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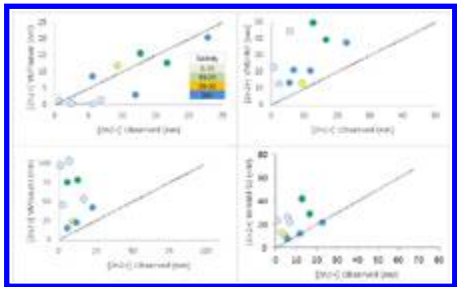
1    **Determination and prediction of zinc speciation in estuaries**

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12    # Abstract

13    Lowering of the estuarine Environmental Quality Standard for zinc in the UK to 121 nM
14    reflects rising concern regarding zinc in ecosystems and is driving the need to better
15    understand its fate and behaviour and to develop and parameterise speciation models to
16    predict the metal species present. For the first time, an extensive dataset has been gathered
17    for the speciation of zinc within an estuarine system with supporting physico-chemical
18    characterization, in particular dissolved organic carbon. WHAM/Model VII and Visual
19    MINTEQ speciation models were used to simulate zinc speciation, using a combination of
20    measured complexation variables and available defaults. Data for the five estuarine
21    transects from freshwater to seawater endmembers showed very variable patterns of zinc
22    speciation depending on river flows, seasons, and potential variations in metal and ligand
23    inputs from *in situ* and *ex situ* sources. There were no clear relationships between free zinc
24    ion concentration [$Zn^{2+}$] and measured variables such as DOC concentration, humic and
25    biological indices. Simulations of [$Zn^{2+}$] carried out with both models at high salinities or by
26    inputting site specific complexation capacities were successful, but overestimated [$Zn^{2+}$] in
27    low salinity waters, probably owing to an underestimation of the complexation strength of the
28    ligands present. Uncertainties in predicted [$Zn^{2+}$] are consistently smaller than standard
29    deviations of the measured values, suggesting that the accuracy of the measurements is
30    more critical than model uncertainty in evaluating the predictions.
31

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

2

## 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.

68    Furthermore, the predictive ability of WHAM VII and Visual MINTEQ[14] for Zn speciation was

69    tested and compared. The combination of Zn speciation determination and modeling

70    presented here represents a stepping stone to more effective regulation of this metal within

71    saline environments.

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## 2.    Methods

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### 2.1 Tamar catchment and sampling sites

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75    The Tamar estuary (16 km in length) runs from Gunnislake Weir to the English Channel and

76    comprises two significant tributaries of the Lynher and Tavy (Figure S1).[15] The estuary has

77    been contaminated with metals from a variety of sources including previous mining of arsenic,

78    copper, zinc and lead;[16] from the dockyards and marinas using zinc anodes and antifoulant

79    paints;[17] and effluents from sewage works containing metals as well as potentially complexing

80    ligands (Figure S1).[18] Other sources of metal complexing ligands include spring and summer,

81    phytoplankton blooms in the lower estuary as observed via chlorophyll 'a' measurement in this

82    work (Figure S2) and previously.[19] Five full estuary transects (typically 8 sampling stations)

83    were undertaken between July 2013 and February 2013 covering different seasons with

84    variations in river flow, salinity, and the presence of phytoplankton blooms during the spring

85    and summer months. Not all surveys have a complete data set owing to practicalities and

86    sampling based on trying to achieve a representative range of salinities, which is reflected in

87    the representation of surveys in some graphs and tables.

### 2.2    Chemicals and reagents

88

89    As previously reported[20] all chemicals used were of analytical grade or higher and ultrahigh

90    purity (UHP) water (Elga Process Water, resistivity = 18.2 MΩ cm) and trace metal

91    specification hydrochloric acid (6 M, ROMIL SpA) was used throughout to minimise

92    contamination from Zn. Zn standards were prepared from Romil PrimAg reference solutions.

93    All samples were buffered (pH 7.8 (+/- 0.1) using HEPES buffer (1 M) prepared from N-

94    hydroxyethylpiperazine-N'-2'-ethanesulphonic acid (Biochemical grade, BDH Laboratory

95    Supplies). The competitive ligand; ammonium pyrrolidine dithiocarbamate (APDC; Fisher

96    Scientific) was made up as a stock 1 M solution prior to dilution to 40 and 4 μM for

97    complexation capacity titrations. Samples for total dissolved Zn concentration ($[Zn_{TD}]$) were

98    acidified with hydrochloric acid (6 M, ROMIL SpA).

### 2.3 Sampling protocol

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100   Section S1 (ESI) details the sampling protocol. Prior to use, all sampling and filtration

101   equipment was acid washed (10% HCl) and rinsed with UHP water. Water samples for metal

102   speciation were filtered through 0.4 μm polycarbonate membranes (Whatman Nuclepore

103   Track-Etched) onsite and frozen at -20°C in low density polyethylene bottles (Nalgene) prior

104   to analysis. [$Zn_{TD}$] samples were refrigerated and determined within 48 h at room temperature.

105   Samples for DOC were filtered (0.7 µm GF/F) within 24 h of collection, acidified to ca. pH 2,

106   and refrigerated in glass vials.

107   **2.4 Analytical methods, procedures and calculations**

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

109   **2.4.1    Total dissolved Zn**

110   Prior to [$Zn_{TD}$] determinations, acidified samples were UV irradiated after the addition of

111   hydrogen peroxide (final concentration of 15 mM). Sample pH was adjusted to ca. 6 with

112   ammonia solution (SpA, ROMIL) prior to addition of the HEPES buffer and APDC for

113   determination using voltammetry described in section S2. Certified reference materials

114   (CRMs) were used for every batch of samples. Recoveries were between 90 and 109%,

115   with a typical precision of $\leqslant$ 10% RSD. The limit of detection (LOD) was typically 0.33 nM Zn

116   under optimum conditions.(maximum drop size, stirring speed and 60 s deposition time).

117   **2.4.2    Free Zn ion, complexation capacity and conditional stability constants**

118   Complexation capacity titrations (CCT) were performed using the same procedures as

119   described elsewhere.[20] Briefly, titrations (at pH 7.8 using HEPES buffer) were carried out

120   at two competitive ligand strengths (4 and 40 µM APDC) providing detection windows of

121   $\log \alpha_{\mathrm{ZnAPDC}} =$   $3.01 - 4.59$ and $3.03 - 5.29$, respectively, matchning that expected for

122   estuarine samples. A total of 10 Zn additions were performed on duplicate samples, with

123   overnight equilibration. Each sample aliquot was determined in triplicate by CLE-AdCSV

124   using analytical parameters provided in Section S2.   Data were transformed using a

125   method reported by van den Berg/Ruzic[21,22] to quantify the sample ligand concentration [

126   $L_x$] (in nM), the conditional stability constant of the Zn-natural ligand complexes ($\log K'\mathrm{Zn}_{L_x}$

127   ) and [$Zn^{2+}$], with data reported as nM or $pZn^{2+}$ (the negative logarithm of the Zn

128   concentration, rather than activity).

129

130   High temperature catalytic combustion (Shimadzu TOC V analyser)[23] was used to determine

131   Dissolved Organic Carbon (DOC) with appropriate certified reference materials[20] providing a

132   limit of detection of 4 µM. Characterisation of the DOC was carried out using 3-D fluorimetry

133   with a Hitachi F-4500 FL spectrophotometer. Sigma Aldrich humic acid (55.1% C; Sigma

134   Aldrich, UK) and Nordic aquatic fulvic acid reference material supplied by the International

135   Humic Substances Society (45% C) were used as standards. Although the limitations of using

136   commercial humic acids have been reported,[24] the Sigma Aldrich material is well characterized,

137 available and being terrestrially derived, matched the likely sources of humic material likely to

138 be present in the Tamar catchment and so was considered fit for purpose.

### 2.5 Thermodynamic equilibrium speciation calculations

#### 2.5.1  Visual MINTEQ

141 Calculations were undertaken using the Visual MINTEQ (VM) version 3.1,[14] chemical

142 equilibrium model for the calculation of metal speciation, solubility equilibria, sorption etc. for

143 natural waters. It offers the benefit of a Windows interface and the ability to introduce new

144 ligands (in this case $\log K'_{ZnL_x}$ values and ligand concentrations derived from the CCT field

145 data at two separate artificial ligand strengths) into its database. Input major cation and anion

146 concentrations were generated from determined parameters (salinity, pH) using an ion-pairing

147 model.[25]

148  $[Zn^{2+}]$ predictions were generated using the following input parameters (further default

149 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).

161 All modeling was carried out assuming a pH 7.8 to match the conditions under which the

162 experimental work was carried out and at a fixed temperature of 15 °C. The Davies method

163 was used for activity correction, without other organic ligands present at an assumed

164 thermodynamic equilibrium. Sensitivity analyses (not shown reported) for temperature and pH

165 within VM using natural sample values showed negligible difference in predicted $[Zn^{2+}]$.

#### 2.5.2  WHAM/Model VII

167 WHAM/Model VII was used to predict Zn speciation under the assumption that the dissolved

168 organic matter (DOM) in the estuarine water behaves in the same way as if composed of soil

169 and freshwater fulvic and humic acid extracts in competition with inorganic ligands. WHAM VII

170 comprises two components, the inorganic speciation code WHAM[8] and Humic Ion Binding

171 Model VII, described in detail in elsewhere.[9]

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172    To run the model, the pH used for the laboratory Zn speciation measurements and determined

173    Zn concentrations were applied. Major ion concentrations (except carbonate) were obtained

174    using a combination of salinity and freshwater endmember major ion measurements. Modeling

175    was done under two scenarios of differing carbon dioxide equilibration between the

176    atmosphere and water. In Scenario S1, carbonate speciation was determined using the default

177    equilibria and binding constants, assuming equilibrium with the atmosphere and a partial

178    pressure of $CO_2$ of 400 ppm. In Scenario S2, in order to maintain consistency with typical

179    methods for evaluating the carbonate system in marine environments, $[CO_3^{2-}]$ was calculated

180    using the CO2SYS model[27] with the constants describing the carbonate and sulphate

181    equilibrium with hydrogen ions[28] and refitted as described elsewhere[29,30] respectively. The pH

182    was on the total scale and the total boron concentration from Uppström, (1974). This approach

183    was used recently,[31] implementing the carbonate system with measured conditional stability

184    constants into Visual MINTEQ. Detailed information regarding WHAM VII in section S4.

185    Uncertainty in predictions was modelled using repeated estimates of input measurements and

186    model parameters made using the assumption of a normal or log–normal distribution around

187    the measured or default value, coupled with an absolute or relative error value.[32] Here

188    previously reported error values were applied of: ±0.1 absolute error on measured pH, ±5%

189    relative error on measurements except for DOC, ±9% relative error on DOC, ±1K absolute

190    error on temperature, and ±0.3 absolute error on the intrinsic metal–fulvic binding constant.[32]

191    The number of repeat calculations was 2000 and uncertainties were computed as the 15.9[th]

192    and 84.1[th] percentiles of the population of predicted free ion concentrations. This range of

193    percentiles corresponds to ±1 standard deviations from the mean for a normally distributed

194    dataset.

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## 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.
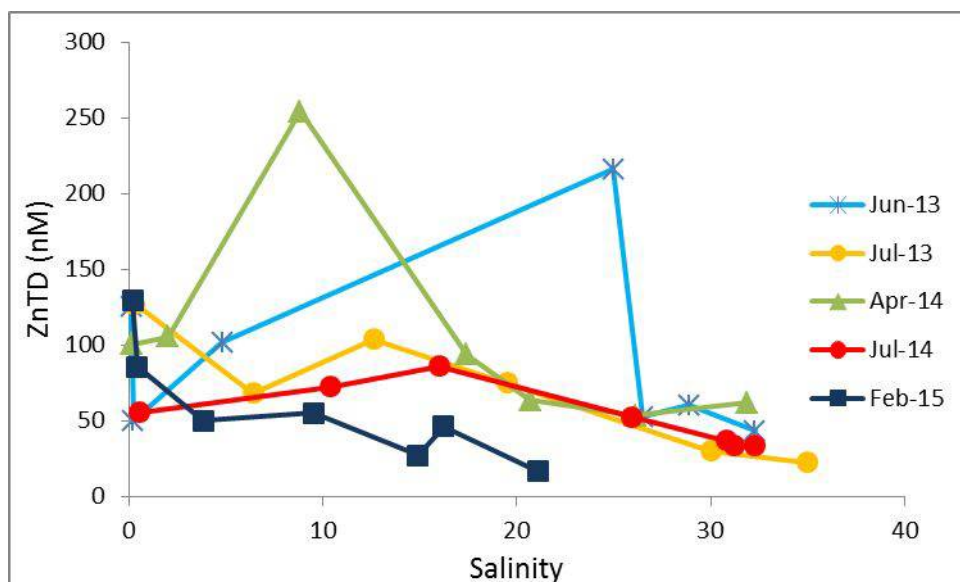


**Figure 1. Total dissolved Zn concentrations determined for the four transects. Note: In April 2015, a sample was taken at low salinity within the estuary, but logistical constraints prevented the sampling of a fresh water endmember in the river.**

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222 **3.2. Zinc speciation and ligand characteristics**

223 Concentrations of $Zn^{2+}$, determined using the two competing ligand strengths, ranged from 0.3

224 – 109 nm (Figure 2) whereby the lowest range of concentrations occurred in July 2013 and

225 the highest in April 2014. These concentrations are of the same order as the very limited data

226 for $[Zn^{2+}]$ provided in literature.[34] The fresh and seawater endmembers contained the lowest

227 $[Zn^{2+}]$ and during all surveys concentrations increased at some location between these two

228 endmembers. Although the trends are broadly similar, results from determinations with the

229 weaker competitive ligand strength (4 µM APDC) returned higher $[Zn^{2+}]$ than determinations

230 using 40 µM APDC. This is a clear indication that differences in the analytical detection window

231 introduce bias into the determination of $[Zn^{2+}]$.

232 Notwithstanding this artefact, in terms of toxicity, $[Zn^{2+}]$ determined in this study are potentially

233 harmful to sensitive aquatic organisms along the whole of the estuary, even at the lower $[Zn^{2+}]$

234 concentrations determined with 40 µM APDC. For example, the growth rate of marine

235 phytoplankton *Synechococcus* sp. has been reported to decline at concentrations of $[Zn^{2+}]$ >

236 0.4 nM and this value is $[Zn^{2+}]$ > 3.2 nM for *Thalassiosira weissflogii*.[35]

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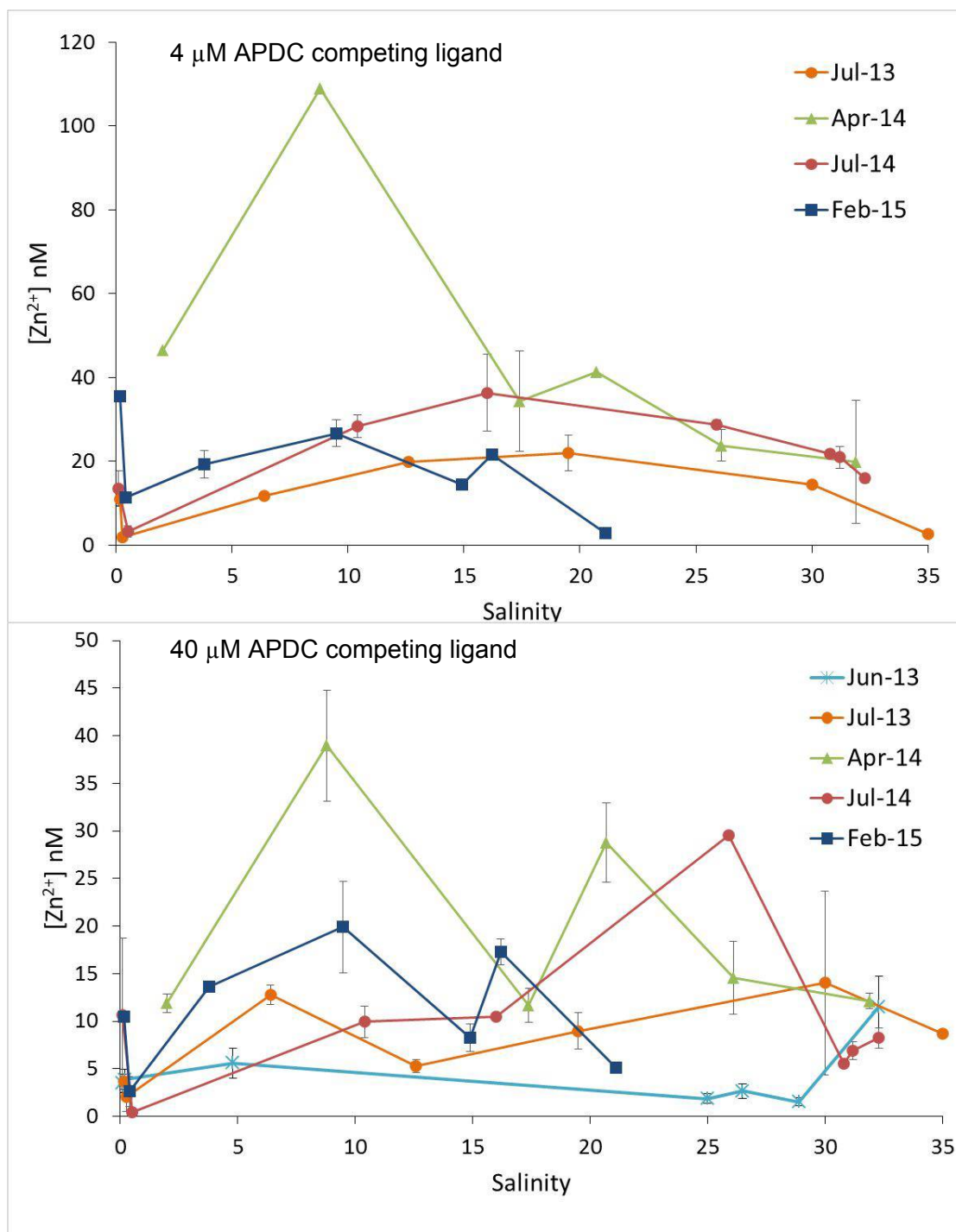


**Figure 2. Free Zn concentrations ([Zn$^{2+}$] determined using various concentrations of APDC) plotted as a function of salinity for the Tamar transects. Each data point is the average of two duplicate analyses (one on each filter fraction) with error bars representing the range. Where error bars are 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

247      (Figure 3), and, to a degree, reflects the observed Lx:$Zn_{TD}$ ratio (Figure S3). Increasing salinity

248      introduces high cation (e.g. calcium, magnesium) concentrations that compete with Zn

249      complexing sites on ligands, therefore raising the potential for more Zn to be less strongly

250      complexed.

251      Concentrations of zinc complexing ligands ([Lx]) between 3 and 412 nM were determined

252      (Figure 3), well within the range reported for other estuarine and coastal studies (5 – 220

253      nM).[34,36] Although some inputs of Zn ligands mid-estuary were apparent in June 2013, April

254      2014 and to some extent July 2014, the highest concentrations, and hence main inputs, were

255      at the FWEM. Zn ligand concentrations, in most cases, tracked the total dissolved metal

256      concentration profiles with salinity, indicating that both the FWEM and tidally induced mid-

257      estuarine sediment processes were sources for $Zn_{TD}$ and Lx (Figures 1 and 3). The degree of

258      Zn complexation varied between detection windows, with higher proportions of [$Zn^{2+}$] present

259      at the weaker analytical competition strength. Variation between surveys was also apparent.

260      In February 2015 the proportion of [$Zn^{2+}$] in samples was high (32 – 49 %) in the mid- and

261      lower estuary, even though $Zn_{TD}$ concentrations were similar to those during other surveys.

262      This reflects the low complexation capacity determined at both APDC concentrations, lack of

263      ligand excess (Figure S3) and low DOC concentrations (31 – 123 µm C) observed during this

264      survey (Table S2).

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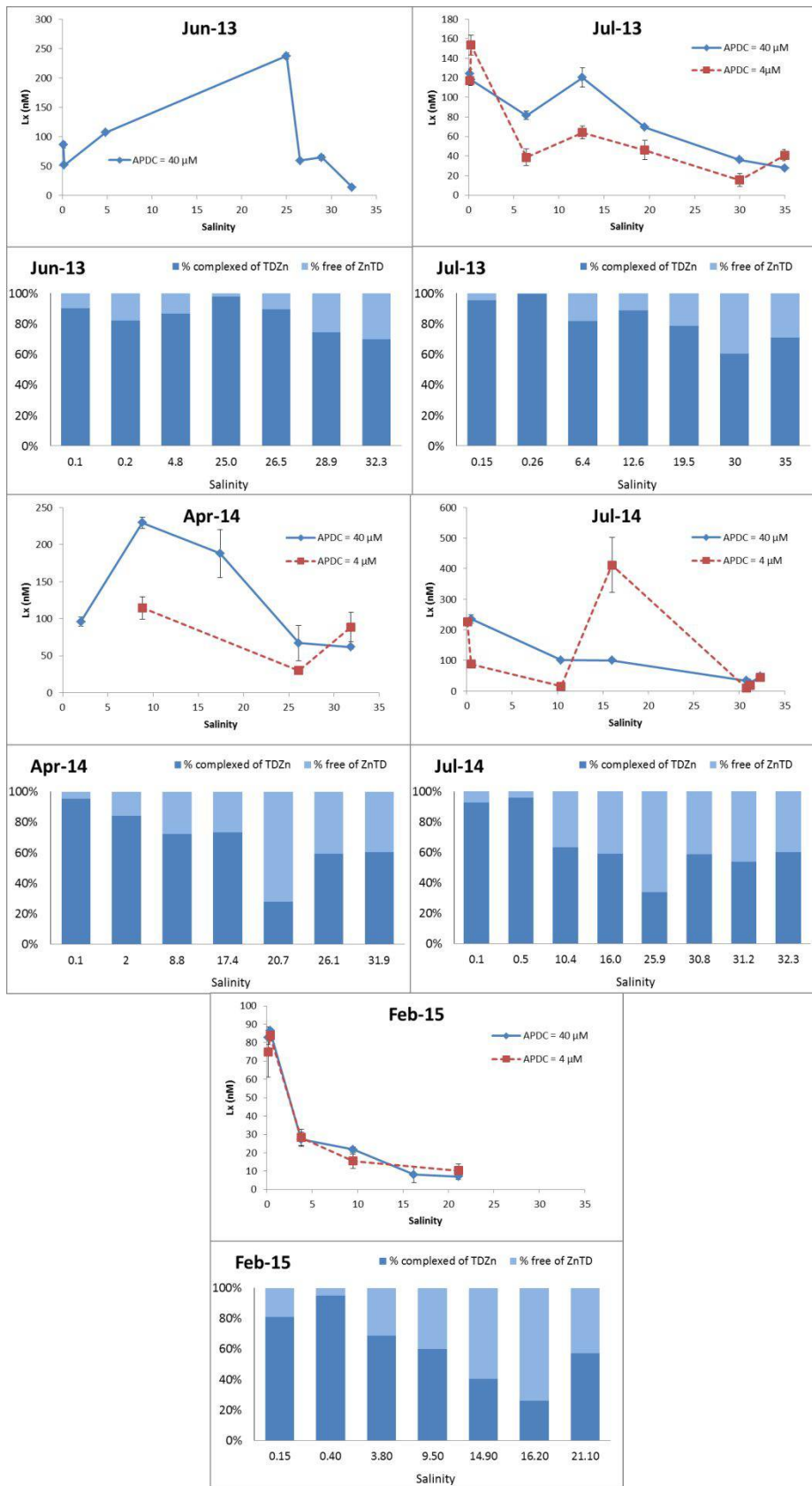
**Figure 3. Ligand concentrations ([Lx]) and proportion of Zn complexed (organically) versus proportion free Zn$^{2+}$ ion as a percentage of total dissolved Zn for each sampling occasion. The x-axes represent salinity in all cases. Error bars on [Lx] plots represent ± an average uncertainty.**

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271    The ratio [Lx]:[$Zn_{TD}$] indicates the significance of $Zn^{2+}$ present in the water, whereby [Lx]:[$Zn_{TD}$]

272    > 1 signifies an excess of Zn complexing ligands and therefore suppression of the more

273    bioavailable and toxic $Zn^{2+}$ species. Saturation of ligands is indicated by [Lx]:[$Zn_{TD}$] < 1, with

274    [$Zn^{2+}$] likely to be more prevalent as the ratio decreases. The ratio showed no relation to salinity

275    in any of the surveys. [Lx]:[$Zn_{TD}$] was consistently low (average 0.5) in February 2015 - only

276    at Cotehele (S = 0.4) did it approach 1 (Figure S3), a profile that indicates ligand saturation

277    throughout the estuary, concomitant with the high proportion of $Zn^{2+}$ observed. In June and

278    July 2013 and April 2014, ratios between 0.4 and 2.1 indicated varied profiles with most

279    samples exhibiting little excess ligand. In July 2014, ratios varied between 0.2 and 4.8, with

280    highest values at Morwellham (4.2, S=0.5) and Cotehele (4.8, S=16), where observed ligand

281    concentrations far outweighed the presence of $Zn_{TD}$. Overall, complexing capacity is exceeded

282    (ratio <1) in 30 of the 53 measurements depending on titration competition strength and time

283    of year, which explains the occurrence of [$Zn^{2+}$] in the samples.

284

285    **3.3 Prediction of [$Zn^{2+}$] in estuarine waters**

286    Measuring [$Zn^{2+}$] within any given estuary provides an indication of possible bioavailable

287    concentrations. However, to develop a BLM or to be able to extrapolate data to other estuaries

288    then the ability to accurately predict the Zn speciation is essential.

289    Figure 4 provides a detailed analysis of VM outputs using the input parameters detailed in the

290    methodology section and Table S3 of the ESI. VM provides three options for converting DOC

291    concentrations to metal complexing ligands, the Nica-Donnan, Gaussian and Stockholm

292    Humic Models). The theory associated with these calculations is provided in S3 of the ESI.

293    For six sites of varying salinity from 0 to 33 where a full dataset was available, the model was

294    run using the three options for predicting metal complexation based on inputted DOC

295    concentration (Gaussian, Nica Donnan and Stockholm Humic Model).

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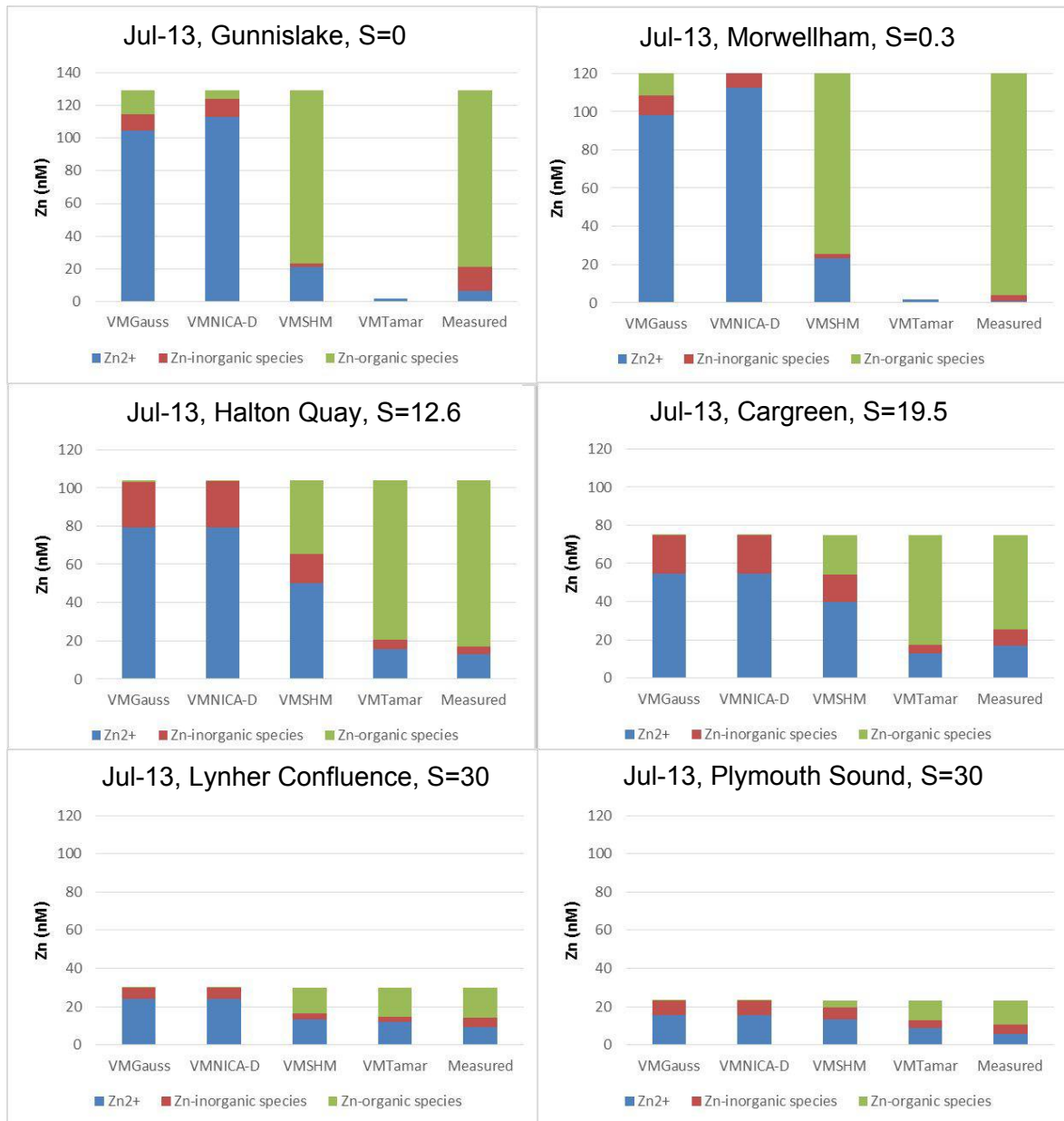


299  **Figure 4. Comparison of measured and predicted Zn speciation (free ion, inorganic and**
300  **organic) using the ligand predicting models within Visual MINTEQ (VM).**
301  **VMGauss = Gaussian model; VMNICA-D = Nica-Donnan model; VMSHM =**
302  **Stockholm Humic Model; VM Tamar = VM inputs of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

305  The distribution of Zn between free ion, inorganic and organically complexed species varied

306  considerably among the different approaches to estimating complexing ligand strength and

307  concentrations. When site-specific measured complexation capacity and ligand concentration

308  were entered into VM, the best fit between measured and calculated $Zn^{2+}$ and organically

309  bound Zn was achieved (Figure 5 and S3). Where only DOC concentration was entered and

310  VM was used to predict Zn complexation using the three options available, $[Zn^{2+}]$ was

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311    overestimated in all samples, compared to measured values. At low to moderate salinities the

312    Gaussian and Nica-Donnan methods significantly overestimated [$Zn^{2+}$], and consequently

313    underestimated the organically-bound Zn. The Stockholm Humic Model (VMSHM) offers a

314    much closer agreement. At higher salinity, total dissolved concentrations and Zn complexing

315    ligands present in the samples were lower, and consequently the fraction of $Zn^{2+}$ increased

316    (Figure 3). The pattern between the different methods of calculating Zn complexation remains

317    the same with only the VMSHM approach predicting significant organic ligand complexation

318    and hence, corresponding reductions in [$Zn^{2+}$].

319    For the WHAM/Model VII, Scenario S2 generates a significantly greater quantity of $ZnCO_3$ and

320    $ZnHCO_3^+$, and average of 21.4 ± 4 nM SD and 21.5 ± 4 nM SD (n = 44), respectively. This has

321    the effect of significantly reducing [$Zn^{2+}$] and bringing it more in line with the measured values

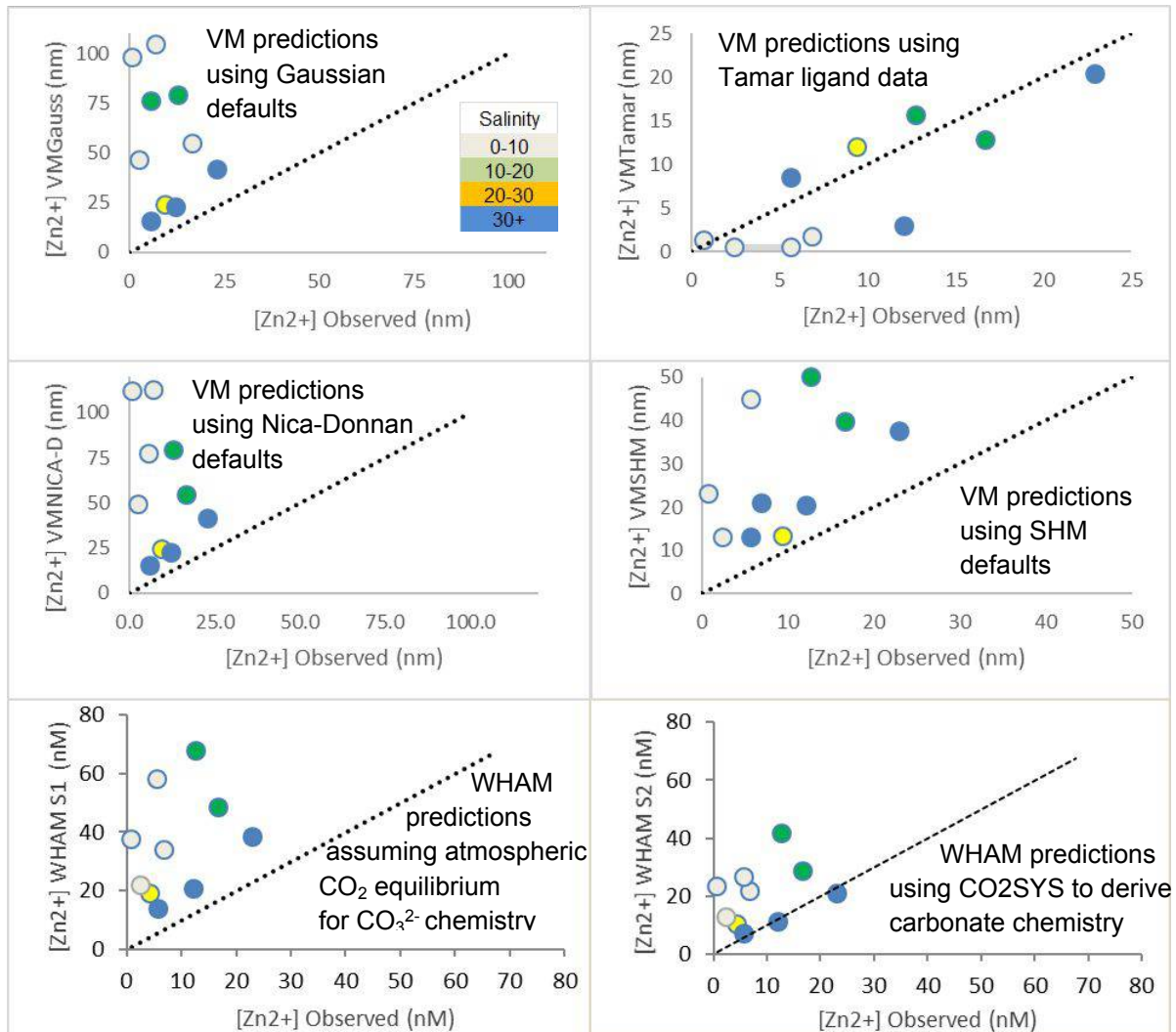322    using voltammetry (Figures 5 and S4).

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

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

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

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

368    Whilst this research shows reasonable predictions can be achieved for estuarine/seawater, it

369    has been shown that as the waters get more saline the trend is for a greater discrepancy

370    between measurement and modeling.[38]

371    Uncertainties for WHAM VII predictions are shown in Figure S6. Predictions include estimates

372    of uncertainty due to uncertainty in measurements and parameters, using the Monte Carlo

373    approach.[9] The prediction uncertainties cover the 15.9–84.1 percentile range of model outputs,

374    equivalent to ±1 standard deviation. Patterns of goodness-of-prediction against salinity are

375    generally similar to those for the other models. Measurements in samples of salinity $\geqslant$ 30‰

376    (n = 4) are reproduced well by the modeling, with all predictions within the ±1 standard

377    deviation range of measurement. In salinities of 1–30‰ ($n$ = 3), predictions are reasonable.

378    Predictions in samples of salinity $\leqslant$ 1‰ underestimate of the observed extent of Zn

379    complexation. Uncertainties in predicted [$Zn^{2+}$] are consistently smaller than standard

380    deviations of the measured values, suggesting that the accuracy of the measurements is more

381    critical than model uncertainty in evaluating the predictions.

382    An important observation is that in terms of potentially using these models for implementing

383    an EQS, that none of the default modes lead to over-prediction of measurements and therefore

384    will be conservatively protective.

385    **3.4 Relationships between free Zn ion and DOC**

386    The results of VM$_{Tamar}$ calculations show that it is possible to use VM to predict the [$Zn^{2+}$] to

387    within an order of magnitude when details for site-specific complexation parameters (ligand

388    concentrations and conditional stability constants of metal-ligand complexes) are entered into

389    the model. This provides a certain degree of confidence in the agreement between the

390    speciation programme outputs for Zn and measured ligand parameters that can be factored

391    into a safety margin with respect to setting EQSs. However, the model outputs for predicted

392    free metal ion concentration based on inputs of DOC concentrations alone gave a poorer

393    prediction of the free ion concentration, and therefore the most potentially bioavailable and

394    toxic metal fraction. The possible reasons for this have been explored further below.

395    DOC concentrations of 30 to 500 µM C in the samples were consistent with those observed

396    previously in the Tamar.[39] In comparison, zinc complexation capacities for the samples were

397    in the region of 10 to 500 nM, typically three orders of magnitude lower (Figure 3). This

398    demonstrates the complexity and challenges associated with attempting to predict the

399    complexing ligands based on such a gross measure of what is a group of compounds with

400    wide variability in physico-chemical characteristics and sources.

401    Plotting [$Zn^{2+}$] versus DOC (Figure 6) shows no specific trends either for the whole dataset or

402    individual sampling occasions. A similar conclusion has been drawn for $Cu^{2+}$ for estuarine

403    samples.[20] The lack of data for Zn speciation in saline waters means there are no data to

404    corroborate these findings, which in itself was a key incentive to undertake this research.

405    Plotting Zn complexation capacity (Lx) against DOC showed a weak positive correlation, but

406    at higher DOC concentrations the data scatter became pronounced. The only firm conclusion

407    which may therefore be drawn is that at low DOC concentrations, complexation capacity is

408    low, with complexation capacity likely to be higher where DOC concentrations exceed 300 µM.

409    For the purposes of predicting [$Zn^{2+}$], there is no obvious relationship that could be applied.

410    The fact that the complexation, and therefore [$Zn^{2+}$], is controlled by both the ligand

411    concentration and its strength, explains the lack of comparability between the plots of [$Zn^{2+}$]

412    and DOC, and [$Zn^{2+}$] and complexation capacity.

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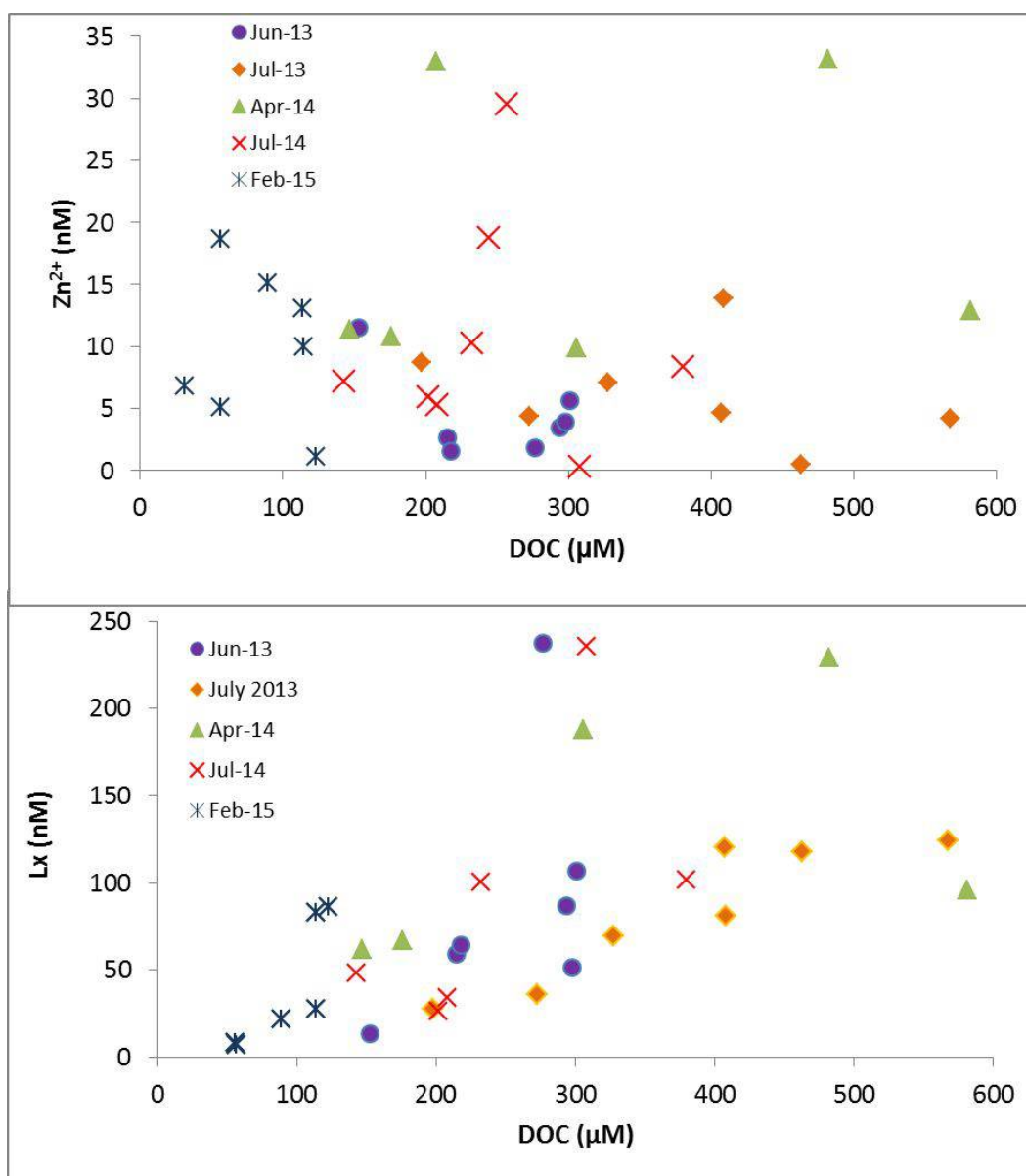
**Figure 6. [Zn$^{2+}$] (top) and Zn complexation capacity (Lx, bottom) versus DOC for the Tamar estuary samples.**

417

**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-

20

427    Em380)/(Ex310-Em430) it has been observed that high ratios (0.8–1.0) corresponded

428    to a predominantly autochthonous origin of DOC from recent aquatic and bacterial

429    activity freshly released into water.[13]

430    In samples from the Tamar surveys, HIX values generally decreased towards the sea

431    water end member (Figure 7, no data for July 2013). Important humic character was

432    indicated for the fresh water endmember (HIX > 10), while DOC generated by *in situ*

433    biological processes increased in importance towards the sea water end member (BIX

434    > 0.7). It is likely that the DOC in the lower estuary was at least partially derived from

435    phytoplanktonic activity and/or sewage effluents from works present in the lower

436    estuary.
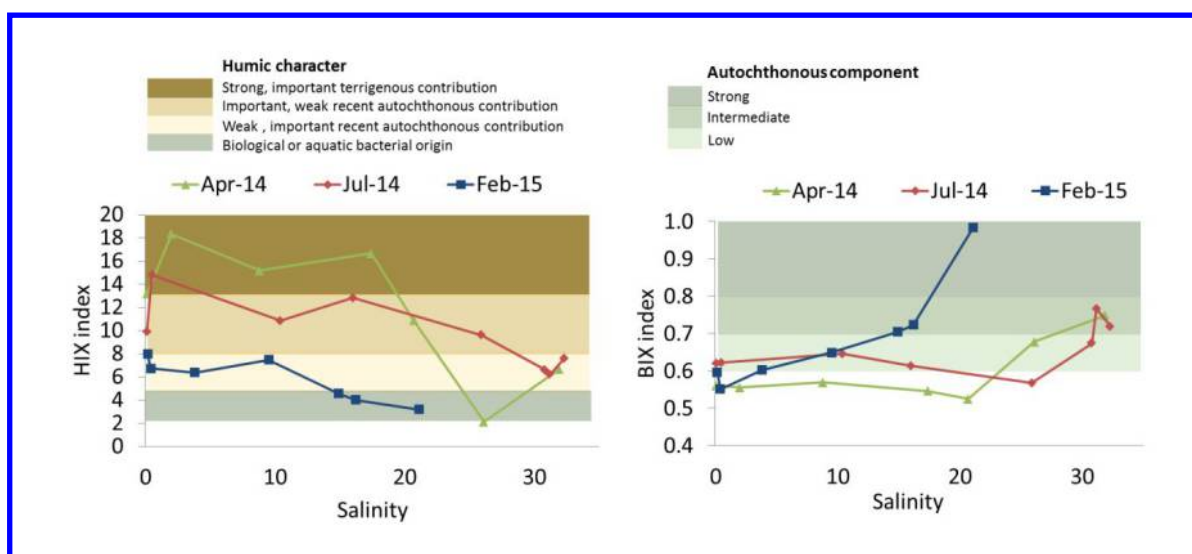


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439    **Figure 7. The humification (HIX) and biological (BIX) indices for each seasonal transect**
440    **against salinity (no data was available for 2013).**

441

442    Upper estuary and riverine HIX indices were > 10 during three surveys, supporting the

443    hypothesis that the DOC was of mainly terrestrial origin, comprising mostly humic and

444    fulvic acids. The BIX index corroborated this, with values increasing towards the sea

445    water end member, demonstrating the autochthonous origin of the DOC present.

446    A plot of [Lx] against HIX and BIX shows weak positive and negative correlations,

447    respectively (Figure S5). Highest HIX values were associated with higher complexation

448    capacities, which suggests a significant affinity of Zn for humic and fulvic acids,[40] which

449    dominate the lower salinity regions in the upper estuary (Figure S5). Any correlation

450    with BIX indices is hampered by the relatively small range of BIX (typically 0.6 to 0.8)

21

451     in the Tamar Estuary. Based on this dataset, it suggests that neither of these indices

452     would be sufficiently robust to improve greatly on the existing use of DOC concentration

453     as a surrogate for Zn complexation characterization.

454     This result corroborates the findings that there is no apparent simply defined relationship

455     between DOC concentration and [$Zn^{2+}$] and lends support to the argument that assuming a

456     fixed "active" portion (50 %) of DOC in a model such as WHAM or VM may be inappropriate,

457     at least in regards to [$Zn^{2+}$]. VM has been reported to consistently underestimate free Cu ion[41]

458     by between 2 and 5 orders of magnitude in comparison to measured values (in the range $10^{-12}$

459     $-$ $10^{-14}$ M) which are potentially controlled via multiple ligand sources at varying

460     concentrations as discussed earlier. A key issue is that whether optimized ratios of measured

461     DOC to 'active' FA fall within previously observed ranges. If not then this might suggest a role

462     for ligands stronger than those humics can provide. The modeling using default data

463     consistently overestimates [$Zn^{2+}$], which suggests that there may be strong specific ligands in

464     the estuary.

465     In the case of the over-prediction of [$Zn^{2+}$] by VM, the effect of synthetic ligands present in

466     sewage effluent discharged to natural waters, such as ethylenediaminetetraacetic acid (EDTA)

467     is not accounted for in the model, were discounted as a possible cause. Stockdale et al.,[1]

468     tested EDTA concentrations within the WHAM/Model VII as low as $5 \times 10^{-8}$ M and there was

469     a small but noticeable effect for Zn (no effect for Cd and a greater effect for other metals) at

470     this low concentration. Although concentrations of 0.1 and 1 µM EDTA caused a considerable

471     reduction (63% and 96% respectively) in calculated [$Zn^{2+}$], these effective EDTA

472     concentrations are unlikely to exist in the Tamar estuary. Although not quantified in this study,

473     significant dilution of EDTA from the likely predominant sources (Ernesettle, Central, Marsh

474     Mills and Camels Head WwTW, serving a combined population of 290,000) near the mouth of

475     the estuary is probable. If the sewage effluent discharge in the Tamar is estimated at 72 million

476     litres a day,[42] setting this against an average river discharge of 2333 million litres a day[43]

477     equates to a 32 times dilution on river flow alone, without allowing for seawater flushing of the

478     estuary. Based on recently published median effluent EDTA concentrations of 0.44 µM[44] such

479     a dilution would reduce the ETDA concentration to well below the effective concentration of

480     ligands observed in this study. This does not exclude the possibility there are other

481     anthropogenic synthetic ligands present within the estuary unaccounted for, but given the

482     strength and typically high concentrations of EDTA present in effluents which have been

483     discounted above, it seems unlikely more powerful ligands may be present at sufficient

484     concentrations to impact on the Zn speciation.

485    Overall, the data presented here for Zn speciation along transects of the Tamar estuary were

486    carried out to investigate the influence of dissolved organic ligands over the course of a

487    calendar year, with the aim of attempting to model Zn speciation based on a limited dataset.

488    It was not possible to attribute observed trends in metal speciation to any single measurable

489    physico-chemical parameter, which was unsurprising as the complexity of the estuarine

490    environment means observations are the result of a combination of many factors which are

491    subject to constant change. In cases where rainfall has been abnormally high (e.g. February

492    2015 survey), the expected trends and concentrations of constituents (e.g. DOC) can change

493    dramatically, and therefore, mixing and physico-chemical parameters, such as turbidity, are

494    likely the more important controls on speciation, rather than time of year.

495    Ligand abundance and excess, type, and binding strength appear to be important factors in

496    controlling the proportion of complexed metal. Although these parameters are measurable

497    with laboratory instrumentation there is no simple relationship between these factors and

498    easily determined variables such as salinity, DOC or even further characterization of the DOC

499    present using 3-D fluorimetry.

500    Modeling using VM generated reasonably accurate estimates of $Zn^{2+}$, provided site specific

501    values for ligand strength and concentration were entered. Inputting DOC concentration and

502    allowing the model's in-built algorithm to estimate complexation capacity generated over-

503    estimates of $Zn^{2+}$, particularly at low salinities, where default fulvic acid log K assumptions

504    appear to under-estimate complexation. A similar trend was observed for the WHAM VII model

505    with much improved agreement between predicted and observed free metal ion at higher

506    salinities, and uncertainties of measured values exceeding those of predictions.

507    Although models generating over-estimates of the more toxic Zn species is a conservative

508    approach to risk assessments, to develop BLMs for estuarine waters it is necessary to be able

509    to accurately predict free metal ion concentrations. The Tamar data presented here for Zn

510    speciation (which are scarce in comparison with Cu speciation data) for the first time provide

511    vital metal-ligand complexing strengths and ligand concentrations detected at various

512    competitive ligand strengths across full salinity ranges which may be used for future modeling

513    and regulatory purposes.

518 Tamar, Dr. Aga Kosinska and David Deruytter for assistance in the laboratory and Dr. Alan

519 Tappin for DOC analysis.

## Supporting Information

521 The following material is supplied in the Supporting Information:

522 • Sample collection and storage

523 • Detailed analytical methodology

524 • Visual Minteq modeling

525 • WHAM modeling

526 • Additional figures and tables

527

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