

1 **Equilibrium calculations of iron speciation and apparent iron solubility**
2 **in the Celtic Sea at ambient seawater pH using the NICA-Donnan**
3 **model**

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32 **Abstract**

33

34 We used a combined ion pairing - organic matter speciation model (NICA-Donnan) to predict the
35 organic complexation of iron (Fe) at ambient pH and temperature in the Celtic Sea. We optimized our
36 model by direct comparison with Fe speciation determined by Adsorptive Cathodic Stripping
37 Voltammetry using the added Fe-binding ligand 1-nitroso-2-naphthol (HNN) in the presence and
38 absence of natural organic matter. We compared determined Fe speciation with simulated titrations
39 obtained via application of the NICA-Donnan model with four different NICA parameter sets
40 representing a range of binding site strengths and heterogeneities. We tested the assumption that
41 binding sites scale to dissolved organic carbon (DOC) concentrations in marine waters. We found
42 that a constant low DOC concentration resulted in an improved fit of our titration data to the
43 simulated titrations, suggesting that inputs of autochthonous marine DOM may not increase the
44 heterogeneity or concentrations of Fe binding sites. Using the optimal parameter set, we calculated
45 $pFe(III)' (-\log(\sum Fe(OH)_i^{3-l}))$ and apparent Fe(III) solubility ($SFe(III)_{app}$) at ambient pH and
46 temperature in the water column of the Celtic Sea. $SFe(III)_{app}$ was defined as the sum of aqueous
47 inorganic Fe(III) species and Fe(III) bound to DOM formed at a free Fe (Fe^{3+}) concentration equal to
48 the limiting solubility of Fe hydroxide ($Fe(OH)_3(s)$). $SFe(III)_{app}$ was within range of the determined
49 dissolved Fe concentrations observed after winter mixing on the shelf and in waters >1500 m depth at
50 our most offshore stations. Our study supports the hypothesis that the ocean dissolved Fe inventory is
51 controlled by the interplay between Fe solubility and Fe binding by organic matter, although the
52 overall number of metal binding sites in the marine environment may not be directly scalable to DOC
53 concentrations.

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60 **Keywords:** trace metals, ocean acidification, intrinsic binding constants.

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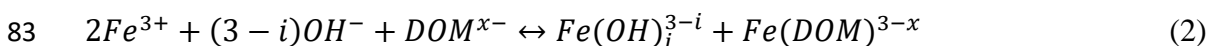
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64 **Introduction**

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66 Iron (Fe) is an essential micronutrient for marine phytoplankton growth, and its low supply and
67 solubility limits primary productivity in large parts of the world's ocean (Boyd and Ellwood, 2010).
68 Iron limitation mostly occurs in high-nitrate, low-chlorophyll (HNLC) regions, which make up
69 approximately 30% of the surface ocean (Boyd et al., 2007). However, both Fe limitation and the
70 potential for seasonal Fe limitation have also been reported for coastal regions and shelf seas,
71 including European shelf seas (Birchill et al., 2017; Hogle et al., 2018; Hutchins & Bruland, 1998).
72 The bioavailability and solubility of Fe in seawater is a function of its chemical speciation (Boyd &
73 Ellwood, 2010; Gledhill & Buck, 2012; Hutchins et al., 1999). Inorganic Fe(III) is the
74 thermodynamically favoured form of Fe in oxygenated seawater but, as a result of hydrolysis
75 (equation 1), it has a low solubility that reaches a minimum between pH 7 and 9 (Byrne et al., 2000;
76 Kuma et al., 1996; Liu & Millero, 2002). Hydrolysis competes with binding by organic matter
77 (equation 2), thus complexation by dissolved organic ligands (i.e. those <0.2 µm in size) has the
78 potential to reduce free Fe³⁺ concentrations and consequent formation of insoluble iron hydroxides
79 (Fe(OH)₃(s)) and thereby increase the concentration of Fe(III) observed in the dissolved fraction
80 (<0.2 µm) (Kuma et al., 2000, 1996; Liu and Millero, 2002).

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85 Reduction to Fe(II), via e.g. photolysis or biological activity, can also change Fe speciation,
86 potentially increasing both the bioavailability and solubility of Fe (Barbeau, 2006; Rose and Waite,
87 2005; Schlosser et al., 2018). Complexation by organic matter, hydrolysis, and redox speciation thus
88 all play important roles in ocean Fe biogeochemistry, and as a result the global Fe cycle is influenced
89 by ocean acidification, water column stratification, warming and deoxygenation (Hutchins & Boyd,
90 2016). Given the role of Fe as an essential micronutrient, there is thus a need to develop reliable
91 approaches that can be used to predict the impact of environmental change on oceanic Fe speciation
92 and biogeochemistry (Ye et al., 2020). Ideally, such approaches would be based on a set of intrinsic
93 (i.e. independent of the physico-chemical characteristics of the water sample such as temperature, pH
94 and ionic strength) thermodynamic and kinetic constants that would describe the chemical speciation

95 and rates of reaction of all Fe species in seawater according to ambient temperature, salinity and pH
96 (Turner et al., 2016; Ye et al., 2020).

97

98 With respect to Fe(III) speciation in seawater, the work of Liu and Millero (1999) and Byrne et al.
99 (2000) has provided a set of intrinsic thermodynamic constants that describe Fe hydrolysis and the
100 formation of fresh Fe(III)-hydroxide colloidal precipitates (retained on a 0.02 μm filter). In contrast,
101 for organic complexation, determination of metal speciation in seawater has traditionally adopted an
102 approach where the observed strength and concentrations of metal-binding ligands were related to
103 specific conditions of the sample (i.e. salinity, dissolved Fe concentration) and analysis (i.e. pH
104 typically 8.0-8.2 depending on the method employed). Ocean sections of conditional ligand
105 concentrations published as part of the GEOTRACES research programme (Buck et al., 2018, 2015;
106 Gerringa et al., 2015) showed that, at pH 8 and room temperature, average conditional ligand
107 concentrations range from 1-2 equivalents of Fe binding sites (nmol L^{-1}), and typically correlate with
108 dissolved Fe concentrations, exceeding them by an average of ca.1 equivalents of Fe binding sites
109 (nmol L^{-1}) (Caprara et al., 2016). This covariance can at least partially be explained by application of
110 analytical experimental designs and mathematical transformations that simplify a heterogeneous
111 group of binding sites to an “average” site that can be observed under the applied experimental
112 conditions (for further information see e.g. Gledhill and Buck, (2017); Town and van Leeuwen,
113 (2005)). Thus, whilst the conditional approach demonstrates that organic complexation is important
114 for the biogeochemistry of Fe, the conditional nature of the obtained results constrains our ability to
115 predict how Fe(III) speciation is likely to change in a future ocean, since it provides no mechanistic
116 knowledge of how Fe(III) binding to organic matter is influenced by pH or temperature.

117

118 Exactly how Fe(III) binding to organic matter changes as a function of pH depends on the functional
119 group characteristics of the metal binding components of marine dissolved organic matter (DOM)
120 (Shi et al., 2010; Zhang et al., 2019). Dissolved organic matter is a highly diverse mix of compounds
121 (Koch et al., 2008) that will also potentially change in a future ocean (Lønborg et al., 2020). Metal
122 binding components likely make up only a minor subset of the overall DOM pool (Zhang et al.,
123 2019). Previous studies have shown that bacteria and phytoplankton can release Fe binding ligands,
124 including siderophores and polysaccharide exudates into their environment (Hassler et al., 2011a;
125 Hassler et al., 2011b; Mawji et al., 2011; Vraspir and Butler, 2009). In addition, ligands can be
126 released following viral lysis (Poorvin et al., 2011) or delivered by terrigenous sources in the form of

127 humic-like substances (Laglera et al., 2019; Muller, 2018). Terrigenous DOM has furthermore been
128 shown to dominate Fe binding in certain oceanic regions like the Arctic Ocean (Laglera et al., 2019;
129 Slagter et al., 2019; Sukekava et al., 2018). The organic ligand pool thus shows an intrinsic chemical
130 heterogeneity, which is still not well understood (Gledhill and Buck, 2012), but is likely analogous to
131 metal binding to natural organic matter in terrestrial and freshwater environments (Lodeiro et al.,
132 2020).

133

134 Binding models for describing metal binding to organic matter using intrinsic constants that account
135 for heterogeneity are widely applied in terrestrial and freshwater environments. Perhaps the most
136 widely used models are the Non-Ideal Competitive Adsorption (NICA)-Donnan model (Kinniburgh
137 et al., 1999), Windermere humic acid model (WHAM) (Tipping et al., 2011), and Stockholm humic
138 model (SHM) (Gustafsson, 2001). A primary assumption in these models is that binding sites scale
139 proportionally to the concentration of dissolved organic carbon (DOC; ‘dissolved’ in this context is
140 typically defined as $<0.7 \mu\text{m}$ in size). The appeal of such an approach lies in the potential for
141 describing the influence of Fe(III) binding to organic matter as a function of ambient pH and DOC
142 concentrations, using a limited set of constants that could be applied to the estimation of Fe
143 speciation across the whole ocean (Hiemstra and van Riemsdijk, 2006; Stockdale et al., 2016).

144 Indeed, a step in this direction has recently been made in Ye et al. (2020), where the NICA-Donnan
145 model has been used to parameterise the impact of future changes in ocean pH on ocean productivity
146 in a global biogeochemical model. The NICA-Donnan model describes the binding behavior of metal
147 ions to a heterogeneous mix of binding sites using a continuous bimodal distribution based on the
148 Langmuir-Freundlich adsorption isotherm (Kinniburgh et al., 1999), while both the WHAM and
149 SHM models rely on a set of empirically derived relationships and a set number of binding sites with
150 different affinities to calculate metal speciation (Gustafsson, 2001; Tipping et al., 2011). A further
151 key difference between the three approaches relates to the application of electrostatic sub-models to
152 describe the impact of ionic strength on binding of metals to the organic matter phase. In the NICA-
153 Donnan model, the Donnan component is used to describe non-specific electrostatic interactions on
154 metal binding to DOM, while the SHM model uses the Basic Stern model (Gustafsson, 2001) and
155 WHAM uses a correction based on the Debye-Hückel and Gouy-Chapman theory (Tipping et al.,
156 2011). All three approaches have been successfully used to predict metal speciation in seawater
157 (Avendaño et al., 2016; Hiemstra & van Riemsdijk, 2006; Ndungu, 2012; Stockdale et al., 2011,

158 2015; Tipping et al., 2016). However, since a direct intercomparison study has yet to be undertaken,
159 it is not known if one model is superior to the others in seawater applications.

160

161 In order to further test the applicability of such heterogeneous models to Fe(III) speciation in the
162 marine environment, we wished to examine predicted and observed relationships between DOC
163 concentrations and Fe speciation in more detail. In this study, we tested the underlying assumption
164 that Fe speciation determined with a given set of intrinsic NICA constants could be scaled to DOC
165 concentrations, at least within the range of DOC concentrations typically observed in marine waters.
166 In coastal waters, average DOC concentrations are ca. 300 $\mu\text{mol L}^{-1}$ because of enhanced
167 productivity or localized DOC inputs from terrestrial sources (Barrón and Duarte, 2015), while in the
168 open ocean DOC concentrations are lower and vary by at most a factor of two (40-80 $\mu\text{mol L}^{-1}$,
169 Hansell, 2013). Since DOM composition changes with DOC concentration (Hansell, 2013), we also
170 implicitly tested a second assumption, that the changes in DOM composition resulting from
171 microbial production and utilization of organic matter does not significantly impact the binding
172 properties of DOM when expressed relative to DOC concentrations. We used samples collected on
173 three cruises in the Celtic Sea during three different seasons. The Celtic Sea is a productive,
174 temperate sea located on the northwest European shelf (Carr et al., 2018; Muller-Karger et al., 2005).
175 Our three cruises transected from a productive shelf environment out to the open ocean and our
176 samples therefore incorporated a range of DOC concentrations and DOM compositions from
177 autochthonous marine DOM produced during phytoplankton bloom conditions to aged DOM from
178 deep waters (>500 m). We compared the concentrations of observed Fe species to those predicted
179 using four sets of NICA constants representative of different degrees of heterogeneity and overall
180 binding strength. Two sets of NICA constants were previously described in the literature (Gledhill et
181 al., 2015; Hiemstra and van Riemsdijk, 2006) and two sets were re-derived from raw titration data
182 obtained in a previous study in our region (Avendaño et al., 2016) and were thus more specific to
183 DOM in our research area. We used the NICA-Donnan model to calculate the equilibrium speciation
184 of Fe(III) at ambient pH and temperature in our region. We estimated the impact of Fe bound to
185 organic matter on the inorganic Fe fraction (Fe^{inorg}) in our study region. Since Fe(III) solubility is also
186 directly related to Fe speciation, we also examined the saturation state of Fe^{3+} with respect to
187 $\text{Fe}(\text{OH})_3(\text{s})$ at ambient pH and temperature in our study region by calculating the apparent Fe(III)
188 solubility ($\text{SFe}(\text{III})_{\text{app}}$). We define $\text{SFe}(\text{III})_{\text{app}}$ as the sum of aqueous inorganic Fe(III) species and
189 Fe(III) bound to DOM formed at a free Fe (Fe^{3+}) concentration equal to the limiting solubility of Fe

190 hydroxide ($\text{Fe}(\text{OH})_3(\text{s})$; Zhu et al., 2021). We discuss the observed trends in the context of observed
191 total dissolved Fe concentrations in order to understand the relative importance of different physico-
192 chemical drivers that influence Fe speciation.

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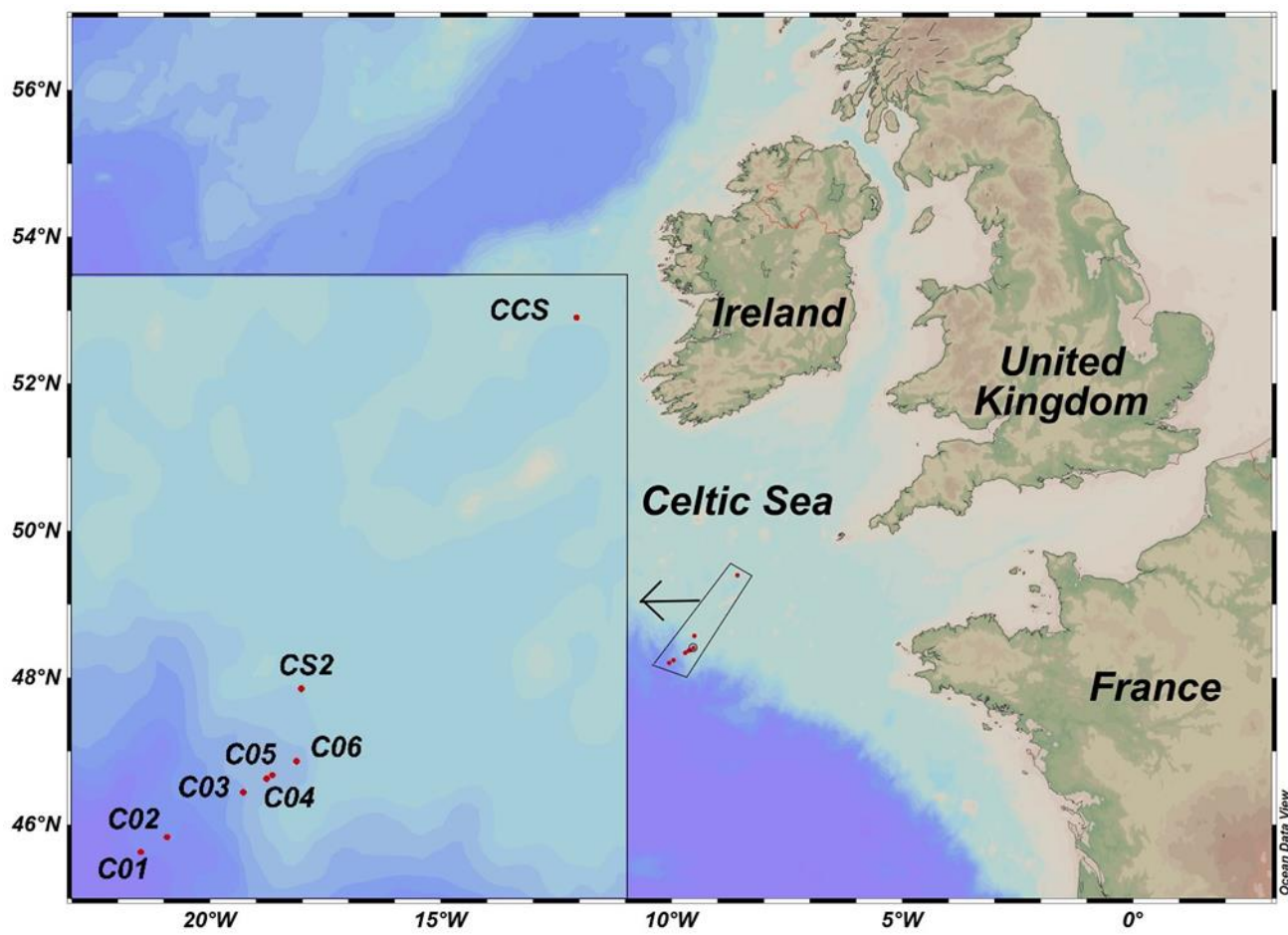
194 **Materials and Methods**

195

196 Sampling

197 Samples were collected during three cruises: DY018 in autumn (November 2014), DY029 in spring
198 (April 2015) and DY033 in summer (July 2015) in the Celtic Sea on board the RRS Discovery as part
199 of the UK Shelf Sea Biogeochemistry programme (Birchill et al., 2017; Rusiecka et al., 2018). Here,
200 we examine Fe speciation at the central Celtic Sea site (CCS), a shelf edge station (CS2) and an off-
201 shelf transect through a submarine canyon (C01-06) (Figure 1). Salinity, depth and temperature were
202 measured using a Seabird CTD attached to a titanium rosette frame equipped with 24 x 10 L Ocean
203 Test Equipment bottles (Birchill et al., 2017). Trace metal samples were collected following
204 GEOTRACES protocols (Cutter et al., 2017). Samples for the determination of Fe speciation were
205 filtered (0.2 μm cartridge filters; Sartobran-300, Sartorius) into acid-cleaned 250 ml low density
206 polyethylene (LDPE) bottles (Nalgene) and frozen immediately ($-20\text{ }^\circ\text{C}$). Samples were subsequently
207 analyzed in a trace metal clean laboratory at GEOMAR.

208



209

210 *Figure 1. Map of study area with stations indicated by red dots. Map generated using Ocean Data View*
 211 *(Schlitzer, 2015).*

212

213 Determination of dissolved Fe, dissolved organic carbon and pH

214 Samples for DFe analysis were collected after filtration through 0.2 µm cartridge filters. The samples
 215 were stored in acid cleaned LDPE bottles (Nalgene) and acidified to pH 1.7 (0.024 mol L⁻¹ HCl,
 216 Romil-UpA). Dissolved Fe concentrations were determined using flow injection with
 217 chemiluminescence detection (Birchill et al., 2017; Obata et al., 1993). The accuracy and analytical
 218 uncertainty of the method was assessed by applying the top down NordtestTM approach to the
 219 analysis of SAFe and GEOTRACES consensus materials, the combined uncertainty was calculated to
 220 be 9.5 % (Worsfold et al., 2019).

221

222 Samples for the determination of DOC were collected after filtration (ashed glass fibre filters, 0.7 µm
 223 nominal pore size, Whatman), and acidified to pH 2 using hydrochloric acid. The DOC samples were
 224 analyzed onshore using high temperature catalytic oxidation on a Shimadzu TOC-VCPN. Consensus

225 reference materials (CRM; University of Miami) were used to determine accuracy and precision of
226 analysis daily, which were both better than 4 %.

227

228 Samples for dissolved inorganic carbon (C_T) and total alkalinity (A_T) were collected via silicone
229 tubing into 250 ml borosilicate glass bottles following established protocols (Dickson, 2010). For the
230 off-shelf transect, samples for C_T and A_T were collected only during DY018 and DY033. Each bottle
231 was sealed shut with a greased ground glass stopper after introducing a 2.5 ml air headspace and
232 sterilising the sample with 50 μ l of saturated mercuric chloride solution. All samples were stored in
233 the dark until analysis with VINDTA 3C instruments (Marianda, Germany). The C_T and A_T
234 measurements were calibrated using measurements of certified reference material obtained from Prof
235 A. G. Dickson (Scripps Institution of Oceanography, USA) (Humphreys et al., 2019). The pH of our
236 seawater samples was calculated on the IUPAC/NBS scale (pH_{NBS}) from C_T and A_T using CO2SYS
237 (Pierrot et al., 2006). In CO2SYS, the constants describing the carbonate and sulphate equilibrium
238 with hydrogen ions were from Mehrbach et al. (1973) (refitted by Dickson and Millero (1987)) and
239 Dickson, (1990), respectively, and the total boron concentration was estimated from salinity
240 following Uppström, (1974). We used the NBS pH scale because it is consistent with the speciation
241 constants in the applied NICA-Donnan and ion pairing models.

242

243 Determination of iron speciation via adsorptive cathodic stripping voltammetry

244 Iron speciation was determined by competitive ligand equilibrium with adsorptive cathodic stripping
245 voltammetry (CLE-AdCSV), using 1-nitroso-2-naphthol (HNN) as the added ligand (van den Berg,
246 1995). HNN (Sigma-Aldrich) was diluted in methanol (Fisher, HPLC grade) to make a stock
247 solution. To clean the stock buffer solution of N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic
248 acid) (HEPES; Sigma-Aldrich), HNN was added and equilibrated with the buffer overnight. HNN
249 and $FeNN_3$ were subsequently removed using a pre-activated C18 SepPak column (Whatman). The
250 pH_{NBS} of the buffer solution was adjusted to 8 prior to the titration work with ammonium hydroxide
251 (20-22%) (Optimal, Fisher Scientific), and the pH_{NBS} of each buffered sample was determined to be
252 between 7.9 and 8.1, with an overall average of 8.00 ± 0.08 (n=93).

253

254 Since the speciation measurements are thermodynamic, it is important that voltammetric peaks are
255 stable and equilibrium is achieved (Laglera & Filella, 2015; Van Leeuwen & Town, 2005). In
256 previous studies, a reaction time of > 6 h was assumed to be sufficient to reach equilibrium

257 conditions (Avendaño et al., 2016; Boye et al., 2001; Boye et al., 2003; Gledhill & van den Berg,
258 1994). However, Wu and Luther (1994, 1995) waited 24 h to reach the equilibrium condition
259 between FeL (i.e. Fe bound to natural ligand) and HNN. Here, we tested equilibration time prior to
260 analyzing Fe speciation in our seawater samples. Our test indicated that a reaction time > 12h was
261 needed to obtain consistent, reproducible peak heights, which we took to approximate equilibrium
262 conditions between FeL and HNN for our method and we therefore allowed for a 16 h equilibration
263 period.

264

265 Our speciation measurements are based on establishing an equilibrium between HNN, Fe³⁺, binding
266 sites (L⁻) and the remaining inorganic Fe species (e.g. hydroxides) in the solution. The ratio of free to
267 complexed species gives the side reaction coefficient (α) for the reaction (Ringbom and Still, 1972),
268 which is also related to the conditional stability constant ($k_{FeNN_3,Fe^{3+}}^{cond}$) and the concentration of
269 ligand not bound to Fe ([NN⁻]), as shown for the formation of FeNN₃ in equation (3).

270

$$271 \frac{[FeNN_3]}{[Fe^{3+}]} = \alpha'_{FeNN_3,Fe^{3+}} = k_{FeNN_3,Fe^{3+}}^{cond} \times [NN^-]^3 \quad (3)$$

272

273 Species can only compete when their side-reaction coefficients are within an order of magnitude of
274 each other, hence ligands detectable in a CLE-AdCSV titration are restricted to those with side
275 reaction coefficients (α'_{FeL}) within this “detection window” (Apte et al., 1988; Hudson et al., 2003;
276 Nimmo et al., 1989; Voelker and Kogut, 2001). However, there may be a considerable range of
277 ligand strengths in seawater and the use of at least two detection windows has previously been
278 recommended to ensure the full range of ligand strengths can be accounted for (Buck et al., 2012;
279 Pižeta et al., 2015; Sander et al., 2011). We therefore used three different total HNN concentrations,
280 [HNN_T] = 1, 5 and 20 μmol L⁻¹.

281

282 We combined our different HNN concentrations with seven different Fe additions between 0 and 5
283 nmol L⁻¹ at the two lower HNN concentrations (1 and 5 μmol L⁻¹) and 3 concentrations (5, 10, 15
284 nmol L⁻¹) at the highest HNN concentration (20 μmol L⁻¹) to create a matrix of 18 titration points. All
285 titration data for one sample were obtained on the same day. Our aim was to estimate the slope using
286 our highest HNN concentration and calculate [FeNN₃] according to the “overload titration” method
287 (Kogut and Voelker, 2001). Examination of the sensitivity observed for each HNN concentration in
288 seawater in our samples at Fe concentrations ≥ 3 nmol L⁻¹ showed no significant difference between

289 sensitivity at 5 and 20 $\mu\text{mol L}^{-1}$ HNN (details in supplementary information, Figure S1). On the other
290 hand, the titration point with the highest added Fe concentration (15 nmol L^{-1}) was often lower than
291 expected, suggesting non-linearity in the titration at higher Fe concentrations, possibly caused by
292 adsorption of the hydrophobic FeNN_3 complex on the walls of the voltammetric cell (Supplementary
293 Figure S2). We thus used the data with 5 and 20 $\mu\text{mol L}^{-1}$ HNN and added Fe concentrations from 3
294 to 10 nmol L^{-1} to calculate the sensitivity of our analysis and determine the FeNN_3 concentration.

295
296 The concentration of HNN not complexed by Fe ($[\text{NN}^-]$) and the conditional stability constant
297 ($k_{\text{FeNN}_3, \text{Fe}^{3+}}^{\text{cond}}$) of the FeNN_3 complex were used to derive the free Fe^{3+} concentrations in the sample at
298 the fixed titration pH_{NBS} of 8.0 over the range of Fe concentrations according to equation (3). Since
299 $[\text{HNN}_T] \gg [\text{Fe}]$, we assumed that $[\text{HNN}_T] = [\text{NN}^-]$. The cumulative random error for Fe^{3+} is largely
300 dependent on the random error in the FeNN_3 concentration, as the 95 % confidence interval for the
301 estimation of $k_{\text{FeNN}_3, \text{Fe}^{3+}}^{\text{cond}}$ was 0.2 % of the determined value (see results). We estimated an average
302 analytical precision for our determined FeNN_3 concentrations of 9 % based on the mean variability of
303 observed peak areas. However, we note this estimate does not account for errors incurred during
304 calculation of the sensitivity, which will result in an additional random error between titrations, or the
305 potential increase in error that is likely to occur as peak heights decrease.

306
307 The difference between the total Fe present in the solution and $[\text{FeNN}_3]$ were used to determine the
308 non-labile dissolved Fe concentration (DFe^*):

$$309 \quad \text{DFe}^* = [\text{TFe}] - [\text{FeNN}_3] \quad (4)$$

310
311
312 where $[\text{TFe}]$ is the concentration of total Fe (i.e. $\text{DFe} + \text{added Fe}$). DFe^* is subject to error
313 propagation from the determinations of both FeNN_3 (9 %) and dissolved Fe (7 %) and thus will be
314 subject to the combined error of 11.4 %. We therefore only report values of DFe^* where $[\text{FeNN}_3]$ is
315 at least 11.4 % less than $[\text{TFe}]$.

316 Derivation of equilibrium constant for FeNN_3 for application in ion pairing models for seawater

317
318 To ensure consistency between our observed FeNN_3 concentrations and our speciation calculations
319 we derived an equilibrium constant valid for seawater ($S=35$) between pH_{NBS} 7.2-8.5 that accounts
320 for competition between Fe and hydrogen ions for NN^- .

321



323

324 We distinguish this constant from previously derived conditional stability constants ($\log k_{FeNN_3,Fe^{3+}}^{cond}$)
325 by denoting it $\log k_{FeNN_3,H^+}$. We used the equilibrium constant for HNN of $10^{7.9}$ (NIST, Smith et al.
326 2004). Derivation was carried out by combining the chemical speciation program ORCHESTRA
327 (Meeussen, 2003) with the parameter estimation software PEST (Doherty, 2019). Speciation
328 calculations in ORCHESTRA were set up with input, chemistry and objects files as described
329 previously (Janot et al., 2017; Zhu et al., 2021). Further details can be downloaded from protocols.io
330 (dx.doi.org/10.17504/protocols.io.brc4m2yw). We used the Minteqv4 database for thermodynamic
331 constants, which is consistent with the database used previously in visual MINTEQ (Avendaño et al.,
332 2016; Gledhill et al., 2015) and we also verified that calculations in ORCHESTRA and visual
333 MINTEQ were comparable. For the derivation of $\log k_{FeNN_3,H^+}$ we specified an initial estimate of 31
334 (Avendaño et al., 2016; Gledhill et al., 2015), with an allowed range of 28 to 32. Parameter
335 derivation is performed by calculation of the $FeNN_3$ concentration in ORCHESTRA for each
336 measurement, which is then passed to PEST and compared to the observed values. PEST provides a
337 new value for $\log k_{FeNN_3,H^+}$, which is then passed back to ORCHESTRA for a fresh calculation of
338 $FeNN_3$. The procedure is iterated to minimize the residuals between observed and calculated $FeNN_3$
339 calculations via the Levenberg-Marquardt algorithm. The PEST output comprises a value for
340 $\log k_{FeNN_3,H^+}$ with 95% confidence intervals, together with a full record of the optimization in the
341 output file. Consistency was then further assessed by comparison between observed and calculated
342 $FeNN_3$ and Fe^{3+} in UV irradiated seawater as a function of HNN concentration, within the HNN
343 concentration range applied in this study.

344

345 Assessment of relationship between observed and calculated concentrations of iron species to DOC
346 concentrations assuming binding sites behave according to the NICA-Donnan model.

347 The NICA-Donnan model was used to calculate the speciation of Fe at equilibrium for each titration
348 point at pH_{NBS} 8.0, via speciation calculation tool ORCHESTRA (Meeussen, 2003). We tested the
349 assumption that one set of NICA-Donnan parameters could describe variability in $[FeNN_3]$ and $[Fe^{3+}]$
350 by adding the “Fulvic acid” NICA-Donnan adsorption model to the dissolved ion pairing model used
351 for the derivation of $\log k_{FeNN_3,H^+}$. Marine DOM was thus considered analogous to terrestrial and
352 freshwater DOM (Gledhill et al., 2015; Laglera & Van Den Berg, 2009; Lodeiro et al., 2020). The

353 applied NICA model assumes a continuous Sips bimodal distribution of binding sites. The
 354 distribution of the affinities of the two groups of binding sites (Denoted (1): Carboxylic-type groups,
 355 and (2): Phenolic-type groups) are described by three constants per binding site group: the width of
 356 the binding site distribution (p_1 and p_2), NICA affinity constant ($\log K_{Me1}$ and $\log K_{Me2}$ for a metal
 357 cation or $\log K_{H1}$ and $\log K_{H2}$ for the protonation constants) which represents the median of the
 358 distribution, and non-ideality constant which represents non-ideal behavior of ion adsorption (n_{Me1} ,
 359 n_{Me2} , n_{H1} , n_{H2}), where n_{Hi} is <1 (Kinniburgh et al., 1999). The binding of a metal by marine DOM,
 360 Q_{Me} is then described with reference to proton binding by marine DOM according to the following
 361 equation:

$$\begin{aligned}
 363 \quad Q_{Me} = & Q_{max1,T} \frac{n_{Me,1}}{n_{H1}} \cdot \frac{(K_{Me,1} \cdot C_{Me})^{n_{Me,1}}}{(K_{H,1} \cdot C_H)^{n_{H,1}} + (K_{Me,1} \cdot C_{Me})^{n_{Me,1}}} \cdot \frac{\{(K_{H,1} \cdot C_H)^{n_{H,1}} + (K_{Me,1} \cdot C_{Me})^{n_{Me,1}}\}^{p_1}}{1 + \{(K_{H,1} \cdot C_H)^{n_{H,1}} + (K_{Me,1} \cdot C_{Me})^{n_{Me,1}}\}^{p_1}} + \\
 364 \quad & Q_{max2,T} \frac{n_{Me,2}}{n_{H2}} \cdot \frac{(K_{Me,2} \cdot C_{Me})^{n_{Me,2}}}{(K_{H,2} \cdot C_H)^{n_{H,2}} + (K_{Me,2} \cdot C_{Me})^{n_{Me,2}}} \cdot \frac{\{(K_{H,2} \cdot C_H)^{n_{H,2}} + (K_{Me,2} \cdot C_{Me})^{n_{Me,2}}\}^{p_2}}{1 + \{(K_{H,2} \cdot C_H)^{n_{H,2}} + (K_{Me,2} \cdot C_{Me})^{n_{Me,2}}\}^{p_2}} \quad (6)
 \end{aligned}$$

365
 366 where $Q_{max1,T}$, $Q_{max2,T}$ refer to the total number of proton binding sites per binding site type, and C_H
 367 and C_{Me} are the concentrations of protons and metal, respectively.

368
 369 In the NICA-Donnan model, electrostatic interactions are described by the Donnan component of the
 370 model which is based on the Boltzmann equation (Benedetti et al., 1996). However, at the ionic
 371 strength of seawater the apparent Donnan volume becomes very small and concentrations of metals
 372 electrostatically associated with DOM become negligible (Lodeiro et al., 2020; Pinheiro et al., 2021).

373
 374 In this study, we used two previously published sets of NICA constants and two new NICA
 375 parameter sets (Table 1). The previously published sets were derived from surface waters collected in
 376 the Sargasso Sea (Set A: Hiemstra and van Riemsdijk, 2006) and surface waters obtained from an
 377 estuarine system on the English south coast (Set B: Gledhill et al. 2015), whilst the new parameter set
 378 C was re-derived from surface waters in the Northwest European Shelf Sea based on titration data
 379 obtained in Celtic Sea samples first reported in Avendaño et al. (2016). We re-derived the set C
 380 values because the original reported values were empirically estimated using a $\log k_{FeNN3,H+}$ of 32.5,
 381 which was considerably higher than the value we derived in this study (see results). We further
 382 examined the impact of n_i by increasing the value of n_1 and n_2 (set D) to consider the possibility that
 383 marine DOM is less heterogeneous than typically observed for terrestrial organic matter (Lodeiro et

384 al., 2020; Zhu et al., 2021). We used PEST-ORCHESTRA to re-derive the NICA constants following
385 a similar procedure used for the derivation of $\log k_{FeNN_3, H^+}$ and described in Zhu et al., (2021). Since
386 this work was focused on the Celtic Sea, we only used the titration data obtained from Celtic Sea
387 samples in this derivation (samples collected at stations 1, 3, 4, 5, 6, 18, 19, 20 from Avendaño et al.
388 (2016)). We provide the raw titration data, required input files and a description of the protocol used
389 in this derivation on protocols.io ([dx.doi.org/10.17504/protocols.io.brc4m2yw](https://doi.org/10.17504/protocols.io.brc4m2yw)). We followed the
390 PEST-ORCHESTRA approach that was first used to derive NICA constants for Cd and Zn binding to
391 Laurentian fulvic acid by Janot et al. (2017). Typically, both equilibrium constants and non-ideality
392 constants are derived from experimental data. However, we found during preliminary derivations that
393 since titrations were undertaken at only three pH_{NBS} values (7.2, 7.6, 8) and encompassed a relatively
394 narrow pH range, data from Avendaño et al. (2016) were not sufficiently well constrained in pH
395 space to reproducibly derive all four parameters. We therefore fixed $n_{Fe(III)1}$ and used the
396 relationship between n_1 and n_2 from Milne et al. (2003) ($n_2 = 0.76 \times n_1$) to calculate $n_{Fe(III)2}$. We
397 then estimated $\log K_{Fe(III)1}$, $\log K_{Fe(III)2}$ using initial estimates of 3 and 9, and allowed ranges of 2 to
398 4 and 8 to 10, respectively. Generic parameters from Milne et al. (2003), (2001) were used to
399 describe binding of proton and major cations (H^+ , Ca^{2+} , Mg^{2+} , Sr^{2+}) to be consistent with parameter
400 sets A and B.

401

402 To investigate goodness of fit at different ambient DOC concentrations, we compared our observed
403 $FeNN_3$ concentrations with $FeNN_3$ concentrations calculated in ORCHESTRA. We then compared
404 Fe^{3+} calculated from observations using equation (3) with those calculated in ORCHESTRA and
405 observed versus calculated DFe^* calculated using equation (4). For speciation calculations, pH was
406 set to the analysis pH_{NBS} ($= 8.00 \pm 0.08$).

407

408 Prediction of apparent Fe(III) solubility and inorganic Fe concentrations at ambient pH and 409 temperature in our study region

410 We predicted Fe speciation in our study area at ambient pH and temperature using the NICA
411 constants with the best fit to our observed titration data. To calculate $SFe(III)_{app}$ we set our total
412 Fe(III) concentration to 10 nmol L^{-1} and allowed for the formation of $Fe(OH)_3(s)$ (ferrihydrite) within
413 ORCHESTRA. We use a solubility product of $\log^* K_s = 3.2$, derived from (Liu and Millero, 1999) to
414 determine iron solubility according to equation (7).

415

416 $*K_S = [Fe^{3+}] \div [H^+]^3 = 10^{3.2}, \Delta H_r = -100.4 \text{ kJ mol}^{-1}$ (7)

417

418 We therefore consider organically bound Fe as soluble, but Fe(OH)₃(s) as insoluble. We compare our
419 SFe(III)_{app} with observed dissolved Fe concentrations. However, given the potential size of both
420 freshly formed Fe(OH)₃(s) (defined in Liu and Millero (1999) as >0.02 μm) and organic matter
421 (determined in the < 0.7 μm fraction), the Fe associated with both DOM and Fe(OH)₃(s) may both be
422 colloidal in nature (>0.02 but <0.2 μm) and this should be kept in mind when comparing the absolute
423 values.

424

425 We calculated the sum of soluble inorganic species and express these concentrations as pFe(III)'
426 using:

427

428 $pFe(III)' = -Log ([FeOH^{2+}] + [Fe(OH)_2^+] + [Fe(OH)_3] + [Fe(OH)_4^-])$ (8)

429

430 In these calculations, we set the total Fe concentration to be equal to the determined DFe
431 concentration, but Fe(OH)₃(s) was also allowed to form to account for possible formation of
432 insoluble iron hydroxides when Fe³⁺ becomes oversaturated, according to equation (7).

433

434 **Results and Discussion**

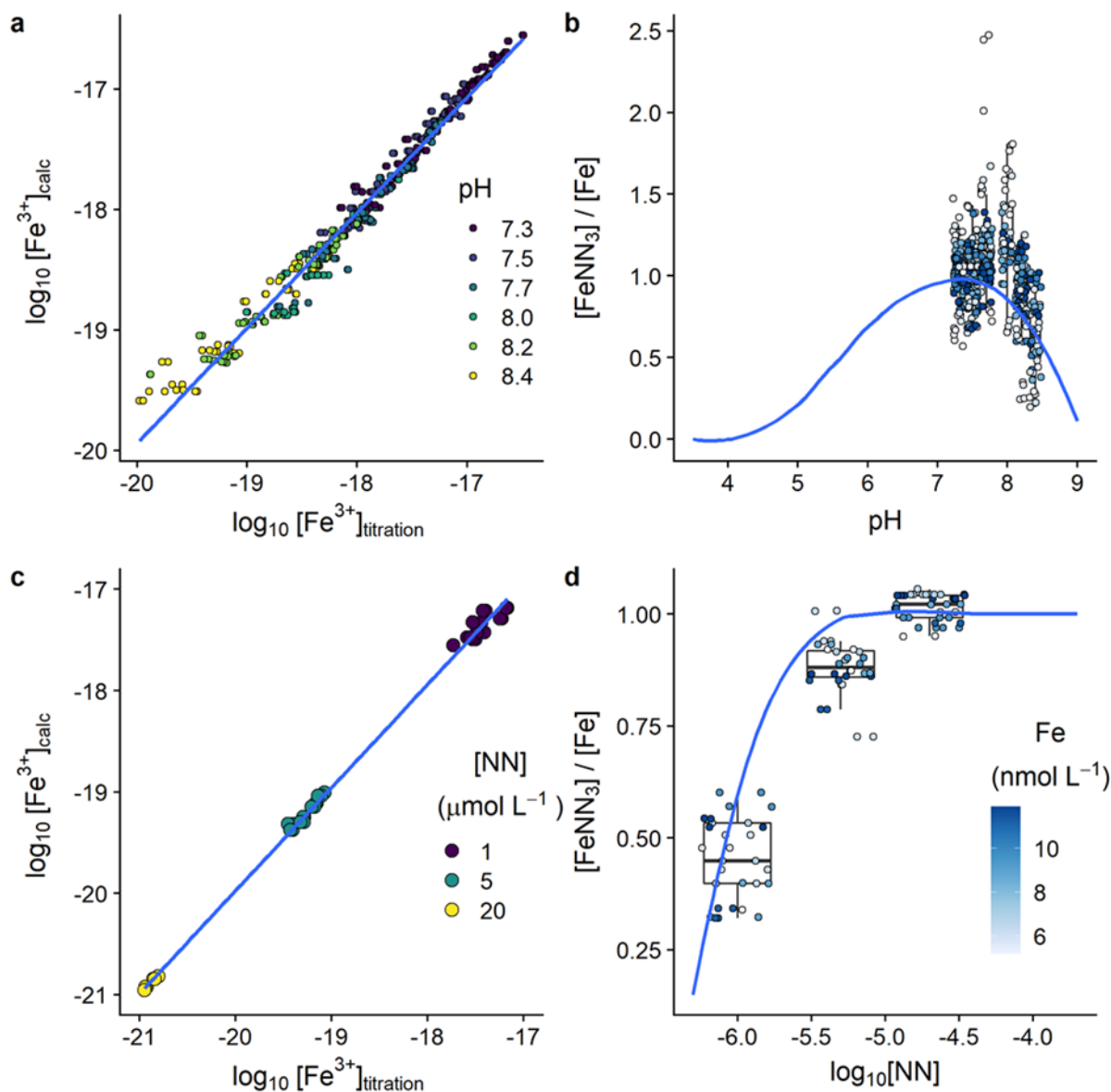
435

436 Establishing consistency between observations and calculations in the absence of organic matter.

437 An understanding of how pH and temperature might influence trace element speciation at equilibrium
438 can be obtained via iterative algorithms based on thermodynamic principles using sets of
439 thermodynamic constants valid for the physico-chemical conditions to be explored in the study. We
440 applied “off the shelf” ion pairing software packages in our study that incorporate ionic strength
441 corrections based on the extended Debye-Hückel equation, but we highlight this is not fully optimal
442 and warn that absolute values predicted via our speciation calculations will be affected by systematic
443 bias as a result of overestimation of activities. The impact of the ion pairing approach is illustrated by
444 an approximate 15% underestimation in ionic strength in our calculation (*I*=0.6 M compared to *I*=0.7
445 M typically assumed for seawater), which is consistent with previous estimates of the error
446 introduced by application of the Debye-Hückel equation (Stockdale et al., 2016). Nevertheless,
447 valuable information – with respect to the extent that changes in physico-chemical properties such as

448 pH and temperature may have on metal speciation – can be obtained if a system can be calibrated
449 such that its observed and calculated values are consistent for a given critical species. In our study,
450 we used a value for $\log k_{FeNN_3,H^+}$ within an ion pairing model, which would account for competition
451 between NN^- , H^+ , Fe^{3+} and OH^- at the ionic strengths and pH relevant to our study. In previous work,
452 a first attempt at such a system was made by manually changing constants to obtain an empirical
453 estimate for $\log k_{FeNN_3,H^+}$ (Avendaño et al., 2016). In this study, we sought to improve on this by
454 first calibrating $\log k_{FeNN_3,H^+}$. We particularly focused on establishing consistency between
455 determined and calculated Fe^{3+} and $FeNN_3$ concentrations, since $FeNN_3$ is the measured species from
456 titrations and Fe^{3+} is the Fe species that reacts with the added ligand, hydroxide ion and natural
457 organic matter.

458 Estimation of $\log k_{FeNN_3,H^+}$ using the parameter estimation software package PEST (Doherty, 2019)
459 in combination with the ion pair speciation program ORCHESTRA (Meeussen, 2003) resulted in a
460 $\log k_{FeNN_3,H^+}$ of 29.5 ± 0.1 . With this value, the Pearson correlation coefficient between observed and
461 calculated $\log[FeNN_3]$ was 0.962 with a root mean squared error (RMSE) of 1.12 nmol L^{-1} over the
462 pH_{NBS} range 7.2-8.5 and at an HNN concentration of $2 \text{ } \mu\text{mol L}^{-1}$. Predicted Fe^{3+} concentrations
463 (Fe^{3+}_{calc}) correlated with Fe^{3+} calculated from the observed $FeNN_3$ concentrations ($Fe^{3+}_{titration}$)
464 ($\log[Fe^{3+}]_{calc} = 0.95 \times \log[Fe^{3+}]_{titration} - 0.83$, $r^2 = 0.97$, $n=456$) (Figure 2a). The modelled distribution
465 of the relative proportion of Fe present as $FeNN_3$ as a function of pH suggests that $FeNN_3$ will be the
466 dominant species between pH_{NBS} 7 and 8, with a maximum predicted response at pH_{NBS} 7.5 (Figure
467 2b), which is consistent with the relationship between pH and the voltammetric response for $FeNN_3$
468 previously reported by van den Berg (1991). However, our derived value of 29.5 for $\log k_{FeNN_3,H^+}$ is
469 three orders of magnitude lower than the empirical estimate of 32.5 given by Avendaño et al. (2016).
470 Further comparison with literature values showed that our calculated conditional stability constant at
471 pH_{NBS} 8 is within the reported range after calibration against hydroxide and EDTA but lower than
472 obtained at pH_{NBS} 6.9 (Supplementary Table 1). The difference between the calibrated constants
473 could be explained by the ionic strength corrections applied during the calculations, the choice of
474 conditional constants for Fe binding to EDTA, and the applied inorganic side reaction coefficient for
475 Fe (Laglera et al., 2011).



476

477 Figure 2 Predicted Fe^{3+} and FeNN_3 for seawater after ultra violet irradiation to destroy organic matter. (a)
 478 Predicted Fe^{3+} is plotted versus determined Fe^{3+} from titrations using HNN concentrations at 2 and 5 $\mu\text{mol L}^{-1}$
 479 over the pH_{NBS} range 7.2-8.5 (number of observations, $n = 456$). (b) The proportion of FeNN_3 relative to total Fe
 480 from titrations using HNN concentrations at 2 and 5 $\mu\text{mol L}^{-1}$ is shown over the pH_{NBS} range 7.2-8.5, where
 481 measured FeNN_3 is indicated as colored points and predicted FeNN_3 is shown by the solid line. The colours
 482 represent different Fe concentrations (see legend in d) and $n = 456$ (c) Predicted Fe^{3+} is plotted versus
 483 determined Fe^{3+} from titrations undertaken at constant pH_{NBS} (~8.0) over the range of HNN concentrations. The
 484 line shows the 1:1 relationship ($n=98$). (d) The proportion of FeNN_3 relative to total Fe from titrations
 485 undertaken at constant pH_{NBS} (~8.0), is shown over the range of HNN concentrations, where measured FeNN_3
 486 concentrations are shown as colored points and predicted FeNN_3 is shown by the solid line ($n=98$).

487

488 We next examined the relationship between calculated and determined FeNN_3 and Fe^{3+}

489 concentrations using the derived $\log k_{\text{FeNN}_3, \text{H}^+}$ over the range of HNN concentrations (1, 5 and 20

490 $\mu\text{mol L}^{-1}$) employed in this study at pH_{NBS} 8.0 using the ‘overload titration’ method. We obtained a
491 linear relationship between observed and calculated $[\text{Fe}^{3+}]$ ($\log[\text{Fe}^{3+}]_{\text{calc}} = 1.07 \pm 0.03 \times \log[\text{Fe}^{3+}]_{\text{titration}}$
492 $+ 8.7 \times 10^{-20} \pm 6.5 \times 10^{-20}$, $r^2 = 0.93$, $n = 98$, Figure 2c). The positive intercept implies a slight systematic
493 overestimate of FeNN_3 by the ion pairing model, which is supported by the relationship between the
494 proportion of Fe bound to FeNN_3 and the HNN concentration (Figure 2d). The observed proportion
495 of Fe(III) that was detected as FeNN_3 at both 1 and 5 $\mu\text{mol L}^{-1}$ HNN was thus slightly lower (by an
496 average of 10 and 15 % respectively) than predicted by the ion pairing model. Our calculated side
497 reaction coefficients were $\log \alpha'_{\text{FeNN}_3, \text{Fe}^{3+}} = 9.1, 11.2$ and 13 for 1, 5 and 20 $\mu\text{mol L}^{-1}$ HNN,
498 respectively. These values compared to a $\log \alpha'_{\text{Fe}}$ of 8.95 calculated by the ion pairing model at
499 pH_{NBS} 8.0. The similarity between $\log \alpha'_{\text{FeNN}_3, \text{Fe}^{3+}}$ and $\log \alpha'_{\text{Fe}}$ at an HNN concentration of 1 μmol
500 L^{-1} means that hydroxide ions will compete with HNN at our lowest HNN concentration (Figure 2c).
501 Given the low solubility of Fe hydroxides (at pH_{NBS} 8.0 and 293 K, $\text{Fe}(\text{OH})_3(\text{s})$ is predicted to form
502 at an $[\text{Fe}^{3+}]$ concentration of $7.58 \times 10^{-20} \text{ mol L}^{-1}$, equivalent to $\text{pFe}(\text{III})' = 10.2$), the relatively high
503 proportion of Fe^{3+} (maximum calculated Fe^{3+} in UV irradiated seawater = $5.7 \times 10^{-18} \text{ mol L}^{-1}$) should
504 theoretically result in formation of $\text{Fe}(\text{OH})_3(\text{s})$ at both 1 and 5 $\mu\text{mol L}^{-1}$ HNN concentrations.
505 Nevertheless, the linear relationship between observed and calculated Fe^{3+} suggests that $\text{Fe}(\text{OH})_3(\text{s})$
506 formation did not impact on the determination of FeNN_3 , possibly because the equilibration time was
507 not long enough to detect a reduction due to $\text{Fe}(\text{OH})_3(\text{s})$ (a week was used for determination of *K_5
508 (Liu and Millero, 1999)). If we assume no formation of $\text{Fe}(\text{OH})_3(\text{s})$ occurred, then Fe^{3+}
509 concentrations are consistent over the range of pH and HNN values examined here.

510

511 We concluded that our experiment - speciation calculation framework was adequately consistent
512 within the time frame of our titration experiments. However, we caution that our experiments are
513 likely not at true equilibrium, and while it was not detectable over the <24-hour equilibration period
514 of our titrations, we cannot completely rule out formation of $\text{Fe}(\text{OH})_3(\text{s})$. Although our calculations
515 simplify the complex kinetic and thermodynamic processes that influence chemical Fe speciation in
516 aqueous solutions, we argue that they are sufficiently consistent to be used to investigate the
517 relationship between DOC concentration and Fe speciation predicted by the NICA-Donnan model.

518

519 As a final step in the development of our experimental framework for examining the relationship
520 between DOC concentrations and the fit of observed Fe speciation to different sets of NICA
521 parameters, we re-derived the NICA constants from Avendaño et al. (2016). We carried out this re-

522 derivation to improve upon the empirical nature of the original estimates and to account for the
523 difference in $\log k_{FeNN3,H^+}$ used to generate the estimates for the NICA affinity constants reported by
524 Avendaño et al. (2016). When fitting for four parameters ($n_{Fe(III)1}$, $n_{Fe(III)2}$, $\log K_{Fe(III)1}$,
525 $\log K_{Fe(III)2}$) we found that repeated estimations ($n > 3$) using the same initial arbitrary parameter
526 values did not produce reproducible results, likely as a result of overfitting the data set. The value of
527 n_i and its relationship to n_H as described in equation (6) have been related to reaction stoichiometry
528 between H^+ and the metal ion (Hiemstra and van Riemsdijk, 2006), thus determination of n_i requires
529 experimental data with sufficient density and range in pH space (Zhu et al., 2021). Unfortunately, we
530 found that this criterion was not satisfied by the data of Avendaño et al. (2016), since titrations at
531 only 3 pH values within a relatively restricted range (less than one pH unit) were undertaken. We
532 therefore initially set the value for $n_{Fe(III)1}$ to 0.31 based on previously reported values available for
533 marine organic matter (Avendaño et al., 2016; Gledhill et al., 2015; Hiemstra and van Riemsdijk,
534 2006). The value of $n_{Fe(III)2}$ was calculated using the formula $n_2 = 0.76 \times n_1$ which has previously
535 been shown to describe the covariance between n_1 and n_2 observed for multiple cations (Milne et al.,
536 2003). Our re-derived NICA affinity constants (set C) are presented in Table 1 along with a further
537 two sets of constants (sets A and B) taken from the literature (Gledhill et al., 2015; Hiemstra and van
538 Riemsdijk, 2006). As expected, the combination of fixing n_i , the mathematical rederivation and the
539 change in $\log k_{FeNN3,H^+}$, resulted in differences in the derived $\log K_{Fe(III)1}$ and $\log K_{Fe(III)2}$ used in
540 this study compared to the values empirically estimated (0.26, 3.6, 0.23 and 8.3 for $n_{Fe(III)1}$,
541 $\log K_{Fe(III)1}$, $n_{Fe(III)2}$, $\log K_{Fe(III)2}$ respectively) by Avendaño et al. (2016). We further examined the
542 impact of n_i by increasing the value of n_1 and n_2 (set D) to consider the possibility that marine DOM
543 is less heterogeneous than typically observed for terrestrial organic matter (Lodeiro et al., 2020; Zhu
544 et al., 2021). As well as influencing the effective competition between the metal and protons (Milne
545 et al., 2003), the non-ideality constant influences the relationship between the free metal ion
546 concentration and the total dissolved metal concentration (also termed the concentration dependency,
547 Milne et al. 2003). Incorporation of heterogeneity results in an exponential increase in Fe^{3+} as DFe
548 concentrations increase, which arises because stronger binding sites in the distribution are occupied
549 first. Higher values of n_i result in a shallower exponential curve for the relationship between Fe^{3+} and
550 DFe concentrations.

551

552

553

554

555 Table 1. Four sets of constants for Fe(III) binding to the two dissolved organic matter binding site
 556 types of the NICA-Donnan model. Parameter sets A and B were taken from the literature, Hiemstra
 557 and van Riemsdijk (2006) and Gledhill et al. (2015), respectively. Parameter sets C and D were re-
 558 derived for this study based on raw titration data obtained in Celtic Sea samples previously reported
 559 in Avendaño et al. (2016). We fixed the non-ideal constants ($n_{Fe(III)}$) to derive the binding affinity
 560 ($\log K_{Fe(III)}$) for both parameter sets C and D. The goodness of fit is indicated as root mean square
 561 error (RMSE).

562

Fe(III) NICA constants	set A	set B	set C	set D
Carboxylic-type groups				
$\log K_{Fe(III)1}$	2.8	3.6	2.81±0.36	3.16±0.001
$n_{Fe(III)1}$	0.36	0.3	0.31	0.4
Phenolic-type groups				
$\log K_{Fe(III)2}$	8.3	11.2	9.04±0.01	9.80±0.01
$n_{Fe(III)2}$	0.23	0.15	0.24	0.3
RMSE for parameters rederived in this study	NA		0.7908	0.2149

563

564 Influence of dissolved organic carbon concentration on determined and calculated Fe speciation at 565 constant pH

566 In this work, we analyzed 106 samples from three cruises undertaken in November (DY018, 47
 567 samples), April (DY029, 34 samples) and July (DY033, 28 samples) by CLE-AdCSV and present
 568 raw titration data in the SI (Supplementary Figure S2). We first compared FeNN₃ concentrations
 569 calculated with the NICA-Donnan model using parameter sets A-D with the observed FeNN₃
 570 concentrations for the whole data set (Table 2, Supplementary Figure S3). Simulated FeNN₃ using
 571 parameter set B systematically underestimated the observed FeNN₃ concentrations, resulting in a
 572 larger RMSE in comparison to sets A, C and D (Table 2). Parameter set B thus overestimated the
 573 binding strength of organic matter in our study region. The stronger binding represented by

574 parameter set B could reflect the estuarine nature of the samples used for the parameter estimation,
 575 which might be more strongly influenced by terrestrial organic matter. However, we caution that the
 576 data set used for the estimation in Gledhill et al. (2015) was based on analysis of one sample and the
 577 authors of that study emphasized that it was intended as a proof of concept.

578

579 Table 2. Relationships between calculated (y) and observed (x) FeNN₃ concentrations obtained using
 580 four sets of NICA constants. Sets A and B and are taken from the literature, Hiemstra and van
 581 Riemsdijk (2006) and Gledhill et al. (2015), respectively. Sets C and D were re-derived for this study
 582 based on titrations data taken from Avendaño et al. (2016). The number of observations (n) was 643.
 583 The goodness of fit is indicated as root mean square error (RMSE).

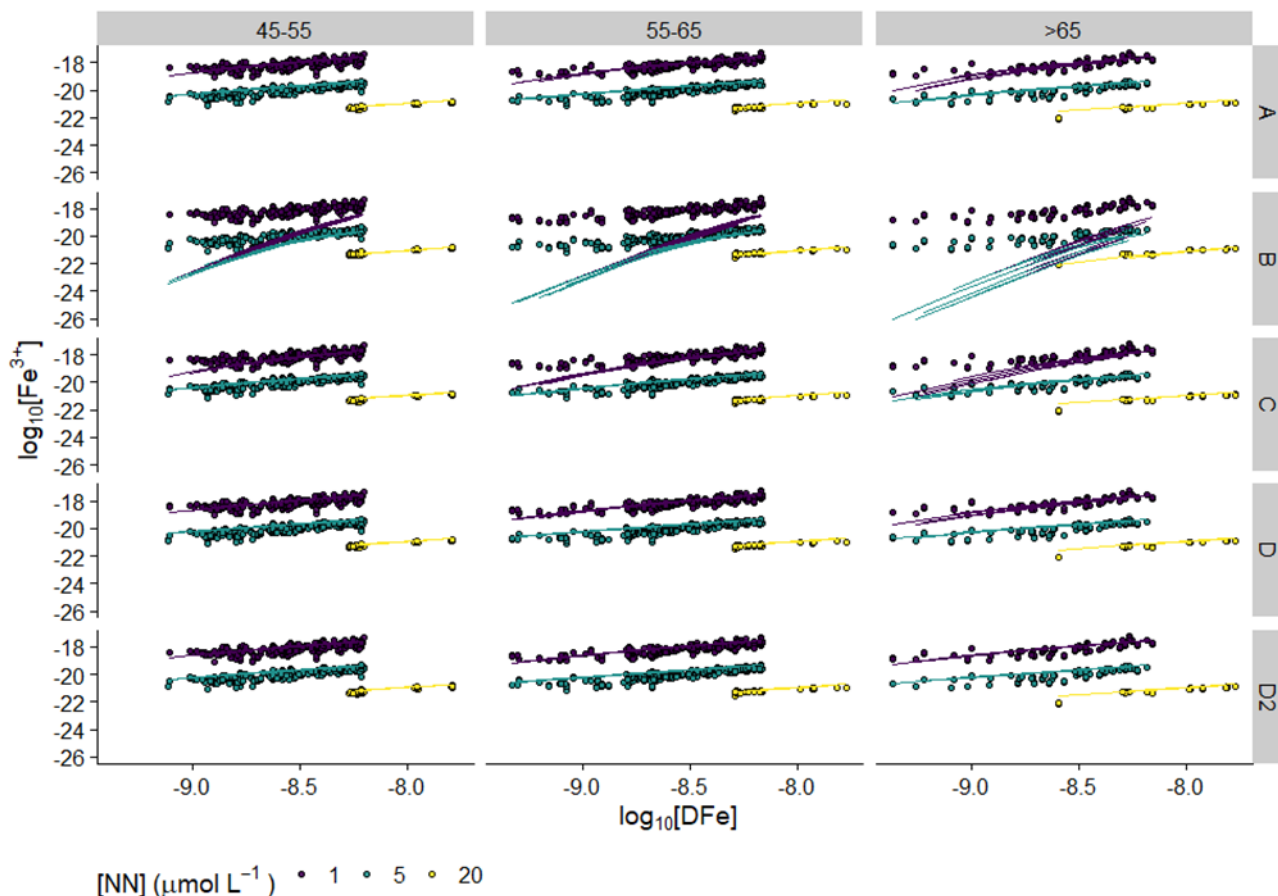
584

Fe(III) NICA constants	set A	set B	set C	set D
Linear equation	$y=1.03.x+0.36$	$y=8.05x-0.79$	$y=1.03x+0.50$	$y=1.03x+0.39$
r^2	0.85	0.78	0.84	0.85
RMSE (nmol L ⁻¹)	0.95	1.52	0.89	0.98

585

586 FeNN₃ is a dominant species at 5 and 20 μmol L⁻¹ HNN in our titration experiments, and variability
 587 in less abundant species might be expected to be more sensitive to changes in binding site
 588 concentrations and better highlight systematic bias with respect to DOC concentrations. Therefore,
 589 we next compared the relationship between [Fe³⁺]_{titration} and total Fe with calculated values for Fe³⁺
 590 obtained from combining the ion-pairing and NICA model ([Fe³⁺]_{NICA}) using our four sets of NICA
 591 constants for samples binned into three different DOC concentrations: 45-55, 55-65, and >65 μmol L⁻¹
 592 ¹ (Figure 3). The DOC bins broadly align with concentrations typically observed for semi-refractory,
 593 semi-labile and labile DOC respectively (Hansell, 2013), although the division between the different
 594 DOC fractions is likely less well defined than implied here. Figure 3 shows that the relationship
 595 between [Fe³⁺]_{titration} and total Fe was quite well described by A, C and D, but not well described by
 596 parameter set B, although some differences between Fe³⁺ at 1 μmol L⁻¹ HNN at low total Fe
 597 concentrations was evident for all sets at DOC concentrations > 55 μmol L⁻¹. Accurate determination
 598 of Fe³⁺ concentrations at low total Fe concentrations appears to be an issue for other voltammetric
 599 methods used for analysis of Fe speciation (Gerringa et al., 2021) and could be especially

600 problematic when using HNN as an added ligand because of its lower sensitivity (Ardiningsih et al.,
 601 2021). Results of correlation between $[\text{Fe}^{3+}]_{\text{titration}}$ and $[\text{Fe}^{3+}]_{\text{NICA}}$ are given in Table 3. Calculated
 602 $[\text{Fe}^{3+}]_{\text{NICA}}$ using parameter sets A, C and D again showed better agreement with $[\text{Fe}^{3+}]_{\text{titration}}$ (Table 3)
 603 than parameter set B. Combining information from intercept, slope and r^2 and Akaike Information
 604 Criteria (AIC), A and D were found to be a better fit to the data than C.



605

606 *Figure 3. Plots of Fe^{3+} versus total Fe concentrations obtained for titrations binned into three DOC*
 607 *concentration ranges (45-55, 55-65 and $>65 \mu\text{mol L}^{-1}$). Points show Fe^{3+} concentrations obtained from*
 608 *measured FeNN_3 concentrations at three different HNN concentrations: 1, 5 and $20 \mu\text{mol L}^{-1}$. Lines show Fe^{3+}*
 609 *concentrations calculated using the NICA-Donnan model combined with an ion-pairing model. Four different*
 610 *NICA parameter sets were applied: parameter set A was reported in Hiemstra and van Riemsdijk, (2006), B in*
 611 *Gledhill et al. (2015), whilst C and D were re-derived for this study based on titration data from Avendaño et*
 612 *al. (2016) (Table 1). Scenario D2 used NICA parameter set D, but assumed that DOC concentrations were*
 613 *constant at $43.7 \mu\text{mol L}^{-1}$. Total number of observations = 1489.*

614 We noted that goodness-of-fit of $[\text{Fe}^{3+}]_{\text{NICA}}$ to $[\text{Fe}^{3+}]_{\text{titration}}$ tended to decrease with increasing DOC
 615 concentration (Table 3). We therefore further examined the scenario that binding sites did not scale
 616 with DOC concentration by calculating the Fe speciation using parameter set D and fixing the DOC
 617 concentration to the lowest value observed in our study ($43.7 \mu\text{mol L}^{-1}$). We found similar goodness-
 618 of-fit results for this fixed-DOC scenario (D2) across the whole range of DOC concentrations

619 observed in our study, suggesting that binding sites are not necessarily more abundant at higher DOC
 620 concentrations.

621
 622

623 Table 3. Correlations of $\log[\text{Fe}^{3+}]_{\text{titration}}$ (x) observed in titrations undertaken at different HNN
 624 concentrations with $\log[\text{Fe}^{3+}]_{\text{NICA}}$ (y) calculated using a combined ion-pair/NICA-Donnan model.
 625 Sets A and B and are taken from the literature, Hiemstra and van Riemsdijk (2006) and Gledhill et al.
 626 (2015), respectively. Sets C and D were rederived for this study based on titration data taken from
 627 Avendaño et al. (2016). The D2 scenario used parameter set D but assumed a constant DOC
 628 concentration of $43.7 \mu\text{mol L}^{-1}$.

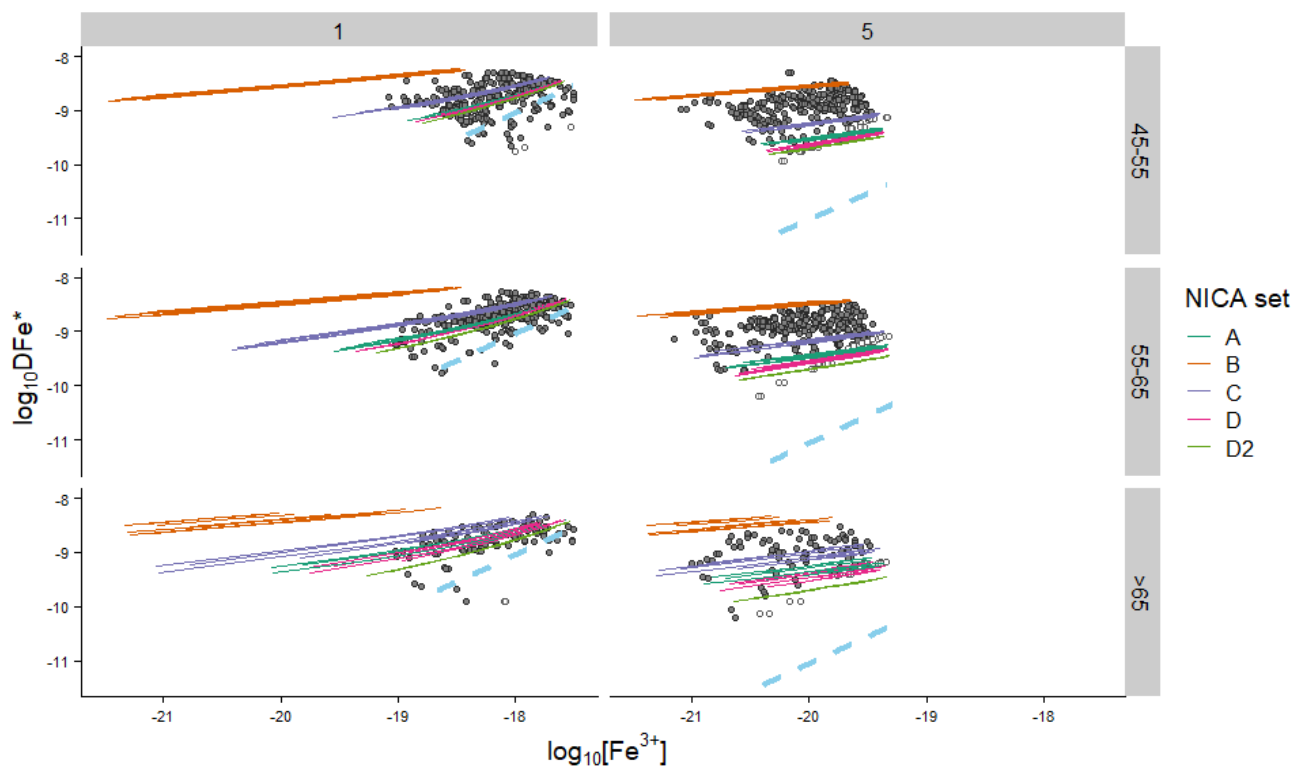
629

DOC concentration ($\mu\text{mol L}^{-1}$)	NICA parameter set	Intercept	Slope	R ²	AIC
45-55 (n=612)	A	-1.21±0.16	0.93±0.01	0.95	-55
	B	-10.8±0.6	0.50±0.03	0.27	1655
	C	-2.67±0.18	0.85±0.01	0.93	116
	D	-1.24±0.16	0.93±0.01	0.96	-80
	D2	-1.05±0.16	0.93±0.01	0.96	-89
55-65 (n=643)	A	-1.34±0.14	0.92±0.01	0.96	-138
	B	-10.0±0.8	0.55±0.03	0.23	2008
	C	-2.83±0.2	0.85±0.01	0.91	289
	D	-1.38±0.14	0.92±0.01	0.97	-202
	D2	-1.04±0.13	0.93±0.01	0.97	-249
65-200 (n=234)	A	-2.06±0.32	0.89±0.02	0.92	117
	B	-13.1±1.7	0.43±0.09	0.09	881
	C	-4.20±0.48	0.79±0.03	0.80	303
	D	-2.04±0.29	0.89±0.01	0.94	62
	D2	-1.18±0.25	0.92±0.01	0.96	-16

630

631 For our final assessment employing our titration data, we examined the relationship between the non-
 632 labile Fe concentrations (DFe^*) and $[\text{Fe}^{3+}]_{\text{titration}}$ observed in our titrations and compared the

633 relationship to values calculated using the NICA model. For both titration data and simulated results
634 using the NICA-Donnan model, $D\text{Fe}^*$ was calculated using equation (4); as the HNN concentration
635 decreases, the portion of the Fe bound to hydroxides becomes an increasingly more important
636 component of $D\text{Fe}^*$. Figure 4 shows the relationship between $D\text{Fe}^*$ and Fe^{3+} of measured
637 ($D\text{Fe}^*_{\text{titration}}$) and calculated data ($D\text{Fe}^*_{\text{NICA}}$), binned according to DOC concentration. The overload
638 titration method assumes that $D\text{Fe}^*$ will be negligible at $20 \mu\text{mol L}^{-1}$ HNN, and indeed we rarely
639 observed $D\text{Fe}^*$ values greater than 11 % of total Fe (the threshold of uncertainty) at this HNN
640 concentration and so $D\text{Fe}^*$ values are only shown for 1 and $5 \mu\text{mol L}^{-1}$ HNN values. We observed
641 larger scatter in the calculations of $D\text{Fe}^*$ at each HNN concentration compared to that observed for
642 $\log_{10}(\text{Fe}^{3+})$ (Fig. 3) and weak correlations ($r^2 < 0.2$, data not shown) between $D\text{Fe}^*_{\text{titration}}$ and
643 $D\text{Fe}^*_{\text{NICA}}$, which likely reflects increased error propagation for the calculation of $D\text{Fe}^*$. However, for
644 the most part, observed $D\text{Fe}^*_{\text{titration}}$ overlapped with parameter sets A, C and D and predicted values
645 were thus in the range of observed values. We note that at $5 \mu\text{mol L}^{-1}$ HNN, concentrations of
646 $[\text{FeNN}_3]_{\text{calc}}$ were overestimated in our UV seawater experiments (Figure 2d), which could contribute
647 further to discrepancies between $D\text{Fe}^*_{\text{titration}}$ and $D\text{Fe}^*_{\text{NICA}}$. The analytical limitations of CLE-
648 AdCSV should also be considered here, since its results are known to be influenced by the estimation
649 of sensitivity, lack of equilibrium conditions, and the number and distribution of titration points
650 (Gledhill and Gerringa, 2017; Hudson et al., 2003; Pižeta et al., 2015; Town and Filella, 2000). In
651 particular, the calculation of $D\text{Fe}^*$ is sensitive to bias in estimation of the slope (Hudson et al., 2003),
652 and the ability to detect significant concentrations of $D\text{Fe}^*$ is strongly influenced by the sensitivity of
653 the method. In our case, we note that HNN is one of the least sensitive ligands that can be used to
654 detect Fe by CLE-AdCSV (Ardiningsih et al., 2021), although it has the advantage that it forms one
655 dominant species (Waska et al., 2016), which simplifies application over a range of added ligand
656 concentrations (Abualhaija and van den Berg, 2014). Importantly, the FeNN_3 complex can also be
657 detected over a relatively wide pH range (van den Berg, 1991), allowing speciation analysis to be
658 applied over a range of pH values (Avendaño et al., 2016; Gledhill et al., 2015).



659

660 *Figure 4. Plot of DFe^* (i.e. total Fe - $FeNN_3$) versus Fe^{3+} concentrations. Calculated DFe^* and Fe^{3+} from*
 661 *titrations are shown as grey points. Only data from 1 and 5 $\mu\text{mol L}^{-1}$ HNN are shown since, in the overlaid*
 662 *titration method, DFe^* is assumed to be negligible at 20 $\mu\text{mol L}^{-1}$ HNN. Open symbols show values where DFe^**
 663 *was below detection and are set to the value of the detection limit. Solid Lines show values predicted for each*
 664 *titration using the NICA-Donnan parameter sets (A-D), at pH 8.0 and temperature 20° C with ambient DOC*
 665 *concentrations. Scenario D2 is the same as set D but a DOC concentration of 43.7 $\mu\text{mol L}^{-1}$ was applied to all*
 666 *samples. The dashed line shows the calculated DFe^* in the absence of DOM for reference. The horizontal*
 667 *facet corresponds to the HNN concentration ($\mu\text{mol L}^{-1}$) and vertical facet bins DFe^* and Fe^{3+} concentrations*
 668 *observed and calculated under the different scenarios according to their ambient DOC concentration ($\mu\text{mol L}^{-1}$).*
 669

670 Taken together, the calculated $[Fe^{3+}]_{NICA}$ values in our titrations suggest parameter sets A and D
 671 provide the best approximations of $[Fe^{3+}]_{titration}$. Examination of DFe^* suggests that NICA parameters
 672 A, C and D predict DFe^* within the range of observed values. Considering NICA sets A and D,
 673 binning the data into three different DOC concentrations showed that goodness of fit decreased
 674 slightly with increasing DOC concentration (Table 3). The increase in negative intercept with
 675 increased DOC concentration suggests that this was because the NICA model slightly overestimated
 676 Fe binding to DOM at higher DOC concentrations, and this effect was largely eliminated by
 677 assuming a constant DOC concentration of 43.7 $\mu\text{mol L}^{-1}$ with scenario D2. The overestimation of
 678 the impact of increasing DOC concentrations could point to dilution of the Fe-binding functional
 679 groups by input of autochthonous marine DOM with a lower binding site density. Since the main
 680 source of autochthonous marine DOM in our study area is phytoplankton (Carr et al., 2018; Davis et

681 al., 2018), this would imply that the overall binding affinity of DOM produced by phytoplankton is
682 lower than the aged DOM pool. There is a paucity of data investigating the acid-base binding
683 characteristics of marine DOM, so we recommend further investigation of total binding site
684 concentrations and binding site heterogeneity as a function of DOM mass (Lodeiro et al., 2020),
685 particularly with respect to the changes in DOM composition as a function of productivity.
686 Furthermore, we recommend that alternative experimental designs for titrations are explored for their
687 ability to derive intrinsic, rather than conditional, metal binding constants (e.g. titrations over a wider
688 range of pH values (Avendaño et al., 2016; Gledhill et al., 2015) or with higher pH resolution (Zhu et
689 al., 2021)).

690

691 **Prediction of Fe(III) speciation in the Celtic Sea using ambient pH and dissolved organic** 692 **carbon concentrations**

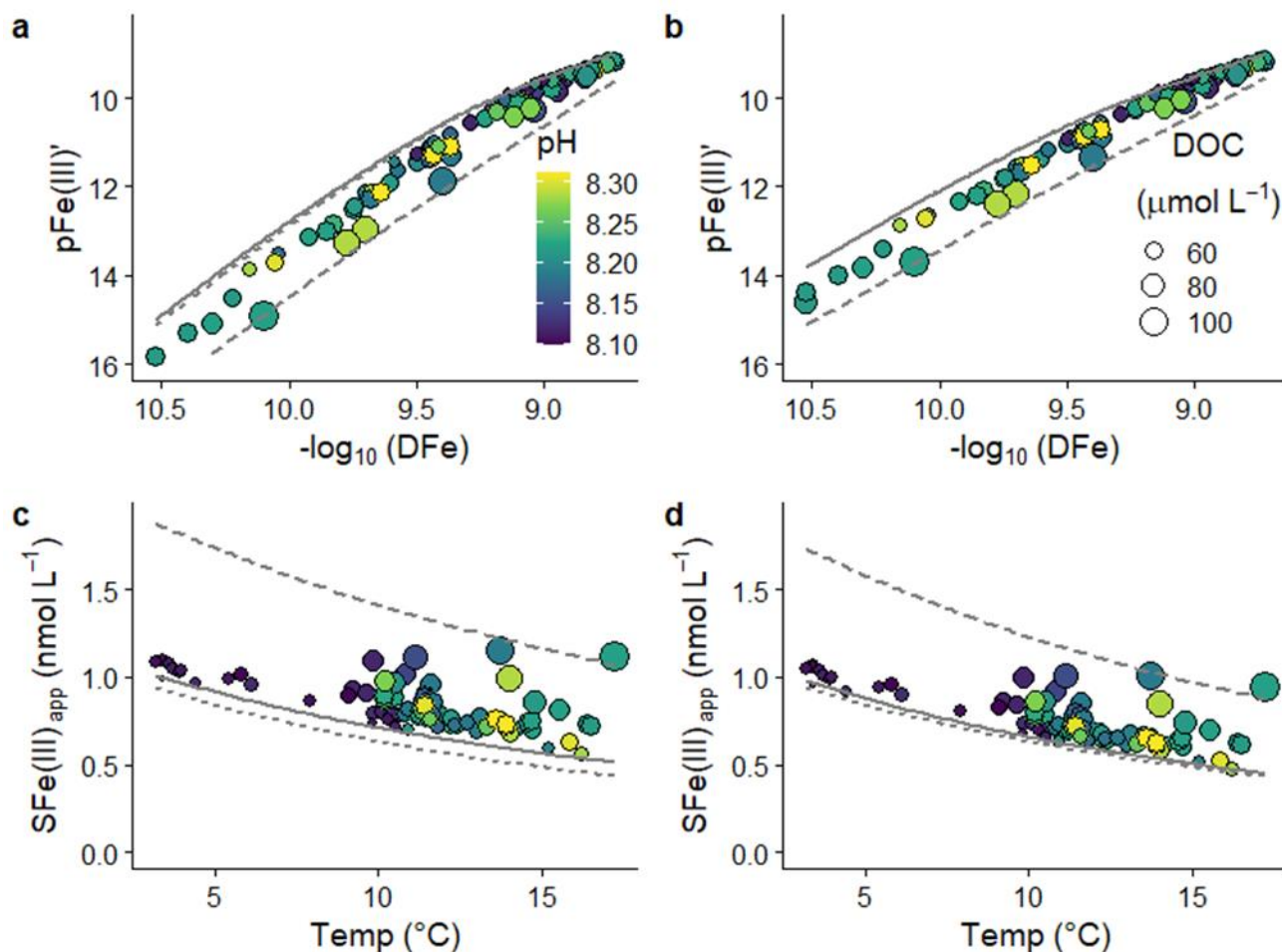
693

694 The combined impact of variability in pH and DOC concentration and choice of NICA constants on
695 calculated Fe speciation.

696 For a heterogeneous group of binding sites, DFe, pH, and DOC all influence $p\text{Fe(III)}'$. We illustrate
697 the relative importance of the key parameters for driving variability in $p\text{Fe(III)}'$ with model
698 experiments (Figure 5a and b). We calculated $p\text{Fe(III)}'$ with three scenarios based on the minimum
699 and maximum observed values for pH and DOC we encountered in our study area: i) $\text{pH}_{\text{NBS}} = 8.1$,
700 $\text{DOC} = 45 \mu\text{mol L}^{-1}$ ii) $\text{pH}_{\text{NBS}} = 8.3$, $\text{DOC} = 45 \mu\text{mol L}^{-1}$ and iii) $\text{pH}_{\text{NBS}} = 8.3$, $\text{DOC} = 150 \mu\text{mol L}^{-1}$.
701 We compare these scenarios with values calculated using ambient pH and DOC, without considering
702 the formation of $\text{Fe(OH)}_3(\text{s})$. The different scenarios show that the DOC range encountered in our
703 study area has a greater potential impact on $p\text{Fe(III)}'$ than pH does, especially for parameter set D,
704 where scenarios (i) and (ii) overlap. The low impact of pH arises because pH did not vary greatly in
705 the study region (range of ~ 0.2) and because the lower heterogeneity described by parameter set D
706 reduced the impact of pH.

707 We compared the data points and the solid curve in Figure 5 (a) and (b) and observed a decrease in
708 $p\text{Fe(III)}'$ of approximately 1 and 0.5 log units at our lowest DFe concentrations and 2 and 1 log units
709 at our highest DOC concentrations for parameter sets A and D, respectively. These values provide an
710 estimate of the likely error in $p\text{Fe(III)}'$ introduced by scaling to DOC and, not surprisingly, show that
711 the greatest impact will occur at the highest DOC concentrations (Table 1). The differences between
712 the magnitude of the estimates for sets A and D relate to the degree of heterogeneity, as described by

713 the non-ideality constant, with set D describing a less heterogeneous distribution of binding sites than
 714 set A.



715
 716 *Figure 5 (a), (b) Plots of $p\text{Fe(III)}$ ' as a function of dissolved Fe concentration (DFe) and (c),(d) apparent Fe(III)*
 717 *solubility ($S\text{Fe(III)}_{\text{app}}$) as a function of temperature for NICA parameter sets A and D respectively. The impact of*
 718 *pH and DOC concentration are shown by the colour and size of the points, respectively. The lines show the*
 719 *trend if pH and DOC are assumed constant at (i) solid line: $\text{pH} = 8.09$ and $\text{DOC} = 43.7 \mu\text{mol L}^{-1}$ (ii) short dashes:*
 720 *$\text{pH} = 8.31$ and $\text{DOC} = 43.7 \mu\text{mol L}^{-1}$ and (iii) long dashes: $\text{pH} = 8.31$ and $\text{DOC} = 150 \mu\text{mol L}^{-1}$.*

721
 722 Impact of pH, DOC and temperature on apparent Fe(III) solubility in the Celtic Sea

723 Fe(III) solubility strongly influences the overall Fe inventory in the ocean (Johnson et al., 1997) and
 724 in the absence of ligands the oceanic DFe inventory would be significantly lower (Hunter and Boyd,
 725 2007; Liu and Millero, 2002). Previous work has suggested that the ocean is saturated with respect to
 726 Fe(III) hydroxide (Byrne & Kester, 1976; Kuma et al., 1996, 1998, 2003). However, CLE-AdCSV
 727 determinations suggested that ligand concentrations are in excess of DFe, which implies that Fe(III)
 728 hydroxide might be undersaturated at the pH of the measurement (Caprara et al., 2016). The
 729 saturation state of Fe in the ocean is thus subject to some uncertainty. Furthermore, the interplay

730 between scavenging and solubility is poorly constrained (Tagliabue et al., 2016), and the potential
731 impact of changes in ambient seawater pH on Fe solubility has rarely been considered (Millero et al.,
732 2009; Ye et al., 2020).

733

734 Our calculations of $[\text{Fe}^{3+}]$ at the ambient pH and DOC concentrations described above resulted in a
735 maximum value of $4.5 \times 10^{-19} \text{ nmol L}^{-1}$ for both parameter sets A and D, obtained at the highest DFe
736 concentration of 1.9 nmol L^{-1} . At $\text{pH}_{\text{NBS}} 8.0$ and 20°C , our ion pairing model predicts formation of
737 $\text{Fe}(\text{OH})_3(\text{s})$ at an Fe^{3+} concentration of $7.58 \times 10^{-20} \text{ mol L}^{-1}$. Therefore our predicted Fe^{3+}
738 concentrations were oversaturated with respect to $\text{Fe}(\text{OH})_3(\text{s})$. We therefore used iterative speciation
739 calculations to investigate the potential interaction between Fe(III) solubility, temperature, pH and Fe
740 binding to DOM in our study area. We calculated apparent Fe(III) solubility ($\text{SFe(III)}_{\text{app}}$) by setting
741 the total Fe(III) concentrations to 10 nmol L^{-1} for all samples in the model, thereby ensuring
742 formation of the insoluble $\text{Fe}(\text{OH})_3(\text{s})$ species. $\text{SFe(III)}_{\text{app}}$ was then expressed as the sum of the
743 concentrations of aqueous inorganic Fe(III) species and Fe(III) bound to DOM.

744

745 Figure 5 (c) and (d) shows the variation of calculated $\text{SFe(III)}_{\text{app}}$ plotted as a function of temperature
746 for parameter sets A and D respectively. Trends for $\text{SFe(III)}_{\text{app}}$ for both parameter sets were similar
747 and the highest $\text{SFe(III)}_{\text{app}}$ was observed at maximum DOC concentrations. Whilst DOC was an
748 important influence on $\text{SFe(III)}_{\text{app}}$, decreased temperature and pH also both lead to increasing
749 $\text{SFe(III)}_{\text{app}}$ as a result of changes in the hydrolysis according to equation (1) (Figure 5, c and d). We
750 assessed the relative importance of pH and temperature by calculating $\text{SFe(III)}_{\text{app}}$ using the same
751 scenarios described for calculation of $\text{pFe(III)}'$ (section 4.3.2). We observed that pH had a greater
752 impact on $\text{SFe(III)}_{\text{app}}$ than on $\text{pFe(III)}'$. However, in the scenario where Fe binding does scale with
753 DOC concentration, DOC was more important than pH for our study area.

754

755 Comparisons between NICA parameter sets A and D showed that $\text{SFe(III)}_{\text{app}}$ was 0.05 nmol L^{-1}
756 higher for parameter set A than for parameter set D at our lowest DOC concentration ($43.7 \text{ } \mu\text{mol L}^{-1}$)
757 and 0.2 nmol L^{-1} higher at our highest DOC concentration ($111 \text{ } \mu\text{mol L}^{-1}$) (Figure 5, c and d). The
758 difference was driven by changes in the affinity constant and the relative non-ideality of the binding
759 sites, which effectively results in a lower binding affinity for parameter set D in comparison to
760 parameter set A.

761

762 Both parameter sets predict maximum $SFe(III)_{app}$ values (1.2 and 1.1 $nmol L^{-1}$) that are lower than
763 the determined maximum DFe concentrations (1.9 $nmol L^{-1}$). We emphasize that absolute values
764 have to be compared with caution because of systematic errors in the calculations from e.g. ionic
765 strength corrections. Here, we also need to consider the influence of physical size and filter size cut-
766 off, since the solubility product used in this study was determined using 0.02 μm filter cut off (Liu
767 and Millero, 1999), whilst Fe binding characteristics were determined with a 0.2 μm filter cut off
768 range and DOC concentrations used in this study were determined in the $<0.7 \mu m$ fraction. We note
769 that fresh Fe hydroxide nanoparticles can be as small as 2-3 nm (Cismasu et al., 2011; Janney et al.,
770 2000) and would thus be classed as dissolved when a 0.02 μm filter cut off is employed, although Fe
771 rich inorganic colloids are potentially negligible in seawater due to rapid flocculation at seawater
772 ionic strength (Gunnars et al., 2002; Krachler et al., 2012). In addition, scavenging processes in
773 which DFe is potentially reversibly adsorbed onto solid phases present in the water column are
774 thought to be an important influence on DFe concentrations (Achterberg et al., 2018; Fitzsimmons et
775 al., 2017) but are not considered in our approach. It is therefore difficult to precisely map our
776 predicted $SFe(III)_{app}$ onto DFe concentrations. Nevertheless, we considered being able to predict
777 $SFe(III)_{app}$ to within 58 % of the determined DFe concentration as encouraging and hence further
778 examined the temporal and spatial variability of Fe species calculated at ambient pH, DFe and DOC
779 in the Celtic Sea.

780

781 Calculated Fe speciation at ambient pH and temperature in the Celtic Sea

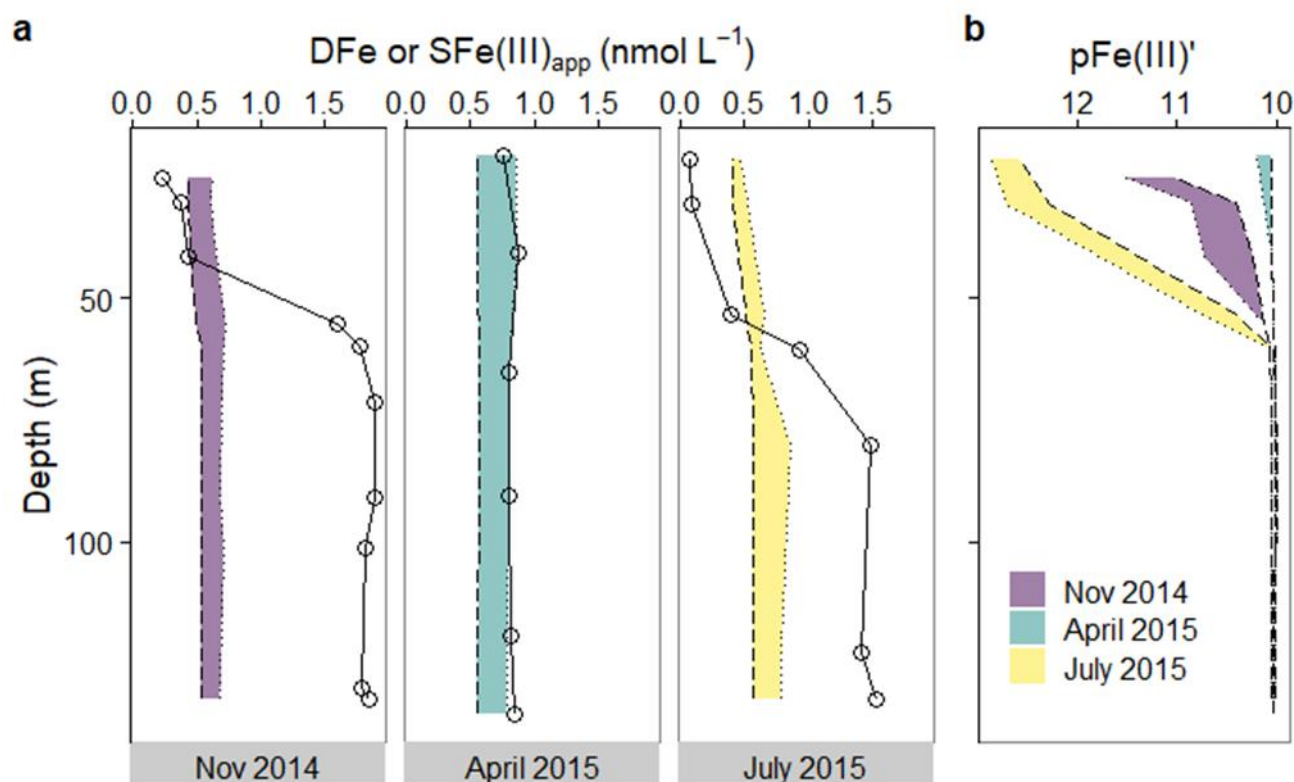
782 We examined spatial and temporal variability in Fe speciation that results from changes in DFe and
783 pH in our study region using parameter set D. However, we note that the differences in both
784 calculated $pFe(III)'$ and $SFe(III)_{app}$ between A and D were limited (maximum for $pFe(III)'$ of 1.2 log
785 units and 0.2 $nmol L^{-1}$ for $SFe(III)_{app}$), especially at low DOC concentrations (negligible for $pFe(III)'$
786 and 0.05 $nmol L^{-1}$ for $SFe(III)_{app}$). We re-calculated Fe speciation using ambient DFe concentrations
787 and allowed for formation of $Fe(OH)_3(s)$ where $DFe > SFe(III)_{app}$. We first examined the temporal
788 variability in $SFe(III)_{app}$ and $pFe(III)'$ on the Celtic Sea Shelf and then spatiotemporal variation
789 across the shelf break using i) ambient DOC and ii) a fixed DOC concentration set to the lowest
790 deep-water DOC concentration observed in our study area (43.7 $\mu mol L^{-1}$).

791

792 Seasonal variability in the Central Celtic Sea (site CCS) on the shelf.

793 The hydrography and the seasonal cycles of DFe, DOC and pH of the Celtic Sea during our sampling
794 period has been described in detail elsewhere (Birchill et al., 2017; Carr et al., 2018; Humphreys et
795 al., 2019; Rusiecka et al., 2018). Briefly, DFe concentrations varied both in the surface mixed layer
796 and deeper waters, with the spring bloom resulting in significant drawdown of DFe in surface waters
797 ($0.08 \pm 0.01 \text{ nmol L}^{-1}$, $n=2$) to levels similar to observations in open ocean regions, while in deeper
798 waters DFe increased from 0.82 ± 0.02 , $n=3$ (April) to $1.48 \pm 0.06 \text{ nmol L}^{-1}$, $n=3$ (July) (Birchill et
799 al., 2017). In the surface mixed layer, DOC concentrations were highest in April ($73.3 \pm 2.9 \text{ } \mu\text{mol L}^{-1}$,
800 $n=3$) and lowest in July ($57.7 \pm 4.0 \text{ } \mu\text{mol L}^{-1}$, $n=5$) (Figure S4). DOC concentrations tended to
801 decrease with increasing depth in April and November, and concentrations in all three samples below
802 the thermocline were $64.8 \pm 0.0 \text{ } \mu\text{mol L}^{-1}$ ($n=3$) in April and $58.7 \pm 1.8 \text{ } \mu\text{mol L}^{-1}$ ($n=4$) in
803 November. In July, the trend of DOC was opposite, such that above the thermocline DOC decreased
804 with increasing depth, whilst higher DOC was observed below the thermocline ($67.6 \pm 4.6 \text{ } \mu\text{mol L}^{-1}$,
805 $n=2$). pH was higher in surface waters compared to deeper waters during all three sampling
806 campaigns (Figure S4). A vertical gradient in pH was observed in November with a difference of
807 0.11 between the surface mixed layer and below the mixed layer.

808 The changes in DFe, pH and temperature throughout the seasonal cycle resulted in changes in both
809 $\text{SFe(III)}_{\text{app}}$ and $\text{pFe(III)}'$ (Figure 6). In surface waters in July and November, DFe was consistently
810 lower than calculated $\text{SFe(III)}_{\text{app}}$ and as expected, DFe was thus undersaturated with respect to
811 $\text{Fe(OH)}_3(\text{s})$ formation in our calculations (Figure 6a). Below the mixed layer ($>75 \text{ m}$),
812 remineralization of sinking organic matter in the bottom mixed layer resulted in increased DFe
813 (Birchill et al., 2017) and formation of $\text{Fe(OH)}_3(\text{s})$ in our calculations (Figure 6a). With the constant
814 DOC scenario, $\text{SFe(III)}_{\text{app}}$ changed by $< 0.03 \text{ nmol L}^{-1}$ at station CCS. For the ambient DOC scenario
815 $\text{SFe(III)}_{\text{app}}$ was overall higher by 0.3 nmol L^{-1} as a result of the increased DOC concentrations
816 (Figure S4), however variability was also low ($< 0.02 \text{ nmol L}^{-1}$). We found that the $\text{SFe(III)}_{\text{app}}$
817 determined by our speciation model for the bottom mixed layer were very similar ($0.54\text{-}0.87 \text{ nmol L}^{-1}$,
818 Figure 6a) to the concentrations of DFe determined throughout the well-mixed water column in
819 April ($0.82 \pm 0.04 \text{ nmol L}^{-1}$, $n=6$). Our results therefore suggest that when the water column is
820 stratified, water below the mixed layer is oversaturated with Fe as a result of constant supply of DFe
821 by remineralization. Winter mixing subsequently resets the DFe inventory to one that our results
822 suggest could be based on Fe solubility.



824

825 Figure 6. (a) Seasonal changes in the vertical distribution of observed dissolved Fe ($<0.2 \mu\text{m}$, point and solid line) and calculated
 826 apparent Fe(III) solubility ($S\text{Fe(III)}_{\text{app}} = \text{Fe(III)}' + \text{Fe bound to DOM}$) using NICA parameter set D. Lines show values calculated using a
 827 fixed DOC concentration of $43.7 \mu\text{mol L}^{-1}$ (long dashes) or ambient DOC concentration (dotted line) and the shaded area highlights the
 828 difference between the two scenarios. For calculation of $S\text{Fe(III)}_{\text{app}}$, we assumed a total Fe(III) concentration of 10 nmol L^{-1} in our
 829 calculations and DFe was therefore not an input parameter in the calculations. (b) $p\text{Fe(III)}'$ ($-\log_{10}(\text{Fe(III)}')$) was calculated allowing for the
 830 formation of ferrihydrite when DFe was greater than $S\text{Fe(III)}_{\text{app}}$ with ambient (dots) and fixed (dashes) DOC concentration scenarios.
 831 Calculations were performed using ambient pH and temperature for samples collected on the shelf in autumn (November 2014),
 832 spring (April 2015) and summer (July 2015).

833

834 Above the mixed layer, $p\text{Fe(III)}'$ was primarily dependent on DFe concentrations, with pH having a
 835 minor influence (Figure 6b) because of the low degree of heterogeneity predicted by parameter set D
 836 (Figure 5b). Below the surface ($>75 \text{ m}$), the over-saturation of Fe in July and November meant that
 837 $p\text{Fe(III)}'$ was rather constant (10.04 ± 0.02) and controlled by the formation of $\text{Fe(OH)}_3(\text{s})$ in our
 838 calculations rather than by the strength of binding to organic matter. In April, surface water ($<75 \text{ m}$)
 839 $p\text{Fe(III)}'$ was similar to those in deeper waters as the water column was well-mixed, whilst a marked
 840 increase of $p\text{Fe(III)}'$ was observed from surface to deeper waters in July and November (Figure 6b).
 841 $p\text{Fe(III)}'$ was thus predicted to increase in surface waters from summer through to spring in both
 842 constant and ambient DOC scenarios. The increase in $p\text{Fe(III)}'$ was thus largely driven by the
 843 drawdown of DFe in April resulting from phytoplankton productivity (Birchill et al., 2017). After
 844 July, the slight increase in vertical exchange due to mixing and the on shelf circulation pattern

845 resulted in a decrease surface water $p\text{Fe(III)}$ from July to November, even though the water column
846 remained stratified.

847

848 Spatiotemporal variation in key variables and Fe speciation over the Shelf break

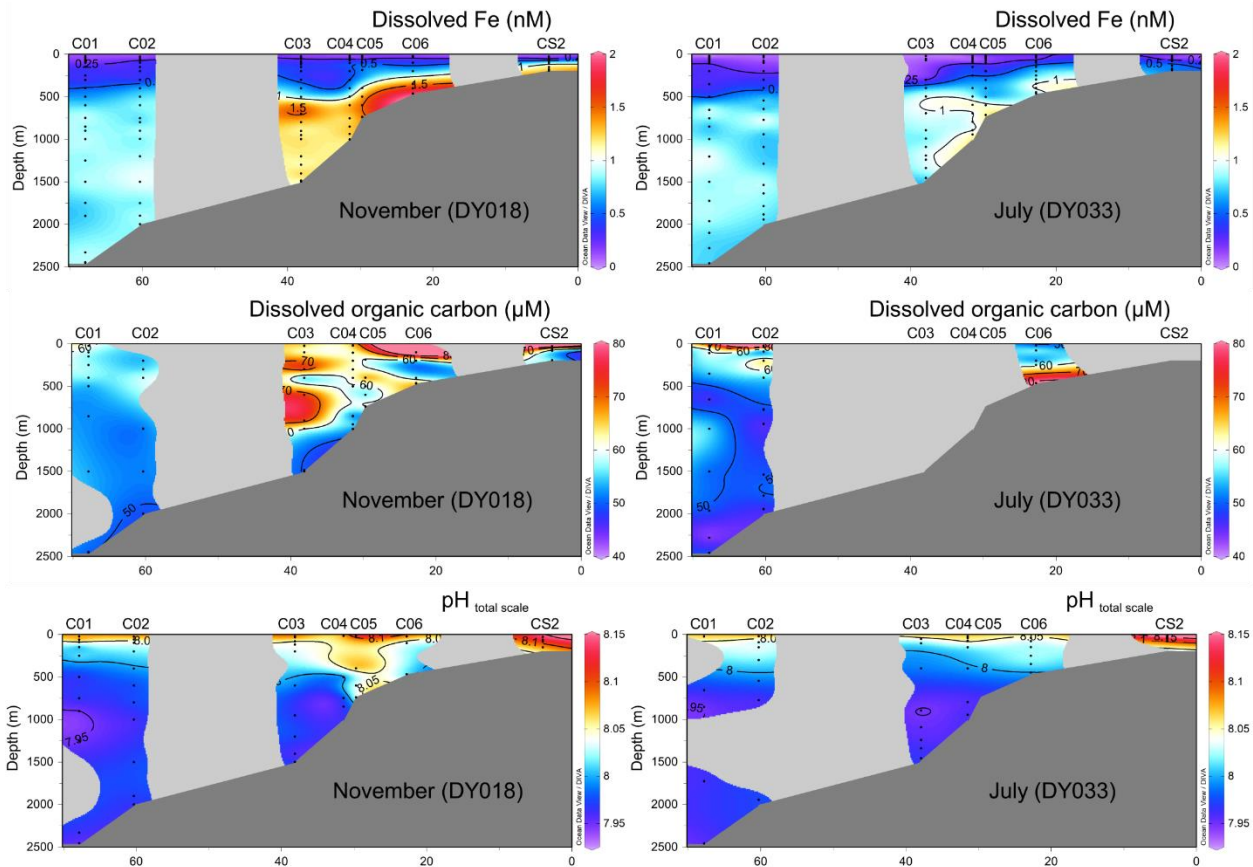
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850 Temperature and salinity data over the shelf break are provided in Figure S5. Dissolved Fe ranged in
851 concentration between 0.03-1.90 nmol L^{-1} along the transect and was lower in surface waters
852 ($0.22 \pm 0.12 \text{ nmol L}^{-1}$ in November, $0.20 \pm 0.28 \text{ nmol L}^{-1}$ in July), and enhanced in deeper waters below
853 ~500 m ($1.04 \pm 0.24 \text{ nmol L}^{-1}$ in November, $0.87 \pm 0.14 \text{ nmol L}^{-1}$ in July, Figure 7). The distribution
854 and concentration of DFe are broadly consistent with previous observations in the Celtic Sea
855 (Nedelec et al., 2007) and neighbouring Bay of Biscay (Laès et al., 2007; Ussher et al., 2007). A
856 notable exception is that the DFe observed in this study during July 2015 in the surface mixed layer
857 include the lowest reported DFe concentrations ($< 0.1 \text{ nmol L}^{-1}$) for waters in this region. These are
858 attributed to biological Fe uptake during the spring bloom coupled with low external inputs to the
859 surface mixed layer (Birchill et al., 2017). In contrast to surface waters, concentrations of DFe in
860 excess of 1.00 nmol L^{-1} (to a maximum of 1.90 nmol L^{-1}) were observed at inner shelf stations in
861 November (C03-C06, CS2) and July (C04-06) at depths $> 500 \text{ m}$, which we attribute to a lateral flux
862 of DFe from the Celtic Sea shelf slope (Nedelec et al., 2007).

863

864 Average DOC concentrations of $60.1 \pm 9.2 \text{ } \mu\text{mol L}^{-1}$ ($n= 48$) were observed in November, and $58.4 \pm$
865 $14.2 \text{ } \mu\text{mol L}^{-1}$ ($n= 28$) in July (Figure 7). Higher DOC concentrations were occasionally observed in
866 surface waters (station C06 in November ($98.23 \text{ } \mu\text{mol L}^{-1}$) and station C02 in July ($111 \text{ } \mu\text{mol L}^{-1}$)).
867 Between 200-1000 m, DOC was higher at C03 station than at other stations in November, a feature
868 that partly coincided with higher DFe concentrations. In the deep ocean ($> 1000 \text{ m}$), DOC was
869 slightly lower in July ($49.2 \pm 3.19 \text{ } \mu\text{mol L}^{-1}$) than in November ($52.3 \pm 3.3 \text{ } \mu\text{mol L}^{-1}$).

870



871

872 *Figure 7. The distribution of dissolved Fe (DFe), dissolved organic carbon (DOC), pH_{total} (on the total scale) during autumn (DY018,*
 873 *November 2014) and summer (DY033, July 2015) over the shelf break.*

874

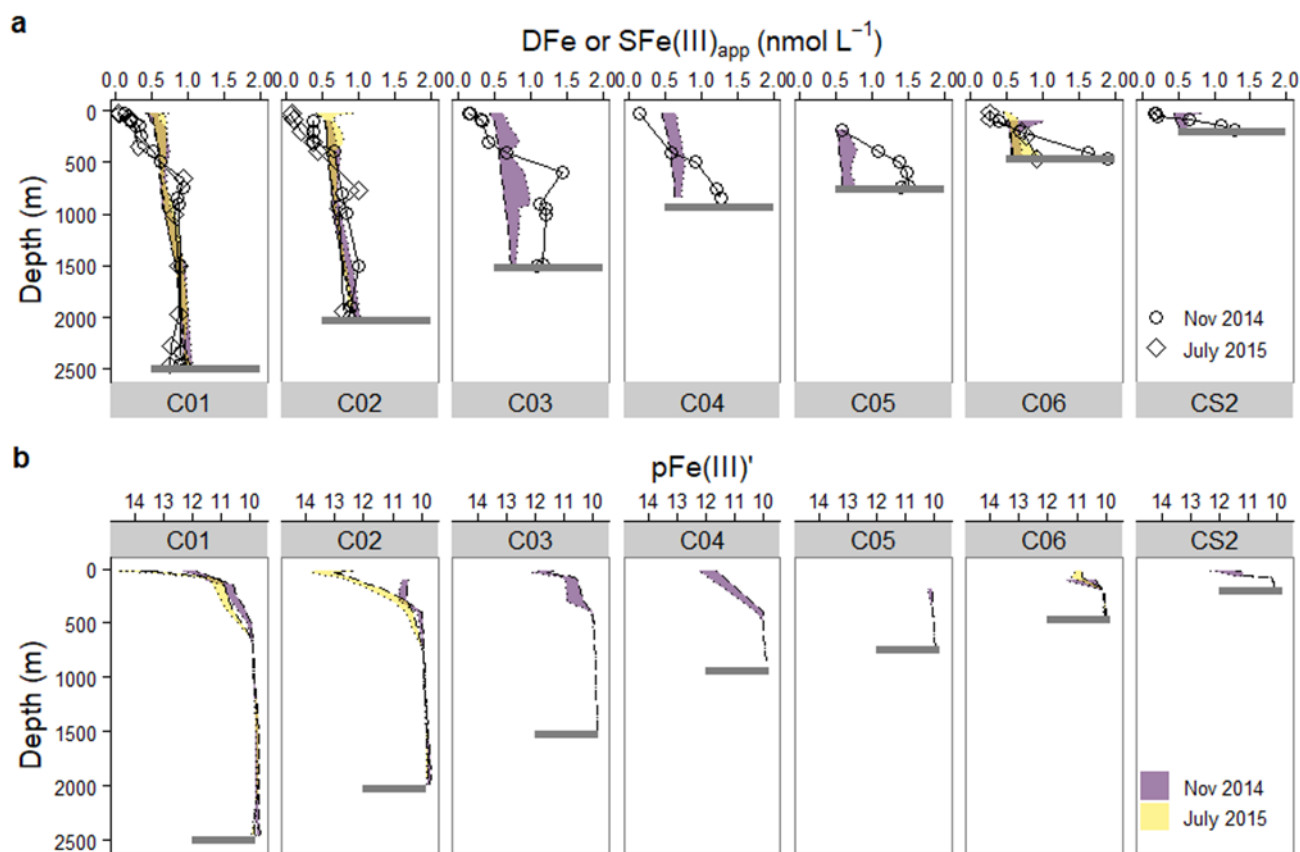
875 Surface waters in the area exhibited higher, relatively uniform pH (Figure 7). Higher surface water
 876 pH at C04-06 stations coincided with higher DOC in autumn, which suggest that both of these
 877 features were driven by increased productivity as observed at CCS. At depth (> 1000 m), changes in
 878 pH corresponded to changes in salinity and temperature and were thus likely influenced by water
 879 mass circulation and the biological carbon pump (Figure S5).

880

881 For both the ambient and constant DOC scenarios, $SFe(III)_{app}$ was >0.8 $nmol L^{-1}$ in the deep ocean
 882 (>1500 m) at stations C01 and C02 in November and July (Figure 8). Mean $SFe(III)_{app}$ (0.96 ± 0.08
 883 and 0.88 ± 0.08 $nmol L^{-1}$ for the ambient and constant DOC scenarios respectively) was again
 884 remarkably close to the mean observed DFe concentrations (0.9 ± 0.1 $nmol L^{-1}$). We emphasize here
 885 that the DFe concentration is not a parameter included in the calculations of $SFe(III)_{app}$, since the
 886 total Fe concentration is set to 10 $nmol L^{-1}$ for all samples and our calculated $SFe(III)_{app}$
 887 concentrations are thus independent of DFe. The potential impact of scaling to DOC concentration is
 888 illustrated by increases in the difference between $SFe(III)_{app}$ calculated where ambient DOC

889 concentrations were high ($>70 \mu\text{mol L}^{-1}$) relative to the assumed constant concentration scenario of
890 $43.7 \mu\text{mol L}^{-1}$ at stations C03-CS2 in November or at station C06 in July (Figure 8). Nevertheless the
891 difference between $\text{SFe(III)}_{\text{app}}$ calculated at constant DOC and ambient DOC was always less than
892 0.54 nmol L^{-1} (Figure 8) and scaling to DOC thus has a limited overall impact on determined
893 $\text{SFe(III)}_{\text{app}}$ in shelf waters.

894 In surface waters, DFe was consistently lower ($<0.25 \text{ nmol L}^{-1}$) than $\text{SFe(III)}_{\text{app}}$ predicted using both
895 DOC scenarios and Fe^{3+} was thus undersaturated with respect to $\text{Fe(OH)}_3(\text{s})$ formation in our
896 calculations (Figure 8), as observed for surface waters at CCS. The depth at which DFe became less
897 than $\text{SFe(III)}_{\text{app}}$ shoaled with the DFe concentration (Figure 7 and 8). In waters close to the seafloor
898 on the inner shelf (C03-C06), DFe concentrations were in excess of the $\text{SFe(III)}_{\text{app}}$ concentration. As
899 described for station CCS and observed on the Peruvian Shelf (Zhu et al., 2021), we suggest that
900 these waters were influenced by non-equilibrium processes. Our speciation calculations are
901 considered to be at equilibrium and thus do not account for any non-equilibrium processes that may
902 be occurring in the water column, such as remineralization, scavenging, inputs from sediments or
903 changes in redox state. Our study region is known to experience inputs of DFe along with other
904 metals in nepheloid layers that propagate offshore from the sediments over the shelf break (Laës et
905 al., 2007; Rusiecka et al., 2018), and previous work found that the authigenic or scavenged fraction
906 of particulate Fe becomes increasingly important close to the seafloor (Marsay et al., 2017). Such
907 sediment-derived benthic inputs can be expected to be scavenged from the water column and
908 adsorptive processes are likely to depend on particle concentrations (Bergquist and Boyle, 2006;
909 Fitzsimmons et al., 2013; John et al., 2018). However, the mechanisms and processes governing
910 scavenging in the ocean are poorly constrained (Boyd and Ellwood, 2010; Tagliabue et al., 2014) and
911 scavenging rates are effectively treated as “free” parameters in biogeochemical models and thus
912 tuned to achieve realistic Fe concentrations (Tagliabue et al., 2014). Our work confirms previous
913 studies (Hiemstra and van Riemsdijk, 2006) suggesting that the solubility of Fe is an important
914 constraint on the extent of Fe scavenging in the ocean.



915

916 *Figure 8. (a) Changes in the vertical distribution of calculated $SFe(III)_{app}$ using NICA parameter set D - observed dissolved Fe ($<0.2 \mu\text{mol L}^{-1}$, point/diamond and solid line) are provided for reference. Lines show values calculated using a fixed DOC concentration of $43.7 \mu\text{mol L}^{-1}$ (long dashes) or ambient DOC concentration (dotted line) and the shaded area highlights the difference between the two scenarios. (b) $pFe(III)'$ ($-\log_{10}(Fe(III)')$) was calculated from DFe, or $SFe(III)_{app}$ when $DFe > SFe(III)_{app}$. Calculations applied the ambient pH and temperature for samples collected over the shelf break in autumn (November 2014) and summer (July 2015) seasons. DOC concentrations were not determined for C03-C05 and CS2 in July 2015. Grey bars show the depth of the water column at each station.*

924 Calculated $pFe(III)'$ was lowest in deep waters and highest in surface waters indicating an increase in
 925 $Fe(III)'$ in deeper waters (Figure 8b, $pFe(III)'$ scale is reversed). Below $\sim 500\text{m}$, $pFe(III)'$
 926 (10.04 ± 0.02) was relatively constant throughout the water column in November and July and
 927 irrespective of the DOC scenario, largely because it is set by the solubility product of $Fe(OH)_3(s)$ in
 928 our calculations (i.e. $pFe(III)' \propto [Fe^{3+}]$ which in turn is limited by $Fe(OH)_3(s)$). In surface waters,
 929 when $pFe(III)'$ is dependent on the DFe concentration, $pFe(III)'$ increased towards the open ocean
 930 from a minimum of 10.8 (constant DOC) or 11 (ambient DOC) at C06 to a maximum of 13.2
 931 (constant DOC) or 14.6 (ambient DOC) at C01 (Figure 8). For the stations furthest offshore, the
 932 potential impact of scaling to DOC for calculation of $pFe(III)'$ was more important. For example, a
 933 $30 \mu\text{mol L}^{-1}$ increase in DOC resulted in an increase of two units in $pFe(III)'$ in surface waters at
 934 station C01. We found that values of $pFe(III)'$ predicted using parameter set D under both constant

935 and variable DOC scenarios encompassed the range of values found to support both iron-replete and
936 iron-limited growth in families of phytoplankton including cyanophytes, haptophytes and diatoms
937 which have been observed in our study area (Blain et al., 2004). For example, reduction in growth of
938 *Synechococcus* sp. was shown to begin at pFe' values of 14 (Timmermans et al., 2005), close to the
939 lowest values predicted by parameter set D with ambient DOC concentrations, while Sunda &
940 Huntsman (1995) found onset of growth was limited at 20 pmol L^{-1} ($pFe(III)'$ = 10.7) for the small
941 haptophyte *Emiliania huxleyi* and at 160 pmol L^{-1} ($pFe(III)'$ = 9.8) for the diatom *Thalassiosira*
942 *weissflogii*. However, we have not considered the role of redox chemistry in our calculations. Fe(II)
943 is known to be more readily available to phytoplankton (Shaked and Lis, 2012) and significant
944 concentrations of Fe(II) can be formed via photochemical reduction in surface waters, with Fe(II)
945 concentrations of up to 175 pmol L^{-1} previously reported for surface waters (<50 m) in this region
946 (Ussher et al., 2007).

947

948 **Conclusions**

949

950 In this work, we combined analysis of Fe speciation by AdCSV with an ion-pairing/NICA-Donnan
951 model to determine Fe(III) speciation at equilibrium in the Celtic Sea. We first calibrated our
952 competing added ligand (HNN) in the absence of organic matter for the experimental conditions
953 applied in our study. We then compared titration data obtained by varying both Fe concentrations and
954 HNN concentrations with calculations of Fe speciation predicted via the NICA-Donnan model with
955 four sets of parameters and found that the parameter sets that predicted relatively weak binding with
956 low heterogeneity best described our titration data. We further found that fits improved on
957 application of a constant low DOC concentration of $43.7 \text{ } \mu\text{mol L}^{-1}$ across the data set, rather than
958 assuming that binding scaled to ambient DOC concentrations. This suggests that binding sites may be
959 more strongly linked to the refractory component of marine DOM and autochthonous marine inputs
960 of DOM that result from phytoplankton productivity may not result in increased binding site
961 concentration or heterogeneity.

962 We used the NICA-Donnan parameters that fitted most closely to our titration data to predict
963 $SFe(III)_{app}$ and $pFe(III)'$ at ambient seawater pH and temperature with both ambient and fixed DOC
964 concentrations. Calculated $SFe(III)_{app}$ concentrations (ca. 0.9 nmol L^{-1}) were within the range of the
965 water column DFe concentrations observed on the shelf after winter mixing and also the furthest off-
966 shore deep water DFe concentrations. In surface waters DFe concentrations were lower than

967 SFe(III)_{app} as result of the drawdown of DFe by phytoplankton. On the shelf in July and November
968 and over the shelf break DFe exceeded SFe(III)_{app} in deeper waters close to the seafloor, which could
969 potentially be ascribed to inputs of DFe from remineralization and/or release from sediments.
970 Although the proximity of our calculated SFe(III)_{app} to the observed DFe concentrations is very
971 encouraging, we highlight that our calculations are a simplification of the real system since we do not
972 account for non-equilibrium processes, and the physical size of our SFe(III)_{app} fraction may not map
973 directly onto the DFe concentration. Comparing the fixed and ambient DOC scenarios suggests that
974 scaling binding site concentrations to DOC concentrations has a limited overall impact on Fe
975 speciation and the impact was mostly restricted to surface waters where DFe concentrations are lower
976 than SFe(III)_{app}. Since SFe(III)_{app} is controlled by the solubility of Fe(OH)₃(s), relative changes in
977 SFe(III)_{app} will depend on both pH and temperature. In our study region, changes in temperature
978 resulted in a potential 0.5 nmol L⁻¹ change in SFe(III)_{app}, whilst the pH range observed in our study
979 area was too limited to detect a strong pH effect. The impact of temperature on Fe speciation
980 therefore deserves further consideration in future studies.

981 We also calculated pFe(III)′ in our study region and predicted values between 10 and 14, a range
982 which encompasses the range of pFe(III)′ shown to limit growth in phytoplankton. The lower limit
983 on pFe(III)′ was set by the solubility of Fe(OH)₃(s). The upper limit and changes in pFe(III)′ were
984 strongly influenced by the DFe concentration, although DOC concentrations also had an impact if
985 binding site concentrations are scaled to DOC. The limited pH range and low binding site
986 heterogeneity meant that pH did not have a strong influence on pFe(III)′ in this study region.

987 We suggest that the use of intrinsic binding parameters for Fe binding to DOM has the potential to
988 improve understanding of the influence of organic matter on Fe solubility at ambient pH and
989 temperatures and allow for more confident disentangling of the different processes affecting the DFe
990 inventory, although further work is required to refine NICA constants for Fe in seawater.

991 Furthermore, our results suggest it may be possible to further simplify calculations of Fe speciation in
992 marine waters by assuming a constant binding site concentration, at least in waters remote from
993 terrestrial influences, although this finding should be confirmed in further work employing more
994 sensitive analytical approaches for determination of Fe speciation than we applied in this study. A
995 robust parameterization of the relationship between pH, DOC, temperature and DFe with respect to
996 both Fe bioavailability and solubility also has the potential to provide for a more mechanistic
997 description of Fe binding in global biogeochemical models.

998

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1000

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1384 **Abbreviations**

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Terms	Description
$k_{FeNN_3, Fe', or Fe^{3+}}^{cond}$	Conditional stability constants describing the strength of a complex FeNN ₃ relative to inorganic Fe concentration or free Fe ³⁺ concentrations
$\alpha_{FeNN_3, Fe', or Fe^{3+}}$	Side reaction coefficient for FeNN ₃ expressed relative to inorganic Fe concentration or free Fe ³⁺ concentrations
k_{FeNN_3, H^+}^{cond}	Stability constants of HNN used in an ion pairing model, that would account for competition between NN ⁻ , H ⁺ , Fe ³⁺ and OH ⁻ at the ionic strengths and pH relevant to our study
Detection window	The detection window describes the range over which competition between NN ⁻ and binding sites (L ⁻) can be detected. It is traditionally defined as ± 1 or 1.5 log units of $\alpha_{FeNN_3, Fe'}$ (Apte et al., 1988)
$k_{FeL, Fe'}^{cond}$	Conditional stability constants describing the strength of a complex FeL relative to inorganic Fe concentration
$\alpha_{FeL, Fe'}$	Side reaction coefficient for metal-natural ligand expressed relative to inorganic Fe concentration
$\log K_{Fe(III)1 or 2}$	The median value of distribution of binding affinity of Fe(III) binding to organic matter in the NICA-Donnan model
$n_{Fe(III)1 or 2}$	The non-ideal constants describe the ratio of Fe(III) to binding sites
$[Fe^{3+}]_{titration}$	Free Fe concentrations determined in titrations
$[Fe^{3+}]_{cal}$	Free Fe concentrations calculated using an ion pairing model in the absence of organic matter
$[Fe^{3+}]_{NICA}$	Free Fe concentrations calculated using the NICA-Donnan model in the presence of organic matter
DFe* _{titration}	The non-labile fraction of Fe determined in titrations (i.e. total Fe – FeNN ₃)
DFe* _{NICA}	The non-labile fraction of Fe calculated using the NICA-Donnan model in the presence of organic matter (i.e. total Fe – FeNN ₃)

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