0	±	5.5	86.1	55.1	±	0.2	134	33.73	3.69	7.80
	200	33.2	±	8.8	222.1	±	5.2	85.0	171.2	±	0.0	83.4	34.14	4.65	7.78
	250	62.5	±	3.3	299.5	±	13.7	79.1	295.9	±	2.4	68.5	34.34	5.08	7.74
	300	99.3	±	6.8	294.9	±	9.8	66.3	473.3	±	3.4	59.6	34.46	5.33	7.74
	340	289.2	±	15.4	295.1	±	16.5	2.0	1073	±	11.2	54.8	34.49	5.41	7.75
	Overlying	2930	±	25.0	2930	±	25.0	0.0							
SAG30	10	29.8	±	3.5	85.2	±	4.8	65.0	5.9	±	0.3	287	24.77	7.15	7.96
	20	41.4	±	2.6	82.3	±	2.8	49.7	13.7	±	0.3	298	28.16	5.38	8.02
	50	39.6	±	9.2	55.6	±	1.4	28.8	18.1	±	0.3	293	29.09	3.56	8.00
	100	164.3	±	2.2	206.2	±	2.2	20.3	13.3	±	0.3	281	29.60	2.55	7.97
	125	135.7	±	4.3	170.6	±	15.0	20.5	15.5	±	0.3	276	29.87	2.84	7.97
	150	152.0	±	5.2	175.7	±	8.6	13.5	18.3	±	0.3	272	30.03	2.20	7.94
	200	269.5	±	10.1	297.7	±	6.7	9.5	21.6	±	0.3	251	30.60	1.86	7.89
	250	207.8	±	7.7	691.9	±	7.6	70.0	12.8	±	0.3	230	30.83	1.79	7.84
	Overlying	2280	±	19.5	2280	±	19.5	0.0							

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Table 3 A comparison of the original speciation results from the shipboard porphyrin method analysis ($dMn_{T(unacidified)}$ and $Mn(II)_{unacidified}$) to samples analyzed 13 months later, after freezing, thawing, acidification, and centrifuging ($dMn_{T(acidified)}$); all concentrations are reported in nM. The $\% Mn(III)-L = 100 * (original\ dMn_{T(unacidified)} - Mn(II)_{unacidified})$ shipboard measurements. The $\% Humic = 100 * (original\ shipboard\ dMn_{T(unacidified)} - dMn_{T(acidified)})$ analyzed 13 months later after freezing, thawing, and acidifying. The $\% Mn(III)-L_{Humic} = 100 * (\% Mn(III)-L / \% Humic)$ and indicates what % of the Mn(III)-L is the result of humic complexation.

		<i>Original, shipboard, unacidified</i>			<i>13 Months later, frozen, acidified</i>		
Station	Depth (m)	$dMn_{T(unacidified)}$	$Mn(II)_{unacidified}$	$\% Mn(III)-L$	$dMn_{T(acidified)}$	$\% Humic$	$\% Mn(III)-L_{Humic}$
23	3	80.9 ± 3.4	66.7 ± 1.9	17.6	66.7 ± 7.2	17.6	99.9
	100	102.8 ± 4.8	47.0 ± 3.5	54.3	49.7 ± 3.7	51.6	95.1
	300	294.9 ± 9.8	99.3 ± 6.8	66.3	99.6 ± 6.8	66.2	99.8
	340	295.1 ± 16.5	289.2 ± 15.4	2.0	288.1 ± 1.8	2.4	120.2
SAG30	250	691.9 ± 7.6	207.8 ± 7.7	70.0	322.6 ± 26.6	53.4	76.3

Table 4 Comparison of dMn_T (nM) from shipboard analyses (Sept. 2014) following filtration through a 0.20 µm membrane, laboratory analyses 13 months later after filtration through a 0.20 µm membrane, and laboratory analyses one year later following filtration through a 0.02 µm membrane.

Station	Depth (m)	dMn _T 0.2 µm	dMn _T 0.2 µm - Oct. 2015	dMn _T 0.02 µm - Oct. 2015
23	3	80.9 ± 3.4	71.2 ± 1.8	73.7 ± 9.6
	100	102.8 ± 4.8	99.9 ± 1.7	92.7 ± 3.7
	340	295.1 ± 16.5	310.4 ± 5.6	306.9 ± 19.2
SAG30	50	55.6 ± 1.4	55.2 ± 13.2	59.0 ± 2.0
	250	691.9 ± 7.6	683.0 ± 4.5	635.2 ± 59.5

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