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Abstract

The impact of copepod grazing on Fe regeneration was investigated in a naturally iron fertilised area during KEOPS (Kerguelen Ocean and Plateau compared Study, Jan.-Feb. 2005). ^{55}Fe labelled natural plankton assemblages (< 200 µm) were offered as food to copepod predators sampled in the field (*Calanus propinquus*, *Rhincalanus gigas*, *Metridia lucens* and *Oithona frigida*). Diatoms (*Eucampia antarctica*, *Corethron inerme* and *Navicula* spp.) constituted the bulk of the protists whereas microzooplankton (i.e. ciliates and dinoflagellates) were in very low abundance. Copepod grazing on phytoplankton ranged from 0.3 to 2.6 µgC ind^{-1} d^{-1} and reflected low utilisation of the food stocks (1-10% of total Chlorophyll *a* d^{-1}) and low daily rations (0.2-3.3 % body C d^{-1}). Copepod grazing resulted in a 1.7-2.3-fold increase in Fe regeneration. Fe speciation determined by extraction onto C18 columns showed that less than 1% of the regenerated Fe was complexed with hydrophobic organic ligands. This suggests that Fe was regenerated as inorganic species and/or bound to freely soluble organic ligands. The biogenic Fe budget established from our study and literature based data indicates that most of the primary production is recycled through the detrital pool, which represents the largest Fe pool (49% of total Fe). Our iron budget further indicates that mesozooplankton and diatoms represent the dominant Fe biomasses above the Kerguelen plateau. The rate of Fe regeneration accounts for half of the Fe demand, strengthening the need for new Fe sources to sustain the massive phytoplankton bloom above the Kerguelen plateau.

Key words: Iron, regeneration, food web, copepod, grazing, phytoplankton bloom.

Regional index terms: Southern Ocean, Kerguelen Island, Kerguelen plateau

1. Introduction

Our knowledge of the relationships linking phytoplankton to iron (Fe) availability has vastly improved over the last decade. The biological availability of Fe has indeed been shown to limit primary production in up to 50% of oceanic waters (Moore et al., 2002). Mesoscale artificial iron infusions in high-nutrient, low-chlorophyll (HNLC) regions, such as the subarctic Pacific (Tsuda et al., 2003; Boyd et al., 2004), the Equatorial Pacific (Coale et al., 1996), and the Southern Ocean (Boyd et al., 2000; Coale et al., 2004), have highlighted the strong coupling between Fe and carbon (C) biogeochemical cycles. Fe supply controls many aspects of algal physiology (Boyd, 2002): it can alter phytoplankton productivity and, when high, induce a shift in species composition towards large diatoms (Cavender-Bares et al., 1999; de Baar et al., 2005).

Fe exists under various chemical forms in the ocean rendering the determination of its concentration, its bioavailability, and thus its overall importance within a given ecosystem difficult (Sunda, 2001). Between the two oxidation states of Fe in oxic waters (Fe(II) and Fe(III)), Fe(III) is the thermodynamically favoured form although weakly soluble (Byrne and Kester, 1976). Additionally, more than 90% of total dissolved Fe is bound to organic ligands (Gledhill and van den Berg, 1994; Rue and Bruland, 1995), which can increase the Fe solubility in seawater (Kuma et al., 1996), but it can also dramatically reduce the inorganic Fe fraction (i.e. ionic Fe and Fe-hydroxides). Whether these organic ligands increase or decrease the availability of Fe for individual phytoplankton species is still a question of debate (Hutchins et al., 1999a, 1999b; Maldonado and Price, 1999). Hutchins et al. (1999b), for instance, report that prokaryotic and eukaryotic phytoplankton have different abilities to obtain iron bound to siderophores and porphyrin complexes, suggesting a competitive advantage for those algal species that can obtain iron from specific ligands. Particulate iron can also be transformed into dissolved or bioavailable iron by ligand promoted dissolution

(Croot and Johanson, 2000) and by interactions with the planktonic food web (Hutchins et al., 1993; Barbeau et al., 1996; Poorvin et al., 2004). A number of studies have highlighted the role of protist grazers in rapidly regenerating Fe from ingested prey into the dissolved phase (Hutchins et al., 1993; Hutchins and Bruland, 1994; Barbeau et al., 1996; Chase and Price, 1997; Barbeau et al., 2001; Xu and Wang, 2003). Assimilation of Fe by bacteria and subsequent viral induced lysis may also play a critical role in Fe recycling (Gobler et al., 1997; Maranger et al., 1998; Poorvin et al., 2004; Mioni et al., 2005). Virus-mediated Fe regeneration can support as much as 90% of the primary production in recycling based HNLC systems (Poorvin et al. 2004). In HNLC regions, Fe regeneration could thus be a key parameter in ecosystem productivity (Landry et al., 1997), since new sources of Fe are suggested to support only 4-20% of the total primary production (Hutchins et al., 1995). The quantification of Fe within the various biogenic Fe pools as well as the magnitude of the fluxes through the planktonic food web appear to be absolute prerequisites to improve our understanding of the biologically mediated Fe cycle in the ocean. However, to our knowledge, these Fe pools and fluxes were only quantified twice using field measurements in the open ocean; the first time in artificially Fe fertilised HNLC waters (Bowie et al., 2001) and the second time in unperturbed HNLC waters (Strzepek et al., 2005).

Within the Southern Ocean, the Kerguelen plateau offers an opportunity to study natural iron fertilisation and its impact on the planktonic food web structure and functioning. This area is characterized by recurrent and persistent (~ 3 months) phytoplankton blooms during austral summer (November to February) within the HNLC Southern Ocean (Blain et al., this issue a). During the KEOPS (Kerguelen Ocean and Plateau Study)-cruise, this bloom event was dominated by large diatoms (Armand et al., this issue) and accompanied by high mesozooplankton stocks (Carlotti et al., this issue). This suggests that zooplankton grazing had the potential to control the accumulation of the Kerguelen bloom as observed previously

for iron-induced blooms (Rollwagen Bollens and Landry, 2000; Zeldis, 2001). It further suggests that mesozooplankton represented a dominant pool of biogenic Fe and that grazing activity possibly had important consequences for the regeneration of Fe.

Our major objectives during the KEOPS program were 1) to shed new light on the impact of copepod grazing on Fe regeneration, 2) to assess whether natural copepod assemblages inhabiting the high Chl *a* waters (~ 2.0 µg L^{-1}) of the Kerguelen plateau were efficient grazers of the phytoplankton bloom and 3) to determine whether copepod grazing was sufficient to account for their daily needs of carbon.

Feeding of dominant copepods on natural prey assemblages (< 200 µm) was investigated via bottle incubation experiments during the intense bloom period and its decline over time. Focus on Fe regeneration was possible due to ^{55}Fe labelled prey assemblages and simultaneous measurements of Fe stocks in the dissolved and particulate phases, with special emphasis on organic Fe speciation through C18 column extraction. Based on our determination of the main biogenic Fe pools and fluxes we constructed a ‘‘steady state’’ Fe budget for the planktonic food web above the Kerguelen plateau.

2. Materials and methods

2.1. Study area

The study area was located South-East of the Kerguelen Islands in the Indian sector of the Southern Ocean where phytoplankton blooms are observed each summer. Experiments were carried out during the KEOPS cruise from 19 January to 13 February 2005 aboard the R.V. Marion Dufresne II (TAAF/ IPEV). Experiments were performed at Station A3 (50.38°S/72.05°E) located above the Kerguelen plateau in the core of the bloom and defined as the reference station for the plateau (Blain et al., this issue a). Data presented herein were acquired during the first (A3-1, January 19), fourth (A3-4, February 2) and last visit (A3-5,

February 12) to A3, corresponding to the bloom maximum and its subsequent decline over time.

2.2. Prey collection and Fe labelling

All sample manipulations were carried out in a class 100 laminar flow bench. All sampling and incubation bottles were thoroughly acid-cleaned before use with three successive baths of HCl 10%, 1% and 0.1%. The two last baths were done with suprapur® HCl (MERCK). Natural seawater containing prey assemblages was collected in the surface mixed layer (~ 30 m) using 12-L Go-Flo bottles, mounted on a poly-urethane coated frame (Timmermans et al., this issue) attached to a 2500-m Kevlar wire. Bottles were triggered by a Teflon messenger. Our experimental protocol is detailed on Fig. 1. Seawater was immediately pre-screened on a 200 µm Polyester mesh sieve by slow reverse filtration in a cooled laboratory (3-4°C) and dispensed in 10 L polycarbonate bottles. This pre-screening step avoided the introduction of large grazers within the prey assemblages.

Prey preparation consisted in adding to the < 200 µm prey assemblages either ^{55}Fe ($FeCl_3$ in 10 mM HCl, 1.7 nM final concentration, 63 kBq L^{-1} final activity) for radioactive experiments (referred as RadEX thereafter) or ^{56}Fe ($FeCl_3$ in 10 mM HCl, 1.7 nM final concentration) for non radioactive experiments (NRadEX thereafter). This permitted to start both experiments in the same Fe conditions. Major nutrients, $Si(OH)_4$, NO_3^- and PO_4^{3-}, were simultaneously added to give final concentrations of 19 µM, 50 µM, and 3.2 µM, respectively, thus avoiding prey growth limitation. Nutrient-enrichment stock solutions were purified of trace metals using Chelex-100 ion exchange resin (Bio-Rad Laboratories), according to Price et al. (1988/1989). Prey assemblages were incubated on deck for 29-40 hours under 50 % incident irradiance (Blain et al., 2004) representing the intensity of photosynthetic active radiation (PAR) at 6 to 9 m (mixed layer depth: 51-84 m ; see Christaki et al., this issue, Table 4). Although

incubation time varied between each experiment, prey assemblages were experiencing similar light and dark conditions with light period representing 70, 75 and 82 % of the total incubation duration for A3-5, A3-4 and A3-1 experiments, respectively. Thus, the impact of incubation conditions on Fe stock and uptake rates were assumed to be negligible. A continuous running seawater system supplied water from the sea surface, allowing the maintenance of *in situ* surface temperature as well as the perpetual motion of the free floating bottles which prevented any settling of material.

After the prey enrichment phase (RadEX), triplicate 100-mL aliquots were filtered through 0.2 µm porosity Nuclepore membranes to recover total particulate iron (TPFe*). For the determination of the intracellular Fe (IntraFe*), additional 100 mL were filtered through 0.2 µm porosity nuclepore membranes and subsequently rinsed with the trace metal clean oxalate reactant to remove extracellular Fe (Tovar-Sanchez et al., 2003). Total dissolved iron concentrations in the incubations were calculated as the sum of the radiolabelled iron added (1.7 nM) and *in situ* dissolved iron concentrations (< 0.2 µm filtered seawater) measured on board by Flow Injection Analysis with chemiluminescence detection (Blain et al., this issue b). TPFe* and IntraFe* were calculated by the following equations:

$$\text{TPFe*} = A \times \text{dpm total } {}^{55}\text{Fe on filter} \quad (1)$$

$$\text{IntraFe*} = A \times \text{dpm intracellular } {}^{55}\text{Fe on filter} \quad (2)$$

where

$$A = (\text{pmol } {}^{55}\text{Fe added} + \text{pmol } \textit{in situ} \text{ Fe}) / (\text{dpm } {}^{55}\text{Fe added}) \quad (3)$$

Fe uptake rates (total and intracellular; pM d^{-1}) were calculated using the ratio between particulate Fe (TPFe* and IntraFe*, respectively) and incubation duration.

The remaining volume of prey assemblages (~ 9 L) was then gently filtered onto 0.2 µm polycarbonate membranes (Nuclepore). Before running dry, filters were rinsed with the oxalate reagent (Tovar-Sanchez et al., 2003). Prey assemblages were then resuspended in 0.2 µm filtered seawater collected at 30 m as described above.

2.3. Predator collection and grazing experiments.

Copepods were caught on the day of the grazing experiments. Short oblique hauls (< 1 m s^{-1}, mean duration less than 15 min) were performed through the upper 200 m of the water column using a 330 µm WP-2 Bongo net fitted with a 1-L filtering cod-end. The samples were immediately diluted in a 30 L isotherm tank with surface seawater, and transferred to the cooled laboratory for sorting. The choice of grazers used in the various experiments depended on their *in situ* numerical abundance and on the availability of sufficient numbers of healthy individuals. Fifty copepods were sorted using a dissecting microscope and then acclimated for 5 h in 2.4-l 0.2-µm filtered seawater in the dark. This acclimation of copepods aimed to reduce stress, permitted omission of injured individuals and reduced the "hunger effect" (Runge, 1980).

After the acclimation phase, copepods were carefully collected over a 200 µm mesh sieve, re-sorted under a dissecting microscope to eliminate dead or injured individuals and transferred to 2.4 L polycarbonate experimental bottles filled to the rim with the prey suspension. Copepod assemblages used for both types of grazing experiments (i.e. RadEX and NRaDEX) were composed of identical mixtures of CIV to CVI stages of the species *Calanus propinquus, Metridia lucens, Oithona frigida* and *Rhincalanus* gigas (Table 1). Twenty to thirty individuals were added per experimental bottle according to their dominance within the plankton catches. The RadEX and NradEx were both performed in triplicate. Control treatments consisted of resuspended prey in 0.2 µm filtered seawater and were used to correct for prey assemblages growth rates (see below) and to correct Fe regeneration for processes other than copepod grazing in our incubation bottles (e.g. phytoplankton release, viral lysis, bacterial degradation of dying phytoplankton, microzooplankton grazing). All bottles (controls and experimentals) were topped-up and screw-capped with plastic film to insure that

prey assemblages remained undisturbed (Gifford, 1985). They were then placed in the on-deck incubator (see section 2.2) for 24 h.

2.4. Sampling and analyses

2.4.1. Phytoplankton biomass, composition and F_v / F_m

Phytoplankton biomass was assessed by Chl *a* determinations, at the end of the prey labelling period as well as at the beginning (T_0) and end (T_f) of the grazing experiments (see Fig. 1). Triplicate 100-200 ml seawater samples were filtered on GF/F glass fibre filters (Whatman) and immediately frozen (-20°C). Chlorophyll pigments were extracted in 100% methanol (30 min; 4°C) and quantified according to Raimbault et al. (1988). Pigment supernatants were analysed onboard using a Turner Design fluorometer (TD Fluorometer 10.005R).

In order to define the biological context of our experiments and to determine whether < 200 µm prey assemblages used in our experiments were similar to those sampled in the field, prey enumeration was carried out on T_0 samples of the grazing experiments (Utermöhl, 1958). For prey enumeration, 130-150 ml sub-samples from the prey suspension were collected in triplicates and fixed with acid lugol solution (2% final concentration). One hundred millilitre sub-samples were settled in Hydro-bios counting chambers and settled slides (settling time 24 h) observed by reverse microscopy under phase contrast illumination (Nikon; magnification X 200 and X 400). Cells were counted on one-half of the whole slide resulting in a mean of 780 ± 220 cells per slide (minimum 234 cells, maximum 1730 cells). Diatoms were identified when possible to the species level and measured (length and width). Most of the dinoflagellates counted (*Gyrodinium* sp. and *Protoperidinium* sp.) appeared to be heterotrophic (HDino hereafter) and were treated separately from diatoms. Phytoplankton carbon biomasses were calculated by multiplying cell abundance by cell carbon biomass (pgC

cell^{-1}) given for each identified taxa by Cornet-Barthaux et al. (subm.). No significant difference in cell abundance was observed between triplicate sub-samples within controls (Kruskal Wallis test, $p<0.05$).

A PAM fluorometer (Pulse Amplitude Modulated-CONTROL Universal Control Unit, WATER-mode, Walz, Germany) was used to determine the physiological states of the prey communities (Geider and LaRoche, 1994; Behrenfeld and Kolber, 1999). Parameters F_0 (Chl *a* autofluorescence), F_m (maximum Chl *a* fluorescence) and F_v/F_m (photochemical quantum efficiency, where $F_v=F_m-F_0$) were determined on 20 ml sub-samples at T_0 and T_f. Samples were stored in the dark for 15 min prior to the actual PAM measurements.

2.4.2. Copepods and grazing measurements

At the end of grazing experiment (T_f), copepods from NRadEX were collected on a 200 µm mesh sieve, checked for mortality and preserved in buffered formaldehyde (4% final concentration). Back in the home laboratory, individuals were measured (total length, mm), identified and staged according to Razouls (1994) under a dissecting microscope.

Apparent prey growth rates (µ, d^{-1}) and copepod grazing rates (g, d^{-1}) were calculated from Chl *a* removal according to Frost (1972). Prey growth rates were obtained from:

$$\mu = \ln (C_{fc}/C_0) / (T_f - T_0) \quad (4)$$

where C_0 and C_{fc} are the Chl *a* concentrations in controls at the beginning (T_0) and end of the incubation (T_f). Copepod grazing rates were obtained by the equation:

$$g = [(\ln C_0 - \ln C_{fe}) / (T_f - T_0)] + \mu \quad (5)$$

where C_{fe} is the Chl *a* concentrations in experimental bottles at the end of incubation. The clearance rate F (mL copepod^{-1} d^{-1}) defined as "the volume swept clear" by copepods (Frost, 1972) was calculated by the following equation:

$$F = Vg / N \quad (6)$$

where V(mL) is the volume of the incubation bottle and N, the number of copepods in the bottle (Table 1).

Individual ingestion rates (I) were calculated by multiplying positive single clearance rate by mean Chl *a* concentration <C> in experimental bottles (Frost, 1972). Grazing rates were calculated only when Chl *a* concentration in experimental bottles was significantly smaller than in controls at T_f (Wilcoxon Mann Whitney test, WMW hereafter, with significant set either at $P<0.05$ or <0.01). Ingestion rates were converted to carbon using the *in situ* measured particulate organic carbon (POC):Chl *a* ratio at the depth of sample collection (Garcia, pers. com.). Statistical comparisons of grazing rates (clearance and ingestion rates) were made using non-parametric Kruskal-Wallis tests (KW hereafter with significant set at either at $P<0.05$ or $P<0.01$) because of highly heterogeneous variances in the data sets.

Copepod body carbon content (in μgC ind^{-1}) was estimated from species-specific length-weight relationships from the literature, assuming that carbon content is 43.6% of dry weight for *C. propinquus* (Ikeda, 1988), 45.3% for *M. lucens* (Ikeda, 1988), 47.3% for *R. gigas* (Conover and Huntley, 1991) and 44.7% for *Oithona* spp. (Båmstedt, 1986). The equations employed came from Godlewska (1989) for *R. gigas* and *C. propinquus*, from Hay et al. (1991) for *M. lucens* and from Uye and Sano (1998) for O*ithona* sp.. Copepod daily rations (% body C d^{-1}) were estimated from the ratio of ingestion rate to copepod body carbon content.

Copepod ingestion rates were compared to estimates of basic respiratory requirements for copepods based on empirical relationships published in the literature (Mayzaud et al., 2002). Oxygen values were converted into respiratory carbon (μgC $copepod^{-1}$ d^{-1}) after calculating respiration rates (μL O_2 ind^{-1} d^{-1}) and assuming a respiratory quotient of 0.9 (Mayzaud et al., 2002). These values were compared to carbon ingestion rates derived from each of the incubation experiments. Grazing pressures (% standing stock d^{-1}, % s.s. d^{-1} hereafter) were

obtained by dividing copepod ingestion rates (expressed in ngChl *a* L^{-1} d^{-1}) by Chl *a* standing stocks.

2.4.3 Heterotrophic bacteria

For bacterial abundance, a 1 ml sub-sample from each incubation bottle (at T_0 and T_f) was fixed with 2% formaldehyde (final concentration), quick-frozen in liquid nitrogen and stored at -80 °C until flow cytometric analysis according to Lebaron et al. (1998; see Obernosterer et al., this issue, for more details).

2.4.4 Fe partitioning

Following the incubation (T_f), ^{55}Fe labelled copepods from RadEX (copepods*) were checked for mortality, recovered on a 200 µm mesh sieve, and gently rinsed with < 0.2 µm filtered seawater before being transferred to 20-ml scintillation vials.

For the determination of Fe in the different pools, triplicate 400-ml subsamples were then filtered at T_0 and T_f through 0.2 µm Nuclepore membranes and treated as described above for TPFe* and IntraFe* measurements. Six-millilitres of the < 0.2 µm filtrate were collected for the determination of total dissolved regenerated iron (TDFe*). For Fe speciation, extractions were performed onto Sep Pak C18 cartridges (Waters). The C18 column is a hydrophobic reverse-phase column and collects mainly hydrophobic, lypophilic and nonpolar organic iron complexes (HphOFe*, Oztürk et al., 2002). It is commonly used to collect organic-metal complexes (Watanabe et al., 1981; Donat et al., 1986; Haraldsson et al., 1993) and serves as a pre-concentration column for iron after complexation with added organic ligands in seawater and freshwater (King et al., 1991). Prior to use, the C18 cartridges were cleaned with 25 mL of methanol-water (1:1) and successively rinsed with (i) 100 mL Milli-Q water, (ii) 25 mL HCl (suprapur®, MERCK, 1M) and (iii) with 100 mL of Milli-Q water (twice). Four hundred

millilitres of the < 0.2 µm filtered samples were passed through the column and eluted back with 6 mL of methanol.

Copepods*, TDFe* and HphOFe* were calculated by the following equations:

$$\text{Copepods*} = A \times \text{dpm } {}^{55}\text{Fe of copepods} \quad (7)$$

$$\text{TDFe*} = A \times \text{dpm } {}^{55}\text{Fe in the filtrate} \quad (8)$$

$$\text{HphOFe*} = A \times \text{dpm } {}^{55}\text{Fe extracted on C18 column} \quad (9)$$

where A is defined in eq. 3.

Fe regeneration rates (pM d^{-1}) were calculated using the ratio between TDFe* (eq. 8) and grazing experiment duration.

All the samples for ^{55}Fe analyses were transferred to 20 ml scintillation vials containing Ultima Gold AB scintillation cocktail (Packard). The radioactivity of the ^{55}Fe samples was counted back at the laboratory using a Wallac 1414 Winspectral DSA analyzer after correction for radioactive decay and quenching. Counting times were adjusted to enable propagated counting errors less than 5%.

3. Results

3.1. Prey assemblage composition and carbon biomass distribution

In terms of abundance, the relative contribution of the different prey (autotrophs and HDino) to the total prey pool was similar between experiments A3-4 and A3-5 (Fig. 2). *Eucampia antarctica* always represented the main contributor to total prey cell abundance (85-92% Fig. 2A). The cylindrical diatom *Corethron inerme* followed in decreasing order of importance constituting 2-7% of total abundance. Other diatoms such as *Navicula* spp., *Pseudonitzschia* sp., *Fragilariopsis kerguelensis* and *Leptocylindrus danicus* sporadically contributed to the prey pool and accounted for 2-9% relative abundance. The same observation holds for the abundance of HDino that never exceeded 200 cell L^{-1} i.e. < 2% of total prey abundance.

Biomass distribution followed a distinct pattern (Fig. 2B). Either due to their numerical dominance (e.g. *E. antarctica*) or large size (e.g. *C. inerme, Odontella weissflogii*; Cornet-Barthaux et al., subm.), diatoms constituted the bulk of carbon biomass, representing ~ 78-98% of the total prey carbon biomass. Large HDino (e.g. *Ceratium* sp. and *Gonyaulax* sp.) also contributed to total carbon biomass, particularly at A3-5, reaching a relative contribution of up to 22%.

Ciliates were rare in the 100-200 ml samples, reflecting the low *in situ* ciliate abundance at 30 m (296, 158 and 160 cells L^{-1} at A3-1, A3-4 and A3-5, respectively). Moreover, screening, filtration and recovering of the organisms from the filter is known to damage soft cells such as nanoflagellates and ciliates (Gifford, 1985; Gifford and Caron, 2000).

The abundance of heterotrophic bacteria in our incubation bottles varied between 0.6-1.6 $x10^8$ cells L^{-1}, thus representing 9-25% of the *in situ* abundance in surface waters above the Kerguelen plateau (Christaki et al., this issue).

3.2. Copepod grazing

In two out of the five experiments (A3-1 RadEX and A3-5 NradEX), a clear balance between prey growth rates (0.09 ± 0.01 d^{-1} to 0.31 ± 0.13 d^{-1}, respectively) and copepod grazing on phytoplankton (0.09 ± 0.01 d^{-1} to 0.21 ± 0.07 d^{-1}) was observed (Table 2). By contrast, for both A3-4 experiments and the A3-5 RadEX no significant growth of phytoplankton prey was detectable over the incubation period (WMW test, $P>0.05$).

For the A3-1 and A3-4 experiments, Chl *a* concentrations were reduced by 8-11% of the initial value, while for the A3-5 experiments, the Chl *a* reduction was more important (30%). This resulted in a significant increase of Chl *a* based clearance rates from A3-1 (9.9 ± 0.9 mL ind^{-1} d^{-1}) to A3-5 (18.4 ± 3.9 mL ind^{-1} d^{-1} i.e. 460.0 ± 97.5 mL $mgDW^{-1}$ d^{-1}; Table 2). It was interesting to note that clearance rates were negatively correlated with Chl *a* concentrations

determined at the beginning of the grazing experiment (Pearson test, $P<0.01$, $n=18$, $r^2=0.90$ and 0.96 for A3-4 and A3-5, respectively; Tables 1 and 2), suggesting that copepods adapted to food concentrations by maintaining and/or increasing their feeding activity under low Chl *a* concentration and slowing down their clearance rates with increasing food quantity.

Highest carbon ingestion was recorded during the A3-1 experiments (2.6 ± 0.3 µgC ind^{-1} d^{-1}) and lowest ingestion was observed during the A3-4 experiments (0.3 – 0.4 µgC ind^{-1} d^{-1}, Table 2). The corresponding grazing pressure on Chl *a* (% s.s. d^{-1}) varied between 1-2 % for the A3-4 experiments and reached ~10% for the A3-1 and A3-5 experiments (Table 2). While grazing on phytoplankton, copepods ingested 0.2-0.3 % their body C d^{-1} during the A3-4 experiments (Table 2). This value increased up to 2.3 – 3.3 % of body C d^{-1} for the A3-5 copepods. Based on the relationships between body weight and respiration, copepods from the A3-4 experiments (median body weight 0.2 mg DW ind^{-1}) needed to ingest 3.5% of their body C d^{-1} just to balance their respiratory needs (range of variation 0.5 – 7.0 µgC ind^{-1} d^{-1}). Therefore, the carbon derived from Chl *a* was not enough to cover the copepod metabolic demand during the A3-4 experiments. Smaller copepods from the A3-5 experiments (median body DW= 0.04 mg DW ind^{-1}) ingested food with 10 times higher weight-specific rates than those from the A3-4 experiments (Table 2). This was countered by 1.6-fold higher daily respiratory requirements reaching 5.6% of body C d^{-1} for the A3-5 copepods. When compared to carbon daily ration, copepods from the A3-5 experiments were 'better off' as ~ 45% (i.e. ratio of daily ration to respiration rate) of their metabolic requirements were covered by phytoplankton ingestion.

3.3. Fate of biogenic Fe

3.3.1 Total and intracellular Fe uptake rates and Fe:C ratios

Total Fe uptake rates ranged from 7.0 ± 0.8 pM d^{-1} (A3-4) to 9.3 ± 1.0 pM d^{-1} (A3-1), with a mean value of 8.0 ± 3.8 pM d^{-1} (Table 3). Intracellular uptake rates varied between 4.4 ± 0.5 pM d^{-1} (A3-4) and 6.2 ± 0.1 pM d^{-1} (A3-5), with a mean value of 5.3 ± 1.2 pM d^{-1}, giving an intracellular Fe content between 63% and 80% of total Fe (mean value 66 ± 46%).

Based on measurements of intracellular Fe (pM), concentrations of Chl *a* determined in our experiments, and *in situ* C:Chl *a* ratios at 20-30 m (i.e. 78.1 g/g and 122.5 g/g for A3-4 and A3-5, respectively), calculated Fe:C ratios reached 3.0 to 5.7 µmol/mol, with a mean value of 4.4 ± 0.1 µmol/mol (n=10).

Stoichiometric Fe:C uptake ratios were also estimated using intracellular ^{55}Fe uptake rates and *in situ* primary production (^{13}C incorporation, Mosseri and Quéguiner, this issue) estimated at the depth of prey collection (20-30 m). The mean Fe:C uptake ratio equalled 5.0 ± 2.6 µmol/mol (n=6).

3.3.2 Fe regeneration: pools and fluxes

The partitioning of Fe between the different Fe pools (dissolved fraction < 0.2 µm, particulate fraction > 0.2 µm, including intracellular Fe and copepods) are reported on Table 4. For each experiment, the sum of all the fractions was not significantly different between T_0 and T_f (KW test, P>0.05), suggesting that no Fe loss occurred (e.g. through bottle wall adsorption).

Intracellular Fe represented the largest Fe pool with values between 51 ± 2 % (Fig. 3C) and 78 ± 3% of total Fe (Fig. 3B). Extracellular Fe (estimated as the difference between TPFe* and IntraFe*) represented 1 ± 1% (A3-4; Fig. 3B) to 39 ± 3% (A3-5; Fig. 3C) of TPFe* (1-33% of total Fe). A very small amount of Fe was retained by copepods (Table 4; Figure 3). Overall, the highest proportion of Fe retained within copepods (4%) coincided with the highest grazing pressure on phytoplankton standing stocks (9-10 % d^{-1}) during the A3-1 and

A3-5 experiments, whereas the smallest values were related to lowest grazing pressures during the A3-4 experiment (1-2% d^{-1}).

Within the dissolved phase, the fraction that was extracted onto C18 column represented less than 1% (Table 4). For all the experiments, a significant increase in dissolved Fe was observed in both experimental and control bottles (KW test, $P<0.01$), indicating a significant regeneration of Fe. During experiments A3-1 and A3-5, regeneration rates were significantly higher in the presence of copepods compared to controls (KW test, $P<0.01$). Fe regeneration rates ranged from 1.4 ± 0.5 pM d^{-1} to 3.4 ± 0.7 pM d^{-1} in the control treatments (mean value 2.1 ± 1.1 pM d^{-1}) and from 2.7 ± 0.5 pM d^{-1} to 3.6 ± 0.6 pM d^{-1} in the experimental treatments (mean value 3.2 ± 0.5 pM d^{-1}). Thus, on average, the presence of copepods increased the Fe regeneration rates by 48%.

3.4. Construction of the biogenic Fe budget

A steady state biogenic Fe budget (Table 5) was constructed using cell abundances, biomasses, growth rates and regeneration rates obtained during the KEOPS cruise or from the literature. Our budget is based on previous work by Bowie et al. (2001) during the SOIREE experiment and by Strzepek et al. (2005) during the FeCycle experiment. Standing stocks, steady-state Fe assimilation and Fe regeneration rates are reported in Table 5. The biogenic Fe pool was partitioned into six plankton classes: phototrophic microplankton (here diatoms), phototrophic nanoplankton (PNAN hereafter; see Christaki et al., this issue), heterotrophic bacteria (Bacteria) and flagellates (HNAN), microzooplankton (ciliates; Christaki et al., this issue) and mesozooplankton. The phototrophic picophytoplankton (< 2 µm) represented less than 1% of total phototrophic plankton biomass (Christaki et al., this issue) and was therefore not taken into account. Carbon biomasses for phytoplankton components are from Armand et al. (this issue), from Christaki et al. (this issue) for PNAN, heterotrophic bacteria,

heterotrophic nanoflagellates (HNAN) and ciliates, and from Carlotti et al. (this issue) for mesozooplankton. Fe quotas for microphytoplankton were those estimated during this study. Values for the other plankton classes were taken from steady state Fe-limited laboratory cultures: autotrophic flagellates (Twinning et al., 2004; Maranger et al., 1998), heterotrophic bacteria (Tortell et al., 1996), heterotrophic flagellates and ciliates (Chase and Price, 1997; Twining et al., 2004; Strzepek et al., 2005), and copepods (Chase and Price, 1997; Schmidt et al., 1999). Growth rates for microphytoplankton and heterotrophic bacteria reported here are those determined during the KEOPS cruise (Timmermans et al., this issue; Obernosterer et al., this issue) or from the literature (Bowie et al., 2001). The steady state Fe assimilation rates were calculated for the producers which directly assimilate Fe from solution, and thus included diatoms, PNAN, and heterotrophic bacteria. Computation was done by multiplying individual producer biogenic Fe content by their corresponding growth rate, taking into account cell mortality for diatoms (Armand et al., this issue).

The biological Fe cycle in the upper 100 m of the Kerguelen plateau is presented in Fig. 4. Biogenic Fe pools and fluxes were extracted from Table 5. Dissolved and particulate Fe (DFe and PFe) were determined during the KEOPS cruise and averaged 90 ± 34 pM and 357 ± 177 pM in the surface mixed layer, respectively (Blain et al., this issue b; Bowie et al., unpublished data). This gives a total Fe pool of 447 ± 211 pM. PFe represents the sum of lithogenic (LithFe), detrital (DetriFe) and biogenic (BioFe) iron. LithFe can be estimated using values of particulate aluminium (Bowie et al., unpublished data) and a Fe:Al ratio of 0.19 for lithogenic material (Wedepohl, 1995). Thus, the mean value for LithFe equals 57 ± 32 pM. Taking into account the estimated BioFe pool (80 ± 9 pM), DetriFe pool (DetriFe = PFe – LithFe – BioFe) equalled 219 ± 134 pM.

Total Fe demand of the producers equalled 6.04 ± 0.62 pM d^{-1}. Total Fe supply by regeneration (3.07 ± 0.48 pM d^{-1}) was determined from our ^{55}Fe incubation experiments after

correction by the ratio between *in situ* biomasses to experimental biomasses of both prey and predators.

These fluxes gave a mean value for the net Fe demand, calculated as the difference between the total demand and the total supply of 2.97 ± 1.10 pM d^{-1}.

4. Discussion

4.1. Experimental set up

The predator abundance in our grazing experiments (8 - 12 copepods L^{-1}) was in the same order of magnitude as the *in situ* abundance above the Kerguelen plateau (e.g. maximum zooplankton abundance = 6.2 ind L^{-1}; Carlotti et al., this issue) and it was also congruent with other field grazing experiments performed on similar predator assemblages (Zeldis et al., 2002; Mayzaud et al., 2002; Schultes et al., 2006). Predator composition (e.g. *C. propinquus, R. gigas, M.lucens* and *O. frigida*, see Table 1) reflected well the *in situ* assemblage above the Kerguelen plateau (Carlotti et al., this issue).

The reverse filtration procedure represented a gentle process for separating predators from their potential prey. With regards to our experimental protocol, the 200 µm sieving step represented a compromise between the need to limit the introduction of mesozooplanktonic grazers during the enrichment phase of prey and the need to determine zooplankton grazing on prey assemblages that are as close as possible (in abundance and composition) to *in situ* conditions. The importance of ciliates in our experiments was likely negligible, as *in situ* ciliate abundances were low and ciliate grazing on phytoplankton prey was estimated to be weak (3% of primary production at Station A3; Christaki et al., this issue). By contrast, large proportions of nauplii were recorded at Stations A3-1, A3-4 and A3-5 (32-48 ind L^{-1}, Christaki et al., this issue; 50-70 ind L^{-1}, Carlotti et al., this issue) and they were probably included in our prey labelling experiment. These naupliar stages are likely to compete for

food with older copepod stages (Turner et al., 2001; Schultes et al., 2006). The sieving step resulted in a reduction of phytoplankton biomass (by factors of 1.5-5), probably due to the loss of chain-forming diatoms that characterized the phytoplankton bloom at Station A3 (e.g. *Thalassiothrix antarctica*, *Proboscia* spp.; Armand et al., this issue). Despite these phytoplankton losses, prey composition at the beginning of our grazing experiments (T_0) was similar to that observed *in situ*, except for *T. antarctica*. Abundance losses were equal in all bottles and they were accounted for in grazing rate calculations. Therefore, the grazing rates presented in this study should be viewed as conservative since nauplii grazing on phytoplankton was included in our grazing calculation. Finally, due to the exclusion of the major part of heterotrophic bacteria from our incubation bottles, our results do not allow any firm conclusions on their importance on Fe-regeneration.

4.2. Phytoplankton growth and Fe uptake

Our phytoplankton growth rates (0.09 – 0.21 d^{-1}; Table 2) were congruent with those determined independently during the KEOPS cruise and based on a similar approach (Timmermans et al., this issue) and they were in the range of those reported previously for the Southern Ocean (Landry et al., 1995; Froneman and Perissinotto, 1997; Gall et al., 2001). Fe uptake rates, determined during the Fe labelling phase (4.4-6.2 pM d^{-1}, mean 5.3±1.2 pM d^{-1}, Table 3) or estimated from biogenic Fe and turnover rates (5.5-6.5 pM d^{-1}, mean 6.0±0.7 pM d^{-1}, Table 5) yielded similar values, as observed in previous studies (Bowie et al., 2001; Maldonado et al., 2001; Maldonado and Price, 1999; Tortell et al., 1999). In the present study, 20 to 37 % of Fe was surface-associated, a result that compares well with field data from the Equatorial Pacific, the Polar front and the Antarctic Circumpolar Current (36-39%, Hutchins et al., 1993; Tovar-Sanchez et al., 2003). The same observation holds for laboratory cultures where cells growing under low-Fe conditions exhibit lower percentages of surface-associated

Fe (20–30%, Sunda and Huntsman, 1995; Ho et al., 2003). When normalised to C, Fe contents and Fe uptake rates agreed well with Fe:C ratios measured during low Fe laboratory studies of open ocean diatom species (see Sarthou et al., 2005 and references herein). They were also consistent with values observed during the SOIREE experiment for > 5-µm eukaryotic phytoplankton (3 µmol/mol, Bowie et al., 2001) or for diatoms during the SOFeX experiments at the unfertilized stations (6 µmol/mol, Twining *et al.*, 2004). These results along with our observations confirm that our natural prey assemblages were experiencing low Fe concentrations (Blain et al., 2007; Blain et al., this issue b), which prevented them from any Fe luxury uptake.

4.3. Copepod feeding and grazing impact

The reverse relation between clearance rates and Chl *a* concentrations suggests that copepods have maintained and/or increased their *in situ* feeding activity despite lower prey concentrations in the feeding suspension (compared to *in situ* abundances). Indeed, active copepod feeding in low food environments has previously been reported for *C. propinquus*, *M. gerlachei* and *Oithona* sp. in the Bellingshausen Sea (0.1 µg Chl *a* l^{-1}) and resulted in an increase of copepod feeding effort (i.e. clearance) despite low carbon intake (Atkinson and Shreeve, 1995).

Our ingestion rates (0.3 to 2.6 µgC ind^{-1} d^{-1}) compared well to *in situ* gut measurements carried out at the same time on large (> 3 mm) and small copepods (Carlotti et al., this issue; Table 2). Carbon ingestion rates revealed that copepods ingested 0.3% and 3.3 % of body C d^{-1} during the A3-4 and A3-5 experiments, respectively. These rates correspond to 3.5 and 6.9 % of body C d^{-1} during the A3-4 and A3-5 experiments, respectively, and they cover only 8% (A3-4) and 45% (A3-5) of respiratory requirements (Mayzaud et al. 2002). This carbon deficit has been reported previously (Atkinson 1996; Dagg and Walser, 1987) and could be

due to high respiration rates and active growth and development (Carlotti et al., this issue). Moreover, Southern Ocean copepods are often qualified as 'omnivorous', feeding on ciliates (Atkinson, 1996; Zeldis, 2001; Schultes et al., 2006), detritus (Poulet, 1976; Heinle et al., 1977), fecal pellets (Weisse, 1983; Head, 1988; Noji et al., 1991) or small zooplankters (e.g. nauplii, copepodites; Atkinson, 1995; Pasternak and Schnack-Schiel, 2001). Although our experimental design did not allow us to determine whether protozoans and metazoans were strongly linked, the observed low ciliate abundances above the Kerguelen plateau are hypothesized to result from a very strong top-down control by copepods (Christaki et al., this issue). Large aggregates (0.3-0.4 mm) along with large fecal pellets (> 100 µm) dominated the particle export at station A3 (Ebersbarch and Trull, subm.). These aggregates and fecal pellets are within the size spectra exploitable by copepods and could have supplemented copepod C requirements above the Kerguelen plateau (Widder et al., 1999).

Using the ingestion rates of phytoplankton measured during our experiments and the Fe:C ratio of our prey assemblages (4.4 µmol/mol), copepod iron ingestion rates equalled 8.7 ± 1.0, 1.2 ± 0.6, and 4.1 ± 1.4 pM Fe d^{-1}, for experiments A3-1, A3-4 and A3-5, respectively. The percentage of ingested Fe retained in the copepods varied between 13% (A3-1 and A3-5) and 25% (A3-4). Fe not retained within the copepods will be either in the dissolved or particulate phase (fecal pellets or as by-products of grazing). Fe regenerated in the dissolved phase due to mesozooplankton grazing ranged from 0.2 to 1.8 pM d^{-1}, suggesting that more than 70% of the phytoplankton Fe that transited through the copepod was recovered in the particulate phase. This is congruent with microscopic observations of fecal pellets produced during the A3-4 and A3-5 grazing experiments, where the diatom *E. antarctica* was often packaged in a nearly intact stage in fecal pellets and in rather high proportion (Fig. 5). The incorporation into fecal pellets will result in a rapid export to the underlying water column (Fowler et al. 1986).

Overall our results of low removal of phytoplankton standing stocks by copepods are consistent with previous observations in Antarctic Ocean Waters (Atkinson, 1996; Razouls et al., 1998; Rollwagen-Bollens and Landry, 2000; Zeldis, 2001). With the exception of A3-4 experiment, grazing rates expressed in percentage of *in situ* primary production (Garcia, pers. com) resulted in much higher values (30.2 to 50.6 % d^{-1}) and strongly suggest that copepods had the potential to control primary production. Using the same approach developed by Zeldis (2001), grazing pressures on primary production would have to increase by a factor 11.0 (A3-4) and 1.6 (A3-5) to cope with copepod respiratory needs. Given that feeding was removing 1.1 % s.s. d^{-1} (A3-4) to 9-10% s.s. d^{-1} (A3-1 and A3-5), this increase will still not constitute a large exploitation of the Chl *a* standing stock.

4.4. Fate of labelled Fe during grazing experiments

In our grazing experiments, the presence of copepods increased Fe regeneration by a factor of 1.7-2.3 as compared to the control treatment. This suggests that grazing increased the Fe residence time in the surface waters, making it potentially available for the producer community.

The A3-4 experiments were different in that the physiological status of phytoplankton prey was low (Fv/Fm ~ 0.30) and that the grazing pressure was very low (see Table 2). This resulted in no significant differences between the control and experimental treatments. This station was therefore not considered in the biogenic Fe budget (Table 5).

Within the dissolved phase, the HphOFe* represented less than 1% of the dissolved Fe pool. This value might be underestimated due to the exclusion from our incubation bottles of the major part of heterotrophic bacteria, which are known to secrete siderophores. In the Trondheim Fjord, Öztürk et al. (2002) observed values from less than 1% to values as high as 35% during phytoplankton blooms, likely due to complexation with lipophilic endogenic

cellular exudates. The low values observed in our experiments suggested that Fe was regenerated as inorganic Fe(II) and Fe(III) species and/or that it was bound to non-hydrophobic organic compounds. The acidic conditions which initiate the digestive process in most grazers are exceptionally well suited to solubilize Fe. The pH within copepod guts can fall to 5 during digestion (Dall and Moriarty, 1983). During intracellular phagotrophic digestion of some protozoans, the initial pH can drop to 2 (Fok et al., 1982), and during subsequent steps at pH 5 hydrolytic enzymes attack bacterial membrane components (Fok et al., 1984). The combination of enzymatic degradation and low pH is ideal for returning organically bound cellular metals to inorganic dissolved forms (Hutchins and Bruland, 1994). Bacterial degradation of dissolved and particulate organic matter through exoenzymatic activity (Martinez et al., 1996) may also help to transform organic Fe into inorganic Fe. Measurements of organic speciation in the water column during the KEOPS cruise showed that organic ligands were present in excess of dissolved Fe concentration (Gerringa et al., this issue). In our grazing experiments, Fe regenerated as inorganic species were probably rapidly bound to freely soluble organic ligands.

4.5. Biogenic Fe budget

4.5.1 Partitioning of biogenic Fe during KEOPS

Our biogenic Fe budget suggested that the detrital Fe pool was the largest Fe pool above the Kerguelen plateau (49% of the total Fe pool). However, one should notice that this Fe pool is calculated by difference (see section 3.4) and not directly measured, so its precise definition is uncertain. This suggested that, above the Kerguelen plateau, much of the primary production was recycled through the detrital pool (i.e. through dead phytoplankton cells, mesozooplankton fecal pellets, and detrital aggregates). Our biogenic Fe pool (80 ± 9 pM) was higher than those previously estimated for polar HNLC waters at the beginning of the

SOIREE experiment (15 pM, Bowie et al., 2001) or in the subarctic Pacific (38 pM, Price and Morel, 1998). By contrast, during the FeCycle experiment in Sub-Antarctic HNLC waters the biogenic Fe pool was substantially higher (115 pM, Strzepek et al., 2005). In the latter case, mesozooplankton biomass was not accounted for but the high biogenic Fe was attributed to the high *Synechococcus* biomass. In our study, mesozooplankton represented the largest stock of biogenic Fe (44% of the total biogenic pool), thus playing a key role in the Fe biogenic budget. Diatoms represented the second largest biogenic Fe pool (36% of the total biogenic pool), and due to their high carbon biomass (83% of the producer C biomass), they represented 65% of the total Fe producer pool. Heterotrophic bacteria represented a relatively small biogenic Fe pool at station A3 (9% of the Fe producer pool). This highly contrasts with HNLC waters, where heterotrophic bacteria account for a large part of Fe (29 - > 50%, Price and Morel, 1998; Bowie et al., 2001; Strzepek et al., 2005). These results indicate the importance of the classical phytoplankton – herbivorous food web within the Kerguelen bloom, with mezosooplankton and diatoms dominating the biogenic Fe pools.

4.5.2 Fe demand and supply during KEOPS

Fe requirements of the producers during KEOPS was higher than that estimated at station Papa (3.0 pM d^{-1}, Price and Morel, 1998), equivalent to the Fe demand during SOIREE after 5 days of fertilisation (5.0-11.9 pM d^{-1}, Bowie et al., 2001), but lower than the Fe demand estimated during FeCycle (26-100 pM d^{-1}, Strzepek et al., 2005). In addition to their substantial contribution to the biogenic Fe standing stock, diatoms consumed the largest part of the dissolved Fe (62% of the total Fe uptake, Table 5). Despite their minor contribution to the biogenic Fe pool, heterotrophic bacteria accounted for 17-27% of the overall Fe-uptake, indicating their potential role in the cycling of Fe. The dominance of diatoms in Fe uptake was also observed at the end of the SOIREE experiment, where large pennate diatoms

accounted for 57% of the total Fe assimilation (Bowie et al., 2001). Both the KEOPS study and the SOIREE study confirm that Fe induces a shift in the ecosystem structure, favouring the growth of diatoms (de Baar et al., 2005).

Our Fe budget estimations suggested that 42-61 % of the total Fe demand was supplied by Fe regeneration (Table 5). The recycled supply of Fe as determined from our control treatments (including direct release from phytoplankton, viral lysis, bacterial degradation of dying phytoplankton, and microzooplankton grazing) represented between 28 and 50% of the Fe demand. This was equivalent to the microzooplankton grazing supply during the SOIREE experiment (28-62% of the Fe demand, Bowie et al., 2001). During KEOPS, the grazing pressure exerted by ciliates was very low, whereas grazing pressure by microplankton metazoans (e.g. copepod nauplii) could have been significant (see section 4.1. and Brussaard et al., this issue). Due to the low bacterial abundance in our experiments, their contribution to Fe regeneration is probably underestimated. Obernosterer et al. (this issue) estimated that the release of iron from heterotrophic bacterial cells due to viral lysis and grazing by heterotrophic nanoflagellates could result in a Fe regeneration flux of ~ 1 pM d^{-1}, corresponding to ~ 30% of total regeneration flux. Mesozooplankton grazing resulted in an additional flux of 11-14% of the Fe demand.

Our estimation of Fe supply and regeneration allowed us to estimate a *fe* ratio as defined by Boyd et al. (2005). During KEOPS, the *fe* ratio equalled 0.49, which was comparable to the average *f*-ratio for nitrogen (0.41, Mosseri et al., this issue), indicating that both NH_4^+ and regenerated Fe could support export production. The KEOPS *fe* ratio was much higher than the *fe* ratio estimated during FeCycle (0.17, Boyd et al., 2005). Such a high *fe* ratio suggests that new sources of Fe must have been available. Blain et al. (2007) and Blain et al. (this issue b) showed that a combination of winter mixing, diapycnal diffusive flux and particulate Fe

dissolution was sufficient to fuel the surface waters with Fe and to sustain the net Fe demand of the massive bloom above the Kerguelen plateau over roughly three months.

5. Conclusions

Our results suggest a low grazing impact on the Kerguelen bloom, allowing the build-up of a large phytoplankton standing stock. Grazing on phytoplankton left copepods in high nutritional deficits since carbon uptake represented only 8 to 45% of their respiratory carbon requirements, not accounting for growth or egg production. This raises the possibility that copepods were feeding on other food resources to cope with their carbon dietary needs (i) by exerting a serious top-down control on ciliates preventing them to build-up large biomasses (see Christaki et al, this issue), (ii) by grazing on large aggregates or fecal pellets encountered in high concentration in the field (Ebersbach and Trull, 2007) or (iii) by increasing their feeding activity (clearance rates) under low food concentration.

Copepod grazing had a strong impact on the fate of Fe above the Kerguelen plateau. More than 70% of phytoplankton-bound Fe that transited through copepods were recovered in the particulate phase (e.g. as fecal pellets), probably resulting in rapid export of detrital Fe to deeper water layers or the sediment. Fe regeneration accounted for 42-61 % of the total Fe demand and the presence of copepods increased Fe regeneration by 48 %. This suggests that copepods increased the Fe residence time in surface waters where it is potentially available for the producer community. Other processes, such as direct phytoplankton release, viral lysis, bacterial activity and microzooplankton grazing, are also likely to play a key role in Fe regeneration.

Our “steady state” budget of biogenic Fe highlighted that mesozooplankton and diatoms were the predominant biogenic Fe pools within the Kerguelen bloom. The estimated *fe* ratio of 0.49 above the Kerguelen plateau suggests that new sources of Fe, such as winter mixing,

diapycnal diffusive flux, and particulate Fe dissolution (Blain et al. 2007; this issue b) are available to sustain the massive bloom.

Acknowledgements

We are grateful to the project coordinator Stéphane Blain for giving us the opportunity to participate to this cruise, to Bernard Quéguiner, the chief scientist, and to the captain and crew of the R.V. Marion Dufresne for their support on board. We thank Nicole Garcia and Valérie Sandroni for Chl *a* and POC measurements, Leanne Armand and Véronique Cornet-Barthaux for data and discussion on diatom abundances and biomasses. We also want to acknowledge Andrew Bowie for providing us particulate Fe and Al data, Thibault Wagener for his help with sampling clean seawater, and Philippe Catala, for flow cytometry analyses. This work was supported by the Institut National des Sciences de l'Univers (INSU) and the French Polar Institute (Institut Polaire Emile Victor, IPEV).

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Table 1. Experiments conducted during the KEOPS cruise. Conditions of RadEX and NRadEX experiments including incubation duration (h), seawater temperature at the depth of prey collection (°C), mean initial prey concentrations (cell L^{-1}; Chl*a* µg L^{-1}; ± SD) and biomass (µgC l^{-1}; ± SD), copepod genera, stages, mean length (mm, ± SD), *in situ* proportion and number of grazers per experimental bottle.

Experiments	Dates	Incubation duration (h)	Temperature (°C)	Initial prey concentration ($x10^3$ Cell l^{-1})	Chla (µg l^{-1})	Initial prey biomass (µgC l^{-1})	Copepod assemblages	Stages	Mean length (mm, ± SD)	*In situ* species proportion (% of total copepods)	Number incubated (per 2.4 L bottle)
A3.1 RadEx	19 Jan.	29.5	3.5	nd	1.93 ± 0.04	nd	nd	nd	nd	nd	22
A3.4	4 Feb.	32-34	3.6								
RadEX				17.9 ± 4.2	0.50 ± 0.03	7.5 ± 1.8	*C. Propinquus*	CIV - CV	2.4 ± 0.5	54	20
							M. lucens	CV - CVI	2.8 ± 0.2	6	
NRadEX				14.3 ± 2.5	0.36 ± 0.01	6.6 ± 1.0	*R. gigas*	CIV - CVI	3.6 ± 0.4	5	
A3.5	12 Feb.	38-40	3.9								
RadEX				14.8 ± 6.7	0.62 ± 0.03	7.7 ± 2.5	*C. Propinquus*	CIV - CVI	2.7 ± 0.6	27	30
							M. lucens	CIV - CVI	1.8 ± 0.4	18	
NRadEX				7.6 ± 1.8	0.47 ± 0.04	3.6 ± 0.8	*O. frigida*	CIV - CV	2.2 ± 0.7	6	

Table 2. Grazing measurements of copepod assemblages based on Chl*a* removal during the five experiments carried out at A3. Indicated are phytoplankton prey growth rates (d^{-1}) in controls, F_v/F_m ratio in controls at the end of incubation, copepod grazing coefficient (d^{-1}), clearance rates (mL ind^{-1} d^{-1} and mL $mgDW^{-1}$ d^{-1}), ingestion rates (µgC ind^{-1} d^{-1} and µgC $mgDW^{-1}$ d^{-1}), copepod grazing pressures on Chl*a* standing stocks (% s.s. d^{-1}) and daily rations (DR; % body C d^{-1}). All values are mean values ± SD. n.s.: not significant; n.d.: no data.

	Phytoplankton prey growth rates (d^{-1})	F_v/F_m	Grazing coefficient (d^{-1})	Clearance rates (mL ind^{-1} d^{-1})	Clearance rates (mL $mgDW^{-1}$ d^{-1})	Ingestion rates (µgC ind^{-1} d^{-1})	Ingestion rates (µgC $mgDW^{-1}$ d^{-1})	Grazing Pressure (% s.s. d^{-1})	DR % Body C d^{-1}
A3.1 RadEX	0.09 ± 0.01	n.d.	0.09 ± 0.01	9.9 ± 0.9	n.d.	2.6 ± 0.3	n.d.	9.0 ± 1.0	n.d.
A3.4 RadEX	n.s		0.11 ± 0.05	13.3 ± 6.5	66.5 ± 32.5	0.4 ± 0.2	2.0 ± 1.0	1.7 ± 0.7	0.3
A3.4 NRadEX	n.s	0.30 ± 0.03	0.09 ± 0.02	11.1 ± 2.7	55.5 ± 13.5	0.3 ± 0.05	1.5 ± 0.25	1.1 ± 0.2	0.2
A3.5 RadEX	n.s		0.19 ± 0.07	15.8 ± 5.8	395.0 ± 145.0	0.9 ± 0.3	22.5 ± 7.5	9.1 ± 2.9	2.3
A3.5 NRadEX	0.21 ± 0.07	0.48 ± 0.06	0.23 ± 0.00	18.4 ± 3.9	460.0 ± 97.5	1.0 ± 0.1	25.0 ± 2.5	10.4 ± 1.8	3.3

Table 3. Mean Fe uptake rates (pM d^{-1}), ± SD, calculated during the ^{55}Fe enrichment phase of natural prey assemblages.

	A3-1	A3-4	A3-5	Mean value
Total	9.3 ± 1.0	7.0 ± 0.8	7.7 ± 0.8	8.0 ± 3.8
Intracellular	-	4.4 ± 0.5	6.2 ± 0.1	5.3 ± 1.2
% intracellular Fe	-	63 ± 14	80 ± 22	66 ± 46

Table 4. Fe partitioning within the different Fe pools involved in the ^{55}Fe grazing experiments. Fe contents are in pM (10^{-9} M) and fluxes are in pM d^{-1}. Values annoted § for Fe contents are in fM (10^{-15} M). HphOFe* corresponds to the fraction extracted on C18 column (see text for further details). TPFe* and IntraFe* correspond to total particulate Fe and intracellular Fe, respectively. The sum of fractions stands for (TDFe*) + (TPFe*) + (copepods*). Depicted are mean values ± SD.

Experiments	Fe pool	Fe content (pM)			Regeneration flux (pM d^{-1})	
		T_0	T_f Control	T_f Experimental	Control	Experimental
A3-1	TDFe* (<0.2 µm)	5.4 ± 0.1	7.0 ± 0.5	9.1 ± 0.4	1.4 ± 0.5	3.2 ± 0.4
	Dissolved HphOFe*	33.2 ± 0.6§	57.1 ± 4.1§	90.4 ± 4.0§		
	TPFe* (> 0.2 µm)	35.0 ± 2.2	31.8 ± 2.8	24.6 ± 2.0		
	"IntraFe*"	26.0 ± 1.7	22.9 ± 2.1	23.3 ± 1.5		
	% of TPFe* as IntraFe*	74%	72%	95%		
	Copepods*	-	-	1.3 ± 0.2		
	% retained	-	-	4%		
	Sum of fractions	**40.4 ± 2.3**	**38.8 ± 3.3**	**35.0 ± 2.6**		
A3-4	TDFe* (<0.2 µm)	2.9 ± 0.4	7.3 ± 0.5	7.5 ± 0.4	3.4 ± 0.7	3.6 ± 0.6
	Dissolved HphOFe*	19.9 ± 2.7§	46.5 ± 3.2§	55.3 ± 3.0§		
	TPFe* (> 0.2 µm)	20.7± 0.7	15.5± 0.1	15.1± 0.2		
	"IntraFe*"	18.3 ± 0.6	12.7 ± 0.7	14.9 ± 0.7		
	% of TPFe* as IntraFe*	88%	82%	99%		
	Copepods*	-	-	0.4 ± 0.0		
	% retained	-	-	2%		
	Sum of fractions	**23.6 ± 1.0**	**22.8 ± 0.6**	**23.0 ± 0.7**		
A3-5	TDFe* (<0.2 µm)	4.3 ± 0.4	6.8 ± 0.6	8.6 ± 0.5	1.6 ± 0.6	2.7 ± 0.5
	Dissolved HphOFe*	28.4 ± 2.6§	49.7 ± 4.4§	74.8 ± 4.3§		
	TPFe* (> 0.2 µm)	24.4 ± 0.7	23.0 ± 0.3	19.0 ± 0.7		
	"IntraFe*"	15.0 ± 0.8	17.3 ± 1.3	14.6 ± 0.4		
	% of TPFe* as IntraFe*	62%	75%	77%		
	Copepods*	-	-	0.9 ± 0.1		
	% retained	-	-	4%		
	Sum of fractions	**28.7 ± 1.1**	**29.8 ± 0.9**	**28.6 ± 1.3**		

Table 5. Biogenic Fe budget of the KEOPS cruise.
Standing stocks (pM), steady-state Fe assimilation rates (pM d^{-1}) and Fe regeneration rates are shown for station A3-1 and A3-5. Data extracted from literature are in italics. See materials and method section for further details and references.

Stations	Plankton Class	Biomass[a] (µM C)	Fe quota[b] [µmol Fe (mol C)$^{-1}$]	Biogenic Fe (pM)	Growth Rate[c] (d^{-1})	Steady-state Fe uptake rate[d] (pM d^{-1})	Regeneration rate of Fe[e] (pM d^{-1})	Total Biogenic Fe demand (pM d^{-1})	Total Biogenic Fe supply (pM d^{-1})	Net Biogenic Fe demand (pM d^{-1})
A3-1	**Autotrophs**									
	Microphytoplankton (diatoms)	6.50 ± 0.33	4.4	28.60 ± 1.45	0.13	3.72 ± 0.19		6.48 ± 0.57	2.73 ± 1.10	3.74 ± 1.67
	PNAN	0.80 ± 0.24	*8.0*	6.40 ± 1.92	*0.16*	1.02 ± 0.31				
	Heterotrophs									
	Bacteria	0.49 ± 0.02	*7.5*	3.69 ± 0.15	0.47	1.73 ± 0.07				
	HNAN	0.55 ± 0.30	*11.6*	6.38 ± 3.48						
	Microzooplankton (ciliates)	0.08 ± 0.05	*11.6*	0.92 ± 0.58			1.84 ±0.66			
	Mesozooplankton	4.42 ± 1.50	*8.0*	35.36 ± 12.00			0.90 ±0.45			
	Total	**12.84 ± 2.44**		**81.35 ± 19.58**						
A3-5	**Autotrophs**									
	Microphytoplankton (diatoms)	6.50 ± 1.57	4.4	28.60 ± 6.93	0.13	3.72 ± 0.90		5.60 ± 1.33	3.41 ± 1.54	2.19 ± 2.87
	PNAN	0.72 ± 0.31	*8.0*	5.73 ± 2.48	*0.16*	0.92 ± 0.40				
	Heterotrophs									
	Bacteria	0.58 ± 0.02	*7.5*	4.38 ± 0.15	0.22	0.96 ± 0.03				
	HNAN	0.31 ± 0.02	*11.6*	3.58 ± 0.23						
	Microzooplankton (ciliates)	0.04 ± 0.02	*11.6*	0.50 ± 0.23			2.81 ±1.05			
	Mesozooplankton	4.42 ± 1.50	*8.0*	35.36 ± 12.00			0.60 ±0.49			
	Total	**12.57 ± 3.44**		**78.15 ± 22.02**						
	Mean value	**12.71 ± 0.19**		**79.75 ± 2.26**				**6.04 ± 0.62**	**3.07 ± 0.48**	**2.97 ± 1.10**

[a] Carbon biomass was estimated using the upper 100-m average cell abundance and published conversion factors.
[b] Fe quotas are either taken from direct field measurements or laboratory cultures.
[c] The growth rates reported here were estimated during KEOPS or taken from literature.
[d] Steady state Fe assimilation rates were calculated as the product of biogenic Fe and growth rate.
[e] Regeneration rates were determined from our ^{55}Fe radiolabelled plankton incubation experiments after correction of the actual biomass of prey and predators *in situ*.

Figure caption

Fig 1: Experimental protocol and analyses performed during the KEOPS cruise. See text for used abbreviations.

Fig. 2: Prey composition at the beginning of the grazing experiments (T_0). Relative abundance (A) and biomass (B) distribution between main prey throughout the four visits to station A3. No data were available for A3-1.

Fig. 3: Time course evolution of Fe partitioning between Dissolved Fe, Extracellular Fe, Intracellular Fe and copepods for the three ^{55}Fe grazing experiments at A3-1 (A), A3-4 (B) and A3-5 (C). Depicted are mean values ± SD in controls at T_0 and T_f and in experimental bottles at T_f.

Fig. 4: Biological Fe cycle in the surface mixed layer of the Kerguelen plateau. The cycle is portrayed in steady state with inputs balancing outputs. Fe pools are in pM and rates in pM d^{-1}. The biogenic pool is comprised of three pools: the producers (microphytoplankton, autotrophic flagellates, heterotrophic bacteria), the microzooplankton (heterotrophic flagellates and ciliates), and the mesozooplankton (copepods). We assume that the biogenic Fe cycle is in steady state. Grazing rate of heterotrophic protozoa on the producers was taken from Christaki et al. (this issue). Flows among the Fe pools were determined assuming that all daily production was consumed by higher trophic levels. See text and Table 5 for more details on the biogenic cycle. The flux between heterotrophic protozoa and dissolved Fe (dotted array) is supposed to be negligible (see text). The rates of transfer between the detrital, lithogenic, and dissolved pools are not known at this time.

Fig. 5: Light microscope photographs of fecal pellets containing *E. antarctica* in grazing experiments on the <200 µm natural plankton community during KEOPS (A3-4 and A3-5 experiments). Scale bar stands for the two photographs.

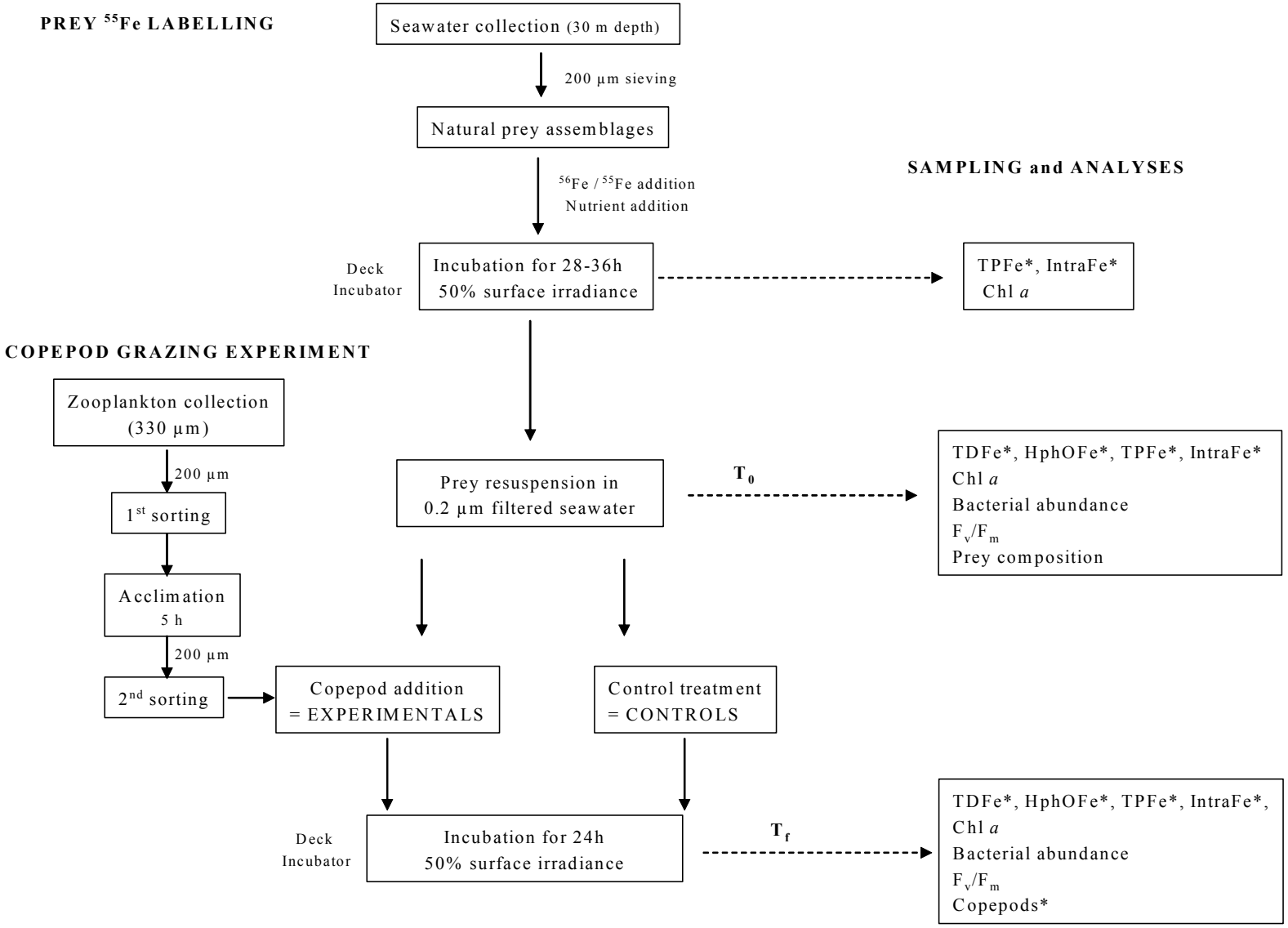
PREY 55Fe LABELLING
Seawater collection (30 m depth)
200 µm sieving
Natural prey assemblages
56Fe / 55Fe addition
Nutrient addition
SAMPLING and ANALYSES
Deck Incubator
Incubation for 28-36h
50% surface irradiance
TPFe*, IntraFe*
Chl a
COPEPOD GRAZING EXPERIMENT
Zooplankton collection
(330 µm)
200 µm
1st sorting
Acclimation
5 h
200 µm
2nd sorting
Prey resuspension in
0.2 µm filtered seawater
T0
TDFe*, HphOFe*, TPFe*, IntraFe*
Chl a
Bacterial abundance
Fv/Fm
Prey composition
Copepod addition
= EXPERIMENTALS
Control treatment
= CONTROLS
Deck Incubator
Incubation for 24h
50% surface irradiance
Tf
TDFe*, HphOFe*, TPFe*, IntraFe*,
Chl a
Bacterial abundance
Fv/Fm
Copepods*

Figure 1

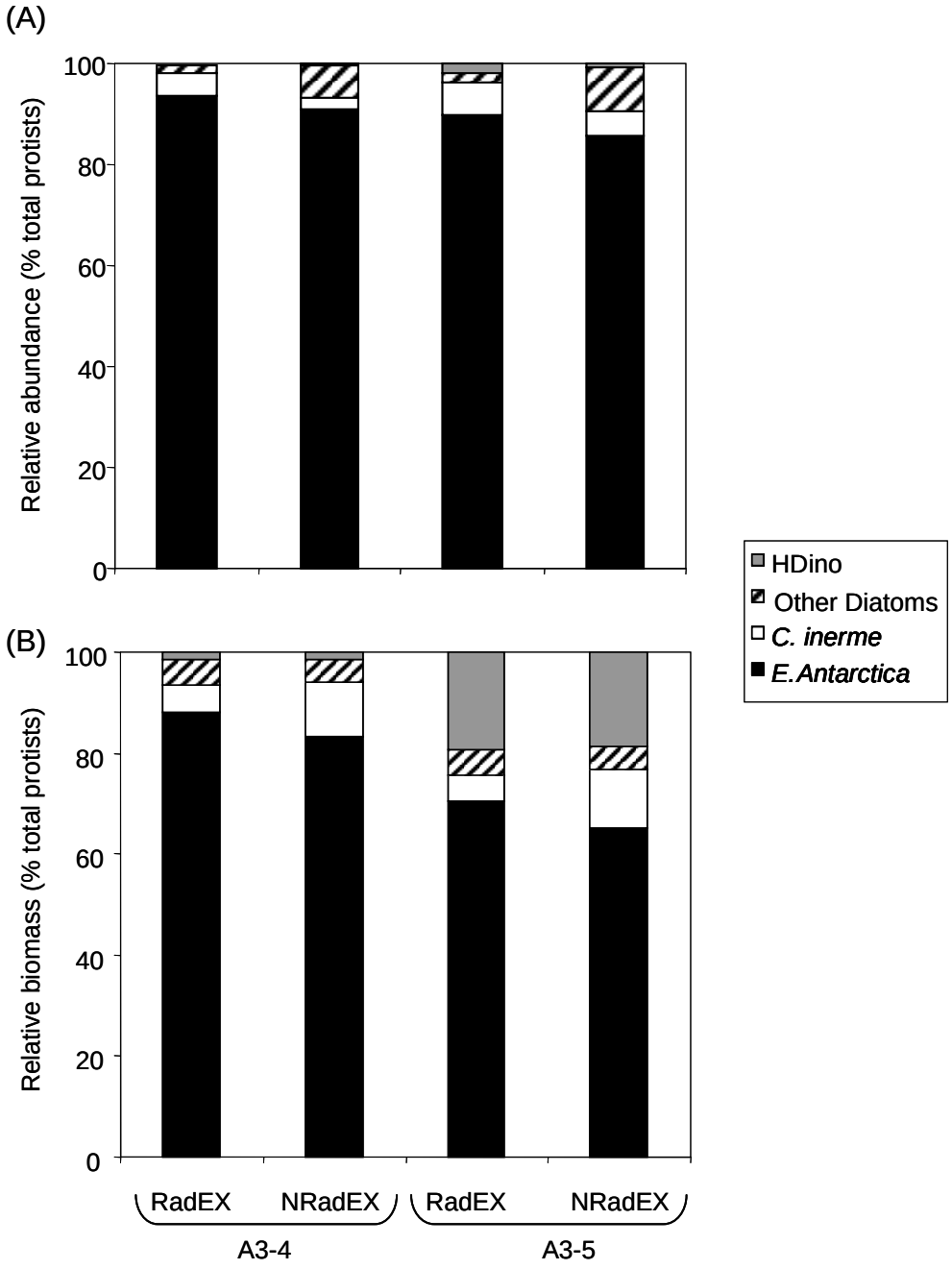
(A)
Relative abundance (% total protists)
100
80
60
40
20
0
(B)
Relative biomass (% total protists)
100
80
60
40
20
0
RadEX
NRadEX
RadEX
NRadEX
A3-4
A3-5
HDino
Other Diatoms
C. inerme
E.Antarctica

Figure 2

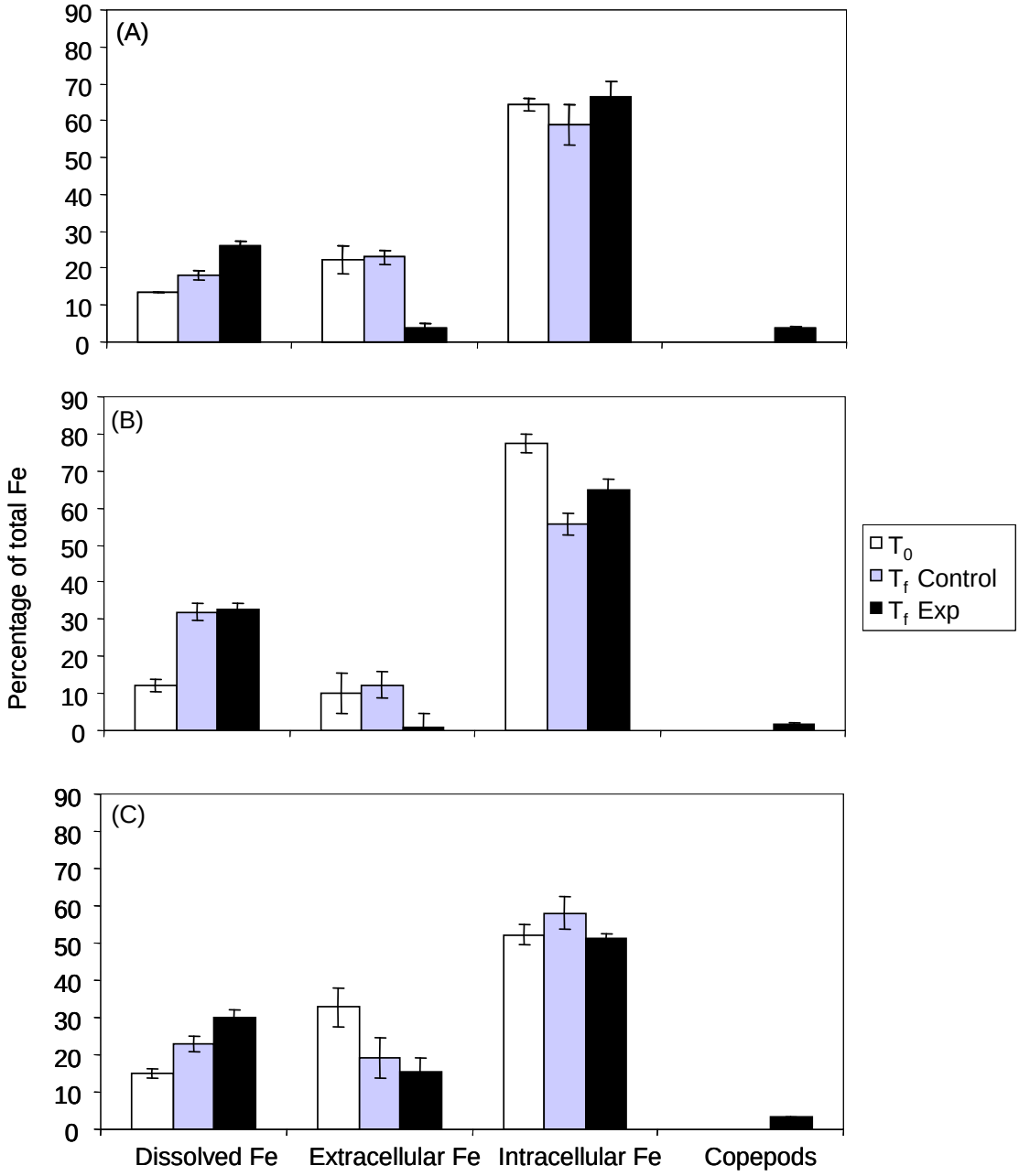
(A)
(B)
(C)
Percentage of total Fe
90
80
70
60
50
40
30
20
10
0
Dissolved Fe
Extracellular Fe
Intracellular Fe
Copepods
T0
Tf Control
Tf Exp

Figure 3

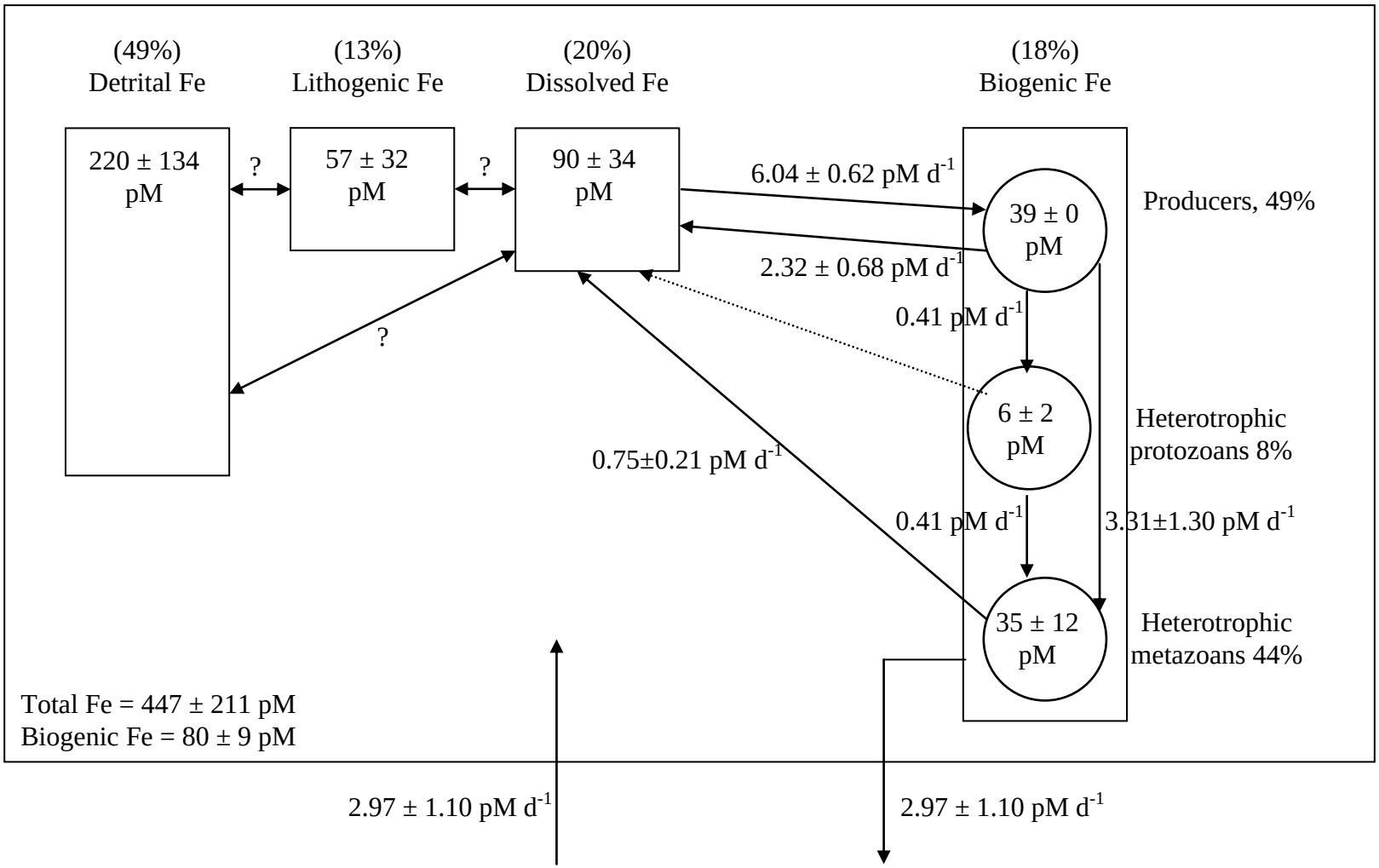
(49%)
Detrital Fe
(13%)
Lithogenic Fe
(20%)
Dissolved Fe
(18%)
Biogenic Fe
220 ± 134
pM
?
57 ± 32
pM
?
90 ± 34
pM
6.04 ± 0.62 pM d-1
39 ± 0
pM
Producers, 49%
2.32 ± 0.68 pM d-1
0.41 pM d-1
?
6 ± 2
pM
Heterotrophic
protozoans 8%
0.75±0.21 pM d-1
0.41 pM d-1
3.31±1.30 pM d-1
35 ± 12
pM
Heterotrophic
metazoans 44%
Total Fe = 447 ± 211 pM
Biogenic Fe = 80 ± 9 pM
2.97 ± 1.10 pM d-1
2.97 ± 1.10 pM d-1

Figure 4

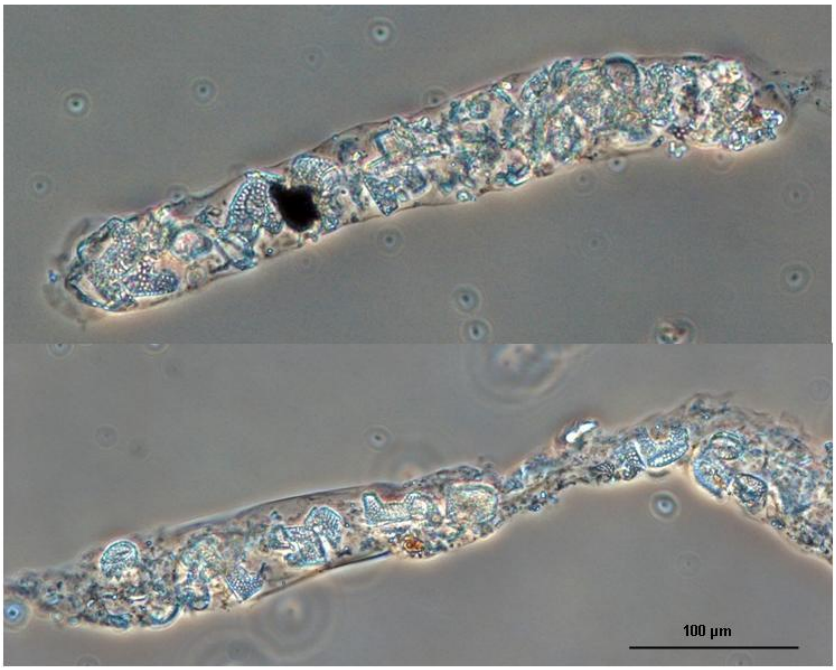

Figure 5