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Environmental Modeling

Determination and prediction of zinc speciation in estuaries

Holly Beverley Clare Pearson, Sean David W. Comber, Charlotte
Braungardt, Paul J. Worsfold, Anthony Stockdale, and Stephen Lofts

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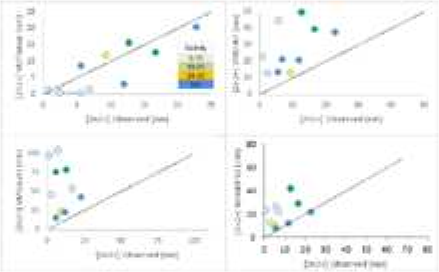

TOC Art

Determination and prediction of zinc speciation in estuaries

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Abstract

Lowering of the estuarine Environmental Quality Standard for zinc in the UK to 121 nM reflects rising concern regarding zinc in ecosystems and is driving the need to better understand its fate and behaviour and to develop and parameterise speciation models to predict the metal species present. For the first time, an extensive dataset has been gathered for the speciation of zinc within an estuarine system with supporting physico-chemical characterization, in particular dissolved organic carbon. WHAM/Model VII and Visual MINTEQ speciation models were used to simulate zinc speciation, using a combination of measured complexation variables and available defaults. Data for the five estuarine transects from freshwater to seawater endmembers showed very variable patterns of zinc speciation depending on river flows, seasons, and potential variations in metal and ligand inputs from *in situ* and *ex situ* sources. There were no clear relationships between free zinc ion concentration [Zn^{2+}] and measured variables such as DOC concentration, humic and biological indices. Simulations of [Zn^{2+}] carried out with both models at high salinities or by inputting site specific complexation capacities were successful, but overestimated [Zn^{2+}] in low salinity waters, probably owing to an underestimation of the complexation strength of the ligands present. Uncertainties in predicted [Zn^{2+}] are consistently smaller than standard deviations of the measured values, suggesting that the accuracy of the measurements is more critical than model uncertainty in evaluating the predictions.

Keywords: Zinc, speciation, estuary, WHAM, model, Visual MINTEQ

1. Introduction

Zinc (Zn) is ubiquitous in the aquatic environment and, whilst an essential element for all organisms, can be toxic in excess. In 2013 the UK Environmental Quality Standards (EQS) for Zn in saline waters was lowered from 612 nM (40 µg L^{-1}) to 121 nM (7.9 µg L^{-1}, including a 17 nM (1 µg L^{-1}) allowance for background concentrations) dissolved metal. Such a revision reflects the awareness that Zn is a potential pollutant, yet there has been a paucity of data published on its speciation in estuarine and coastal waters, particularly regarding the free metal ion concentration.[1] The reasons for this are related to low concentrations (relative to freshwaters) present in estuarine and coastal waters, relatively weak complexation of Zn with dissolved organic ligands and a saline matrix, leading to significant analytical challenges.[2]

EQS have been set or proposed for a number of trace elements including Cu, Ni and Zn in freshwaters taking account of the bioavailability of metals by using either Biotic Ligand Models (BLMs) or statistical models by using factors such as pH, calcium and dissolved organic carbon (DOC).[3-6] In estuarine and coastal waters, however, the development of BLM has been more challenging owing to the complex matrix and the constantly changing physico-chemical characteristics, such as salinity, suspended solids and organics (both natural and anthropogenic). Recently, a BLM approach been proposed for Cu in saline waters by the US EPA[7] based on USEPA 2007 information,[3] but no BLM or BLM-influenced statistical model approach as of yet has been developed for Zn in salt waters.

The ability to predict the free metal ion concentration is pre-requisite to setting a scientifically robust EQS that takes metal speciation into account. Thermodynamic equilibrium models have been developed (e.g. WHAM/Model VII,[8,9] FIAM[10] and Visual MINTEQ[11]) for calculating the speciation of trace elements in fresh waters and these can also be applied to saline waters successfully.[1] However, in many cases there are fewer data available for validating model outputs particularly complexing ligand concentrations and strength. A recent review showed that, since 1984, only four papers reported [Zn^{2+}] in estuaries across the USA, Netherlands and SE Asia[2] and not all of them covered the full salinity range expected in an estuary.

Five surveys carried out on a temperate flooded river valley estuary in the SW of England (Tamar) have generated comprehensive Zn speciation data, including free metal ion concentrations, complexation capacity and natural ligand dissociation constants, using competitive ligand exchange cathodic stripping voltammetry (CLE-AdCSV).[2]

The aim of this study was to provide essential information required for the development of an estuarine BLM for Zn. To this end, the Zn speciation within the Tamar estuary was appraised against physico-chemical parameters, such as salinity and DOC, and the latter was characterized further using fluorimetric analysis[12,13] of the organic components present.

Furthermore, the predictive ability of WHAM VII and Visual MINTEQ[14] for Zn speciation was tested and compared. The combination of Zn speciation determination and modeling presented here represents a stepping stone to more effective regulation of this metal within saline environments.

2. Methods

2.1 Tamar catchment and sampling sites

The Tamar estuary (16 km in length) runs from Gunnislake Weir to the English Channel and comprises two significant tributaries of the Lynher and Tavy (Figure S1).[15] The estuary has been contaminated with metals from a variety of sources including previous mining of arsenic, copper, zinc and lead;[16] from the dockyards and marinas using zinc anodes and antifoulant paints;[17] and effluents from sewage works containing metals as well as potentially complexing ligands (Figure S1).[18] Other sources of metal complexing ligands include spring and summer, phytoplankton blooms in the lower estuary as observed via chlorophyll 'a' measurement in this work (Figure S2) and previously.[19] Five full estuary transects (typically 8 sampling stations) were undertaken between July 2013 and February 2013 covering different seasons with variations in river flow, salinity, and the presence of phytoplankton blooms during the spring and summer months. Not all surveys have a complete data set owing to practicalities and sampling based on trying to achieve a representative range of salinities, which is reflected in the representation of surveys in some graphs and tables.

2.2 Chemicals and reagents

As previously reported[20] all chemicals used were of analytical grade or higher and ultrahigh purity (UHP) water (Elga Process Water, resistivity = 18.2 MΩ cm) and trace metal specification hydrochloric acid (6 M, ROMIL SpA) was used throughout to minimise contamination from Zn. Zn standards were prepared from Romil PrimAg reference solutions. All samples were buffered (pH 7.8 (+/- 0.1) using HEPES buffer (1 M) prepared from N-hydroxyethylpiperazine-N'-2'-ethanesulphonic acid (Biochemical grade, BDH Laboratory Supplies). The competitive ligand; ammonium pyrrolidine dithiocarbamate (APDC; Fisher Scientific) was made up as a stock 1 M solution prior to dilution to 40 and 4 μM for complexation capacity titrations. Samples for total dissolved Zn concentration ($[Zn_{TD}]$) were acidified with hydrochloric acid (6 M, ROMIL SpA).

2.3 Sampling protocol

Section S1 (ESI) details the sampling protocol. Prior to use, all sampling and filtration equipment was acid washed (10% HCl) and rinsed with UHP water. Water samples for metal speciation were filtered through 0.4 μm polycarbonate membranes (Whatman Nuclepore

Track-Etched) onsite and frozen at -20°C in low density polyethylene bottles (Nalgene) prior to analysis. [Zn_{TD}] samples were refrigerated and determined within 48 h at room temperature. Samples for DOC were filtered (0.7 µm GF/F) within 24 h of collection, acidified to ca. pH 2, and refrigerated in glass vials.

2.4 Analytical methods, procedures and calculations

All dissolved Zn analysis was undertaken by CLE-AdCSV (Section S2).

2.4.1 Total dissolved Zn

Prior to [Zn_{TD}] determinations, acidified samples were UV irradiated after the addition of hydrogen peroxide (final concentration of 15 mM). Sample pH was adjusted to ca. 6 with ammonia solution (SpA, ROMIL) prior to addition of the HEPES buffer and APDC for determination using voltammetry described in section S2. Certified reference materials (CRMs) were used for every batch of samples. Recoveries were between 90 and 109%, with a typical precision of $\leqslant$ 10% RSD. The limit of detection (LOD) was typically 0.33 nM Zn under optimum conditions.(maximum drop size, stirring speed and 60 s deposition time).

2.4.2 Free Zn ion, complexation capacity and conditional stability constants

Complexation capacity titrations (CCT) were performed using the same procedures as described elsewhere.[20] Briefly, titrations (at pH 7.8 using HEPES buffer) were carried out at two competitive ligand strengths (4 and 40 µM APDC) providing detection windows of $\log \alpha_{\mathrm{ZnAPDC}} =$ 3.01 – 4.59 and 3.03 – 5.29, respectively, matchning that expected for estuarine samples. A total of 10 Zn additions were performed on duplicate samples, with overnight equilibration. Each sample aliquot was determined in triplicate by CLE-AdCSV using analytical parameters provided in Section S2. Data were transformed using a method reported by van den Berg/Ruzic[21,22] to quantify the sample ligand concentration [L_x] (in nM), the conditional stability constant of the Zn-natural ligand complexes ($\log K'\mathrm{Zn}_{\mathrm{L_x}}$) and [$Zn^{2+}$], with data reported as nM or pZn^{2+} (the negative logarithm of the Zn concentration, rather than activity).

High temperature catalytic combustion (Shimadzu TOC V analyser)[23] was used to determine Dissolved Organic Carbon (DOC) with appropriate certified reference materials[20] providing a limit of detection of 4 µM. Characterisation of the DOC was carried out using 3-D fluorimetry with a Hitachi F-4500 FL spectrophotometer. Sigma Aldrich humic acid (55.1% C; Sigma Aldrich, UK) and Nordic aquatic fulvic acid reference material supplied by the International Humic Substances Society (45% C) were used as standards. Although the limitations of using commercial humic acids have been reported,[24] the Sigma Aldrich material is well characterized, available and being terrestrially derived, matched the likely sources of humic material likely to be present in the Tamar catchment and so was considered fit for purpose.

2.5 Thermodynamic equilibrium speciation calculations

2.5.1 Visual MINTEQ

Calculations were undertaken using the Visual MINTEQ (VM) version 3.1,[14] chemical equilibrium model for the calculation of metal speciation, solubility equilibria, sorption etc. for natural waters. It offers the benefit of a Windows interface and the ability to introduce new ligands (in this case $\log K'_{\mathrm{ZnL_x}}$ values and ligand concentrations derived from the CCT field data at two separate artificial ligand strengths) into its database. Input major cation and anion concentrations were generated from determined parameters (salinity, pH) using an ion-pairing model.[25]

$[Zn^{2+}]$ predictions were generated using the following input parameters (further default parameters in Table S3):

1) Major ion concentrations were predicted from assumed conservative mixing of Tamar freshwater (mean concentrations from Gunnislake sample from present surveys and available Environment Agency data) and sea water[26] end member data obtained from an ion-pairing seawater model (Table S3).
2) $[Zn_{TD}]$ determined as part of this work.
3) Ligand concentrations:
   a. Taken from this work using 4 and 40 μM APDC with their accompanying conditional stability constants.
   b. Ligands predicted by VM 3.1 using measured DOC concentrations from this study combined with the SHM, Gaussian and Nica-Donnan model options (see section S3).

All modeling was carried out assuming a pH 7.8 to match the conditions under which the experimental work was carried out and at a fixed temperature of 15 ^{o}C. The Davies method was used for activity correction, without other organic ligands present at an assumed thermodynamic equilibrium. Sensitivity analyses (not shown reported) for temperature and pH within VM using natural sample values showed negligible difference in predicted $[Zn^{2+}]$.

2.5.2 WHAM/Model VII

WHAM/Model VII was used to predict Zn speciation under the assumption that the dissolved organic matter (DOM) in the estuarine water behaves in the same way as if composed of soil and freshwater fulvic and humic acid extracts in competition with inorganic ligands. WHAM VII comprises two components, the inorganic speciation code WHAM[8] and Humic Ion Binding Model VII, described in detail in elsewhere.[9]

To run the model, the pH used for the laboratory Zn speciation measurements and determined Zn concentrations were applied. Major ion concentrations (except carbonate) were obtained using a combination of salinity and freshwater endmember major ion measurements. Modeling was done under two scenarios of differing carbon dioxide equilibration between the atmosphere and water. In Scenario S1, carbonate speciation was determined using the default equilibria and binding constants, assuming equilibrium with the atmosphere and a partial pressure of CO_2 of 400 ppm. In Scenario S2, in order to maintain consistency with typical methods for evaluating the carbonate system in marine environments, $[CO_3^{2-}]$ was calculated using the CO2SYS model[27] with the constants describing the carbonate and sulphate equilibrium with hydrogen ions[28] and refitted as described elsewhere[29,30] respectively. The pH was on the total scale and the total boron concentration from Uppström, (1974). This approach was used recently,[31] implementing the carbonate system with measured conditional stability constants into Visual MINTEQ. Detailed information regarding WHAM VII in section S4.

Uncertainty in predictions was modelled using repeated estimates of input measurements and model parameters made using the assumption of a normal or log–normal distribution around the measured or default value, coupled with an absolute or relative error value.[32] Here previously reported error values were applied of: ±0.1 absolute error on measured pH, ±5% relative error on measurements except for DOC, ±9% relative error on DOC, ±1K absolute error on temperature, and ±0.3 absolute error on the intrinsic metal–fulvic binding constant.[32] The number of repeat calculations was 2000 and uncertainties were computed as the 15.9th and 84.1th percentiles of the population of predicted free ion concentrations. This range of percentiles corresponds to ±1 standard deviations from the mean for a normally distributed dataset.

3. Results and Discussion

3.1. Total dissolved zinc

In the Tamar estuary, [Zn_{TD}] ranged from 11 - 225 nM, with typically, highest concentrations observed at the historically mine-influenced freshwater end member (FWEM) (Figure 1). The non-conservative mixing profiles (Figure 1) show removal of Zn at the freshwater-seawater interface (FSI) for all the surveys, and further reduction of Zn concentrations toward the seawater end member (SWEM). Even with the observed non-conservative behaviour of Zn_{TD} and dilution with seawater within the estuary, there were some exceedances of the new EQS (104 nM). This was particularly noticeable within the mid-estuarine area, where distinct inputs of Zn were observed during the July 2013 and April 2014 surveys. These inputs were the likely result of Zn desorption from sediment, resuspended by tidal forces from an expanse of mud flats extending from the road bridge at Saltash to Pentillie Castle (typical salinity 10 to 15), where the estuary channel narrows. Kinetic experiments conducted by previously[33] demonstrated a pulse of Zn released from sediments (estuarine and riverine) within 10 min of its exposure to seawater, and attributed mid-estuarine dissolved Zn maxima partially to desorption. Therefore, the peak in dissolved Zn observed at Pentillie Castle during the April survey and at Halton Quay during July 2013 may be explained by desorption, combined with tidally induced porewater infusions.

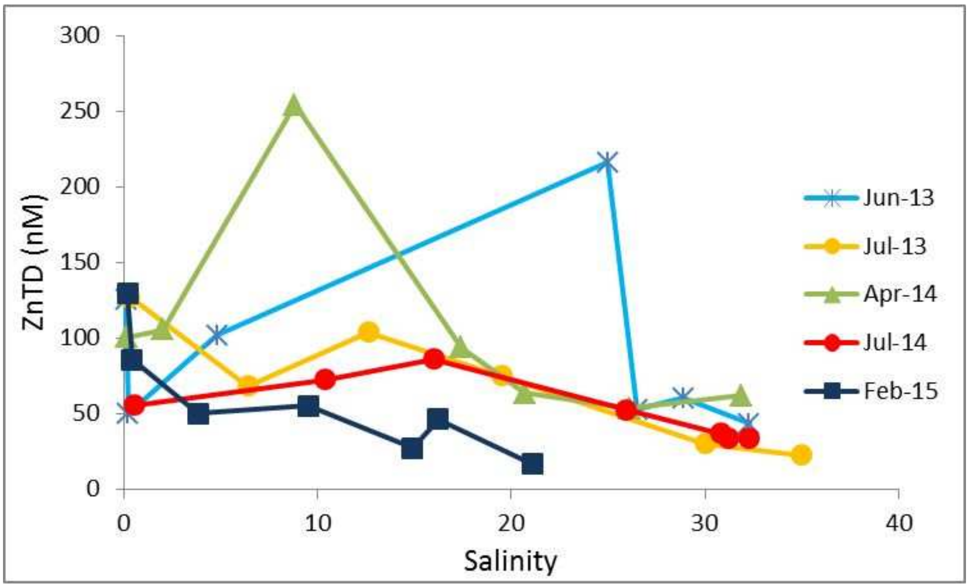

Figure 1. Total dissolved Zn concentrations determined for the four transects. Note: In April 2015, a sample was taken at low salinity within the estuary, but logistical constraints prevented the sampling of a fresh water endmember in the river.

3.2. Zinc speciation and ligand characteristics

Concentrations of Zn^{2+}, determined using the two competing ligand strengths, ranged from 0.3 – 109 nm (Figure 2) whereby the lowest range of concentrations occurred in July 2013 and the highest in April 2014. These concentrations are of the same order as the very limited data for $[Zn^{2+}]$ provided in literature.[34] The fresh and seawater endmembers contained the lowest $[Zn^{2+}]$ and during all surveys concentrations increased at some location between these two endmembers. Although the trends are broadly similar, results from determinations with the weaker competitive ligand strength (4 μM APDC) returned higher $[Zn^{2+}]$ than determinations using 40 μM APDC. This is a clear indication that differences in the analytical detection window introduce bias into the determination of $[Zn^{2+}]$.

Notwithstanding this artefact, in terms of toxicity, $[Zn^{2+}]$ determined in this study are potentially harmful to sensitive aquatic organisms along the whole of the estuary, even at the lower $[Zn^{2+}]$ concentrations determined with 40 μM APDC. For example, the growth rate of marine phytoplankton *Synechococcus* sp. has been reported to decline at concentrations of $[Zn^{2+}] > 0.4$ nM and this value is $[Zn^{2+}] > 3.2$ nM for *Thalassiosira weissflogii*.[35]

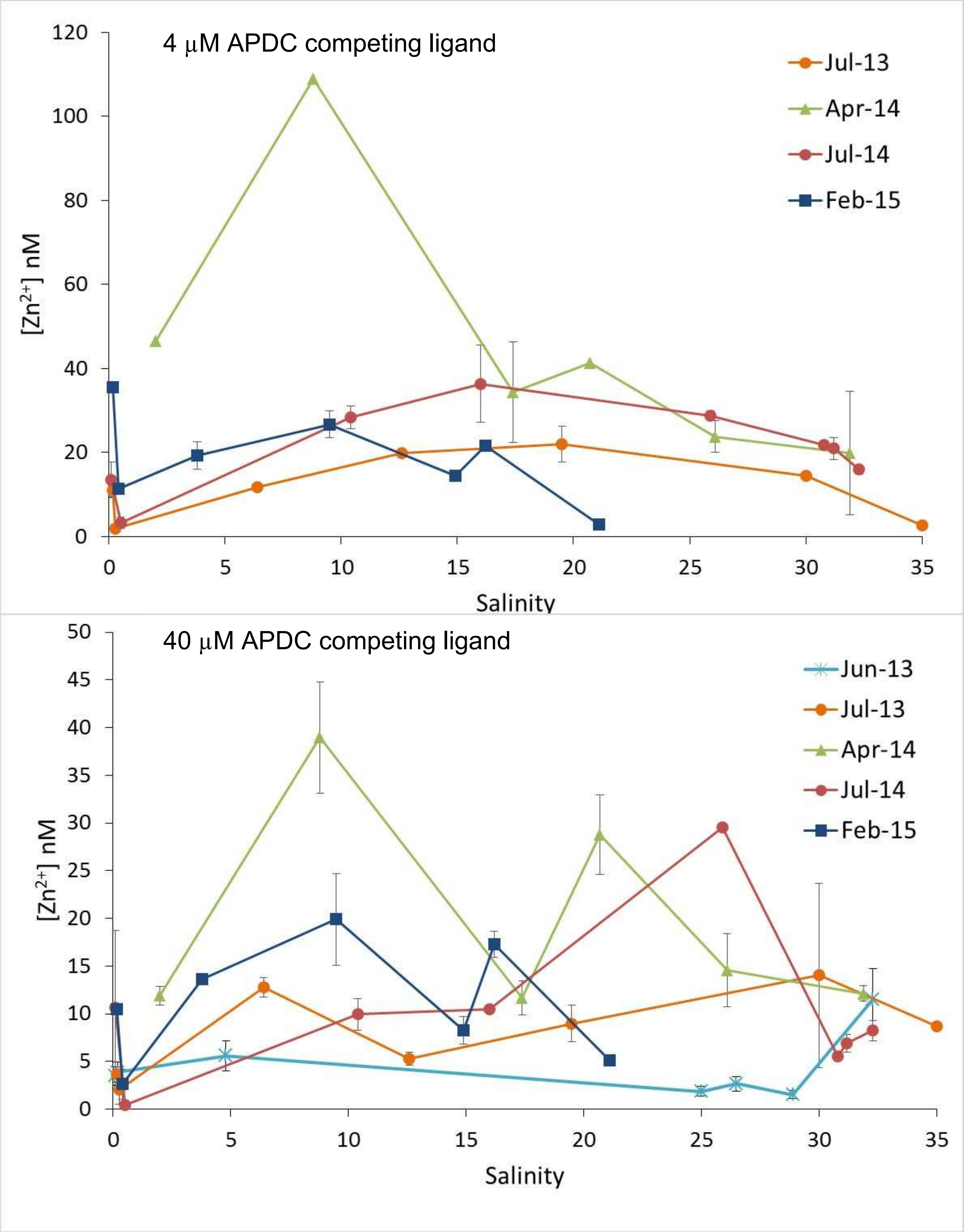

Figure 2. Free Zn concentrations ([Zn^{2+}] determined using various concentrations of APDC) plotted as a function of salinity for the Tamar transects. Each data point is the average of two duplicate analyses (one on each filter fraction) with error bars representing the range. Where error bars are absent, only one data point was obtained. Nb. There was no determination of [Zn^{2+}] for the Jun-13 survey for 4 µM competing APDC ligand

In general, the percentage of [Zn^{2+}] as a fraction of the [Zn_{TD}] increased with salinity for both ligand strengths (4 and 40 µM APDC) used to determine complexation capacity by Zn titration

(Figure 3), and, to a degree, reflects the observed Lx:Zn_{TD} ratio (Figure S3). Increasing salinity introduces high cation (e.g. calcium, magnesium) concentrations that compete with Zn complexing sites on ligands, therefore raising the potential for more Zn to be less strongly complexed.

Concentrations of zinc complexing ligands ([Lx]) between 3 and 412 nM were determined (Figure 3), well within the range reported for other estuarine and coastal studies (5 – 220 nM).[34,36] Although some inputs of Zn ligands mid-estuary were apparent in June 2013, April 2014 and to some extent July 2014, the highest concentrations, and hence main inputs, were at the FWEM. Zn ligand concentrations, in most cases, tracked the total dissolved metal concentration profiles with salinity, indicating that both the FWEM and tidally induced mid-estuarine sediment processes were sources for Zn_{TD} and Lx (Figures 1 and 3). The degree of Zn complexation varied between detection windows, with higher proportions of [Zn^{2+}] present at the weaker analytical competition strength. Variation between surveys was also apparent. In February 2015 the proportion of [Zn^{2+}] in samples was high (32 – 49 %) in the mid- and lower estuary, even though Zn_{TD} concentrations were similar to those during other surveys. This reflects the low complexation capacity determined at both APDC concentrations, lack of ligand excess (Figure S3) and low DOC concentrations (31 – 123 µm C) observed during this survey (Table S2).

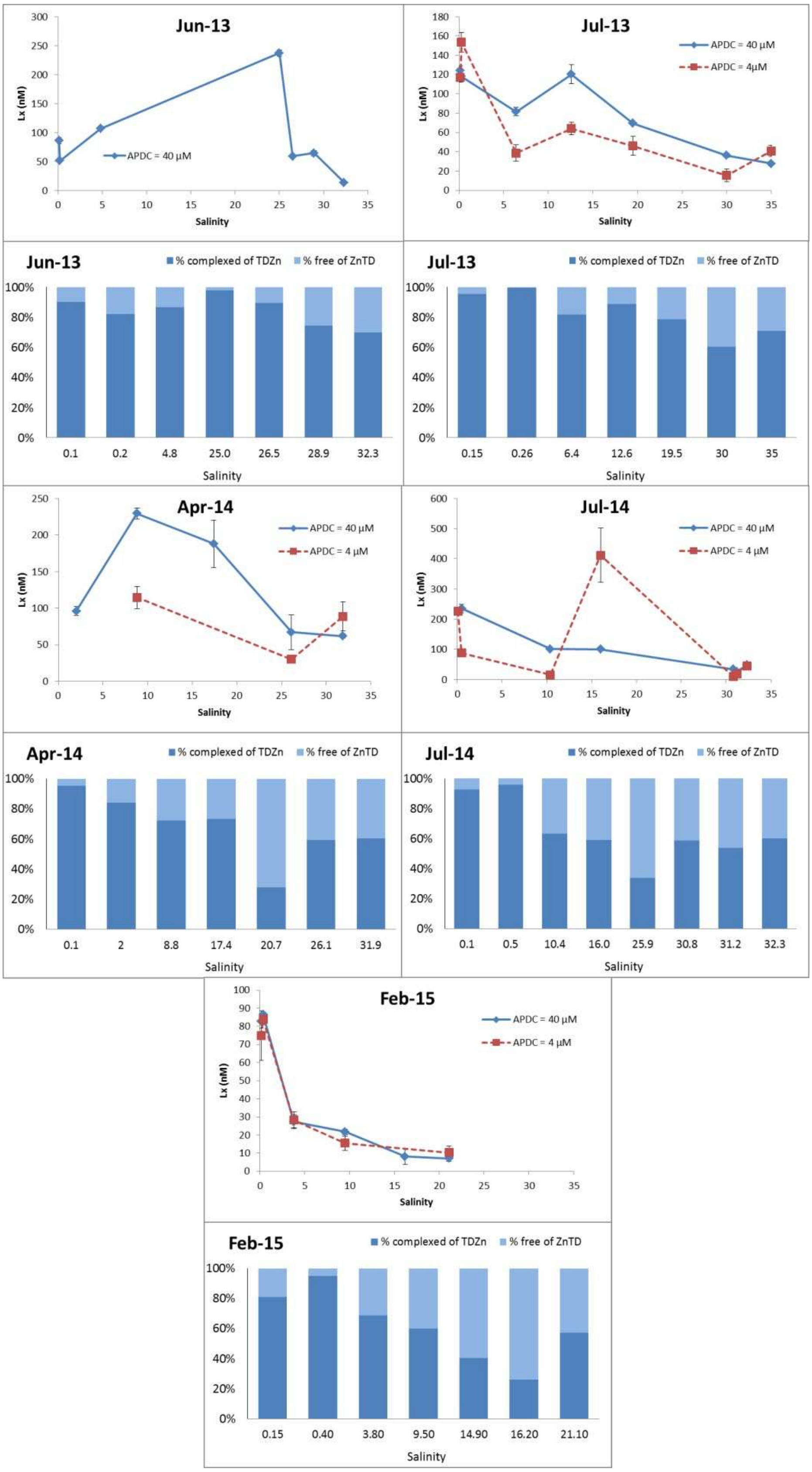

Figure 3. Ligand concentrations ([Lx]) and proportion of Zn complexed (organically) versus proportion free Zn^{2+} ion as a percentage of total dissolved Zn for each sampling occasion. The x-axes represent salinity in all cases. Error bars on [Lx] plots represent ± an average uncertainty.

The ratio [Lx]:[Zn_{TD}] indicates the significance of Zn^{2+} present in the water, whereby [Lx]:[Zn_{TD}] > 1 signifies an excess of Zn complexing ligands and therefore suppression of the more bioavailable and toxic Zn^{2+} species. Saturation of ligands is indicated by [Lx]:[Zn_{TD}] < 1, with [Zn^{2+}] likely to be more prevalent as the ratio decreases. The ratio showed no relation to salinity in any of the surveys. [Lx]:[Zn_{TD}] was consistently low (average 0.5) in February 2015 - only at Cotehele (S = 0.4) did it approach 1 (Figure S3), a profile that indicates ligand saturation throughout the estuary, concomitant with the high proportion of Zn^{2+} observed. In June and July 2013 and April 2014, ratios between 0.4 and 2.1 indicated varied profiles with most samples exhibiting little excess ligand. In July 2014, ratios varied between 0.2 and 4.8, with highest values at Morwellham (4.2, S=0.5) and Cotehele (4.8, S=16), where observed ligand concentrations far outweighed the presence of Zn_{TD}. Overall, complexing capacity is exceeded (ratio <1) in 30 of the 53 measurements depending on titration competition strength and time of year, which explains the occurrence of [Zn^{2+}] in the samples.

3.3 Prediction of [Zn^{2+}] in estuarine waters

Measuring [Zn^{2+}] within any given estuary provides an indication of possible bioavailable concentrations. However, to develop a BLM or to be able to extrapolate data to other estuaries then the ability to accurately predict the Zn speciation is essential.

Figure 4 provides a detailed analysis of VM outputs using the input parameters detailed in the methodology section and Table S3 of the ESI. VM provides three options for converting DOC concentrations to metal complexing ligands, the Nica-Donnan, Gaussian and Stockholm Humic Models). The theory associated with these calculations is provided in S3 of the ESI. For six sites of varying salinity from 0 to 33 where a full dataset was available, the model was run using the three options for predicting metal complexation based on inputted DOC concentration (Gaussian, Nica Donnan and Stockholm Humic Model).

Figure 4. Comparison of measured and predicted Zn speciation (free ion, inorganic and organic) using the ligand predicting models within Visual MINTEQ (VM). VMGauss = Gaussian model; VMNICA-D = Nica-Donnan model; VMSHM = Stockholm Humic Model; VM Tamar = VM inputs of measured ligand strength and concentration for July 2013 samples in the Tamar estuary from a range of salinities (except 0 and 2.3).

The distribution of Zn between free ion, inorganic and organically complexed species varied considerably among the different approaches to estimating complexing ligand strength and concentrations. When site-specific measured complexation capacity and ligand concentration were entered into VM, the best fit between measured and calculated Zn^{2+} and organically bound Zn was achieved (Figure 5 and S3). Where only DOC concentration was entered and VM was used to predict Zn complexation using the three options available, [Zn^{2+}] was overestimated in all samples, compared to measured values. At low to moderate salinities the Gaussian and Nica-Donnan methods significantly overestimated [Zn^{2+}], and consequently underestimated the organically-bound Zn. The Stockholm Humic Model (VMSHM) offers a much closer agreement. At higher salinity, total dissolved concentrations and Zn complexing ligands present in the samples were lower, and consequently the fraction of Zn^{2+} increased (Figure 3). The pattern between the different methods of calculating Zn complexation remains the same with only the VMSHM approach predicting significant organic ligand complexation and hence, corresponding reductions in [Zn^{2+}].

For the WHAM/Model VII, Scenario S2 generates a significantly greater quantity of $ZnCO_3$ and $ZnHCO_3^+$, and average of 21.4 ± 4 nM SD and 21.5 ± 4 nM SD (n = 44), respectively. This has the effect of significantly reducing [Zn^{2+}] and bringing it more in line with the measured values using voltammetry (Figures 5 and S4).

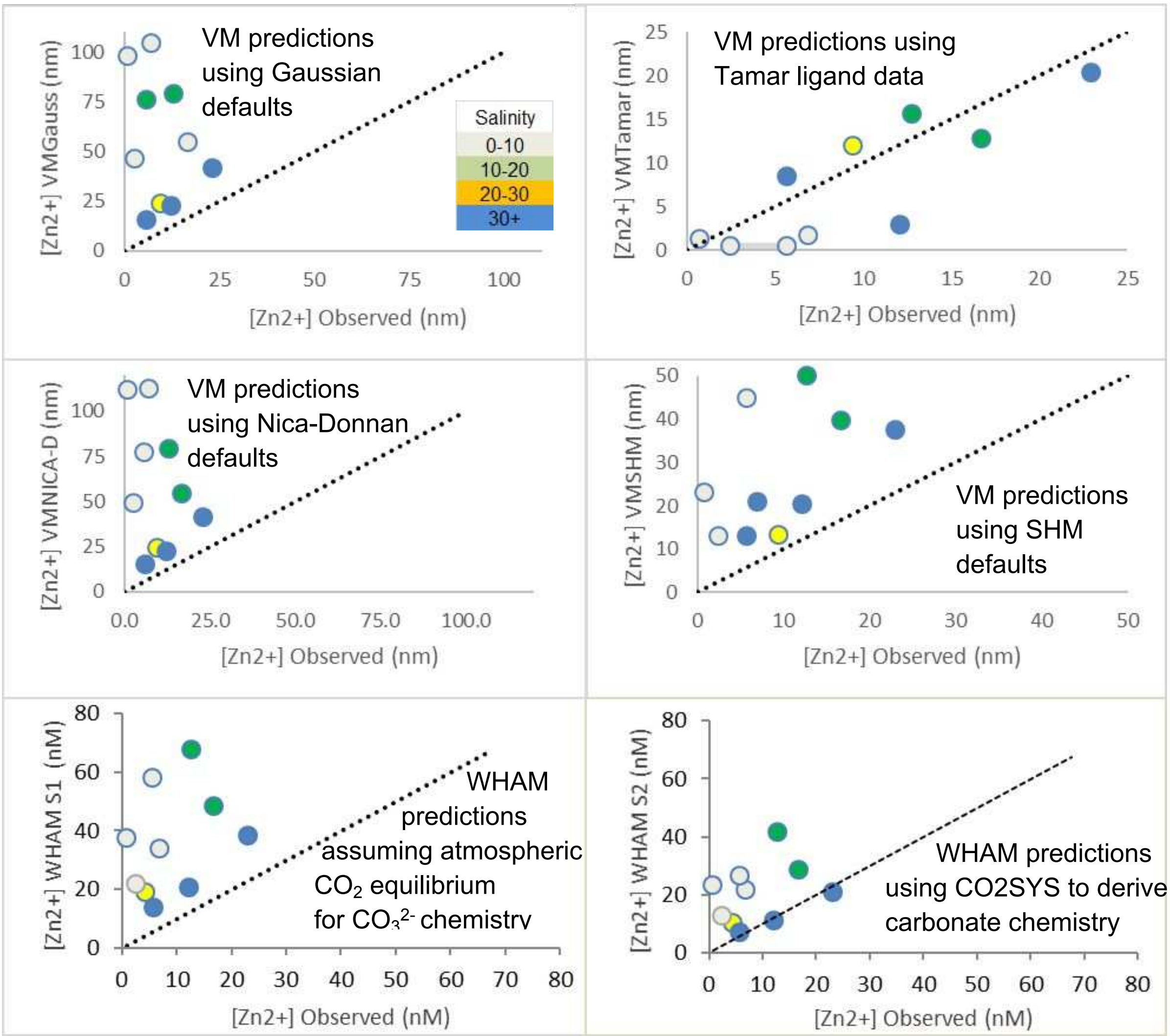

Figure 5. Predicted versus measured Zn^{2+} concentrations (nM) using the VM and WHAM/ModelVII models.

The data points representing the largest discrepancy in [Zn^{2+}] (the most over- or under-predicted by the model) between predicted and measured values are for samples located at low salinity zones (< 1) in the upper estuary (Gunnislake, two samples from Morwellham Quay, Cotehele, Figure 5) for both, VM and WHAM. In these locations, humic and fulvic type ligands were dominant (see below) and present at relatively high concentrations. Measured log *K* values for Zn-organic complexes at these sites ranged between 7.74 and 9.66, which is much higher than, for example, the assumption used for the $VM_{NICA\text{-}D}$ calculations. $VM_{NICAD\text{-}D}$ assumes complexation by fulvic acid alone (log *K* = -3.84 for carboxylic and logK = 0.73 for phenolic functional groups, respectively).

The measured log Ks are conditional binding constants, which are specific to the water composition, as they do not consider the influence of competition for binding to organic matter – particularly competition from the H^+ ion. The log Ks in the models (at least in WHAM and the SHM) are thermodynamic constants for the binding of the metal to a single binding site on a humic molecule. Competition is provided by having similar constants for other cations (including H^+). More importantly from the point of view of the apparent binding strength, in WHAM and the SHM pairs and triplets of single binding sites can form bidentate and tridentate sites, respectively. These have log Ks that are the sum of the log Ks for the sites that make them up and thus have higher metal binding affinities, closer to those of the ligands identified by measurement. The better agreement between observed and computed Zn^{2+} when using the measured ligand concentrations is due to the fact that there is a strong element of fitting involved, i.e. the initial determination of ligand concentration and binding strength. It ought to be equally possible to fit any of the other models to the titration data and obtain improved agreement with the field data.

As with the SHM, NICA–Donnan and Gaussian models, assumptions regarding the humic composition of DOC are required to perform computations in WHAM if only DOC concentration is available as a measure of the organic matter. In past applications of WHAM this has been done by assuming a proportion of the DOC to behave as model fulvic acid (FA) and the remainder to be inert with respect to ion binding. Lofts and Tipping[32] computed a mean DOC to “active” FA ratio of 1.27, based on previous work.[37] However, the ratios obtained by Bryan and co–workers[37] from 14 freshwaters showed an inter sample variation by a factor of over two, from 0.80 to 1.82. Results demonstrate the importance of using an appropriate approach for handling carbonate speciation and show predictions for marine systems are improved with this approach. Furthermore, if there were more comprehensive data on the nature of marine DOM and transitions across salinity gradients these could be built into generic models. However, the (current) reliance on freshwater and soil fulvic acid/humic acid data means some discrepancy is likely if the two end member DOMs are different in character (see Section 3.5).

Whilst this research shows reasonable predictions can be achieved for estuarine/seawater, it has been shown that as the waters get more saline the trend is for a greater discrepancy between measurement and modeling.[38]

Uncertainties for WHAM VII predictions are shown in Figure S6. Predictions include estimates of uncertainty due to uncertainty in measurements and parameters, using the Monte Carlo approach.[9] The prediction uncertainties cover the 15.9–84.1 percentile range of model outputs, equivalent to ±1 standard deviation. Patterns of goodness-of-prediction against salinity are generally similar to those for the other models. Measurements in samples of salinity $\geqslant$ 30‰ (n = 4) are reproduced well by the modeling, with all predictions within the ±1 standard deviation range of measurement. In salinities of 1–30‰ (*n* = 3), predictions are reasonable. Predictions in samples of salinity $\leq$ 1‰ underestimate of the observed extent of Zn complexation. Uncertainties in predicted [Zn^{2+}] are consistently smaller than standard deviations of the measured values, suggesting that the accuracy of the measurements is more critical than model uncertainty in evaluating the predictions.

An important observation is that in terms of potentially using these models for implementing an EQS, that none of the default modes lead to over-prediction of measurements and therefore will be conservatively protective.

3.4 Relationships between free Zn ion and DOC

The results of VM_{Tamar} calculations show that it is possible to use VM to predict the [Zn^{2+}] to within an order of magnitude when details for site-specific complexation parameters (ligand concentrations and conditional stability constants of metal-ligand complexes) are entered into the model. This provides a certain degree of confidence in the agreement between the speciation programme outputs for Zn and measured ligand parameters that can be factored into a safety margin with respect to setting EQSs. However, the model outputs for predicted free metal ion concentration based on inputs of DOC concentrations alone gave a poorer prediction of the free ion concentration, and therefore the most potentially bioavailable and toxic metal fraction. The possible reasons for this have been explored further below.

DOC concentrations of 30 to 500 µM C in the samples were consistent with those observed previously in the Tamar.[39] In comparison, zinc complexation capacities for the samples were in the region of 10 to 500 nM, typically three orders of magnitude lower (Figure 3). This demonstrates the complexity and challenges associated with attempting to predict the complexing ligands based on such a gross measure of what is a group of compounds with wide variability in physico-chemical characteristics and sources.

Plotting [Zn^{2+}] versus DOC (Figure 6) shows no specific trends either for the whole dataset or individual sampling occasions. A similar conclusion has been drawn for Cu^{2+} for estuarine samples.[20] The lack of data for Zn speciation in saline waters means there are no data to corroborate these findings, which in itself was a key incentive to undertake this research. Plotting Zn complexation capacity (Lx) against DOC showed a weak positive correlation, but at higher DOC concentrations the data scatter became pronounced. The only firm conclusion which may therefore be drawn is that at low DOC concentrations, complexation capacity is low, with complexation capacity likely to be higher where DOC concentrations exceed 300 μM. For the purposes of predicting [Zn^{2+}], there is no obvious relationship that could be applied. The fact that the complexation, and therefore [Zn^{2+}], is controlled by both the ligand concentration and its strength, explains the lack of comparability between the plots of [Zn^{2+}] and DOC, and [Zn^{2+}] and complexation capacity.

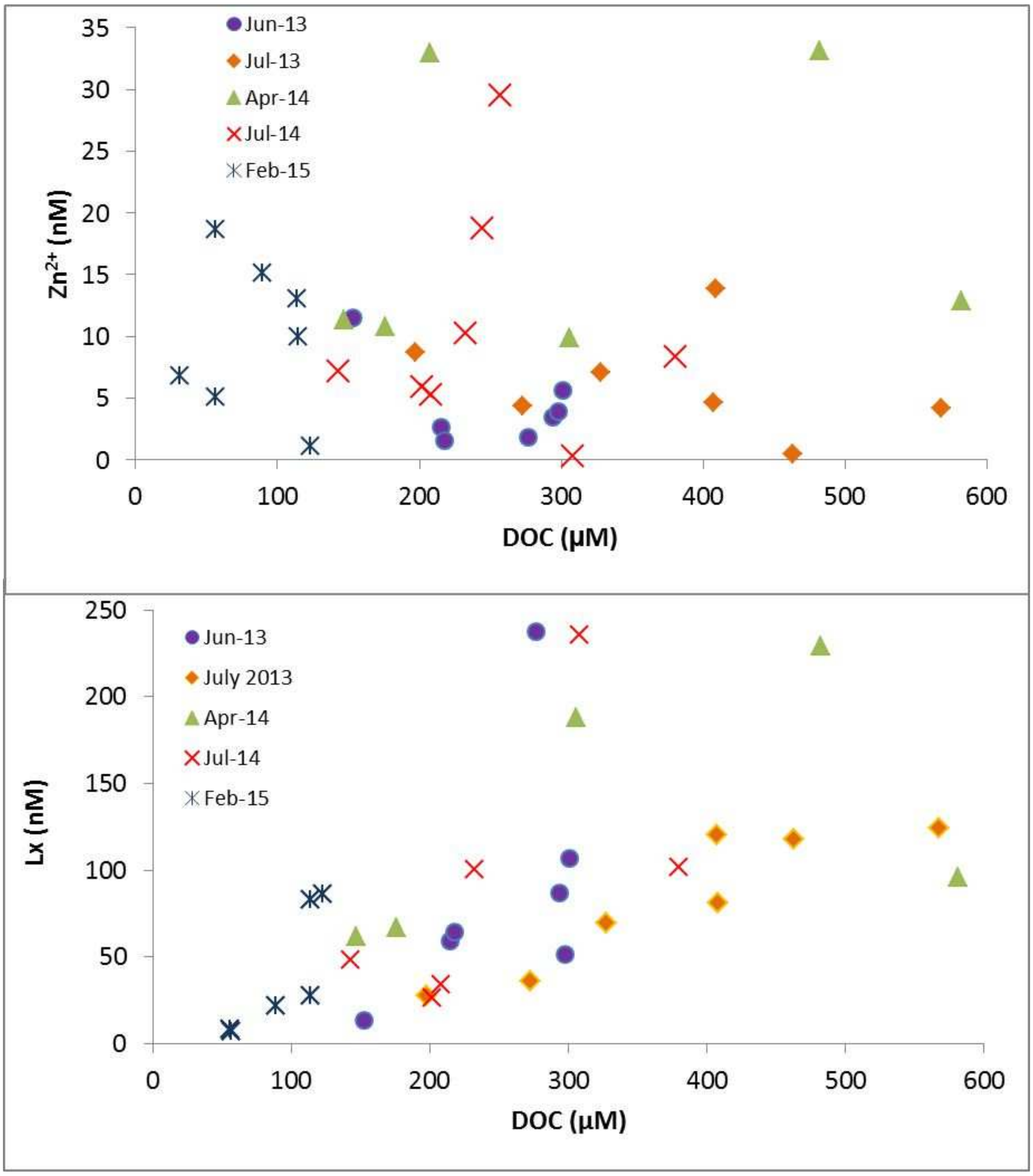

Figure 6. [Zn^{2+}] (top) and Zn complexation capacity (Lx, bottom) versus DOC for the Tamar estuary samples.

3.5 Further DOC characterization

There are techniques available to better characterize dissolved organic matter using UV and fluorimetric methods. 3-D fluorimetry allows a semi-quantitative assessment of the characteristics of compounds making up the DOC present in a sample. The ratios of observed fluorescence peaks can be used to categorise the organic carbon as humic and fulvic, terrestrial or *in situ* generated material using the humification (HIX) and biological (BIX) indices (S2.4). HIX ratios (Ex260-Em320)/(Ex260-Em460) <4 suggest biological or aquatic bacterial origin, while increasing humic character up to ratios >16 show progressively stronger humic character (Figure 7).[12] For BIX (Ex310-

Em380)/(Ex310-Em430) it has been observed that high ratios (0.8–1.0) corresponded to a predominantly autochthonous origin of DOC from recent aquatic and bacterial activity freshly released into water.[13]

In samples from the Tamar surveys, HIX values generally decreased towards the sea water end member (Figure 7, no data for July 2013). Important humic character was indicated for the fresh water endmember (HIX > 10), while DOC generated by *in situ* biological processes increased in importance towards the sea water end member (BIX > 0.7). It is likely that the DOC in the lower estuary was at least partially derived from phytoplanktonic activity and/or sewage effluents from works present in the lower estuary.

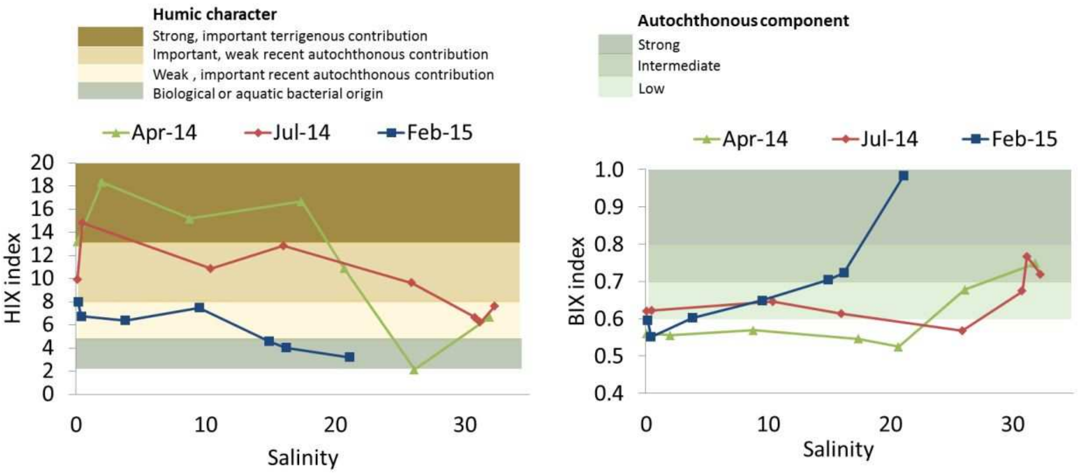

Figure 7. The humification (HIX) and biological (BIX) indices for each seasonal transect against salinity (no data was available for 2013).

Upper estuary and riverine HIX indices were > 10 during three surveys, supporting the hypothesis that the DOC was of mainly terrestrial origin, comprising mostly humic and fulvic acids. The BIX index corroborated this, with values increasing towards the sea water end member, demonstrating the autochthonous origin of the DOC present.

A plot of [Lx] against HIX and BIX shows weak positive and negative correlations, respectively (Figure S5). Highest HIX values were associated with higher complexation capacities, which suggests a significant affinity of Zn for humic and fulvic acids,[40] which dominate the lower salinity regions in the upper estuary (Figure S5). Any correlation with BIX indices is hampered by the relatively small range of BIX (typically 0.6 to 0.8) in the Tamar Estuary. Based on this dataset, it suggests that neither of these indices would be sufficiently robust to improve greatly on the existing use of DOC concentration as a surrogate for Zn complexation characterization.

This result corroborates the findings that there is no apparent simply defined relationship between DOC concentration and [Zn^{2+}] and lends support to the argument that assuming a fixed “active” portion (50 %) of DOC in a model such as WHAM or VM may be inappropriate, at least in regards to [Zn^{2+}]. VM has been reported to consistently underestimate free Cu ion[41] by between 2 and 5 orders of magnitude in comparison to measured values (in the range 10^{-12} – 10^{-14} M) which are potentially controlled via multiple ligand sources at varying concentrations as discussed earlier. A key issue is that whether optimized ratios of measured DOC to ‘active’ FA fall within previously observed ranges. If not then this might suggest a role for ligands stronger than those humics can provide. The modeling using default data consistently overestimates [Zn^{2+}], which suggests that there may be strong specific ligands in the estuary.

In the case of the over-prediction of [Zn^{2+}] by VM, the effect of synthetic ligands present in sewage effluent discharged to natural waters, such as ethylenediaminetetraacetic acid (EDTA) is not accounted for in the model, were discounted as a possible cause. Stockdale et al.,[1] tested EDTA concentrations within the WHAM/Model VII as low as 5×10^{-8} M and there was a small but noticeable effect for Zn (no effect for Cd and a greater effect for other metals) at this low concentration. Although concentrations of 0.1 and 1 μM EDTA caused a considerable reduction (63% and 96% respectively) in calculated [Zn^{2+}], these effective EDTA concentrations are unlikely to exist in the Tamar estuary. Although not quantified in this study, significant dilution of EDTA from the likely predominant sources (Ernesettle, Central, Marsh Mills and Camels Head WwTW, serving a combined population of 290,000) near the mouth of the estuary is probable. If the sewage effluent discharge in the Tamar is estimated at 72 million litres a day,[42] setting this against an average river discharge of 2333 million litres a day[43] equates to a 32 times dilution on river flow alone, without allowing for seawater flushing of the estuary. Based on recently published median effluent EDTA concentrations of 0.44 μM[44] such a dilution would reduce the ETDA concentration to well below the effective concentration of ligands observed in this study. This does not exclude the possibility there are other anthropogenic synthetic ligands present within the estuary unaccounted for, but given the strength and typically high concentrations of EDTA present in effluents which have been discounted above, it seems unlikely more powerful ligands may be present at sufficient concentrations to impact on the Zn speciation.

Overall, the data presented here for Zn speciation along transects of the Tamar estuary were carried out to investigate the influence of dissolved organic ligands over the course of a calendar year, with the aim of attempting to model Zn speciation based on a limited dataset. It was not possible to attribute observed trends in metal speciation to any single measurable physico-chemical parameter, which was unsurprising as the complexity of the estuarine environment means observations are the result of a combination of many factors which are subject to constant change. In cases where rainfall has been abnormally high (e.g. February 2015 survey), the expected trends and concentrations of constituents (e.g. DOC) can change dramatically, and therefore, mixing and physico-chemical parameters, such as turbidity, are likely the more important controls on speciation, rather than time of year.

Ligand abundance and excess, type, and binding strength appear to be important factors in controlling the proportion of complexed metal. Although these parameters are measurable with laboratory instrumentation there is no simple relationship between these factors and easily determined variables such as salinity, DOC or even further characterization of the DOC present using 3-D fluorimetry.

Modeling using VM generated reasonably accurate estimates of Zn^{2+}, provided site specific values for ligand strength and concentration were entered. Inputting DOC concentration and allowing the model's in-built algorithm to estimate complexation capacity generated over-estimates of Zn^{2+}, particularly at low salinities, where default fulvic acid log K assumptions appear to under-estimate complexation. A similar trend was observed for the WHAM VII model with much improved agreement between predicted and observed free metal ion at higher salinities, and uncertainties of measured values exceeding those of predictions.

Although models generating over-estimates of the more toxic Zn species is a conservative approach to risk assessments, to develop BLMs for estuarine waters it is necessary to be able to accurately predict free metal ion concentrations. The Tamar data presented here for Zn speciation (which are scarce in comparison with Cu speciation data) for the first time provide vital metal-ligand complexing strengths and ligand concentrations detected at various competitive ligand strengths across full salinity ranges which may be used for future modeling and regulatory purposes.

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Supporting Information

The following material is supplied in the Supporting Information:

- Sample collection and storage
- Detailed analytical methodology
- Visual Minteq modeling
- WHAM modeling
- Additional figures and tables

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