

# Current Biology

## Salp fecal pellets release more bioavailable iron to Southern Ocean phytoplankton than krill fecal pellets

### Highlights

- Salps recycle iron in a more bioavailable form than krill
- Per mol fecal pellet carbon, salps release more iron than krill
- Possibly, salps increase the carbon fixation potential of the Southern Ocean

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### In brief

Böckmann et al. show that salp fecal pellets release more iron than krill fecal pellets. Additionally, the iron recycled from salp fecal pellets is more bioavailable to Southern Ocean phytoplankton than iron recycled from krill fecal pellets. Increasing salp populations might increase the carbon fixation potential of the Southern Ocean.

Article

# Salp fecal pellets release more bioavailable iron to Southern Ocean phytoplankton than krill fecal pellets

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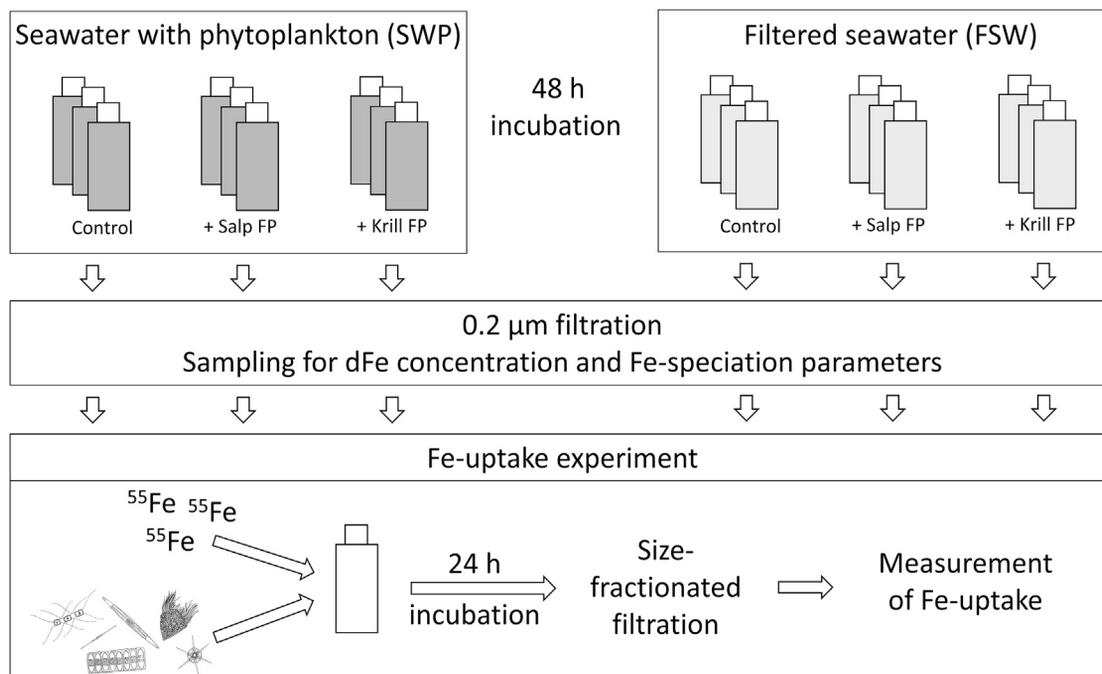
## SUMMARY

Over the last decades, it has been reported that the habitat of the Southern Ocean (SO) key species Antarctic krill (*Euphausia superba*) has contracted to high latitudes, putatively due to reduced winter sea ice coverage, while salps as *Salpa thompsoni* have extended their dispersal to the former krill habitats. To date, the potential implications of this population shift on the biogeochemical cycling of the limiting micronutrient iron (Fe) and its bioavailability to SO phytoplankton has never been tested. Based on uptake of fecal pellet (FP)-released Fe by SO phytoplankton, this study highlights how efficiently krill and salps recycle Fe. To test this, we collected FPs of natural populations of salps and krill, added them to the same SO phytoplankton community, and measured the community's Fe uptake rates. Our results reveal that both FP additions yielded similar dissolved iron concentrations in the seawater. Per FP carbon added to the seawater,  $4.8 \pm 1.5$  times more Fe was taken up by the same phytoplankton community from salp FP than from krill FP, suggesting that salp FP increased the Fe bioavailability, possibly through the release of ligands. With respect to the ongoing shift from krill to salps, the potential for carbon fixation of the Fe-limited SO could be strengthened in the future, representing a negative feedback to climate change.

## INTRODUCTION

In vast areas of the Southern Ocean (SO), phytoplankton growth is limited by iron (Fe) availability. Fe input from *de novo* sources, including upwelling, dust deposition, melting sea ice, and resuspension of coastal sediments,<sup>1–4</sup> is strong in the vicinity of land. In contrast, for the open ocean, upwelling and Fe recycling in the water column is of paramount importance.<sup>5,6</sup> Previous studies suggested that grazers<sup>7,8</sup> and predators<sup>9</sup> contribute to the recycling of Fe in the water column. In some open ocean regions, up to 50% of the soluble Fe pool is turned over on a weekly basis, mediated by biological recycling,<sup>10</sup> with Antarctic krill (*Euphausia superba*), hereafter referred to as krill, being particularly important.<sup>11</sup> So far, few studies have quantified the amount of Fe

released by krill<sup>8,12–14</sup> and the dominant SO salp species *Salpa thompsoni*,<sup>15</sup> hereafter referred to as salps. Because krill and salps form high biomass aggregates, it is believed that both play a substantial role in Fe recycling via acidic and anaerobic digestion of phytoplankton cells.<sup>14</sup> Krill foraging on the seabed<sup>16</sup> was also identified as a vector of new Fe from abyssal depths, hence sustaining phytoplankton productivity in the euphotic zone by recycling and importing Fe from below. Maldonado et al.<sup>17</sup> calculated that krill and salps can release 3.4–14.4 kg Fe km<sup>-2</sup> year<sup>-1</sup> and 1.3–12.1 kg Fe km<sup>-2</sup> year<sup>-1</sup>, respectively, making them the third and fourth most important Fe recycling organisms in the SO, after microzooplankton and carnivorous zooplankton. However, the actual bioavailability of this recycled Fe to SO phytoplankton has never been tested.



**Figure 1. Experimental setup**

Natural Southern Ocean seawater was filled into 18 bottles. Nine were filled with seawater that contained phytoplankton (SWP), and 9 were filled with filtered seawater (FSW). Three bottles were left untreated (controls); to 3 bottles, salp fecal pellets (salp FPs) were added, and to the other 3 bottles, krill fecal pellets (krill FPs) were added. After 48 h, all bottles were filtered through a 0.2  $\mu\text{m}$  filter, and the filtrate was used to determine the dissolved iron concentration (dFe) and Fe-speciation parameters. Afterward, the filtrate from each bottle was used to determine Fe-uptake rates. A concentrated natural phytoplankton community and  $^{55}\text{Fe}$  as a tracer label were added to the preconditioned water aliquots. After a 24-h incubation phase, the Fe uptake into the 0.2–2  $\mu\text{m}$  fraction and the >2  $\mu\text{m}$  fraction were measured in a scintillation counter.

It has been reported that, between 1926 and 2016, krill distributions shifted from the northern part of the southwest Atlantic sector of the SO to the Antarctic shelf.<sup>18</sup> Concurrently, Pakhomov et al.<sup>19</sup> reported that, due to global warming, salps increasingly inhabit krill habitats. In light of these climate-driven ecosystem changes, the biogeochemical consequences for the Fe supply from salps and krill to the SO need to be quantified.

The objective of this study was to assess the bioavailability of recycled Fe from krill and salp fecal pellets (FPs) to a natural SO plankton community. Our incubation experiment showed that the concentrations of Fe dissolved from salp and krill FPs were similar although the dissolved Fe (dFe) from salp FPs was taken up 1.7 times more effectively by phytoplankton, suggesting a higher bioavailability for dFe released from salp FPs.

## RESULTS AND DISCUSSION

To investigate the role of salp and krill FPs on SO Fe biogeochemistry, a two-step incubation experiment was performed close to the Western Antarctic Peninsula (STAR Methods; Figure 1) 45 km north-east of Elephant Island, a location which experienced increasing salp and decreasing krill densities in the past 5 decades.<sup>20</sup>

Freshly collected FPs were added to natural seawater (including the ambient phytoplankton community; seawater with plankton [SWP]) and to 0.2  $\mu\text{m}$  filtered seawater (FSW) (no phytoplankton) in concentrations matching typical *in situ*

situations during a bloom period.<sup>20–25</sup> Amended (salp FPs and krill FPs) and unamended (control) samples were incubated for 48 h at ambient light and temperature conditions. At the end of the experiment, dFe concentrations and the ligand complexing capacity were determined. In a second step, the Fe bioavailability was determined with  $^{55}\text{Fe}$  as a tracer, by adding a concentrated, natural phytoplankton community to the 0.2  $\mu\text{m}$  filtered water from the different treatments (Figure 1; STAR Methods).

### FPs as a source of Fe

The amount of FP additions per liter for salps was chosen to mimic natural conditions based on reported salp densities in the Lazarev Sea of 4 individuals  $\text{m}^{-3}$  and a projected doubling of salp density every decade.<sup>20,21</sup> At the beginning of the experiments, an amount of  $3.4 \pm 0.5 \mu\text{g FP C L}^{-1} \text{d}^{-1}$  (Table 1) was added once, corresponding to the release of FP by 13.4 salps  $\text{m}^{-3}$ , defecating at a rate of  $\sim 250 \mu\text{g C salp}^{-1} \text{day}^{-1}$ ,<sup>22–24</sup> representing realistic *in situ* densities of present-day SO waters. For krill, an average density of 16–64 individuals  $\text{m}^{-2}$  at the tip of the Western Antarctic Peninsula<sup>20</sup> and a defecation rate of  $146 \mu\text{g C krill}^{-1} \text{d}^{-1}$  (estimated from Atkinson et al.<sup>26</sup>) were reported. We added  $9 \pm 1 \mu\text{g FP C L}^{-1} \text{d}^{-1}$  (Table 1), representing a density of one individual krill in 16 L, which equals 62.5 krill individuals  $\text{m}^{-3}$ . Our krill FP addition thus exceeded the expected average krill FP density of the region but stayed well below the density of FPs estimated for krill swarms of  $1.3 \text{ g C m}^{-3} \text{d}^{-1}$ .<sup>25</sup>

**Table 1. Iron and carbon in FP**

Parameter	Unit	Salp FP treatment	Krill FP treatment
C addition by FP	$\mu\text{g L}^{-1} \text{d}^{-1}$	$3.4 \pm 0.5$	$9 \pm 1$
Dry weight addition by FP	$\mu\text{g L}^{-1} \text{d}^{-1}$	$80 \pm 0.2$	$142 \pm 3.8$
tFe in FP	$\text{nmol Fe } \mu\text{g dw}^{-1}$	$0.73 \pm 0.0004$	$0.75 \pm 0.001$
Leached dFe from total added tFe	%	$0.6 \pm 0.2$	$0.3 \pm 0.03$
Leached dFe per FP C	$\text{nmol dFe } \mu\text{g FP C}^{-1} \text{d}^{-1}$	$0.1 \pm 0.05$	$0.03 \pm 0.005$
Fe uptake by phytoplankton per added FP C	$\text{nmol dFe } \mu\text{g FP C}^{-1}$	$0.08 \pm 0.02$	$0.02 \pm 0.004$

Carbon (C) addition by fecal pellets (FPs), addition of FP dry weight, total iron content (tFe) inside the FP, leached dissolved iron (dFe) from added tFe, leached dFe per FP C, and iron (Fe) uptake into phytoplankton cells per added FP C.

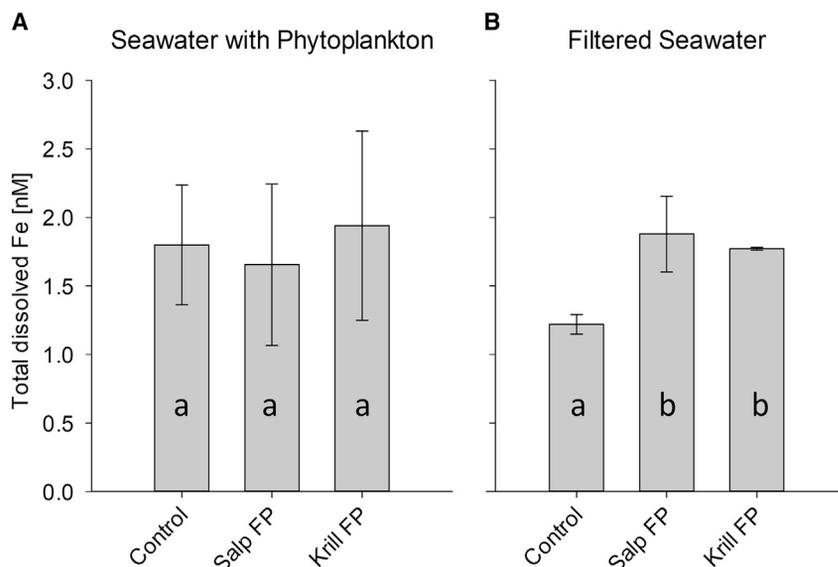
Thus, measurements were obtained at conditions that represent the effect of FP material in the extended surrounding area of a krill swarm event. In order to fuel phytoplankton production, the FPs have to release the dFe in the euphotic zone. Thus, even though FPs can develop high sinking velocities,<sup>27</sup> it has been reported that two-thirds of salp FPs produced do not reach 100 m depth,<sup>28</sup> putatively because of zooplankton coprophagy (feeding of zooplankton organisms on FPs), which causes the FPs to loosen or break up. It is possible that this effect is transferable to krill FPs as well.

To our knowledge, this study is the first time that dFe release rates from FPs of these two major zooplankton species under ambient ocean conditions (without an additional acidification step) have been measured (Table 1). Irrespective of the FP type that was added, no significant differences in dFe were detected in the SWP (Figure 2A). This is in stark contrast to the FSW, where salp and krill FP additions significantly increased the dFe pool (salp FP:  $p = 0.007$  and krill FP:  $p = 0.021$ ) by  $1.54 \pm 0.10$ - and  $1.45 \pm 0.04$ -fold, respectively, indicating Fe fertilization from both FP types (Figure 2B). We hypothesize that, in the SWP treatments, the phytoplankton cells present during the 48 h of incubation removed most of the Fe leached from the FP either by absorption or adsorption to their cell surface.

It has been reported that the FP of krill contain higher concentrations of Fe than any other part of the krill's body.<sup>12</sup> Also, krill guts have the potential to release originally lithogenic Fe in a reactive form.<sup>14</sup> Furthermore, the Fe:C ratio in FPs of marine animals is generally enriched in comparison to their food, due to (1) low assimilation rates of Fe in animals and (2) the respiratory conversion of particulate organic carbon to dissolved organic and inorganic carbon.<sup>29</sup> Schmidt et al.<sup>14</sup> report an Fe content in krill FPs of  $0.94 \text{ nmol Fe } \mu\text{g}^{-1} \text{ FP dry weight}^{-1}$ ,<sup>14</sup> which is very similar to the  $0.75 \text{ nmol Fe } \mu\text{g}^{-1} \text{ FP dry weight}$  of this study (Table 1). For salps, the observed increase of dFe in FSW contradicts previous results by Cabanes et al.,<sup>15</sup> who used whole salp FP, and did not measure a significant amount of dFe leaching from them. In the study presented here, the FPs were broken up, destroying their peritrophic membrane, mimicking natural exposure to grazers (i.e., through coprophagy [ingestion of FPs], coprophagy [fragmentation of FPs], and coprochaly [loosening of FPs]).<sup>30,31</sup> This activity facilitates a disintegration of the FPs into small, slow-sinking fragments.<sup>28</sup>

From the total Fe added in form of FP,  $0.3\% \pm 0.03\%$  and  $0.6\% \pm 0.2\%$  were released in a dissolved form for krill and salp FP, respectively (Table 1). These values lie at the low end of a previously reported range of  $0.05\%$ – $6.33\%$  as leachable particulate Fe in krill FPs<sup>13</sup> and krill stomachs.<sup>32</sup> The latter two studies, however, measured the leachable Fe fraction by acidification with a 25% acetic acid solution and a  $\text{NH}_4\text{Ac}$  buffer, respectively, therefore reaching a lower pH than in our study. Similarly, Cabanes et al.<sup>15</sup> acidified their salp FPs to  $\text{pH} = 2$  in order to measure leachable Fe. No study so far has measured dFe release from FPs under natural conditions without an additional acidification step. It is therefore not surprising that all previous studies report dFe release values, which differ from our study. What is consistent across all (this study and Schlosser et al.,<sup>13</sup> Cabanes et al.,<sup>15</sup> and Schmidt et al.<sup>32</sup>) is that  $<7\%$  of the total Fe content was leached from the FPs, suggesting that most of the Fe was refractory.

Even though the amount of krill FPs added (FP C given in  $\mu\text{g}$  of carbon) was 2.6 times higher than the amount of salp FP C added (Table 1), both additions increased the dFe concentration similarly in the FSW treatments (Figure 2B). Furthermore, normalized to FP C, the salp FPs released 3.3 times as much dFe as the krill FPs (Table 1). This higher leachability could be explained as follows: in contrast to krill FPs, salp FPs are fragile,<sup>28</sup> less dense than those of pelagic crustaceans,<sup>33</sup> and lack a peritrophic membrane.<sup>34</sup> In contrast, krill FPs are known to be dense and physically robust.<sup>26</sup> Salp FPs disintegrate easier than krill FPs, which results in a larger surface area to volume ratio of the FP fragments. Thus, an increased probability for exchange with seawater and naturally present Fe binding ligands exists, facilitating remineralization, both by leaching and microbial degradation.<sup>35</sup> Additionally, pH and the presence of oxygen inside the animal's guts as well as diet composition and amount<sup>36,37</sup> have been proposed as factors influencing Fe release in copepods. Also, the microbial community composition has been reported as an important factor governing the mobilization of particulate Fe from sinking biogenic particles.<sup>6</sup> Measurements of pH, oxygen, and microbial community compositions inside krill and salp guts are lacking, but it is evident that the two animals rely on different digestion strategies. Although krill cut and grind food items with their mandibles and their stomach before the food enters



**Figure 2. FPs as a source of iron**

Total dissolved Fe contents were determined after 48 h of incubation. SWP FP treatments and untreated control (A) and FSW FP treatments and untreated control (B) are shown. Significant differences ( $p < 0.05$ ) among treatments were tested using the Fisher-least significant difference (LSD) post hoc test and are indicated by small letters (a and b).  $n = 3$  except for (A): krill FP as well as control and (B): krill FP ( $n = 2$ ). Values represent means  $\pm$  SD.

the digestive gland,<sup>38</sup> salps have no mechanical apparatus to disintegrate their food before digestions<sup>39</sup> and rely only on chemical and enzymatic digestion. It is possible that, compared to krill, salp guts maintain a lower pH in order to compensate for less mechanical breakdown of the food. A lower pH inside a salp gut would enhance the dissolution of colloidal Fe into the dFe pool<sup>14,40</sup> compared to a krill gut. Any one of these factors could be responsible for salp FP leaching more dFe than krill FP, as reflected by our data (Table 1). Our data suggest that, due to the projected climate-change-driven population shift from krill to salps in the SO, more dFe could be released in surface waters, thereby potentially enhancing phytoplankton productivity and impacting the strength of the biological pump.

### Bioavailability of released Fe

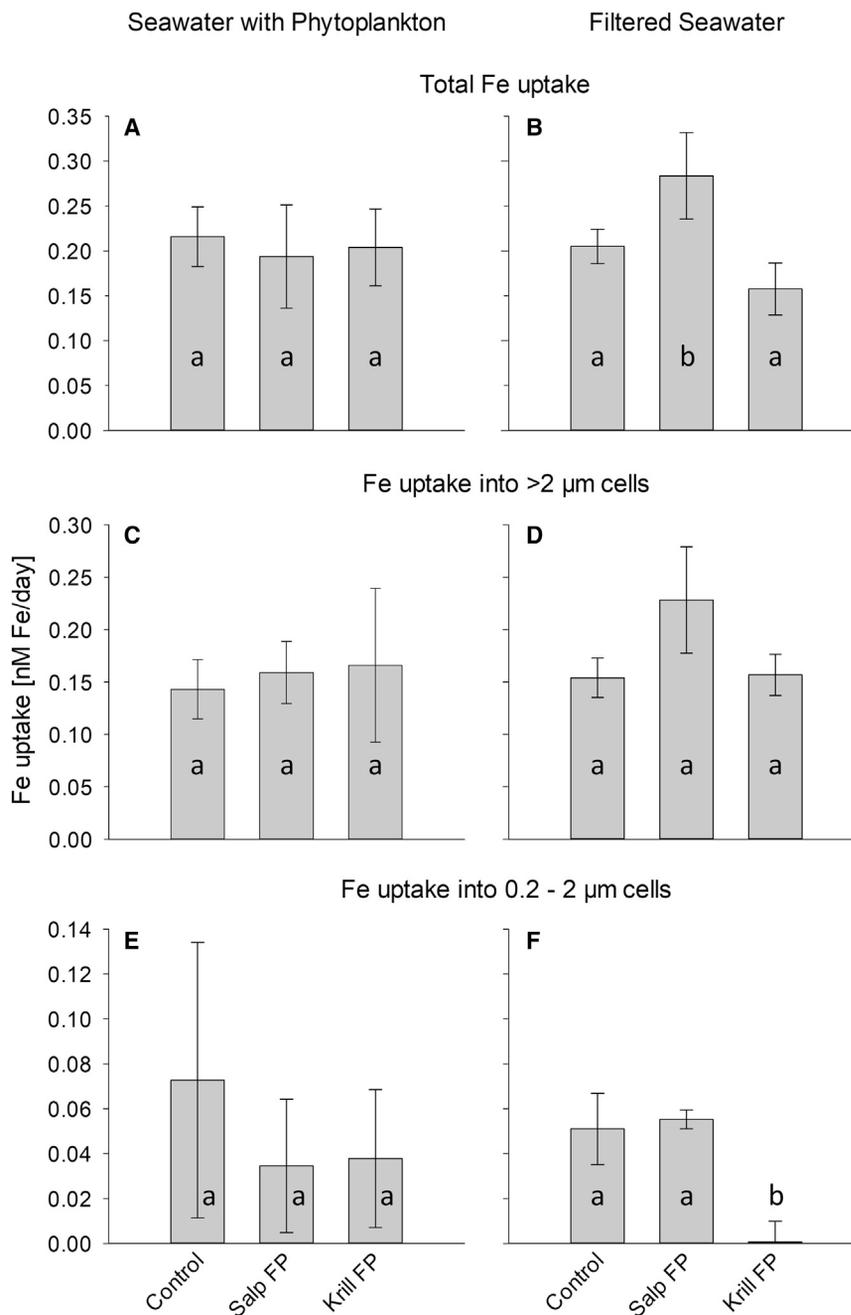
In order to assess the bioavailability of the Fe released from krill versus salp FPs, each treatment bottle was filtered (0.2  $\mu\text{m}$ ) after the initial 48 h of incubation and amended with a pre-concentrated, natural plankton community. After 24 h incubation in the presence of 0.03 nM <sup>55</sup>Fe, size fractionated (0.2–2  $\mu\text{m}$  and >2  $\mu\text{m}$ ) intracellular Fe uptake rates (Figure 1; STAR Methods) were measured. dFe concentrations for each treatment were considered in the calculation of the Fe uptake rates, enabling a direct comparison among treatments.

In all SWP treatments, Fe uptake rates of the two size classes (0.2–2  $\mu\text{m}$  and >2  $\mu\text{m}$ ) were not significantly different (Figures 3A, 3C, and 3E), likely due to regeneration and recycling of Fe and organic ligands during the initial incubation, as has been previously reported for diatoms<sup>41</sup> and bacteria.<sup>42</sup> Flowcytometric analysis revealed that the bulk of the 0.2–2  $\mu\text{m}$  size fraction consisted of heterotrophic bacteria, with less than 1% being photoautotrophic picoplankton (data not shown). In the FSW treatment, where no Fe recycling during the incubation occurred, the total Fe uptake in the salp FP treatment was significantly higher than in the krill FP and the control treatments ( $p = 0.011$  and  $0.041$ , respectively; Figure 3B). Surprisingly, in comparison to the FSW salp FP treatment and the FSW control, the picoplankton fraction (0.2–2  $\mu\text{m}$ ;

filtrates of the FSW salp FP treatment in comparison to the FSW krill FP treatment cannot be explained by differences in the dFe concentrations of the treatments because the dFe concentrations were similar in both treatments (Figure 2B). Rather, they must be related to the presence of highly bioavailable inorganic or labile organic Fe in the salp FP treatment.

Ligands play a significant role in the biogeochemistry of Fe in seawater<sup>43,44</sup> by increasing its solubility and modulating its bioavailability. In our study, we observed that salp and krill FPs released significant quantities ( $p = 0.004$  and  $p = 0.028$ , respectively, compared to the control) of ligands into the FSW of our experiment. The complexing capacity (STAR Methods) was increased by  $1.25 \pm 0.38$  nM and  $0.78 \pm 0.33$  nM by the addition of salp and krill FPs, respectively, in comparison to the control, an increase of 78% and 49%, respectively. Neither the complexing capacity nor the stability constant of Fe-ligand complexes differed between the FSW salp and krill FP treatments (Supplemental information; Table S1). Nevertheless, in this study, the greatest Fe uptake was measured in the salp FP treatments, suggesting that the quality of salp-FP-associated ligands enhanced the bioavailability of Fe. Similarly, the grazing of copepods has been hypothesized to release rapidly degradable Fe-ligand complexes,<sup>45</sup> and bacterial exopolysaccharides,<sup>46</sup> as well as the monosaccharide glucuronic acid and polysaccharide dextran,<sup>47</sup> have been reported to act as ligands that enhance Fe bioavailability.

In laboratory experiments, unchelated Fe showed the highest bioavailability to phytoplankton, although the bioavailability of Fe bound to the siderophore desferrioxamine B was 1,000 times lower.<sup>48</sup> In the ligand soup of natural seawater, the availability of Fe to plankton lies within a narrow range (factor of four) between these two extremes.<sup>49</sup> Similar to our study, Fe bioavailability did not clearly correlate with dFe concentrations, Fe-binding ligand concentrations, or their stability constants.<sup>49</sup> However, the bioavailability may depend on the specific chemical nature of ligands and environment interactions inside the natural ligand soup.<sup>48</sup> The ligand species inside a FP are likely



**Figure 3. Bioavailability of released iron**

Fe uptake rates were determined for all cells (A and B), for large cells (>2  $\mu\text{m}$ ; C and D), and for small cells (0.2–2  $\mu\text{m}$ ; E and F) from the filtrate of SWP (A, C, and E) and FSW (B, D, and F), to which no addition (control), salp, or krill FPs were given. For determination of Fe uptake, a 10 mL concentrate of the same phytoplankton community was added to each treatment. Please note that, before addition of the cell concentrate, all treatments were 0.2  $\mu\text{m}$  prefiltered. Significant differences ( $p < 0.05$ ) among treatments were tested using Fisher-LSD post hoc tests and are indicated by different small letters (a and b).  $n = 3$  except for FSW: krill FP and SWP: control as well as krill FP ( $n = 2$ ). Values represent means  $\pm$  SD.

differences in their digestion mechanisms and assimilation efficiencies. Thus, in our study, it is plausible that the set of ligands leaching from the salp FPs was different from the ligands released by the krill FPs.

Krill digestion and excretion can influence the availability of dissolved organic carbon (DOC),<sup>51</sup> which in turn can positively influence the Fe-uptake<sup>52</sup> and the growth of heterotrophic bacteria.<sup>53</sup> Additionally, a recent study has extended the range of the known capacity for Fe storage in marine bacteria by an order of magnitude,<sup>54</sup> suggesting high capacities for luxury Fe uptake. In our study, heterotrophic bacteria made up the small size fraction (0.2–2  $\mu\text{m}$ ) of plankton. As in our study, DOC concentrations remained unaltered in any treatment over the whole experiment, being on average  $49.4 \pm 2 \mu\text{M}$ ; this demonstrates clearly that, in our study, DOC was neither consumed nor released (data not shown).

### Impacts on the ecosystem

It has been reported that the recent warming in the SO may be responsible for a 2-fold decrease of krill numbers per decade after 1976 in the sampling area of this study.<sup>20</sup> Additionally, between 1926 and 2003, salp numbers have increased by 2-fold per decade.<sup>20</sup> Increasing temperatures and reduced sea ice cover are proposed as factors negatively influencing krill egg production and the survival of larval krill<sup>18</sup> and in turn thought to favor salp growth. Krill spawning is closely related to the intensive diatom bloom in spring and summer,<sup>38</sup> which follows the seasonal retreat of sea ice, and its intensity is closely related to the duration of winter sea ice cover.<sup>55</sup> On the other hand, salp blooms can occur in spring and summer<sup>56,57</sup> and are known to be favored by diminished sea ice coverage.<sup>19</sup> In regions where salps and krill co-occur, salps influence krill abundance in two ways: (1) indirectly by competing for the same limited food

dependent on the animal's nutrition and digestion. Although salp and krill guts contain a similar composition of ingested food items, the content of salp and krill FPs differed significantly from each other (N.-C. Pauli, personal communication). Salp FPs were shown to contain more whole diatoms in their FPs than in their stomachs, most likely due to a lack of mandibles to destroy the diatom silica frustule (N.-C. Pauli, personal communication). Extracellular cracking of diatoms by foraminifera without any mandibles has been observed,<sup>50</sup> but no such mechanism has yet been described in salps. In krill, the percentage of diatoms in the stomach and in the FP was very similar (N.-C. Pauli, personal communication). This suggests

between 1926 and 2003, salp numbers have increased by 2-fold per decade.<sup>20</sup> Increasing temperatures and reduced sea ice cover are proposed as factors negatively influencing krill egg production and the survival of larval krill<sup>18</sup> and in turn thought to favor salp growth. Krill spawning is closely related to the intensive diatom bloom in spring and summer,<sup>38</sup> which follows the seasonal retreat of sea ice, and its intensity is closely related to the duration of winter sea ice cover.<sup>55</sup> On the other hand, salp blooms can occur in spring and summer<sup>56,57</sup> and are known to be favored by diminished sea ice coverage.<sup>19</sup> In regions where salps and krill co-occur, salps influence krill abundance in two ways: (1) indirectly by competing for the same limited food

**Table 2. Contribution of krill and salps to iron flux**

Species	Limits	Density (ind. 1,000 m <sup>-3</sup> )	FP egestion rate (μg C ind. <sup>-1</sup> d <sup>-1</sup> )	dFe release (μmol Fe m <sup>-2</sup> d <sup>-1</sup> )	Source
Krill	mean	1 ± 2	146 (Clarke et al. <sup>25</sup> )	0.001 ± 0.001	this study
	max	5.7	146 (Clarke et al. <sup>25</sup> )	0.005	this study
	min	0	146 (Clarke et al. <sup>25</sup> )	0	this study
Krill	max			0.076	Ratnarajah et al. <sup>30</sup>
	min			0.002	Ratnarajah et al. <sup>30</sup>
Krill	max			0.7	Maldonado et al. <sup>17</sup>
	min			0.17	Maldonado et al. <sup>17</sup>
Salps	mean	1,385 ± 820	239 (Huntley et al. <sup>22</sup> )	5.5 ± 4.1	this study
	max	2,707	239 (Huntley et al. <sup>22</sup> )	10.8	this study
	min	103	239 (Huntley et al. <sup>22</sup> )	0.4	this study
Salps	max			0.59	Maldonado et al. <sup>17</sup>
	min			0.06	Maldonado et al. <sup>17</sup>

Density of krill and salps in individuals 1,000 m<sup>-3</sup> measured during this study at 8 stations in the study area northeast of Elephant Island using IKMT net trawls, assumed FP egestion rates based on literature sources, and dFe release measured in this study in comparison to the literature.

supply, phytoplankton, and thus affecting krill gonadal development<sup>55</sup> and (2) directly via predation by salps on krill larvae.<sup>57</sup> Conversely, krill have been shown to feed on salps.<sup>58</sup> In light of our results, a shift from a dominance of krill to a dominance of salps in some areas of the SO may have significant implications for the Fe recycling in the SO ecosystem.

### Fe recycling in a salp-dominated area

Northeast of Elephant Island, between 60° to 60°44.4 S and 54° to 55°31.8 W, where this study was conducted, the abundance and distribution of salps and krill were observed as 1,385 ± 820 salps and 1 ± 2 krill per 1,000 m<sup>3</sup> (Table 2).

Egestion rates of 239 μg C salp<sup>-1</sup> d<sup>-1</sup> and of 146 μg C krill<sup>-1</sup> d<sup>-1</sup> (estimated from Atkinson et al.<sup>26</sup>) were reported.<sup>23</sup> Our observed dFe release of 0.1 ± 0.05 nmol dFe μg salp FP C<sup>-1</sup> d<sup>-1</sup> and 0.03 ± 0.005 nmol dFe μg krill FP C<sup>-1</sup> d<sup>-1</sup> (Table 1) was integrated over a 170-m-depth interval, resulting in a release of 0.4–10.8 μmol dFe m<sup>-2</sup> d<sup>-1</sup> (average 5.5 ± 4.1) by salp FPs and 0.002–0.004 μmol dFe m<sup>-2</sup> d<sup>-1</sup> (average 0.001 ± 0.001) by krill FPs (Table 2). Compared to the literature on dFe release from krill,<sup>8,17,30,32</sup> our values are much lower (Table 2), which is not surprising for two reasons: first, our study is the first to have directly measured dFe release from krill FPs under *in situ* conditions (without acidification), which is probably why less Fe was leached from the individual FPs than in previous studies. Second, the release of dFe is strongly dependent on the amount of FP material produced. Although we measured 1 ± 2 krill individuals 1,000 m<sup>-3</sup> in the study area and used this value for our calculations, other studies used higher krill biomass.<sup>17</sup>

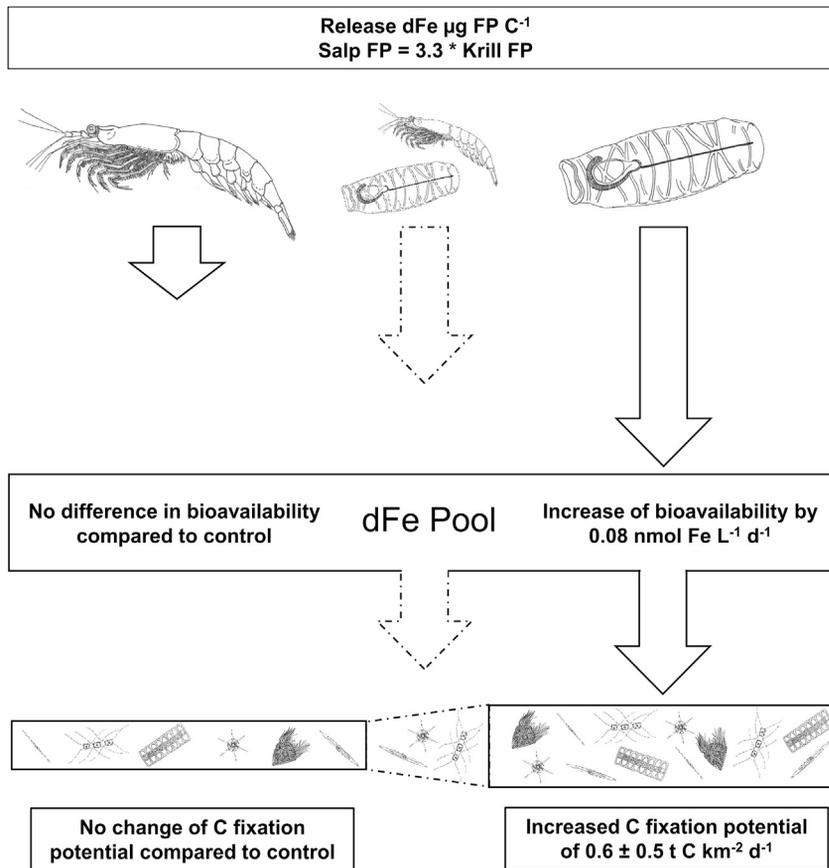
Schmidt et al.<sup>32</sup> acidified the krill's stomach content with NH<sub>4</sub>Ac buffer (pH 4.5) for 2 h, in order to analyze labile Fe, thus driving the reported higher release of dFe. Tovar-Sanchez et al.<sup>8</sup> used whole animals and their exudates for their experiments, which is why their values are higher than ours. For salps, on the other hand, our numbers are much higher than values previously estimated by Maldonado et al.<sup>17</sup> This is not surprising, because their numbers were based on mass-balanced Ecopath models, in which they used information on Fe content of sea squirts from Strohal et al.<sup>59</sup> as an approximation of the Fe

content of salps bodies. To our knowledge, we, for the first time ever, measured the direct release of dFe from salp FPs under ambient *in situ* conditions. According to this study, the regional dFe supply from salp FPs exceeds the lateral flux of 1.8 μmol dFe m<sup>-2</sup> d<sup>-1</sup> at Elephant Island that was estimated to support a production of 0.84–1.32 g C m<sup>-2</sup> d<sup>-1</sup>.<sup>60</sup> Additionally, the release of dFe into the water is no guarantee that it is bioavailable and utilized by phytoplankton cells. As we have seen in this study and the literature,<sup>48,61,62</sup> the chemical environment, into which the Fe is released, can affect its bioavailability greatly. Furthermore, our Fe uptake experiment allows for a calculation of the carbon (C) fixation potential generated by the Fe released from both FP types. The addition of 3.4 ± 0.5 μg salp FP C L<sup>-1</sup> d<sup>-1</sup> (Table 1) resulted in an increased Fe uptake of 0.08 ± 0.05 nmol Fe L<sup>-1</sup> d<sup>-1</sup> by phytoplankton (Figure 3B) in the salp FP treatment in comparison to the control, although the krill FP treatment did not show any significant difference. An average salp FP egestion rate of 56 273 ± 33 258 μg FP C m<sup>-2</sup> d<sup>-1</sup> in the sampling area is realistic, based on salp abundances and the individual egestion rate.<sup>23</sup> Using Equation 1 (details in STAR Methods: Quantification and statistical analysis), an estimate of the C fixation potential, assuming an Fe:C ratio of 27 ± 4 μmol Fe mol C<sup>-1</sup>, which has been reported for a phytoplankton community in the SO,<sup>63</sup> was obtained. Salp FPs increase the C fixation potential by 0.6 ± 0.5 g C m<sup>-2</sup> d<sup>-1</sup> (t C km<sup>-2</sup> d<sup>-1</sup>), in comparison to the control, although krill FPs did not increase the dFe uptake by the plankton community (Figure 4).

$$C \text{ fixation potential} = \frac{Fe \text{ uptake} * FP \text{ production} * molar \text{ mass}_C}{Added \text{ FP } C * Fe : C \text{ ratio}_{phytoplankton}}$$

### Effects on Fe recycling if salps replaced krill

In comparison to the already salp-dominated region around Elephant Island, in the Weddell Sea, krill dominated with 14–449 krill individuals 1,000 m<sup>-3</sup> at 5 different stations between 63°39.906 S 54°38.184 W and 63°55.046 S 55°28.518 W although no salps were observed. Assuming an egestion rate of 146 μg C krill<sup>-1</sup> d<sup>-1</sup> (estimated from Atkinson et al.<sup>26</sup>) and



**Figure 4. Impact on the ecosystem**

Input of dissolved iron (dFe) and changes in bioavailability of Fe to phytoplankton from krill and salp FPs, as well as calculated increase in carbon fixation potential by phytoplankton. Arrow length proportional to amount of Fe released by krill and salp FPs is shown. According to the results of this study, FPs from salps released 3.3 times more dFe  $\mu\text{g FP C}^{-1}$  than krill FPs. Additionally, in the dFe pool fueled by salp FPs, bioavailability of Fe to a natural phytoplankton community was increased by  $0.08 \text{ nmol Fe L}^{-1} \text{ d}^{-1}$  compared to the control, although in the dFe pool fueled by krill FPs, no change in bioavailability was detected. All FPs used in this study were sampled around Elephant Island within a time frame of a few days. Based on our results, we deduce that, with a shift from a krill- to a salp-dominated community in the future, the bioavailable dFe pool as well as the production of chlorophyll and carbon fixation will increase—particularly in HNLC areas. In krill-dominated areas of the SO, the recycling of bioavailable Fe might be less efficient than in salp-dominated regions. The response of phytoplankton in areas where salp and krill populations merge requires further research (broken lines).

an integration depth of 170 m, at which krill were found, a production of  $347\text{--}11\,144 \mu\text{g krill FP C m}^{-2} \text{ d}^{-1}$  is realistic. Given a dFe uptake into plankton of  $0.02 \pm 0.004 \text{ nmol dFe } \mu\text{g krill FP C}^{-1}$  (Table 1), this amount of krill FP material results in a C fixation potential of  $0.003 \pm 0.0007$  to  $0.09 \pm 0.02 \text{ g C m}^{-2} \text{ d}^{-1}$ . If this krill population was replaced by salps, which produced the same amount of FP C, the C fixation potential through Fe from salp FP would be  $4.8 \pm 1.5$  times higher, reaching  $0.01 \pm 0.004$  to  $0.4 \pm 0.1 \text{ g C m}^{-2} \text{ d}^{-1}$ . Although these numbers are strongly dependent on the regional salp and krill densities, it is striking how close they fall to the net air-sea carbon flux in the SO south of  $44^\circ \text{ S}$  of  $0.007\text{--}0.01 \text{ g C m}^{-2} \text{ d}^{-1}$  (estimation based on  $0.16 \pm 0.18 \text{ Pg year}^{-1}$  in the period between 2015 and 2017).<sup>64</sup> The relative increase of C fixation potential by Fe released from both salp and krill FPs by a factor of  $4.8 \pm 1.5$ , however, is not dependent on the animals' densities and likely a general phenomenon. For the majority of the Fe-poor SO south of  $44^\circ \text{ S}$ , this increased C fixation potential is likely to strongly enhance phytoplankton biomass and carbon fixation wherever krill is replaced by salps.

### Conclusions

Our study highlights that, per  $\mu\text{g FP C}$  added to the FSW treatments, salp FPs released 3.3 times as much dFe as krill FPs did (Table 1) and increased the bioavailability of the dFe (Figure 3). In contrast to the absolute dFe release in the study area by salp and krill FP, respectively (Table 2), which is strongly dependent

on the local FP density, we hypothesize that the relatively higher release, normalized to FP C, and the higher bioavailability could be species-specific values, influenced by the digestion capabilities and resulting ligand compositions of the animals' FPs. Therefore, the observed elevated Fe bioavailability from salp FPs in comparison to krill FPs may be applicable to other oceanic regions in a similar way. In the future, particularly in HNLC waters of the SO, the rising abundances of salps will likely increase the amount and bioavailability of recycled Fe, potentially increasing phytoplankton productivity. One could expect that a potential increase in bioavailable Fe could enhance phytoplankton biomass.<sup>65</sup> However, it remains unclear whether the fixed C could be transferred to higher trophic levels, potentially increasing the biomass of grazers and predators. Still, a potential increase in primary production is likely to enhance the efficiency of the biological carbon pump, thus acting in this case as a stronger sink of anthropogenically released  $\text{CO}_2$ .

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- EXPERIMENTAL MODEL AND SUBJECT DETAILS
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  - Fe speciation
- QUANTIFICATION AND STATISTICAL ANALYSIS

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2021.02.033>.

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## AUTHOR CONTRIBUTIONS

Conceptualization, S.T., F.K., and C.H.; Investigation, S.B., F.K., F.P., M.I., L.M.L., and R.D.; Writing – Original Draft, S.B., S.T., F.K., B.M., and C.H.; Writing – Review & Editing, all authors; Supervision, S.T. and F.K.; Funding Acquisition, B.M. and S.T.

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The authors declare no competing interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
55FeCl3	Perkin Elmer, MA, USA	LOT 021714C
2-(2-Thiazolylazo)-p-cresol	Alfa Aesar	LOT 30549
Deposited data		
Original dataset	PANGAEA	<a href="https://doi.org/10.1594/PANGAEA.931631">https://doi.org/10.1594/PANGAEA.931631</a>
Software and algorithms		
SigmaPlot	Systat Software GmbH	<a href="https://systatsoftware.com/downloads/download-sigmaplot/">https://systatsoftware.com/downloads/download-sigmaplot/</a> ; RRID:SCR_003210

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Sebastian Böckmann ([sebastian.boeckmann@awi.de](mailto:sebastian.boeckmann@awi.de)).

#### Materials availability

This study did not generate new unique reagents.

#### Data and code availability

The dataset generated during this study is available at <https://doi.org/10.1594/PANGAEA.931631>. Complexing capacities and stability constants of the iron-ligand complexes are available in the supplementary material in [Table S1](#).

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

No models or cell strains were used in this study. The study was an *in vivo* study performed with a natural community of phytoplankton.

### METHOD DETAILS

In the framework of the Polarstern cruise PS112, Antarctic seawater was sampled in the vicinity of the Western Antarctic Peninsula (60° 44.455 S 54° 30.477 W, 04/11/2018) from a depth of 25 m using a polyethylene line connected to a teflon ALMATEC membrane pump. The water was pumped directly into a trace metal free laboratory container for processing. Prior to use, all lab ware was cleaned according to GEOTRACES cookbook<sup>66</sup> at the Alfred Wegener Institute in Bremerhaven, Germany.

#### Experimental set-up

Ambient untreated seawater with phytoplankton (SWP) was filtered through an acid cleaned 200 μm mesh to avoid mesozooplankton contamination, used to rinse and fill 9 acid cleaned 4.2 L polycarbonate bottles ([Figure 1](#)). 9 other bottles were rinsed and filled with 0.2 μm filtered ambient seawater (FSW) using acid cleaned Acropak capsule (PALL). 3 SWP and FSW bottles were immediately sealed and served as controls. 3 bottles of each set (SWP and FSW) were amended with homogenized salp FP, while the last 3 bottles of each set were amended with homogenized krill FP material ([Table 1](#)).

FP material of krill and salps used in the experiment was produced by animals that had fed on an *in situ* plankton community and were caught at several stations around Elephant Island. The FP were frozen until use in experiment. The FP were rinsed once in ambient filtered seawater from the studied station and before addition to the incubation bottles, homogenized using a pipette to emulate zooplanktonic coprophagy, coprorhexy and coprochaly in the water column. To determine the carbon and Fe content of each FP material added, 1 mL of each FP suspension was kept and its Fe content determined. Using a representative amount of FP material collected in the same region as the FP used in the actual incubations, the particulate organic carbon amendments to each bottle was calculated.

All incubation bottles were put in front of halogen culture lamps with daylight spectrum which were set to a light intensity of  $30 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  under a light-dark cycle of 10:14 hours at  $2^\circ\text{C}$ . After 48 hours of incubation the dFe concentration of all bottles was measured. The 48 h incubation time reflects the residence time of FP material in the upper ocean after it has been loosened or broken up by zooplankton coprorhexy and coprochaly.<sup>28</sup> In order to assess the photophysiological efficiency ( $F_v/F_m$ ) of the *in situ* phytoplankton community, an indicator of Fe stress, a fast repetition rate fluorometer (FRRF) in combination with a FastAct Laboratory system (FastOcean PTX), both from Chelsea Technologies Group Ltd. was used. All measurements were taken at  $2^\circ\text{C}$  following a 10 min dark acclimation period, assuring that all photosystem II (PSII) reaction centers were fully oxidized and nonphotochemical quenching was relaxed.<sup>67</sup> Iterative algorithms for the induction and relaxation phases were applied to estimate minimum Chl *a* fluorescence ( $F_0$ ) and maximum Chl *a* fluorescence ( $F_m$ ).<sup>63</sup> The apparent maximum quantum yield of photosynthesis of PSII ( $F_v/F_m$ ) could then be calculated according to the equation

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m}$$

The measured  $F_v/F_m$  value of 0.32 at the station was in the range of values, reported for open ocean communities in the vicinity of the Western Antarctic Peninsula.<sup>68</sup> The high initial dFe value of 4.11 nM together with the initial complexing capacity for Fe of 3.31 nM hints toward a saturated Fe solubility at this station.

### Dissolved and particulate Fe concentrations

Samples for the determination of total dFe concentrations were taken in a clean room container. 100 mL from each treatment bottle were filtered on two trace metal clean filtration racks over a  $0.2 \mu\text{m}$  filter with a negative pressure of 200 mbar applied. The filtrate was used to sample for dFe. In between samplings all equipment involved was rinsed 30 minutes in 1 M HCl and MilliQ (18.2 M $\Omega$ .cm). To determine the Fe content of the FP material, the remainder of each FP addition used in the incubation experiment was rinsed with 9 mL of MilliQ water to reduce salt content. Afterward the particulate material was transferred to a 1.5 mL Eppendorf cup and dried at  $60^\circ\text{C}$  in an oven over night. The FP material's dry weight was determined. After that, the material was transferred in 30 mL PTFE vials and dissolved in a mixture of 5 mL subboiled  $\text{HNO}_3$  (distilled 65%, p.a., Merck) and 0.5 mL HF (ROTIPURAN Ultra 48%, Carl Roth) in a pressure digestion system (PicoTrace, DAS 30) at a temperature of  $180^\circ\text{C}$  over 16 hours. Followed by the addition of 1 mL of Milli-Q water. The volume of the FP extract was then evaporated on a  $140^\circ\text{C}$  hot plate and the evaporate was passed through a NaOH solution, which effectively neutralized it. 0.2 mL of subboiled  $\text{HNO}_3$  and 0.8 mL Milli-Q water was then added and the solution was heated to  $50^\circ\text{C}$  for 4 hours to resuspend the FP extract before it was transferred into 10 mL trace metal cleaned polypropylene (PP) vials. Finally,  $10 \mu\text{L}$  of Rh ( $1 \text{ mg L}^{-1}$ ) was added as an internal standard and the volume was brought up to 10 mL using Milli-Q water before subsequent analysis on a high resolution ICP-MS (Attom, Nu Instruments).

Back at AWI Bremerhaven prior to analysis, all seawater samples were acidified to pH 1.7 with sub-boiled  $\text{HNO}_3$  (distilled 65%  $\text{HNO}_3$ , pro analysis, Merck). dFe concentrations in seawater samples were analyzed via standard addition using a SeaFAST system (Elemental Scientific) coupled to an Element2 (Thermo Scientific) mass spectrometer. Therefore, each seawater samples was separated into 4 aliquots and spiked with commercially available ICP-MS single element standards (SCP Science  $1000 \text{ mg L}^{-1}$ ). Standards for external calibration were prepared from seawater spiked with commercially available ICP-MS single element standards (SCP Science;  $1000 \text{ mg L}^{-1}$ ). The SeaFAST system eliminates matrix components, such as the major ions in seawater (Na, Mg, and Cl) and preconcentrates the samples by a factor of 40. This procedure reduces possible interferences by the matrix and enables to analyze expected low concentrations of elements of interest.

The Nass-7 reference material was used to validate the quality of the analysis of trace elements in seawater at the beginning and end of a batch run. Because the element concentrations of the reference material were much higher than the concentrations expected in the seawater samples, the reference material was analyzed in a 1:10 dilution. The analysis of the Nass-7 reference material ( $n = 6$ ) showed good results. Certified  $351 \pm 26 \text{ ng Fe} \cdot \text{L}^{-1}$  measured  $365.97 \pm 14.15 \text{ ng Fe} \cdot \text{L}^{-1}$ .

### Fe-uptake assay

In order to assess the bioavailability of the dFe pool, 50 mL from each experimental treatment was filtered over an acid cleaned  $0.2 \mu\text{m}$  polycarbonate filter, removing all of the biotic and abiotic particles. A concentrate of a natural plankton community, collected at the same station from 25 m depth as the experimental water was then added into this filtrate. Briefly: 10 L of whole seawater was concentrated with gravity filtration onto a  $0.2 \mu\text{m}$  polycarbonate filter (WhatmanNucleoporeTrack-EtchMembrane, 90 mm) using an acid-cleaned AMICON 8400 filtration unit (Millipore). The AMICON was used to keep the plankton community in suspension by gently mixing it during the concentration step. A small aliquot of the concentrate was then added back to the filtrate from each treatment, ensuring that the final plankton biomass matched initial sampling conditions. This permitted the exposure of the same plankton community to each treatment without diluting the conditioned water. To each bottle containing the filtered treatment water and plankton concentrate,  $0.03 \text{ nmol L}^{-1}$  (final concentration)  $^{55}\text{FeCl}_3$  (150 Bq; Perkin Elmer, MA, USA) was added. After an incubation period of 24 hours at  $1^\circ\text{C}$  under  $30 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , the cells were size fractionated by filtering them onto 0.2 and  $2 \mu\text{m}$  filters, allowing for the determination of size class specific uptake of the tracer.<sup>69</sup> Each filter was rinsed 3 times with oxalate solution that was gravity-filtered for approx. 2 min between each rinsing step, followed by 3 rinses with natural  $0.2 \mu\text{m}$  filtered seawater.<sup>47</sup> Finally, each filter was collected in a scintillation vial, amended with 10 mL scintillation cocktail (Ultima Gold, Perkin Elmer) and mixed thoroughly (Vortex). Counts per minute were estimated for each sample on the shipboard scintillation counter (Tri-Carb2900TR). Counts per minute were

then converted into disintegrations per minute taking into account the radioactive decay and custom quench curves.  $^{55}\text{Fe}$  uptake was calculated taking into account the nominal  $^{55}\text{Fe}$  concentration and the total dFe concentration (background and added). To separately infer Fe bioavailability, the Fe uptake rates were then normalized to their respective total dFe concentrations and compared among treatments.

### Fe speciation

From the initially sampled seawater and at the end of both experiments, ligand concentrations and stability constants were determined by the competitive ligand exchange adsorptive cathodic stripping voltammetry method on the basis of the competitive ligand 2-(2-Thiazolylazo)-p-cresol (TAC, LOT 30549, Alfa Aesar) according to Croot and Johansson<sup>70</sup> on a Metrohm 663 VA stand. Defrosted samples were split into 10 mL subsamples and buffered with 5 mM EPPS to obtain a pH of 8.1. Variable amounts of a 0.75  $\mu\text{M}$  Fe standard were added in order to obtain concentrations between 0 and 7 nM added Fe. After 1–2 hours of equilibration, the TAC was added and sample analysis was conducted 24 hours later on a bioanalytical system consisting of an EC epsilon potentiostat and a controlled growth mercury electrode. As a working electrode, a medium sized mercury drop was used in conjunction with the static mercury drop electrode setting, an Ag/AgCl reference electrode and a platinum wire counter electrode. The ligand concentrations and stability constants, as well as complexing capacities were calculated using the Van den Berg/Ruzić linearization.

### QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical significance between samples were in all cases determined by the use of the computer program SigmaPlot (Systat Software). A One Way ANOVA was run for all factors and treatments (post hoc test: Fisher-LSD (Least Significant Difference) test, level of significance  $p = 0.05$ , pairwise comparison). Statistical significance was always tested within the same filtration mode (FSW or SWP). Relevant  $p$  values are given in the sections “FP as a source of Fe” and “Bioavailability of released Fe.” Figures 2 and 3 give means  $\pm$  SD. The respective  $n$  (number of values from replicate bottles) are given in the last two lines of the figure legends. Where  $n$  deviates from 3, in Figure 2 extreme values have been excluded based on a deviation from the mean by at least 5 standard deviations. These values were considered contaminations or machine errors. In Figure 3 values were excluded if a dFe value, necessary for the calculation of the dFe uptake had been excluded based on the principle of deviation from the mean by at least 5 SD.

Equation 1, calculating the carbon fixation potential through iron uptake of phytoplankton from salp or krill FP uses the following units: Carbon fixation potential [ $\text{g m}^{-2} \text{d}^{-1}$ ], Fe uptake [ $\mu\text{mol Fe L}^{-1} \text{d}^{-1}$ ], FP production [ $\mu\text{g FP C m}^{-2} \text{d}^{-1}$ ], molar mass of carbon = 12 [ $\text{g mol}^{-1}$ ], added FP carbon [ $\mu\text{g C L}^{-1} \text{d}^{-1}$ ] and Fe:C ratio of phytoplankton [ $\mu\text{mol Fe mol C}^{-1}$ ].