Elevated iron to nitrogen recycling by mesozooplankton in the Northeast Atlantic Ocean

Sari L. C. Giering,¹ Sebastian Steigenberger,¹ Eric P. Achterberg,¹ Richard Sanders,² and Daniel J. Mayor³

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[1] Low dissolved iron (DFe) concentrations limit primary production in most high-nutrient low-chlorophyll (HNLC) regions. Increased recycling of iron (Fe) relative to nitrogen (N) by zooplankton may help to sustain phytoplankton production in these conditions. We concurrently determined rates of DFe and ammonium (NH₄⁺) recycling by natural mesozooplankton communities in HNLC conditions of the Northeast Atlantic. NH₄⁺ excretion remained constant and ranged between 14.2–54.1 nmol NH₄⁺ mg dry weight⁻¹ h⁻¹. Fe recycling ranged between 6–138 pmol DFe mg dry weight⁻¹ h⁻¹ during the first hour and decreased thereafter, reflecting the transition from the loss of phytoplankton-derived Fe to basal DFe excretion. Mesozooplankton-driven nutrient recycling was estimated to support 6–59% and <1–13% of the respective phytoplankton requirements for DFe and N; DFe:N regeneration ratios were 5–26 times larger than those required by phytoplankton. Our data suggest that Fe recycling by grazing organisms has the potential to reduce the intensity of HNLC conditions. Citation: Giering, S. L. C., S. Steigenberger, E. P. Achterberg, R. Sanders, and D. J. Mayor (2012), Elevated iron to nitrogen recycling by mesozooplankton in the Northeast Atlantic Ocean, Geophys. Res. Lett., 39, L12608, doi:10.1029/2012GL051776.

1. Introduction

[2] Phytoplankton play a fundamental role in biogeochemical cycles, accounting for approximately 50% of global annual carbon fixation [Field et al., 1998]. Their growth in large parts of the oceans is limited by a shortage of nutrient elements such as nitrogen (N), phosphate and iron (Fe). ‘New production’, based on nutrients that are introduced into the photic zone of the oceans via upwelling and atmospheric deposition, typically represents a relatively minor fraction of total production. Phytoplankton growth plays an important role in biological carbon sequestration, export-Ing 36–100 g C m⁻² y⁻¹ [Sanders et al., 2005]. The modest residual nitrate pool in this region at the end of summer is suggestive of seasonal Fe limitation [Sanders et al., 2005], which has been confirmed using shipboard bioassay experiments [Nielsdottir et al., 2009].

2. Methods

[6] Release rates of DFe and ammonium (NH₄⁺) by mesozooplankton were investigated at five sites in July/August 2010 on board the RRS Discovery (cruise D354) in the Irminger Basin (Station R1), west and east of the Reykjanes Ridge (R3 and R4, respectively) and in the Iceland Basin (R5 and R6) (Figure 1 and Table 1). This cruise targeted the post spring bloom period when earlier observations suggested that HNLC conditions would be well established.

[7] All equipment was acid cleaned with 10% hydrochloric acid before use. Ambient seawater was collected using trace metal clean procedures and filtered using a 0.2-μm pore size filter capsule (Sartobran P300, Sartorius). Mesozooplankton were collected using a 200-μm, 1-m diameter WP2 net fitted with a non-filtering cod-end, hauled vertically from 20–30 m. All animals were immediately washed and briefly held in
0.2-μm filtered seawater (Table 1). Mesozooplankton were then transferred into 10 L of 0.2-μm filtered seawater and incubated for ≤5 h in a class 100 laminar flow hood in darkness at the ambient sea surface temperature of 12°C. No damaged or dead animals were observed during our incubation. However, we cannot exclude the possibility that some animals had been damaged and contributed to DFe and NH₄ release via the leakage of intracellular fluids. The densities of incubated mesozooplankton in the incubations ranged between 1.1–124.0 mg DW L⁻¹ (Table 2). There was no correlation between density and individual NH₄ excretion rates (R² = 0.04, p = 0.75), in agreement with previous work [Huntley and Nordhausen, 1995]. We are thus confident that crowding did not significantly affect release rates. [s] Samples for NH₄ (10 mL) analysis were collected before the introduction of experimental mesozooplankton (t₀) and thereafter 18 times at intervals of increasing length (5–60 min) with the last sample taken after 300 min. Samples for DFe (15 mL) analysis were collected at t₀ and 20, 40, 60, 120, 180 and 260 min after transfer. Samples were obtained using a peristaltic pump fitted with silicone tubing. No animals were removed during the sampling procedure. The samples for DFe were filtered using 0.2-μm membrane filters (polycarbonate 25 mm diameter, Whatman Nuclepore). All experiments were conducted using trace metal clean protocols following Achterberg et al. [2001]. [9] In parallel, a control incubation of filtered seawater was undertaken in which NH₄ concentrations were monitored over time. A small increase in NH₄ concentration was observed (3.3 ± 2.8% of the concentration increase observed in the experimental incubations) and our NH₄ release data was therefore corrected to reflect this. A similar suite of control experiments was not carried out for DFe release as it is well established that DFe concentrations in filtered seawater decrease over time [Fischer et al., 2007] as DFe rapidly adsorbs to the walls of the incubation container. Our estimates of Fe release rates therefore represent lower limits for this biogeochemically important process. However, in future it would clearly be preferable to undertake a control. [10] Samples for DFe were acidified (pH ~ 2) with nitric acid (Romil UpA) and analyzed on board following Obata et al. [1993]. The detection limit for this technique was 20 pM, with a precision of <5%. NH₄ in the release experiments was determined fluorometrically on ship using orthophthalate (OPA) following Holmes et al. [1999]. Samples for chlorophyll analysis (100–200 mL) were filtered using GF/F filters (Whatman) and then extracted in 90% acetone for 24 h in the dark before analysis with a fluorometer (TD70; Turner Designs) with Welschmeyer filters. [11] Mesozooplankton from each experiment were preserved in 4% saline formaldehyde. On shore, preserved samples were counted, identified, and analysed for dry weight (DW). Copepods dominated at all stations, with large calanoids constituting 50.1–98.7% of the total biomass (Figure S1 in the auxiliary material). Biomass values were corrected for a DW loss of 40% of fresh weight due to formaldehyde preservation (37–43% in mixed zooplankton [Giguère et al., 1989]). [12] The total volume of incubation water was reduced by sequential subsampling. The total quantity of NH₄ or DFe (Fe₄, nmol mg DW⁻¹) released by mesozooplankton per mg DW of biomass until time t was thus calculated as

\[
Fe = Fe_{t-1} + V_{t-1} \times (c_t - c_{t-1}) \times B_{incub}^{-1}
\]

where \(V\) is the volume (L) of incubation water, \(c\) is the concentration (nM), and \(B_{incub}\) is the total mesozooplankton biomass (mg DW) in the incubation container. The increase in NH₄ and DFe over time was fitted using linear regression and an exponential model with asymptote, respectively. The initial release rate (nmol mg DW⁻¹ h⁻¹) was estimated from the models as total release after one hour. Basal DFe release rate was estimated as the rate 3 h after onset of starvation. Hourly release rates were extrapolated to daily rates using 24 h for NH₄ and 7 h initial release plus 17 h basal release for DFe. Values are presented ±s.d. All statistics were carried out in the R programming environment v. 2.10.0 [R Development Core Team, 2009].

3. Results and Discussion

[13] This study presents the first parallel NH₄ and DFe release rates by mesozooplankton communities in the North Atlantic Ocean. The observed NH₄ excretion rates of 14.2–
54.1 nmol NH$_4^+$ mg DW$^{-1}$ h$^{-1}$ (Table 2) are in good agreement with previous estimates derived from boreal copepods (1.5–50 nmol NH$_4^+$ mg DW$^{-1}$ h$^{-1}$ [Ikeda et al., 2001]) and from a mixed zooplankton community (0.1–65.2 nmol NH$_4^+$ mg DW$^{-1}$ h$^{-1}$ [Ikeda et al., 2000]).

Our estimates of basal and digestion-derived release rates of DFe varied between <0.01–12 and 6–138 pmol DFe mg DW$^{-1}$ h$^{-1}$, respectively (Table 2). Daily release estimates ranged from 0.04–1.18 nmol DFe mg DW$^{-1}$ d$^{-1}$, the same order of magnitude as reported for Antarctic krill, Euphausia superba (0.04–0.17 nmol DFe mg DW$^{-1}$ d$^{-1}$ [Schmidt et al., 2011]). Sarthou et al. (2008) presented shipboard measurements of DFe recycling by copepods incubated in seawater containing radiolabelled phytoplankton. Assuming that regenerated Fe equals the sum of Fe uptake and the increase in DFe at the end of their incubation, copepods regenerated 0.004–0.019 nmol DFe mg DW$^{-1}$ d$^{-1}$ [Sarthou et al., 2008]. These values are an order of magnitude smaller than our estimates, possibly reflecting that their budget calculations do not account for continuous recycling. Tovar-Sanchez et al. (2007) observed release rates of 0.5–17 nmol Fe mg DW$^{-1}$ d$^{-1}$ by krill in the Southern Ocean. These rates included the production of both DFe and acid-leachable particulate Fe, possibly explaining why they are more than an order of magnitude greater than our observations and those of Schmidt et al. (2011).

### 3.1. Controls Over NH$_4^+$ and DFe Release

The increase of DFe and NH$_4^+$ concentrations in the incubations over time followed different trends indicating fundamentally different release pathways. The near constant increase in NH$_4^+$ concentrations during our incubations (Figure 2a) reflects the accumulation of excretory waste products; crustacean zooplankton catabolise nitrogenous products and thus excrete NH$_4^+$ even during starvation [Mayor et al., 2011]. Previous starvation incubations studies, conducted over several days, have reported NH$_4^+$ excretion rates to decline over time [Atkinson and Whitehouse, 2001]. This effect was not apparent in our short (<5 h) incubation experiments; mesozooplankton excretion rates have been shown to be fairly constant during the initial hours of starvation [Huntley and Nordhausen, 1995].

In contrast, DFe concentrations increased rapidly during the first half hour, and the release rate slowed down thereafter and became negligible after ~2 h (Figure 2b). This likely reflects the initial, rapid increase of DFe being caused by the elimination of phytoplankton-derived Fe from zooplankton guts. Previous work has demonstrated that an increase of DFe concentrations during zooplankton grazing originates from phytoplankton cells [Hutchins et al., 1995], with the rate of increase being consistent with the removal of chlorophyll or radiolabelled Fe from zooplankton guts [Wang and Det, 2001]. We suggest that the apparent decline in the rate at which DFe accumulated in our incubations reflects the transition from digestion-derived DFe release towards basal DFe excretion.

The observed biomass-specific release rates of NH$_4^+$ and DFe differed considerably between stations. A previous study suggested a positive relationship between Fe release by E. superba and ambient chlorophyll concentrations [Tovar-Sanchez et al., 2007]. We did not find a relationship between ambient chlorophyll concentrations and the release of either NH$_4^+$ or DFe (R$^2$ < 0.01, p > 0.85 in both cases), reflecting the relatively constant concentrations of chlorophyll across all stations (Table 1). Proximity to hydrothermal
observed that most DFe was released during the initial 2 h of our incubation. As average sinking speed of copepod fecal pellets is <10 m h\(^{-1}\) (review by Turner [2002]), DFe was likely released <20 m below the depth of egestion and thus available to of phytoplankton.

[20] Assuming the products released by mesozooplankton were bioavailable and representative of the region, mesozooplankton-derived NH\(_3\) therefore had the potential to support 3.0 ± 5.5% (0.2–12.9%) of the N uptake by primary production (Table 2). This is in good agreement with the estimate of 3.9% in the North Atlantic region between 60–80\(^\circ\)N [Hernández-León et al., 2008], Fe recycling appeared to be more important, with the potential to support 22.4 ± 24.5% (6.3–58.7%) of the daily requirements for primary production across the region of study (Table 2). Sources of uncertainty for the latter estimate are potential variability of community composition and thus phytoplankton Fe:C ratios. The community showed little variation across the study sites and was dominated by small (<5 \(\mu m\)) flagellates (A. J. Poulton, unpublished data, 2012). Using the above mentioned Fe:C ratios of 2 and 10 \(\mu mol\) mol\(^{-1}\) as upper and lower limit, relative support ranged from 10 ± 11% to 48 ± 53%.

[21] The utilization of Fe sources by phytoplankton depends on factors such as lability of DFe and species of Fe-binding ligands [e.g., Hutchins et al., 1999], hence not all released DFe may contribute to primary production. Conversely, there is evidence that a significant fraction of particulate Fe may become available to phytoplankton via, e.g., ligand-assisted dissolution and photochemical processes [Lippiatt et al., 2010, and references herein]; a possible source of Fe that we have not accounted for in this study.

[22] It is noteworthy that the site where mesozooplankton-mediated NH\(_3\) and DFe recycling was most important also showed the highest mesozooplankton abundance. This suggests that areas experiencing heavy grazing pressure due to high mesozooplankton abundance, as being the case in the Irminger Basin [Gislason et al., 2008], are sites of extensive nutrient recycling.

[23] The DFe:N regeneration ratios ranged between 129–745 \(\mu mol\) mol\(^{-1}\) and were 5–26 times larger than the calculated Fe:N ratios in phytoplankton (42.4 \(\mu mol\) mol\(^{-1}\)). This regeneration ratio is possibly a slight overestimate as it does not include the release of urea and amines, although their release is reported to occur in relatively small amounts (14–26% of total N excreted) [Miller and Roman, 2008]. The high DFe:N regeneration ratio is consistent with copepod physiology: copepods absorb 60–79% of ingested N [Vincent et al., 2007; Mayor et al., 2011] and only 5–16% of ingested Fe [Wang and Dei, 2001]. This suggests that mesozooplankton recycle Fe into the dissolved phase much faster than N, a decoupling that results in considerably more Fe being available to support primary production than would be predicted from a simple consideration of Fe:N ratios in the upper ocean and an estimate of Fe supply.

[24] Relatively high Fe:N ratios observed in sinking particulate matter [Frew et al., 2006], including copepod fecal pellets, suggest that mesozooplankton defecation drives pelagic ecosystems towards Fe limitation. The notion that mesozooplankton rapidly recycle, and thus retain DFe in surface waters is at odds with this interpretation. However, it is strongly supported by the observation that the copepods release \(\sim 50\%\) of ingested Fe into the dissolved phase and only \(\sim 30\%\) as particulate matter [Schmidt et al., 1999]. Our

Figure 3. Relationship between average body mass and release of NH\(_3\) (circles) and DFe (triangles) during the first hour at stations R1–R6. Linear regressions: \(\log(N) = -0.97 \cdot \log(DW) + 1.34\) (\(p = 0.01\), \(R^2 = 0.91\)) and \(\log(DFe) = -2.18 \cdot \log(DW) (p = 0.03, R^2 = 0.94)\).
study clearly highlights the need for a better understanding of the role of mesozooplankton in nutrient recycling, particularly with regards to the relative partitioning of Fe and N into the dissolved and particulate phases; this has important implications for the role that Fe plays relative to N in regulating marine primary production.

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References


