Dear Gwenaël Abril

Thank you for giving us the opportunity to improve our manuscript ("Organic Iron Complexes Enhance Iron Transport Capacity along Estuarine Salinity Gradients" by Herzog et al.). All the referee's comments have been carefully addressed with a detailed description followed by a suggestion for improvements. Further, the suggested changes have been implemented into a revised version of the manuscript.

The responses to the referee's comments are structures as follows: (1) comments from referees, (2) *author's response (italic)* and *author's suggested changes in manuscript (italic and blue font colour)*.

Response to comments by referee #1:

Briefly, the manuscript presents the use of X-ray spectroscopy for the characterization of iron species in freezed-dried samples collected in a range of Scandinavian rivers ending in the Baltic Sea (except one in the Skagerrak). The study is complemented with mixing experiments with artificial seawater to imitate natural estuarine processes. The research group has a broad experience in the use of this specific technique and has published a series of interesting paper on iron speciation in Baltic rivers. The paper is a fine piece of work and brings forward many interesting conclusions about iron transition in riverine waters of boreal rivers. I really wish the authors would extend their area of study and produce similar works in rivers from other areas covering catchments of different characteristics. I also think the authors should start collaborations with other groups that could provide other analytical techniques due to the limitations of the analytical approach shown in their work for species quantification. Overall, this is a very interesting work that brings a lot of qualitative information about the wide range of iron speciation that can be found in fairly similar estuaries. The manuscript supports the recent hypothesis that iron can scape high latitude estuaries in a percentage substantially higher than previously thought. The data has good quality and increases our understanding of estuarine processes. I recommend its publication after moderate revision mainly based on the need to increase the revision of literature (especially of literature referred to other analytical techniques) and improvable description of Fe changes under the increase of salinity. Sampling handling should be polished but I do not think this invalidates the manuscript.

Thank you for these positive remarks and constructive and interesting suggestions for further research within this theme. We have included literature as suggested and polished the description of sampling handling. The detailed response to each comment is listed below (in italic).

I have a few major concerns (nothing cannot be fixed):

1. Data cooking is quite complicated and in principle very difficult to evaluate for an external reviewer and takes a few arguable assumptions. In principle, I have no doubts about the skills of the authors about this process. However, the result is a series of values without any indication of uncertainty or variability. This is a major issue in this kind of studies and in the few number of their papers I went through to prepare this review, there is no indication/description/estimation of the uncertainty associated to the values presented. I would appreciate a paragraph where the topic is addressed for a non initiated in the use of X-ray spectroscopy. So the reader can have an idea of the confidence can be given to the numbers presented in tables. It is also clear that trends obtained with different variables match but it is not clear how pecentaged of org/inor compare using different

ratios.

As noted by the referee, the approach used in this study to analyse and present XAS data has been used in previous work (Karlsson and Persson 2012; Sundman, et al. 2014). The two data evaluation techniques used in this study (CN- and LCF-ratios) are based on different modelling approaches of the XAS data to show the contribution of organic matter to the Fe phases. The CN-ratio is based on the analysis of the X-ray absorption fine structure (EXAFS) region, providing information about the local coordination environment of Fe by quantitative modeling with input structures related to the natural samples. The LCF-ratio is based on a linear combination fitting (LCF) analysis by using a set of reference spectra, allowing to estimate the proportion of the two dominating Fe phases (Fe-OM complexes and Fe (oxy)hydroxides). There was agreement between the two ratios, i.e. a significant correlation between the CN- and the LCF-ratio was observed.

While XAS data is informative to the local structure around the selected element, in this case Fe, it is not strictly quantitative because of the large uncertainty in fitting the amplitude of the spectra, which mainly contain information about the contribution of each component. We therefore prefer to use the ratios to identify trends in the relative contribution of Fe-OM vs Fe (oxy)hydroxides and refrain from presenting exact percentages. For clarification the following has been added to the manuscript (Line 276):

"The XAS data contains information on the local structure around the selected element (Fe). It it is not strictly quantitative. Therefore, the ratios were merely used to identify trends in the relative contribution of Fe-OM vs Fe (oxy)hydroxides among the samples."

The confidence limits on the obtained distances and coordination numbers were estimated by Sundman, et al. (2013) by a procedure recommended by the International XAFS Society Standards and Criteria Committee (Sayers 2000). Each parameter was varied in a stepwise fashion away from its optimal value, while varying all other parameters until $\Delta\chi 2$ increased 1.0 above its minimum value. This resulted in the following confidence limits: CN Fe–O: ±0.6; CN Fe–C: ±0.7; CN Fe–Fe: ±0.6; R Fe–O: ±0.01; R Fe–C: ±0.07; and R Fe–Fe: ±0.06. As we used the same experimental setup and modeling approach as Sundman et al. 2013, the same confidence limits apply to our data. The confidence limits have been added to the caption in Table S1, as follows:

"Confidence limits on the obtained distances and coordination numbers estimated by a procedure recommended by the International XAFS Society Standards and Criteria Committee (Sayers 2000) performed by (Sundman, et al. 2013) CN Fe–O: ±0.6; CN Fe–C: ±0.7; CN Fe–Fe: ±0.6; R Fe–O: ±0.01; R Fe–C: ±0.07; and R Fe–Fe: ±0.06."

2. My second concern refers to the description of iron estuarine processes during the discussion. At the end of the discussion there is an approach to the real complexity of processes but in the first 3 sections there are oversimplifications. Example: "complexed Fe (Fe-OM) can "survive" the salinity gradient, while Fe (oxy)hydroxides are prone to aggregation and selectively removed". No possibility of FeO(OH) remaining in solution, OM is always described as a unique species where the real case is an extraordinary heterogeneity, ternary FeO(OH)-OM are only considered to the end of discussion, fulvic vs humic , OM flocculation and precipitation, etc. I wonder how ternary phases FeO(OH)-OM would show in the WT contour plots. Would those separate on its components or create a third patch?

The XAS technique captures local structures and this means that the method assigns Fe into either organically complexed or Fe(oxy)hydroxides. While it is very likely that molecules/colloids/particles in the suspension include ternary phases, the method does not distinguish this.

3. Third, the use of artificial seawater for mixing experiments. This is an interesting experiment here to see the effect of ions but it is expected that marine OM plays a role in all these processes. So no surprise that the empirical transport parts ways from the "theoretical" value. The experiments presented here are perfectly valid and offer interesting results but the possibility that marine OM plays a role is not considered in the

discussion.

We will expand the discussion on the validity of the artificial seawater mixing experiments and bring in the comparison between the in situ and theoretical values of Fe concentration along salinity gradient into the manuscript, which was presented in the supplementary information. In this context we will also acknowledge the possible role of marine organic matter. The following text addition is suggested to better describe the strengths and weaknesses of the artificial mixing experiments (Line 421):

" Results regarding Fe transport capacity derived from the artificial seawater mixing experiments were in good agreement with the estuarine transects sampled. Theoretically calculated Fe concentrations, based on Fe loss in artificial seawater mixing experiments with river water and the dilution factor, showed only minor deviations from Fe concentrations measured in the Gullmar Fjord. For the Öre estuary on the other hand, measured Fe concentrations were somewhat higher than the theoretical calculations (Figure S3). In the low-salinity mixing regime present in the northern Baltic (Bothnian Bay), aggregation may occur without significant sedimentation (Forsgren and Jansson 1992). This has been observed in the plume of nearby River Kalix, and was hypothesized to result from a high organic component of the aggregates, where low specific density may lead to transport of these aggregates far away from the river mouth (Gustafsson, et al. 2000). Thus, the centrifugation used to efficiently separate aggregates in the mixing experiments, may overestimate estuarine particle loss in this context. Despite the agreement between measured and theoretically estimated Fe concentrations, the artificial mixing experiments are unlikely to capture all processes that affect the loss of Fe along the natural salinity gradient. In the estuary, photoreduction may affect Fe speciation and affect its fate, as well as the occurrence of ligands produced by marine biota which may also influence the behaviour of riverine Fe. Indeed, the artificial mixing experiments capture the response of riverine Fe to increasing salinity in isolation, and how that depends on Fe speciation."

4. Fourth, there is a lot of literature not considered in the introduction. We have information about the fate of iron ligands in estuarine waters from recent work with voltammetric techniques (Buck, Lohan et al. 2007, Laglera and van den Berg 2009, Bundy, Abdulla et al. 2015, Su, Yang et al. 2015, Su, Yang et al. 2016, Yang, Su et al. 2017, Su, Yang et al. 2018). Fe transport is not specifically calculated but can be inferred from data. It is not about citing them all but at least acknowledging their existence and the hypotheses included. There is also interesting mixing work done on iron transport capacity with isotopic labelled iron (Krachler group) (Krachler, Jirsa et al. 2005, Krachler, Krachler et al. 2015) that is not referred. The manuscript relays too much in X-ray and partitioning techniques and does not cite the existence of other analytical approaches; it should include them in my opinion. There is also a recent paper that is much on the direction of this paper where it is determined the concentration of iron specifically bound to humics inclluding a profile of humic-rich Arctic waters (Sukekava, Downes et al. 2018).

The introduction includes studies using other analytical approaches, including papers by the Krachler group (Krachler et al., 2005, Krachler et al., 2010) based on isotopically labelled Fe, and papers using FIFFF (Hassellöv et al., 1999;Andersson et al., 2006; Stolpe and Hassellöv 2007). However referee#1 is right no studies based on voltammetric techniques are currently present in the introduction. The following addition to the introduction has been made (Line 77):

"Further, studies using cathodic stripping voltammetry (CSV) have underlined the importance of complexation by ligands to keep Fe in suspension in saline waters (Laglera and van den Berg 2009; Sukekava, et al. 2018)."

Specific and minor comments:

5. Title: the title is generic and seems to be referring to global processes. The authors made a good effort sampling many rivers and repeating samplings in different seasons. The problem is that all rivers are from a small geographical region and refer to similar

catchments, have similar conditions and end in the same regional sea. This is related to my opinion that the authors should use this interesting analytical approach in rivers from other locations. I think that the title should conceal the relevance of the study to the area where it can be applied. I suggest "Organic Iron Complexes Enhance Iron Transport Capacity along Salinity Gradients of Baltic Estuaries".

Suggestion implemented (Line 1):

"Organic Iron Complexes Enhance Iron Transport Capacity along Estuarine Salinity Gradients of Baltic Estuaries"

6. Fe speciation is not clearly defined as Fe transport is. Fe speciation sometimes refers to organic vs inorganic species and sometimes relates to the oxidation state of iron. My advice is to use organic speciation (or perhaps overall speciation) when org vs inorg is discussed and redox speciation when the Fe(II)/Fe(III) is discussed.

In this paper we assess both what you refer to as organic speciation (organic vs. inorganic) (EXAFS) and redox speciation (HERFED data). We have now clarified that (Line 93):

"The Fe speciation, (organic speciation (organic vs. inorganic) and redox speciation, of all river samples was characterized by XAS."

7. How different noise in Fig 3 spectra translates in uncertainties at the time to report: example Helge river

We are not entirely sure how to understand this comment. The HERFED spectra and $Kb_{2,5}$ *X-ray emission (XES) spectra in Figure 3 of the river mouth are noisy. This is a result of the low Fe concentration, which translates into higher uncertainties in determining peak positions and relative peak intensities. Nevertheless, the difference between the Helge river and the Fe(II) spectra remains evident. We have included this observation in the discussion (Line 289):*

"Due to low Fe concentration there was more noise in the river mouth sample, but the deviation from the Fe(II) spectra was still clear."

Abstract

8. Lines 16-17. Example of oversimplification. All FeO(OH) precipitates and Fe-OM survives. Please add "a fraction" of the organically complexed. I never heard of a study that suggest that all organic Fe "survives" the estuarine transit.

The sentence is not saying that all Fe-OM is surviving the estuarine transit, but rather that Fe-OM ***can*** *survive the salinity gradient, while Fe (oxy)hydroxides* ***are more prone*** *to aggregation.*

9. Low-order stream? Not sure what is the meaning of the expression.

The wording was changed to "upstream" and reads as followed (Line 24):

"We further found that that Fe-OM was more prevalent at high flow conditions in spring than at low flow conditions during autumn, and that Fe-OM was more dominant upstream in a catchment than at the river mouth."

Introduction:

10. 28 iron is the fourth most abundant element in the earth crust and mantle, on earth it is possibly the most abundant element.

Thank for spotting this mistake. We have corrected it (Line 37):

"While Fe is the fourth most abundant element in the earth crust (Taylor, 1964), Fe concentrations in oxygenated aquatic systems are generally low (Johnson et al., 1997;Kraemer, 2004), while they can be higher during high flow conditions and in boreal waters with high dissolved organic carbon (DOC) concentrations (Kritzberg et al., 2014;Ekström et al., 2016)."

11. 29 systemS.

Thank you for pointing out this mistake (Line 37 see comment 10).

12. Please give range. I am not sure whether the authors refer here to freshwater systems or fresh and seawaters.

This statement is general to fresh- and marine waters and the exception to that is illustrated in the continuation of the sentence. Thus providing a range of Fe concentration in aquatic systems serves no purpose.

13. 33 please add reference (Liu and Millero 2002). This is the main work on iron solubility in seawater and this paper is dedicated mostly to seawater. The Lofts paper is dedicated to freshwater. This paper should be the reference to discuss solubility

The reference (Liu and Millero 2002) was added. It is indeed relevant to give a marine reference here as our study spans from freshwater to marine water conditions (Line 42).

14. 38 "suggesting that Fe export from soils are increasing." Check grammar, export is increasing

Thank you for spotting this mistake (Line 48).

15. 40 the Fe requirement in coastal waters is high but since it is not limiting I do not know if it plays a key biological role

Fe influences the mobility, availability and biogeochemistry of numerous other elements, especially it can play a key role in affecting phosphorous availability not only by limiting primary production. Further, Fe limitation in the Baltic Sea has been suggested by several studies, to clarify this the following section has been added into the conclusion (Line 558):
"This would suggest that high and rising concentrations of Fe from boreal rivers (Kritzberg and Ekstrom, 2012;Björnerås et al., 2017) may indeed result in increasing export of bioavailable Fe to the Baltic Sea and open waters, where it may limit N-fixation and primary production (Stal et al., 1999;Stolte et al., 2006;Martin and Fitzwater, 1988)."

16. 48 "much higher than generally observed". This phrasing accepts several interpretations. I think the authors mean higher than predicted from prior works (the reference to 95% precipitation)

Thank your for this input this is a much clearer way to express it (Line 60):
"Fe transport capacity – the fraction of riverine Fe remaining in suspension at higher salinity – has been shown to vary widely and is in some instances much higher than predicted from prior works (Kritzberg et al., 2014;Krachler et al., 2005)."

17. 50 "Fe in natural waters is known to occur in two main phases, mononuclear organic complexes (Fe-OM) and Fe rich Fe (oxy)hydroxide colloids associated with chromophoric organic matter (Breitbarth et al., 2010;Hassellöv et al., 1999;Andersson et al., 2006)." In my opinion this is oversimplification, as it reads it seems that CDOM only can be found associated to Fe(OH)O and not forming soluble complexes. Electrochemical measurements have proved that fulvic and humic components of CDOM bind iron forming complexes that can migrate freely to the electrode, i.e.: mononuclear (van den Berg and Laglera works on humics). This phrasing also assumes that associated to FeO(OH) there are no non coloured substances. This interpretation comes from the cited paper that include a peculiar description of iron complexation in rivers: "iron transport in rivers is associated with two types of carrier phases (besides detrital particles), an oxyhydroxide phase with associated CDOM (chromophoric dissolved organic matter, mostly consisting of humic acids) and an organic carbon (fulvic) phase (e.g. Lyv0en et al., 2003; Andersson et al., 2006)." From Breitbarth etal 2010). There is a huge body of literature that proves that fulvics belong to the CDOM fraction of DOM. Actually, the oversimplification that is found in the first 3 sections of this manuscript is diluted in the discussion section and there is a recount of the real complexity of the problem. Please rewrite this paragraph.

Thank you for taking the time to explain why this phrasing was problematic. We entirety *agree that there is oversimplification and a degree of sloppiness in how the characteristic of the organic components are described. Our paper is focusing on the iron and it's speciation more than targeting the characteristics of the organic matter. We have therefore removed chromophoric as a descriptor of the organic matter interacting with the Fe (oxy)hydroxides (Line 65).*

18. 56 found "in" aggregates Materials and methods

Thank you for pointing this out, it has been corrected (Line 84).

19. 83 I am going to give a piece of advice to the authors for future work. Do not sample by hand in estuaries unless you have a system to open the bottle once it is at depth. Dipping an open bottle across the surface opens the possibility to collect a lot of surfactants and floating debris from the surface microlayer. I saw samplings ruined by this strategy.

Thank you for this valuable advice, we will keep this in mind.

20. Fair paragraph at 90. I am interested in the concentration factor and should be included. Line 90 is a bit tricky because speciation here is obtained from the physical and not chemical properties of the sample (centrifugation= size partitioning prior to analysis). The warning is interesting because later on the manuscript the authors try to argue about the form of FeO(OH) crystallization.

We are not sure how to understand this comment. This part described how samples were treated that were later analyzed by XAS for Fe speciation of total Fe in the river samples. There was no separation prior to this.

21. 94 how was the pH meter calibrated? TRIS or NBS solutions?

The pH meter was calibrated daily using a three point calibration with three buffer solutions (pH 4.01/7.00/10.00) purchased together with pH meter by Mettler Toledo. The pH calibration was not adjusted to the changing ionic strength. This may have an impact on the pH reading of the high salinity samples, however it should not have an affect on the freshwater samples on which our discussion builds. For further work we will take this valuable input into consideration when working with changing ionic strength.

Section 2.2.

22. No problems with the approach. However I strongly recommend that for future studies they either obtain cleaner reagents or consider to remove metals from their working solutions. 150 nM Fe is a huge contamination and could interfere with some of the mixing experiments (obviously, the speciation of this contamination is different to the speciation of the sample).

Thank you for this comment, we will consider this for our future work. Since this contamination is between 0.1-3.8% of the Fe concentration in our river waters, we do not think this has affected our results.

23. Please convert the blank concentration to mg Fe /L since this is the unit used throughout the paper.

Thank you for this input the blank (0.15 µM) has been converted into mg/l (0.0025 mg/l) as it is used in this unit throughout the paper (Line 148).

24. 110 24 h is a good compromise but has to be put into context. From (Liu and Millero 2002) work on Fe solubility in seawater "In our first series of measurements, we examined the solubility as a function of time. The results of iron solubility over 4 days at pH 3 and 8 are shown in Fig. 1. The iron concentrations decreased significantly from 3 h to 1 day and continued to decrease. After 1 week, the solubility did not decrease significantly. These results agree with our work in NaCl (Liu and Millero, 1999) and Kuma et al. (1996) in seawater. The subsequent solubility measurements were conducted with an equilibration time of at least 1 week. Our results represent the quasi-equilibration

with iron solubility with particle size greater than 0.02 mm. Such a definition is in accordance with those of Byrne and Kester (1976a) and Kuma et al. (1996, 1998a,b). It may take several years for the solid to reach equilibrium (Schindler et al., 1963). The causes for the decrease in the solubility of Fe(III) with time has been recently been examined in more detail by Kuma et al. (1992, 1996, 1998a,b)." Please make a back of the envelop calculation about the fraction that is not removed in 24 h (I agree should be small) and cite Liu and Millero work. Also consider that with longer equilibrium times (as those residence times verified in estuaries, FeO(OH) aging could shift crystallization.

Salinity induced aggregation of Fe consists of sequential reactions. Nowostawska, et al. (2008) showed that a significant fraction of Fe (~80 %) is aggregated immediately within a few seconds after mixing river water and sea salt solution. Furthermore, Hunter and Leonard (1988) demonstrated that aggregation of riverine Fe after the addition of sea salt is well described second-order kinetics where the rate of aggregation decline with time. The figure below (Figure 1) exemplifies how a clear increase in aggregation of riverine Fe was observed for the first ~100 minutes but additional aggregation after 2 hours was minor. Thus, while aggregation is continuing at a slow rate also after 24 hours, the first 24 hours should incorporate the dominant part of the aggregation.

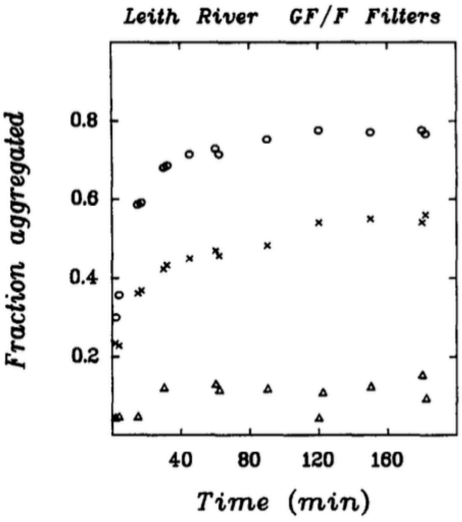

Figure 1 from *Hunter and Leonard (1988)*

25. 111 why 3000 rcf for 8 h?????? there is no bibliography attached as justification. What is approximately the size you discriminate?

A similar protocol has been used in previously published work by Krachler et al 2005 and by our group (Herzog et al. 2019, Kritzberg et al. 2014) and as well. We have no estimate of the size discriminated, bur from other work we see that filtration through 0.2 µm- filters removal a large fraction of reactive Fe that remains stable in the water column. Thus. Separating by size may not be the best way to reflect loss from the water column. Centrifugation acts on gravitational particles, which may be of different size and density.

26. Was any reference material analysed? Or at least in a previous work using exactly the same analytical settings? This is always required for oceanic studies.

We don't know to what analytical method the referee is referring too. If it was DOC, the same analytical setting has been used in previous work from our group and many others.

27. 118 problem with measuring pH with an electrode with a single calibration at changeable ionic strength. Again, how was this electrode calibrated?

This has already been addressed (Comment 21)

28. 2.4 and 2.5 I congratulate the authors for the degree of detail used to describe the analysis

and data treatment steps. Again, the only thing I missed is a rough estimation of the uncertainty associated to the technique (specially the complex data treatment of the signals).

Thank you, the estimation of the uncertainty associated to the technique has been addressed in response to comment 1.

29. 125 recorded?

Thank you for pointing this out, it has been adjusted (Line 172).

30. 140 Here I have a question. What is he concentration of Fe(III) added per mg of SRFA? Several recent reports state that the actual complexation is in the order of 15 nmol Fe per mg SRFA (Yang, Su et al. 2017, Slagter, Laglera et al. 2019). It could be that if iron was added greatly in excess, it was partially chemically bound and partially attached with other type of weaker interaction. I think a short explanation is important and future work could help to elucidate these binding capacities reported by voltammetric methods.

The Fe(III) concentrations in Suwannee River natural organic matter for the reference material used in this study was 6489 µg g^{-1} on a dry mass basis, used in Karlsson and Persson (2012). For clarification the two techniques (voltammetric methods and XAS) measure different aspects to distinguish Fe speciation. The basic principles behind XAS is that X-rays strike and excite core electrons of an atom, which in turn get either promoted to an unoccupied level, or ejected from the atom and consequently create a core hole. Dissociation of the electron will produce an excited ion as well as a photoelectron. The scattering of the photoelectron will modulate the absorption coefficient, and both the local transitions and the effects from the out-going photoelectron can be measured and analyzed. As XAS provides information on the local coordination environment of Fe, the concern by refree#1 about what is measured (chemically bound or partially attached with other type of weaker interaction) is not of concerns

31. 175 please rewrite “were close to saturated with dissolved oxygen (85 – 118%)”.

Thank for pointing this out, the sentence has been corrected as follow (Line 235):

“At the time of sampling all river mouths were close to saturation with dissolved oxygen (85 – 118%) and pH values close to neutral (Table 2).”

32. 178 was this higher pH caused by biological or geochemical processes?

The higher pH was the result of the low level of DOC measured during autumn. This is further discussed in the manuscript in section 4.3 (Control of spatial variation and flow conditions on Fe speciation.).

33. 180 oxygen saturation suspiciously low. Was the temperature effect properly accounted for?

The oxygen saturation at the upstream sample (Svineö) is correct and the temperature effect was accounted for as the OxyGuard probes have built-in temperature compensation. Low oxygen is often found in waters that drain peatbogs, since the organic matter degradation in the standing water is consuming oxygen.

34. This paper would greatly benefit of some sort of visual library (supplementary file?). Not clear from the text whether ferrihidrate and goethite show exactly the same contours.

We assume this comment refers to the section 3.2 (XAS characterization). The main purpose of XAS characterization was to distinguish between organically complexed Fe and Fe (oxy)hydroxides and it was not the goal to completely resolve the structure of the Fe (oxyhydroxide fraction. When we talk about Fe (oxy)hydroxides, we compare it to reference material from both ferrihydrite and goethite as both have a similar Fe-bond distances. To make the XAS results easier to understand we added WT plots of the model compounds ferrihydrite, goethite and two plots showing a mixture of goethite and trisoxalatoiron(III) to the supplement (Figure S2) and referred to them in section 3.2 (Line 249, 250 and 253). We *hope these additions will make the XAS analyses and interpretations easier to grasp.*

Addition to the Supplement:

"For the quantitative modeling of the EXAFS spectra two input structures were used, goethite for the Fe-Fe paths and trisoxalatoiron(III) for the Fe-O, Fe-C and Fe-C/O. WT plots for the model compounds (Figure S2), show that the different paths in the model compounds are in good agreement with the ones found in our samples (Figure 2). The Fe (oxy)hydroxides ferrihydrite and goethite (Figure S2 a and b) are represented in the same area of the WT plots, so that distinction between the two is difficult."

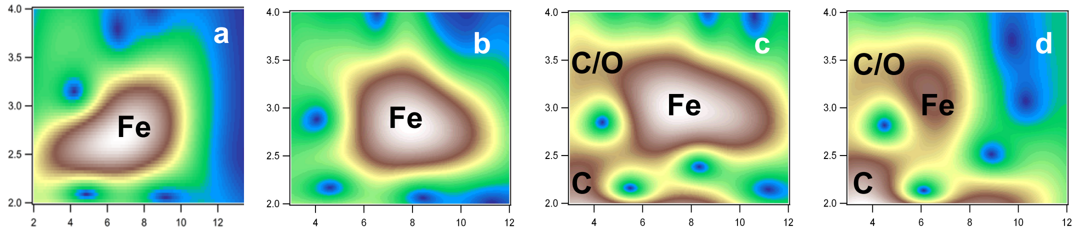

Figure 2 Figure S2. Morlet wavelet transforms for a ($\eta = 10$, $\sigma = 1$) and for b-c ($\eta = 8$, $\sigma = 1$) of EXAFS data displaying the second and third coordination shells collected on model compounds: (a) 6-line ferrihydrite by Sundman et al. (2014), (b) goethite, (c) goethite/trisoxalatoiron(III) mixture (50:50), and (d) goethite/trisoxalatoiron(III) mixture (25:75) by Karlsson and Persson (2010).

35. 190 what compounds? Humics or mononuclear ligands?

As our approach was aiming to distinguish between the two Fe phases - organically complexed Fe and Fe (oxy)hydroxide – the purpose of this section was to point out the difference between these two with WT plots. We are not characterizing the organic matter.

36. 193-198. Not sure tables S1 and S2 are correctly cited. Not in order for sure

Thank you for pointing this out, the reference to Table S2 on line 194 was wrong and it should have been referring to Table S1, this has been corrected.

37. 208- it is clear there is a good correlation between the CNFe-C/CNFe-Fe and LCF ratios but my question is, are values comparable? Please add an statistic or visual comparison (for instance, if values of two different analytical approaches to the same parameter are close, the correlation should have slope close to one and Y-axis close to zero).

The CN- and LCF-ratios are obtained by two different data evaluation techniques and are two different ways measuring the contribution of organic matter in the Fe phases. As requested by referee #1 a visual comparison (see comment 1) has been added into the supplementary.

38. 227- Are the authors referring to the Helge river here? In Helge pH is not low and O2 is not low

Thank you for pointing this out. In this section we are referring to the upstream samples of Helge River, which was not entirely clear in the current sentence. It has been adjusted as follows (Linie 305):

"Finally, comparing the various EXAFS analyses with the HERFED and $Kb_{2,5}$ *emission spectroscopy results show that Fe(II) in the upstream samples of the Helge river system is present as Fe-OM complexes. These complexes are favored by low pH and low oxygen concentrations, as expected."*

39. 233-238 this paragraph is very difficult to evaluate without a rough idea of the uncertainty of the approach. Since the technique clearly struggles with quantification (although fantastic for qualitative analysis of multiple species) I would recommend for

future work the combination with other speciation techniques. It could be that correlations have been hindered by the low number of data and uncertainty.

This comment has already been addressed (comment 1).

40. 243 high removal but here the point would be, the remaining concentrationis high or close to seawater concentrations in the Baltic sea (not reported here)?

The high riverine input of Fe and OM, in combination with the relatively low salinity, render Fe concentrations in the Baltic Sea high (15–144 nmol L-1; Bothian Sea –Baltic proper, (Gelting et al. 2010) compared to the open sea. The values obtained by the mixing experiment at high salinity (35) by not considering the dilution factor are much higher (0.4-9.0 µmol L-1) compared to values measure in the Baltic Sea.
This information has been implemented into the manuscript, as also referee#2 has commended on a similar issues (Line 382):
"Nevertheless, the high values of Fe remaining in suspension due to complexation with organic matter at high salinity (0.02 mg/l – 0.50 mg/l) supports that the importance of rivers as a source of Fe into the Baltic Sea with an Fe concentration of around 1 µg/l (Baltic proper) (Gelting, et al. 2010)."

41. 243-244 This is very surprising and requires further discussion. Fe precipitation is usually the consequence of coprecipitation with organic phases after flocculation of organic matter (mostly humics) due to the increase of the ionic strength that cancels the negative charge of organic matter at natural pH (addition: this is discussed further in the discussion section but still: : :.). This is something described since Sholkovitz/Boyle papers. Therefore, the lack of OC precipitation is very surprising and not a result that mimics natural conditions. Here the authors need to elaborate much more in this result. If the experimental procedure somehow precluded the flocculation/coagulation of organic matter, then the precipitation of Fe was severely underestimated. This is for me the most worrying result in the manuscript.

The results presented in this study go along with previous studies from similar system within the Baltic Sea catchment (Herzog et al., 2017;Forsgren et al.(Kritzberg, et al. 2014), 1996). It is indeed very likely that there is co-precipitation with organic matter, but since the DOC pool is so large, this does not result in a significant loss.

42. 251-252. This should have not been done like this. Seawater has a minimum pH of 7.8 (function of course of temperature and salinity and local biochemical conditions); the perform of dilution experiments with pH at the high saline end member as low as 7.1 is not realistic. The authors should had forced the pH to realistic values _8. This could have modulated the precipitation of species during mixing experiments.

It is correct that pH has an effect, since higher pH promotes colloid formation, due to increased Fe(III) hydrolysis and Fe(II) oxidation (Ilina, et al. 2013; Karlsson and Persson 2012; Pullin and Cabaniss 2003).However, such an effect is mainly important when moving within a lower pH range (3.0−6.7)(Karlsson and Persson 2012; Neubauer, et al. 2013) than we have in this study (>pH 7.1), and it has been previously verified that pH alone does not significantly affect Fe stability in the pH range of the current experiment (Kritzberg, et al. 2014).

43. 265 I would rephrase to "in contrast to the thermodynamic modeling suggested by (Wällstedt et al., 2010) for these systems that predicted a dominance of ferrihydrite (_97 %).". This prediction is subjective and depends in the parameters fed to the SHM model. I think that other research group could had obtained different results with the same model. Actually, I could not find the DOM concentration used in that specific paper.

The suggestion was implemented (Line 389).

44. 268 I had concerns about the preservation of the redox speciation during sample

processing but the result of Figure 3 is very revealing of the power of the analytical approach here. Kudos to the authors. Again, I would recommend for future work support from other ex-situ techniques more suited for quantification (spectrophotometry or chemiluminescence).

Thank you for this comment. We were also very pleased to see the high FeII measured in the upstream samples supporting that redox state is preserved. Supporting this with other techniques is a good suggestion.

45. 284 I do not think Sholkovitz was ever supportive of reduced Fe aggregation in any type of estuary independently of its latitude. The Powell paper shows complete iron precipitation (Figure 1). The idea of effective iron transport off high latitude rivers and humic rich streams was first put forward (to my knowledge) by Krachler and coauthors and it is deeply discussed in a recent review (Muller 2018).

Thank you for this input, the references has been changes accordingly (Line 378):

"The high Fe transport capacity for most of those Swedish rivers studied go along with the existing literature showing that high-latitude DOC-rich rivers exhibit higher Fe-carrying capacities (Krachler et al., 2005;Muller, 2018)."

46. 285"DOC was little affected by increasing salinity as previously observed (Linkhorst et al., 2017;Herzog et al., 2017)." This assertion is against prior observation by Sholkovitz, Boyle and other authors (I referred to this before), please discuss this finding and discrepancies among authors if exist or refer to the type of estuaries were this specific behavior was observed.

The sentence has been adjusted and reads now as follows (381):

"DOC was little affected by increasing salinity as previously observed in such high latitude rivers with high DOC concentrations (Herzog et al., 2017;Forsgren et al., 1996)."

47. 289 these authors do not argue that the whole iron complexed to organic matter survives the estuarine transition. It is a bit more subtle although not against findings in this paper. The assertion is that against prior reports that sustained that all Fe coprecipitates with OM, a significant percentage of iron bound to DOM (in some works they specify to humic substances) "survives" estuarine mixing. Laglera and van den Berg argue that coprecipitation takes place down to a Fe/humics ratio when both stabilize (or "learn to survive" if we continue with the metaphor). I advise rephrasing this section correcting the interpretation of prior literature and putting it into context with findings in this work.

The section has been changed as follows (Line 389):

"In contrast, Fe complexed by terrigenous organic matter is supposedly less affected and to a larger extent "surviving" estuarine mixing and can thereby be a source of bioavailable Fe to marine waters (Batchelli, et al. 2010; Krachler, et al. 2010; Laglera and van den Berg 2009)."

48. 294-295. First I would remove Sander's reference since this is a description of interactions at pH 4 under complete protonation of carboxylic groups. At pH 7-8 negative charges are dominant.

The reference has been removed (Line 425).

49. 296-298. This is not exactly the common description of the estuarine transition of DOM and their interaction with inorganic iron. As cations increase and neutralize the surface groups of DOM, repulsion forces decrease and DOM starts flocculation. Many non charged colloids (such as Fe colloids) get trapped during this formation of bigger aggregations and coprecipitate eventually. Basically, the result is the same described in the paper but the authors suggest independent precipitation and the literature is full with text about combined precipitation. Actually, $FeCl_3$ addition for organic matter coprecipitation and removal is a common procedure used in water treatment plants.

We are not suggesting independent precipitation. The Fe (oxy) hydroxides lost are most likely

in association with organic matter and "bring this down", it is only that that OM is only a minor fraction of the total OM. This is now clarified in the manuscript (Line 425):
"With increasing salinity, the surface charge gets neutralized resulting in reduced colloidal repulsion (Mosley, et al. 2003) and formation aggregates containing both Fe and OM."

50. 306 this assertions ignores a whole body of literature. Iron speciation at the concentrations found at the saline end member of estuarine is available after cathodic voltammetric methods (Stan van den Berg, Kristen Buck, Loes Gerringa, Han Su and many others). With those methods it is possible to measure the iron ligand concentrations and concentration of humic substances. It is true that it is not clear whether those methods may discern between stable Fe oxyhidroxydes and Fe-OM complexes but the reported ligand concentrations in excess of iron concentration can only be ascribed to the presence of organic ligands. It would be fair to do a short summary of findings and add to the discussion that organic ligands in excess of iron concentrations have been found by this technique.

Thank you for pointing this out. The following section was added (Line 446):
"Moreover, based on cathodic stripping voltammetry (CSV), ligand concentrations have been found to be in excess of iron concentration, suggesting that organic ligands are complexing the Fe and keeping it in suspension in saline waters (Gledhill and Buck 2012; Laglera, et al. 2011)."

51. 310-315 there is a factor not considered. In the estuary, there is production of iron ligands by biota that could be used to explain why dissolved Fe in the estuary was higher than the predicted after experimentation with ligand free seawater.

Thank you for pointing this out. The production of ligands by the biota will be mentioned in an expanded text that addresses the validity of our artificial mixing experiments (see comment 3).

52. 323-324. Please add reference to Liu and Millero 2002. Some of the observations here could be easily predicted.

Thank you for this input, the reference was added (Line 487).

53. 340. there could be other Fe(II) sources. For instance, at higher flow conditions probably there is more turbidity and less light penetration limiting Fe(II) photoproduction. Let alone biological production of Fe(II). The subject is very complex.

It is true that there are a different of sources and processes promoting Fe(II) in freshwater. This section is discussing the seasonal variation of the two main sources of Fe-OM and Fe (oxy)hydroxides into the rivers, especially the formation of Fe (oxy)hydroxide based on the rapid oxidation of Fe(II) from groundwater input. These systems have a minimum of turbidity and phytoplankton. Thus elaborate more on other may be relevant in other systems, however in the context it is not relevant.

54. 360 which amounts?

The word "amounts" has been exchanged with "quantity" to make it clearer that it is refers to Fe exported from the catchment. The sentence reads now accordingly (Line 530):
"While characteristics such as land-cover and soil type are most likely affecting both quantity and speciation of Fe exported from the catchment, the limited number of rivers and sampling occasions of this study cannot accurately discern such relationships."

55. 370 this sentence has to be toned bown. First, this study is carried out in rivers which impact is never going to reach iron limited areas. Second, the number of iron limited areas in high latitudes of the northern hemisphere is not so extended (areas of the Bering Sea and perhaps after bloom in the Northern Atlantic). Third, the two studies referenced mention Arctic rivers, which are completely different catchment areas to those presented here since those are affected by permafrost melting. Not because there are iron limited

areas and Fe from Baltic rivers is expected to increase, the Arctic Ocean is going to be fertilized.

Thank you for pointing this out. We have made the sentence specific to the Baltic only and refer to papers that indicate periods of Fe limitation in the Baltic (Line 540):

"This would suggest that high and rising concentrations of Fe from boreal rivers (Kritzberg and Ekstrom, 2012;Björnerås et al., 2017) may indeed result in increasing export of bioavailable Fe to the Baltic Sea and open waters, where it may limit N-fixation and primary production (Stal et al., 1999;Stolte et al., 2006;Martin and Fitzwater, 1988).

In regard to the third point, two studies with rivers in the catchments of the Baltic Sea have been added, showing the same trends as the Arctic Rivers mentioned previously. The sentence reads now as follows (Line 543):

"Major hydrological events like spring floods and heavy storms have been observed to increase of the Fe concentration by up to a factor of 20 and alter the annual Fe load in northern rivers (Hölemann et al., 2005;Rember and Trefry, 2004;Dahlqvist et al., 2007;Herzog et al., 2019)."

56. 377. I would remove last sentence. Although possible, it is very speculative and brings the focus out the main topic of the paper.

The sentence has been removed (Line 549).

57. Is there a first column missing in table S1?

No there is no column missing. The table size was too large for the page format. This has been changed and the table is now fully visible.

References from referee#1:

Buck, K. N., M. C. Lohan, C. J. M. Berger and K. W. Bruland (2007). "Dissolved iron speciation in two distinct river plumes and an estuary: Implications for riverine iron supply." Limnology and Oceanography 52(2): 843-855. Bundy, R. M., H. A. N. Abdulla, P. G. Hatcher, D. V. Biller, K. N. Buck and K. A. Barbeau (2015). "Iron-binding ligands and humic substances in the San Francisco Bay estuary and estuarine-influenced shelf regions of coastal California." Marine Chemistry 173(0): 183-194. Krachler, R., F. Jirsa and S. Ayromlou (2005). "Factors influencing the dissolved iron input by river water to the open ocean." Biogeosciences 2(4): 311-315. Krachler, R., R. F. Krachler, G. Wallner, S. Hann, M. Laux, M. F. Cervantes Recalde, F. Jirsa, E. Neubauer, F. von der Kammer, T. Hofmann and B. K. Keppler (2015). "River-derived humic substances as iron chelators in seawater." Marine Chemistry 174: 85-93. Laglera, L. M. and C. M. G. van den Berg (2009). "Evidence for geochemical control of iron by humic substances in seawater." Limnology and Oceanography 54(2): 610-619. Liu, X. W. and F. J. Millero (2002). "The solubility of iron in seawater." Marine Chemistry 77(1): 43-54. Muller, F. L. L. (2018). "Exploring the Potential Role of Terrestrially Derived Humic Substances in the Marine Biogeochemistry of Iron." Frontiers in Earth Science 6(159). Slagter, H. A., L. M. Laglera, C. Sukekava and L. J. A. Gerringa (2019). "Fe-binding Organic Ligands in the Humic-Rich TransPolar Drift in the Surface Arctic Ocean using Multiple Voltammetric Methods." Journal of Geophysical Research: Oceans 124: 1491-1508. Su, H., R. Yang, Y. Li and X. Wang (2018). "Influence of humic substances on iron distribution in the East China Sea." Chemosphere 204: 450-462. Su, H., R. Yang, I. Pižeta, D. Omanovi´c, S. Wang and Y. Li (2016). "Distribution and Speciation of Dissolved Iron in Jiaozhou Bay (Yellow Sea, China)." Frontiers in Marine Science 3(99). Su, H., R. Yang, A. Zhang and Y. Li (2015). "Dissolved iron distribution and organic complexation in the coastal waters of the East China Sea." Marine Chemistry 173(0): 208-221. Sukekava, C., J. Downes, H. A. Slagter, L. J. A. Gerringa and L. M. Laglera (2018). "Determination of the contribution of humic substances to iron complexation in seawater by catalytic cathodic stripping voltammetry." Talanta 189: 359-364. Yang, R., H. Su, S. Qu and X.Wang (2017). "Capacity of humic substances to complex with iron at different salinities in the Yangtze River estuary and East China Sea." Scientific Reports 7(1): 1381.

Response to comments by referee #2:

The authors present new data characterizing iron speciation in Scandinavian rivers together with Fe stability experiments aiming at estimating Fe transport across the salinity gradient to reach oceanic waters. While the work about Fe speciation seems rather well described and of high quality (for a non-specialist like I am), the work about Fe transport across the salinity gradient deserves more attention in my opinion. In addition, the authors seems to excessively generalize their findings. For instance the first sentence of the abstract is about 'open marine waters', while the most saline sample analyzed here has a salinity of 25 (seawater has a salinity of 35). Moreover, most studied rivers (7 out of 8) flow into the Baltic sea (typical salinities of 5 to 10) that is not proper seawater. Finally, the manuscript really lacks quantification (the authors state that fluxes could be 'significant' but no quantification is provided). The topic is extremely interesting. I recommend publication in Biogeosciences only after the points below have been addressed.

Nice to hear that the topic is found to be interesting and that we are given the opportunity to address points raised. As the comments above are further elaborated by the referee in the list of major points, we respond and describe suggested changes to each specific comment below.

Major points:

58. Excessive generalization of results obtained mainly along the Baltic Sea. Authors should make clear from the title and abstract (and discussion and conclusion) that their study is regional, mainly along a sea with especially low salinity, and based on lab experiments (for the transport capacity).

Thanks for this comment. Our intention was not to suggest that our results can be generalized to all systems, but rather to put the topic in a general context. While the systems we work in are indeed atypical given the low salinity in the Baltic Sea, the general response of riverine Fe to increasing salinity is likely to be comparable in other regions. The response is probably more influenced by the water chemistry of the rivers than the salinity gradient, considering that the loss of Fe from suspension appear to occur at salinities below 15. Nevertheless, it is important that the results are not overstated and that the reader is not mislead about how far conclusions can be drawn. We have gone through the manuscript with this in mind and suggest the following changes:

In the abstract we clarify the geographical region in which the study is performed (Line 20):

*"In this study, we directly identified, by X-ray absorption spectroscopy, the occurrence of these two Fe phases across **eight boreal rivers draining into the Baltic Sea**, and confirmed a significant but variable contribution of Fe-OM in relation to Fe (oxy)hydroxides among river mouths."*

We also clarify that Fe transport capacity was assessed by lab experiments (Line 25):

*"The stability of Fe to increasing salinity, **as assessed by artificial mixing experiments**, correlated well to the relative contribution of Fe-OM, confirming that organic complexes promote Fe transport capacity."*

Moreover, we removed the reference to marine waters in the concluding sentence of the abstract (Line 27):

*"This study suggests that boreal rivers may provide significant amounts of potentially bioavailable Fe **beyond the estuary**, due to organic matter complexes."*

In the introduction we also clarify the geographic region of the study and the fact that the rivers drain into the brackish Baltic Sea (Line 90):

"To this purpose, we sampled eight river ***mouths that drain at the Swedish coast into the brackish Baltic Sea****."*

The low salinity, particularly of the northern Baltic is now explicit in the Discussion (Line 426):

"In the ***low-salinity mixing regime present in the northern Baltic (Bothnian Bay),*** *aggregation may occur without significant sedimentation (Forsgren and Jansson 1992)."*

Finally in the conclusion (Line 573):

"This would suggest that high and rising concentrations of Fe from boreal rivers (Björnerås, et al. 2017; Kritzberg and Ekstrom 2012) may indeed result in increasing export of bioavailable Fe ***to the Baltic Sea*** *and open waters, where it may limit N-fixation and primary production (Martin and Fitzwater 1988; Stal, et al. 1999; Stolte, et al. 2006)."*

59. Lack of quantification of the potential Fe source the authors talk about (L 23 'potentially bioavailable Fe' from rivers) compared to other Fe sources to the surface ocean. The authors should provide estimations of the different Fe sources to the ocean, so that the reader can make an opinion about the significance of the source discussed in the present paper compared to other sources. This is necessary to support for in-stance the 2 following sentences (L13-14 and L 23-24 below). - 'Rivers discharge a notable amount of Fe (1.5x10 9 mol yr−1) to coastal waters, but are still not considered important sources of bioavailable Fe to open marine waters' - 'This study suggests that boreal rivers may provide significant amounts of potentially bioavailable Fe to marine waters beyond the estuary, due to organic matter complexes.' The authors should remove assertions such as 'Fe loading from boreal rivers to estuaries is increasing substantially [...] this is a finding with major implications' (L 35 - 40) if they cannot present data showing that river dissolved Fe stabilized by organic ligands is indeed a significant flux compared to others for the surface ocean.

Thank you for this comment. In our view, the first sentence of the abstract is there to provide a general context. The elaboration on quantifying different sources as suggested by referee#2 is complex and would require more text than can be fitted into an abstract. Furthermore, the increasing Fe loading from boreal waters will likely have major implications also if not stabilized by organic ligands. Studies have shown that Fe of riverine origin is a phosphorus sink in coastal sediments, for instance. The reasoning is that what the specific implications may be depending on the fate of Fe across the salinity gradient. To underline the importance of river a a Fe source to the Batic Sea, the following text has been added (Line 382):

"Nevertheless, the high values of Fe remaining in suspension due to complexation with organic matter at high salinity (0.02 g/l – 0.50 g/l) supports that the importance of rivers as a source of Fe into the Baltic Sea with an Fe concentration of around 1 µg/l (Baltic proper) (Gelting et al., 2010)."

60. The core of the paper, in my opinion, reside in the fact that 2 main characteristics are studied, 1) Fe speciation and 2) Fe transport capacity, and that these 2 characteristics are compared to each other. However, while the first point, Fe speciation is well de-scribed in the ms (notably with 3 figures), the transport capacity experiment is hardly presented in the main part of the ms (data are almost only shown in the supplementary materials), so that the reader cannot really make an idea about the validity of the author assertions. This is really a problem, because all the work about speciation is much less useful (at least in the presented context), if the transport capacity experiments are not validated. I believe that much more attention should be

given to this part of the paper, with a proper discussion about the validity of the experiments, especially using the in situ data. In the main part of the ms, not in the supplement.

In the original submission, the Fe transport capacity was presented in Figure 5 and Table 2 of the main manuscript, and the comparison of in situ Fe concentration along estuarine salinity gradients and theoretically estimated concentration based on the artificial salinity experiments were presented in Figure S3 in the supplementary information. In response to the above comment, we moved the latter Figure into the main manuscript and expand the discussion on the validity of the artificial mixing experiments (Figure 6). We added a section (Line 421), see response to comment 62 below.

61. Unfortunately, from what is shown in the supplement, I am not convinced that the mixing experiments do simulate accurately what would happen in situ. My opinion in that this dataset is insufficient to validate the transport capacities illustrated in Fig. 5 for instance. At least the authors should try to estimate error bars on the transport capacities (Table 2) and on the concentrations presented in Fig. 5.

It is important that mixing experiments are initiated as soon as possible after sampling, to make sure that Fe speciation is not altered. Moreover, the number of samples that can be included and processed in the experiment within a reasonable timeframe after sampling, is limited by centrifugation capacity. In the trade-off between running experimental replicates at a few selected salinity levels and including a wide gradient with many levels of salinity, we chose the latter, as we believe this provides more information. The consistency in the gradual loss of Fe in suspension with increasing salinity is in itself a validation of the Fe transport capacity measured at high salinity. We agree that the artificial mixing experiments are unlikely to capture exactly the loss of Fe along the natural salinity gradient, where for instance photoreduction may play a role, as well as the occurrence of organic matter of marine origin which may interact with riverine Fe and influence its behaviour. We also agree that these limitations should have been clearly recognized. Indeed, the experimental setup we apply capture the response of riverine Fe to increasing salinity in isolation. We added a section (Line 421), see response to comment 62 below.

62. They should also mention that organic matter of oceanic origin (not reproduced in the lab mixing experiment) may also take part to the process.

We agree that this should be mentioned. The following text addition is suggested to better describe the strengths and weaknesses of the artificial mixing experiments (Line 421):

"Results regarding Fe transport capacity derived from the artificial seawater mixing experiments were in good agreement with the estuarine transects sampled. Theoretically calculated Fe concentrations, based on Fe loss in artificial seawater mixing experiments with river water and the dilution factor, showed only minor deviations from Fe concentrations measured in the Gullmar Fjord. For the Öre estuary on the other hand, measured Fe concentrations were somewhat higher than the theoretical calculations (Figure S3). In the low-salinity mixing regime present in the northern Baltic (Bothnian Bay), aggregation may occur without significant sedimentation (Forsgren and Jansson 1992). This has been observed in the plume of nearby River Kalix, and was hypothesized to result from a high organic component of the aggregates, where low specific density may lead to transport of these aggregates far away from the river mouth (Gustafsson, et al. 2000). Thus, the centrifugation used to efficiently separate aggregates in the mixing experiments, may overestimate estuarine particle loss in this context. Despite the agreement between measured and theoretically estimated Fe concentrations, the artificial mixing experiments are unlikely to capture all processes that affect the loss of Fe along the natural salinity gradient. In the estuary, photoreduction may affect Fe speciation and affect its fate, as well as the occurrence of ligands produced by marine biota which may also influence the behaviour of riverine Fe.

Indeed, the artificial mixing experiments capture the response of riverine Fe to increasing salinity in isolation, and how that depends on Fe speciation."

63. In addition, I think that the comparison between the 2 characteristics (speciation, transport) is also not sufficiently presented and described. L 245-247 'For the river mouth samples, the Fe transport capacity at 35 salinity correlated positively with the Fe speciation ratios (CN Fe- 245 C /CN Fe-Fe : r = 0.675, p = 0.023; LCF ratio:0.78, p = 0.005). Further, Fe transport capacity at 35 salinity were negatively correlated to pH (r = -0.730, p = 0.007)' and L 291-293 ' The positive correlation between the contribution of Fe-OM (as determined by XAS) and Fe transport capacity (determined in artificial mixing experiments) adds a direct support that organic complexation of Fe is enhancing the stability across salinity gradients.'. I think that if the authors could provide a graphical representation of these correlations, this would be much easier for the reader and more convincing.

Thanks for this input. To follow this advice, we added a figure to the supplementary that visually demonstrates the relationship between Fe transport capacity and the contribution of Fe-OM (as determined by XAS) (Figure S5) and refereed to it in Line 328 and Line 616.

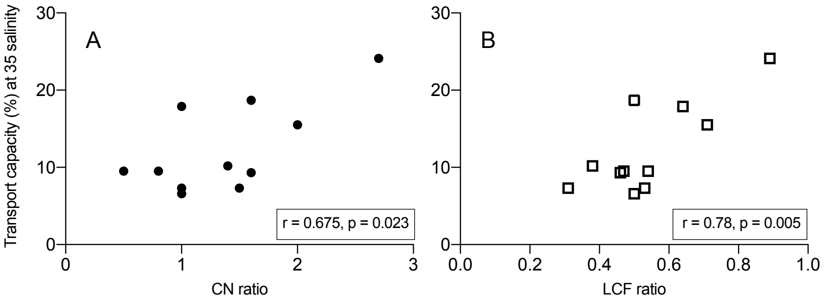

Figure S5 Relationship between Fe transport capacity at 35 salinity and relative contribution of organically complexed Fe as assessed by the CN-ratio (A) and LCF-ratio (B).

Minor points

64. Throughout the ms, the Fe phase the authors are talking about is not always clear. For instance, L 14 'the vast majority of riverine Fe', it seems that this is about dissolved Fe, but it is not mentioned. What's about particulate Fe ? Same for L 12. '1.5x109 molyr-1'. For what phase ? etc. L 13-14. 'Rivers discharge a notable amount of Fe (1.5x10 9 mol yr−1) to coastal waters, but are still not considered important sources of bioavailable Fe to open marine waters'. This is not totally true in my opinion, because, since papers such as Radicet al 2011 or Labatut et al 2014, remobilization of particulate iron river discharges is presented as a major source. This comment is related to the preceding one.

While size distributions are not a focus of this manuscript - organically complexed Fe and Fe (oxy)hydroxides are overlapping in size and can span from dissolved to particulate – we have gone over the manuscript to avoid unclarity as to the Fe phase referred to. Moreover, it is correct that iron that has settled to the sediment may be remobilized. While we cannot elaborate on this in the abstract, we have included this in the introduction (Line 58):

"Moreover, benthic release of Fe and subsequent lateral transport, was recently found to be a significant source of dissolved Fe to open marine waters (van Hulten, et al. 2016)."

65. L47. ’fraction of riverine Fe remaining in suspension’. A discussion about the phases involved would help clarify the ms. what about colloids, very small particles etc.

This comment has already been address (see above).

66. L56 ’aggregates’. Check English

Thanks – corrected (Line 84).

67. L63. XAS. Define

Thank you for pointing this out the abbreviation will be written out (Line 83):

“A preferential loss of Fe (oxy)hydroxides by aggregation was shown by Herzog et al. (2017) using X-ray absorbance spectroscopy (XAS).”

68. L86. ’cold’. What temperature?

The samples were stored in cooling boxes with freezing blocks during transport to keep the water from warming, This will be clarified (Line 126):

“Samples were stored cold and dark in a cooling box with freezing elements until return to the lab.”

69. L 128 ’were according’. Check English

The phrasing has been changed and reads now (Line 176):

“… was performed according to …”

70. L283. FeTC. Define.

Thank you for pointing this out, FeTC has been replaced by “Fe transport capacity” (Line 379).

71. L 378. ’ the increases in Fe discharge is also likely to alter e.g. P retention in coastalsediments’. Again, this assertion should be supported by quantification.

The sentence has been removed based on a comment by referee #1 (Comment 56).

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Organic Iron Complexes Enhance Iron Transport Capacity along Estuarine Salinity Gradients of Baltic Estuaries

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Abstract. Rivers discharge a notable amount of dissolved Fe (1.5×10^9 mol yr^{-1}) to coastal waters, but are still not considered important sources of bioavailable Fe to open marine waters. The reason is that the vast majority of particular and dissolved riverine Fe is considered to be lost to the sediment due to aggregation during estuarine mixing. Recently however, several studies demonstrate relatively high stability of riverine Fe to salinity induced aggregation, and it has been proposed that organically complexed Fe (Fe-OM) can "survive" the salinity gradient, while Fe (oxy)hydroxides are more prone to aggregation and selectively removed. In this study, we directly identified, by X-ray absorption spectroscopy, the occurrence of these two Fe phases across eight boreal rivers draining into the Baltic Sea, and confirmed a significant but variable contribution of Fe-OM in relation to Fe (oxy)hydroxides among river mouths. We further found that that Fe-OM was more prevalent at high flow conditions in spring than at low flow conditions during autumn, and that Fe-OM was more dominant upstream in a catchment than at the river mouth. The stability of Fe to increasing salinity, as assessed by artificial mixing experiments, correlated well to the relative contribution of Fe-OM, confirming that organic complexes promote Fe transport capacity. This study suggests that boreal rivers may provide significant amounts of potentially bioavailable Fe beyond the estuary, due to organic matter complexes.

1 Introduction

Iron (Fe) mobility from the litho- and pedosphere into the hydro- and biosphere is controlled by physical, chemical, and biological processes. While Fe is the fourth most abundant element in the earth crust (Taylor, 1964), Fe concentrations in oxygenated aquatic systems are generally low (Johnson et al., 1997;Kraemer, 2004), while they can be higher during high flow conditions and in boreal waters with high dissolved organic carbon (DOC) concentrations (Kritzberg et al., 2014;Ekström et al., 2016). The more soluble form of Fe - Fe(II) - is favored under strongly reducing or highly acidic conditions (Waychunas et al., 2005). At circumneutral pH and oxic conditions Fe(II) gets oxidized to Fe(III), which has a low solubility and precipitates as Fe (oxy)hydroxides (Lofts et al., 2008;Liu and Millero, 2002). Thus, mobilization of Fe to surface waters requires either water flow through anoxic soil layers, favoring Fe(II), or that Fe is complexed by organic ligands and becomes mobile also in oxic soil layers (Tipping, 1981;Stumm and Morgan, 1970).

Several studies report rising Fe concentrations in surface waters, especially in Northern Europe (Neal et al., 2008;Kritzberg and Ekstrom, 2012;Sarkkola et al., 2013;Weyhenmeyer et al., 2014;Björnerås et al., 2017), suggesting that Fe export from soils is increasing. As a consequence, Fe loading from boreal rivers to estuaries is increasing substantially (Kritzberg and Ekstrom, 2012;Kritzberg et al., 2014;Björnerås et al., 2017). Given the key role that Fe plays in both local and global biogeochemical cycles in coastal and marine systems, this is a finding with major implications to the receiving systems. What the consequences may be, depend first and foremost on the fate of Fe in the estuarine salinity gradient. To the extent that Fe is stable to salinity induced aggregation and sedimentation, it may provide potentially bioavailable Fe to the marine system. However, Fe is known to behave non-conservatively in estuaries, and it has been suggested that at least 95 % of Fe is aggregated and lost to the sediments in the early part of estuarine mixing (Sholkovitz et al., 1978;Haese, 2006). Fe can play an important role in the sediment *e.g.* by acting as a C and P sink (Lalonde et al., 2012;Lenstra et al., 2018). Moreover, benthic release of Fe and subsequent lateral transport, was recently found to be a significant source of dissolved Fe to open marine waters (van Hulten et al., 2016).

Fe transport capacity – the fraction of riverine Fe remaining in suspension at higher salinity – has been shown to vary widely and is in some instances much higher than predicted from prior works (Kritzberg et al., 2014;Krachler et al., 2005). Thus, the riverine Fe source to marine waters may be underestimated, especially for boreal rivers, where high DOC concentrations can affect Fe speciation. Fe in natural waters is known to occur in two main phases, mononuclear organic complexes (Fe-OM) and Fe-rich Fe (oxy)hydroxide colloids associated with organic matter (Breitbarth et al., 2010;Hassellöv et al., 1999;Andersson et al., 2006). It has been suggested that variability in Fe transport capacity between rivers (1% to 55%) may be explained by a varying proportion of these Fe phases (Kritzberg et al., 2014). Further, studies using cathodic stripping voltammetry (CSV) have underlined the importance of complexation by ligands to keep Fe in suspension in saline waters (Laglera and van den Berg, 2009;Sukekava et al., 2018). However, the postulated link between Fe-OM and Fe transport capacity requires direct assessment of Fe speciation to verify previous interpretations based on Fe:OC ratios and molecular size (Stolpe and Hassellöv 2007; Krachler et al. 2010; Kritzberg et al. 2014). A preferential loss of Fe (oxy)hydroxides by aggregation was shown by Herzog et al. (2017) using X-ray absorbance spectroscopy (XAS). While this infers that Fe-OM is more stable to salinity induced aggregation, some Fe-OM was also found in aggregates, indicating that the control of Fe stability is more complex.

Based on the previous findings, the aim of the current study was to better understand what controls the fate of Fe from boreal rivers across estuarine salinity gradients by exploring 1) if variability in relative contribution of Fe-OM and Fe (oxy)hydroxides can explain variation in Fe transport capacity, and 2) if the relative contribution of Fe-OM and Fe (oxy)hydroxide is controlled by spatial factors and flow conditions, within and among rivers.

To this purpose, we sampled eight river mouths that drain at the Swedish coast into the brackish Baltic Sea. The rivers were chosen to encompass a wide geographical and climatic gradient, as well as a range in Fe and DOC concentrations. The Fe speciation, organic speciation (organic vs. inorganic) and redox speciation, was characterized by XAS. To be able to link Fe speciation to Fe transport capacity, the same river waters were exposed to artificial salinity gradients (mixing experiments). Four of the rivers were sampled under high (spring) and low flow conditions (autumn). To reveal differences along the flow path, two upstream sites were sampled in addition to the river mouth in one river catchment.

2 Materials and Methods

2.1 Site description and sampling

Eight rivers around the Swedish coast, with distinct differences in climate and catchment characteristics (Figure 1), were selected for this study. The annual temperature ranged from 5.8°C for the most northern (Öre) and 9.8°C for the most southern river mouth (Helge). Forest is the most dominant land cover and peat soils are present to a varying extent in all the catchments (Table 1). While six of these river mouths were sampled for previous studies exploring Fe dynamics in response to increasing salinity (Kritzberg et al., 2014;Herzog et al., 2017), relating the XAS assessed contribution of Fe-OM complexes to Fe transport capacity, was not previously done. Moreover, to investigate differences in Fe speciation along a river, two upstream locations in river Helge were included. The most upstream sample site, Svineö, is draining from a peat bog, with a high percentage of peat soil. The second site along the river path, Biveröd, is a small 2nd-order stream in a predominantly forested landscape. In addition, to understand the impact of high and low flow conditions on Fe speciation and transport capacity, sampling was carried out twice – during autumn and spring – in four of the river mouths

(Emån, Lyckby, Mörrum and Helge). A major difference in the discharge between the autumn and spring sampling was observed (Table 1). Finally, for two rivers (Öre and Örekil), transects were sampled starting at the river mouth and extending over the estuarine salinity gradient, to facilitate comparison of Fe transport capacity by mixing experiments and *in situ* Fe concentrations along the natural salinity gradient.

Water was sampled by hand from half a meter below the surface into acid cleaned polyethylene containers through a 150-µm nylon mesh. The mesh was used to ensure homogeneous samples free of large detritus. No further filtration steps were applied, to ensure that all suspended Fe was included, which is critical when studying the stability of riverine Fe across salinity gradients. Samples were stored cold and dark in a cooling box with freezing elements until return to the lab.

For the XAS analysis, a 1-L sample of water was frozen as soon as possible and never more than 5 hours after collection. The samples were later freeze-dried and stored dry in the dark until analyzed. Freeze-drying is commonly applied for preservation and pre-concentration of XAS samples (Karlsson, et al. 2008; Vilgé-Ritter, et al. 1999). While the freezing may lead to changes in the physical properties of colloids (Raiswell, et al. 2010), the chemical composition should be less affected. For instance, drying/freeze-drying could increase the rate of crystallization of Fe (Bordas and Bourg 1998), reducing the amorphous Fe phase compared with native samples, but this would not affect the distinction between organically complexed Fe and Fe (oxy)hydroxides which was the focus here.

Oxygen and pH were measured in situ with OxyGuard MkIII and SevenGo Duo pH meter (Mettler Toledo), respectively. Only acid washed material was used for sample handling, and for XAS and total Fe measurement polycarbonate bottles/containers were used.

2.2 Artificial Seawater Mixing Experiments

Experiments mixing river water and artificial seawater were initiated as soon as possible, and no later than 3 hours after sampling. Water samples were mixed with artificial sea salt solutions in a 6:1 ratio (vol:vol) in 50-ml Falcon tubes to 8 or 16 levels of salinity ranging from 0 to 35. To achieve the desired final salinities the added concentration of the sea salt solution varied. These were made from an artificial sea salt stock solution produced using reagent grade salts (Sigma Aldrich) following a standard protocol (Kester et al., 1967) (mass fraction given in %: Cl^- (55.05), Na^+ (30.62), SO_4^{2-} (7.68), Mg^{2+} (3.69), Ca^{2+} (1.15), K^+ (1.10), HCO_3^- (0.40), Br^- (0.19), H_3BO_3 (0.07), Sr^{2+} (0.04), F^- (0.003)). The Fe contamination from the salts used was negligible, e.g. the addition of salt to produce salinity 35 added a maximum of 0.0025 mg/l Fe, which corresponds to 0.1-3.8 % of the Fe concentration in the river waters studied. The stock solution was diluted to the desired concentration by Milli-Q water (Millipore, 18.2 MΩ). After mixing the river water with the salt solution, the samples were kept in the dark on a shaker for at least 24 h to allow aggregation. Salinity induced aggregation of Fe consists of several reactions with a significant fraction aggregating within a few seconds (Nowostawska et al., 2008). While aggregation then continues at a slower rate after the first few hours (Nowostawska et al., 2008;Hunter and Leonard, 1988), the first 24 h should include the largest fraction of the Fe removal.

The aggregates were separated by centrifugation at 3000 rcf for 8 h at 4°C. After centrifugation Fe, DOC, pH and salinity were measured in the supernatant. Fe transport capacity was calculated as the Fe concentration in the supernatant divided by the *in situ* Fe concentration and multiplied by 100 (%).

2.3 Standard Analytical Methods

An ICP-AES Optima 3000DV (Perkin Elmer) was used to determine total Fe concentration on acidified samples (1% vol, HNO_3). A Shimadzu TOC V-CPN was used to analyze organic carbon by high temperature catalytic-oxidation, using the Non-purgeable Organic Carbon (NPOC) method. For calibration a five-point standard curve was used and blanks and standards were included in all runs. The pH of the mixing experiment samples was measured with a 913 pH Meter (Metrohm) and salinity was determined by a WTW inoLab Cond730.

2.4 XAS Data Collection and Analysis

Synchrotron data was collected at the beam line I811 MaxII ring; Max Lab, Lund University. Fe K-edge XAS spectra were collected on the river samples at fluorescence mode at room temperature. A Lytle detector with an Mn-filter (3µx) was used to minimize unwanted scattering and fluorescence contributions. Aligned samples at 45° relative to the incident beam guaranteed an optimal fluorescence signal. Depending on the Fe concentration 15 to 40 spectra for each samples were recorded taking approximately five minutes each. Transmission scans of a reference Fe foil were collected simultaneously during all scans for energy calibration.

Data treatment and analysis for the extended X-ray absorption fine structure (EXAFS) and wavelet transform (WT) spectra was performed according to Herzog et al. (2017). A subset of the samples (6 out of 14) was previously analyzed for EXAFS (Herzog et al., 2017), and reanalysis was performed with consistent fitting parameters for the whole data set to allow comparison among all samples. In short, all scans were checked for beam damage before averaged with SixPack (Webb, 2005). The averaged scans were normalized and the background was removed by subtracting a spline function in Viper (Klementiev, 2000). The same program was used for investigation of self-absorbance and shell by shell fitting of the EXAFS data. For the WT analysis Igor Pro script was used (Funke et al., 2005). The k^3-weighted spectra were modeled in k-space from 2.8-12.0 Å^{-1} using theoretical phase and amplitude functions from FEFF7 (Zabinsky et al., 1995). Goethite (O'day et al., 2004) and the trisoxalatoiron(III) complex (Persson and Axe, 2005) were used as input structures for calculations with FEFF. While fitting, the amplitude reduction factor (S_0^2) was set to 0.70. Further, with values found in the literature, the numbers of free variables were restriction by correlating coordination numbers and fixing the

Debye–Waller factors (σ^2). Also, the threshold energy (ΔE_0) beyond the first was assumed to be identical for all shells.

A linear combination fitting (LCF) analysis was applied to the river mouth samples using SixPack. k^3-weighted EXAFS spectra (k 3.0 to 12.0 Å^{-1}) were used in the LCF analysis. Reference spectra of ferrihydrite, goethite, hematite, lepidocrocite and a Fe(III) complexed to Suwannee Rives fulvic acid were used as model compounds. This model provided good fits for all samples and further allowed us to distinguish between organically complexed Fe and Fe (oxy)hydroxide. During the LCF analysis E_0 was allowed to float, a non-negative boundary condition was applied and the sum of species was not forced to equal 100%. Components with a contribution less than 5% were excluded from the models.

Fe K-edge high-energy resolution fluorescence detection (HERFD) XANES spectra and Fe $Kb_{2,5}$ emission spectra were collected at the high-brilliance X-ray absorption and X-ray emission spectroscopy undulator beamline ID26 of the European Synchrotron Radiation Facility (ESRF, Grenoble) (Signorato et al., 1999). The incident X-ray beam was monochromatized with a pair of cryogenically cooled Si(111) crystals. The sample, analyzer crystal, and photon detector (silicon drift diode) were arranged in a vertical Rowland geometry. The intensity was normalized to the incident flux. Both the HERFD and the Fe $Kb_{2,5}$ emission spectra were measured using the X-ray emission spectrometer (Glatzel and Bergmann, 2005;Kvashnina and Scheinost, 2016), and the HERFD measurements were performed by recording the intensity of the Fe Kb_1 emission line (7058 eV) as a function of the incident energy using five Ge (620) crystal analyzers at 79° Bragg angle.

2.5 Data treatment

The contribution of the single Fe-C and the multiple Fe-O/C scattering paths, was statistically analyzed in the EXAFS fits by an F-test with Viper (Klementev, 2001).

As a measure of the relative contribution of Fe-OM and Fe (oxy)hydroxides in the water samples, two different approaches using the k^3-weighted EXAFS spectra were applied: 1) a ratio of the coordination numbers of the fitting results, between the Fe-C path and the shortest (edge-sharing) Fe-Fe path (i.e. $CN_{Fe\text{-}C}/CN_{Fe\text{-}Fe}$); 2) a ratio of the Fe-OM fraction and the sum of Fe-oxide fractions from the LCF analysis.

Relationships for the river mouth samples between Fe transport capacity at 35 salinity (corresponding to the salinity of the open sea), $CN_{Fe\text{-}C}/CN_{Fe\text{-}Fe}$ ratios, LCF ratio, Fe:OC ratios, total Fe, and DOC were tested by Pearson correlations. Assumptions of normality were verified by Shapiro-Wilk tests. Differences in Fe transport capacity between spring and autumn samples, as well as DOC concentrations at *in situ* and 35 salinity in the mixing experiment, were tested by paired t-tests.

Expected, or theoretical, values of *in situ* Fe across the estuarine salinity gradient were calculated by accounting for the dilution of riverine water by sea water (estimated by salinity) and the stability of Fe to aggregation as assessed by the artificial mixing experiments. The following equation Eq. (1) was used to calculate the expected Fe concentration [Fe_{exp}] at a given salinity:

$$[Fe_{exp}] = \frac{Fe_{river} \times salinity}{Fe_{stability} \times salinity_{marine\ end-member}} + [Fe_{marine\ end-member}] \times \left(1 - \frac{salinity}{salinity_{marine\ end-member}}\right) \quad , \tag{1}$$

where [Fe_{river}] is the Fe concentration at the river mouth, $salinity_{marine\ end\text{-}member}$ is the highest salinity in the estuarine *in situ* gradient, [$Fe_{marine\ end\text{-}member}$] is the Fe concentration at the highest salinity in the estuarine gradient, and $Fe_{stability}$ is the fraction that remained in suspension in the artificial sea water mixing experiments at the given salinity.

3 Results

3.1 Water chemistry

At the time of sampling all river mouths were close to saturation with dissolved oxygen (85 – 118%) and pH values close to neutral (Table 2). For the river mouths that were sampled twice, pH was consistently lower during spring than during autumn sampling, i.e. lower during higher discharge. Total Fe concentrations in the river mouths varied from 0.22 to 2.28 mg/l and DOC concentrations from 8.8 to 24.2 mg/l. Water chemistry in the two upstream samples from the Helge catchment differed strongly from those of the river mouth. Oxygen saturation and pH were lower in the upstream sites, especially in Svineö (dissolved oxygen saturation 41% and pH 4.4), while Fe and DOC concentrations were markedly higher. Across all samples Fe and DOC concentrations were strongly correlated ($r = 0.96$; $p < 0.001$), but since Fe was more variable (32-fold) than DOC (6-fold) there was a wide range in Fe:DOC molar ratios, from 0.005 to 0.035.

3.2 XAS characterization

The XAS analyses identified two main Fe phases, namely Fe (oxy)hydroxide, and Fe ions associated with organic matter as Fe-OM complexes. Both phases were qualitatively identified in the WT contour plots (Figure 2). The feature in the WT plots at ca. 7.5 $Å^{-1}$, 2.8 Å, originate from Fe-Fe scattering paths (denoted in the first plot in Figure 2 as Fe) and are similar to that of ferrihydrite (Figure S2) (Sundman et al., 2014;Yu et al., 2015) and goethite (Figure S2) (Karlsson and Persson, 2010;Sundman et al., 2014). Features for the Fe-OM complexes occurred at ca. 3 $Å^{-1}$, 2.5 Å and 3 $Å^{-1}$, 3.2-3.7 Å, caused by single Fe-C and multiple Fe-C-C(O or N) scattering, respectively (denoted in the first plot in Figure 2 as C and C/O) and are in good agreement with previously identified Fe-OM complexes(Figure S2) (Karlsson and Persson, 2010). In Figure 2 only a selection of the WT plots are shown, all WT plots of the remaining samples can be found in Figure S1.

Guided by the WT results, the EXAFS spectra were quantitatively modeled by a shell-by-shell non-linear least squares fitting procedure (Table S2) including five paths (Fe-O, two Fe-Fe, Fe-C and a Fe-C/O multiple scattering path). The two Fe-Fe paths were used to describe the contribution of Fe (oxy)hydroxide and the Fe-C and the Fe-C/O multiple scattering paths were used for the Fe-OM component. This modeling approach corroborated the qualitative WT analyses and provided good fits to all spectra (Figure S3). For the river mouth samples the Fe-Fe edge- and corner-sharing distances were determined to 3.05-3.11 Å and 3.41-3.46 Å, respectively (Table S1). The coordination number (CN) of the short Fe-Fe path varied between 1.0 and 2.7, indicating significant contribution from Fe (oxy)hydroxide, as corroborated by the WT plots. The Fe-C distances varied between 2.85 and 3.00 Å and the CN of the Fe-C path varied between 0.9 and 2.8 Å. To verify the contribution of the Fe-C path, an F-test comparing EXAFS models with and without the Fe-C and the Fe-C/O multiple scattering paths was performed, showing a significant contribution at the 92 % confidence level or better. While the EXAFS fitting analyses confirmed the presence of both Fe (oxy)hydroxide and Fe-OM complexes in all river mouth samples, the CN values indicated a large variation in the relative contribution of these Fe species (Table S1).

LCF analysis supported the variable contribution of the two Fe phases in the water samples (Table S2) and was in good agreement with the WT data. LCF analysis assigned the main components in the river mouth samples to Fe-OM, ferrihydrite and lepidocrocite. The contributions from goethite and hematite were below 5% in all samples and therefore excluded from the final analysis.

The XAS data contains information on the local structure around the selected element (Fe) that it is not strictly quantitative. Therefore, the ratios were merely used to identify trends in the relative contribution of Fe-OM vs Fe (oxy)hydroxides among the samples. Differences in the relative contribution of Fe-OM and Fe (oxy)hydroxide across the samples were obvious from the WT plots (Figure 2 and Figure S1) and supported by the $CN_{Fe\text{-}C}/CN_{Fe\text{-}Fe}$ and LCF ratios (Table 2). There was agreement between the two ratios as indicated by correlations across all samples ($r = 0.58$; $p = 0.047$) and river mouth samples only ($r = 0.64$; $p = 0.033$) (Figure S4). Low ratios, i.e. low contributions of Fe-OM, were indicated by both approaches for Rivers Öre, Alster, $\text{Lyckeby}_{\text{autumn}}$, and Örekil, for which the Fe (oxy)hydroxide feature was dominating in the WT plots. This was contrasted by River $\text{Lyckeby}_{\text{spring}}$ and $\text{Mörrum}_{\text{spring}}$, with high ratios and particularly strong Fe-OM signals in the WT plots. For the other river mouth samples a more even contribution of the two Fe species was found.

Fe speciation in the two samples taken upstream in the River Helge catchment was distinctly different from the river mouth. The HERFD and $Kb_{2,5}$ emission spectra indicated a gradual change from low to high Fe oxidation state when approaching the river mouth. Due to low Fe concentration there was more noise in the river mouth sample, but the deviation from the Fe(II) spectra was still clear. In the HERFD spectra the presence of Fe(II) was shown by a low energy shoulder at 7.1135 kEv, which was most pronounced for the Svineö sample (Figure 3). This effect was even more obvious in the $Kb_{2,5}$ emission spectra where the peak at 7.105 kEv provided direct evidence for the presence of Fe(II), based on comparison with the model compounds. The emission spectrum of the Svineö sample is very similar to the organic Fe(II) complex Fe(acac)$_2$, which suggests that Fe(II) was the predominant oxidation state at this site.

The WT results of the Svineö sample indicated no Fe-Fe scattering (Figure 4), whereas further downstream at Biveröd both Fe-C and Fe-C-C(O or N) features were present. In the river mouth Fe-Fe was even further pronounced. Thus, the Fe-Fe signal increased in strength the further downstream the sample was taken.

The LCF analysis corroborated the WT results; i.e. for Svineö no Fe-oxides were identified, whereas in Biveröd both Fe-OM and Fe-oxides were present and in the Helge river mouth Fe-oxides were dominating (Table S2). This trend was also obvious from the quantitative shell-by-shell fitting results (Table S1). Finally, comparing the various EXAFS analyses with the HERFED and Kb$_{2,5}$ emission spectroscopy results show that Fe(II) in the upstream samples of the Helge river system is present as Fe-OM complexes. These complexes are favored by low pH and low oxygen concentrations, as expected.

For the river mouths that were sampled twice, samples taken during high flow regime in spring displayed consistently higher CN$_{Fe-C}$/CN$_{Fe-Fe}$ and LCF ratios than samples taken in the same river in autumn during lower discharge, indicating a higher contribution of Fe-OM complexes during high flow conditions (Table S1).

Considering all samples, the relative contribution of Fe-OM and Fe (oxy)hydroxide was not predicted by the molar Fe:OC, i.e. no correlation between the Fe:OC and the Fe speciation ratios was found (CN$_{Fe-C}$/CN$_{Fe-Fe}$ ratio: $r = 0.182$, $p = 0.570$; LCF ratio: $r = 0.53$; $p = 0.077$). Instead, the LCF ratio was negatively correlated to pH ($r = -0.69$; $p = 0.019$). Among all samples, no significant relationships were found between CN$_{Fe-C}$/CN$_{Fe-Fe}$ or LCF ratios and variables related to catchment size, land cover or soil type.

3.3 Fe transport capacity.

The general pattern of the artificial seawater mixing experiments was a non-conservative behavior of Fe with increasing salinity (Figure 5). Fe removal took place already at low salinities, with more than 50% of the Fe removed at salinity 2 for some river samples (Öre, Alster, Mörrum$_{autumn}$ and Helge$_{spring}$, Helge$_{autumn}$). At a salinity corresponding to the open ocean (35) between 76 and 93% of Fe was removed. High Fe transport capacity was measured for river Lyckeby with 24% remaining in suspension at salinity 35. No significant loss of OC in response to increasing salinity was found for rivers where OC was analyzed in the mixing experiment (Öre, Örekil, Helge, Mörrum, Emån and Lyckeby; $t_5 = 1.38$ $p = 0.17$).

For the river mouth samples, the Fe transport capacity at 35 salinity correlated positively with the Fe speciation ratios (CN_{Fe-C}/CN_{Fe-Fe}: r = 0.675, p = 0.023; LCF ratio: 0.78, p = 0.005) (Figure S5). Further, Fe transport capacity at 35 salinity were negatively correlated to pH (r = -0.730, p = 0.007).

When comparing the samples taken within the Helge catchment, upstream samples showed a higher Fe transport capacity than the river mouth sample (Figure 5). For the four river mouths that were sampled twice, Fe transport capacity (at 35) was consistently higher in spring than in autumn (t_3 = 4.696, p = 0.0183).

In addition to raising the salinity, also the pH in the samples increased due to the experimental treatments from 6.4 - 7.5 in the river mouths to 7.4 - 8.3 in the high-salinity treatments, and for the upstream samples from pH 4.7 - 6.5 to pH 7.1 - 7.7.

In situ concentrations of total Fe declined with increasing salinity and distance from the river mouths (Figure 6). The theoretical values for Fe, calculated based on salinity induced aggregation in the artificial seawater mixing experiments and dilution estimated by salinity, were only slightly lower than the measured *in situ* values for River Örekil. The deviation was larger for River Öre, where the Fe measured *in situ* was substantially higher than the theoretical values at the lower levels of salinity.

4 Discussion

4.1 In situ speciation of Fe

Two carrier phases associated with Fe transport in freshwater in boreal system - Fe-OM and Fe (oxy)hydroxides (Andersson et al., 2006;Hassellöv et al., 1999) - have been previously verified by XAS (Sundman et al., 2013;Herzog et al., 2017) and were also identified and detected in all river mouth samples in this study. The results of the quantitative modeling of the EXAFS spectra and LCF analysis correlated, which is satisfying considering the potential sources of error of both analyses. Results showed a wide variation in the relative contribution of the Fe phases across river mouths, with some dominated by Fe-OM, *e.g.* River $\text{Lyckeby}_{\text{spring}}$, and some by Fe (oxy)hydroxide, *e.g.* River Öre. The significant contribution of Fe-OM in the river mouth samples is in contrast to the thermodynamic modeling suggested by (Wällstedt et al., 2010) for these systems that predicted a dominance of ferrihydrite (97 %).

The contribution of the Fe phases changed markedly along the Helge river catchment. In the water draining from a peat bog (Svineö), Fe(II) was predominant and only Fe-OM was present. Neither EXAFS fitting nor LCF identified Fe-Fe paths to support the presence of Fe (oxy)hydroxide. The sample showed similar features as groundwater in a northern boreal catchment (Sundman et al., 2014) or Fe(II) loaded onto peat humic acid (Yu et al., 2015). Further downstream (Biveröd), a mixture of the two phases could be seen, similar to samples from soil waters from organic layers close to a boreal stream (Sundman et al., 2014). The Fe(III) and Fe (oxy)hydroxide fraction was more prominent along the river path and was highest at the river mouth.

In addition to variation across river mouths and within the Helge river catchment, XAS analyses revealed a clear variation between high and low flow regime in the river mouth samples. Samples collected during high flow in spring showed a higher contribution of Fe-OM than the autumn samples, which is a direct support for previous interpretations made by ultra filtration (Pokrovsky et al., 2010;Stolpe et al., 2013). This variation is likely to be driven by changes in the source of the Fe depending on seasonal dynamics in hydrology (Dahlqvist et al., 2007).

4.2 Fe transport capacity and the link to Fe speciation

Non-conservative behavior of Fe was seen in the artificial mixing experiments as well as in the estuarine gradients sampled, and is consistent with existing literature (Gustafsson et al., 2000;Boyle et al., 1977;Sholkovitz, 1976). Fe transport capacity varied from 0.7 to 24 % among the rivers. The high Fe transport capacity for most of the rivers studied go along with the existing literature showing that high-latitude DOC-rich rivers exhibit higher Fe-carrying capacities (Krachler et al., 2005;Muller, 2018). DOC was little affected by increasing salinity as previously observed in high latitude rivers with high DOC concentrations (Herzog et al., 2017;Forsgren et al., 1996). Nevertheless, the high values of Fe remaining in suspension due to complexation with organic matter at high salinity (0.02 g/l – 0.50 g/l) supports that the importance of rivers as a source of Fe into the Baltic Sea with an Fe concentration of around 1 µg/l (Baltic proper) (Gelting et al., 2010).

Previous studies using size separation and spectrometric methods (Stolpe and Hassellöv, 2007), but also XAS (Herzog et al., 2017), have suggested that mainly Fe (oxy)hydroxide is affected by salinity and selectively lost from suspension by aggregation and sedimentation (Herzog et al., 2017). In contrast, Fe complexed by terrigenous organic matter is supposedly less affected and to a larger extent "surviving" estuarine mixing and can thereby be a source of bioavailable Fe to marine waters (Krachler et al., 2010;Laglera and van den Berg, 2009;Batchelli et al., 2010). The positive correlation between the contribution of Fe-OM (as determined by XAS) and Fe transport capacity (determined in artificial mixing experiments) adds a direct support that organic complexation of Fe is enhancing the stability across salinity gradients.

In freshwater Fe (oxy)hydroxide is stabilized by surface interactions with organic matter (OM), providing a negative surface charge (Tiller and O'Melia, 1993). With increasing salinity, the surface charge gets neutralized resulting in reduced colloidal repulsion (Mosley et al., 2003) and formation aggregates containing both Fe and OM. Marine cations, like magnesium and calcium, which neutralize the negatively charged surface groups of the OM, weaken the interaction between colloidal Fe (oxy)hydroxide and OM (Turner and Millward, 2002) and further promote the destabilization of Fe (oxy)hydroxides at increasing salinity. The same cations may favor release and hydrolysis of Fe-OM, by competing for the binding sites of the organic ligands (Fujii et al., 2008). The stability of organically complexed Fe may also be reduced at high ionic strength leading to compression of water and consequent "salting out" of the organic complexes (Turner and Millward, 2002;Turner et al., 2004). Accordingly, Herzog et al. (2017) also identified organically complexed Fe in salinity-induced aggregates especially at high salinities. However, both the selective loss of Fe (oxy)hydroxide at saline conditions, and the positive correlation between the relative contribution of Fe-OM and transport capacity in the current study underpin the role of Fe speciation in controlling the fate of Fe across salinity gradients. It would be an advantage to directly measure Fe speciation remaining in suspension in saline samples, to see if Fe (oxy)hydroxide is present, but this is currently hindered by methodological limitations. It has been suggested that Fe isotopic ratios may reflect Fe speciation (Ingri et al., 2006;Ilina et al., 2013), however this remains to be confirmed. Moreover, based on cathodic stripping voltammetry (CSV), ligand concentrations have been found to be in excess of iron concentration, suggesting that organic ligands are complexing the Fe and keeping it in suspension in saline waters (Gledhill and Buck, 2012;Laglera et al., 2011).

Results regarding Fe transport capacity derived from the artificial seawater mixing experiments were in good agreement with the estuarine transects sampled. Theoretically calculated Fe concentrations, based on Fe loss in artificial seawater mixing experiments with river water and the dilution factor, showed only minor deviations from Fe concentrations measured in the Gullmar Fjord. For the Öre estuary on the other hand, measured Fe concentrations were somewhat higher than the theoretical calculations (Figure 6). In the low-salinity mixing regime present in the northern Baltic (Bothnian Bay), aggregation may occur without significant sedimentation (Forsgren and Jansson, 1992). This has been observed in the plume of nearby River Kalix, and was hypothesized to result from a high organic component of the aggregates, where low specific density may lead to transport of these aggregates far away from the river mouth (Gustafsson et al., 2000). Thus, the centrifugation used to efficiently separate aggregates in the mixing experiments, may overestimate estuarine particle loss in this context. Despite the agreement between measured and theoretically estimated Fe concentrations, the artificial mixing experiments are unlikely to capture all processes that affect the loss of Fe along the natural salinity gradient. In the estuary, photoreduction may affect Fe speciation and affect its fate, as well as the occurrence of ligands produced by marine biota which may also influence the behaviour of riverine Fe. Indeed, the artificial mixing experiments capture the response of riverine Fe to increasing salinity in isolation, and how that depends on Fe speciation.

4.3 Control of spatial variation and flow conditions on Fe speciation.

The results of this study showed that Fe speciation is highly variable across spatial scales and during different flow conditions and is further linked to Fe stability across salinity gradients. It is therefore imperative to understand what factors govern Fe speciation. The largest variability in Fe speciation was observed between samples taken along the flow path of River Helge. In the most upstream location, which drain a major peat bog (Svineö), Fe(II) and Fe-OM dominated. This site also showed low oxygen and pH but high DOC concentration – conditions that favor complexation over Fe(III) hydrolysis (Neubauer et al., 2013;Liu and Millero, 2002). The Fe speciation in the stream water close to the Fe sources is thus determined by the properties of the in-flowing terrestrial Fe(II)/Fe(III)-OM complexes and of the conditions when anoxic, hydrated Fe(II) meets oxygenated DOC-rich waters (Sundman et al., 2014). The contribution of Fe(III) and Fe (oxy)hydroxide increased with pH and oxygen saturation along the flow path. This is in agreement with Neubauer et al. (2013), who argued that pH and OM were the main factors controlling Fe speciation in a boreal catchment, and explaining the dominance of Fe-OM in wetland-influenced headwaters and increasing Fe (oxy)hydroxide downstream (based on molecular size and chemical equilibrium modeling). The difference in speciation along the flow-path may in part be due to organically complexed Fe precipitating as Fe (oxy)hydroxide due to strong hydrolytic tendencies (Karlsson and Persson, 2012) as pH increases and OM declines, and due to photo reduction of Fe(III)-OM (Fujii et al., 2011;Waite and Morel, 1984;Neubauer et al., 2013). But the difference may also reflect different sources of Fe to the river along the flow-path. Thus while runoff from organic soil layers may bring predominantly Fe-OM to low order streams (Lydersen et al., 2002;Abesser et al., 2006;Dahlqvist et al., 2007;Sundman et al., 2014), groundwater inflow is more significant further downstream and may bring small Fe (oxy)hydroxides or Fe(II), which is rapidly hydrolyzed in the riparian zone when anoxic groundwater and oxic waters mix (Vasyukova et al., 2010). Hence, the speciation of Fe at the river mouth is determined both by the properties of the inflowing water and the chemical processing along the river flow path.

A consistent pattern was that samples taken in spring, when discharge was higher, showed a larger contribution of Fe-OM than autumn samples from the same river mouths. Temporal variation in river runoff is tightly linked to different hydro-geological pathways (Andersson et al., 2006;Pokrovsky et al., 2006;Neff et al., 2006). During autumn, dominance of groundwater input, and longer residence time of ground water, should promote input of Fe(II), which rapidly oxidizes to form Fe (oxy)hydroxides in surface water in the absence of high OM concentrations (Dahlqvist et al., 2007). During high discharge on the other hand, like during spring flood or high precipitation events, organically complexed Fe gets mobilized from the upper soil layers into the river, due to raising water tables and surface runoff (Grabs et al., 2012;Dahlqvist et al., 2007). The lower pH and higher DOC values in our spring samples agrees with this reasoning – that during higher discharge flow through organic-rich soil layers has a higher influence on river water chemistry and allows for the formation of more Fe-OM.

It was notable how Fe speciation and Fe stability matched pH across the entire dataset. A high contribution of Fe-OM and high stability coincided with low pH, across river mouths, along the flow path of the Helge catchment, and in the spring samples compared to the autumn samples. pH should exert a strong control on Fe speciation and increasing pH favors precipitation of Fe (oxy)hydroxide due to the strong hydrolytic tendency of Fe(III) (Karlsson and Persson, 2012). Moreover, a low pH may reflect strong influence from organic soils where Fe prevails as Fe-OM on water chemistry, as seen in low order systems and under high flow conditions (Dahlqvist et al., 2007;Neubauer et al., 2013). On the contrary, a high pH in the river mouth in these boreal systems may reflect a higher influence of groundwater input further downstream, possibly increasing the contribution of Fe (oxy)hydroxide. The increasing pH downstream in parallel with photoreduction and declining DOC concentration, may lower the stability by promoting release and hydrolysis of organically complexed Fe originating from organic soils further upstream (Neubauer et al., 2013;Waite and Morel, 1984;Fujii et al., 2011).

The temporal variability within rivers suggests that Fe speciation at a given time is not well predicted by catchment characteristics only. While characteristics such as land-cover and soil type are most likely affecting both quantity and speciation of Fe exported from the catchment, the limited number of rivers and sampling occasions of this study cannot accurately discern such relationships.

5 Conclusions, implications and future perspectives

The collective results from this study confirmed the existence and wide variability in the contribution of two Fe phases – Fe-OM complexes and Fe (oxy)hydroxides – among the boreal rivers included. It further confirmed that the response of river-borne Fe to increasing salinity differed widely. Interestingly, the differences in stability towards salinity-induced aggregation matched well the differences in relative contribution of Fe-OM across the river mouths, between high and low flow conditions, and along the flow path of a river catchment. Thus, by assessing the Fe speciation by XAS, this study provides direct evidence that Fe-OM enhances survival over estuarine salinity gradients. This would suggest that high and rising concentrations of Fe from boreal rivers (Kritzberg and Ekstrom, 2012;Björnerås et al., 2017) may indeed result in increasing export of bioavailable Fe to the Baltic Sea and open waters, where it may limit N-fixation and primary production (Stal et al., 1999;Stolte et al., 2006;Martin and Fitzwater, 1988). Major hydrological events like spring floods and heavy storms have been observed to increase of the Fe concentration by up to a factor of 20 and alter the annual Fe load in northern rivers (Hölemann et al., 2005;Rember and Trefry, 2004;Dahlqvist et al., 2007;Herzog et al., 2019). The hydro-geological conditions during such events (Dahlqvist et al., 2007), may promote a higher contribution of Fe-OM and thus a higher stability during estuarine mixing, resulting an increase of Fe export into the open waters. However, sampling with higher temporal resolution would be required to substantiate such an extrapolation.

Author contribution

SH and EK conceived and designed the study. SH carried out the fieldwork and lab work. SH and PP performed the XAS analyses and subsequent data treatment. SH, PP and EK analyzed the data. SH wrote the manuscript with support from PP and EK.

Competing interests

The authors declare that they have no conflict of interest.

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The supporting information contains: High resolution WT modulus of EXAFS data river Emån, Alster and Ljungby not shown in the manuscript (Figure S1). k^3-weighted EXAFS spectra and Fourier transformations of all samples and corresponding model fits of all samples (Figure S3). Relationship between the LCF-ratio and CN-ratio (Figure S4). Relationship between Fe transport capacity at 35 salinity and relative contribution of organically complexed Fe as assessed by the CN-ratio and LCF-ratio Figure (S5). Table S1 showing the k^3-weighted Fe K-edge EXAFS fit results for all samples and Table S2, containing the EXAFS LCF results for the river mouth samples.

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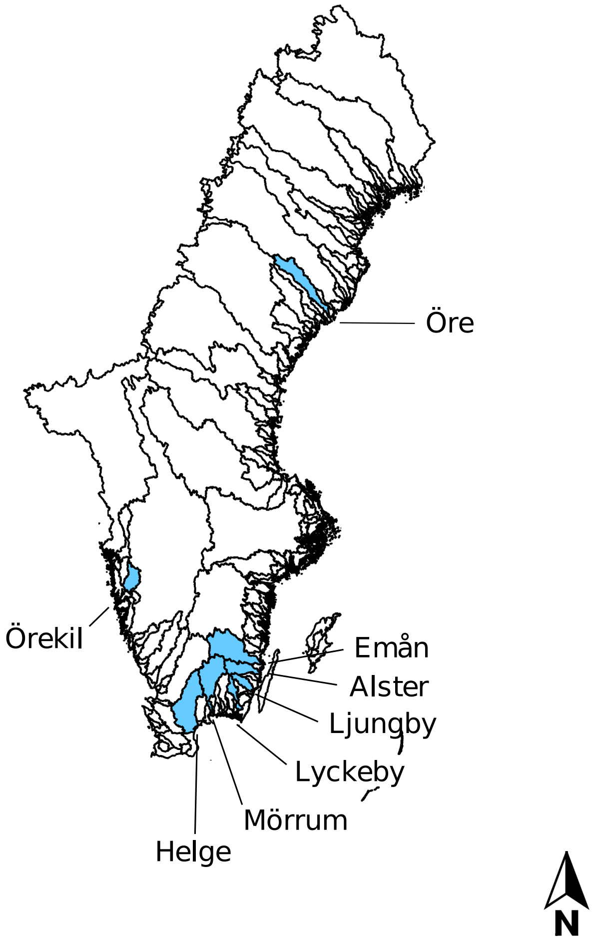


Figure 1: Map of all river catchments in Sweden with the ones considered in this study named and marked blue.

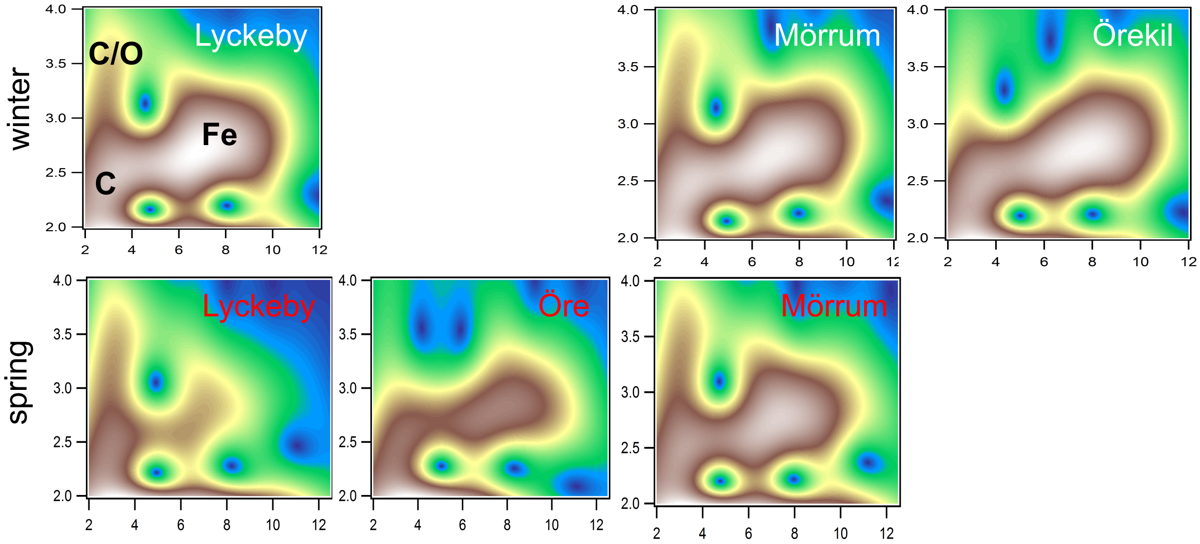


Figure 2: Morlet wavelet transforms (η= 4, σ= 2) of EXAFS data collected on samples of river Lyckeby, Mörrum, Örekil and Öre (white=autumn, red spring) and are plotted as a function of k (Å^{-1}) on the x-axis and R (Å) on the y-axis. In the top left plot (Lyckeby) areas representing the different Fe scattering paths are indicated by C (Fe−C), C/O (Fe−C−C/O), and Fe (Fe−Fe).

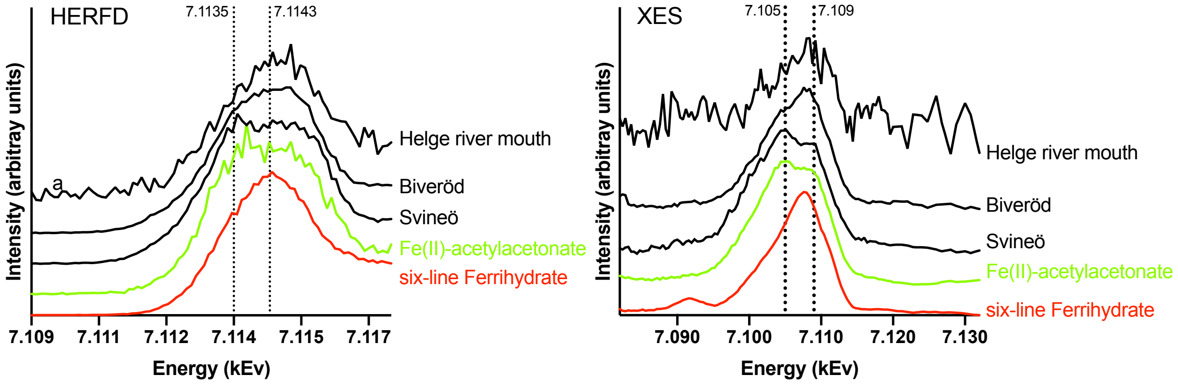


Figure 3: Fe K-edge HERFD pre-edge spectra (left) and Kb$_{2,5}$ X-ray emission (XES) spectra (right) of the Helge river mouth sample, the Biveröd sample, the Svineö sample, Fe(II)-acetylacetonate (Fe(acac)$_2$) (green), and six-line Ferrihydrite (red). The spectra were normalized to the maximum intensities. The dotted lines are included for visual guidance.

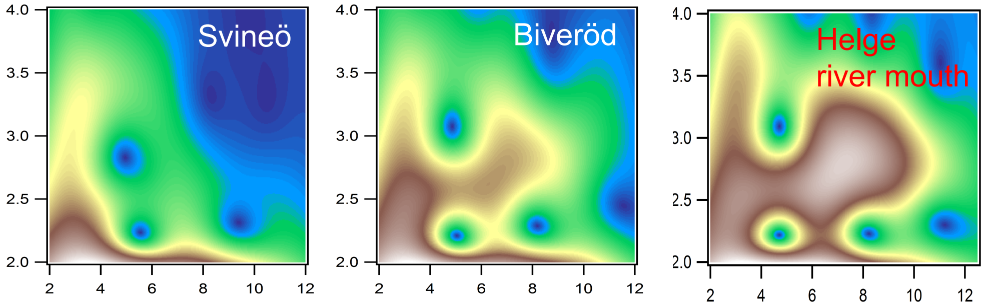
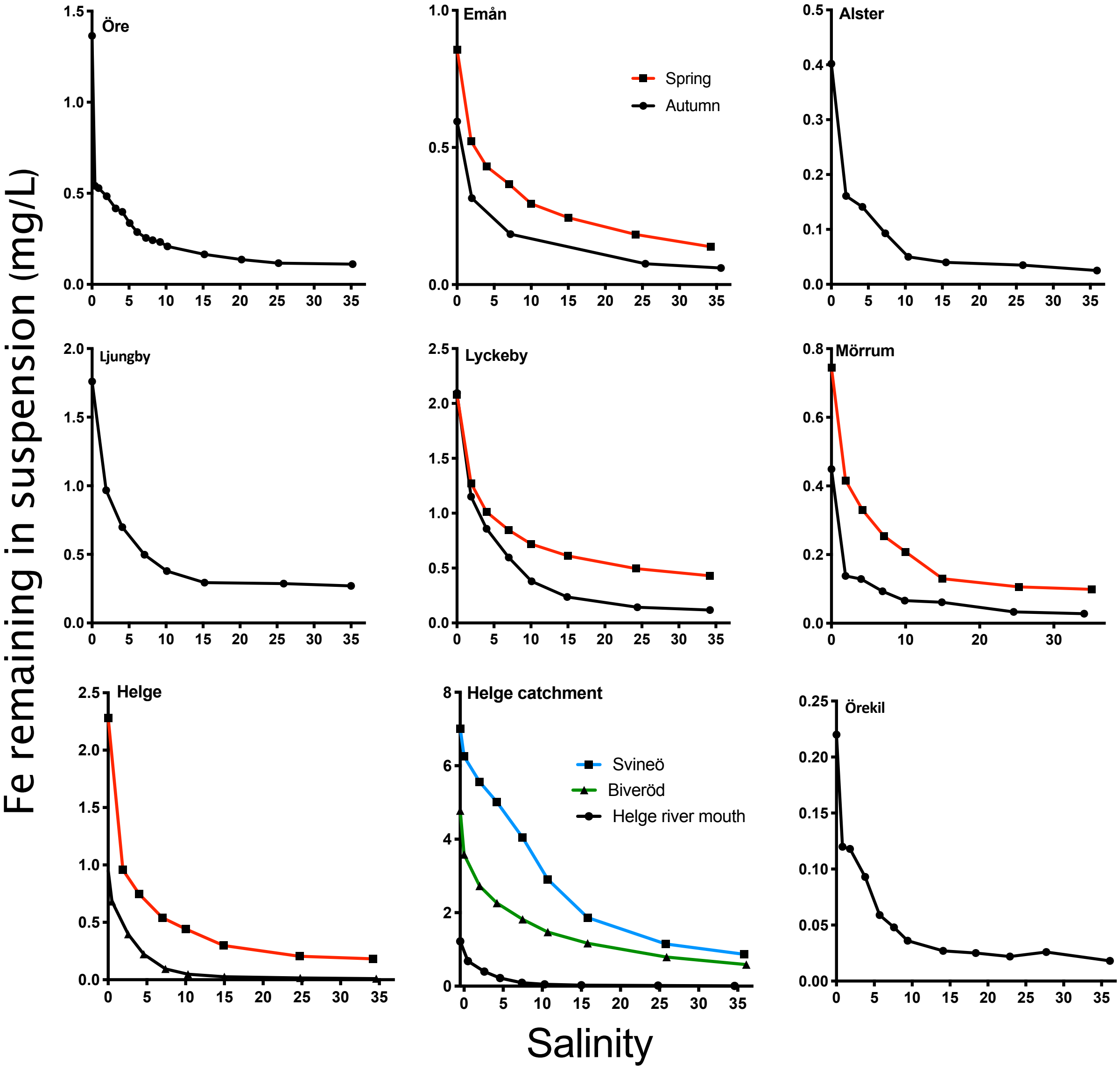


Figure 4: Morlet wavelet transforms (η= 4, σ= 2) of EXAFS data of the two upstream samples Svineö and Biveröd and river mouth of the Helge catchment (white=autumn, red spring), plotted as a function of k (Å-1) on the x-axis and R (Å) on the y-axis.



685 **Figure 5: The change in Fe in suspension in response to increasing salinity (0-35). Black lines denote sampling in autumn and red lines denote sampling in spring. For river Helge, the blue line denotes the most upstream sample (Svineö), the green line the other upstream sample (Biveröd) and the black line the river mouth.**

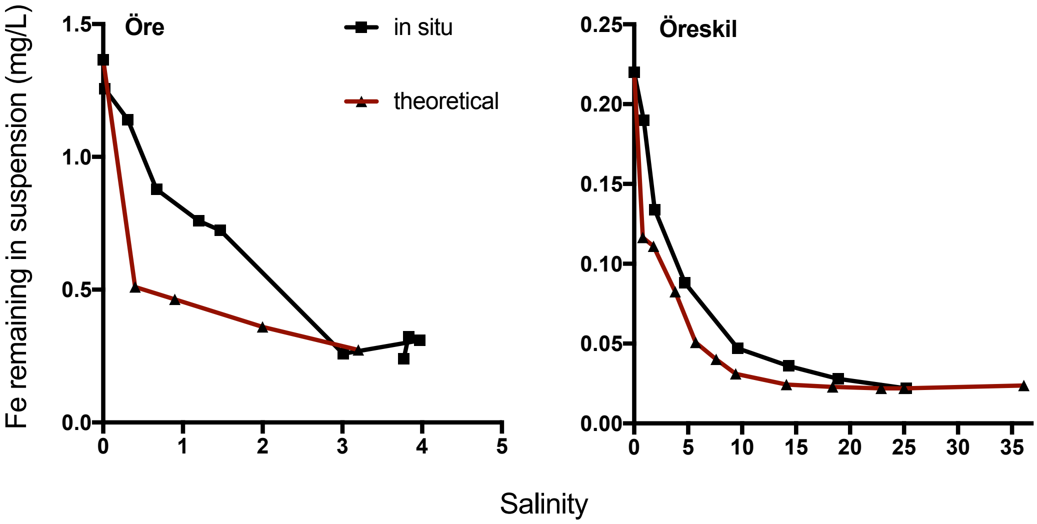


Figure 6: Fe remaining in suspension in response of increasing salinity of *in situ* samples along a transect and theoretical for river Öre and Örekil. The black line denote the *in situ* samples and the brown the theoretical values.

Table 1: Catchment characteristics.

Site	Catchment area[a]	River length[a]	Discharge[a] (autumn/ spring)	Water retention time[b]	Forest cover[a]	Water cover[a]	Peat soil[a]
	km^2	km	m^3/s	year	%	%	%
River Öre	3029	225	8.2	0.5	71.8	3.1	25.3
River Emån	4471	220	24.1/65.4	1.4	73.3	6.1	8.4
River Alster	1525	100	4.5	1.0	79.7	5.1	9.6
River Ljungby	758	62	2.6	0.2	71.9	0.6	8.5
River Lyckeby	810	90	6.4/13.3	0.6	75.0	4.2	7.5
River Mörrum	3369	175	26.6/47.6	2.0	69.9	12.7	9.2
Svineö*	28		0		83.5	1.01	33.8
Biveröd*	44		1		92.6	0.9	11.6
River Helge	4724	190	14.9/36.9	0.5	57.5	4.8	14.4
River Örekil	1340	70	3.9	0.4	53.0	3.9	11.1

*Upstream sites in the Helge catchment. [a]Data obtained from http://vattenweb.smhi.se.[b]Data from Lindström et al. (2018).

Table 2: Water chemistry and transport capacity at salinity 35 (corresponding to the salinity of the open sea) of collected samples.

Site	Sampling Date	pH	O_2	Total Fe	DOC	Transport capacity at 35 salinity	CN ratio[a]	LCF ratio[b]
			%	mg/l	mg/l	%		
River Öre	20.04.2015	7.45	-	1.365	10.6	9.5	0.5	0.54
River Emån	03.11.2014	7.36	98	0.595	11.3	10.2	1.4	0.38
	09.03.2015	7.24	-	0.857	12.8	18.7	1.6	0.50
River Alster	03.11.2014	7.11	87	0.402	9.8	7.3	1.0	0.31
River Ljungby	20.10.2014	7.01	118	1.76	24.2	17.9	1.0	0.64
River Lyckeby	29.10.2014	6.99	101	2.095	19.4	6.6	1.0	0.50
	09.03.2015	6.55	-	2.082	19.4	24.1	2.7	0.89
River Mörrum	29.10.2014	7.43	103	0.449	10.2	7.3	1.5	0.53
	23.03.2015	7.05	105	0.745	13.6	15.5	2.0	0.71
Svineö*	06.11.2014	4.44	41	7.011	49.3	12.4	-	1.00
Biveröd*	06.11.2014	6.38	84	4.777	29.6	12.3	1.7	-
River Helge	06.10.2014	7.58	86	1.22	-	0.7		-
	23.03.2015	7.47	101	2.280	15.8	9.3	1.6	0.46
River Örekil	14.07.2014	7.28	85	0.220	8.8	9.5	0.8	0.47

* Upstream sites in the Helge catchment. [a]Ratio of the coordination numbers of the fitting results, between the Fe-C path and the shortest (edge-sharing) Fe-Fe path (i.e. $CN_{Fe\text{-}C}/CN_{Fe\text{-}Fe}$). [b]Ratio of the Fe-OM fraction and the sum of Fe-oxide fractions from the LCF analysis.