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Temporal development and vertical distribution of major components of the plankton assemblage during an iron fertilization experiment in the Antarctic Polar Frontal Zone

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1. Zusammenfassung

Obwohl der Südozean eines der größten ozeanischen Gebiete unseres Planeten darstellt, ist noch wenig über die Biologie der einzelligen Planktonarten bekannt, die dieses polare Ökosystem besiedeln. Das mangelnde Wissen über die Biologie dieser Arten ist die Konsequenz einer veränderten Gewichtung: von einem traditionellen, Arten orientierten Ansatz hin zu einem modernen Massenparameter (wie z.B. Chlorophyll und partikulärer organischer Kohlenstoff) orientierten Ansatz. Um dieses Missverhältnis auszugleichen sollten die Arten und ihre Eigenschaften wieder stärker in den Fokus der Planktonökologie gerückt werden, da die Evolution auf Artenebene unweigerlich die Evolution der Ökosysteme regelt, die wiederum die biogeochemischen Stoffkreisläufe antreiben. Die Untersuchung zurückliegender und zukünftiger Veränderungen dieser Stoffkreisläufen sollte daher wieder stärker auf dem Artgedanken basiert sein. Diese Dissertation liefert eine umfassende Beschreibung der Reaktion und Vertikalverteilung der Hauptkomponenten des pelagischen Ökosystems und hebt die Artenwechselbeziehungen hervor, die diesen ozeanische Lebensraum und die damit verbundenen biogeochemischen Stoffkreisläufe formen.

Während des Fahrtabschnitts ANT XVIII-2 (EisenEx) auf der PFS Polarstern wurde die Reaktion der Planktongemeinschaft auf Eisendüngung erfolgreich über drei Wochen im Südsommer 2000 verfolgt. Ein Ozeanwirbel (etwa 120 km breit), der sich von der Antarktischen Polar Front abgeschnürt hatte, wurde als Untersuchungsgebiet ausgewählt und sein Zentrum mit einer Treibboje markiert. Eine Fläche von ca. 40 km^2 im Umkreis der Boje wurde dreimal im Abstand von 8 Tagen mit jeweils 4 Tonnen angesäuerter Eisensulphatlösung ($FeSO_4$) gedüngt (Bathmann und Smetacek 2001). Schwefelhexafluorid (SF_6) diente als inerter Marker bei der ersten Düngung und erleichterte es den eisengedüngten „Fleck“ im Laufe des Experiments wieder zu finden. So genannte „Innen-Stationen“ wurden in Gebieten mit den höchsten gemessenen SF_6 Konzentrationen durchgeführt, während „Außen-Stationen“ im Umgebungswasser mit Hintergrundkonzentrationen von SF_6 gelegen waren. Wasserproben wurden in sieben Tiefenstufen zwischen 10 und 150 m an 14 Innen- und 5 Außen-Stationen genommen. Die mikroskopische Auswertung der Proben umfasste Diatomeen, andere Phytoplankter, intakte leere und zerbrochene Diatomeenschalen, Nano- und Microprotozoen sowie Proto- und Metazoen-Kotpellets. Große Proto- und kleine Metazoen, leere Tintinnen-Loricae und Kolonien von *Phaeocystis antarctica* wurden in aufkonzentrierten Proben, wobei der gesamte Inhalt einer Niskin-Flasche (ca. 12 L) über 10

µm Gaze abfiltriert wurde, gezählt. Die konzentrierten Proben wurden an den gleichen Stationen wie die Wasserproben genommen, jedoch in einem anderen Beprobungslauf.

Innerhalb der Diatomeenvergesellschaftung konnten vier Hauptreaktionstypen innerhalb des gedüngten Wasserkörpers unterschieden werden.

Reaktionstyp I war durch schnell wachsende und schwach verkieselte Diatomeen ausgezeichnet, die exponentielles Wachstum über den gesamten Zeitraum des Experiments zeigten. Die zwei charakteristischsten Vertreter dieses Reaktionstyps waren *Pseudo-nitzschia lineola* und *Chaetoceros curvisetus*. Diese beiden Arten zeigten die höchsten Akkumulationsraten während des Experiments. *P. lineola*, die dominante Diatomeenart am Ende des Experiments, ist von ursprünglich $3*10^3$ Zellen l^{-1} auf $234*10^3$ Zellen l^{-1} angestiegen und machte 25% der Biomasse und 53% der Abundanz nach drei Wochen aus. Aufgrund der geringen Ausgangskonzentration blieb *C. curvisetus* eine geringfügigere Komponente des Gesamtdiatomeenbestandes, trotz der hohen Akkumulationsraten.

Arten charakteristisch für Reaktionstyp II zeigten innerhalb der ersten Woche nur eine geringfügige Zunahme, in deren Anschluss jedoch eine Phase linearen Anstiegs folgte. Die stark verkieselten Arten *Fragilariopsis kerguelensis* und *Thalassionema nitzschioides* gehörten zu dieser Kategorie. Beide Arten zeichneten sich durch hohe Ausgangspopulationen aus, fielen jedoch im Laufe des Experiments hinter Arten des Reaktionstyps I zurück. Des weiteren hat sich die Kettenlänge von *F. kerguelensis* während des Experiments etwa verdoppelt, was auf einen verbesserten physiologischen Zustand der Art durch die Eisenzugabe hindeutet.

Arten die dem Reaktionstyp III angehören waren durch große, schwach verkieselte Einzelzellen vertreten und zeigten einen linearen Anstieg ohne anfängliche Stagnationsphase. Typischste Diatomeenarten für diesen Reaktionstyp waren: *Haslea* sp. und *Corethron pennatum*. Beide Arten waren zwar eine zahlenmäßig unbedeutende Komponente, trugen jedoch durch ihre Größe erheblich zur Gesamtbiomasse bei.

Reaktionstyp IV waren durch einen anfänglich linearen Anstieg und eine Abnahme während der zweiten Hälfte des Experiments ausgezeichnet. Dieser Reaktionstyp bestand aus eher kleinen Arten charakterisiert durch *Nitzschia* sp. und *Cylindrotheca closterium*. Die Abnahme in Abundanz sowie in Biomasse gegen Ende des Experiments ist wahrscheinlich auf Fraßdruck zurückzuführen.

Alle anderen Diatomeenarten und –gruppen, die während EisenEx identifiziert wurden, konnten entweder einer der oben genannten Reaktionstypen zugeordnet werden oder zeigten keine Reaktion auf Eisendüngung.

Die Reaktion anderer Phytoplankter, die nicht den Diatomeen angehören, waren weniger stark ausgeprägt. Diese Gruppe spielte eine untergeordnete Rolle in Bezug auf Gesamtbiomasse und war durch *Phaeocystis antarctica*, phototrophe Dinoflagellaten, Coccolithophoriden und den Silikoflagellaten *Dictyocha speculum* vertreten.

Die Coccolithophoriden, von denen *Emiliania huxleyi* als Einzige unter dem Lichtmikroskop bis zur Art bestimmt werden konnte, überstiegen die Diatomeenvergesellschaftung anfänglich in Zellzahlen. Die Abundanzen der Coccolithophoriden nahmen jedoch sowohl innerhalb als auch außerhalb des gedüngten Wasserkörpers dramatisch ab, was darauf hindeutet, dass Eisenzugabe wenig oder keinen Effekt auf ihre Wachstumsraten hatte und das Fraß und/oder andere ungünstige Wachstumsbedingungen den Rückgang der Population zur Folge hatten.

P. antarctica zeigte einen ursprünglichen Anstieg nach Eisendüngung, fiel danach jedoch wieder auf annähernd Ausgangsabundanzen zurück. Der Großteil der *P. antarctica* Population war durch Einzelzellen vertreten, die leicht vom Mikrozooplankton gefressen werden. Dieser Fraß mag den Rückgang während der zweiten Hälfte des Experiments erklären. Eine ähnliche zeitliche Entwicklung wurde für phototrophe Dinoflagellaten, die von der Gattung *Prorocentrum* dominiert waren, beobachtet. Bis zur Mitte des Experiments wurde ein Anstieg und danach eine Abnahme beobachtet. Der Rückgang mag ebenfalls auf Fraßdruck durch Mikro- and Mesozooplankton zurückzuführen sein.

Der Silikoflagellat *D. speculum* reagierte mit einem schwachen Anstieg und es konnte kein signifikanter Unterschied zum Umgebungswasser ausgemacht werden, was darauf hindeutet, dass Eisendüngung keinen maßgeblichen Einfluss auf die Wachstumsraten hatte.

Neben lebenden Diatomeenzellen wurden weiterhin intakte leere und zerbrochene Diatomeenschalen als Indikatoren für Diatomeenmortalität gezählt. Zerbrochene Diatomeenschalen werden durch Fraß von Crustaceen, die in der Lage sind die Silikatschale zu knacken, produziert. Der Aufschluss von Kotpellets durch koprophage Copepoden scheint der Hauptmechanismus zu sein, wie Bruch wieder ins Wasser zurückgelangt. Die Abundanz von Diatomeenbruch hat sich innerhalb des Fleckens verdreifacht und außerhalb verdoppelt. Dies deutet auf einen verstärkten Fraßdruck durch herbivores Zooplankton hin. Intakte leere Schalen können unterschiedlichen Ursprungs sein und die Bandbreite der Ursachen reicht von Proto- and Metazoen Fraß (Sieburth et al. 1978), natürlicher Mortalität, sexueller Reproduktion, viraler Infektion bis hin zu Befall durch Parasitoide (Marchant et al. 2000, Pierce and Wilson 2003). Während EisenEx schien Protozoenfraß und wahrscheinlich zu einem geringeren Maße Metazoenfraß die Hauptursache für die Akkumulation von leeren

Diatomeenschalen in der Wassersäule zu sein. Leere Schalen haben sich innerhalb des gedüngten Gebietes verdoppelt, wohingegen außerhalb keine Veränderungen beobachtet wurden, was auf einen erhöhten Fraßdruck im gedüngten Wasserkörper hindeutet.

Die Vertikalverteilung von passiven Partikeln und Planktonorganismen beinhaltet einen weiteren wichtigen Aspekt dieser Arbeit. Innerhalb der durchmischten Oberflächenschicht waren die verschieden Variablen heterogen verteilt und der Einfluss unterschiedlicher physikalischer, chemischer und biologischer Faktoren auf diese Unregelmäßigkeiten innerhalb der Deckschicht wurde untersucht.

Passive Partikel umfasste Proto- und Metazoen Kotpellets, intakte leere und zerbrochene Diatomeenschalen und leere Tintinnen-Loricae. Die Vertikalverteilung der Planktonorganismen wurde anhand wichtiger heterotropher Organismengruppen untersucht, die thekate and athekate Dinoflagellaten, loricate and aloricate Ciliaten sowie juvenile und adulte Copepoden kleiner Arten. Verschiedene Prozesse beeinflussen die Zusammensetzung und das Ausmaß von passiven Partikeln biologischen Ursprungs, von denen Fraß die wichtigste Rolle während EisenEx gespielt zu haben schien. Kotmaterial verschiedenster Größenordnungen wurde in Wasserproben gezählt, dass sowohl von Proto- als auch Metazoen produziert wurde. Metazoen Kotpellets sowie Diatomeenbruch akkumulierten am Boden der Deckschicht insbesondere gegen Ende des Experiments, was auf eine erhöhte Fraßaktivität von koprophagen Copepoden hindeutet und einer damit einhergehenden erhöhten Recyclingeffizienz von Kotpellets. Im Gegensatz dazu wurden die höchsten Konzentrationen an leeren Diatomeenschalen und heterotrophen Dinoflagellaten in der Nähe der Wasseroberfläche gefunden, ein weiteres Indiz für eine Nahrungsbeziehung zwischen Protozoen und Diatomeen.

1. Summary

Although the Southern Ocean comprises one of the largest oceanic realms of our planet little is known about the biology of the protistan species inhabiting this polar ecosystem. This lack of species specific knowledge is the consequence of a shift in attention of plankton ecologists from the traditional species oriented approach to research based on bulk parameters (e.g. chlorophyll and particulate organic carbon) during more recent studies. Evolution at the species level results in the evolution of ecosystems that drive the biogeochemical cycles. Hence understanding past and predicting future changes in these cycles will have to be based on the species concept. This thesis provides a detailed assessment of the composition, succession, temporal development and vertical distribution of major components of the pelagic ecosystem and highlights the species interactions shaping this open ocean environment and its associated biogeochemical cycles.

During the *R/V Polarstern* cruise ANT XVIII-2 (EisenEx) the response of the plankton assemblage to iron fertilization was successfully followed for three weeks. A cyclonic eddy (approximately 120 km wide) shed by the Antarctic Polar Front (APF) was chosen as the experimental site and its centre marked with a drifting buoy. An area of about 40 km^2 around the buoy was fertilized with 4 tonnes of acidified iron sulphate solution ($FeSO_4$) on three occasions at intervals of 8 days (Bathmann and Smetacek 2001). Sulphurhexafluoride (SF_6) was added as an inert tracer to the first iron infusion in order to relocate the iron fertilized "patch". So called "in-stations" were situated at the highest observed SF_6 concentrations, whereas "out-stations" were within adjacent waters of the fertilized patch with background SF_6 concentrations. The microscopical enumeration of diatoms, intact empty and broken diatom frustules, non-diatom phytoplankton, nano- and microprotozoa, proto- and metazoan fecal pellets was conducted in water samples collected during 14 in- and 5 out-patch stations taken at seven discrete depths during November 2000. Large proto- and small metazoans, empty tintinnid loricae as well as colonies of *Phaeocystis antarctica* were counted in concentrated samples by gently pouring the whole content of a Niskin bottle through 10 µm gauze. The concentrated samples were taken at the same stations and depths as the water samples although from another cast.

Within the diatom assemblage four major response types could be distinguished inside the fertilized patch.

Response type I was characterised by fast growing and weakly silicified diatoms with sustained exponential growth rates throughout the experiment. This response type was best

exemplified by *Pseudo-nitzschia lineola* and *Chaetoceros curvisetus* that exhibited the highest accumulation rates observed during the experiment. *P. lineola*, the dominant diatom by the end of the experiment, emerged from initially $3*10^3$ cells l^{-1} to $234*10^3$ cells l^{-1} accounting for 25% of biomass and 53% of abundance of the total diatom assemblage after three weeks. Due to its low initial concentration *C. curvisetus* remained a minor portion of the total diatom standing stock despite its high accumulation rates.

Response type II species showed an initial phase with negligible growth during the first week followed by a linear increase in abundance thereafter. The heavily silicified diatom species *Fragilariopsis kerguelensis* and *Thalassionema nitzschioides* belonged to this category. Both species were characterised by high initial seeding populations but lagged behind the faster growing species of response type I during the course of the experiment. Furthermore the chain length of *F. kerguelensis* roughly doubled over the course of the experiment indicating an improved physiological status of this species due to iron enrichment.

Species belonging to response type III exhibited a linear growth with no initial lag phase. Two large solitary and weakly silicified diatom species were most characteristic for this response type: *Haslea* sp. and *Corethron pennatum*. Both species were a numerically unimportant component but had an important contribution to the total diatom standing stock due their large size.

Response type IV species were characterised by an initial linear increase and a decline during the second half of the experiment. This response type comprised rather small species exemplified by *Nitzschia* sp. and *Cylindrotheca closterium*. The decrease in abundance as well as biomass towards the end of the experiment was most likely due to grazing.

All other diatom species and groups identified during EisenEx could be either assigned to one of the described response types or showed no response to iron addition.

The response of major components of the non-diatom phytoplankton assemblage to iron addition was less pronounced than that of the diatom assemblage. Non-diatom phytoplankton played a minor role in terms of biomass as compared to diatoms and comprised the haptophyte flagellate *Phaeocystis antarctica*, phototrophic dinoflagellates, coccolithophores and the silicoflagellate *Dictyocha speculum*.

Only coccolithophores, of which *Emiliania huxleyi* was the only species identified under light microscopy, initially exceeded the diatom assemblage in terms of abundance. Nevertheless coccolithophores decreased inside and outside the fertilized patch indicating that iron addition had but a little effect on their growth rates and that grazing and/or other unfavourable growth conditions caused the population to decline.

P. antarctica showed an initial increase in response to iron fertilization but declined thereafter to almost initial concentrations. The majority of the *P. antarctica* population was represented by solitary flagellate cells that are easily grazed upon by microzooplankton what may explain the decline during the latter half of the experiment.

A similar temporal development inside the patch was observed for phototrophic dinoflagellates, dominated by the genus *Prorocentrum*, with an increase until the middle of the experiment and decrease thereafter. The decline might also be explained by intense grazing of micro- and mesozooplankton.

The silicoflagellate *D. speculum* exhibited only a slight increase inside the patch with no significant difference to control waters indicating that iron addition had little or no effect on its growth rates.

In addition to life cells intact empty and broken diatom frustules were also accounted for in this study as indicators of diatom mortality. Broken frustules are produced by crustacean grazers that are capable of crushing the silica cell walls. Disintegration of fecal pellets by coprophagic copepods seems to be the major source of freely suspended broken frustules. The abundance of broken diatom frustules tripled inside the patch and doubled outside the patch indicating an increased grazing pressure by herbivorous crustacean zooplankton. Intact empty frustules on the other hand may derive from various sources encompassing proto- and metazoan grazing (Sieburth et al. 1978), natural mortality, sexual reproduction, viral infection and parasitoids (Marchant et al. 2000, Pierce and Wilson 2003). During EisenEx protozoan and maybe to a lesser extent metazoan grazing seemed to be chiefly responsible for the accumulation of empty frustules in the water column. Empty frustule numbers doubled inside the patch whereas in control waters no change could be observed indicating an enhanced grazing pressure in fertilized waters.

The vertical distribution of non-motile particles and planktonic organisms comprises another important aspect of this study. Within the mixed layer vertical heterogeneities of the different variables were detected and the impact of various physical and biological factors on these irregularities was investigated. Non-motile particles included proto- and metazoan fecal material, intact empty and broken diatom frustules and empty tintinnid loricae whereas life organisms were composed of major groups of the heterotrophic community including thecate and athecate dinoflagellates, loricate and aloricate ciliates as well as juvenile and adult copepods of small species. Various processes affect the composition and magnitude of non-motile particles of biological origin of which grazing seemed to have played the major role during this study. Fecal material spanning over a wide size range was detected in water

samples that was produced by both proto- and metazoan grazers. Metazoan fecal pellet numbers as well as broken diatom frustules accumulated at the bottom of the mixed layer towards the end of the experiment suggesting an increased feeding activity of coprophagic copepods and thus an enhanced recycling efficiency of fecal material. In contrast highest concentrations of intact empty frustules and heterotrophic dinoflagellate abundances were predominantly detected at the surface indicating another important link in the trophic food web.

2. General introduction

Thus far the study of marine plankton ecology has largely focussed on growth rates of ecosystem components and how they affect biogeochemical cycles. The quantities and rates, based on summary parameters such as size classes, pigments or 14C uptake, rather than the species composition of pelagic ecosystems took centre stage in plankton ecology. The complex structure underlying pelagic ecosystems has only been marginally addressed. This structure comprises the composition of the plankton community and the species interactions. The detailed study of the plankton and the proximate (nutrient uptake, grazing) and ultimate (defence mechanisms, life cycle) factors shaping the composition and magnitude of plankton communities will shed new light on ecosystem processes. In future studies it will be essential to identify specific attributes of planktonic key species and thereby determine their respective roles in biogeochemical cycles and pelagic food chains.

T. John Hart (1934, 1942) summarized the results of the *R.R.S. Discovery* cruises from 1925-1939 and provided one of the first comprehensive reports on Southern Ocean phytoplankton ecvology. Hart was the first plankton ecologist working in the Southern Ocean who, apart from merely describing phytoplankton species distribution, tried to integrate these patterns within an ecosystem context. Hart (1942) realised the role of bottom-up factors such as nitrate and silicate in determining phytoplankton productivity and further suggested that the availability of micronutrients like iron may have a great influence on the rather poorer pastures of the open ocean. He also noted the role of grazing (top-down factor) as an important loss term of phytoplankton standing stocks. Considering the limited methodology available at the time, Hart´s insights are remarkably close to the modern view.

Despite high nutrient concentrations phytoplankton biomass is usually low throughout the year in the land remote Antarctic Circumpolar Current (ACC). This Antarctic paradox has now been solved: iron availability limits phytoplankton growth in “high-nutrient, low-chlorophyll” (HNLC) regimes like the Southern Ocean. Iron fertilisation experiments in the equatorial and subarctic Pacific and the Southern Ocean have all resulted in a dramatic increase of phytoplankton biomass (Coale et al. 1996, Behrendfeld et al 1996, Boyd et al. 2000, Gervais et al. 2002, Tsuda et al. 2003). Neritic environments like the Antarctic Peninsula exhibit extensive phytoplankton blooms during the growth season indicating that iron from continental shelves significantly increases algal growth rates (Sullivan et al. 1993). In contrast the dissolved iron concentrations in surface waters of the ACC are low with somewhat elevated concentrations in the Polar Frontal region (Löscher et al. 1997). The only

source of iron in these land remote regions of the Southern Ocean comes from up-welling of Circumpolar Deep Water (CDW). Increased iron input from Patagonian dust and sea ice and iceberg melting may therefore have a stimulating effect on growth rates of oceanic species. Model simulations generate a much higher dust deposition over the open ACC during the Last Glacial Maximum (LGM) (Mahowald et al. 1999, Petit et al. 1999) implying that the oceanic environment was more productive during the LGM. These evidence support the "Iron Hypothesis" proposed by late John Martin (1990): the much higher aeolian dust outfall during glacials will have provided sufficient iron to significantly stimulate ocean productivity over much of the Southern Ocean. However if the increased productivity will eventually result in an enhanced strength of the biological carbon pump (BCP), the vertical flux of biogenic matter from the surface layer, and a subsequent greater oceanic sequestration of atmospheric CO_2 still remains to be shown.

2.1 The role of diatoms in the Southern Ocean

Diatom blooms are a common phenomenon during periods of new production. They drive the BCP and hence play a major role in the oceans carbon cycle (Falkowski et al. 1998). Furthermore the silica cycle of the ocean is largely dominated by diatoms that incorporate dissolved silica into their frustules (Ragueneau et al. 2000).

The majority of diatom species described for the Antarctic Circumpolar Current (ACC) and the seasonal ice zone (SIZ) remain at low concentrations throughout the year and only a few species contribute the bulk of biomass. Throughout the open, iron-limited ACC long chain-forming (*Fragilariopsis kerguelensis*, *Pseudo-nitzschia* spp. and *Chaetoceros* spp.) and large-celled (*Corethron pennatum* and *Thalassiothrix antarctica*) diatoms are the dominant species (Hart 1934 and1942, Hustedt 1958, van der Spoel et al. 1973, Laubscher et al. 1993, Smetacek et al. 1997, Zielinski and Gersonde 1997, Kemp et al. 2000, Smetacek et al. 2002) and this oceanic cold water flora is marked by the enormous preponderance of the *Fragilariopsis-Pseudo-nitzschia* and *Corethron-Chaetoceros* associations which are characteristic of the Southern Ocean. These more important species show a completely circumpolar distribution (Hart 1942). Most have thick silica frustules or robust spines with silica to nitrogen (Si:N) ratios above 2. The frustules of *F. kerguelensis* and *T. antarctica* are among the main contributors of the diatom ooze accumulating under the ACC (Zielinski and Gersonde 1997, Taylor et al. 1997, Smetacek 2000). In contrast the dominant diatoms in near-shore, iron-rich environments are more similar to diatoms from the continental shelves

of the world ocean: weakly silicified species of the genera *Thalassiosira* and *Chaetoceros* together with the ice algae *Fragilariopsis cylindrus* and *F. curta* (Kang and Fryxell 1992, Kang and Fryxell 1993, Leventer and Dunbar 1996, Taylor et al. 1997, Crosta et al. 1997, Goffart et al. 2000, Gersonde and Zielinski 2000, Suzuki et al. 2001). Colonies of the haptophyte flagellate *Phaeocystis antarctica* also dominate biomass under bloom conditions in coastal environments (Arrigo et al. 2000, Goffart et al. 2000). The diatoms of the continental margins tend to have lower Si:N ratios around 1. As a result, silica burial under the productive regions is much less but carbon burial is significantly higher than in the land-remote ACC.

We hypothesize that the dominance of large, robust diatoms in the open ACC is a result of heavy grazing pressure relative to the low, iron-limited primary production which leads to selection and persistence of species with effective defences. Despite their heavy frustules, the life cycle strategy of these species is to persist in the surface layer where their carbon is recycled but from which their silica frustles sink out. In productive environments on the other hand, fast-growing species build-up biomass till iron depletion. Similar species from other regions tend to form aggregates of living cells, resting spores and phytodetritus that sink out en masse from the surface layer following nutrient depletion (Smetacek 1985). Such mass sinking events have been observed or recorded by sediment traps in some regions such as the Bransfield Strait and the southern Weddell Sea (von Bodungen et al. 1986, Smetacek et al. 1992).

It follows that three broad categories of diatoms can be differentiated in the Southern Ocean: the background species, the fast-growing, boom-and-bust, carbon sinkers of iron-replete regions and the slower growing, persistent silica sinkers of the iron-limited ACC. Hence the intrinsic properties of the key diatom species ultimately shape the biogeochemical cycles of the respective biogeographical regions in the Southern Ocean.

2.2 Diatoms of the iron limited system

Pico- and nanophytoplankton of the microbial community contribute the bulk of chlorophyll biomass in the iron limited system of the land remote ACC (Detmer and Bathmann 1997). Nevertheless large robust diatom species are a persistent and characteristic component of this system, albeit at low concentrations (Fig. 1).

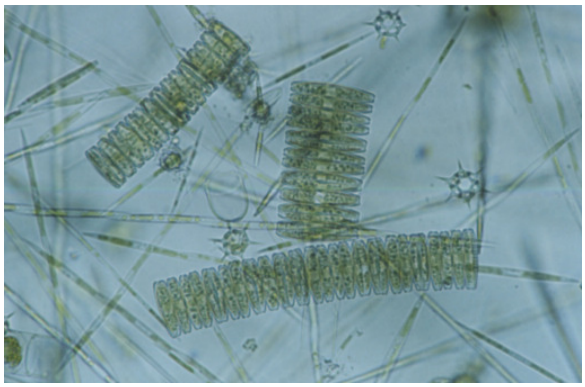

Fig. 1: Overview picture of the giant diatom flora encountered during EisenEx.

These “giant diatoms” are largely dependent on the recycling efficiency of the microbial community that makes iron bioavailable to them. Thus under typical ACC conditions diatoms hardly reach bloom proportions and chlorophyll concentrations remain below 1 mg m^{-3}. Their persistence and dominance under unfavourable (iron limited) growth conditions prevailing throughout most of the ACC can thus only be explained by low mortality. Most of the carbon associated with these “giant diatoms” is recycled by the regenerating system within the mixed layer. However silica concentrations are considerably depleted in a northward direction from the continent towards to Subantarctic Front (SAF) and 75% of today’s global biogenic silica deposition accumulates in the siliceous girdle around the Antarctic continent (Ledford-Hoffman et al. 1986) although only 20% of the global biogenic silica production occurs in the surface waters of the Southern Ocean (Treguer et al. 1995). Large heavily silicified diatoms typical of the ACC are responsible for this silica deposition. Among those are: *Fragilariopsis kerguelensis*, *Thalassiothrix antarctica* and *Thalassiosira lentiginosa*. Furthermore chain-forming or large celled diatoms are important components of the giant diatom flora including various *Chaetoceros* species of the *Phaeoceros* type, *Pseudo-nitzschia* spp. and *Corethron pennatum*. The silica frustules of theses species usually dissolve before reaching the sediment. The pennate diatom *F. kerguelensis* represents one of the most abundant and the most characteristic endemic diatom species in the Southern Ocean (Hustedt 1958, Hasle 1965, Van der Spoel et al. 1973, Zielinski and Gersonde 1997). *F. kerguelensis* is a true oceanic form and dominates the diatom assemblages throughout the ACC (Karsten 1906, Hendey 1937, Zielinski and Gersonde 1997). *F. kerguelensis* occurs in long ribbon-shaped chains of two to more than 100 cells which can be as long as 2000 µm (Fig. 2). The shape of the frustule and the cell size can be quite variable (10 to 90 µm) (Hustedt 1958) and empty cells are frequent within chains of *F. kerguelensis* (El-Sayed and Fryxell 1993).

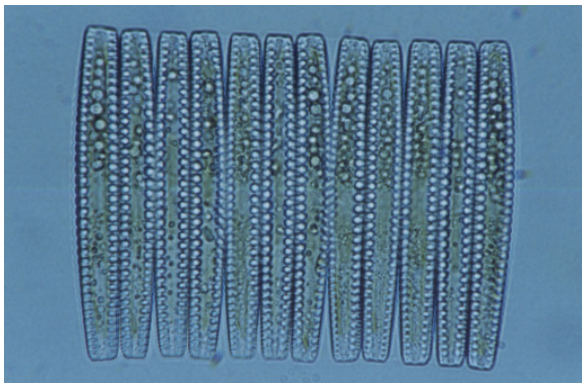

Fig. 2: A chain of *Fragilariopsis kerguelensis.*

Hart (1934) describes *F. kerguelensis* as a typical spring and autumn form which especially dominates the phytoplankton community in spring. Because of its high abundance and heavily silicified cell walls which are very resistant to dissolution *F. kerguelensis* is the main contributor to the opal belt of the Southern Ocean (Zielinski and Gersonde 1997). *F. kerguelensis* is therefore strongly enriched in surface sediments of the Southern Ocean even in areas like the Weddell Basin where this species contributes only a minor portion of the diatom assemblages originally produced in the euphotic zone (Zielinski and Gersonde 1997). The deposition of its frustules has continued unabated through glacial and interglacial cycles (Verity and Smetacek 1996).

Thalassiothrix antarctica is often one of the most important of the larger species in the northern part of the Antarctic zone, and, more rarely, farther south. The larger individuals of this robust oceanic species are amongst the longest diatoms known with up to 5 mm in length (Hart 1942) although being only about 5 µm in diameter (Fig. 3). The cell wall is extremely thick and covered with small marginal spines. It frequently forms colonies of up to twenty-four individuals and clogs even large-meshed plankton nets when abundant (Hart 1942, El-Sayed and Fryxell 1993).

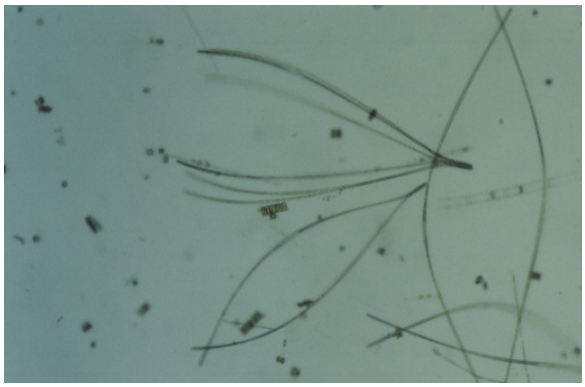

Fig. 3: Colonies of *Thalassiothrix antarctica*.

The distribution pattern of *T. antarctica* shows highest abundances during the austral summer especially in the december-january period (Hart 1942, Van der Spoel 1973). Hart (1934) describes *T. antarctica* as being co-dominant with *Corethron* spp. in comparatively cold water. Due to its strongly silicified frustules *T. antarctica* is an abundant species in the sediments of the Southern Ocean. Although the remains are mostly fragmentary (Hart, 1942) mass sedimentation of *Thalassiothrix* spp. has been documented in the fossil record (Kemp and Baldauf 1993).

Thalassiosira lentiginosa represents an oceanic species with a widespread distribution in Antarctic waters (Hart 1942, Johansen and Fryxell 1985, Zielinski and Gersonde 1997). *T. lentiginosa* is a large discoid species with a valve diameter of 29-120 µm (Johansen and Fryxell 1985; Fig. 4).

Fig. 4: Valve face of *Thalassiosira lentiginosa*.

Relative abundances are highest in the Permanent Open Ocean Zone (POOZ) and the Polar Frontal Zone (PFZ) whereas in coastal areas around the Antarctic Peninsula and the Weddell Sea only low abundances were encountered or the species was absent (Zielinski and Gersonde

1997). Due to its robust valves *T. lentiginosa* is enriched in sediments and represents one of the prominent open ocean species in the Southern Ocean surface sediments (Hustedt 1958, Zielinski and Gersonde 1997). It seems that the temperature range of *T. lentiginosa* is somewhat smaller than that of *F. kerguelensis* and that the taxon is primarily dwelling in waters with temperatures ranging from 0-7°C in the POOZ and the PFZ (Zielinski and Gersonde 1997).

Cheatoceros species of the subgenus *Phaeoceros* are large often chain forming species with spines of up to a millimetre in length. They are widely distributed throughout the Southern Ocean and a prominent feature of the open ACC (Fig. 5). Prominent members of this subgenus are even frequent invaders of the Subantarctic Zone (SAZ), e.g. *C. atlanticus*, and to the south both in the Weddell and Bellingshausen Sea, e.g. *C. dichaeta* (Hart 1934).

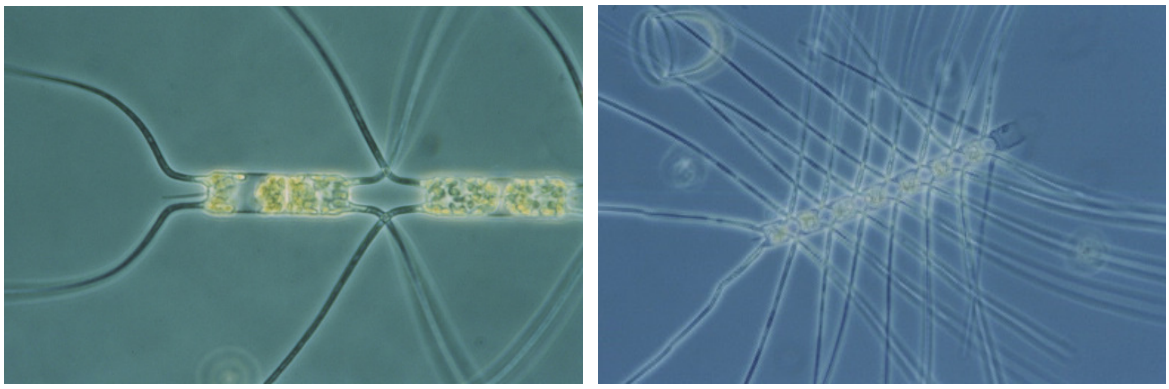

Fig. 5: End cell of a chain of *Chaetoceros dichaeta* (left) and a chain *of Chaetoceros atlanticus* (right).

Since these species are not known to form resting spores they are usually not preserved in the sediment record. However there seem to be seasonal differences in the degree of silification of *C. dicheata* and *C. atlanticus*. The summer forms tend to be only slightly silicified, whereas the winter forms showed a heavily silicified cell wall.

The genus *Pseudo-nitzschia* shows a widespread distribution throughout the world ocean with *Pseudo-nitzschia prolongatoides* and *P. turgiduloides* being the only species reported from ice and likely the only endemic species to the Antarctic (Medlin and Priddle 1990). Despite their widespread distribution *Pseudo-nitzschia* spp. have often been neglected by phytoplanktonologists doing routine work (Hasle 1965). A characteristic feature of these pennate diatoms is the chain formation by overlapping of the cell ends, i.e. stepped colonies (Hasle 1965, Medlin and Priddle 1990; Fig. 6).

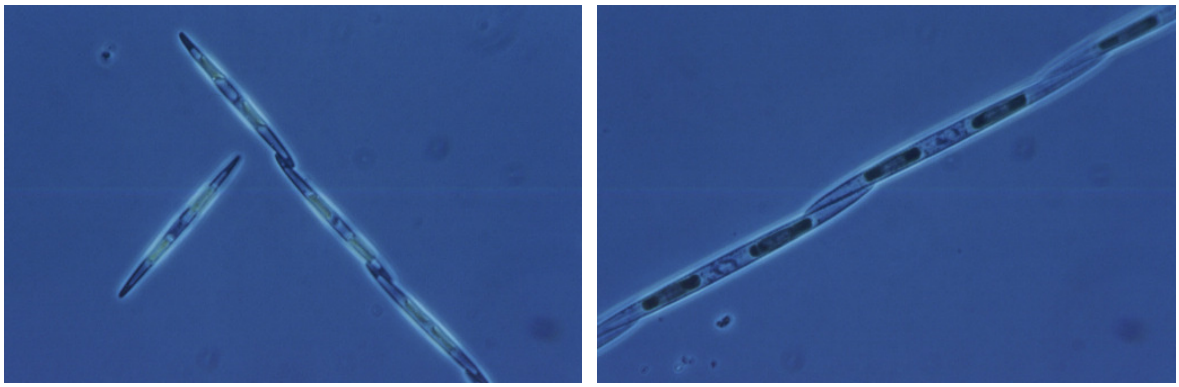

Fig. 6: Stepped colonies of *Pseudo-nitzschia* species: *P. turgidula* (left) and *P. lineola* (right).

P. heimii was recorded in the South Atlantic in plankton from 44°S-61°S with highest abundances at 51°S (Hustedt 1958) but records from the North Atlantic suggest that this species has a much wider distribution (Hasle 1965). Another *Pseudo-nitzschia* species, *P. lineola*, shows a similar widespread distribution throughout the world ocean (Hasle 1965). Despite its widespread distribution this species belongs to the most numerous planktonic species in the Atlantic sector of the Southern Ocean from 51°S-66°S. *P. turgidula* appeared in moderate numbers between 53°S and 62°S at temperatures from about 6.0 to 2.3 °C. The sporadic findings south of the Antarctic Convergence indicates that *P. turgidula* belongs to a subantarctic flora (Hasle 1965). *P. turgiduloides* was almost exclusively observed in the Antarctic zone with greatest abundances near the ice-edge. *P. turgiduloides* has been recorded together with *P. lineola* being the more abundant species of the two in more southerly regions (Hasle 1965).

Corethron pennatum forms solitary cylindrical cells that can vary quite considerably in size (Hendey 1937). Two types of spines can be distinguished in this species, the hooked spines and the long barbed spines (Medlin and Priddle 1990; Fig. 7).

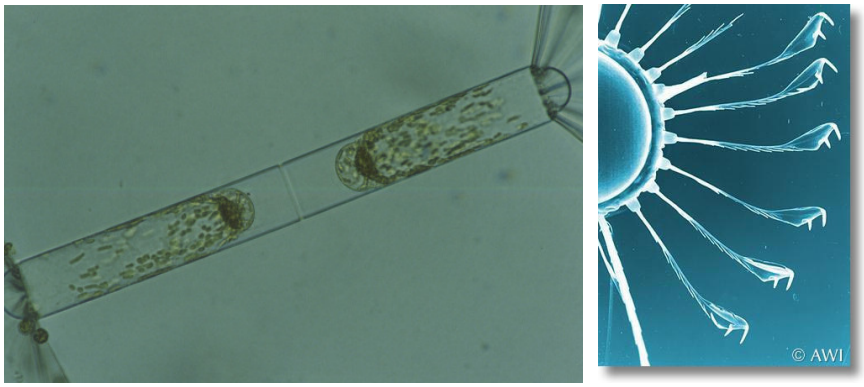

Fig. 7: Dividing *Corethron pennatum* cell (left) and hooked spines of this species (right).

It is proposed that the spines serve to move apart and maintain separation of the cells in the ocean. *C. pennatum* is a thinly silicified species which is only occasionally found in the sedimentary record of the Southern Ocean (El-Sayed and Fryxell 1993, Crawford 1995) but there is evidence of deposits of almost monospecific layers of *C. pennatum* in the Weddell Sea as well as in the Ross Sea (Jordan et al. 1991, Leventer et a. 1993). These monospecific layers are most likely due to a mass sexual phase of *C. pennatum* which triggers downward transport of empty diatom cell walls and seems therefore significant for the vertical silica flux in the Southern Ocean (Crawford 1995). During austral summer *C. pennatum* was found in sediment traps near King George Basin in low but consistent numbers. However, *C. pennatum* is strongly underrepresented in sediment traps and in the sediments because its frustules are mechanically destroyed during krill feeding (Abelmann and Gersonde 1991). *C. pennatum* is among the most common diatoms of the marginal ice zone (MIZ) in austral summer (Kang and Fryxell 1993). Hart (1934) describes *C. pennatum* as one of the most important of Antarctic plankton diatoms, being almost universally present but more abundant south of the Polar Front. Close to the MIZ *Corethron* is often associated with *Phaeocystis* where both forms multiply rapidly when liberated in the summer. However, *Phaeocystis* soon decreases and the *Corethron* cells, already near the lower size limit for the species, begin to form auxospores (Hart 1942).

2.3 Diatoms of the iron replete system

Near the Antarctic continent or island plumes chlorophyll concentrations regularly exceed background levels and reach bloom proportions (Sullivan et al. 1993). These coastal blooms are either dominated by fast growing, weakly silicified diatoms or colonies of the haptophyte flagellate *Phaeocystis antarctica* (Leventer and Dunbar 1996, Crosta et al. 1997, Arrigo et al. 2000, Goffart et al. 2000). Due to the higher iron input in these neritic environments growth rates are not resource limited and species with high intrinsic growth rates benefit most under these conditions. The more robust and hence better defended but slower growing species lag behind the “bloom-and-bust” species that eventually out grow the former and bloom. After nutrient depletion the bloom eventually collapses and accumulates in large phytoaggregates that rapidly settle out of the surface layer (Smetacek et al. 1992). These mass sedimentation events carry a substantial amount of carbon with them and hence fuel the BCP. Silica deposition on the other hand is less pronounced due the light silification of the abundant

neritic diatom species that are much more prone to dissolution than their oceanic counterparts. An important life cycle strategy of many neritic species involves a resting stage that can endure periods of nutrient limitation or low irradiance and germinate again when growth conditions are favourable (Smetacek 1985).

Species of the diatom genus *Thalassiosira* are often a dominant component of the annual phytoplankton bloom in many coastal areas around the globe. *Thalassiosira antarctica* is an abundant species within the Antarctic ecosystem and largely related to near-shore and ice associated environments (Hart 1942, Johansen and Fryxell 1985, Zielinski and Gersonde 1997). *T. antarctica* is somewhat smaller than its oceanic counterpart *T. lentiginosa* with a valve diameter of 16-56 µm (Johansen and Fryxell 1985). *T. antarctica* shows a different distribution pattern than *T. lentiginosa* with significant amounts in the coastal areas of the Weddell Sea. Hart (1934) describes *T. antarctica* as a spring form with highest abundances early in the season. *T. antarctica* is a very variable form sometimes occurring in vast masses of gelatinous colonies (Hart 1934, El-Sayed and Fryxell 1993). Karsten (1906) observed *T. antarctica* often associated with *Phaeocystis*. Due to their rapid vegetative growth in austral spring both *Phaeocystis* and *Thalassiosira* form gelatinous colonies (El-Sayed and Fryxell 1993).

The smaller and more delicate *Cheatoceros* species belonging to the subgenus *Hyalochatae* exhibit a cosmopolitan distribution also penetrating into the Antarctic Zone. Many of the species form chains that are connected by thin and hyaline spines (Fig. 8).

Fig. 8: Chains of *Chaetoceros curvisetus*. Copyright © 1996-2000, Mats Kuylenstierna & Bengt Karlson All rights reserved.

The majority of members belonging to this subgenus are composed of neritic species that form resting stages under unfavourable growth conditions. This is documented by Hargraves and French (1983) and Nöthig (1988) who found the highest abundances of *Cheatoceros*

spores in Southern Ocean waters in neritic environments influenced by sea ice or in the vicinity of shelf ice areas. *Chaetoceros curvisetus*, *C. neglectus* and *C. socialis* are among the most dominant of this subgenus in the Southern Ocean and considerably contribute to costal blooms.

Fragilariopsis curta and *F. cylindrus* represent two small sized pennate diatom species with *F. cylindrus* being one of the smallest known diatom species with a cell size often not exceeding 4 µm in length (Hustedt 1958). *F. curta* is known to form short chains but usually occurs solitary (Hendey 1937). Both species are among the most common diatoms of the MIZ in austral summer (Kang and Fryxell 1993) and dominate the surface sediments along the continental shelf (Kang and Fryxell 1991, Cunningham and Leventer 1998, Gersonde and Zielinski 2000). Thus it can be asserted that *F. curta* and *F. cylindrus* are neritic species which frequently dominate under the ice and near the continent (Hendey 1937, Hart 1942, El-Sayed and Fryxell 1993; Fig. 9).

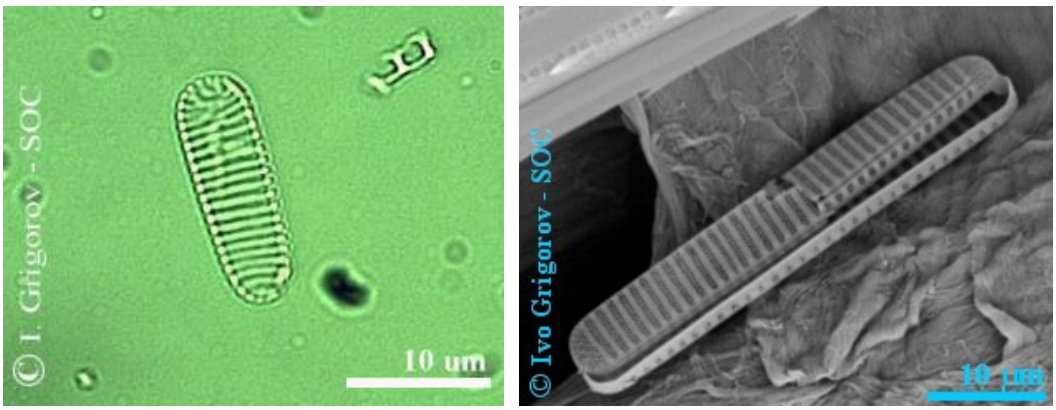

Fig. 9: Valve face of *Fragilariopsis curta* (left) and SEM image of *Fragilariopsis cylindrus* (right).

The environmental significance of *F. curta* has been well established since the species is common in fast and pack ice, as well as in the meltwater-stratified surface layer associated with a retreating ice edge (Gleitz et al. 1996). Therefore *F. curta* may be used as a proxy for meltwater stratification resulting from fast and pack ice melt-out (Cunningham and Leventer 1998). Gersonde and Zielinski (2000) have documented the importance of the sea-ice related taxa *F. curta* and *F. cylindrus* for the reconstruction of Antarctic winter sea-ice extent and its variation through the Pleistocene.

Pheaocystis antarctica is a small (< 10µm) flagellate haptophyte, which occurs solitary as well as in large spheroidal colonies (Fig. 10). The colonies are due to their sheer size and

surprisingly tough and plastic skin less susceptible to grazing than the small nanoflagellate form and hence blooms of *P. antarctica* are always dominated by the colonial stage.

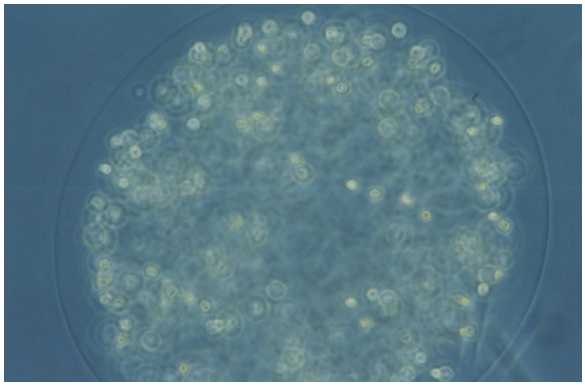

Fig. 10: Colony of *Phaeocystis antarctica*.

When reaching peak concentration in austral spring *P. antarctica* blooms cause serious clogging of fine meshed nets (El Sayed and Fryxell 1993). This species is the only non-diatom phytoplankton known to form blooms in the Southern Ocean, in particular the Ross Sea (Arrigo et al. 1999). *P. antarctica* is also often found under the pack-ice and near the continent, for example in the MIZ of Prydz Bay and the Weddel Sea (El-Sayed and Fryxell 1993, Waters et al. 2000).

2.4 The role of grazing in shaping Southern Ocean ecosystems

Thus far the main focus was on bottom-up factors, namely iron in the Southern Ocean, in shaping the two different productive regimes described above. Grazing as a top-down control however, has largely been neglected by plankton ecologists. The fact that grazing must play a decisive role in shaping ecosystem structure becomes evident by looking at the "giant diatoms" of the land remote, iron-limited ACC. These diatoms have evolved an array of defence mechanisms ranging from extremely thick silica frustules to long barbed spines that deter grazers. In a nutrient limited system persistent and hence well-defended species are able to keep mortality low and sustain viable populations throughout the year.

The most characteristic diatom of the open ACC, *Fragilariopsis kerguelensis*, is due to its very robust frustules less susceptible to the destructive pressure exerted by the mandibles and gizzards of crustacean zooplankton (Hamm et al. 2003). Furthermore the formation of chains with sometimes over one hundred cells per chain is an effective defence against smaller metazoan but especially protozoan grazers. The same applies to the stepped chains of species

of the genus *Pseudo-nitzschia* that can reach over a millimetre in length and pose despite the weak silica armature characteristic to this genus a major impediment to most grazers. Besides its strong silification and comparably large size *Thalassiosira antarctica* features no further obvious defence morphologies and is a rather minor component of the oceanic "giant diatom" flora. However due to their robustness frustules of this species are enriched in sediments underlying the ACC (Taylor et al. 1997). Another oceanic species of the Southern Ocean, *Thalassiothrix antarctica*, is next to its enormous size and heavily silicified cell walls armed with minute bristles. These bristles facilitate the formation of large aggregates or mats of this species that carry even smaller particles that get entangled with them to depth. On the other hand the barbed surface of *T. antarctica* may deter salps, common filter feeders in the ACC, by clogging their feeding apparatus. Although *Corethron pennatum* and large *Chaetoceros* species of the *Phaeoceros* type are a weakly silicified their hooked and barbed spines and sheer size may serve a similar function and most proto- and small metazooplankton are unlikely to be able to tackle these large diatom species. All these "giant diatoms" are perfectly adapted to the grazer community of the regenerating system that mainly consists of protozoans, salps and small copepods.

Although all species occur year round in more or less predictable patterns only a few species from disparate genera, of which many are cosmopolitan, dominate blooms of the iron replete, coastal system. These bloom-forming species must therefore have different properties than the background species. Whereas the latter seem to be better defended at the expense of fast growth the former are geared to high growth rates and have evolved "boom-and-bust" life cycles that are characterised by variations in population size over several orders of magnitude. The "boom-and-bust" species are therefore able to grow out of the grazer gauntlet and build up biomass. Nevertheless even these species have evolved ways to defend themselves against grazers. Many form long chains connected by spines (*Chaetoceros* species of the subgenus *Hyalochaetae*) or threads (species of the genus *Thalassiosira*) that makes them less susceptible to smaller predators. Especially many protozoans that can exhibit growth rates similar to those of phytoplankton are deterred by long spiny chains (Sommer 1989, Strom 1991, Bjoernsen and Kuparinen 1991, Hall and Safi 2001). Another example of adaptation to grazing is *Phaeocystis antarctica*, the only non-diatom phytoplankton reported to bloom in the Southern Ocean. This small solitary flagellate forms large multicellular colonies under favourable growth conditions that provide due to their though and plastic skin protection (Hamm et al. 1999) against a whole array of grazers ranging from viruses to copepods.

Next to mainly physical factors mortality induced by pathogens, parasitoids and predators will also effect the vertical distribution of detritus and planktonic organisms in the water column and the flux of inorganic and organic matter from the surface. Metazoo- as well as some protozooplankton release fecal material that will settle out faster than individual particles and hence drive the BCP (Komar et al. 1981, Gowing and Silver 1985, Klaas 1997). However feeding on these fecal pellets and strings by various uni- and multicellular grazers may significantly retard the vertical flux and affect the vertical distribution (Gonzalez and Smetacek 1994). The vertical distribution of intact empty and broken diatom frustules may be tightly geared to the respective grazer community. Whereas broken frustules only stem from crustacean grazing intact empty frustules may derive from multiple sources including pathogens, parasitoids and proto- and metazoan grazing.

It can be concluded that next to bottom-up factors grazing as a top-down control plays a decisive role in structuring the Southern Ocean pelagic ecosystems and biogeochemical cycles. The “mortality environment” as termed in Manuscript 5 should thus receive the same significance in pelagic ecology than the “growth environment”.

3. Aims and outline of the dissertation

Thus far the study of marine phytoplankton ecology has tended to be based on summary parameters such as size classes or pigments and little attention has been paid to the species interactions underpinning the large-scale ecological processes. In contrast the aim of this study is to give a detailed assessment of the planktonic community during an iron fertilization experiment in the Atlantic sector of the Southern Ocean (EisenEx) and derive ecological principles for the interpretation of pelagic ecology and its impact on nutrient cycling.

This dissertation is based on five individual manuscripts that examined the following topics:

Manuscript 1 focuses on the composition and succession of the diatom assemblage and other components of the phytoplankton community during the experiment.

Manuscript 2 examines the influence of physical, chemical and biological factors on vertical distribution of non-motile particles and proto- and small metazooplankton groups.

Manuscript 3 analyses the response of the proto- and small metazooplankton assemblage to an iron-induced phytoplankton bloom.

Manuscript 4 investigates the response of larger protozooplankton to an iron-induced phytoplankton bloom.

Manuscript 5 gives a review of the role of grazing in structuring the Southern Ocean pelagic ecosystem and biogeochemical cycles.

The following sections give a short overview of the aims and outlines of the individual manuscripts.

Temporal development of the diatom assemblage and other components of the phytoplankton community

Previous iron fertilization experiments have all resulted in phytoplankton blooms that were dominated by diatoms (Coale et al. 1996, Boyd et al. 2000, Gervais et al. 2002, Tsuda et al. 2003). The study therefore mainly focused on the species composition and succession of this important group. Nevertheless other components of the phytoplankton community that could be satisfactory identified with light microscopy were also accounted for. **Manuscript 1** therefore deals with the composition and succession all diatom species and groups identified during EisenEx and other major components of the phytoplankton assemblage including phototrophic dinoflagellates (mainly *Prorocentrum* spp.), *Phaeocystis antarctica*, coccolithophores (mainly *Emiliania huxleyi*) and *Dictyocha speculum*. Hitherto, studies in biological oceanography in the Southern Ocean usually targeted on bulk parameters, e.g. chlorophyll, or size classes, e.g. pico, nano and micro, that contained no species specific information. Some reports provided information with low taxonomic resolution (diatoms, dinoflagellates etc.) or lists of species identified during the study however the ecological significance of individual species and their impact on the entire ecosystem was not revealed. The microscopic analysis of the phytoplankton community described herein is unparalleled in its floristic details compared to previous iron fertilization experiments and was aimed to further our understanding of the processes governing community composition and species succession in the pelagic environment.

Water samples were taken at major CTD stations at nearly daily intervals to achieve a good temporal resolution. Unfortunately gale force winds prevented daily sampling intervals during much of the second half of the experiment. In order to obtain a comparison between unperturbed waters and the fertilized patch water samples were taken both inside and outside the patch. The identification of phytoplankton was carried out when possible down to species level and an optimum number of cells were counted for the more abundant species, taking the time constraints of microscopical investigations into consideration, to achieve significant trends. Intact empty and broken diatom frustules were also enumerated. This approach yields valuable information on species specific mortality and only a limited number of studies have previously investigated this aspect (Kang et al. 1991, Klaas 1997). Furthermore the chain

length of the prominent Antarctic diatom *Fragilariopsis kerguelensis* was monitored over the course of the experiment as an indicator of the physiological status of this species.

All these detailed species information were combined and discussed in the context of the whole EisenEx data set, compared to previous iron fertilization experiments and integrated into larger ecological principles.

Vertical distribution of non-motile particles and proto- and small metazooplankton groups

Mesoscale *in situ* fertilization experiments provide an ideal case study for the processes structuring the pelagial because a distinct patch of ocean can be tracked in space and time and all the processes inherent to it can be followed. Various factors, including physical, chemical and biological ones, influence the vertical distribution of planktonic organisms in the water column. **Manuscript 2** investigates the influence of these factors on the vertical distribution of intact empty and broken diatom frustules, empty tintinnid loricae, protozoan and crustacean fecal material and various components of the heterotrophic community, including athecate and thecate dinoflagellates, aloricate and loricate ciliates and juvenile and small adult copepods.

At each major CTD station the water column was sampled at 7 discrete depths down to 150 m. Despite this rather coarse vertical resolution distinct concentration peaks within the mixed layer were detected and the factors influencing these vertical heterogeneities investigated. Due to their low occurrence in water samples empty tintinnid loricae and juvenile and small adult copepods were counted in concentrated samples as compared to the other variables. Zooplankton fecal pellets together with phytoplankton aggregates are thought to dominate vertical fluxes in the ocean (Honjo 1990). It is therefore essential to investigate their distribution in the water column and assess their impact on the BCP. Furthermore to determine the significance of proto- and metazooplankton grazing on the distribution of intact empty and broken diatom frustules, empty tintinnid loricae, protozoan and crustacean fecal material will shed new light on species interactions in the plankton.

The vertical distribution of non-motile particles of biological origin, e.g. fecal pellets, is largely determined by physical processes, e.g. current velocities, the turbulent field in the mixed layer and Stoke´s law. However biological processes may significantly alter their distribution. Disintegration of fecal material by coprophagic copepods for example may retard the vertical flux quite substantially (Gonzalez and Smetacek 1994). On the other hand many

planktonic organisms are able to actively regulate their position in the water column irrespective of the local flow field (Smayda and Bienfang 1983, Yamazaki and Squires 1996). Their vertical distribution may well reflect a specific depth preference or orientation towards a food source. Partial correlations for the various variables were carried out in order to elucidate the role of the different vectors affecting the vertical distribution of non-motile particles and planktonic organisms and interpreted in the context of the variable physical environment encountered during EisenEx.

Temporal development of the proto- and metazooplankton and vertical distribution of larger protozoa

Results on the temporal development of abundance, carbon standing stock and composition of nano-, micro- and mesoprotozoa and small metazoa as well as the importance of these groups compared to other compartments of the pelagic community were discussed in the light of literature data (**Manuscript 3** and **4**). Factors influencing the vertical distribution of large protozoans were compared and discussed as a function of physical, chemical and biological parameters of the water column (manuscript 4).

Till this day only a limited amount of studies were conducted on protozoan grazing impact in the cold waters of the Southern Ocean (Bjørnsen und Kuparinen 1991; Burkill et al. 1995; Archer et al. 1996; Klaas 1997). They all point out the important role of protozoan grazing in regulating phytoplankton stocks in the Southern Ocean. In order to extend the knowledge of Antarctic protozoan feeding behaviour to the microprotozoan component of the protozoan assemblage, and give estimates of microprotozoan clearance rates of the iron-stimulated phytoplankton community in the field, dilution experiments to determine grazing impact of the microprotozoan assemblage to natural food composition were carried out. Grazing impact of microprotozoa during EisenEx was determined by combining these experimental studies to the field data on microprotozoan temporal development and composition. Since diatoms are the dominant phytoplankton group in new production and additionally played major role in vertical flux during former iron fertilization experiments (Nodder and Waite 2001), it is necessary to separate microprotozoa capable of feeding on diatoms from those only capable feeding on nanophytoplankton. Therefore, microprotozoa counted in the field samples were grouped as a function of feeding behaviour and size classes (manuscript 3). These results enable the assessment of microprotozooplankton grazing impact of the different size classes on diatoms and other phytoplankton, and thus on the development and structure of the iron-induced phytoplankton bloom.

The mortality environment

Manuscript 5 deals with the role of grazing in structuring the Southern Ocean pelagic ecosystem and biogeochemical cycles. It is obvious that the properties of the key species must differ significantly from those of the background ones. So far most of the research has focused on the growth environment of the dominant phytoplankton groups and grazer defences have not been adequately addressed in marine plankton although they must play a central role in species evolution purely on the basis of theoretical considerations (Smetacek 2001). It has been shown that the colonies of *Phaeocystis* are protected by a tough skin (Hamm et al. 1999) and that the frustules of *Fragilariopsis kerguelensis* are remarkably strong and can resist pressures exerted by mandibles or gizzards of copepods and euphausiids respectively (Hamm et al. 2003). Furthermore some phytoplankton species, especially diatoms, show a remarkable resilience to grazing even after being ingested. Fowler and Fisher (1983) showed that some marine phytoplankton even survive in fecal pellets collected from sediment traps and can undergo cell division when placed in either nutrient enriched or un-enriched seawater. Preliminary epifluorescent investigations of fecal pellets from the Weddel Sea indicated that about 10% of the cells in some fecal pellets could have been alive, and germination experiments confirmed that at least *Fragilariopsis cylindrus* is capable of growth after removal from fecal pellets (El-Sayed and Fryxell 1993). Hence the dominance of these species could be explained by their effective defence mechanisms. Under unfavourable growth conditions prevailing throughout most of the ACC the morality environment rather than the growth environment shapes the pelagic ecosystem. Species that invest in effective grazer defence mechanisms are less prone to considerable stock reductions and hence are able to maintain sizeable seeding populations. Under favourable growth conditions however less well defended species can eventually grow out of the grazer gauntlet due to their high growth rates and bloom. These converse production regimes are characterised by different grazer assemblages that can be best exemplified by the distribution of two major groups of metazooplankton grazers in the Southern Ocean: krill and salps. Attention generally focuses on krill, *Euphausia superba* which is regarded to be the dominant herbivorous species in Antarctic waters and known to preferentially feed under high phytoplankton densities underneath the sea ice and during coastal blooms. Euphausiids are the only known grazers to specifically target large diatoms, and where present, their high grazing rates can completely alter phytoplankton community (Waters et al. 2000). The hypothesis that grazing may be in favour to smaller cell sizes seems incorrect, as *E. superba* proved to graze mainly on cells

with a diameter of 20 to 25µm, which is the most abundant size class in the whole area where *E. superba* is assumed to be one of the most important grazers. Weber and El-Sayed (1985) also showed that feeding efficiency of krill decreases with size. Therefore it seems that krill is adapted especially to feed on the most frequent size of prey cells in the environment (van der Spoel et al. 1973). The seasonal rise and fall of the krill population appears to lag behind that of the phytoplankton biomass. Early investigations indicate that areas of high krill concentration are usually noted for their standing crop of phytoplankton (Hart 1934, Hardy and Gunther 1935, Hart 1942). Rakusa-Suszczewski (1982) and Uribe (1982) found that areas of dense krill concentration in the central parts of the Bransfield Strait exhibited low chlorophyll values at the surface (<0.5mg m^3) and in the water column (<50mg m^2). According to Uribe (1982), the indigence of the phytoplankton was not due to nutrient limitation, but probably to intensive krill feeding. Nast and Gieskes (1986) demonstrate the inverse relationship between phytoplankton and krill in the waters around Elephant Island during early November 1983.

In contrast to krill, salps (e.g. *Salpa thompsoni*) are capable of retaining particles and cells over a wide size range (4-1000µm) with a high efficiency. Furthermore salps are able to quickly respond to improved food concentration by asexual budding. However there is some evidence of the exclusion of *S. thompsoni* in regions of elevated chlorophyll concentration, due to the clogging of the salps feeding apparatus (Perissinotto and Pakhomov 1998). Salps are therefore assumed to be important gazers in the open, iron-limited ACC where plankton concentrations are low throughout the year.

Copepods comprise another major group of metazooplankton grazers that are important herbivores affecting phytoplankton density in the Southern Ocean (Bathmann et al. 1997). They are abundant grazers near the continent at high plankton concentrations as well as in HNLC waters of the open ACC. Schnack et al. (1985) found that copepods were the single most important grazers during a spring phytoplankton bloom in the Brainsfield Strait. High copepod abundances have also been recorded for the PFZ in the Atlantic sector of the Southern Ocean (Dubischar et al. 2003). However the copepod assemblage is usually numerically dominated by small copepod genera like *Oithona* that will less effectively graze on the giant diatoms prevailing in oceanic regions of the Southern Ocean.

Moreover microzooplankton, including mainly ciliates, heterotrophic nano flagellates (HNF) and heterotrophic dinoflagellates, are also reported to graze on phytoplankton (Capriulo et al. 1991). More recently, the importance of heterotrophic dinoflagellates in Antarctic waters has been reported by Archer et al. (1996a) with the activity of coastal dinoflagellate populations

equivalent to the ingestion of 4.8% of autotrophic biomass and 25% of daily production consumed per day. Especially thecate dinoflagellates have evolved a formidable array of feeding strategies (Jacobson 1999) that enables them to feed on larger prey items, e.g. diatoms.

It can be concluded that high grazing pressure of small copepods and salps keep the regenerating communities at low concentrations characteristic of the iron-limited Southern Ocean and leads to the accumulation of large, heavily silicified diatoms that drive the silica pump. Under iron replete conditions however fast growing, weakly silicified diatoms build up blooms that are grazed by crustacean zooplankton and drive the BCP (Manuscript 5). Hence the “mortality environment” plays a decisive role in shaping pelagic ecosystem structure and exerts a strong influence on the elemental cycles.

Manuscripts

This dissertation is based on five individual manuscripts prepared for submission. The contributions of authors are noted.

1. P. Assmy and J. Henjes (to be submitted) Response of a diatom community to iron fertilization in the Polar Frontal Zone of the Southern Ocean (EisenEx).

   P. Assmy wrote the manuscript. Sample collection, processing and interpretation of data were conducted by both authors.

2. P. Assmy and J. Henjes (to be submitted) Vertical distribution of non-motile particles and major components of the heterotrophic community during an iron fertilization experiment in the Polar Frontal Zone of the Southern Ocean (EisenEx).

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   P. Assmy wrote manuscript. Sample collection, production and interpretation of data were conducted by both authors.

3. J. Henjes and P. Assmy (to be submitted) Response of the protozoo- and metazooplankton assemblage to an iron-induced phytoplankton bloom during EisenEx.

   J. Henjes wrote manuscript. Sample collection, production and interpretation of data were conducted by both authors.

4. J. Henjes and P. Assmy (to be submitted) Response of larger protozooplankton assemblage to an iron-induced phytoplankton bloom in the Polar Frontal Zone of the Southern Ocean (EisenEx).

   J. Henjes wrote the manuscript. Sample collection, production and interpretation of data were conducted by both authors.

5. Smetacek, V., Assmy, P. and Henjes, J. (submitted). The role of grazing in structuring Southern Ocean ecosystems and biogeochemical cycles.

V. Smetacek wrote the manuscript. P. Assmy and J. Henjes contributed to the discussion of the ideas, their formulation and to literature research. The figures were prepared by both co-authors.

Manuscript 1

Response of the phytoplankton assemblage to iron fertilization in the Polar Frontal Zone of the Southern Ocean (EisenEx)

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In preparation for submission

Abstract

All *in situ* iron fertilization experiments in HNLC-regions of the Equatorial and Subarctic Pacific and the Southern Ocean resulted in phytoplankton blooms that were dominated by diatoms. Here we present data from a detailed analysis of the diatom assemblage and some other major non-diatom phytoplankton components, including *Phaeocystis antarctica*, photosynthetic dinoflagellates, *Emiliania huxleyi* and *Dictyocha speculum*. Iron addition led to a dramatic floristic shift from a community dominated by pico- and nanophytoplankton to one that was dominated by diatoms. Initially the diatom assemblage was numerically dominated by small delicate forms as well as by heavily silicified pennates of which *Fragilariopsis kerguelensis* contributed most in terms of biomass. The weakly silicified, chain-forming pennate diatom *Pseudo-nitzschia lineola* exhibited the strongest response to iron addition and accounted for 53% of total diatom abundance and 25% of total diatom biomass by the end of 3 weeks. A similar marked response was observed for the small centric diatom *Chaetoceros curvisetus*. Furthermore intact empty and broken diatom frustules doubled and tripled inside the fertilized patch respectively indicating an increased grazing pressure by proto- and metazooplankton. Components of the non-diatom phytoplankton assemblage showed only a minor or no increase and were most likely controlled by grazers. This iron-mediated shift in the autotrophic community has major implications for the biological carbon pump, nutrient cycling and interpretation of the sediment record of the Southern Ocean and sheds new light on the ecology of pelagic ecosystems.

Introduction

Ocean biogeochemistry is driven by the photosynthesis of phytoplankton and a large body of knowledge pertaining to environmental control of growth rates has accumulated in the last decades (Falkowski et al. 1998). The suite of factors regulating the build-up of phytoplankton biomass range from size of the seed population, light climate and nutrient availability to grazing pressure by various proto- and metazooplankton groups. Field observations (De Baar et al. 1995), bottle experiments (Martin et al. 1991) and *in situ* fertilization experiments (Coale et al. 1996; Boyd et al. 2000; Smetacek 2001; Gervais et al. 2002; Tsuda et al. 2003) have now established beyond doubt that iron availability limits growth rates of oceanic phytoplankton in "high-nutrient, low-chlorophyll" (HNLC) regimes. In the land-remote ocean the supply of iron is mediated by atmospheric transport and subsequent outfall of dust from the continents. Therefore *in situ* fertilization can be regarded as simulating natural events such as dust input from the atmosphere or from melting icebergs.

The ocean's biological carbon pump (BCP) – the vertical flux of biogenic matter from the surface layer – is one of the major processes regulating atmospheric CO_2 concentrations (Falkowski et al. 1998) and it is therefore essential to understand the suite of factors are regulating the pump. Iron fertilization experiments now enable the study of the impact of phytoplankton species composition on the pump. Five iron fertilization experiments carried out so far (Martin et al. 1994; Coale et al. 1996; Boyd et al. 2000; Gervais et al. 2002, Tsuda et al. 2003) have all resulted in phytoplankton blooms. The first artificially generated phytoplankton bloom in the Southern Ocean persisted in the surface for over 40 days after fertilization as shown by satellite images (Boyd et al. 2000), suggesting that sinking losses were low. The bloom was reported to be dominated by the same heavily silicified diatom species *Fragilariopsis kerguelensis* also observed to dominate a natural assemblages (Bathmann et al. 1997). Sediments from the Atlantic sector of the Southern Ocean confirm this finding (Zielinski and Gersonde 1997). However, the factors determining the composition and succession of the iron-induced blooms have so far only been marginally addressed.

Over 100 diatom species are common in the Southern Ocean covering a wide range of sizes and shapes. Some of these species are cosmopolitan but compared to other ocean regions the Southern Ocean has the largest percentage of endemic species (Priddle and Fryxell 1985; Medlin and Priddle 1990). Despite this diversity only a few species contribute the bulk of biomass. The dominant species must thus differ from the background ones by a specific adaptation to the environment. The diatom assemblage of the land-remote Antarctic

Circumpolar Current (ACC) is characterised by large and often robust species. These giants of their respective genera are a persistent feature in oceanic regions of the ACC, albeit at low concentrations. Among those, chain-forming (Fragilariopsis kerguelensis, Pseudo-nitzschia spp. and Chaetoceros spp. of the subgenus Phaeoceros) and large-celled (Corethron pennatum and Thalassiothrix antarctica) diatoms are the major species (Hart 1934 and 1942, Hustedt 1958, van der Spoel et al. 1973, Laubscher et al. 1993, Smetacek et al. 1997, Zielinski and Gersonde 1997, Kemp et al. 2000, Smetacek et al. 2002). Near continental margins and island plumes where the iron input is much higher chlorophyll concentrations regularly exceed background levels and reach bloom proportions (Sullivan et al. 1993). These coastal blooms are either dominated by small, weakly silicified diatoms (Thalassiosira spp. and Chaetoceros spp. of the subgenus Hyalochaetae) or colonies of the haptophyte flagellate *Phaeocystis antarctica*.

Thus far the study of marine plankton ecology has tended to be based on summary parameters such as size classes or pigments and little attention has been paid to the ecological processes underpinning the large-scale variations in ocean productivity. The microscopic analysis of the diatom assemblage and other major components of the phytoplankton community described herein is unparalleled in its floristic details compared to previous iron fertilization experiments and is aimed to further the understanding of the mechanisms underlying species succession and bloom dynamics. Mesoscale in situ perturbation experiments like EisenEx now enable to follow the waxing and waning of plankton blooms in space and time under controlled experimental conditions and help to derive ecological principles that could improve ecosystem models.

Material and Methods

Results of an *in situ* mesoscale iron fertilization experiment (EisenEx) conducted in the Atlantic Sector of the Southern Ocean (~21°E, 48°S) in austral spring (6-29 November 2000) during the cruise ANT XVIII/2 of R.V. *Polarstern* are presented here. A cyclonic eddy (approximately 120 km wide) shed by the Antarctic Polar Front (APF) was chosen as the "container" for the experiment and its centre marked with a drifting buoy. An area of about 40 km^2 around the buoy was fertilized with 4 tonnes of acidified iron sulphate solution ($FeSO_4$) on three occasions at intervals of 8 days (Bathmann and Smetacek 2001). Sulphurhexafluoride (SF_6) was added as an inert tracer to the first iron infusion in order to relocate the iron fertilized "patch". Since SF_6 can be rapidly measured on board, measurements of the fertilized (inside the patch) and reference (outside the patch) waters can be made online while the ship is steaming. Inside and outside stations were chosen according to SF_6 concentration measured along areal surveys of surface properties. So called "in-stations" were situated at the highest observed SF_6 concentrations situated close to the centre of the iron-fertilized patch. "Out-stations" were located in waters with background SF_6 concentrations usually located in the vicinity of patch. However each time different water masses were sampled that differed in several of the chemical and biological parameters and concentration differences between outside stations should therefore not be interpreted as temporal trends (Riebesell et al. to be submitted). Two of the in-stations were, in fact, situated at the edge of the iron-enriched patch ("edge-stations") as indicated by intermediate SF_6 concentrations. The two edge stations are not discussed as long as they do not considerably deviate from outside values. The station occupied two days prior to fertilization within the centre of the eddy was chosen as the initial reference station for a better comparison of in- and outside stations. During the three weeks of the experiment the fertilized patch circled within the eddy and increased its area to about 950 km^2 (Watson et al. 2001). During EisenEx the response to iron fertilization was analysed in detail and compared with processes in the surrounding water for three weeks.

For quantitative assessment of the phytoplankton assemblage water samples were obtained from Niskin bottles attached to a Conductivity Temperature Depth (CTD) rosette from 7 discrete depths between 10 and 150 m at 19 in, edge and out patch stations. Water samples of 200 ml were preserved with hexamine buffered formaline solution at a final concentration of 2% and stored at 4°C in the dark for subsequent counting back in the home laboratory. Cells were identified and enumerated using inverted light and epifluorescence microscopy

(Axiovert 25, Axiovert 135 and IM 35) according to the method of Utermöhl (1958). Water samples were settled in 50 ml Hydrobios sedimentation chambers for 48 hours. Organisms were counted at magnifications of 200-640× according to the size of the organisms examined. Very abundant species were counted in stripes, abundant species in a quarter or half a chamber and less abundant species in the whole chamber. Each sample was examined until at least 400 cells had been counted.

Cell sizes of species were measured and their biovolume calculated from equivalent geometrical shapes (Edler 1979). Cell volume was converted to cellular carbon content through recommended carbon conversion equations using the following carbon to volume relationships (Menden-Deuer and Lessard 2000): for small diatoms (<3000 μm^3), C $cell^{-1}$ = $0.288 \times V^{0.811}$; for large diatoms (>3000 μm^3), C $cell^{-1}$ = $0.117 \times V^{0.881}$; for prymnesiophytes; C $cell^{-1}$ = $0.228 \times V^{0.899}$; for dinoflagellates, C $cell^{-1}$ = $0.444 \times V^{0.864}$ and for chrysophytes, C $cell^{-1}$ = $0.020 \times V^{1.218}$; with V representing total cell volume (μm^3) and C the estimated cellular carbon content (pg).

The temporal development of the diatom assemblage inside and outside the fertilized patch is described in detail for total diatoms and eight characteristic species. The complete list of species with abundance and biomass values two days prior to fertilization (day –2) and on day 21 inside as well as outside the patch is given in table 1. Accumulation rates- the balance between growth and mortality rates – were derived for all important diatom species and groups for the period of spurt growth. The temporal development of the diatom standing stock is given in detail for the eight most dominant species and groups in terms of carbon. Abundance and biomass of all species described herein are trapezoidal depth-integrated values for the upper 0-80 m of the water column. Abundance values were back calculated to litre according to:

$$N_t = ((\int^{0-80} N)/z)/1000$$

where N_t is the average abundance at time t, $\int^{0-80} N$ the depth integral and z the depth (80 m).

Net population accumulation rates (k) were calculated from integrated abundances according to the following equation:

$$k(d^{-1}) = \ln(N_t/N_0)/\text{time (in days)}$$

where N_0 is the initial abundance and N_t the abundance at time t.

Furthermore cell size distribution and chain-length spectra of the dominant chain-forming diatom species as well as intact empty and broken diatom frustules were followed in the water samples. For further details on empty and broken diatom frustules see Assmy and Henjes (to be submitted). The cell size of the different species and groups was determined by measuring the diameter for discoid diatoms, the apical axis for pennate diatoms and the length for cylindrical diatoms.

For *Phaeocystis antarctica* colonies the total content of a Niskin bottle (approximately 12 l) was concentrated to a volume of about 50 ml by pouring the water gently through a 10 µm mesh net. Concentrated samples were fixed with hexamine buffered formaline solution at a final concentration of 0.5%. Three ml of each sample were counted at a magnification of 200× in the whole chamber. Cells per colony were estimated from colony size and recognisable cells inside the colony. The mucous carbon of *Phaeocystis* colonies was calculated using the relationship ng C mucus = 213×(Colony volume in mm^3) (Mathot et al. 2000). *Phaeocystis* colonies in the samples were spherical in shape and ranged from 20-300 µm in diameter hence an average colony diameter of 160 µm was assumed.

Fine-meshed hand nets (20 and 55 µm) were used at each in and out patch station to collect plankton samples for total preparations of diatom frustules for confirmation of species identification. Species identification was carried out to species level as far as possible during counting. Otherwise organisms were grouped according to genus or in shape and size classes.

Results

Environmental setting

Horizontal dispersion of the fertilized patch doubled its area every four to five days on average (Watson et al. 2001) and caused an increase from initially 40 km^2 to 950 km^2 by day 21 (Riebesell et al. to be submitted). The mixed layer depth varied considerably over the course of the experiment. At the beginning of the experiment the seasonal mixed layer depth of 10-40 m was relatively shallow for spring both inside and outside the patch providing favourable light climate for phytoplankton growth. Increased wind velocities on day 5 deepened the mixed layer down to 60-70 m depth. After wind relaxation the mixed layer depth shoaled again and the intrusion of fresh water due to precipitation caused a transient shallow mixed layer during the middle of the experiment. During the final phase two strong wind events (up to 25 m s^{-1}) increased vertical mixing and a mixed layer depth of 75-85 m prevailed during the end of the experiment. Surface temperatures increased from initially 3.5 °C to 4.0 °C by day 21. Below 100 m depth the temperature ranged between 1.5 and 2 °C indicative of an eddy shed by the Antarctic Polar Front (APF). Surface salinity was 33.8 ‰ steadily increasing with depth to about 34 ‰ at 200 m. Elevated surface salinities were found outside the patch during the second half of the experiment (Strass et al. 2001). Favourable incident photosynthetic active radiation (PAR) prevailed over the course of the experiment with a mean down-welling irradiance ($\sum E_a$) of 34.2 ± 13.7 mol photons m^{-2} d^{-1}. On six sampling days the daily sum of down-welling irradiance was low (mean $\sum E_a$ = 20.5 ± 7.2 mol photons m^{-2} d^{-1}) and the critical depth, calculated according to Nelson and Smith (1991), (z_c) <100 m. On most other sampling days $\sum E_a$ averaged 45.5 ± 6.9 mol photons m^{-2} d^{-1} and z_c exceeded 100 m. The euphotic depth (z_{eu}) steadily decreased from initially >60 m to 41 m by the end of the experiment inside the patch due to self-shading by phytoplankton accumulation. Outside the patch z_{eu} remained roughly at initial values (Gervais et al. 2002).

The areal daily primary production increased inside the patch and reached its maximum of 790 mg C m^{-2} d^{-1} on day 16, and decreased thereafter. The fast repetition rate fluorometer (FRRF) derived potential photochemical efficiency (F_v/F_m) showed an iron-mediated increase from initially 0.3 to values as high as 0.56 inside the patch with the strongest relative increase in the large cell fraction (> 20 µm). Chlorophyll-a concentrations increased from initially 0.5 mg m^{-3} to maximal values of 2.5 mg m^{-3} in the upper 80 m inside the patch by the end of the experiment (Gervais et al. 2002). Transmission values co-varied with chlorophyll-a

fluorescence both indicating the increase in phytoplankton particle abundance inside the patch. The increase in cellular chlorophyll fluorescence was 2 to 2.5 fold in the small size fraction (<5 µm) but 4 fold in the > 5 µm cells. Hence, the increase in biomass is partly due to the increase in chlorophyll content per cell (ca. 3 fold) in addition to an increase in cell numbers. In contrast to the iron-mediated increase in diatom cell numbers the abundance of the < 5 µm phytoplankton fraction remained more or less constant over the course of the experiment (Veldhuis and Timmermans 2001).

Prior to fertilization ambient dissolved iron (< 0.2 µm) concentrations in the surface mixed layer were less than 0.1 nM (Nishioka et al. 2001) indicating that phytoplankton were iron stressed. All major nutrients decreased during the experiment with nitrate by 2 µM, phosphate by 0.2 µM and silicate by 4 µM but were still abundant by day 21 inside the patch (>21 µM nitrate, > 1.5 µM phosphate, >10 µM silicate; average over 80 m depth) (Hartmann et al. 2001). The 80 m depth integrated particulate organic carbon (POC), particulate organic nitrogen (PON) and biogenic silica (BSi) standing stocks had increased from 439 mmol m^{-2}, 82 mmol m^{-2} and 61 mmol m^{-2} at the beginning of the experiment to 679 mmol m^{-2}, 126 mmol m^{-2} and 227 mmol m^{-2} respectively by day 21 inside the patch (Riebesell et al. to be submitted). Ambient levels of DMS and DMSP were already high at the start of the experiment and only minor changes occurred over the three weeks of the experiment (Turner and Chuck, 2001). Bacterial abundance roughly doubled inside the patch and remained constant outside with no changes in bacterial community composition (Arrieta et al. to be submitted).

Temporal development of the diatom assemblage

Species composition, abundance and accumulation rates

A total of 43 diatoms were identified to species or genus level in samples collected during EisenEx. A variety of centric diatoms with a disc-like shape could not be satisfactorily identified under light microscopy and were hence grouped as discoid diatoms according to size.

The species assemblage two days prior to fertilization was dominated by the two heavily silicified pennate diatoms *Fragilariopsis kerguelensis* and *Thalassionema nitzschioides* as well as by the small *Cylindrotheca closterium* and small discoid diatoms (<30 µm). Following iron fertilization the total diatom abundance increased 7-fold from initially $63*10^3$ cells l^{-1} to

$444*10^3$ cells l^{-1} by day 21 inside the patch (Fig. 1A). This corresponded to an accumulation rate for the total diatom assemblage of 0.10 d^{-1} inside the patch. *Pseudo-nitzschia lineola*, small discoid diatoms and another 8 diatom species accounted numerically for 98% of the increase in cell numbers at the end of the experiment. Outside the patch total diatom abundance stayed more or less constant with slightly higher abundances of $88*10^3$ cells l^{-1} towards the end of the experiment. The accumulation rate of 0.02 d^{-1} for the whole population in iron-limited control waters was considerably lower than inside the patch. The minor increase in out-patch waters was mainly due to the accumulation of *Pseudo-nitzschia* species and large cylindrical diatoms (Table 1, Fig. 1A). Total diatom abundances were significantly higher inside the patch compared to outside the patch after day 8 (unpaired t-test; $p < 0.05$).

Four major response types to iron fertilization can be distinguished between diatom species over the course of the experiment. Response type I is characterised by fast growing and weakly silicified diatoms with sustained exponential growth rates. Response type II species show an initial lag phase with negligible growth for the first week followed by a linear increase in abundance thereafter. Species belonging to response type III exhibited linear growth with no initial lag phase. Response type IV species are characterised by an initial linear increase and a decline during the second half of the experiment.

Response type I species almost exclusively belonged to the genera *Pseudo-nitzschia* and *Chaetoceros* of which *Pseudo-nitzschia lineola* and *Chaetoceros curvisetus* were the most characteristic (Figs. 1B and C). Both species showed an immediate response to iron fertilization with an exponential increase in cell numbers and exhibited the highest accumulation rates of 0.20 d^{-1} and 0.18 d^{-1} respectively observed during the experiment. Other species with a similar growth pattern were *Pseudo-nitzschia turgidula*, *P. turgiduloides*, *P. heimii*, *Chaetoceros dichaeta*, *C. convolutus* and *Corethron inerme*. Especially *P. turgidula, P. turgiduloides* and *P. heimii* showed high accumulation rates of 0.14 d^{-1}, 0.15 d^{-1} and 0.14 d^{-1} respectively until day 19 inside the patch.

During EisenEx *Pseudo-nitzschia lineola* dominated the bloom at the end of the experiment. Initially this species accounted numerically with $3*10^3$ cells l^{-1} for 5% of the total diatom assemblage but increased to $234*10^3$ cells l^{-1} by day 21 accounting for 53% of the whole population (Fig. 1B). This equals roughly a 80-fold increase in abundance within three weeks. Already during the first days of the experiment abundances of *P. lineola* exhibited a strong response to iron addition and further increased after day 11 with an exponential growth curve. Outside the fertilized patch abundances of *P. lineola* also increased to $21*10^3$ cells l^{-1}. Among all diatom species *P. lineola* showed the highest accumulation rate of 0.20 d^{-1} in response to

iron addition. Even outside the patch the net growth of *P. lineola* was relatively high with 0.09 d^{-1}. *Chaetoceros curvisetus* was the most abundant *Chaetoceros* species at the end of the experiment and showed a steep increase in cell numbers after iron addition. Inside the patch abundances increased from initially $<10^3$ cells l^{-1} to $19*10^3$ cells l^{-1} after three weeks (Fig. 1C). Similar to *P. lineola* the increase in cell numbers of *C. curvisetus* was observed immediately after iron fertilization. Outside the patch cell numbers remained low and stayed at initial levels. Over the whole duration of the experiment *C. curvisetus* exhibited the second highest accumulation rate of 0.18 doublings per day in response to iron addition. Outside the patch *C. curvisetus* accumulated with only 0.01 d^{-1}.

Response type II was best characterised by *Fragilariopsis kerguelensis* and *Thalassionema nitzschioides* (Figs. 1D and E). These heavily silicified species showed only very minor increases in abundance during the first week of the experiment. Thereafter both species showed a linear increase with accumulation rates of 0.15 d^{-1} for *F. kerguelensis* and 0.09 d^{-1} for *T. nitzschioides*. *F. kerguelensis* however showed no further increase after day 16 and seemed to have reached a plateau. Species with a similar growth pattern comprised *Navicula* spp., *Thalassiosira oliverana* and *Dactyliosolen antarcticus*.

Initial abundances of *Fragilariopsis kerguelensis* were relatively high with $7.5*10^3$ cells l^{-1} and accounted for 12% of the total diatom assemblage at the start of the experiment. Cell numbers further increased to $23*10^3$ cells l^{-1} by day 16 inside the patch. After day 16 no further increase was observed and cell numbers stayed at roughly $22*10^3$ cells l^{-1} (Fig. 1D). The relative contribution of *F. kerguelensis* to the total diatom population had declined to 5%. Outside the fertilized patch abundances varied between $4*10^3$ cells l^{-1} and $9*10^3$ cells l^{-1} with no clear trend. *F. kerguelensis* showed an accumulation rate of 0.15 d^{-1} during the growth phase of the second week inside the patch. In outside waters this species accumulated with 0.02 d^{-1} over the whole duration of the experiment. Prior to fertilization *Thalassionema nitzschioides* was the second most abundant diatom species with $10*10^3$ cells l^{-1} accounting for 17% of the total diatom assemblage. Cell numbers of *T. nitzschioides* further increased to $48*10^3$ cells l^{-1} by day 21 inside the patch although its relative importance decreased to 11 % of the total diatom abundance (Fig. 1E). Outside the patch a reverse trend could be observed with a decrease to $5*10^3$ cells l^{-1} by day 21. During linear growth inside the patch *T. nitzschioides* accumulated with 0.09 doublings per day whereas in adjacent waters a decline in cell numbers was observed.

Corethron pennatum and *Haslea* sp. were representative species for response type III (Figs. 1F and G). Both species showed an immediate linear increase in cell numbers after iron

addition with accumulation rates of 0.08 d^{-1}. Diatoms with a similar growth strategy were small (<30 µm) and large (>30 µm) discoid diatoms, *Guinardia cylindrus*, *Rhizosolenia chunii*, *Proboscia alata*, *Leptocylindrus mediterraneus*, *Thalassiothrix antarctica*, *Asteromphalus hyalinus*, *Thalassionema nitzschioides* var. *lanceolatum* and *Membraneis imposter*.

The most important cylindrical diatom during EisenEx was *Corethron pennatum*. This species exhibited a linear increase in abundance from initially $0.7*10^3$ cells l^{-1} to $4.6*10^3$ cells l^{-1} by day 21 inside the patch (Fig. 1F). Outside the patch a doubling in cell numbers to values of $1.7*10^3$ cells l^{-1} was observed. *C. pennatum* accumulated with 0.08 d^{-1} inside and 0.04 d^{-1} outside the patch. The pennate diatom *Haslea* sp. showed a similar linear increase in cell numbers from initially $0.5*10^3$ cells l^{-1} to $3*10^3$ cells l^{-1} by day 21 (Fig. 1G). Cell numbers at the edge stations were considerably higher than abundances outside the patch. In out-patch waters abundances stayed constant and even remained below initial values. *Haslea* sp. exhibited a negative slope outside whereas inside the patch an overall accumulation rate of 0.08 d^{-1} was observed.

Two species characteristic of response type IV were an unidentified, small *Nitzschia* sp. and *Cylindrotheca closterium* (Figs. 1H and I). *Nitzschia* sp. and *C. closterium* both showed an initial linear increase until day 11 and 16 with accumulation rates of 0.03 d^{-1} and 0.07 d^{-1} respectively and decreased thereafter to approximately pre fertilization cell numbers by the end of the experiment. Other species with an initial increase and a decline thereafter included *Pseudo-nitzschia prolongatoides*, *Thalassiosira gracilis*, *Azpeitia tabularis*, *Chaetoceros aequatorialis* and *Fragilariopsis rhombica*.

Abundances of *Nitzschia* sp. linearly increased inside the patch from initially $1.6*10^3$ cells l^{-1} to $2.6*10^3$ cells l^{-1} by day 11 and declined thereafter to values of $1.1*10^3$ cells l^{-1} after three weeks (Fig. 1H). The same trend was observed outside the patch with an increase to $2.7*10^3$ cells l^{-1} by day 11 and a decrease to $1.4*10^3$ cells l^{-1} on day 21. Accumulation rates of *Nitzschia* sp. during growth period were recorded with 0.03 d^{-1} inside and 0.04 d^{-1} outside the patch.

Cylindrotheca closterium almost tripled in abundance after iron addition from initially $15*10^3$ cells l^{-1} to $41*10^3$ cells l^{-1} by day 16, thereafter cell numbers dropped to values of $24*10^3$ cells l^{-1} at the end of the experiment (Fig. 1I). Outside the patch abundances, although variable between 9 and $20*10^3$ cells l^{-1}, remained close to initial concentrations. *C. closterium* declined outside the patch and accumulated with 0.07 d^{-1} inside the patch until day 16.

All other species not mentioned above exhibited either no consistent trend in response to iron addition like *Chaetoceros neglectus*, *C. atlanticus*, *Thalassiosira lentiginosa*, *T. oestrupii* and *Guinardia delicatula* or were actually characterized by a linear decrease in abundance inside the patch like *Chaetoceros* spp. and *C. peruvianus*. Rare species like *Actinocyclus curvatulus*, *Chaetoceros bulbosus*, *Eucampia antarctica*, *Fragilariopsis obliquecostata*, *Nitzschia bicapitata*, *Rhizosolenia curvata* or *Rhizosolenia hebetata* f. *semispina* were underrepresented in water samples collected during EisenEx and hence have not been considered further.

Biomass

The total diatom standing stock in the upper 80 m increased by more than a factor of four from 790 mg C m^{-2} to 3508 mg C m^{-2} during the course of the experiment (Fig. 2A). *Dactyliosolen antarcticus* and other large cylindrical diatoms dominated the initial diatom standing stock and together made up 44% of the total biomass. *Pseudo-nitzschia lineola*, *Corethron pennatum*, *Haslea* sp., small discoid diatoms, *D. antarcticus*, *Proboscia alata*, *Chaetoceros curvisetus* and *Fragilariopsis kerguelensis* dominated in terms of carbon and accounted for 70% of the diatom standing stock increase inside the patch at the end of the experiment. Outside the patch diatom biomass roughly doubled to 1550 mg C m^{-2} after three weeks. This diatom biomass increase in outside waters was mainly due to an increase in abundance of large cylindrical diatoms (Table 1, Fig. 2A). By day 16 the diatom biomass was significantly higher inside the patch than outside the patch (unpaired t-test; $p < 0.05$).
The dominant diatom at the end of the experiment *Pseudo-nitzschia lineola* increased 80-fold from initially 11 mg C m^{-2} to 883 mg C m^{-2} by day 21 in response to iron addition (Fig. 2B). This species accounted for 25% of the diatom standing stock after three weeks. Despite elevated biomass of 70 mg C m^{-2} after three weeks the relative contribution of *P. lineola* accounted for 5% of the total diatom crop outside the patch. The second most important species in terms of biomass was *Corethron pennatum* which increased inside the patch from initially 52 mg C m^{-2} to 446 mg C m^{-2} by day 21 and accounted for 13% of the total diatom carbon at the end of the experiment (Fig. 2C). Outside the patch a three-fold increase to 164 mg C m^{-2} after three weeks was recorded. Standing stocks of *Haslea* sp. started with 39 mg C m^{-2} prior to fertilization and reached 227 mg C m^{-2} after three weeks inside the patch (Fig. 2D). Outside the patch biomass stayed at initial levels and reached 30 mg C m^{-2} on day 21. Considerably higher biomass values of *Haslea* sp. compared to control waters were recorded at the edge stations. Inside the patch small discoid diatoms increased from initially 91 mg C m^{-2} to 220 mg C m^{-2} by day 21 (Fig. 2E). Out-patch values were only slightly higher at the

end of the experiment (110 mg C m^{-2}) compared to initial standing stocks. Prior to fertilization *Dactyliosolen antarcticus* was the prominent diatom in terms of carbon. Both in fertilized and non-fertilized waters biomass of *D. antarcticus* exhibited a similar increase from initially 157 mg C m^{-2} to 210 mg C m^{-2} inside and 269 mg C m^{-2} outside the patch by day 21 (Fig. 2F). The same applies to *Proboscia alata*. This species increased from initially 4 mg C m^{-2} to 199 mg C m^{-2} inside and 154 mg C m^{-2} outside the patch (Fig. 2G). *Chaetoceros curvisetus* showed a strong response to iron addition and accumulated from initially 5 mg C m^{-2} to 138 mg C m^{-2} by day 21 inside the patch (Fig. 2H). Outside the patch the biomass of *C. curvisetus* remained at initial levels and stayed below 10 mg C m^{-2}. A threefold increase of *Fragilariopsis kerguelensis* inside the patch from 53 mg C m^{-2} to 162 mg C m^{-2} until day 16 was observed and thereafter biomass decreased to 137 mg C m^{-2} by day 21 (Fig. 2I). Outside the patch no change in the standing stock of *F. kerguelensis* was observed.

Other important diatom species in terms of carbon inside the patch included *Pseudo-nitzschia heimii*, *P. turgidula*, *Rhizosolenia chunii*, *Thalassionema nitzschioides*, *Guinardia cylindrus*, *Corethron inerme*, *Leptocylindrus mediteraneus* and *Thalassiosira tumida*. Together these species accounted for roughly another 20% of the total diatom standing stock at the end of the experiment. Other species than the above mentioned contributed only little to the iron induced diatom biomass increase and were all well below 50 mg C m^{-2} (Table 1).

Size composition of the diatom assemblage

We divided the diatom community into three size classes to assess the role of size in degree of response: small (<30 µm), medium sized (30-60 µm) and large (>60 µm) diatoms. The relative contribution of the different size classes in abundance and biomass values with the respective accumulation rates is given for inside and outside the patch.

Although abundances of all size classes increased inside the fertilized patch the total diatom assemblage shifted from a community initially dominated by small diatoms to one that was dominated by large diatoms at the end of the experiment. This shift was due to the higher accumulation rate of large cells (0.15 d^{-1}) compared to 0.06 d^{-1} for medium and small sized cells (Fig. 3A). Prior to and at the start of the experiment the small size class made up 44-47% (~ $25*10^3$ cells l^{-1}), the medium size class contributed 33-37% (~ $20*10^3$ cells l^{-1}) and the large size class accounted for roughly 19% (~ $11*10^3$ cells l^{-1}) of the total assemblage. At the end of the experiment the large cells had increased to 63% ($254*10^3$ cells l^{-1}) whereas the proportion of small and medium size classes made up 22 ($87*10^3$ cells l^{-1}) and 15% ($60*10^3$

cells l^{-1}) respectively (Figs. 3A and C). At the beginning of the experiment *Cylindrotheca closterium*, *Thalassionema nitzschioides, Fragilariopsis kerguelensis* and small discoid diatoms dominated the total assemblage. The increase in large cells towards the end of the experiment was mainly due to the chain-forming diatom *Pseudo-nitzschia lineola*. This shift in the size composition reflected the changes in diatom abundance and assemblage composition induced by iron fertilization. Outside the fertilized patch only the large sized cells accumulated with 0.06 d^{-1} over the course of the experiment whereas the small and medium size class exhibited no increase in abundance (Fig. 3B). At the start of the experiment the relative contribution of the different size classes was similar to the inside situation with the small cells contributing >40% ($25*10^3$ cells l^{-1}), the medium cells >30% ($21*10^3$ cells l^{-1}) and the large cells roughly 19% ($11*10^3$ cells l^{-1}) to the total diatom assemblage. After three weeks large cells accounted for 50% ($39*10^3$ cells l^{-1}) of the assemblage outside the patch whereas small and medium sized cells made up 27 ($22*10^3$ cells l^{-1}) and 23% ($18*10^3$ cells l^{-1}) respectively (Figs. 3B and D). The increase of large cells outside the patch was mainly due to the accumulation of *Pseudo-nitzschia* species and large cylindrical diatoms.

All diatom size classes showed a biomass increase inside the patch. The large size fraction exhibited the strongest response to iron addition increasing from 489 mg C m^{-2} prior to fertilization to 2675 mg C m^{-2} after three weeks. The small and medium sized cells increased from initially 162 mg C m^{-2} and 139 mg C m^{-2} to 500 mg C m^{-2} and 322 mg C m^{-2} at the end of the experiment respectively. The respective biomass accumulation rates were 0.09 d^{-1} for large and 0.05 d^{-1} for small and medium sized cells (Fig. 3E). The large cells already contributed 62% to the total diatom standing stock prior to fertilization and further increased to 77% by day 21. The smaller size classes therefore declined in relative importance and accounted for less than a quarter of the diatom biomass after three weeks (Fig. 3G). Outside the patch the large size class accumulated 1257 mg C m^{-2} (0.05 d^{-1}) over the investigation period and was solely responsible for the diatom biomass increase accounting for 81% of the total diatom standing stock after three weeks. Both the small and medium size class showed no build up of biomass and remained at initial concentrations (Figs. 3F and H).

Chain length of *Fragilariopsis kerguelensis*

Cells in the ribbon shaped chains of *Fragilariopsis kerguelensis* were counted to estimate whether iron addition increases chain length. All chain lengths are the mean over the upper 80 m surface layer.

Chains of *F. kerguelensis* showed an increase of 0.21 cells chain^{-1} d^{-1} in response to iron addition (Fig. 4). Initially the average chain length consisted of 7.8 cells chain^{-1} two days prior to fertilization and 5.6 cells chain^{-1} on day 0 and increased to 11.3 cells chain^{-1} by day 21. Outside the patch no increase in chain length was observed. Significantly longer chains of *F. kerguelensis* were found inside the patch compared to control waters after day 11 (unpaired t-test; $p < 0.05$).

Empty and broken diatom frustules

Both the total number of empty and broken diatom frustules increased over the course of the experiment (Figs. 5A and B). Despite the increase in absolute numbers of empty and broken diatom frustules the relative contribution of both fractions to the total diatom assemblage decreased with time inside the patch. Initially both empty and broken frustules contributed roughly 25% to total diatom abundance. At the end of the experiment this fraction had declined to 9% inside the patch. This was due to the proportionally stronger increase of live cells. Outside the patch the relative contribution of empty and broken frustules to the total assemblage accounted for 20% after three weeks. The ratio of full cells to empty and broken frustules increased from initially 3:1 to 10:1 inside and 4:1 outside the patch by day 21.

Empty diatom frustules doubled inside the patch from initially $16*10^3$ empty frustules l^{-1} to $32*10^3$ empty frustules l^{-1} whereas constant values were observed outside the patch (Fig. 5A). Initially small discoid diatoms, *Thalassionema nitzschioides* and *Fragilariopsis kerguelensis* contributed most to empty diatom frustules with 35, 30 and 13 % respectively. At the end of the experiment *Pseudo-nitzschia* spp. (mainly *Pseudo-nitzschia lineola*) dominated with 45% of total empty diatom frustules inside the patch. The above-mentioned diatoms still contributed 20, 20 and 8% respectively. Outside the patch empty frustules of *Pseudo-nitzschia* spp. also increased in relative importance from 3% to 24%. Nevertheless the shift was not as strong as inside the patch (Fig. 5C).

The amount of broken diatom frustules tripled inside the patch from $4*10^3$ broken frustules l^{-1} to $13*10^3$ broken frustules l^{-1}. A doubling in broken frustules was detected in control waters where final numbers reached $8*10^3$ broken frustules l^{-1} (Fig. 5B). Other diatoms contributed 50% to total broken diatom frustules two days prior to fertilization. After three weeks the relative contribution of *Pseudo-nitzschia* spp. had increased from initially 0.8% to 47% inside the patch. Broken frustules of *Thalassionema nitzschioides* and *Fragilariopsis kerguelensis* were still a considerable fraction of the total numbers with 16 and 11% respectively. Outside

the patch broken *Pseudo-nitzschia* frustules also showed the strongest increase although the relative contribution of the different species and groups changed but not as dramatically as inside the patch (Fig. 5D).

Temporal development of non-diatom phytoplankton

Abundance and species composition

Depth integrated abundance and biomass of non-diatom phytoplankton includes single cells, cells in colonies and colonies of *Phaeocystis antarctica*, coccolithophores, photosynthetic (plastidic) dinoflagellates and the silicoflagellate *Dictyocha speculum*.

The total abundance of *Phaeocystis antarctica* inside the patch including both solitary and colonial cells, increased from initially $20*10^3$ cells l^{-1} to $138*10^3$ cells l^{-1} by day 16; thereafter cell numbers decreased and were recorded with $49*10^3$ cells l^{-1} by the end of the experiment (Fig. 6A). Cell numbers at the two edge stations resembled the inside trend with higher abundance at the station prior to day 16 and a decrease at the second edge station on day 17. Outside the patch the depth integrated total abundances of *P. antarctica* varied between $11*10^3$ cells l^{-1} and $29*10^3$ cells l^{-1} with no consistent change over the course of the experiment. Solitary cells of *P. antarctica* dominated by far the total abundance. Cells in colonies accounted on average for less than 2% of the total population both inside as well as outside the fertilized patch. Despite their relatively small contribution to the total abundance, the number of *P. antarctica* colonies and cells in colonies increased significantly inside the patch. Colonies accumulated from initially 7 colonies l^{-1} to 39 and 30 colonies l^{-1} on days 19 and 21 respectively (Fig. 6A1). The number of cells in colonies increased from roughly 200-300 cells l^{-1} during the first days of the experiment to $1.6*10^3$ and $1.3*10^3$ cells l^{-1} on days 19 and 21 respectively (Fig. 6A2). A colony contained on average 39 cells $colony^{-1}$ with no consistent increase in the number of cells $colony^{-1}$ observed over the course of the experiment. Outside the patch both colonies and cells in colonies showed no increase over the duration of the experiment with colony numbers ranging from 3-16 colonies l^{-1} and cells in colonies from 50-400 cells l^{-1}. Accumulation rates for single cells, colonies and cells in colonies amounted to 0.07 d^{-1}, 0.08 d^{-1} and 0.10 d^{-1} respectively inside the patch.

Although coccolithophores could not be distinguished to genus or even species level under light microscopy, scanning electron microscopy (SEM) investigations revealed only one species: *Emiliania huxleyi*. Coccolithophores were the most abundant phytoplankton group

apart from the microbial autotrophic community at the beginning of the experiment even exceeding cell numbers of total diatoms. Initial abundances averaged over a 80 m water column were in the range of 165-175*10^3 cells l^{-1}. Thereafter cell numbers steadily decreased with time both inside and outside the patch and exhibited concentrations of about 90*10^3 cells l^{-1} at the end of the experiment (Fig. 6B). Coccolithophore abundances inside and outside the patch were not significantly different.

The group of photosynthetic dinoflagellates comprised athecate and thecate dinoflagellates. With the exception of the genus *Prorocentrum* and the species *Mesoporus perforatus* no further taxonomic identification was possible under light microscopy. Thecate phototrophic dinoflagellates were numerically dominated by the genus *Prorocentrum* accounting on average for 68% inside and 61% outside the patch of total abundance. Depth integrated abundances (0-80 m) of photosynthetic dinoflagellates increased after iron addition from 5*10^3 cells l^{-1} two days prior to fertilization to 15*10^3 cells l^{-1} on day 11. Thereafter cell numbers decreased and accounted for 8*10^3 cells l^{-1} on day 21 (Fig. 6C). The accumulation rate was 0.12 d^{-1} during the first 11 days of the experiment inside the patch. Outside the patch depth integrated abundances varied between 3-7*10^3 cells l^{-1} with higher values two days prior to fertilization and during the first five days compared to the last three out stations.

The 80 m depth integrated abundances of the silicoflagellate *Dictyocha speculum* inside the patch exhibited a moderate increase from initially 2*10^3 cells l^{-1} to 3*10^3 cells l^{-1} on day 21 accounting for an accumulation rate of 0.04 d^{-1} (Fig. 6D). Outside the patch depth integrated abundances varied around 2*10^3 cells l^{-1} with exceptionally high abundances of 4*10^3 cells l^{-1} on day 1.

Biomass

The integrated biomass (0-80 m) of total non-diatom phytoplankton is shown (Fig. 6E) as well as the relative contribution of the different species and groups to the total non-diatom standing stocks (Figs. 6F and G). Total non-diatom phytoplankton biomass increased from 485 mg C m^{-2} two days prior to fertilization to 944 mg C m^{-2} by day 11. Thereafter the standing stock declined to 777 mg C m^{-2} by day 21. Outside the patch a slight decrease in biomass to values of 371 mg C m^{-2} after three weeks was observed. Photosynthetic dinoflagellates contributed most to non-diatom phytoplankton standing stocks. They accounted for 51% (245 mg C m^{-2}) two days prior to fertilization and for 67% (522 mg C m^{-2}) by day 21 of the total non-diatom phytoplankton standing stock inside the patch. Outside the

patch the relative contribution of photosynthetic dinoflagellates to non-diatom phytoplankton standing stock varied between 45% (280 mg C m^{-2}) and 59% (353 mg C m^{-2}). *Prorocentrum* spp. contributed on average 77% to the total photosynthetic dinoflagellate standing stock inside as well as outside the patch. Depth integrated biomass of coccolithophores decreased from initially 23% (112 mg C m^{-2}) to 8% (59 mg C m^{-2}) of the total non-diatom phytoplankton standing stock by day 21 inside the patch. Although the absolute biomass values were the same inside and outside the patch the relative contribution of coccolithophores still accounted for 16% in control waters after three weeks. Biomass of *D. speculum* increased inside the patch from initially 114 mg C m^{-2} to 163 mg C m^{-2} on day 21 whereas outside the patch standing stocks stayed relatively constant at around initial values with the exception on day 1 with 213 mg C m^{-2}. The relative contribution of *D. speculum* to the total non-diatom phytoplankton biomass showed no consistent trend and varied between 11-24% inside and 21-35% outside the patch. In terms of biomass *Phaeocystis antarctica* played only a minor role and accounted on average for less than 10% of the total non-diatom phytoplankton carbon inside as well as outside the patch. Inside the patch *P. antarctica*, including single cells, cells in colonies and colony carbon, increased from 13 mg C m^{-2} two days prior to fertilization to 89 mg C m^{-2} by day 16. Thereafter biomass decreased and reached 33 mg C m^{-2} by the end of the experiment. Outside the patch the depth integrated total biomass of *P. antarctica* varied between 8 mg C m^{-2} and 19 mg C m^{-2} with no consistent change over the course of the experiment. Single cells of *P. antarctica* dominated by far the total biomass. The biomass of cells in colonies and mucus carbon of colonies accounted on average for 4% of the total standing stock of *P. antarctica* both inside as well as outside the fertilized patch. Despite their relatively small contribution to the total biomass colony carbon as well as carbon of cells in colonies increased inside the patch.

Discussion

Pre-conditions of EisenEx

The pre-conditions of EisenEx were characterised by a sparse (maximal $30*10^3$ cells l^{-1}) and variable but species-rich diatom assemblage (Assmy et al. 2001). Despite improving light climate and high macronutrient concentrations characteristic of austral spring as well as relatively shallow mixed layers diatom growth was limited due to low ambient dissolved iron concentrations typical of Southern Ocean HNLC waters. The phytoplankton community was dominated by the ubiquitous pico- and nanophytoplankton that accounted for roughly 90% of the chlorophyll standing stock of 0.5 µg l^{-1} (Gervais et al. 2002) characteristic of oceanic waters. This scenario is reported to be the rule in HNLC waters (Verity and Smetacek 1996; Detmer and Bathmann 1997).

The iron-mediated floristic shift

During the EisenEx experiment the impact of iron fertilization on the pelagic ecosystem of an ocean eddy in the ACC was successfully monitored over a period of three weeks. The iron enrichment resulted in a floristic shift from a pico- and nanophytoplankton dominated community to a distinct phytoplankton bloom that was mainly due to the accumulation of diatoms. Diatoms contributed the bulk of phytoplankton biomass at the end of the experiment (3,5 g C m^{-2} over 80 m depth) as indicated by a 7-fold increase in diatom abundance and a fourfold increase in diatom biomass (Figs. 1A and 2A). Outside the patch a slight spring increase in diatom stocks and abundances was observed. Non-diatom phytoplankton taxa showed differential responses to iron addition with no biomass accumulation in the case of picophytoplankton (Veldhuis and Timmermans 2001; Gervais et al. 2002), only little biomass build up in the case of *Phaeocystis antarctica*, photosynthetic dinoflagellates and *Dictyocha speculum* (Figs. 6A, C and D) or a decrease in biomass in the case of coccolithophores. Even the observed increase of nanophytoplankton (Gervais et al. 2002) can be largely explained by an accumulation of small diatoms like *Chaetoceros curvisetus* and *Thalassionema nitzschioides* (Figs. 1C and E). The most pronounced increase in chlorophyll a concentrations was, however, due to microphytoplankton (Gervais et al. 2002) that was almost exclusively represented by diatoms. This is also reflected in the diatom size class distribution with the greatest increase in large diatoms (> 60 µm) inside the patch (Fig. 3A). A detailed

microscopic analysis of water samples from four small-scale grid surveys (Harjes 2002) that were conducted over the course of the experiment to achieve a better spatial resolution of the fertilized patch revealed overall patchy plankton distributions over the entire grid areas. Nevertheless the central grid stations, according to highest SF_6 concentrations, showed a remarkably similar response of the phytoplankton community to the major in-patch stations and therefore confirm the floristic shift described above. Furthermore these results prove the coherence of the iron-enriched patch and that the processes inherent to it can indeed be interpreted as temporal trends.

Results from the microscopic analysis are also in good agreement with the EisenEx pigment data. Fucoxanthin, a diatom marker, showed a clear increase inside the patch and a slight increase outside consistent with our observations. The decrease in 19`-hexanoyloxyfucoxanthin, a prymnesiophyte marker, inside the patch corresponds with the decline in coccolithophore abundance. Peridinin and chlorophyll b, both markers of photosynthetic dinoflagellates and chlorophytes, were less affected by iron addition with slightly higher concentrations inside the patch. This is in accordance with the slight increase in photosynthetic dinoflagellate biomass observed by our microscopic analysis (Peeken 2001). Furthermore the tight correlation of chlorophyll *a* concentrations and diatom biomass inside the fertilized patch (Fig. 7A) clearly confirm that the iron-induced bloom was chiefly due to an increase in diatom standing stocks. Rising Si/C and Si/N ratios of particulate matter as well as of inorganic nutrient and carbon uptake over the course of the experiment further denote diatoms as the primary source for the observed increase in phytoplankton biomass (Riebesell et al. to be submitted). The ratio of biogenic silica (BSi) to diatom carbon (DC) however decreased inside as well as outside the patch (Fig. 7B). However, from day 7 onwards in-patch values are significantly lower ($P < 0{,}05$; unpaired T-test) than outside the patch. Unfortunately the mechanism underlying this decline in BSi/DC cannot be satisfactorily resolved because of the variable contribution of empty and broken diatom frustules as well as faecal pellets containing diatom frustules to the BSi pool. However, the decrease in the relative contribution of empty and broken frustules to total diatoms inside the patch (Fig. 5E), characteristic of an evolving bloom, was probably compensated by the increase of BSi containing faecal pellets (Henjes and Assmy to be submitted). Therefore the relative contribution of detrital BSi to bulk BSi might not have changed very dramatically over the course of the experiment. Other factors that could influence the BSi/DC ratio under iron-replete conditions are: changes in the diatom community (as had been observed during EisenEx), reduction of frustule thickness (Boyle 1998) and/or increasing C and N content of

the cell. Our results provide evidence that the shift from heavily silicified diatoms to weakly silicified ones probably caused much of the decline in the BSi/DC ratio observed during EisenEx.

Mechanisms regulating diatom succession and dominance

Within the diatom assemblage, species succession and dominance of species can be attributed to three factors: size of the seed population, growth and loss rates.

The seeding stock of a population is a crucial factor determining the dominance of a given species during the annual plankton increase in austral spring. The winter stock in turn will define the size of the inoculum population in the following year (Backhaus et al. 2003). Hence species with low mortality and the ability to remain in the winter mixed layer will exhibit high seeding populations in spring. This is emphasised by the high seeding stocks of heavily silicified and hence defended diatom species, e.g. *Fragilariopsis kerguelensis*, at the start of the experiment in early spring. Younger life stages and adults of small copepod species of the cyclopoid genus *Oithona* are reported to stay near the surface, where they are able utilize food resources throughout the year (Dubischar et al. 2002, Ashjian et al. 2003). Besides seasonal differences highest numbers of larger copepod species were always found beneath the thermocline especially during winter and early spring (Schnack-Schiel et al. 1998). The dominance and preponderance of *Oithona* spp. at shallow depths during our study (Henjes and Assmy to be submitted) indicates that species of this and probably other small copepod genera are indeed persistent grazers in the productive surface layer and hence might exert a critical control on the stock size of their respective prey organisms. Since species of the genus *Oithona* are known to preferentially graze on smaller food items (Atkinson 1996) and to a lesser extent on large diatoms (Dubischar et al. 2002) the latter will eventually be able to maintain a larger stocks over the winter. However all initially dominant diatom species, e.g. *Cylindrotheca closterium*, *Thalassionema nitzschioides* and *Fragilariopsis kerguelensis*, were not dominant at the end of the experiment and accounted for only a minor portion of the iron-mediated biomass increase (Table 1). *Pseudo-nitzschia lineola*, the dominant diatom inside the patch after three weeks, compensated the relatively low initial stock with its fast exponential growth and outgrew all other diatom species (Fig 1B). Hence the dominance of a given species is next to its seeding stock strongly determined by its growth potential.

A suite of loss terms, that include sinking, horizontal dispersion, vertical mixing and grazing, influence phytoplankton standing stocks. Sinking losses were assumed to be low since cells were still actively growing by the end of the experiment and therefore assumed to be iron-saturated. This is in good agreement with results from a previous iron fertilization experiment in the Southern Ocean (SOIREE) where reduced diatom sinking rates in response to iron addition were found and calculated sinking losses of iron-saturated, unaggregated cells were estimated to be ~1% d^{-1} (Waite and Nodder 2001). The persistence of the SOIREE bloom in surface waters of the ACC for several weeks as indicated by satellite images (Boyd et al. 2000) further suggest that aggregation and sinking of ACC phytoplankton is probably not the rule while the bloom is still building up.

Nelson and Smith (1991) made predictions from critical depth/mixing depth relationships and postulated a maximum chlorophyll a concentration of ~ 1 mg m^{-3} in Southern Ocean surface waters even if all macro- and micronutrients are in sufficient supply. Using a simple one-dimensional ecosystem model based on observations Mitchell et al. (1991) concluded that without increased stratification of the ACC surface mixed layer a substantial increase in phytoplankton standing stocks could not be realised and hence iron fertilization would fail to significantly mitigate atmospheric CO_2 derived from fossil fuels in the Southern Ocean. Despite increased wind speeds and vertical mixing causing the mixed layer to deepen to >80 m by the end of the experiment the diatom bloom was still evolving as indicated by the continuous increase in diatom abundance and biomass (Figs. 1A and 1A) as well as chlorophyll a concentration of 2.5 mg m^{-3} (Gervais et al. 2002). This iron mediated increase in phytoplankton standing stocks in a deeply mixed environment was additionally favoured by the high incident PAR and a critical depth well below 80 m during most sampling days (Gervais et al. 2002). It can thus be assumed that loses to mixing were minimal.

Severe storms not only caused the deepening of the surface mixed layer but also resulted in considerable horizontal dispersion of the fertilized patch (Watson, et al. 2001). Surrounding water masses were therefore constantly mixed into the iron enriched patch and diluted the fertilized patch especially at its margins. Abraham et al. (2000) noted the importance of horizontal stirring as a control on bloom development by mixing phytoplankton and iron out of the fertilized patch, but also entraining macronutrients like silica. However the dilution effect was strongest during the initial phase and decreased in importance during the further course of the experiment especially in the centre of the patch due to the increase in patch size. Furthermore the gradient in phytoplankton abundance between inside and outside the patch was initially not as distinct and hence the dilution effect on the assemblage not as pronounced.

It can hence be assumed that horizontal dispersion played a lesser role in the centre of the patch where the in patch stations were situated according to SF_6 concentrations and during the latter phase of the experiment when most of the increase in phytoplankton biomass occurred. The closed heat and fresh water budget indicates that advection of other water masses played a minor role over the course of the experiment (V. Strass personnal communication) and assumingly had no impact on the phytoplankton assemblage. It can also be concluded that phytoplankton growth was not constrained by nutrient limitation since all macronutrients were still in abundant supply by the end of the experiment and the three consecutive iron additions assured that iron was not becoming the limiting factor.

Despite high growth rates and rapid response to iron enrichment, as indicated by elevated F_v/F_m ratios (Gervais et al. 2002), production by small autotrophs was directly channelled to the microzooplankton community as documented by the initial increase of both the small autotrophs and heterotrophs. Within a matter of about a week microzooplankton grazing appeared to have compensated growth of small autotrophs that resulted in a subsequent decline of the latter (Henjes and Assmy to be submitted, Verity et al unpubl. data). This is in accordance with the general concept that biomass of small phytoplankton is rather grazer limited (top down control) than resource limited (bottom up control) (Cullen 1991). During EisenEx copepod grazing pressure increased inside the patch as indicated by the accumulation of empty and broken diatom frustules (Figs. 5A and B) and recognizable copepod faecal pellets as well as high mesozooplankton abundances especially towards the end of the experiment (Henjes and Assmy to be submitted, Krägefsky et al. to be submitted). These data indicate that grazing also played a pivotal role for larger phytoplankton. The dominance of diatom species like *Pseudo-nitzschia lineola*, leading to an increase in chlorophyll a and phytoplankton biomass, is clearly illustrated in the elevated accumulation rates inside the patch compared to outside values (Fig. 1B). This species could only maintain advanced accumulation rates by either reducing grazing induced mortality or increased growth rates. The intensified accumulation of broken and empty frustules of the dominant diatom genus *Pseudo-nitzschia* (Figs. 5C and D) indicates that the species of this genus could compensate grazing loses with high growth rates induced by iron fertilization compared to species that were less heavily grazed. To summarise the above it can be concluded that iron is the prominent factor limiting phytoplankton growth – especially diatom growth - in the ACC and that grazing exerts the strongest control on phytoplankton stocks.

Response of the diatom assemblage to iron fertilization

In order to categorise the diverse diatom assemblage and the response to iron fertilization the various species can be assigned to different categories as exemplified by the characteristic response types (Fig. 1). First there are the background species that are usually large in size and occur in low abundances but are characterised by relatively stable populations in space and time. During our study the background species were represented by large discoid diatoms like *Asteromphalus hookeri*, *Thalassiosira lentiginosa*, *T. tumida* and the large pennate diatom *Pleurosigma atlanticus*. These species showed no response to elevated iron concentration over the course of the experiment. *T. lentiginosa* is probably the best studied because it represents an oceanic species with a widespread distribution in Antarctic waters (Hart 1942, Zielinski and Gersonde 1997) and due to its robust valves *T. lentiginosa* is enriched in sediments and represents one of the prominent open ocean species in the Southern Ocean surface sediments (Hustedt 1958, Zielinski and Gersonde 1997).

Other diatoms exhibited an initial increase in abundance and a decline thereafter (response type IV, Figs. 1H and I). This category was characterised by small to medium sized species like *Nitzschia* sp. and *Cylindrotheca closterium*. Due to their favourable surface to volume ratio small cells are more competitive in the acquisition of nutrients and hence should out-compete large cells under iron-replete conditions. Nevertheless no accumulation of biomass in the case of *Nitzschia* spp. and only a slight accumulation in the case of *C. closterium* was observed for these species inside the patch. The decline in abundance during the second half of the experiment was most likely induced by micro- and more severe mesozooplankton grazing. The small chain-forming *Chaetoceros neglectus* showed a similar pattern with no consistent increase in cell numbers after iron addition although its somewhat larger congener *Chaetoceros curvisetus* showed a strong response despite considerably lower initial abundances. These discrepancies between morphologically similar species of the same subgenus might be due to differences in growth properties or a stronger grazing pressure exerted on *C. neglectus*.

Corethron pennatum and *Haslea* sp. characteristic of response type III (Figs. 1F and G) both showed a linear increase in cell numbers with no initial lag phase. Mainly large solitary diatoms with low initial abundances represented this response type. Despite their unfavourable surface to volume ratio these large diatoms were able to maintain accumulation

rates similar to or above those of many smaller species. This is most likely due to their large size that makes them less susceptible to grazing by microzooplankton especially ciliates. Furthermore they account for a considerable fraction of diatom biomass despite relatively low abundances (Figs. 2C, D, F and G). These large but often rare diatoms can actually contribute considerably to vertical particle flux and hence have a significant impact on ocean biogeochemistry (Kemp et al. 2000; Smetacek 2000). Among the species of response type III *C. pennatum* was the most important one during EisenEx. Hart (1934) describes *C. pennatum* as one of the most prominent of Antarctic plankton diatoms, being almost universally present but more abundant south of the Polar Front. *C. pennatum* is a thinly silicified species which is only occasionally found in the sedimentary record of the Southern Ocean (Crawford 1995) but deposits of almost monospecific layers of *C. pennatum* have been found in the Weddell Sea as well as in the Ross Sea (Jordan et al. 1991, Leventer et al. 1993). These monospecific layers are most likely due to a mass sexual phase of *C. pennatum* that triggers downward transport of empty diatom cell walls and seems therefore significant for the vertical silica flux in the Southern Ocean (Crawford 1995).

The two heavily silicified diatoms *Fragilariopsis kerguelensis* and *Thalassionema nitzschioides* characteristic of response type II (Figs. 1D and E) showed no or only a slight increase during the first week of the experiment respectively and a linear increase thereafter. The high seeding populations in austral spring imply that *F. kerguelensis* and *T. nitzschioides* were able to maintain considerable standing stocks throughout the winter. In the case of *F. kerguelensis* this might be due to low light adaptation (Mock personal communication) and/or reduced mortality. This is in accordance with descriptions of Hart (1934) who reports *F. kerguelensis* as a typical spring and autumn form which especially dominates the phytoplankton community in spring. Furthermore *F. kerguelensis* might reduce grazing induced mortality by its strong silification and as a consequence the frustules are the most abundant recognisable frustules in diatom oozes surrounding the Antarctic continent (Gersonde and Wefer 1987). The average chain length of *F. kerguelensis* had roughly doubled during the course of the experiment (Fig. 4E). These results are in good agreement with a similar increase in chain length for the same species during a previous iron fertilization experiment in the Southern Ocean (Gall et al. 2001). Chain formation decreases diffusive nutrient supply and therefore both high nutrient concentrations and strong turbulence are important prerequisites for chain formation (Pahlow et al. 1997) as was the case during EisenEx. Mann and Bates (2002) proposed to determine the physiological condition of pennate diatoms by simply examining the number of cells per chain because it can be

assumed that actively growing cells form long chains. The increase in chain length of *F. kerguelensis* is thus a further indication that this species was in good physiological condition and actively growing. Numerous observations that *F. kerguelensis* represents one of the most abundant and most characteristic endemic true oceanic diatom species in the Southern Ocean (Hustedt 1958; Hasle 1965; van der Spoel et al. 1973; Zielinski and Gersonde 1997) further support that these robust and persistent but slower growing species are dominant under iron deplete conditions typical of the open ACC. In contrast to this finding *F. kerguelensis* dominated the diatom assemblage with a peak abundance of $44*10^3$ cells l^{-1} by day 12 during the SOIREE bloom (Boyd et al. 2000). Nevertheless this abundance is in the same range of those observed during EisenEx where a maximum abundance of $35*10^3$ cells l^{-1} in 60 m depth on day 16 was observed (data not shown). Furthermore *F. kerguelensis* had by far the highest seeding stock during SOIREE and initially constituted 85-90% of the total diatom assemblage (Gall et al. 2001). The initial situation during EisenEx was characterised by a much more diverse diatom assemblage with no single species being universally dominant. During SOIREE the evolving bloom had to be abandoned during an earlier phase than the EisenEx bloom and therefore the rise of truly bloom forming species might have been missed. The fact that abundances of *F. kerguelensis* stagnated after day 16 of this study further indicates that this species is rather characterised by stable and persistent populations throughout the year.

Species of response type I profited most from iron fertilization as reflected in the highest accumulation rates of *Pseudo-nitzschia lineola* and *Chaetoceros curvisetus* (Figs. 1B and C) and belonged with one exception exclusively to the genera *Pseudo-nitzschia* and *Chaetoceros*. The EisenEx bloom had evolved mainly because of these chain-forming, fast-growing species. An increase in chain length of *Pseudo-nitzschia* species was not as obvious as in the case of *F. kerguelensis* but nevertheless slight increases were still observed especially for *P. heimii* (Fig. 4D). Both genera, *Pseudo-nitzschia* and *Chaetoceros*, have a widespread distribution throughout the world ocean (Hasle 1972; Fryxell and Medlin 1981) and are known to form blooms (Horner, et al. 2000, Booth, et al. 2002). They are also widely distributed throughout the Atlantic sector of the Southern Ocean (Hart 1934; Hustedt 1958) and are often associated with high chlorophyll a concentrations (Tremblay et al. 2002, Smetacek et al. 2002). Furthermore shipboard incubation experiments (Hutchins and Bruland 1998) as well as an *in situ* iron fertilization experiment (Tsuda et al. 2003) were dominated by species of these two genera. *In situ* accumulation rates of *P. lineola* and *C. curvisetus* are in the upper range of those observed for diatoms around the globe (Furnas 1990) indicating that these species are adapted to fast growth. The genus *Chaetoceros* can be distinguished into two

subgenera: *Phaeoceros* and *Hyalochaetae*. Species of the subgenus *Phaeoceros* are usually larger in size and characterised by strong, chloroplast bearing setae often armoured with conspicuous spines whereas species of the subgenus *Hyalochaetae* are typically small and equipped with thin setae containing no chloroplasts inside. Among the *Chaetoceros* species identified during EisenEx only two species, namely *C. curvisetus* and *Chaetoceros neglectus*, belonged to the subgenus *Hyalochaetae* whereas all other species were represented by the subgenus *Phaeoceros*. However, species of the subgenus *Hyalochaetae* seem to exhibit higher growth rates under iron replete conditions as indicated by the superior accumulation rates of *C. curvisetus*. Another *Hyalochaetae* species (*Chaetoceros debilis*) showed exceptionally high accumulation rates during an iron fertilization experiment in the subarctic Pacific (Tsuda et al. 2003). All these observations further support our finding that certain species, especially of certain genera, are dominant under iron-replete conditions and bloom due to their higher growth rates.

Response of non-diatom phytoplankton to iron fertilization

Non-diatom phytoplankton, including *Phaeocystis antarctica*, coccolithophores, photosynthetic dinoflagellates and *Dictyocha speculum*, showed no such clear response to iron fertilization as some of the diatom species. The increase in biomass of total non-diatom phytoplankton inside the patch (Fig. 6E) was relatively minor compared to the increase in diatom standing stocks and was mainly due to the accumulation of photosynthetic dinoflagellates (Fig. 6F). Initially *P. antarctica* and photosynthetic dinoflagellates showed a marked increase in abundance until day 16 and 11 respectively indicating that growth rates were iron limited prior to fertilization (Figs. 6A and C). Nevertheless abundances of both, *P. antarctica* and photosynthetic dinoflagellates, declined during the second half of the experiment. *P. antarctica* appears in two live stages, as a single celled flagellate form (6 µm) and in colonies of up to ca. 10 mm in diameter with several 1000 cells (Hamm 2000). The decline in abundances was only observed for solitary cells of *P. antarctica* whereas the number of colonies and cells in colonies further increased during the latter half of the experiment (Figs. 6A1 and A2). The decrease of solitary cells within the patch could well be due to grazer-induced mortality. Especially ciliate grazers are known to rapidly ingest *Phaeocystis* solitary cells (Verity 2000) and the increase in aloricate ciliate abundance inside the patch (Henjes and Assmy to be submitted) suggests that single cells were likely controlled by ciliate grazers. *P. antarctica* colonies on the other hand are due to their large size less

susceptible to microzooplankton grazing and even ingestion by copepods appears to be strongly dependent upon a proper size match between grazer and prey (Verity 2000). This might explain the increase of *Phaeocystis* colonies.

The decrease in photosynthetic dinoflagellate abundance after day 11 could be due to grazing and to negative effects of turbulence on growth rates. Probably grazing accounted for much of the decline in abundance since remains of *Prorocentrum* spp. were frequently observed in copepod faecal pellets (personal observation). Nevertheless previous studies have shown that growth rates of some photosynthetic dinoflagellates are negatively affected by turbulence (Sullivan and Swift 2003; Berdalet and Estrada 1993) and since the decrease in abundance after day 11 coincides with the second major storm event on day 13, a negative effect of increased turbulence on growth of photosynthetic dinoflagellates cannot be ruled out.

Although a significant component of the initial phytoplankton community coccolithophores decreased during the experiment. The similar decline inside and outside the patch (Fig. 6B) suggests that iron addition seemed to have little effect on coccolithophore production. On the other hand coccolithophores are known to dramatically decrease in diversity as well as abundance in a poleward direction from the Subtropical Convergence to the Polar Front (Findlay and Giraudeau 2000; Nishida 1986). The EisenEx site therefore might have been at the periphery of their natural area of occurrence and coccolithophores were probably exposed to sub-optimal growth conditions. This might explain the decrease in coccolithophore abundance observed during the experiment. Nevertheless coccolithophores have been recorded as far south as 71°S in the Weddell Sea (Winter et al. 1999). Coccolithophore diversity was assumed to be low since only one species, *Emiliania huxleyi*, was identified by SEM investigations. *E. huxleyi* seems to be by far the dominant species in the Southern Ocean especially south of the Polar Front (Eynaud et al. 1999; Winter et al. 1999; Buma et al. 1992). The decline in coccolithophore abundance could also be the result of grazing. Species of the abundant tintinnid genus *Codonellopsis* were observed to almost exclusively agglutinate coccoliths of *E. huxleyi* to their loricae (Henjes and Assmy to be submitted) indicating that they preferentially fed on this species. Bottle incubation experiments conducted during the EisenEx cruise also indicate the importance of microzooplankton grazing on coccolithophore stocks. All treatments exhibited a similar decrease in coccolithophore abundance as was observed in the field except the dilution treatment. In the dilution treatment (unfiltered to filtered seawater ratio of 1:3 (25%)) abundances of coccolithophores showed a distinct increase over the nine days of incubation (unpublished data). Dilution experiments were especially designed to reduce the encounter rate between consumer and prey in order to assess

the impact of microzooplankton grazing (Landry and Hassett 1982). These findings suggest that microzooplankton grazing probably exerted a major control on coccolithophore production.

Silicoflagellates were exclusively represented by *Dictyocha speculum*, a conspicuous species reported to dominate this group in Antarctic waters (Eynaud et al. 1999). The moderate increase in abundance inside the patch and similar cell numbers outside the patch point to a minor effect of elevated iron concentrations on the growth of *D. speculum*. Although *D. speculum* contributes only a minor fraction to total biogenic opal flux (Gersonde and Wefer 1987) it has been suggested as an indicator for Antarctic Circumpolar Water (Romero et al. 2001).

Conclusions

Previous *in situ* iron fertilization experiments clearly demonstrated that iron addition leads to diatom blooms in HNLC regions (Coale et al. 1996; Boyd et al. 2000; Tsuda et al. 2003). Our results from EisenEx, the second *in situ* iron fertilization experiment conducted in the Southern Ocean during austral spring, support these findings and indicate that phytoplankton growth rates were already iron limited at the start of the growth season (November). Although growth rates of all phytoplankton groups increased within days, only diatoms accumulated considerable biomass despite horizontal dispersion, deep vertical mixing and grazing pressure by various proto- and metazoans. Despite this overall floristic shift from a background community dominated by small-celled algae to a superimposed diatom bloom the diatom community itself also changed in size as well as species composition. The diatom community shifted from slower-growing, heavily silicified diatom species (e.g. *Fragilariopsis kerguelensis*, *Thalassionema nitzschioides*) to faster-growing, weakly silicified ones (e.g. *Pseudo-nitzschia lineola*, *Chaetoceros curvisetus*). Despite the larger seed populations of the robust species the delicate species overtook the former under iron-replete conditions due to their higher accumulation rates and thereby in the case of *P. lineola* dominated diatom abundance as well as biomass by the end of the experiment. The initial situation and the iron induced diatom bloom were both dominated by only a few species of disparate genera and the majority of species remained at background concentrations throughout the experiment indicating that the response of phytoplankton assemblages to environmental change is not proceeding in a “free-for-all” manner but each species is characterised by specific adaptations to the pelagic environment. Many of the background species are a universal and widespread phenomenon in the Southern Ocean and are therefore evolutionary as persistent as the

dominant species in terms of carrying ones success over to the next year. These results cast doubt on the selection of fast growth rates as the driving force in plankton evolution and competitive exclusion is therefore probably not the rule in pelagic ecosystems.

This iron-mediated shift has strong implications for the BCP since chain-forming and fast growing r-strategy species like *Pseudo-nitzschia* spp. and small *Chaetoceros* spp. especially of the subgenus *Hyalochaetae* tend to aggregate and sink when nutrients are depleted and the bloom collapses and therefore enhance vertical carbon flux (Alldredge and Gotschalk 1989; Thornton 2002). The rapid sedimentation of *Chaetoceros* resting spores is presumably linked to aggregation, supporting the conclusion of Smetacek (1985) that aggregation and rapid sedimentation represents a transition to a resting stage in the life history of this and also other diatoms. High abundances of *Chaetoceros* resting spores in sediments underlying the highly productive Antarctic Peninsula (Crosta et al. 1997, Ferrario et al. 1998) suggest that these species are a good indicator for regimes of high primary productivity. Increased accumulation of *Chaetoceros* resting spores in Antarctic sediments during the Last Glacial Maximum (LGM) further suggest that enhanced iron input through airborne dust stimulated diatom productivity during the LGM (Abelmann et al. to be submitted). The mechanisms that play a major role when the phytoplankton bloom eventually collapses still remain to be investigated. In future iron fertilization experiments, it will be therefore essential to study the fate of carbon export to mesopelagic layers and the deep sea. Furthermore this iron-mediated shift in diatom dominance raises concern about the interpretation of sedimentary opal accumulation data as a measure of diatom productivity and carbon export from the surface since weakly silicified diatoms that dominate under iron replete conditions are with the exception of *Chaetoceros* resting spores more susceptible to dissolution and not well preserved in the sediment.

Our detailed microscopic analysis of the phytoplankton assemblage revealed species-specific responses to iron enrichment with novel implications for the interpretations of plankton succession and functioning of pelagic ecosystems. Mesoscale *in situ* perturbation experiments like EisenEx now enable to follow the waxing and waning of plankton blooms in space and time and help to elucidate the interplay of ultimate factors (growth rates, genetic inventory) and proximate factors (iron, grazing etc.) in shaping the pelagic ecosystem and the underlying biogeochemical cycles. The Southern Ocean ecosystems especially lend themselves to ecosystem studies like mesoscale fertilization experiments due to their confined extent within a distinct climate zone. Owing to the circumpolar character of the Southern Ocean very few warm water species ever reach far south and become established (Hendey 1937). Therefore the ecosystems of the Southern Ocean are more stable and less prone to expatriation than

other marine systems (Verity and Smetacek 1996) and may provide more general ecological principles that would help to improve ecosystem models.

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Figure legends

Table 1 80m-depth integrated abundance (cells l^{-1}) and biomass (mg C m^{-2}) two days prior to fertilization and after 21 days inside and outside the patch for all diatom species and groups identified during EisenEx.

Fig. 1 Temporal development of 80m-depth integrated abundance in cells l^{-1} of A) total diatoms, B) *Pseudo-nitzschia lineola*, B1) first 10 days enlarged, C) *Chaetoceros curvisetus*, C1) first 10 days enlarged, D) *Fragilariopsis kerguelensis*, E) *Thalassionema nitzschioides*, F) *Corethron pennatum*, G) *Haslea* sp., H) *Nitzschia* sp. and I) *Cylindrotheca closterium*. For a better comparison the first 9 days for *P. lineola* and *C. curvisetus* are enlarged. Only the maximum accumulation rate (*k*) for the period of spurt growth is given.

Fig. 2 Temporal development of 80m-depth integrated biomass in mg C m^{-2}. A) Total diatoms, B) *Pseudo-nitzschia lineola*, C) *Corethron pennatum*, D) *Haslea* sp., E) discoid diatoms <30 µm, F) *Dactyliosolen antarcticus*, G) *Proboscia alata*, H) *Chaetoceros curvisetus* and I) *Fragilariopsis kerguelensis*.

Fig. 3 Size composition of the diatom assemblage. Temporal development of 80m-depth integrated abundances in cells l^{-1} of the three different size classes A) inside, A1) first 8 days enlarged, and B) outside the patch. Relative contribution of different size classes to total diatom abundance C) inside and D) outside the patch. Temporal development of 80m-depth integrated biomass in mg C m^{-2} of the three different size classes E) inside and F) outside the patch. Relative contribution of different size classes to total diatom biomass G) inside and H) outside the patch.

Fig. 4 Chain length distribution of *Fragilariopsis kerguelensis* over the course of the experiment.

Fig. 5 Temporal development of 80m-depth integrated A) intact empty and B) broken diatom frustules l^{-1}. Relative contribution of different diatom species and groups to C) intact empty and D) broken frustules.

Fig. 6 Temporal development of 80m-depth integrated abundance in cells l^{-1} of A) *Phaeocystis antarctica* including A1) cells in colonies and A2) colonies, B) coccolithophores,

C) autotrophic dinoflagellates and D) *Dictyocha speculum*. Only the maximum accumulation rate (*k*) for the period of spurt growth is given. E) Total non-diatom phytoplankton biomass in mg C m^{-2}. Relative contribution of different non-diatom phytoplankton species and groups to total non-diatom phytoplankton biomass F) inside and G) outside the patch.

Fig. 7 A) Correlation between diatom biomass in µg C l^{-1} and chlorophyll a in µg l^{-1} (Gervais et al. 2002) inside the fertilized patch , B) Temporal development of the molar ratio of biogenic silica (BSi) to diatom carbon (DC) over the course of the experiment.

Table 1

	Abundance (cells l^{-1})			Biomass (mg C m^{-2})		
	day -2	day 21 in patch	day 21 out patch	day -2	day 21 in patch	day 21 out patch
Total Diatoms	63336	444294	87642	790	3508	1550
Pseudo-nitzschia lineola (Cleve) Hasle	2927	233586	21417	11	883	69
P. turgidula (Hustedt) Hasle	2086	26944	6105	6	82	17
P. turgiduloides Hasle	569	12504	2063	2	42	7
P. heimii Manguin	542	10362	5742	6	123	62
P. prolongatoides (Hasle) Hasle	1130	396	1628	3	1	5
Thalassionema nitzschioides (Grunow) Grunow ex Hustedt	10522	48357	4907	17	83	11
T. nitzschioides var. *lanceolatum*	451	613	237	7	9	4
Fragilariopsis kerguelensis (O´Meara) Hustedt	7499	22146	9389	53	137	55
F. rhombica (O´Meara) Hustedt	173	8	171	0,5	0	0,5
F. obliquecostata (Van Heurck) Hasle	22	0	0	0,4	1	0
Cylindrotheca closterium (Ehrenberg) Lewin & Reimann	14958	24483	8576	12	21	11
Nitzschia sp.	1573	1117	1447	0,8	0,5	0,7
N. bicapitata Cleve	0	72	0	0	0,2	0
Navicula spp.	921	1694	1161	14	23	16
Thalassiothrix antarctica (Schimper) Karsten	52	113	286	5	11	29
Haslea sp. Simonsen	495	2899	385	39	227	30
Pleurosigma atlanticus	52	8	50	8	1	7
Membraneis imposter Paddock	127	314	33	16	27	1
Rhizosolenia chunii Karsten	165	1658	1887	7	117	137
R. hebetata f. *semispina* (Hensen) Gran	0	52	61	0	11	13
*R. curvata** Zacharias	0	39	0	0	60	0
Proboscia alata (Brightwell) Sundström	8	349	402	4	199	154
Guinardia delicatula (Cleve) Hasle	303	36	22	28	6	2
G. cylindrus (Cleve) Hasle	1419	1587	2409	69	74	117
Corethron pennatum (Castracane) Crawford	690	4623	1672	52	446	164
C. inerme Karsten	25	877	506	2	73	45
Dactyliosolen antarcticus Castracane	465	963	583	157	210	269
Leptocylindrus mediteraneus (H. Peragallo) Hasle	385	855	814	25	71	57
Eucampia antarctica (Castracane) Mangin	19	17	132	0,9	0,8	6
Chaetoceros dichaeta Ehrenberg	0	762	99	0	15	2
C. atlanticus Cleve	344	542	935	8	12	21
C. curvisetus Cleve	745	18917	594	5	138	4
C. neglectus Karsten	1957	2751	1248	3	5	2
C. convolutus Castracane	132	1100	347	5	40	13
C. aequatorialis Cleve	52	91	154	2	4	7
*C. bulbosus** (Ehrenberg) Heiden	0	17	0	0	0,3	0
C. peruvianus Brightwell	344	171	61	8	4	1
Chaetoceros spp.	171	41	22	2	1	0
Thalassiosira lentiginosa (Janisch) G. Fryxell	509	217	286	22	12	30
T. oliverana (O´Meara) Mak.	39	151	99	2	7	5
T. gracilis (Karsten) Hustedt	1722	877	1282	12	7	7
T. oestrupii (Ostenfeld) Hasle	256	616	369	3	7	4
T. tumida (Janisch) Hasle	143	143	50	40	61	21
Asteromphalus hookeri Ehrenberg	44	105	116	8	13	19
A. hyalinus Karsten	778	1180	435	17	36	9
Azpeitia tabularis (Grunow) G. Fryxell & P. A. Sims	388	149	424	8	9	7
*Actinocyclus curvatulus** Janisch	0	22	22	0	6	6
discoid diatoms <30 µm	8055	19541	8991	91	220	101
discoid diatoms >30 µm	80	311	55	7	37	5

*, highligths species with abundance and biomass values taken on days 19 and 17 for inside and outside the patch respectively.

Fig. 1

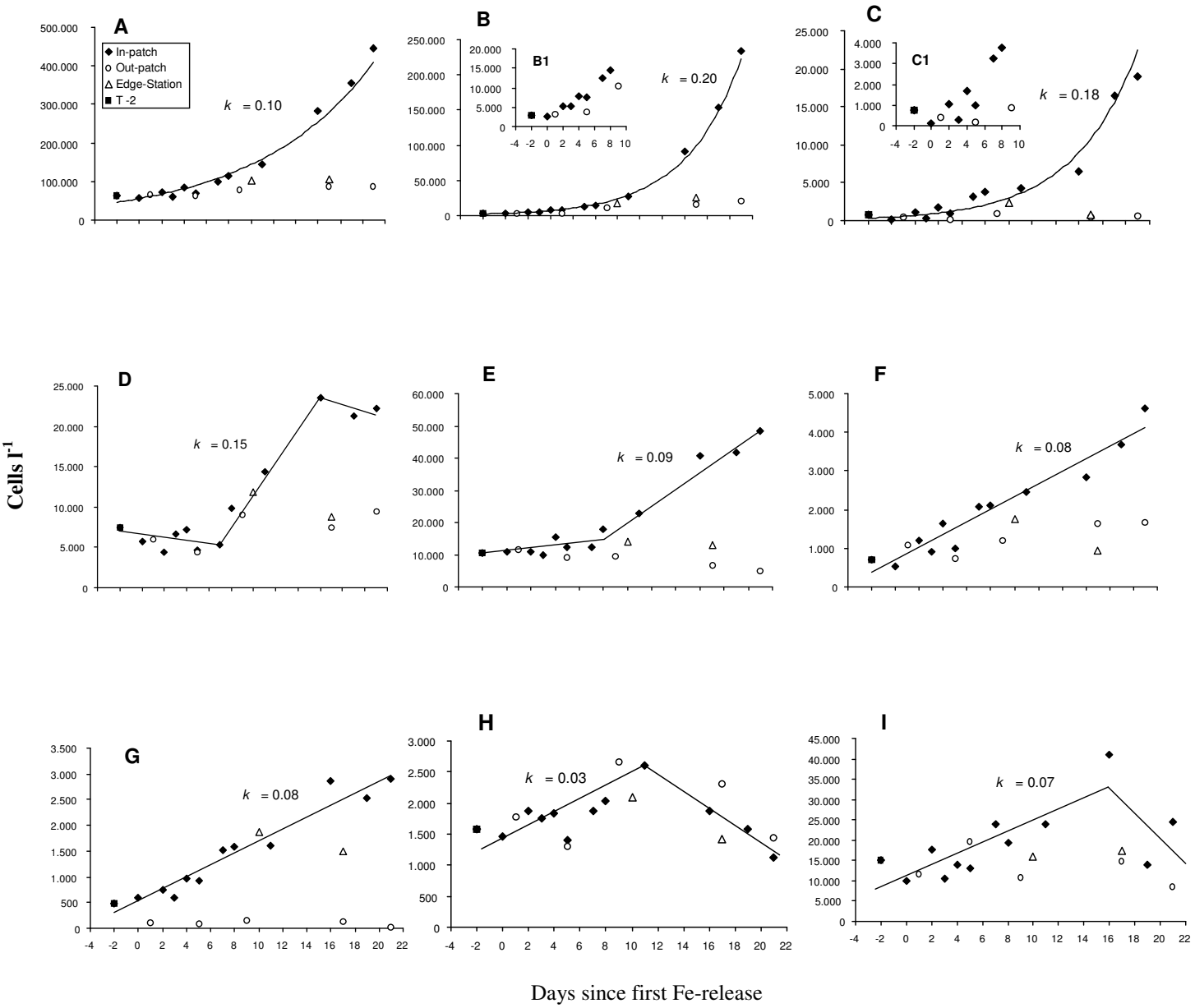

A
In-patch
Out-patch
Edge-Station
T -2
k = 0.10
B
B1
k = 0.20
C
C1
k = 0.18
D
k = 0.15
E
k = 0.09
F
k = 0.08
G
k = 0.08
H
k = 0.03
I
k = 0.07
Cells l-1
Days since first Fe-release

Fig. 2

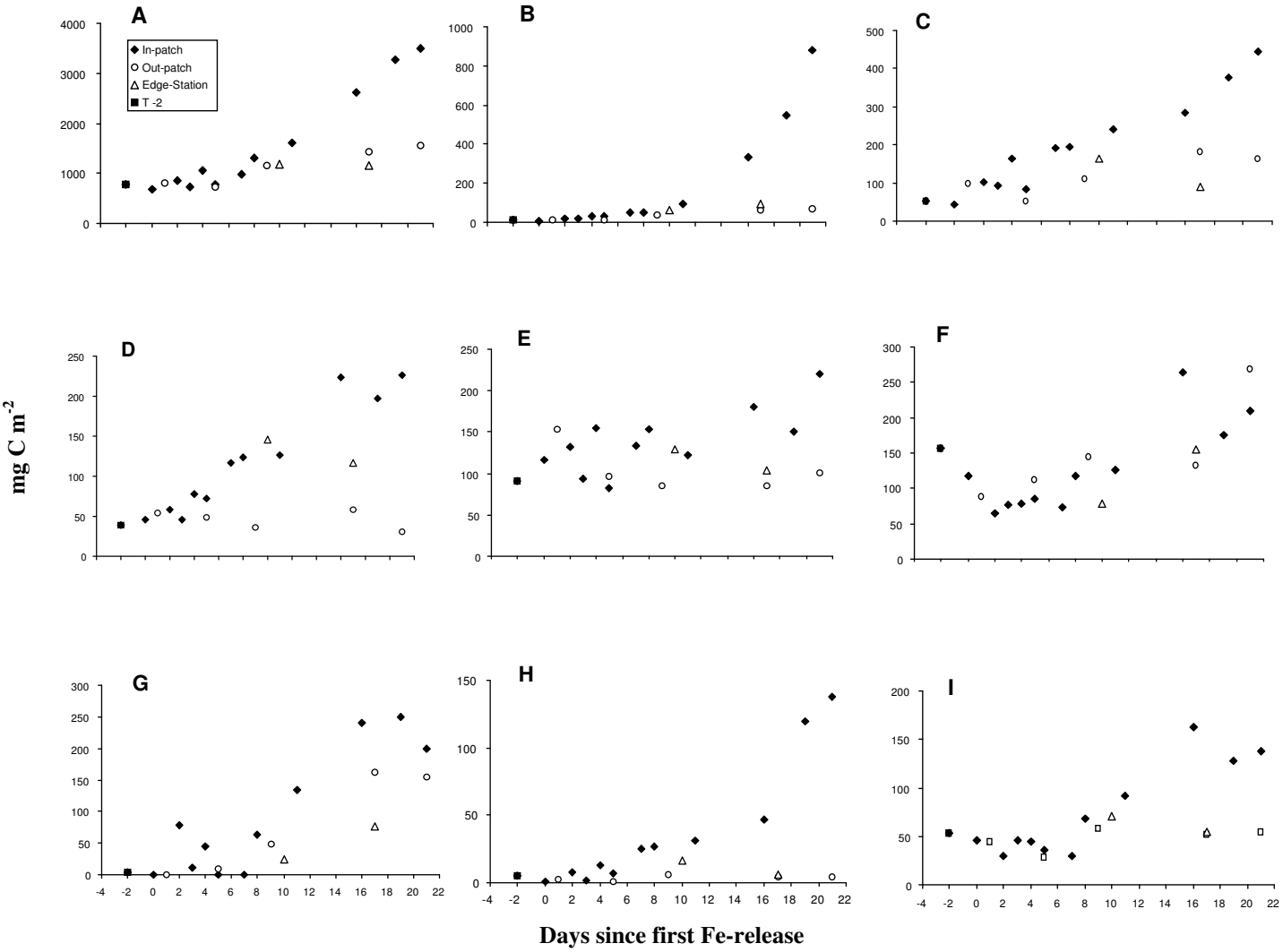

A
B
C
D
E
F
G
H
I
In-patch
Out-patch
Edge-Station
T -2
mg C m-2
Days since first Fe-release

Fig. 3

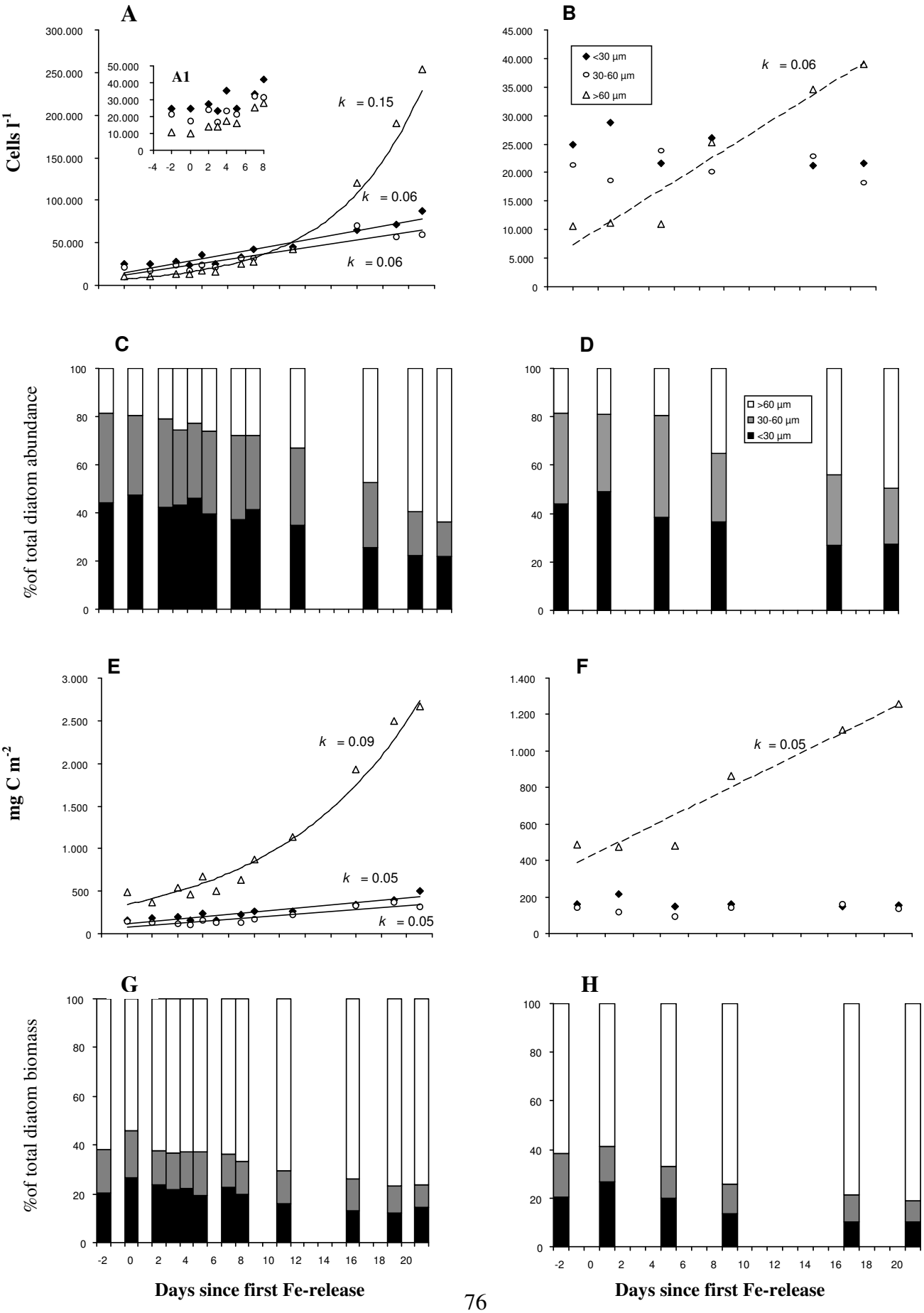

A
A1
B
C
D
E
F
G
H
Cells l-1
%of total diatom abundance
mg C m-2
%of total diatom biomass
k = 0.15
k = 0.06
k = 0.06
k = 0.06
k = 0.09
k = 0.05
k = 0.05
<30 µm
30-60 µm
>60 µm
Days since first Fe-release
Days since first Fe-release

Fig. 4

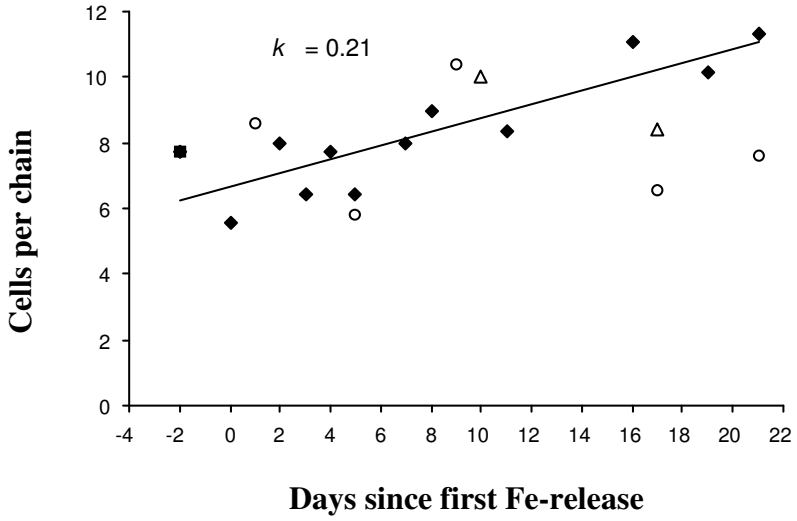

k = 0.21
Cells per chain
Days since first Fe-release
0
2
4
6
8
10
12
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22

Fig. 5

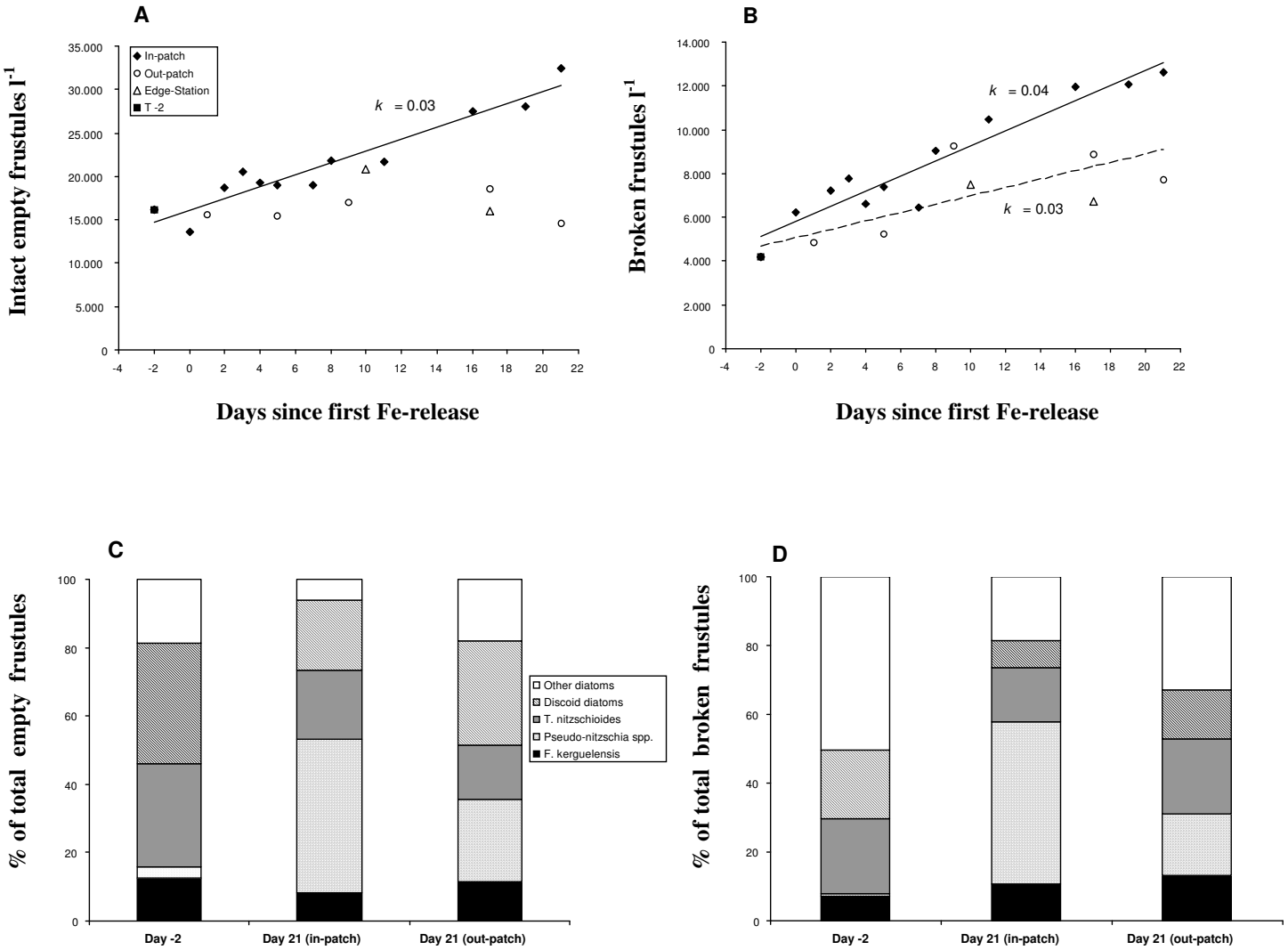

A
Intact empty frustules l-1
In-patch
Out-patch
Edge-Station
T -2
k = 0.03
Days since first Fe-release
B
Broken frustules l-1
k = 0.04
k = 0.03
Days since first Fe-release
C
% of total empty frustules
Other diatoms
Discoid diatoms
T. nitzschioides
Pseudo-nitzschia spp.
F. kerguelensis
Day -2
Day 21 (in-patch)
Day 21 (out-patch)
D
% of total broken frustules
Day -2
Day 21 (in-patch)
Day 21 (out-patch)

Fig. 6

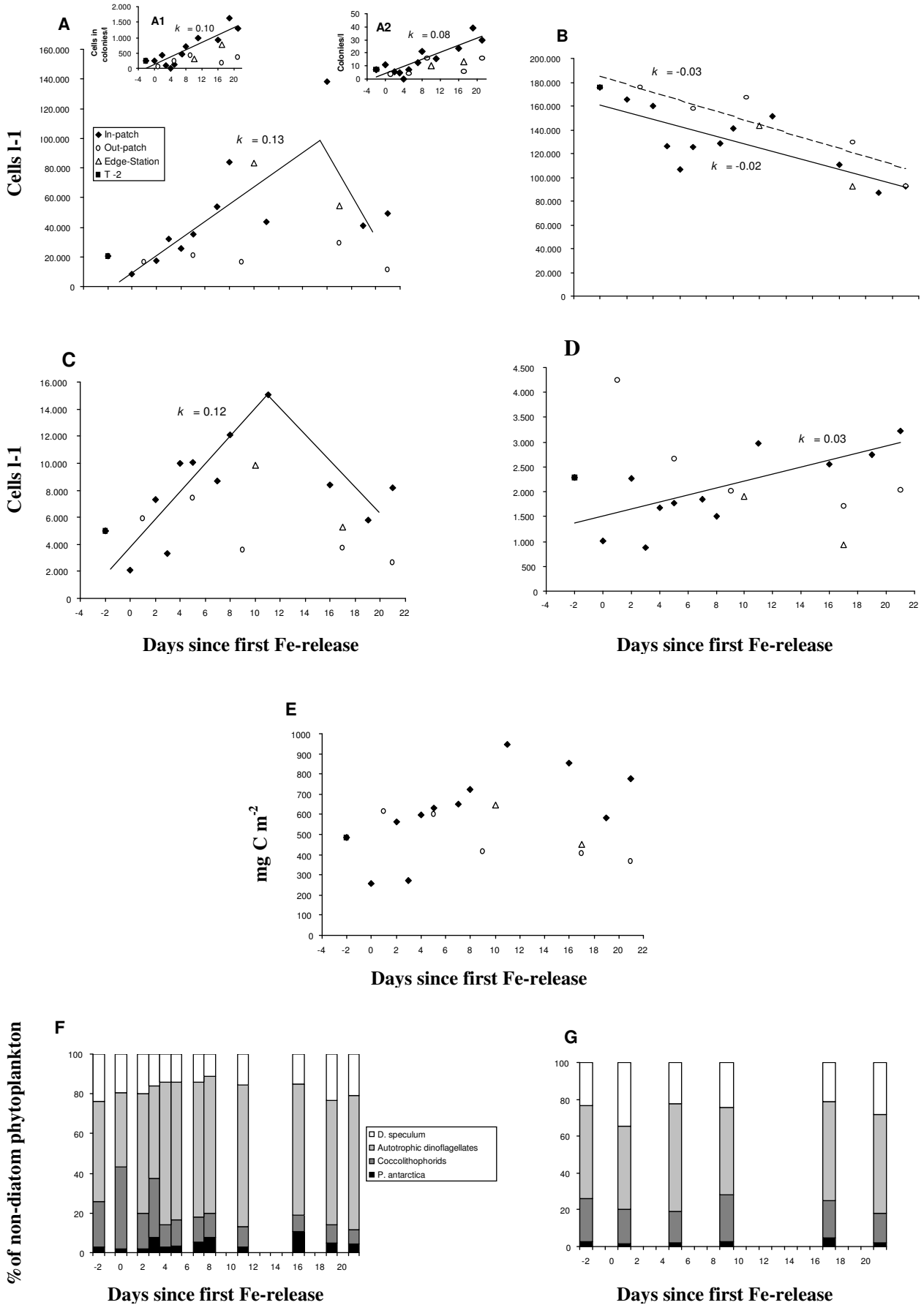
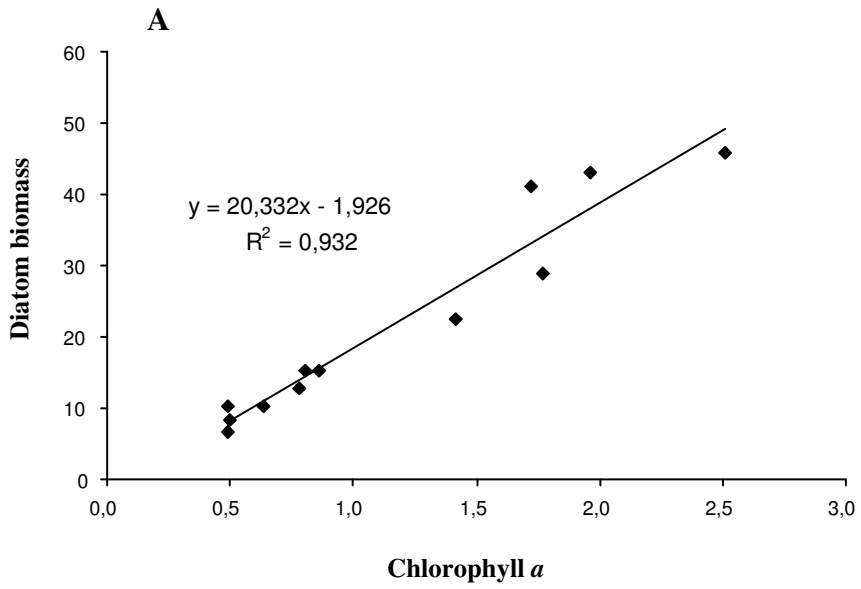
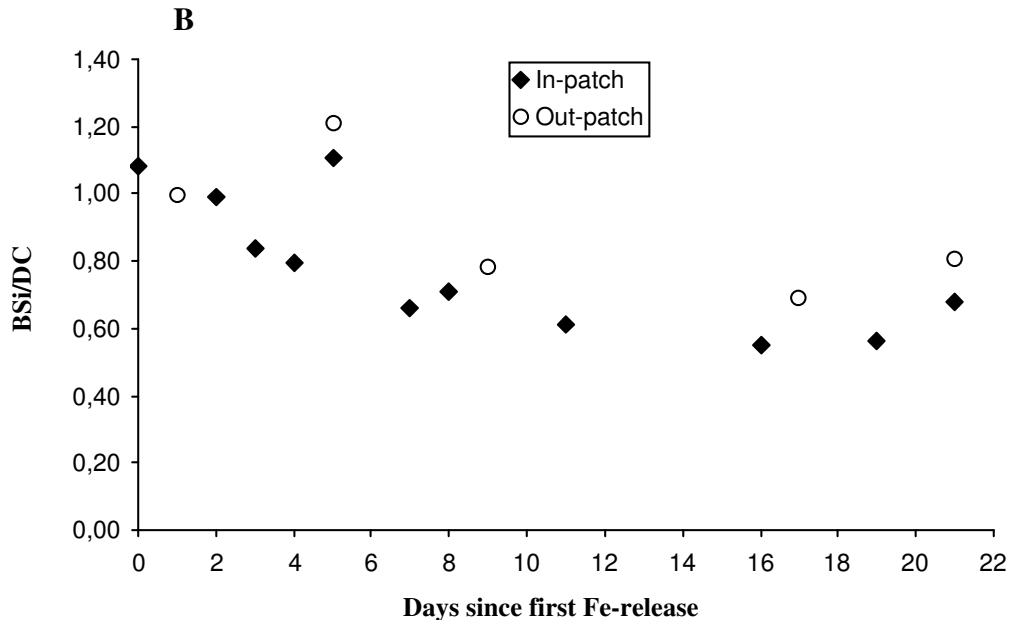

A
A1
k = 0.10
Cells in colonies/l
A2
k = 0.08
Colonies/l
In-patch
Out-patch
Edge-Station
T -2
k = 0.13
Cells l-1
B
k = -0.03
k = -0.02
C
k = 0.12
Cells l-1
D
k = 0.03
Days since first Fe-release
Days since first Fe-release
E
mg C m-2
Days since first Fe-release
F
% of non-diatom phytoplankton
D. speculum
Autotrophic dinoflagellates
Coccolithophorids
P. antarctica
Days since first Fe-release
G
Days since first Fe-release

Fig. 7


A
Diatom biomass
y = 20,332x - 1,926
R2 = 0,932
Chlorophyll a


B
BSi/DC
In-patch
Out-patch
Days since first Fe-release

Manuscript 2

Vertical distribution of non-motile particles and planktonic organisms during an iron fertilization experiment in the Polar Frontal Zone of the Southern Ocean (EisenEx)

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In preparation for submission.

Abstract

We investigated the vertical distribution of intact empty and broken diatom frustules, empty tintinnid loricae, protozoan and crustacean fecal material and various components of the heterotrophic community, including athecate and thecate dinoflagellates, aloricate and loricate ciliates and juvenile and small adult copepods, during an iron fertilization experiment in the Polar Frontal Zone of the Southern Ocean. Despite several deep mixing events over the course of the experiment induced by gale force winds distinct concentration peaks were observed within the mixed surface layer. Broken diatom frustules, empty tintinnid loricae as well as crustacean fecal pellet numbers showed highly significant correlations inside the patch and accumulated at the bottom of the mixed layer at the end of the experiment. An enhanced feeding activity of coprophagic grazers seemed to be the major cause for this accumulation and may have contributed to the recycling of fecal material in the mixed layer. Furthermore the significant correlation between copepodites and small adult copepods and broken frustules highlights the importance of crustacean grazing on the diatom assemblage. Intact empty diatom frustules on the other hand showed a similar distribution to athecate and thecate dinoflagellates and aloricate ciliates with concentration peaks at the surface suggesting that protozoan grazing was a major source of intact empty frustules. Whereas non-motile particles are dedicated by Stoke´s Law, many of the planktonic organisms were able to maintain their position in the water column irrespective of the local turbulent field due to their swimming activities and peaked at distinct depth horizons. These depth preferences have probably evolved to remain at favourable food concentrations or to minimise grazer induced mortality. The interplay of the various physical and biological factors caused these vertical heterogeneities and hence structured the pelagic ecosystem during this study. In this context iron fertilization experiments now enable the detailed study of the various factors influencing plankton distribution and ecology and how they are manifested in ecosystem processes that ultimately drive the biogeochemical cycles of the ocean.

Introduction

Various factors influence the vertical distribution of planktonic organisms in the water column. These include physical (convection, wind energy, light, physical settling dynamics of particles), chemical (nutrient gradient, chemical signals) and biological (grazing, food availability, sexual reproduction, buoyancy regulation) factors. While the nutrient requirements of phytoplankton have been examined in depth, the dependence upon the physical environment has only been poorly addressed by plankton ecologists (Reynolds 1997). Physical processes may affect the vertical distribution of plankton over a wide spectrum of time and space scales. The most obvious vertical gradient can be observed at the seasonal pycnocline separating the productive surface layer from the deep ocean. However plankton variability may also occur at the microscale (<1 m) in the form of thin layers (Franks 1995). Physical processes taking place at the centimetre scale have only been recognised for about the past 20 years by oceanographers due to the difficulties of sampling at these small-scale dimensions in the ocean (Dower and Denman 2001). The effects of small-scale turbulence may be transient and the formation of critical layers (Thorpe 1981) may affect the vertical distribution of particles and planktonic organisms on very short time scales. Another characteristic and persistent feature of certain ocean regions is the deep chlorophyll maximum (DCM). In oligotrophic regions like the Subtropical Gyres phytoplankton accumulates at the depth of the nutricline (Eppley et al. 1988) while deep phytoplankton maxima in high nutrient and low chlorophyll (HNLC) areas like the Southern Ocean are probably related to iron acquisition at depth (Garibotti et al. 2003). Although the depth of the DCM seems to be unaffected by grazing its magnitude is controlled by meta- and protozooplankton grazing (Varela et al. 2003). Subsurface chlorophyll patches are also common to many ocean fronts and the biological and physical dynamics that cause these patches seem to be strongly interrelated (Franks and Walstad 1997). Therefore the interactions of both physical and biological processes and the scales at which theses interactions take place are critical to an understanding of the distribution of plankton (Daly and Smith 1993). When Sverdrup introduced the critical depth model he inter alia assumed a homogeneous distribution of phytoplankton in the water column (Sverdrup 1953). Previous studies have however shown that dominant phytoplankton taxa are able to actively regulate their position in the water column (Waite et al. 1997) and might therefore escape unfavourable growth conditions induced by deep mixing.

Victor Hensen coined the term plankton and defined it as “all particles and materials, which float in the water column, no matter whether they occur in the upper or deeper layers of the water column, or whether they are alive or dead” (Hensen 1887). Today's application of the term plankton comprises live organisms only. However many planktonic organisms not merely float in the water column but are capable of actively swimming against the velocity fluctuations in the seasonal thermocline. Whereas these planktonic organisms are able to actively choose their position in the water column by their respective swimming capabilities the vertical distribution of non-motile particles is strongly affected by the physical settling velocity (Stokes law). The size and density of a particle determine its settling velocity and hence its residence time at the surface. Nevertheless biological interactions, e.g. grazing, may considerably alter the distribution of non-motile particles like fecal pellets and intact empty and broken diatom frustules and hence impact on the vertical particle flux.

In this paper we investigate the vertical distribution of non-motile particles and several components of the heterotrophic community during an iron fertilization experiment in the Polar Frontal Zone (PFZ) of the Southern Ocean. Furthermore the influence of various physical, chemical and biological factors on the vertical distribution of the different components of the pelagic community is discussed.

Material and Methods

For quantitative assessment of protozooplankton, protozoan and metazoan fecal pellets as well as intact empty and broken diatom frustules, water samples were taken at seven discrete depths (10, 20, 40, 60, 80, 100 and 150m) at 19 in and out patch stations using Niskin bottles mounted on a CTD (Conductivity Temperature Depth) rosette. Water samples of 200 ml were preserved with a hexamine buffered formaline solution at a final concentration of 2% and stored at 4°C in the dark for subsequent counting back in the home laboratory. Cells were identified and enumerated using inverted light and epifluorescence microscopy (Axiovert 25, Axiovert 135 and IM 35) according to the method of Utermöhl (1958) following the recommendations of Venrick (1978) and Edler (1979). Before counting water samples were settled for at least 48 hours in 50 ml Hydrobios sedimentation chambers. Organisms, pellets and frustules were counted at magnifications of 200-640x according to the size of the species or particles examined. Protozooplankton and empty and broken frustules were counted according to their frequency of occurrence: very abundant objects were counted in stripes, abundant objects in a quarter or half a chamber and less abundant objects in the whole

chamber. Each sample was examined until a total of 50 to 400 protozoans and at least 400 empty and broken frustules had been counted. During this study the term protozooplankton is used for aplastidic protistan plankton and distinguishes two major groups: ciliates and dinoflagellates. Ciliates were classified as loricate (tintinnids) and aloricate ciliates whereas dinoflagellates were separated in thecate and athecate dinoflagellates. Sheathed protozoans were identified to species or genus level in the case of tintinnids and where possible in the case of thecate dinoflagellates. Protozoans with no obvious armour could not be identified to species level in water samples and were therefore grouped according to size. All unidentified protozoans were categorised into small (<30 µm), medium (30-60 µm) and large (>60 µm) size classes. Aloricate ciliates <20 µm were not accounted for in this study due to poor preservation and hence the small size fraction comprises only species ranging between 20-30 µm. In addition empty tintinnid loricae were enumerated as an indicator of tintinnid mortality. Broken and empty diatom frustules were identified to species or genus level and otherwise grouped into size classes. Only broken diatom frustules of which >50% of the frustule was intact were counted to avoid counting the same individual twice. Broken frustules of particularly weakly silicified diatom species like *Cylindrotheca closterium*, *Chaetoceros neglectus* and *C. curvisetus* were difficult to identify under light microscopy because their frustules easily disintegrate into small debris that cannot be satisfactorily assigned to a single cell. Also intact empty frustules of these delicate species rapidly dissolve and disintegrate and were therefore hardly encountered in water samples.

Fecal pellets counted during this study were characterised by various shapes. We used the recommendations of Klaas (1997) to discriminate between two types of fecal pellets attributed to protozoan origin: olive green ellipsoid to spherical (10-50µm in diameter) pellets as described by Gowing and Silver (1985) containing an matrix of fine material unidentifiable by light microscopy. Pellets of variable size and shape (10-100µm in length) enclosed by a membrane that contained intact empty diatom frustules (Buck et al. 1990, González 1992). Metazoan fecal pellets were classified in four different shapes after the recommendation of Gowing et al. (2001). All metazoan fecal pellets were coated by a membrane and contained mainly unidentifiable organic material, intact empty and broken diatom frustules, thecae of dinoflagellates and loricae of tintinnids. Carbon volume conversion factors from literature were used to estimate proto- and metazooplankton fecal pellet carbon content. For all different protozoan fecal pellet forms mentioned we used 0.0114 pg C μm^{-3}, a value estimated for the cytoplasmic content of an average-sized pellet produced by dinoflagellates in the Weddel Sea ice by Buck et al. (1990).

For all metazoan fecal pellets a conversion factor of 0.016 pg C μm^{-3} was used (Gowing et al. 2001).

Tintinnids, copepod nauplii, copepodites and small copepods were counted in concentrated samples and identified to genus level if possible. The methodology for handling concentrated samples is described in Henjes and Assmy (to be submitted).

The vertical distribution of proto- and metazooplankton, protozoan and metazoan fecal pellets as well as empty and broken diatom frustules is shown for the upper 150 m of the water column. The depth profiles are depicted separately for inside and outside the patch. The edge stations were not included to the in patch profiles in order to achieve a consistent picture of the vertical and temporal development inside the iron-enriched patch. The description of the cruise and the experimental setting of EisenEx can be read elsewhere (Assmy and Henjes to be submitted).

In order to account for correlations between different biological variables a partial correlation was applied. The partial correlation of two variables with respect to a third is the correlation of the two variables after the linear effect of the third has been removed. In our case the effect of depth has been removed to achieve the true relationship of two biological variables.

The degree of heterogeneity was calculated by dividing the peak concentration over the minimum concentration within the mixed layer. Values are always given for the station with the greatest change.

Results

Hydrography

The mixed layer depth varied considerably over the course of the experiment due to wind-induced turbulent vertical mixing events. At the start of the iron fertilization experiment in austral spring the deeply Winter Mixed Layer induced by convection had already stratified and the seasonal mixed layer depth of 10-40 m was relatively shallow for spring both inside and outside the patch providing favourable light climate for phytoplankton growth. This rather calm period prevailed during the initial phase of the experiment. On day 5 the first gale force winds exceeding 20 m s^{-1} caused the deepening of the mixed layer down to 60-70 m depth. After wind relaxation at around day 10 the mixed layer shoaled again at 10-40 m depth. Furthermore the intrusion of fresh water due to precipitation during days 10-12 caused a transient shallow mixed layer. The latter part of the experiment was again interrupted by two strong wind events with top speeds exceeding 25 m s^{-1} that increased vertical mixing and mixed layer depths of 75-85 m prevailed during the end of the experiment (Strass et al., 2001).

Not only the vertical stability of the water column was affected by the wind field but also the horizontal spread of the fertilized patch. Horizontal dispersion of the fertilized patch doubled its size every four to five days on average (Watson et al. 2001) and caused the increase from initially 40 km^2 to 950 km^2 by day 21 (Riebesell et al. to be submitted). The dilution effect was most pronounced during the initial phase of the experiment and decreased in importance as the experiment progressed. Further details on the environmental setting of EisenEx are described in Assmy and Henjes (to be submitted).

Vertical distribution

Protozooplankton

The vertical distribution of dinoflagellates and ciliates, two major components of the protozooplankton, was examined during this study.

Thecate dinoflagellates comprised *Protoperidinium* spp., *Preperidinium meunierii, Oxytoxon* spp. and unidentified species grouped according to size. Despite several deep mixing events over the course of the experiment distinct abundance peaks of thecate dinoflagellates were

restricted to the upper 10-40 m of the water column inside the patch and sharply declined towards greater depths. Highest thecate dinoflagellates abundances of $21*10^3$ cells l^{-1} in 10 m depth on day 11 inside the patch coincided with shallow mixed layer depths during the middle of the experiment. Outside the patch thecate dinoflagellates also predominated in the upper 40 m of the water column and peaked with $14*10^3$ cells l^{-1} in 10 m depth two days prior to fertilization (Fig. 1A). A high degree of heterogeneity was observed for thecate dinoflagellates on day 11 with abundances changes of up to 10.2 times within the mixed layer. Athecate dinoflagellates were not identified to species or genus level and therefore counted in size categories. Inside the patch athecate dinoflagellates exhibited subsurface maxima prior to and at the start of the experiment and predominantly culminated at the surface between 10-40 m depth thereafter. Nevertheless the decline towards greater depth was not as steep as for thecate dinoflagellates (Fig. 1B). This is further indicated by the lower maximum variability of 2.5 on day 0 for athecate dinoflagellate abundances in the mixed layer. A uniform distribution to occasionally 150 m was observed outside the patch. Abundances of athecate dinoflagellates peaked with $50*10^3$ cells l^{-1} in 20 m depth on day 8 inside and with $25*10^3$ cells l^{-1} in 10 m depth on day 5 outside the patch. Protozoan fecal pellet numbers peaked with 484 pellets l^{-1} in 60 m depth on day 11 and changed up to 7 times within the mixed layer on day 21. In control waters protozoan fecal pellets accumulated at the surface as well as in greater depths and peaked with 352 fecal pellets l^{-1} in 80 m depth on day 5 (Fig. 1C). Protozoan fecal pellet carbon initially matched the distribution of fecal pellet numbers quite well inside the patch. From day 5 on however fecal pellet carbon showed elevated concentrations down to 80 m depth until day 11. Thereafter peak concentrations shoaled at the surface until the end of the experiment. Fecal pellet standing stock showed two distinct peaks inside the patch in 10 and 80 m depth on day 8 with 0.9 µg C l^{-1} respectively (Fig. 1D). Loricate ciliates were composed of several tintinnid species: *Cymatocylis vanhoffeni*, *C. calyciformis*, *C. antarctica*, *Codonellopsis pusilla*, *C. gausii*, *Salpingella* spp., *Stenosomella* spp., *Protorhabdonella* spp., *Amphorides* spp. and *Acanthostomella norvegica*. Inside the patch these tintinnids exhibited a rather uniform distribution throughout the experiment down to 60-80 m depth indicated by rather small abundances changes over the MLD. Only towards the end of the experiment distinct maxima were observed at the surface as reflected by abundance changes of up to 9.7 on day 11 within the mixed layer. The highest cell numbers of loricate ciliates inside the patch were recorded prior to fertilization with 235 cells l^{-1} in 60 m depth. Outside the patch deep maxima at 60 m depth prevailed prior to and at the start of the experiment, thereafter cell numbers showed distinct peaks at the surface and

towards the end of the experiment homogenous but low abundances were observed down to 100 m depth. In control waters maximal abundances of 358 cells l^{-1} were detected in 10 m depth on day 5 (Fig. 1E). Inside the patch empty loricae of the afore mentioned tintinnid species accumulated at the surface between 10-20 m depth during the first days of the experiment. Thereafter empty loricae were evenly distributed in the water column down to 60-80 m depth. This trend can also be observed in the degree of vertical variability within the mixed layer with the highest value of 2.2 on day 2 and lower values latter in the experiment. A maximum was observed in 10 m depth on day 8 inside the patch with $1.3*10^3$ empty loricae l^{-1}. Outside the patch high empty loricae numbers sharply declined below 40-60 m during the first half of the experiment. During the latter half of the experiment empty loricae were evenly distributed throughout the upper 100 m of the water column. Peak numbers were found in 40 m depth on day 9 with $1.5*10^3$ cells l^{-1} (Fig. 1F). Aloricate ciliates were dominated by choreotrich species and categorised according to size. Aloricate ciliates exhibited subsurface maxima during the first week of the experiment. Thereafter a shift to higher abundances at the surface was observed for the remainder of the experiment with a peak of $2.6*10^3$ cells l^{-1} in 20 m depth on day 7. Aloricate abundances changed up to 2.8 times on day 21 in the upper mixed layer. In control waters aloricate ciliates were uniformly distributed down to depths of 80 m. Maximum cell numbers in non-fertilized waters were recorded with 10^3 cells l^{-1} in 10 m depth on day 5 (Fig. 1G).

Highly significant correlations for protozoans were only found between athecate dinoflagellates and copepod fecal pellet numbers as well as empty tintinnid loricae inside the patch. Protozoan fecal pellet numbers were highly significant correlated with protozoan fecal pellet carbon inside as well as outside the patch (Table 1).

Small metazooplankton

Small metazooplankton comprised copepod nauplii, copepodites and small adult copepods. These different live stages were represented by unidentified calanoid species and various genera including *Oncaea* spp., *Microsetella* spp., *Ctenocalanus* spp., *Metridia* spp. and *Oithona* spp. of which the latter was by far the most abundant. Nauplii exhibited elevated densities down to 60-80 m depth throughout the experiment with highest abundances usually in the upper 10-40m inside as well as outside the patch. Despite predominantly shallow peaks maximum nauplii abundances were found with approximately $85*10^3$ individuals m^{-3} in 60 m depth on day 5 inside the patch. The highest degree of variability of 3.5 for nauplii

abundances within the mixed layer was observed on day 19. Outside the patch nauplii peaked with $68*10^3$ individuals m^{-3} in 40 m depth on day 1. Below 80 m depth nauplii abundances notably declined and remained low down to 150 m (Fig. 2A). Inside the patch copepodites and small copepods showed a similar vertical distribution to nauplii with peak abundances mainly occurring in the upper 40 m of the water column and a sharp decline below 80 m depth. The maximum change in abundances within the mixed layer occurred on day 4 with 7.2. In control waters somewhat deeper maxima were observed in 20-80 m depth. Highest abundances of copepodites and small copepods were recorded with $79*10^3$ individuals m^{-3} in 40 m depth on day 4 inside and with $36*10^3$ individuals m^{-3} in 60 m depth on day 5 outside the patch (Fig. 2B). In iron enriched waters crustacean fecal pellet numbers were characterised by subsurface maxima between 40-80 m depth until day 11 and a distinct accumulation of fecal pellets in 80 m depth at the bottom of the mixed layer thereafter. Outside the patch a rather uniform distribution of fecal pellets in the upper 80 m was observed. Maximal crustacean fecal pellet numbers of $616*10^3$ fecal pellets m^{-3} were found in 40 m depth on day 11 inside the patch and exhibited highest vertical heterogeneity on day 19 with a factor of 5. Highest numbers in control waters were recorded with $506*10^3$ fecal pellets m^{-3} in 40 m depth on day 1 (Fig. 2C). Crustacean fecal pellet carbon showed a different distribution with highest concentrations predominantly found at the surface both inside and outside the patch. Only at the last two inside stations deeper maxima in 60 m depth prevailed. The highest concentration of fecal pellet carbon inside the patch was detected in 10 m depth on day 16 with 6 mg C m^{-3} whereas in control waters concentrations peaked in 40 m depth on day 21 with 3 mg C m^{-3} (Fig. 2D).

Nauplii abundances were highly significant correlated with copepodite and small copepod abundances inside the patch. Furthermore a highly significant correlation was detected inside the patch between copepod fecal pellet numbers and protozoan fecal pellet numbers as well as empty tintinnid loricae (Table 1).

Intact empty and broken diatom frustules

The vertical distribution of intact empty diatom frustules inside the patch was characterised by concentration maxima below the seasonal mixed layer during the first days of the experiment. Thereafter numbers of empty frustules peaked in shallow depths well within the mixed layer between 10-40 m. The wind-driven deepening of the mixed layer at the end of the experiment was reflected by elevated concentrations down to 60-80 m depth. Nevertheless

peak concentrations of empty frustules were still found at shallow depths between 10-40 m. Highest concentrations of empty diatom frustules inside the patch were recorded with $38*10^3$ empty frustules l^{-1} in 20 m depth on day 21 (Fig. 3A). Intact empty diatom frustules changed up to 1.8 times within the mixed layer two days prior to fertilization. Outside the patch empty frustules were more evenly distributed through the water column. In non-fertilized waters empty frustules peaked with $21*10^3$ empty frustules l^{-1} in 80 m depth on day 1. Four diatom species (*P. lineola*, *P. turgidula*, *F. kerguelensis* and *T. nitzschioides*) accounted for roughly 68% of total empty diatom frustules inside and for about 48% outside the patch by the end of the experiment. Empty frustules of *P. lineola* accounted for about one third of total empty frustules by the end of the experiment inside the patch and were predominantly found at shallow depths. Outside the patch empty *P. lineola* frustules contributed less then 10% to total empty frustules and regularly peaked at greater depths (Fig. 3B). Although highest concentrations of empty *P. turgidula* frustules were observed in 10 m depth on day 21 inside the patch empty frustules of this species usually peaked at greater depth between 40-80 m (Fig. 3C). Concentrations of empty *F. kerguelensis* frustules were more variable with depth both inside and outside the patch but frequently peaked at greater depth between 60-100 m (Fig. 3D). During the first half of the experiment empty *T. nitzschioides* frustules usually accumulated between 40-100 m depth inside the patch. Thereafter peak concentrations were found in 10-20 m depth with the exception of a deep maximum in 150 m depth on day 19. Outside the patch empty frustules of *T. nitzschioides* reached maximal concentrations between 40-100 m depth with no enhanced accumulation at the surface during the latter half of the experiment (Fig. 3E).

The vertical distribution of broken diatom frustules inside the patch exhibited highest values below the mixed layer in 60 m depth until day 3. Thereafter peak concentrations somewhat shoaled until day 11. After day 11 broken frustules culminated in 80 m depth. This depth of maximum broken frustule accumulation agrees well with the bottom of the wind-mixed surface layer (75-85 m) at the end of the experiment. Outside the patch a similar vertical distribution of broken frustules was observed with deep maxima at the beginning, shallow peaks during the middle and again high values at greater depth at the end of the experiment. The highest accumulation of broken frustules inside the patch was observed with $17*10^3$ broken frustules l^{-1} in 80 m depth on day 19. The highest degree of vertical heterogeneity within the mixed surface layer was observed with 2.1 on day 7. In control waters highest concentrations of broken frustules were revealed in 60 m depth on day 17 with $13*10^3$ broken frustules l^{-1} (Fig. 4A). *P. lineola*, *P. turgidula*, *F. kerguelensis* and *T. nitzschioides* together

accounted for 70% of total broken diatom frustules inside and for 50% outside the patch by the end of the experiment. With the exception on day 2 concentrations of broken *P. lineola* frustules peaked at shallow depths between 10-40 m inside the patch until day 11. Thereafter broken frustules clearly accumulated in 80 m depth. Outside the patch broken frustules of *P. lineola* were homogenously distributed throughout the water column during the first half of the experiment and concentrated at greater during the latter half (Fig. 4B). Broken frustules of *P. turgidula* showed a variable distribution throughout the water column over the course of the experiment inside and outside the patch. At the end of the experiment peak concentrations were found at greater depth (day 19) as well as in 20 m depth (day 21) (Fig. 4C). Broken frustules of *F. kerguelensis* reached maximum concentrations in 60 m depth or deeper inside and outside the patch. Only on day 16 inside the patch a peak in broken *F. kerguelensis* frustules was detected in 10 m depth (Fig. 4D). Initially broken frustules of *T. nitzschioides* culminated below 40 m depth until day 4. Thereafter concentration peaks were situated at shallower depths and then again showed deeper maxima towards the end of the experiment (Fig. 4E).

A highly significant correlation was found between intact empty and broken diatom frustules both inside and outside the patch. A highly significant correlation between broken diatom frustules, copepod fecal pellet numbers as well as empty tintinnid loricae could only be established inside the patch (Table 1).

Discussion

Factors influencing the vertical distribution

Despite the relatively coarse vertical resolution of our sampling technique distinct concentration peaks were detected within the mixed surface layer for the different variables. An indication that the particles and planktonic organisms dealt herein were not homogeneously distributed in the water column is exemplified by the degree of vertical heterogeneity. Whereas intact empty and broken diatom frustules exhibited rather minor vertical variability (1.8 and 2.1 times respectively) abundances of thecate dinoflagellates and copepodites and small adult copepods changed up to 10.2 and 7.2 times respectively within the mixed layer. These concentration changes within the wind mixed surface layer are significantly larger than the microscopic counting error at least for abundant objects and can be attributed to various factors that are presented in the following discussion.

Physical factors

During the three weeks of investigation the pelagic ecosystem was exposed to large fluctuations in the physical environment. Variability in wind velocity and hence turbulent kinetic energy was the major mechanism driving the fluctuations in water column structure. Wind-driven changes in mixed layer depth (MLD) therefore strongly regulated the vertical distribution of the plankton community. This is in accordance with the general concept that water column stability is an important environmental factor regulating plankton variability in the Southern Ocean (Hart 1934, Mitchell et al. 1991, Garibotti et al. 2003).
The experiment could be roughly divided into four phases. An initial phase with calm weather and a poorly mixed water column, the first gale force winds and a deepening of the mixed layer, a second phase characterised by shoaling of the mixed layer due to calmer weather and precipitation and the final phase characterised by two strong wind events inducing a deeply mixed surface layer that prevailed for the remainder of the experiment (Strass et al. 2001). Vertically heterogeneous plankton distributions are usually associated with poorly mixed conditions (Lindholm 1992). Within the well-mixed surface layer however the plankton community by definition should be distributed homogeneously. Although reoccurring strong

mixing events homogenised particles in the mixed layer exemplified by elevated concentrations down to the greater depths distinct concentration peaks within the mixed layer indicate that factors other than water column stability play a significant role in controlling plankton variability.

Condie (1999) described in a simple model thermocline concentration maxima as the simple consequence of different physical settling dynamics of non-motile particles in the turbulent surface mixed layer and the density stratified thermocline without necessarily invoking other mechanisms. However this assumption only holds true for large particles with a Peclet number >1, the ratio of the settling speed of a particle to turbulent velocity, and sinking rates within the range of 50-200 m d^{-1} (Condie 1999). For the non-motile particles described herein these sinking rates apply solely for large fecal pellets predominantly of crustacean origin. Settling velocities of cylindrical fecal pellets produced by copepods and euphausiids range from approximately less than 100 m per day to over 1000 m per day depending on particle size and density (Komar et al. 1981). Due to their high sinking rates crustacean fecal pellets may considerably contribute to the export of surface production to greater depths. A highly significant correlation between mixed layer depth and the depth of maximum copepod fecal pellet numbers (Fig. 5, $R^2 = 0.871$, $p < 0.001$) indicates that these pellets accumulated at the bottom of the mixed layer by differential settling. Nevertheless highly significant correlation of copepod fecal pellets with broken diatom frustules and empty loricae (Table 1) suggests that factors other than physical factors also influenced the distribution of large fecal pellets as will be discussed later. Protozoan fecal pellets are usually smaller and decrease more rapidly with depth (González 1992) indicating that sinking rates are considerably lower. Deep concentration peaks are associated with local production (Nöthig and von Bodungen 1989). Intact empty diatom frustules exhibit sinking rates of 0.5 to 3 m d^{-1} (Johnson and Smith 1985) and broken diatom frustules presumably display even lower sinking rates. Taking the low specific biogenic silica dissolution rates of ~ 3 $x10^{-4}$ h^{-1} reported for Antarctic surface waters (Ragueneau et al. 2000) into account 50 to 100% of the frustules will dissolve in the upper 200 m. Estimates from Nelson et al. (1995) indicate that 10 to 100% of the silica produced in the euphotic zone dissolves in the upper 50-100 m. However the accumulation of highly silicified diatom species in the Antarctic opal belt supports evidence for marked differences between taxa in resistance to dissolution. Ryves et al. (2001) found large differences in dissolution susceptibility between various limnic diatom species ranging from 47 to 1513 hours until half of the total number had dissolved. A considerable part of the more robust species like *Fragilariopsis kerguelensis*, especially when occurring in long chains, might

therefore escape dissolution at the surface and eventually reach the sediments. Settling of empty tintinnid loricae was experimentally estimated between 0.25-2.08 m d^{-1} for hyaline loricae and 1.90-15.9 m d^{-1} for agglutinated loricae (Suzuki and Taniguchi 1995). Only a very small fraction (1% or less) of the loricae produced in the upper water column reaches depths of 800-2000 m (Alder 1999). It can thus be assumed that most of these passive particles with the exception of large pellets exhibit long residence times in the surface layer and are likely to be recycled within it.

The considerable horizontal dispersion of the fertilized patch especially during the initial phase of the experiment diluted the plankton assemblage with plankton poor surrounding waters. Nevertheless it can be assumed that the effect of dilution on the vertical distribution was less pronounced as compared to vertical mixing. Advection of water masses played but a minor role during the experiment (V. Strass personnal communication) and therefore should not have impacted on the vertical distribution of particles and planktonic organisms.

Since light attenuates with depth the light conditions experienced by the phytoplankton in the surface layer are strongly influenced by the mixing regime. If the mixing depth exceeds the critical depth (z_c), the depth where photosynthesis is balanced by respiration, no net phytoplankton biomass build up takes place. During our study favourable light conditions for phytoplankton growth prevailed throughout most of the experiment despite deep mixing and z_c exceeded 100 m during most sampling days. For further details concerning the light regime during EisenEx see Gervais et al. (2002).

Chemical factors

Essential micro- and macronutrients are a prerequisite for phytoplankton growth. Klausmeier and Litchman (2001) stressed that the vertical distribution of phytoplankton in a poorly mixed water column is affected by the contrasting gradients of light and nutrients. Nutrient concentrations in the ocean usually increase with depth since they are utilised by phytoplankton at the surface and supplied from below whereas light supplied from above decreases with depth. In contrast to a poorly mixed water column all macronutrients during our study were still in abundant supply showing no marked gradient with depth (Gervais et al. 2002). The trace element iron, the limiting micronutrient in the Antarctic Circumpolar Current (ACC), was injected repetitively into the patch (Bathmann and Smetacek 2001) ensuring sufficient concentrations for phytoplankton growth. Further taking the favourable light regime as described above into account it can be assumed that phytoplankton growth was not limited

by either light nor nutrients and their availability played but a minor role in the vertical distribution of phytoplankton during this study. The same applies to the heterotrophic community since it is indirectly geared to light and nutrient distributions via primary production.

Chemical factors apart from nutrients include a variety of molecules that transmit chemical signals in the pelagic realm and may act as a grazer deterrent or facilitate mate detection. However the chemical signals that determine behavioural interactions are poorly known (Wolfe 2000). The effects of chemical signals on the larval settlement in benthic invertebrates are the best-studied systems thus far. However much less is known about the sensory ecology of the plankton (Zimmer and Butman 2000). Hence no assumptions about the effect of chemical signalling processes on the vertical distribution of planktonic organisms were made.

Biological factors

Highly significant partial correlations of biological variables (Table 1) indicate that next to physical and chemical forcing biological factors influence the zonation of plankton organisms in the water column.

Motile particles

Non-motile particles are dictated by Stokes´ Law, the larger and denser a particle the faster it sinks. Planktonic organisms however, have evolved different means of locomotion to adjust their position in the water column. These range from actively swimming with flagella, undulopodia, cilia or abdominal appendages in the case of bacteria, flagellates, ciliates and copepods respectively to regulation of buoyancy in the case of diatoms and cyanobacteria. Protozoan swimming rates typically range from 100-5000µm s^{-1} (Crawford 1994) and operate at given body lengths of less than 200 µm in a viscosity-dominated realm of low Reynolds numbers (Re <<1) (Wolfe 2000). It has been shown that dinoflagellate swimming rates appear to be considerably greater than their sinking rates (Smayda and Bienfang 1983). Abundance maxima of both thecate and athecate dinoflagellates were mainly restricted to the upper 40 m of the water column with a gradual decline to greater depths (Figs. 1A and B) indicating that these groups were able to largely maintain their position at the surface despite deep mixing. Similar vertical distributions with peaks near the surface have been previously reported for the Weddell Sea and the equatorial Pacific (Kivi and Kuosa 1994, Iriarte and Fryxell 1995). However dinoflagellate distribution was not totally unaffected by turbulent mixing since

somewhat elevated abundances down to 60-80 m depth were observed after the two major wind events on days 5 and 13 (Figs. 1A and B). Furthermore, the prominent surface maxima during the middle of the experiment coincided with a transient, shallow surface layer induced by precipitation. Ciliates have been found to be positively geotaxic (Fornshell 1984) and generally have a low cost for motility (Crawford 1994). However, the cost of motility in fast-moving forms may be significant and many fast-moving ciliates restrict motility to bursts of activity or "jumps" (Crawford 1994). The swimming rates of ciliates may exceed their respective settling velocities with the probable exception of some large, slower swimming and faster settling agglutinated tintinnids. This is documented in the vertical distribution of both loricate and aloricate ciliates. Aloricate ciliates were mainly restricted to the upper 40 m of the water column as has been previously reported for the Weddell Sea (Kivi and Kuosa 1994) suggesting active swimming to remain at the surface. Loricate ciliates however showed a more homogeneous distribution down to 80 m depth. This might be due to different depth preferences of the various tintinnid species encountered (Alder 1999) or an imbalance between sinking and swimming rates of some large presumably agglutinated tintinnids. Copepod swimming speeds, including *Oithona* sp., are higher than velocity fluctuations in the seasonal thermocline and consequently, their motion can be independent of the local flow field (Yamazaki and Squires 1996). Although many copepod species are known to perform large diurnal and seasonal vertical migration juveniles and adults of the small species prevailed in the upper water column and sharply declined below 80 m depth throughout the experiment (Figs. 2A and B). The dominance of *Oithona* spp. at shallow depths during our (Henjes and Assmy to be submitted) and previous studies (Dubischar et al. 2002, Ashjian et al. 2003) indicates that species of this and probably other small copepod genera are persistent grazers in the productive upper zone of the mixed layer throughout the year. The iron-induced phytoplankton bloom provided ample food for the juvenile and small adult copepod assemblage at the surface even supporting an increase in copepodite abundances (Henjes and Assmy to be submitted) despite a probable increase in grazing pressure by various carnivorous predators. Live diatoms, not dealt with in this paper, are able to regulate their buoyancy irrespective of size and may significantly reduce or even counter sinking rates under favourable growth conditions (Waite et al. 1997). Thus all of these planktonic organisms can actively choose their place in the water column often irrespective of the physical environment and it is therefore difficult to make assumptions about their vertical distribution on water column stability alone.

Non-motile particles

The vertical distribution of non-motile particles like fecal pellets or cell remains seems not only be related to physical factors but also to grazing activity. Among the metazoa that graze on diatoms copepods, euphausiids and salps are the major groups in the Southern Ocean (Mayzaud et al. 2002, Smetacek et al. 1990, Dubischar and Bathmann 1997). Already early studies realised the importance of these metazoan grazers on diatom productivity and stressed that *F. kerguelensis*, one of the most abundant diatoms of the open ACC, represents an important food item for euphausiids, salps and copepods and due to their strong silification the frustules are the most plentiful recognisable remains in the faecal pellets of herbivorous zooplankton (Hard 1942, Hustedt 1958, Van der Spoel 1973). Also the dominant diatom during this study, *P. lineola* (Assmy and Henjes to be submitted), was regularly documented within euphausiids and in large quantities within salps at 51°S (Hustedt 1958). Ciliates and dinoflagellates are the two most common groups of larger protozoa and have due to their high growth rates a significant grazing potential on phytoplankton standing stocks (Sommer 1989, Strom 1991, Bjoernsen and Kuparinen 1991, Hall and Safi 2001). The ubiquitous ciliate grazers produce a feeding current and ingest prey items whole and are therefore restricted to an upper size limit. They are important grazers of pico- and nanophytoplankton in Antarctic waters (Froneman and Perissinotto 1996) and have been shown to substantially control the pico and nano size fractions during a previous iron fertilization experiment in the Southern Ocean (Hall and Safi 2001). Due to their feeding mode ciliates are unlikely to substantially graze on large diatoms. In contrast dinoflagellates have evolved a formidable array of feeding strategies (Jacobson 1999) that enables them to feed on larger particles than themselves such as diatoms. The metazoan grazers with the probable exception of salps are able to crush diatom frustules whereas protozoans are incapable of breaking diatom cell walls.

Intact empty diatom frustules might be caused by various processes including recycling and disintegration of protozoan and metazoan fecal material, direct excretion of empty frustules by protozoan grazers, infestation by parasitoids and pathogens, sexual reproduction (Crawford 1995) and natural mortality induced by unfavourable growth conditions (Peters 1996a,1996b). It can be assumed that natural mortality was insignificant during this study since all nutrients were in abundant supply and despite reoccurring deep mixing events a favourable light climate prevailed throughout most of the experiment (Gervais et al. 2002). Information on sexual reproduction of Antarctic diatoms is scarce and it is therefore difficult to make assumptions about its impact on empty frustule distribution. However, mass sexual reproduction has been recorded for *C. pennatum* which triggers downward transport of empty

diatom cell walls and results in almost monospecific layers of this species in Antarctic sediments despite its weakly silicified frustules (Crawford 1995). Empty cells are also frequent within chains of *F. kerguelensis* (El-Sayed and Fryxell 1993) and empty chains and empty frustules within chains of *F. kerguelensis* accounted for 25-94% (70% on average) of total empty frustules of this species during our study. The cause of these empty frustules whether by sexual reproduction, natural or grazer-induced mortality remains obscure. However no massive release of empty frustules that could be associated to a sexual phase was observed during the experiment. The absolute numbers as well as the relative proportion of empty frustules to total cells of *C. pennatum* actually decreased during this experiment indicating that massive sexual reproduction of this species did not take place (Fig. 6). Although release of empty frustules by sexual reproduction and natural mortality cannot be ruled out grazing by proto- and metazoa was likely the major cause of intact empty diatom frustules in the water column. Viral infection of diatoms has to our knowledge not been observed to date, nevertheless pathogens cannot be ruled out as a possible source of intact empty frustules. Diatoms infested with parasitoid protists have been previously reported (Schnepf et al. 1990) and may well cause the accumulation of intact empty diatom frustules in the water column. During our study however no obvious infections by parasitoids were noticed. Occasionally fungi-like protists, possibly Labyrinthulomycetes, some of which are known parasites (Raghukumar 2002), have been observed attached to *Corethron pennatum* spines but they seemed to have no harmful effect on that diatom species.

It is well known that protozoa feed on diatoms (Epstein et al. 1992, Naustvoll 2000) and since protozoa unlike crustacean grazers are incapable of breaking diatom frustules they considerably contribute to the production of empty diatom frustules. Especially dinoflagellates have evolved various feeding modes including tube, pallium and gulp feeding (Hansen and Calado 1999). Peduncular feeding is a common mode of tube feeding and has been reported in a number of athecate and thecate genera. The peduncle typically pierces the cell surface of the prey, sucks its contents and is retracted after the feeding process (Hansen and Calado 1999). Although peduncular feeding on a diatom species has been reported for the parasitic dinoflagellate, Paulsenella sp., (Schnepf et al. 1988) there are to our knowledge no further accounts of this feeding mode on diatoms to date. Pallium feeding is known only from thecate dinoflagellates of the genus *Protoperidinium* and the *Diplopsalis* group. With their "feeding veil" (pallium) these dinoflagellates are able to digest the prey external. After digestion of the prey items the pallium is retracted and the empty frustules, in the case of diatoms, discarded (Hansen and Calado 1999). No correlation was found between empty

diatom frustules and thecate dinoflagellates indicating that pallium feeding was not a major source of intact empty diatom frustules. Gulp feeding is especially common among athecate dinoflagellates that engulf their prey whole (Hansen and Calado 1999) and are therefore restricted to an upper size limit. A significant correlation of empty diatom frustules and athecate dinoflagellates (Table 1) suggests that gulp feeding was an important feeding mode producing intact empty diatom frustules. The much higher abundances of athecate compared to thecate dinoflagellates further indicate that the former exerted a stronger control on diatoms at least in the lower prey size range. However, the highly significant correlation between intact empty and broken diatom frustules (Table 1) inside as well as outside the fertilized patch indicates that grazing by crustaceans might also play a significant role in the distribution of empty frustules. When copepods ingest next to crushed diatoms whole cells and package them intact into fecal pellets these pellets might be a considerable source of empty diatom frustules after disintegration. Although euphausiids are able to grind food particles with their gastric mill (Suh and Toda 1992) intact chains of the heavily silicified diatom *F. kerguelensis* have been observed in euphausiid guts (personnal observation). The accumulation of intact empty frustules of *Pseudo-nitzschia lineola* and *Thalassionema nitzschioides* near the surface where highest abundances of both dinoflagellates and ciliates were found further points to protozooplankton as a significant source of empty frustules of these two species. Although empty frustules of *Pseudo-nitzschia turgidula* peaked at greater depths protist grazers probably accounted for most of their production since the rather delicate frustules of this species are unlikely to outlast crustacean grazing undamaged. Empty frustules of *Fragilariopsis kerguelensis* accumulated at greater depths and were dominated by partly empty and empty chains. Partly empty chains with an unfavourable ratio of live to empty cells might lose buoyancy control and sink. We hypothesise that grazing of protozooplankton was likely the major source of these empty chains.

Broken diatom frustules might originate from “sloppy feeding” by crustacean grazers and/or by recycling and disintegration of diatom containing crustacean fecal pellets. “Sloppy feeding” is rather associated with the release of dissolved organic matter (Banse 1995) than with the production of cell debris and might therefore only be a minor contributor to the pool of broken diatom frustules. Nevertheless Roy et al. (1989) found inefficient feeding by copepods that resulted in diatom cell fragmentation and ingestion of only a fraction of the debris produced. High abundances of juvenile and small adult copepods and the preponderance of the copepod genus *Oithona* (Henjes and Assmy to be submitted), known for its coprophagous feeding behaviour (González and Smetacek 1994), are a strong indication

that recycling of fecal pellets was the major source of diatom debris in the water column. Dubischar et al. (2002) found similar high abundances of small pelagic copepods in the Polar Frontal Zone of the Southern Ocean and concluded that grazing pressure by the small copepod assemblage on small plankton organisms and fecal material led to high recycling efficiencies and low export rates. During EisenEx the small copepod assemblage seemed to have rearranged their vertical distribution towards the chlorophyll maxima and hence performed a diet shift from detritus to phytoplankton (Krägefsky et al. to be submitted). However, highly significant correlation of broken diatom frustules with the number of copepod fecal pellets further indicates that feeding on fecal pellets and their subsequent disintegration led to the accumulation of broken diatom frustules in the proximity of coprophagous feeding activities. This tight coupling is also evident in the similar vertical distribution pattern of both variables especially at the end of the experiment where both broken frustules and crustacean fecal pellet numbers accumulated at the bottom of the mixed layer in 80 m depth (Figs. 2C and 4A). This is consistent with deep maxima of broken frustules of *Pseudo-nitzschia lineola*, *P. turgidula*, *Fragilariopsis kerguelensis* and *Thalassionema nitzschioides* at the end of the experiment. This accumulation of fecal pellets and broken diatom frustules at greater depths and the sharp decline below the pycnocline indicates that despite the mesozooplankton mediated export of organic matter (Krägefsky et al. to be submitted) a part of the fecal pellet production was retained in the surface mixed layer and regenerated within it. Furthermore a significant correlation was established between small copepods and copepodites and broken diatom frustules indicating that copepod grazing accounted for most of the accumulation of broken frustules.

Empty tintinnid loricae were frequently observed in fecal pellets (personnel observation) and highly significant correlations with broken diatom frustules and copepod fecal pellets indicate that empty loricae were re-suspended after disintegration of fecal pellets and that tintinnids constituted an important food item for copepods.

Two main types of small protozoan like fecal pellets were discriminated during this study: olive green ellipsoid to spherical (10-50µm in diameter) pellets similar to those described by Gowing and Silver (1985) and pellets of variable size and shape (10-100µm in length) enclosed by a membrane that mainly contained intact empty diatom frustules. Whereas the former are likely to originate from either re-ingestion and repackaging of larger crustacean pellets by phaeodarian and spumellarian radiolarians, dinoflagellates and coprophagic copepods (e.g. *Oithona*) or from direct production by juvenile and small adult copepods (Gowing and Silver 1985, González 1992, Beaumont et al. 2002) the latter are predominantly

produced by athecate dinoflagellates and pallium feeding thecate dinoflagellates (Nöthig and von Bodungen 1989, Buck et al. 1990, Beaumont et al. 2002). Although planktonic ciliates are known to produce fecal aggregates these aggregates are often loosely packed and disintegrate rapidly (Stoecker 1984) and likely accounted for only a minor fraction of total protozoan fecal pellets. A highly significant correlation between protozoan and copepod fecal pellets suggests that utilisation of crustacean pellets by protozoa and/or coprophagic copepods as well as direct excretion by microcopepods were the major sources of oval opaque pellets. This is further supported by the dominance of this type of pellet in relation to pellets containing solely diatom frustules. However, Beaumont et al. (2002) pointed out that coprophagy and ablation in the water column may significantly fragment larger fecal pellets and therefore it cannot be ruled out that some of the protozoan faeces were actually disintegrated copepod fecal pellets. The membrane-bound pellet type usually contained little amorphic material and was dominated by intact empty frustules of either monospecific origin (mainly chains of *Pseudo-nitzschia* sp.) or of several diatom species. Due to their relatively small contribution to total protozoan fecal pellets this type of pellet is likely produced by specialised protozoan grazers. Buck et al. (1990) described fecal pellets of an athecate dinoflagellate in Antarctic sea ice that were predominantly composed of pennate diatoms and resembles those found during our study. Another source for these membrane-bound, semi-transparent pellets could also be the feeding veil (pallium) of thecate dinoflagellates. Members of the genus *Protoperidinium* spp. are known pallium feeders and their occurrence during this study (Henjes and Assmy to be submitted) indicates that species of this genus were probably responsible for the production of this type of pellet. Especially pellets containing only chains of single diatom species are a strong indicator of the pallium feeding mode. However these pallium like pellets are probably not true fecal pellets since the pallium enclosing the pellet is normally retracted after feeding (Hansen and Calado 1999) and the prey remains discarded. Hence intact “pellets” of this type are probably a product of disruption caused by fixation. The significant correlation between athecate dinoflagellates and protozoan fecal pellet carbon inside the patch (Table 1) together with peak concentrations of both variables on day 8 (Figs. 1B and C) indicates that athecate dinoflagellates contributed considerably to protozoan fecal pellet production. Although not significantly correlated the peak of protozoan fecal pellet numbers coincided with the shallower peak in thecate dinoflagellate abundance on day 11 (Figs. 1A and C). However thecate dinoflagellates are probably not a major source of protozoan fecal pellets assuming that they are predominantly represented by pallium and peduncle feeders that retract their feeding appendages after

feeding and discard the remains of their prey loosely into the water column. Protozoan fecal pellet numbers and carbon showed despite inconsistent concentration peaks on days 11 and 8 respectively (Figs. 1C and D) a highly significant correlation (Table 1) suggesting that a homogeneous size distribution of these pellet types persisted throughout the experiment and no dramatic shifts in feeding modes or grazers occurred.

Faeces produced by large metazoan grazers are a prominent component of the vertical particle flux in the Southern Ocean (Gersonde and Wefer 1987, Bathmann et al. 1991, Dagg et al. 2003). During this study copepods were the main source of large recognisable faeces as indicated by the dominance of compact opaque cylindrical pellets characteristic for copepods. This finding is further supported by the significant correlation of copepodites and small adult copepods with fecal pellet numbers inside as well as outside the patch (Table 1). Large fecal strings mainly produced by euphausiid were occasionally found in water samples and accounted despite their large size for only a minor portion of the total fecal pellet standing stock. No correlation was found between pellet numbers and pellet carbon (Table 1) indicating that maximum numbers were largely represented by small and fragmented pellets. This again points to the importance of coprophagic feeding in the recycling of fecal pellets and the numerical dominance of the genus *Oithona*, known to feed on faeces and to produce small oval pellets (González et al. 1994), further supports this trophic link.

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Figure legends

Fig. 1 Vertical distribution of protozooplankton and protozoan fecal pellets inside and outside the fertilized patch. A) thecate dinoflagellates, B) athecate dinoflagellates, C) protozoan fecal pellet numbers, D) protozoan fecal pellet carbon, E) loricate ciliates, F) empty loricae, G) aloricate ciliates. Abundances are given in cells l^{-1} and protozoan fecal pellet numbers and carbon in numbers l^{-1} and µg C l^{-1} respectively.

Fig. 2 Vertical distribution of small metazooplankton inside and outside the fertilized patch. A) nauplii, B) copepodites and small adult copepods, C) crustacean fecal pellet numbers, D) crustacean fecal pellet carbon. Abundances are given in individuals l^{-1} and crustacean fecal pellet numbers and carbon in numbers l^{-1} and µg C l^{-1} respectively.

Fig. 3 Vertical distribution of intact empty diatom frustules inside and outside the fertilized patch. A) total empty diatom frustules, empty frustules of B) *Pseudo-nitzschia lineola*, C) *P. turgidula*, D) *Fragilariopsis kerguelensis*, E) *Thalassionema nitzschioides*. All numbers are given in empty frustules l^{-1}.

Fig. 4 Vertical distribution of broken diatom frustules inside and outside the fertilized patch. A) total broken diatom frustules, broken frustules of B) *Pseudo-nitzschia lineola*, C) *P. turgidula*, D) *Fragilariopsis kerguelensis*, E) *Thalassionema nitzschioides*. All numbers are given in broken frustules l^{-1}.

Fig. 5 Correlation between the mixed layer depth (MLD) and the depth of maximum crustacean fecal pellet numbers inside the patch. Depths are given in meters.

Fig. 6 Abundances of full, intact empty and broken frustules of *Corethron pennatum* over the course of the experiment inside the patch.

Table 1

	2		3		4		5		6		7		8		9		10		11		12		13	
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
1. Thecate dinoflagellates	0.310	N.S.	N.S.	N.S.	N.S.	N.S.	0.308	0.363	0.256	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.311
2. Athecate dinoflagellates	1.000	1.000	N.S.	0.321	0.218	0.480	N.S.	N.S.	0.326	N.S.	0.342	0.401	0.328	0.407	**0.433**	N.S.	N.S.	N.S.	N.S.	N.S.	0.248	N.S.	**0.476**	0.335
3. Loricate ciliates			1.000	1.000	N.S.	0.305	N.S.	N.S.	N.S.	-0.307	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.366	N.S.
4. Aloricate ciliates					1.000	1.000	N.S.	N.S.	N.S.	N.S.	0.227	N.S.	0.306	0.421	N.S.	N.S.	N.S.	-0.335	N.S.	N.S.	N.S.	N.S.	0.299	N.S.
5. Nauplii							1.000	1.000	**0.497**	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.409
6. Copepodites and small copepods									1.000	1.000	0.294	0.320	N.S.	N.S.	0.312	0.393	N.S.	0.432	N.S.	N.S.	N.S.	N.S.	0.243	0.325
7. Broken diatom frustules											1.000	1.000	**0.679**	**0.608**	**0.480**	N.S.	0.317	N.S.	0.260	N.S.	N.S.	N.S.	**0.379**	N.S.
8. Empty diatom frustules													1.000	1.000	N.S.	0.395	0.236	N.S.	N.S.	N.S.	N.S.	N.S.	0.236	N.S.
9. Copepod fecal pellet number															1.000	1.000	N.S.	N.S.	**0.523**	0.311	0.354	0.360	**0.433**	N.S.
10. Copepod fecal pellet carbon																	1.000	1.000	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
11. Protozoan fecal pellet number																			1.000	1.000	**0.733**	**0.553**	N.S.	N.S.
12. Protozoan fecal pellet carbon																					1.000	1.000	N.S.	N.S.
13. Empty tintinnid loricae																							1.000	1.000

Fig. 1

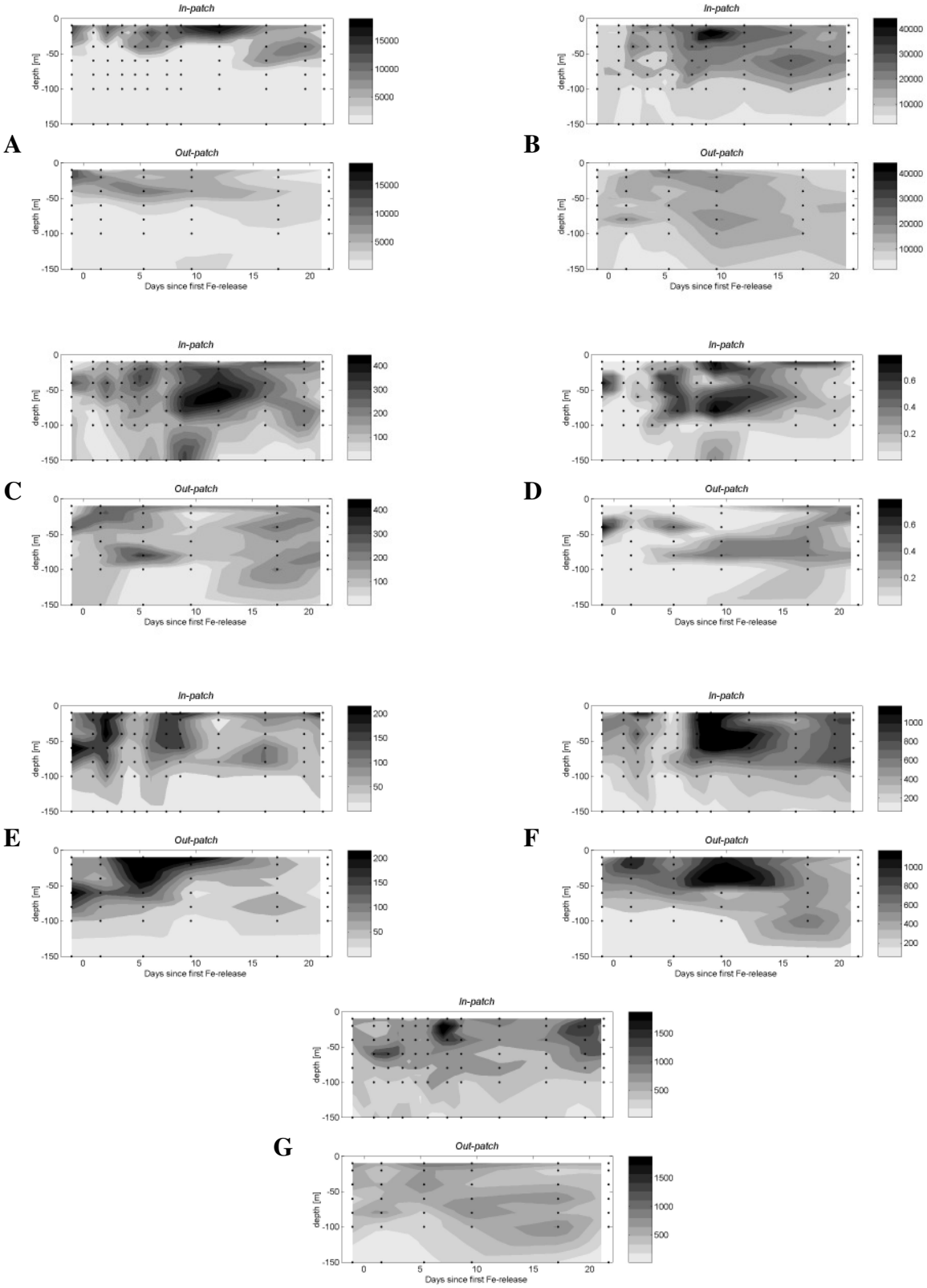

A
In-patch
Out-patch
depth [m]
Days since first Fe-release
B
C
D
E
F
G

Fig. 2

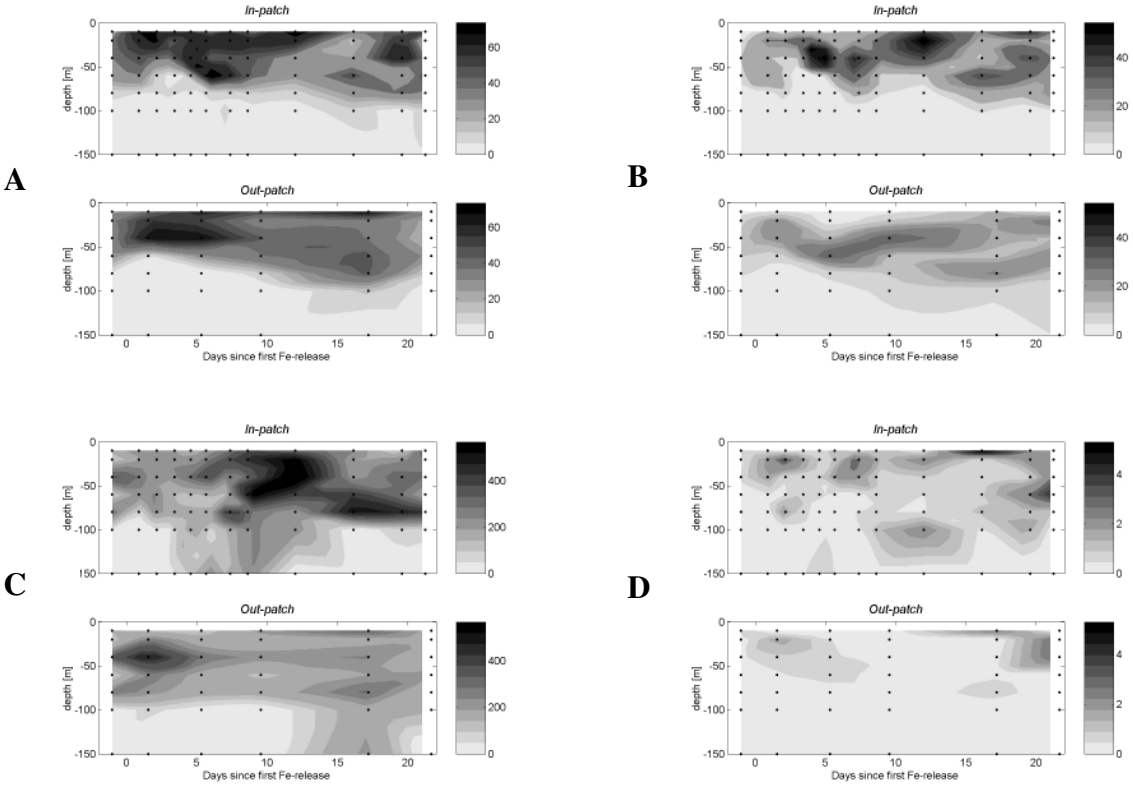


Fig. 3

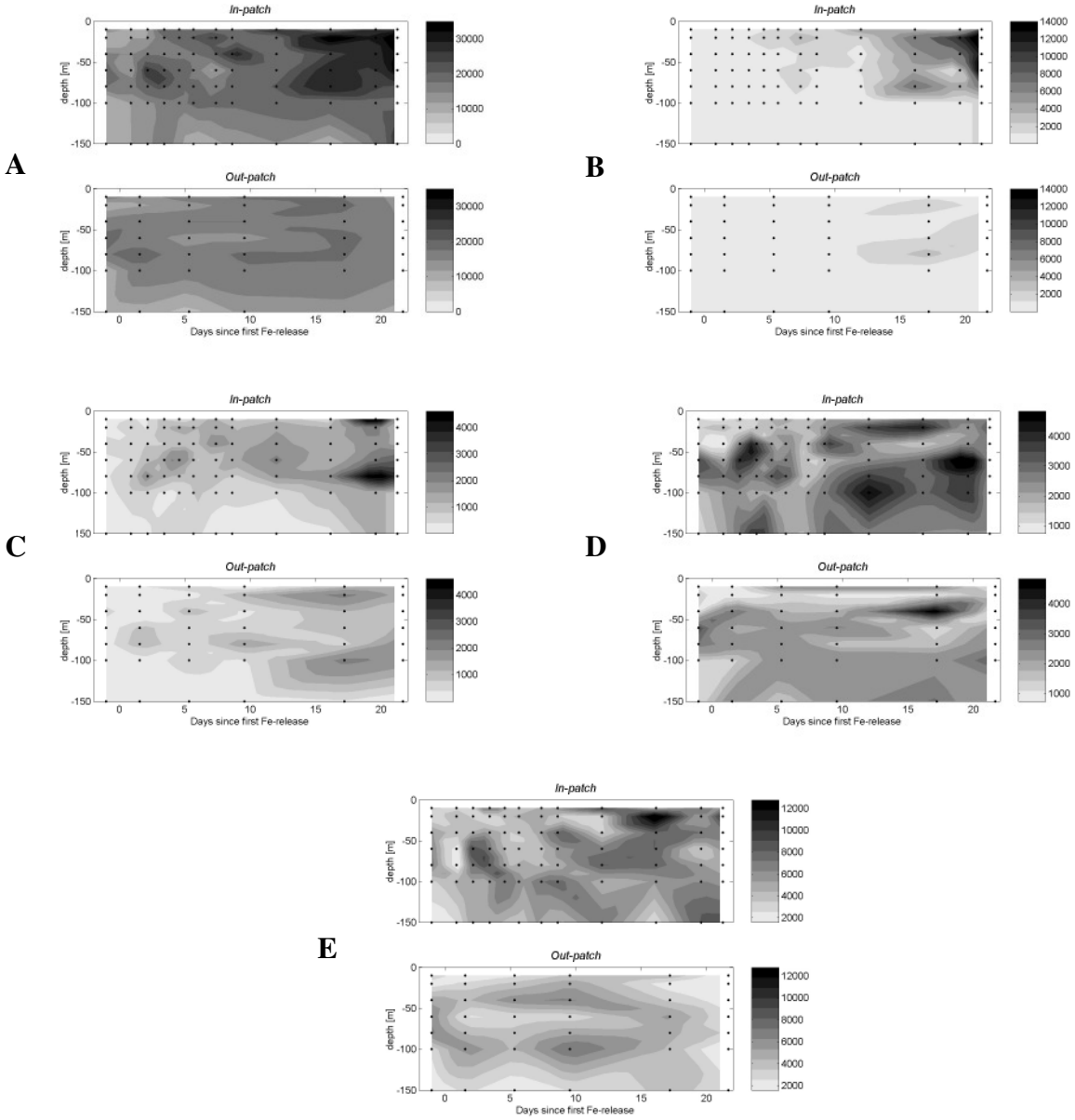


Fig. 4

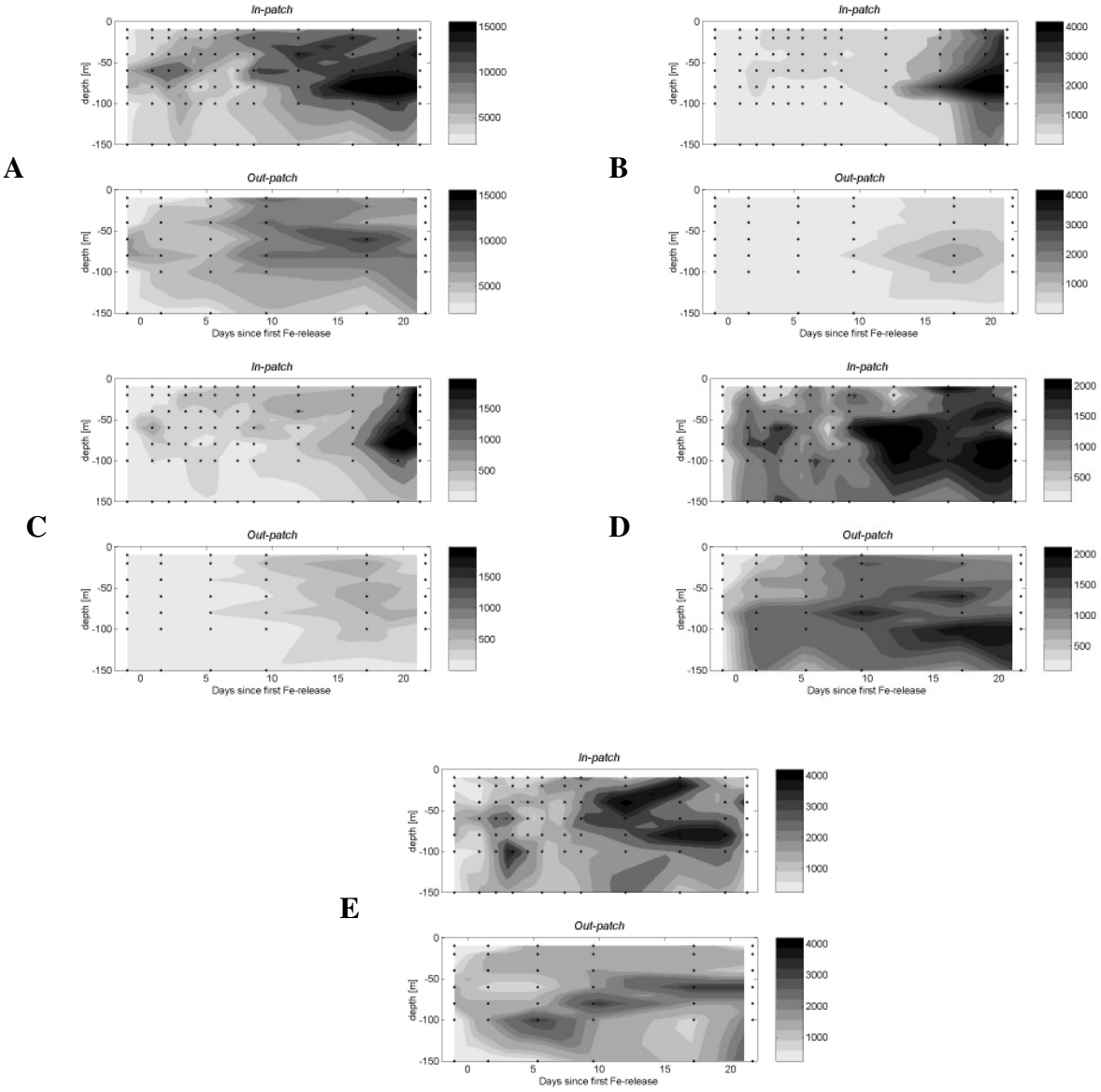


Fig. 5

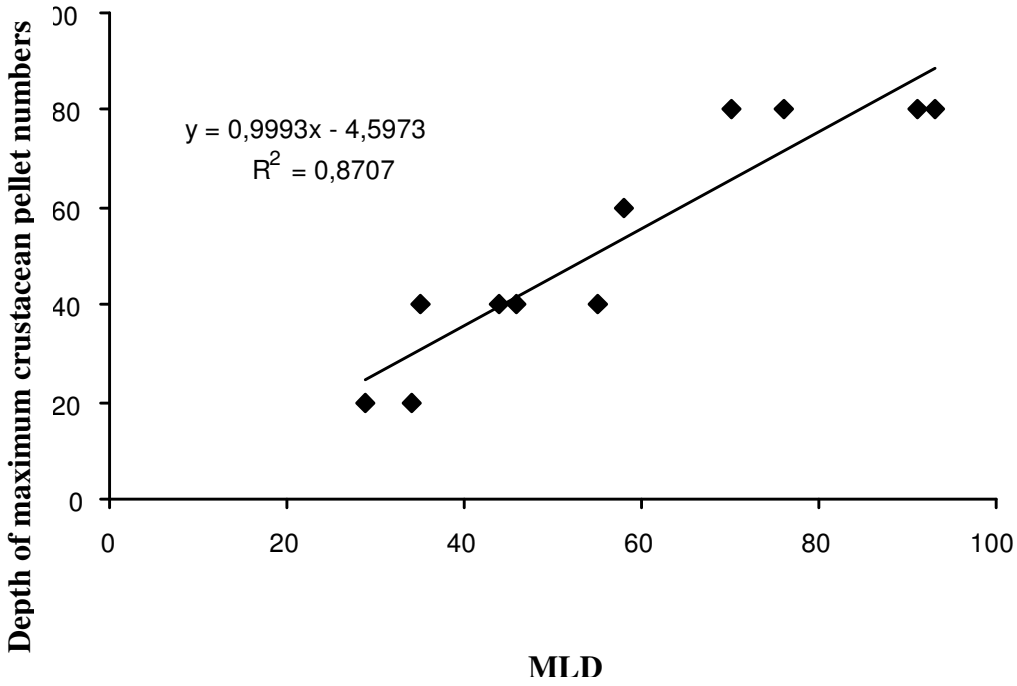
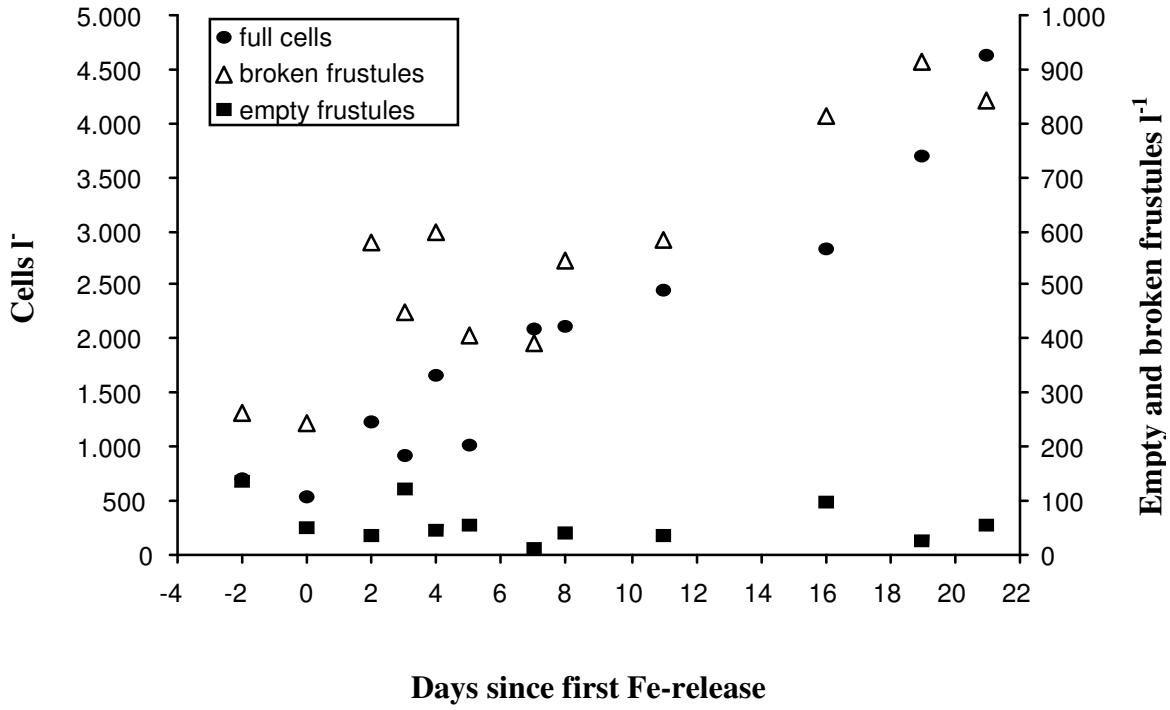

Depth of maximum crustacean pellet numbers
y = 0,9993x - 4,5973
R2 = 0,8707
0
20
40
60
80
100
MLD

Fig. 6


full cells
broken frustules
empty frustules
Cells l-1
Empty and broken frustules l-1
Days since first Fe-release
5.000
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.000
500
0
1.000
900
800
700
600
500
400
300
200
100
0
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22

Manuscript 3

Response of the protozoo- and small metazooplankton assemblage to an iron-induced phytoplankton bloom during EisenEx

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In preparation for submission

Abstract

The dynamics, composition and grazing impact of some components of the pelagic food chain were studied during the *in situ* iron fertilization experiment (EisenEx) in the Polar Frontal Zone of the Southern Ocean in austral spring (November 2000). During the 21 d experiment, protozooplankton (choanoflagellates, aplastidic thecate and athecate dinoflagellates and aplastidic ciliates) and small metazooplankton (copepod nauplii, copepodites and adults of small genera) were determined from the mixed layer inside and outside the patch using Niskin bottle samples. Bacteriovorous choanoflagellates increased slightly inside and outside the patch, indicating that they did not experience iron limitation prior to fertilization. A strong relationship between choanoflagellate and bacteria and the relatively small increase in bacterial concentration further suggests that a close coupling between these two components of the microbial food web was maintained throughout EisenEx. The aplastidic dinoflagellates showed a ~three-fold increase in abundance and biomass in the first 10 d of the experiment, but decreased thereafter to about two-fold higher values compared to pre-fertilization values. The decline after day 10 indicates increasing grazing pressure by copepods. Aplastidic dinoflagellate population was dominated by small sized (20-40µm) athecate dinoflagellates which comprised up to 58% of population stocks suggesting that also food limitation could be a further explanation for the strong decrease since the phytoplankton bloom was dominated by long chain-forming diatoms in the second half of the experiment. Ciliate abundances increased only slightly due to heavy grazing pressure by copepods, but biomass doubled with the majority of the increase caused by a three-fold increase in aloricate choreotrichs. Within the small metazoans, copepod nauplii numbers stayed more or less constant, whereas biomass doubled indicating individual growth during EisenEx. Numbers of small sized copepodites and adults of cyclopoids (predominantly *Oithona similis*) and small calanoid copepods (predominantly *Ctenocalanus citer*) increased ~three-fold suggesting either a enhanced recruitment from naupliar and copepodite stages, respectively, and/or an accumulation of numbers due to migration of individuals from below the mixed layer into the upper water column caused by a diet shift. The changes in the dynamics and structure of the studied components of the pelagic food web during the EisenEx experiment suggest that microprotozooplankton grazing strongly affected bacterial and possibly the pico- and nanoplankton populations, but was low on the diatom assemblage. Thus, the addition of Fe to a small patch of the Southern Ocean had a considerable impact on the food chain components and sheds new light on the interrelationships within the pelagic ecosystem.

Introduction

Pelagic ecosystems are driven by the interaction of organisms that fall into four functional components: the autotrophic phytoplankton and the heterotrophic bacteria, heterotrophic protists and metazoa. The systematic study of the protozooplankton has traditionally lagged behind that of the other components because of their great heterogeneity, despite recognition of their important role in structuring pelagic ecosystems (Burkill et al. 1993, 1995; Becquevort 1997; Klass 1997; Caron et al. 2000). The organisms representing this component are phylogenetically and functionally more diverse than either of the other components. Their study requires specialization and dedication to specific size classes, taxonomic groupings or mineral-bearing taxa with the result that the component as a whole tends not to be regarded on an equal footing with the others of the plankton. Yet, despite their heterogeneity they share common features that identify them as a distinct component: they tend to be of the same size class as their prey and, being unicellular and heterotrophic, can potentially have higher growth rates than the phytoplankton.

It is now well established that grazing pressure of the heterotrophic nanoflagellates (HNF) restricts the biomass of bacteria but also the pico- and nanophytoplankton of the microbial network and prevent them from reaching bloom proportions in the presence of adequate resources (Becquevort 1997; Caron et al. 2000; Hall and Safi 2001). So only larger-celled phytoplankton, in particular diatoms and the colonial *Phaeocystis* contribute to the blooms that fuel the higher trophic levels of pelagic food chains and drive the biogeochemical cycles of elements (Smetacek 1985; Smetacek et al. 1997; Bathmann 1998; Goffart et al. 2000). But this is a paradox because there are various large-celled heterotrophic protists capable of feeding on bloom-forming phytoplankton using various feeding modes. The major groups under study are dinoflagellates and ciliates of which several species are cosmopolitan. The former are able to graze on a large variety of different size classes depending on feeding behavior (Jacobson 1999). More recent studies have shown that several groups of dinoflagellates have complex feeding behaviors and apparatus that enable them to feed via a pallium or to pierce prey much larger than themselves (Inoue et al. 1993; Buskey 1997; Tillmann and Reckermann 2002). Other dinoflagellates, including the athecate species, can engulf prey of similar size meaning that chains and colonies are out of their “reach” (Hansen et al. 1994; Hansen and Calado 1999). Choreotrich ciliates are known to graze organisms of size up to 40% the size of their own oral diameter (Heinbokel 1978; Jonsson 1986). So why are these organisms not capable of checking biomass build-up as are their smaller

counterparts the HNF? One explanation supported by field recent observations is that biomass of this component is kept in check by the selective grazing pressure of metazooplankton, in particular copepods, which feed preferentially on heterotrophic protists rather than phytoplankton (Atkinson 1996; Lonsdale et al. 2000; Turner et al. 2001; Mayzaud et al. 2002). This begs the question why protozooplankton is more palatable than bloom-forming phytoplankton species? Another additional explanation is that the bloom-forming phytoplankton species have developed adequate defences against many of the protozoan grazers implying that the evolutionary arms race is an important factor driving speciation (Smetacek 2001). This would mean that heterotrophic protists and their prey have co-evolved implying that phylogenetic groups comprising the protozooplankton are selective rather than generalist feeders. In the former explanation biomass is controlled by proximate factors and in the latter ultimate, or evolutionary factors pertaining to species-specific adaptations are at work. These explanations are obviously not mutually exclusive but determining their relative roles can only be carried out in the field.

Our approach to study the complex relationships underlying the structure of pelagic food webs with particular reference to the protozoan component is to follow the populations of the various dominant species of protozoplankton in relation to that of their potential prey (phytoplankton) and their predators (metazooplankton) under experimental conditions.

In iron fertilization experiments, the pelagic system is relieved from its limiting resource, which in the Southern Ocean is iron. Following the responses of the various groups and their interrelationships enables keeping track of proximate factors controlling population sizes of the dominant species and sheds light on possible intrinsic controls. A significant part of the Southern Ocean is one of three major oceanic ecosystems, including the eastern equatorial Pacific and subarctic Pacific, in which the inability of phytoplankton to fully utilize high ambient levels of macronutrients is believed to be due to iron limitation (Martin et al. 1991). Recent analysis of these ocean`s high-nutrient, low chlorophyll (HNLC) regions have emphasized the dual regulatory influences of iron limitation and grazing (Cullen 1991; Morel et al. 1991; Price et al. 1994, Landry et al. 1997).

In view of the limitation regarding our knowledge on the interactions within the microbial community and their implication on other components of the pelagic ecosystem in the Polar Frontal Zone of the Southern Ocean, this study is to investigate the response of heterotrophic protists (choanoflagellates, aplastidic dinoflagellates and ciliates) and small metazoans (copepod nauplii, copepodites and small adults) to the addition of iron and hence induced phytoplankton bloom during EisenEx. These data will allow an evaluation of the impact of

iron addition on the microbial community in the Southern Ocean. We also discuss the role of protozooplankton and small metazoans in the ecosystem of the Southern Ocean.

Material and Methods

Sampling took place during an *in situ* mesoscale iron fertilization experiment (EisenEx) conducted in the Atlantic Sector of the Southern Ocean (48°S, 21°E) in austral spring (8-29 November) 2000. A cyclonic eddy (150 km in diameter) shed by the meandering Antarctic Polar Front (APF) and moving northward was chosen as the "container" for the experiment and its centre marked with a drifting buoy. An area of about 50 km^2 around the buoy of was fertilized with 4 tons of acidified iron sulfate solution ($FeSO_4$) on three occasions at 8 day intervals (Strass et al. 2001). The tracer SF_6 was added at the first iron infusion in order to relocate the iron fertilized "patch" (Watson et al. 2001). Inside and outside stations were chosen according to SF_6 concentration measured along horizontal surface surveys. So called "in-stations" were situated at the highest observed SF_6 concentrations, whereas "out-stations" were within adjacent waters of the fertilized patch with background SF_6 concentrations. Two of the in-stations were, in fact, situated at the edge of the iron-enriched patch ("edge-stations") as indicated by intermediate SF_6 concentrations. The station "T-2", which occurred 2 days prior to the first fertilization and/or day 0 were referred to as an reference station. (see Assmy and Henjes, to be submitted).

The areal daily primary production increased inside the patch and reached the maximum of 790 mg C m^{-2} d^{-1} on day 16, and decreased thereafter to almost initial values. Chlorophyll-*a* concentrations increased from initially 0.5 mg m^{-3} to maximal values of 2.5 mg m^{-3} in the upper 80 m inside the patch by the end of the experiment, but stayed more or less constant in adjacent waters (Gervais *et al*., 2002). Diatoms dominated phytoplankton standing stocks in the second half of the experiment increasing to a standing stock of ~3.5 g C m^{-2} (integrated over 80m depth) on day 21 with long-chain forming *Pseudonitzschia* spp. being the most dominant genus (Assmy and Henjes, to be submitted).

During EisenEx the analysis of the proto- and small metazooplankton community and its response to iron fertilization was carried out in detail and compared with processes in the surrounding water for three weeks.

Abundance, biomass and composition of heterotrophic protists and small metazooplankton

For quantitative assessment of the protozooplankton population (heterotrophic nano-, protozooplankton), small metazooplankton (copepod nauplii, copepodites and small adults <1.5mm), protozoan and metazoan fecal pellets water samples were taken at seven discrete depths (10, 20, 40, 60, 80, 100 and 150m) at 19 in and out patch stations using Niskin bottles mounted on a CTD (Conductivity Temperature Depth) rosette. Aliquots of 200 ml were preserved with hexamine buffered formaline solution at a final concentration of 2% and stored at 4°C in the dark for subsequent counting back in the home laboratory. Cells were identified and enumerated using inverted light and epifluorescence microscopy (Axiovert 25, Axiovert 135 and IM 35) according to the method of (Utermöhl, 1958) following the recommendations of Venrick (1978) and (Edler, 1979). Before counting water samples were transferred into 50 ml Hydrobios sedimentation chambers and settled for at least 48 hours. Organisms were counted at magnifications of 200-640x according to the size of the organisms examined. Very abundant species were counted in stripes, abundant species in a quarter or half a chamber and less abundant species in the whole chamber. Each aliquot was examined until at least 50 to 400 cells, depending on taxon, had been counted. Organisms were classified as plastidic or aplastidic on the basis of genus and by the presence of an autofluorescence signal. All aplastidic ciliates, except Mesodinium rubrum, which was not included in the data, were counted as heterotrophs. Aplastidic flagellates were classified as heterotrophic dinoflagellates (thecate and athecate) and other flagellates. The Utermöhl method does not allow a reliable discrimination of plastidic and aplastidic nanoflagellates, especially in the smallest size group (<20 µm). Due to this reason the aplastidic nanoflagellates were not taken into account for the results, except the choanoflagellates which could be reliable identified.

Fecal pellets counted during this study had several different forms. We used the recommendations of Klaas (1997) to discriminate between two types of fecal pellets attributed to protozoan origin: ellipsoid to spherical (10-50µm in diameter) pellets as described by Gowing and Silver (1985) containing an matrix of fine material unidentifiable by light microscopy. Pellets of variable size and shape (10-100µm in length) enclosed by a membrane and containing intact diatom frustules (Buck et al. 1990; Gonzalez 1992). Metazoan fecal pellets were classified in four different shapes after the recommendation of Gowing et al. (2001): cylindrical, ellipsoidal, ovoid and angular pellets. All metazoan fecal pellets were

coated by a membrane and contained mainly unidentifiable amorphous material, intact and broken diatom frustules and thecae of dinoflagellates.

In addition to protozoans and fecal pellets, copepod nauplii, copepodites and small adults were counted because copepod nauplii are the same size as small protozoans, and thus might have a similar function in the microbial food web whereas small copepods are suggested to feed frequently on heterotrophic protists (Atkinson 1996). Copepod nauplii, copepodites and small adults, where the latter are grouped into small copepods <1.5mm, were enumerated from concentrated samples. The methodology for handling concentrated samples is described in Henjes and Assmy (to be submitted).

The sizes of species were measured and their biovolume calculated from equivalent geometrical shapes (Edler, 1979). Biovolumes were calculated from measurements on at least 300 cells for choanoflagellates and on at least 30-40 randomly chosen cells of each size class for heterotrophic dinoflagellates and ciliates, respectively. Cell volume was converted to cellular carbon content through recommended carbon conversion equations using the following carbon to volume relationships (Menden-Deuer and Lessard 2000): for heterotrophic dinoflagellates, $C\ cell^{-1} = 0.444*V^{0.864}$; for small protozoan plankton, $C\ cell^{-1} = 0.216*V^{0.939}$; for aloricate ciliates, $C\ cell^{-1} = 0.230*V^{0.984}$ and for loricate ciliates, $C\ cell^{-1} = 0.679*V^{0.841}$, with V representing total cell volume (μm^3) and C describing cellular carbon content (pg).

Carbon volume conversion factors from literature were used to estimate protozooplankton fecal pellet carbon content. For all different protozoan fecal pellet forms mentioned we used 0.0114 pg C μm^{-3}, a value estimated by Buck et al. (1990) for the cytoplasmic content of an average-sized pellet produced by heterotrophic dinoflagellates in the Weddell Sea ice. For all metazoan fecal pellets a conversion factor of 0.016 pg C μm^{-3} was used (Gowing et al., 2001).

For copepod nauplii at least 30 randomly chosen individuals were measured. Cell volumes were calculated by using the most similar geometrical shapes. To estimate carbon (in pg) the mean volume (in μm^3) of the three taxonomic groups was multiplied by 0.08 (Beers and Stewart 1970). For analyzing carbon content of small copepods a significant amount of individuals were picked out of a 300µm bongo net sample stored at –30°C in a airtight container. Individuals were rinsed quickly in deionised water to wash of salt crystals, stored in tin cups and dried for 24 h in a drying oven at 55°C. Dried samples were analysed by a C/N-Analyzer (Carlo Erba NA-1500 Analyzer) using a standard protocol (Meyer et al. 2003). For genera which were not frequently caught with the Bongo net, a significant amount of individuals were picked out of preserved water samples, rinsed with deionised water,

transferred to a pre-weighted tin cup, dried for 24h in a drying oven and re-weighed using a electronic micro scale (Sartorius, S4) . Carbon content was calculated following the recommendation of Harris et al. (2000). Protozoan, copepod nauplii and small copepod carbon standing stocks were estimated by integrating the biomass in the upper 80 m of the water column for all stations.

Accumulation rates (k) of protozooplankton were calculated according to the following equation:

$k(d\text{-}1) = \ln(N_t/N_0)/\text{time (in days)}$

where N_0 is the initial abundance and N_t the abundance at time t.

Size composition of microprotozooplankton

For determination of size distribution, cells were classified during counting into size groups at 20 µm interval whereas only ciliates >20µm were put into account for this data. Protozoans in size classes larger than 60 µm were pooled so that a statistically significant number of cells for each size class (Edler 1979). Dinoflagellates were sized-classified according to their body and ciliates according to their diameter in order to account for the differences in the prey size spectrum consumed by the two groups. For dinoflagellates prey:predator size ratios (ranging from 0.4:1 to 7:1; Hansen et al 1994) tend to be variable and depend on feeding behaviour. In thecate dinoflagellates of the genera *Protoperidinium*, *Dinophysis* and *Oblea* incline to feed on prey much larger than themselves by either extruding a membrane that can engulf whole diatom chains or by using a peduncle to broach the prey (Jacobson 1999). Athecate dinoflagellates (e.g. *Gyrodinium*) the average size of prey ingested can be considered to be smaller or comply with predator length (Jacobson and Anderson, 1986; Hansen et al, 1994; Jacobson, 1999). Thus, size constraints are difficult to estimate, and these two dinoflagellate groups are therefore treated separately.

The lorica diameter and the peristome diameter set the upper size limit of prey for the dominant ciliates in our samples, the tintinnid ciliates (Rassoulzadan 1978) Heinbockel 1978; Heinbockel and Beers 1979; Fenchel 1980a) and aloricate choreotrichs, respectively (Jonsson 1986).

Grazing impact of microprotozooplankton

Microprotozooplankton clearance rates were determined using a modified methods based on the dilution technique of Landry and Hassett (1982). Water samples were taken from three out

patch (St. 12, 42 and 48) and five in patch stations (St.38, 41, 49, 88 and 91) Experimental water was prepared by Verity (2001) and added gently to dilution water to create five duplicated dilution treatments (10, 30, 50, 70 and 100% of experimental water). One set of bottles were incubated in flowing seawater on deck incubators covered with on layer of neutral density screening to match near surface irradiance. Incubation began at various times of the day, but always during daylight. A parallel set of bottles were incubated in a dark lab container mounted to a plankton wheel. At T_0, T_{24} and T_{48},T_{96},T_{120} hours, respectively, samples were collected and preserved with a final concentration of 2% hexamine buffered formaline solution and stored at 4°C in the dark for subsequent counting back in the home laboratory. Further treatment of the samples were done by the method of Utermöhl (see above) following the recommendations of Venrick (1978) and Edler (1979). To determine clearance rates on phytoplankton, abundances of the different phytoplankton groups from cell counts were used to calculate the clearance rates in µl ind.$^{-1}$ h^{-1} in 100% undiluted water at T_0 and T_{48}.

Results

The data presented in this paper are from the mixed layer only (80m), as this is the section of the water column directly impacted by the Fe addition (Strass et al. 2001). In most cases, the distribution of the components of the microbial community were concentrated to the mixed layer with distinct peaks in the upper 80m and with numbers decreasing below the pycnocline. The data are presented in 80m depth integrated abundances calculated in cells l^{-1}, ind. m^{-3}, respectively, by dividing through the integration depth (80m). Biomasses is given in mg C m^{-2} integrated over 0-80m depth. The temporal development of the protozooplankton (choanoflagellates, aplastidic dinoflagellates and ciliates) and small metazoans (copepod nauplii and small copepods <1.5mm) assemblage inside and outside the fertilized patch is described for all groups identified.

Abundance and biomass of heterotrophic protists and small metazooplankton

The total microprotozooplankton population showed an increase in abundance from 232.0 $\times 10^2$ cells l^{-1} on day 0 to a value of 483.6 $\times 10^2$ cells l^{-1} on day 21 (Fig. 1A). A similar trend was observed for carbon standing stocks of microprotozooplankton, which increased during the experiment from 653 mg C m^{-2} on day 0 to 1,149 mg C m^{-2} on day 21 (Fig. 1C).

Outside the patch, abundances also increased, whereas biomass declined slightly (Figs. 1B, 1D) and were significantly different from inside stations ($P < 0.05$; unpaired t-test).

In patch abundances of choanoflagellates increased slightly over the experiment from 156.3 $\times 10^2$ cells l^{-1} two days prior to fertilization to 244 $\times 10^2$ cells l^{-1} on day 21 (Fig. 2A, Tab. 1). This increase resulted in a accumulation rate of 0.04 d^{-1} over the course of the experiment. The biomass also increased throughout the experiment from 6.3 mg C m^{-2} on day T-2 to 9.8 mg C m^{-2} on day 21 (Fig. 3A, Tab. 1). The cell size (4µm ∅) with mean cell volume of 27μm^3 stayed constant in the course of the experiment. Abundances and biomass in the surrounding waters also showed an increase during the experiment and were not significantly different from in patch waters (Fig. 3A, Tab. 1).

During the experiment, aplastidic dinoflagellate abundances increased markedly from 109.3 $\times 10^2$ cells l^{-1} on day 0 to 373.1 $\times 10^2$ cells l^{-1} on day 10 and decreasing thereafter to a value of 227.4 $\times 10^2$ cells l^{-1} on day 21 (Fig. 2G). A accumulation rate of 0.08 d^{-1} could be calculated for the great increase between day 0 and day 10. A similar patter was observed for biomass of dinoflagellates, which increased from 385 mg C m^{-2} (day 0) to a maximum of 1,046 mg C m^{-2} on day 11 with a value of 787 mg C m^{-2} after 21 days (Fig. 3G). The population was composed of athecate and thecate dinoflagellates with abundances and biomass increasing markedly until days 10 and 11, respectively, and were subsequently decreasing until the end of the experiment (Figs. 2B, 2C and 3B, 3C). In adjacent waters abundance and biomass of dinoflagellates decreased in the course of the experiment and showed a significant difference to in patch station from day 4 ($P < 0.05$; unpaired t-test).

Aplastidic ciliate abundances increased only slightly during the experiment from 8.9 $\times 10^2$ cells l^{-1} on day T-2 to 11.9 $\times 10^2$ cells l^{-1} on day 21, with the highest value of 16.2 $\times 10^2$ cells l^{-1} on day 7 (Fig. 2H). This increase corresponded to an accumulation in abundance of 0.017 d^{-1} in the course of the experiment. Carbon standing stock of ciliates increased from initially 205 mg C m^{-2} two days prior to fertilization to 352 mg C m^{-2} after 3 weeks with maximum value of 366 mg C m^{-2} on day 5 (Fig. 3H). The population was composed of aloricate choreotrich ciliates which increased both in abundances and biomass during the experiment (Figs. 2D, 3D and Tab. 1). Tintinnids and other ciliates showed a clear decrease in the course of the experiment with other ciliates almost disappearing until the end of EisenEx (Figs. 2E, 2F and 3E, 3F, Tab. 1). The abundance and biomass of the ciliate population outside the patch showed a decreased during the experiment and were significantly different from in patch stations ($P < 0.05$; unpaired t-test).

Abundances of copepod nauplii were very high during the experiment increasing from 30,000 ind. m^{-3} from day T-2 to 43,000 ind. m^{-3} on day 21 (Fig. 4A). Still the abundances at most of the in patch stations were between 39,000 and 52,000 ind. m^{-3}, which leads to the conclusion that there was no significant change in nauplii abundance. Copepod nauplii biomass showed a strong increase in the course of the experiment resulting in a doubling of biomass from 252 mg C m^{-2} (day T-2) to 507 mg C m^{-2} (day 21) with the highest value of 613 mg C m^{-2} on day 17 (Fig. 4D). In unfertilized waters abundances and carbon standing stocks of copepod nauplii showed no significant trend and were below initial values after 21 days. However they were not significantly different from in-patch values.

Small copepods <1.5mm (mainly consisting of copepodites stages CI-CV) increased significantly in abundance and biomass (Figs. 4B and 4E). Initial abundances were already very high, starting with 9,000 ind. m^{-3} two days prior to fertilization and ending with 36,000 ind. m^{-3} on day 19. Carbon standing stocks increased from 184 mg C m^{-2} on day T-2 to 742 mg C m^{-2} on day 19 (Fig. 4E). Abundance and standing stocks increased also in surrounding waters reaching 17,000 ind. m^{-3} and 344 mg C m^{-2} on day 21, respectively (Figs. 4B and 4E). Still there was a significant difference in both abundance and biomass between fertilized and non-fertilized waters during EisenEx ($P < 0.05$; unpaired t-test).

Abundances of several of the microprotozooplankton groups were significantly correlated with biological parameters (Tab. 2). Choanoflagellates showed a positive correlation with chlorophyll *a*, bacteria and microprotozooplankton abundance. Total dinoflagellates were positively correlated with primary production, bacteria, nanoheterotrophs and small copepod abundances. Abundances of ciliates showed a significant relationship with chlorophyll *a*, bacteria, microautotrophs and microheterotrophs abundances, whereas nauplii numbers were only correlated with pico- and nanoautotrophs as well as small copepods. Outside the patch, positive correlations was found between choanoflagellates and bacteria, whereas ciliates were negatively correlated with bacteria and small copepod abundances. Dinoflagellates and nauplii showed a positive correlation with pico-and nanoautotroph numbers, the latter also being positively correlated to small copepod abundances.

Proto- and small metazooplankton population composition

The composition of protozooplankton population showed no consistent trend during EisenEx. The dinoflagellates and choanoflagellates contributed up to 66 and 61%, respectively, with the choanoflagellates (mainly *Parvicorbicula socialis*) showing a slight increase of relative importance in the course of the experiment. The ciliate population contributed only 2-4% of

total abundance (Figs. 5A and 5B). Concerning protozooplankton biomass, dinoflagellates were the dominant group at almost all stations (up to 70%) followed by ciliates (18-40%). Choanoflagellates did not exceed 2% of total biomass and was similar inside and outside the patch (Figs. 5C and 5D).

Within the heterotrophic dinoflagellate assemblage, athecate dinoflagellates dominated dinoflagellate abundances (59-83%) and contributed significantly to dinoflagellates biomass inside and outside the patch, making up between 30% two days prior to fertilization and increasing until day 21 to 52% of relative biomass (Figs. 6A, 6B and 6C, 6D). The most important genera represented at all stations were *Gymnodinium*, *Gyrodinium* and *Amphidinium*. Dinoflagellates of the *Protoperidinium* genus and most probably a few species of the "Diplopsalis group" were most important within the thecate dinoflagellates, which made up for 44 to 69% of dinoflagellate carbon standing stocks (Figs. 6C, 6D).

During the experiment, the ciliate assemblage was dominated by aloricate choreotrichs of the genera *Strombidium*, *Strobilidium*, *Tontonia* and *Laboea* (53-93% of total ciliate abundance and biomass) and showed an increase in relative importance inside and outside the patch (Figs. 7A, 7B and 7C, 7D). At a few stations, species of the genus *Didinium*, which feeds on other ciliates, contributed to ciliate carbon standing stock indicating complex food web structures. Still a strong decrease of other ciliates including *Didinium* could be observed in the course of the experiment (from 26% on day T-2 to 1 % on day 21; Figs. 7A, 7B). Tintinnid ciliates also showed a decrease in relative abundance and biomass during the fertilization experiment in- and outside the patch (Figs. 7A, 7B). They were dominated by *Cymatocylis caliciforms* and *C. antarctica* and *Codonellopsis pusilla*. No marked shift within the ciliate and dinoflagellate community composition could be found in any station during the experiment and no significant differences were observed between in- and out-patch water masses. A possible explanation for this could be the taxonomical level at which the dinoflagellates and ciliates were classified was insufficient to detect any variation in assemblages for the period of the differently treated water masses surveyed.

Copepod nauplii comprised 58% to small metazoan carbon standing stock in the beginning of EisenEx, but decreased in relative importance in the course of the experiment (44% on day 19). Within the copepod nauplii, the calanoid nauplii dominated total carbon stocks at most stations (max. 75% of biomass) and showed an increase in relative importance inside the fertilized patch (Fig. 8A). Cyclopoid nauplii, with *Oithona* spp. being the dominant genus, prevailed abundances (up to 73%) and also contributed significantly to total nauplii biomass (19–51% of biomass), but were decreasing inside the patch in the course of the experiment

(Fig. 8A). Calanoid nauplii (mainly nauplii of *Ctenocalanus* spp.) dominated copepod nauplii biomass and accounted for max. 63% of total carbon stocks. Harpacticoid nauplii were represented only by *Microsetella* spp. and made up for only small portion of total nauplii carbon standing stocks (1–14%; Fig. 8B). In surrounding waters no significant trends could be observed.

Among small copepods <1.5mm, cyclopoid copepods dominated the relative abundance and biomass inside and outside the patch and accounted for 58 to 100% of total small copepod carbon standing stocks (Figs. 8C and 8D). *Oithona* spp. (predominantly *O. similis*) was by far the most important genera making up over 70% of all small copepod biomass at most of the stations. However, cyclopoid copepods decreased in relative importance during the experiment in favour of mainly calanoids. The most important calanoid genera were *Ctenocalanus* spp. (predominantly *C. citer*), *Metridia* spp. and *Calanus* spp. and contributed significantly to total biomass (max. 35%). *Microsetella* spp. again made up only a small fraction of total carbon standing stocks and was not present at all stations (Figs. 8C and 8D).

Microprotozooplankton size composition

The size composition of the microprotozooplankton showed consistent trends during EisenEx. Within the athecate dinoflagellates, abundances were dominated by small cells (20-40µm) which increased from 52.0 $\times 10^2$ cells l^{-1} on day T-2 to 220.7 $\times 10^2$ cells l^{-1} on day 10 with a marked decrease until the end of EisenEx (Fig. 9A). They contributed up to 70% of athecate dinoflagellate abundance, but only 18-32% of biomass (Figs. 11A and 12A). Cells of the 40-60µm fraction dominated athecate dinoflagellate biomass and showed an increase during the experiment from 136.3 mg C m^{-2} on day T-2 to 266.2 mg C m^{-2} after 3 weeks (Fig. 10A). The 40-60µm size fraction accounted for 50 to 69% of athecate dinoflagellate biomass (Fig. 12A). There was no significant difference in relative size composition compared to surrounding waters.

Abundances and biomass of thecate dinoflagellates were mainly composed of medium sized cells (40-60µm) The abundances of the 40-60µm size fraction increased from 19.9 $\times 10^2$ cells l^{-1} on day 0 to 38.8 $\times 10^2$ cells l^{-1} after 21 days with the strongest increase occurring between days 0 and 10 (Fig. 9C). Biomass of this fraction increased from 137.5 mg C m^{-2} on day 0 to 265.4 mg C m^{-2} in the same time period (Fig. 10C). The medium sized cells contributed 59-91% of total thecate dinoflagellate abundance and 50-88% of total biomass with no difference in importance of relative size classes compared to outside stations (Fig. 11C and 12 C).

Concerning ciliate size composition, all size fraction were increasing during the experiment in abundances and biomass. The smallest size fraction (20-40µm) was most abundant, but showed no significant change in the course of the experiment. The >60µm size ciliates showed the strongest increase from 27 l^{-1} to 145 l^{-1} over this period (Fig. 9E). During the experiment, the overall size composition of the ciliate population remained unchanged. The 20-40µm size fraction contributed 47-68% of total abundance and 23-49% of ciliate biomass, and showed no real change in relative importance in the course of EisenEx (Figs. 11E and 12E). The larger ciliates (>60µm) increased in relative importance in the course of the experiment both in abundance (from 3 to 12% of total ciliate abundance) and in biomass (from 13 to 39% of total ciliate biomass). Thus, the increase in diatom biomass observed during the experiment (Assmy and Henjes to be submitted) was only accompanied by an increase in the larger ciliate size fraction of the microprotozooplankton.

Microprotozooplankton abundances and biomass of the increased dominant size classes showed no changes or even decreased in some cases in adjacent waters during the experiment (Figs. 9B, 9D, 9F and 10B, 10D, 10F). A significant difference between in-patch and out-patch stations could be observed ($P < 0.05$; unpaired t-test).

Standing stocks of proto- and metazooplankton fecal pellets and empty diatom frustules

The abundance of protozooplankton fecal pellets (<10 µm) showed a similar trend as abundances of heterotrophic dinoflagellates increasing from 0.9 $\times 10^7$ m^{-2} on day T-2 to 2.6 $\times 10^7$ m^{-2} on day 11 and decreasing thereafter to initial values (Fig. 13C). Estimates of protozooplankton pellet carbon standing stocks showed similar pattern increasing from 2.5 mg C m^{-2} on day 0 to 35.9 mg C m^{-2} on day 11 with a decline of biomass to 8.2 mg C m^{-2} on day 21 (Fig. 13D). Fecal pellet abundance was significantly correlated with athecate dinoflagellates ($P < 0.05$; partial correlation). On day 11 protozoan fecal pellet carbon corresponded to only 3% of microautotrophic carbon standing stock or ~5% of primary production (Assmy and Henjes to be submitted; Gervais et al. 2002). Abundances and biomass showed either no trend or in terms of biomass showed an increase in surrounding waters during the experiment.

Metazoan fecal pellets numbers increased only slightly from 1.6 $\times 10^7$ m^{-2} on day T-2 to 2.3 $\times 10^7$ m^{-2} on day 21 (Fig. 4C). Carbon standing stocks, however, increased markedly from 38 mg C m^{-2} two days prior fertilization to 203 mg C m^{-2} after 3 weeks (Fig 4D) and corresponded to ~6% of microautotroph carbon stocks or 79% of primary production (Assmy

and Henjes to be submitted; Gervais et al. 2002). In surrounding waters, numbers of metazoan fecal pellets declined in the course of the experiment, whereas metazoan pellet carbon also increased during EisenEx (Figs. 4C and 4D). Still, numbers and carbon stocks were significantly different from inside stations ($P < 0.05$; unpaired t-test).

In the abundance of empty diatom frustules, which is suggested to be an indicator of protozoan grazing, a marked increase could be observed in the course of the experiment (Assmy and Henjes to be submitted, Fig. 5). Their contribution to total frustules concentration (empty + broken + live) was, however, decreasing in the course of experiment from 19% on day T-2 to 7% after 3 weeks. A significant correlation was found between empty diatom frustule abundance and abundances of ciliates, small copepods, dinoflagellates and live diatoms, respectively ($P < 0.05$; partial correlation). Outside the patch abundances of empty diatom stayed more or less constant, whereas their relative amount is also slightly declining (Assmy and Henjes to be submitted). A positive correlation could only be observed between empty diatom frustules and ciliates.

Microprotozooplankton grazing impact

Microprotozooplankton grazing estimates on nano- and microphytoplankton were derived from clearance rates ranged from 0.1 µl ind.$^{-1}$ h^{-1} on diatoms and 0.3 µl ind.$^{-1}$ h^{-1} on other phytoplankton calculated form the dilution experiments. Daily grazing impacts on total diatom carbon stocks were low (ranging from 3 to 9% d^{-1}), but accounted for a larger fraction of other phytoplankton (9 to 28% d^{-1}) stocks and of the total primary production (PP) (Figs. 13A, 13E and 13F). Grazing impact increased only slightly in the course of the experiment with the highest value recorded on day 10, when more than 18% d^{-1} of PP was consumed by the microprotozooplankton (Fig. 13A). Generally, estimates of clearance rates attributed most of the grazing impact to the smallest size class (20-40µm), however at some stations, the medium size fraction (40-60µm) showed the highest relative effect. At all stations the >60µm size fraction consumed less than 1% d^{-1} of phytoplankton primary production. Outside the patch the highest grazing impact was observed on day 4, but declined thereafter and the 20-40µm size fraction attributed most of the grazing on primary production (Fig. 13B). From day 5 grazing impact inside the patch was significantly higher than in unfertilized waters ($P < 0.05$; unpaired t-test).

Discussion

Microprotozooplankton standing stocks in the Southern Ocean

In the Southern Ocean, early comprehensive studies on protozooplankton assemblage have been generally limited to the western Weddell Sea and the Weddell-Scotia Confluence (Garrison 1991). Furthermore, only a few studies separated the microprotozoa from the smaller members of the protozooplankton community. More recent studies, however, also investigated the protozoan assemblage in the Polar Frontal Zone and concentrated mainly on the microprotozoans (Froneman and Perissinotto 1996; Klaas 1997). During EisenEx, microprotozooplankton constituted an important fraction of microprotist biomass (21-58%) before fertilization and in unfertilized waters. In contrast, Klaas (1997) found significantly lower ratio of microprotozoa to microphytoplankton in the Polar Front than during this study. Klaas (1997) suggested that both lower growth rates of microprotozoa compared to microprotist rates of increase at the beginning of the growth season and grazing pressure by metazoans were responsible for the low ratio. However, Scharek et al. (1994) and Becquevort (1992) found during late winter-early spring in the Weddell Sea that microprotozoan standing stocks ranged from 10 to 90% and 40% of microprotist biomass, respectively. Thus, findings during this study are more in the range of percentages found in other parts of the Southern Ocean for the growth season.

The initial microprotozooplankton biomass (757 mg C m^{-2} integrated over 100m) found during this study in the Polar Frontal Zone are in the range of values reported in this region and in Southern Ocean waters during spring. Klaas (1997) found lower microheterotroph carbon standing stocks (max. 665 mg C m^{-2} at 48°S integrated over 100m depth) in the Polar Front in October and Garrison and Buck (1989a) also reported lower spring carbon standing stocks in the Weddell Sea, whereas Burkill (1994) found carbon standing stocks (max. 2,021 mg C m^{-2} over the upper 100m) that were significantly higher during spring in the Bellingshausen Sea. Nöthig (1988) found values of microprotozoan biomass in the southeastern Weddell Sea in the upper 100m that were similar of values found during EisenEx. In general, this study gives additional evidence that microprotozoan standing stocks from spring to autumn appear highly variable in Southern Ocean waters depending on region, and follow levels of primary production (Garrison 1991). Furthermore, this study extends conclusions by Klaas (1997) that not only in the open waters of the southern Antartic Circumpolar Current but also in the Polar Frontal Zone large surface standing stocks of

protozoa can be sustained through the winter until the beginning of the growth season. This is similar to other HNLC regions such as the subarctic Pacific (Miller 1993).

Response of the microprotozooplankton assemblage

The microprotozooplankton community in the vicinity of EisenEx site in austral spring was dominated initially and during the experiment by the heterotrophic dinoflagellates and ciliates, which comprised nearly all of the microprotozoan standing stock. The composition of the population varied little throughout the experiment (Figs. 5A-D). This is in agreement with observation of Burkill et al. (1995) and Klaas (1997) in the Bellinghausen Sea and in the Polar Front Zone during austral spring where dinoflagellates and ciliates dominated the microprotozooplankton community and Hansen et al. (1996) who reported ciliates and dinoflagellates having approximately equal biomass in the open waters. In contrast, during the Southern Ocean Iron Release Experiment (SOIREE), heterotrophic nanoflagellates accounted for more than 90% of the microprotozooplankton biomass. Estimates of total heterotrophic nanoflagellate carbon standing stocks during this study show that they can make up for about an additional 242 mg C m^{-2} in the upper 100m, which is an underestimation, however, due to the methodical constrains mentioned above. But even if we consider an underestimation of 100%, heterotrophic nanoflagellate biomass would not comprise more than 32% of total protozooplankton carbon stocks during EisenEx.

Microprotozooplankton biomass increased only ~two-fold during EisenEx, with the majority of the increase due to a doubling in heterotrophic dinoflagellate biomass. The small increase in microprotozooplankton stocks in EisenEx is similar to results of SOIREE in the Pacific Sector of the Southern Ocean and Iron Ex I and II in the Equatorial Pacific (Martin et al 1994; Landry et al. 2000; Hall and Safi 2001), where only a small increase in microprotozooplankton biomass was observed. The increased herbivory (Fig. 13A) in the course of the experiment, however, was not reflected by an equally strong increase in DMS inside the patch. This is in contrast to findings during SOIREE and by Wolfe et al. (1997), who suggested herbivory as a mechanism for DMSP release.

The initial choanoflagellate concentration found during EisenEx are within the range of values previously reported from Southern Ocean waters (Fryxell et al 1984; Marchant 1985; Buck and Garrison 1988). The maximum density found during this study (3.3×10^4 cells l^{-1} in 10m), however, is more than an order of magnitude less than the maximum found by Buck and Garrison (1983) at the ice edge during summer with no apparent vertical heterogeneity in the upper water column. In this study, there was more than four-fold difference in abundances

in the upper 80m values. Concerning the role of choanoflagellates among the heterotrophic flagellates, it is difficult to compare with other studies in the Southern Ocean. Buck and Garrison (1988) reported that choanoflagellates comprised 8 to 20% of the total heterotrophic flagellate carbon stocks, but Becquevort (1997) found in his study that choanoflagellates accounted for an average of 4% of total nanoprotozoan biomass. Taking the estimates for total heterotrophic nanoprotist biomass into account, choanoflagellates would comprise 3 to 5% of total nanoprotozoan carbon stocks during EisenEx.

Choanoflagellates increased only slightly and their contribution to heterotrophic microprotist carbon stocks was negligible during EisenEx. The highly significant correlations with bacterial numbers indicate that choanoflagellates were important consumers of bacterial production. Hence, choanoflagellates could be among others responsible that densities of bacteria were able to accumulate only two-fold in response to iron addition (Arietta et al. submitted). Buck and Garrison (1988) found that choanoflagellates could consume up to 14% of the daily bacterial cell production in open water stations. However we have to put into consideration that bacterial densities in the upper 80m during EisenEx (max. value of 5.4×10^5 cells ml^{-1}) were considerably lower than the 1 to 1.5×10^6 bacteria ml^{-1} that Anderson and Fenchel (1985) suggest is needed to support choanoflagellate growth. In fact, the bacterial abundances during this study are in the range to those at which Fenchel (1982b) suggests a cessation of feeding and reduction in body size occurs with the onset of starvation. There was, however, no evidence of cell size reduction during EisenEx that would indicate starvation, and the positive correlation between choanoflagellate and bacterial stocks would rather suggest that the predator population were still lagging the increase in prey numbers. The low abundance and rather small increase of bacterial concentrations concomitant with significant bacterial production and numbers of choanoflagellates and probably also other heterotrophic flagellates capable of consuming a significant fraction of bacterial production, indicates that a close coupling between these two microbial components was maintained throughout EisenEx. A similar coupling can also applied for surrounding waters since choanoflagellates increased in numbers and showed a positive correlation with bacterial concentrations.

Although choanoflagellates consume bacteria primarily, Marchant (1985) reported that they may also take small autotrophic nanoplankton. The fact that choanoflagellates were also positively correlated to chlorophyll *a* suggests that they were consuming also other components of the phytoplankton community, probably nanophytoplankton of which some taxa showed either only little accumulation in numbers (*Phaeocystis*) or even decreased in response to iron addition (coccolithophores). However, there was no direct correlation

between choanoflagellates and pico- and nanophytoplankton concentrations during this study. The possible explanation of iron deficiency of choanoflagellates prior to fertilization as suggested by Chase and Prize (1997) for heterotrophic bacterivorous flagellates in remote oceanic regions can be ruled out during this study, since choanoflagellates showed also an accumulation of stocks outside the patch.

There are also indications that production cycled through the microbial food web is passed to larger pelagic consumers in the food web. The potential importance of choanoflagellates, mainly in form of colonies containing several hundreds individuals, as a food source for the Antartic krill (*Euphausia superba*) has been established from many studies (Tanoue and Hara 1984; Marchant and Nash 1986). Intact costae have also been found in the fecal pellets of metazooplankton during summer and fall in Arctic waters (Urban 1992). During EisenEx, choanoflagellate colonies (~56 µm in Ø) of *Parvicorbicula socialis* deminished in the course of the experiment (unpublished data) suggesting a utilization of these colonies by larger metazooplankton. In addition, other heterotrophic microprotists (predominantly ciliates) also frequently feed on choanoflagellates as shown by several studies in Antarctic waters (reviewed in Garrison and Gowing 1993). This is in good agreement with findings during EisenEx where a positive correlation between choanoflagellates and microprotozooplankton could be observed.

The heterotrophic dinoflagellate assemblage showed a distinct response to the iron-induced phytoplankton bloom with a two-fold increase in abundance and biomass after three weeks. Primarily, athecate dinoflagellates showed a marked increase in abundance until day 10 (Figs. 2G, 9A), indicating that dinoflagellates converted increasing food availability during the phytoplankton bloom into elevated population growth. Dinoflagellates are known to feed on a wide variety of prey including bacteria, small flagellates, other dinoflagellates and nano- and microphytoplankton (Hansen and Calado 1999; Jacobson 1999), which is in good agreement with prey preferences found during this study, although there was no positive correlation between dinoflagellates and microphytoplankton (mainly diatoms). Nevertheless, abundances of both athecate and thecate dinoflagellates declined after day 10 and 11, respectively, indicating that grazing pressure probably by metazoans could be one factor controlling dinoflagellate numbers in the second half of the experiment. The positive correlation between dinoflagellates and small copepods during EisenEx support this conclusion. Estimates of grazing impact of copepods <1.5mm and >1.5mm on dinoflagellate population show that grazing pressure increased from initially 8% of dinoflagellate carbon stocks to 21% d^{-1} on day 19. Many studies support these findings showing that heterotrophic dinoflagellates represent

an important part of metazoan diets (reviewed by Stoecker and Capuzzo 1990; Sanders and Wickham 1993) More recent studies show that heterotrophic dinoflagellates also possess a high importance for smaller copepod species as a diet component (Atkinson 1996; Nakamura and Turner 1997).

The dinoflagellate assemblage was dominated by athecate dinoflagellates throughout the experiment which is consistent with finding of other studies from the Southern Ocean (Archer et al. 1996; Klaas 1997). The size composition showed marked differences between the two forms of dinoflagellates. The athecate dinoflagellates were dominated by smaller cells (20-40µm) which comprised up to 70% of the abundances, but only up to 32% of the biomass. Thecate individuals, however, were dominated by medium sized cells (40-60µm).

Standing stocks of phytoplankton showed a marked increase in the course of the experiment, but the bulk of biomass consisted of colonial pennate diatom species of the genera Pseudo-Nitzschia and colonial cylindrical diatoms (Assmy and Henjes to be submitted). Observation by Assmy and Henjes (to be submitted) indicate that these diatoms built up very long chains and that chain length increased inside the patch. Although a number of thecate genera (e.g. *Protoperidinium*, *Diplopsalis* group) are known to frequently feed on large diatoms and also on diatom chains via their pallium (Hansen and Calado 1999; Jacobson 1999) they only comprised for a smaller fraction of total heterotrophic dinoflagellate numbers. Most of the athecate dinoflagellates (*Gymnodinium, Gyrodinium, Amphidinium*) but also some thecate genera (e.g. *Dinophysis*) are either engulf or tube feeders (Hansen and Calado 1999), and are therefore confined to diatoms that do not surpass their own size (Hansen 1992; Hansen et al. 1994) or their feeding mode (peduncle feeding) does not allow them to prey on diatoms at all. Thus, the composition of the heterotrophic dinoflagellate population and the fact that the larger chain-forming diatom species dominated phytoplankton stocks in the second half of EisenEx indicate that of a large fraction of dinoflagellates growth and grazing might have been food limited later in the experiment. Findings by Bjørnsen und Kuparinen (1991) support this assumption, who reported that saturation food concentrations of heterotrophic dinoflagellates in the Southern Ocean lie around 300 µg C l^{-1} compared to 43 µg C l^{-1} at the end of EisenEx. Hence, a combination of strong grazing pressure by metazoans and food limitation by a large fraction of the dinoflagellates assemblage could have resulted in the strong decline of the heterotrophic dinoflagellate population in the later phase of the experiment.

The ciliate population almost doubled in biomass and was dominated by aloricate choreotrichs, which showed a three-fold increase in carbon stocks during the experiment.

Initial abundances of ciliates were low during EisenEx, but increased slightly despite predation by the metazooplankton, which increased their grazing impact on ciliates in the course of the experiment (Schultes unpubl. data). This is comparable with findings of a previous fertilization experiment (Hall and Safi 2001) and similar trends also have been observed in bottle experiments in other Fe-limited HNLC regions (Buma et al. 1991; Chavez et al. 1991). Tintinnid and other ciliate standing stocks, however, decreased during EisenEx and tintinnids accounted for only 11% of ciliates biomass at the end of the experiment and never reached more than 28% of ciliate carbon standing stocks. A similar composition of the ciliate population has been observed in the Atlantic Sector of the Southern Ocean (Froneman and Perrissinotto 1996), in the Ross Sea (Caron et al. 2000) and an even less importance of tintinnids (<10%) during SOIREE in the Pacific Sector of the Southern Ocean (Hall and Safi 2001). In contrast, during austral spring Klaas (1997) found a clear difference between southern ACC water and the PFr in the ciliate assemblage due to a large fraction of tintinnids which comprised up to 84% of ciliate carbon standing stocks at the PFr. As suggested by Klaas (1997), this marked and early appearance of tintinnids at the PFr could have been a combination of advection from northern waters accompanied by an import of subantartic species, an earlier maturing process of the protozoan assemblage as a result of high productivity and the exhibition of high iron concentrations through input from a large number of melting icebergs. However, the fertilization of iron superimposed on occurrence of subantartic *Codonellopsis* species with coccoliths agglutinated to their loricae (Henjes and Assmy to be submitted) and the dominance of typical Antarctic species such as *Cymatocylis caliciformis* should have favoured tintinnid growth as compared to choreotrich ciliates also during this study. A likely cause why this was not the case might be heavy and selective grazing pressure by copepods (Henjes and Assmy to be submitted). Estimates of grazing impact of total copepods by Schultes (unpubl. data) show that total copepods consumed a maximum of 38% d^{-1} of tintinnid carbon stocks during EisenEx. Preferential grazing by copepods on tintinnids has been demonstrated for several temperate, but also Antarctic copepod species even under conditions where phytoplankton was dominant (reviewed in Gifford 1991; Sanders and Wickham 1993; Lonsdale et al. 2000; Broglio et al 2001). Moreover, the generally low abundances and minor increase of total ciliates indicate that predation by metazooplankton was high during EisenEx, which is comparable to findings during SOIREE. However, no positive correlation between ciliates and small copepods could be found during this study.

The overall size composition of the ciliate population was relatively constant during the experiment, with the < 20µm size fraction making up to 68% of the abundance and up to 73% of the biomass. The strong increase by the >60µm size fraction suggests that probably grazing pressure was reduced on this size class at the expense of the smaller size fractions. Furthermore, grazing of other larger protozoans on ciliates have also reported from temperate waters (Pierce and Coats 1999).

Positive correlations between ciliates and several prey types (Tab. 2) indicate that ciliates were actively grazing during the experiment. Several studies have demonstrated that ciliates in Southern Ocean waters are preferentially feeding on pico- and nanoplankton (Froneman and Perissinotto 1996; Hall and Safi 2001), but also on microphytoplankton (Klaas 1997), and are known to consume bacteria in temperate waters (Bernard and Rassoulzadegan 1990). Findings during EisenEx on protozoan growth rates (unpubl. data) support recent studies that have shown that aloricate choreotrich growth rates are similar to those of tintinnid ciliates, but somewhat higher than those of dinoflagellates also in the Southern Ocean (Bjørnsen und Kuparinen 1991; Hall and Safi 2001). However during EisenEx, the bulk of biomass consisted of colonial pennate diatom species of the genera Pseudo-Nitzschia and large cylindrical diatoms (Assmy and Henjes to be submitted) later in the experiment. The size of prey consumed by choreotrich ciliates and tintinnids does not generally exceed 45% of their peristome and lorica diameter, respectively (Splitter 1973; Jonsson 1986) Therefore, it is likely that long diatom chains would have been difficult to ingest for these ciliates and therefore diatoms probably made only a small fraction of their diets in the later half of the experiment.

Grazing impact of heterotrophic microprotists

The clearance rates calculated from the dilution experiments are within the lower range of values found in the literature. Estimates of clearance rates for heterotrophic dinoflagellates and ciliates found by Capriulo et al. (1991) vary over two orders of magnitude, from 0.1 to >20 µl ind. h^{-1}. Clearance rates on phytoplankton by Antarctic protozoans have been measured using radioactive isotopes and with 0.0 – 0.2 µl ind. h^{-1} are among the lowest estimates found in the literature (Lessard and Swift 1985). During their studies Lessard and Rivkin (1986) also found indications that clearance rates on bacteria and bacterivores are higher and that due to complex trophic interactions a much higher potential grazing impact on primary production can be assumed.

Grazing impact of the microprotozoan assemblage on daily primary production (PP) and other phytoplankton during EisenEx were relatively high with max. grazing impact on day 10 (18% of PP and 28% of other phytoplankton carbon stocks grazed d^{-1}, respectively), and smaller protozoa (20-40µm) constituted a large proportion of the assemblage. The estimates of grazing impact of microprotozooplankton indicate, however, that only a small fraction of diatom carbon stock was removed (max. 9% on day 10) in the course of EisenEx. Estimates of grazing pressure of microprotozoa on the diatom assemblage calculated solely from the empty diatom frustules give an even minor importance (< 2% of diatom standing stock grazed d^{-1}). However, this estimate has several uncertainties due to the high input and output rates into the empty frustule pool. Still these findings are in good agreement with results during SOIREE, where a negligible fraction of the microphytoplankton (mainly diatoms) was grazed by microzooplankton.

Comparison with a study of Klaas (1997) show that grazing impact of heterotrophic microprotist can be significantly larger in the Polar Frontal Zone consuming up to 56% of daily PP and up to 38% of daily microphytoplankton (mainly diatoms) production by assuming a clearance rate an order of magnitude higher than found during this study. In surface waters in the Atlantic Sector of the Southern Ocean up to 46% of primary production was grazed by microzooplankton in summer (Froneman and Perissinotto 1996). However, the very high biomass of copepods, especially the smaller copepod genera, during this study (see also Krägefsky et al. to be submitted) could lead to increased grazing on microprotozoa (~54% of ciliate and dinoflagellate standing stock on day 21); thus, it is likely that their grazing rates are higher than the estimates based on their standing stocks during the experiment. In addition, the grazing impact of nanoprotozooplankton, which can also feed on large diatoms (Schnepf et al. 1990; Kühn 1995) or even diatom chains (Schnepf et al. 1988), is at times higher than microprotozoa with values varying from 5-95% of primary production grazed daily (Becquevort 1997). Furthermore, the larger chain-forming diatom species dominating the phytoplankton stocks in the second half of EisenEx were not suitable prey for most of the heterotrophic dinoflagellates found during EisenEx. Ciliates, which preferentially feed on particles smaller than their oral diameter might also not feed at all on those chains (Jonsson 1986). Additionally, selective feeding behaviour seems to be a rule for most heterotrophic microprotists (Stoecker et al. 1981; Verity et al. 1986; Verity 1991; Naustvoll 2000). Therefore, microprotozooplankton feeding behaviour, heterotrophic and autotrophic microprotist community composition are important factors determining grazing impact of microprotozooplankton on the diatom assemblage.

Furthermore, our data suggest that microprotozooplankton would have consumed mainly bacterio- pico- and nanoplankton. The lack of a substantial increase in the biomass of the bacterial, pico- and nanoplankton populations after iron addition to this iron-limited system (Assmy and Henjes to be submitted) as well as the minor increase of the microprotozoan assemblage indicates a close coupling between the different trophic levels of the pelagic food chain during EisenEx. For the picophytoplankton this indicates a fast control of the stimulated population as suggested by Morel et al. (1991b). Our results are also in agreement with the suggestion of Landry et al. (2000) that even after a significant perturbation the pico- and nano-sized components of the food web will remain relatively constant.

Response of the small metazooplankton community

Maximal initial abundance prior fertilization of 51,000 ind. m^{-3} (in 40m depth) of small metazoans (copepod nauplii and copepods < 1.5mm) was extremely high in the mixed layer and were one of the highest ever recorded in Southern Ocean waters. During during austral summer similar high abundances of small copepods with peaks concentrations up to 49,000 ind. m^{-3} (in the 25-50 depth interval) including naupliar and copepodite stages were found in the Antarctic Polar Front by Dubischar et al. (2002) using Multinet samples equipped with five 100µm mesh nets. Pollard et al. (2002) also found very high numbers (max. 42,000 ind. m^{-3}) of smaller copepods in the Polar Frontal Zone using an optical plankton recorder mounted on a towed profiling SeaSoar. However, most of the recent studies conducted in the Antarctic Polar Frontal (APF) of the Southern Ocean and nearby systems obtained significantly lower densities of small copepods, at times, more than an order of magnitude, from net samples (Fransz and Gonzalez 1997; Atkinson and Sinclair 2000; Froneman et al. 2000; Pakhomov et al. 2000; Urban-Rich et al. 2001). Our findings confirm conclusions by Dubischar et al. (2002) that small copepods with *Oithona* being the most dominant taxa, show their maximum numbers in the vicinity of the Polar Front. The fact that exceptionally high abundances were found during this study can be mainly explained by the sampling gear employed. Niskin bottles seem to enhance catchiness for target organisms in the size range of 50-1000µm (T. Scherzinger pers. com.). Thus, it seems likely that the high numbers of copepod nauplii caught by the Niskin bottle are closer to the actual concentration in the water column since all naupliar stages, even the small sized (50µm) non-calanoid orthonauplii are retained with our sampling gear. The abundances copepodite stages and small adults, however, represent probably still an underestimation of the actual numbers since these

individuals have the ability to escape the Niskin bottle when it is moved through the water column.

Nauplii and copepodites of the genus *Oithona* (predominantly *O. similis*) and, to lesser extent, *Ctenocalanus* were the numerically dominant copepods in the initial phase of EisenEx, which supports findings by Dubischar et al. (2002) in the APF during austral summer. Since *Oithona* spp. undergoes no seasonal migration, but stays year-round in the upper 200m of the water column (Fransz and Gonzalez 1995; Atkinson and Sinclair 2000), egg production possibly starts before onset of spring phytoplankton bloom and enables this genus to increase and build up large concentrations earlier in the growth season than the larger calanoid copepods (Fransz and Gonzalez 1995). This is supported by our study since very high numbers of *Oithona* spp. eggs (max. 8,406 eggs m^{-3} in 60m) were observed in the samples. In addition, *Oithona* spp. is often able to generate a second offspring per year.

Ctenocalanus spp (mainly *C. citer*) also occurred in surprisingly high concentration prior to iron fertilization (5,558 ind. m^{-3} in 80m depth). They were in the range of peak values found by Dubischar et al. (2002) in the APF during austral summer (6,560 ind. m^{-3} in the 25-50m layer). *C. citer* is also suggested to possess highest numbers in the APFr compared to more northern or southern areas (Atkinson and Sinclair unpubl. data). This species is also a moderate seasonal migrant (Atkinson and Sinclair 2000), and is able to grow and reproduce prior to the onset of phytoplankton blooms in the water column (Schnack-Schiel and Mizdalski 1994). Thus, our results suggest that both species, *Oithona* spp. and *Ctenocalanus citer* are able to maintain high numbers of juvenile individuals throughout the winter season and start to grow and proliferate during early spring with favourable growth conditions in the APFr, in terms of high phytoplankton concentrations (Bathmann et al. 1997), continuing during spring and early summer. Ashijan et al. (2003) support this assumption, reporting that *O. similis* utilizes sustained reproduction, based on the presence of naupliar and all copepodite stages over much of the year. These stages of *O. similis* are found near the surface, where they utilize food resources in the relative carbon rich upper water column to obtain reproduction, growth and development throughout the year.

Total small metazoan biomass for the EisenEx water mass was in the range of values observed in other studies of Polar Frontal waters during spring and summer. Initial biomasses were 0.31 g C m^{-2} (integrated over the upper 60 m) during EisenEx which is very a conservative estimate. Still Fransz and Gonzalez (1997) recorded lower values in the Polar Front region during early austral spring, who found 0.4 g and 0.2 g AFDW m^{-2} of copepods 200-1000µm, respectively, over the upper 50m. Estimating a carbon content of ~50% of

AFDW (Fransz and Gonzalez 1997) this is equipollent to 0.2 and 0.1 g C m^{-2}, respectively. Atkinson (1996) found considerably higher values in the Polar Frontal Zone during summer: 3.6 g DW m^{-2} (≈1.8 g C m^{-2}) of copepods in the upper 50m. This may be explained by the different methods applied to calculate copepod stocks since the carbon conversion factor we measured for *Oithona* spp is three times lower than the conversion factor applied by, for example, Fransz and Gonzalez (1997) using AFDW-length relationship for biomass estimations. The consequent build up of populations of large copepodites over summer, which are included in the value of Atkinson (1996) might be another factor.

Although the mean, total biomass of small metazoan determined across the time series inside the patch was higher than outside the patch, the data were variable, but means were significantly different. This was primarily since inside the patch, numbers of copepod nauplii showed only a slight change in the course of the experiment, whereas biomass of nauplii increased by a factor of two. The more or less stable abundances during EisenEx are supported by findings of Huntley and Lopez (1992), who predicted that a generation time of copepods at +2°C is of the order of 100 days. Metz (1996), however, found cyclopoid (*O. similis* and *O. frigida*) naupliar stage durations of less than 10 days per stage in the Bellinghausen Sea with water temperatures of +1.5°C. Thus, the increase of nauplii biomass inside the patch gives indication that individual growth occurred during EisenEx. At the end of the experiment calanoid nauplii were completely absent outside the fertilized patch, but showed high abundances (8,656 ind. m^{-3}) inside the patch. This suggest that individuals of calanoid copepods species were food limited in surrounding waters at the end of EisenEx.

Copepodites and adults of small copepods genera increased by a factor of about three inside the fertilized patch, but showed also a doubling in numbers in adjacent waters. Copepodites and adults of *Oithona* spp. (predominantly *O. similis*) accounted for max. 100% of all small copepod numbers inside the patch followed by small calanoids (predominantly *Ctenocalanus citer*). During SOIREE, numbers of smaller sized copepodites and adults of cyclopoids (predominantly *O. similis*) as well as small calanoid copepods also increased during the first third of the experiment, but then returned to initial values toward the end of that experiment. Similar patterns were reported by Rollwagen-Bollens and Landry (2000) in the equatorial Pacific during IronEx II, where abundances of small taxa increased 3 days after their first iron infusion, but decreased to outside levels thereafter. They attributed the increase in numbers within the fertilized patch principally to elevated recruitment of nauplii, small calanoids and cyclopoids caused by improved feeding conditions through higher phytoplankton densities, and decreased vertical migration brought about lower light penetration.

There are two similar reason, how the increase in small copepod numbers could be explained during EisenEx. First, the relative short naupliar (< 10 d) and copepodite (14-28 d) stage durations of *Oithona* spp. and *Ctenocalanus citer* (Schnack-Schiel and Mizdalski 1994; Metz 1996) and the absence of clear cohorts in both genera during most of the year (Fransz and Gonzalez 1995; Dubischar et al. 2002) suggest that a recruitment from naupliar to early copepodite stages and from late copepodite stages to adults of these two most abundant genera was possible in the course of the 21 day experiment. The improved feeding conditions during EisenEx could result in an additional speedup of growth and hence recruitment of early copepodite stages. Secondly, migration and accumulation by small copepods from deeper layers below 150m into the upper water column where phytoplankton was concentrated is suggested by Kraegefsky et al. (to be submitted), caused by a shift in diet preference. Small copepods are known to feed very actively on smaller particles, faecal material and detritus, but also protozoa (Gonzalez and Smetacek 1994; Atkinson 1996; Dubischar et al. 2000). However, the negative correlation between depth distribution of *Oithona* spp. and fecal pellets and the positive correlation between this genera and diatom standing stocks in the later half of the experiment as well as the gut fluorescence data found by Kraegefsky et al. (to be submitted) support the mentioned diet shift. Nevertheless, both explanations have their constrains and a combination of both processes is also possible to have occurred during EisenEx. Moreover, both suggestions can probably applied for surrounding waters, since an increase in abundance was also observed.

Particle production by proto- and metazooplankton

The trend of protozoan fecal pellet abundance and biomass is very similar to the trend of protozooplankton grazing pressure of total phytoplankton stocks and heterotrophic dinoflagellates and showed a significant correlation with athecate dinoflagellates (Assmy and Henjes to be submitted). This indicates that mainly heterotrophic athecate dinoflagellates were responsible for protozoan fecal pellet production. Although planktic ciliates are known to produce fecal aggregates, these aggregates are often loosely packed and disintegrate rapidly (Stoecker 1984) and therefore likely to account only for a minor fraction of total protozoan fecal pellets. The dominance of olive green ellipsoidal to spherical pellets fecal pellets discriminated during EisenEx suggest that particularly larger protozoan were also actively involved in either re-ingestion and repackaging of larger crustacean pellets (Assmy and Henjes to be submitted). The fact that fecal pellets containing solely diatom frustules, which is typical for the pallium feeding thecate dinoflagellates and gulp feeding large athecate

dinoflagellates, made only a small fraction of total fecal pellet numbers gives further support that these dinoflagellates exerted only a moderate grazing pressure on the diatom community during EisenEx.

Moreover, Klaas (1997) reported abundances of protozoan fecal pellets in the Polar Front during spring that were about an order of magnitude higher than numbers found during this study. Her fecal pellet carbon content, however, was in the same range as during EisenEx using the same conversion factor and thus indicating that protozoan fecal pellets were generally larger during this study.

The increase of metazoan fecal pellets and empty diatom frustules can be mainly referred to the activity of large metazoan and protozoan grazers during EisenEx. Copepods were the main source of large recognisable fecal pellets as indicated by the dominance of compact cylindrical pellets characteristic for copepod faeces (Assmy and Henjes to be submitted) and which is in good agreement with the simultaneous increase of small copepods in the course of the experiment. The highly significant correlation between empty and broken diatom frustules inside as well as outside the fertilized patch suggests that grazing by crustaceans might also play a significant role in the production of empty frustules besides the feeding of protozoans using either pallium or gulp feeding to leave empty intact frustules as remnants of their grazing activity (Assmy and Henjes to be submitted).

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Figure legends

Fig. 1. Temporal development of total protozooplankton abundance and biomass. (A, C) in patch stations; (B, D) out patch stations.

Fig. 2. Temporal development of protozooplankton abundance. (A) choanoflagellates; (B) athecate dinoflagellates; (C) thecate dinoflagellates; (D) aloricate choreotrichs, (E) tintinnid ciliates; (F) other ciliates; (G) total dinoflagellates; (H) total ciliates.

Fig. 3. Temporal development of protozooplankton biomass. (A) choanoflagellates; (B) athecate dinoflagellates; (C) thecate dinoflagellates; (D) aloricate choreotrichs, (E) tintinnid ciliates; (F) other ciliates; (G) total dinoflagellates; (H) total ciliates.

Fig. 4. Temporal development of copepod nauplii abundance (A) and biomass (D), small copepod abundance (B) and biomass (E), metazoan fecal pellet numbers (C) and carbon standing stocks (F).

Fig. 5. Protozooplankton community composition given as percentage of protozoan abundance (cells l^{-1}) and carbon stocks (mg C m^{-2}) respectively. (A, C) in patch stations; (B, D) out patch stations.

Fig. 6. Heterotrophic dinoflagellate community composition given as percentage of protozoan abundance (cells l^{-1}) and carbon stocks (mg C m^{-2}) respectively. (A, C) in patch stations; (B, D) out patch stations.

Fig. 7. Ciliate community composition given as percentage of protozoan abundance (cells l^{-1}) and carbon stocks (mg C m^{-2}) respectively. (A, C) in patch stations; (B, D) out patch stations.

Fig. 8. Small metazoan community composition given as percentage of copepod nauplii and small copepod carbon stocks (mg C m^{-2}). (A, C) in patch stations; (B, D) out patch stations.

Fig. 9. Temporal development of athecate (A, B), thecate dinoflagellate (C, D) and ciliate (E, F) size classes in cells l^{-1}. (A, C, E) in patch stations. (B, D, F) out patch stations.

Fig. 10. Temporal development of athecate (A, B), thecate dinoflagellate (C, D) and ciliate (E, F) size classes in mg C m^{-2}. (A, C, E) in patch stations. (B, D, F) out patch stations.

Fig. 11. Size composition of athecate (A, B), thecate dinoflagellates (C, D) and ciliates (E, F) given the percentage of abundance (cells l^{-1}). (A, C, E) in patch stations. (B, D, F) out patch stations.

Fig. 12. Size composition of athecate (A, B), thecate dinoflagellates (C, D) and ciliates (E, F) given the percentage of biomass (mg C m^{-2}). (A, C, E) in patch stations. (B, D, F) out patch stations.

Fig. 13. Estimates of grazing impact in percent of primary production inside (A) and outside (B) the patch, diatom (E) and other phytoplankton biomass (F) grazed per day by the different size classes of heterotrophic microprotists. Production of heterotrophic protists fecal pellets (C) and fecal pellet carbon stocks (D) respectively

Table 1

Abundances and biomass of protozooplankton from counts of unconcentrated samples 2 days prior (T-2) to first fertilization and after 3 weeks inside and outside the fertilized patch. Values are depth-integrated samples from 0-80m (mg C m^{-2}) and recalculated in cells l^{-1}

Protozooplankton taxa	Abundance (x10^2 cells l^{-1})			Biomass (mg C m^{-2})		
	day T-2	day 21 in patch	day 21 out patch	day T-2	day 21 in patch	day 21 out patch
Choanoflagellates	156.3	244.3	214.3	6.3	9.8	8.6
Aloricate choreotrichs	6.1	12.3	4.6	110.1	320.1	143.9
Tintinnid ciliates	1.4	1.1	0.3	41.9	30.8	7.8
Other ciliates	1.5	0.1	0.1	53.5	2.0	3.5
Gymnodinium/Gyrodinium	4.9	5.5	4.2	61.4	66.4	43.7
Other athecate dinoflagellates	93.3	174.7	100.9	177.3	342.3	183.7
Protoperidinium spp.	1.2*	2.0	1.4	15.7*	30.0	14.3
Other thecate dinoflagellates	24.6*	45.2	20.5	156.0*	348.1	196.2

* values taken on day 0

Table 2

Partial correlation analysis for larger protozooplankton abundances. Values of correlation coefficients for significant correlations are shown. (N.S.) = not significant (P > 0.05); N = 70; 65; 47 (in patch); N = 30; 29; 23 (out-patch). Comparison between discrete depth samples were done with following parameters: Chl. *a* (chlorophyll *a* in µg chl. *a* l^{-1}); Prim. Prod. (primary production in µg C l^{-1} d^{-1}); Microautotrophs (cells l^{-1}); Pico- and Nanoautotrophs (cells ml^{-1}); Nanoheterotrophs(cells l^{-1}); Bacteria (cells ml^{-1}); Small copepod (ind. m^{-3}); Total microzoopl. (cells l^{-1})

Microzooplankton group	Parameter							
	Chl. *a*[§]	Prim. Prod.[$]	Microautotrophs[x]	Bacteria[*]	Pico- and Nanoautotrophs[#]	Nanoheterotrophs	Small copepods	Total microzoopl.
Choanoflagellates	0.317	N.S.	-	0.517	N.S.	-	N.S.	0.352
Ciliates	0.277	N.S.	0.258	0.354	N.S.	0.356	N.S.	-
Dinoflagellates	0.226	0.239	N.S.	0.378	N.S.	0.340	0.230	-
Copepod nauplii	N.S.	N.S.	N.S.	N.S	0.307	N.S.	0.409	-
Total microzoopl.	0.228	0.242	N.S.	0.385	N.S.	0.352	0.231	-

[$] Riebesell *et al*. (to be submitted)

[§] Gervais *et al*. (2002)

[x] Assmy and Henjes (to be submitted)

[*] Arietta *et al*. (2003)

[#] Veldhuis (unpubl. data)

Fig. 1

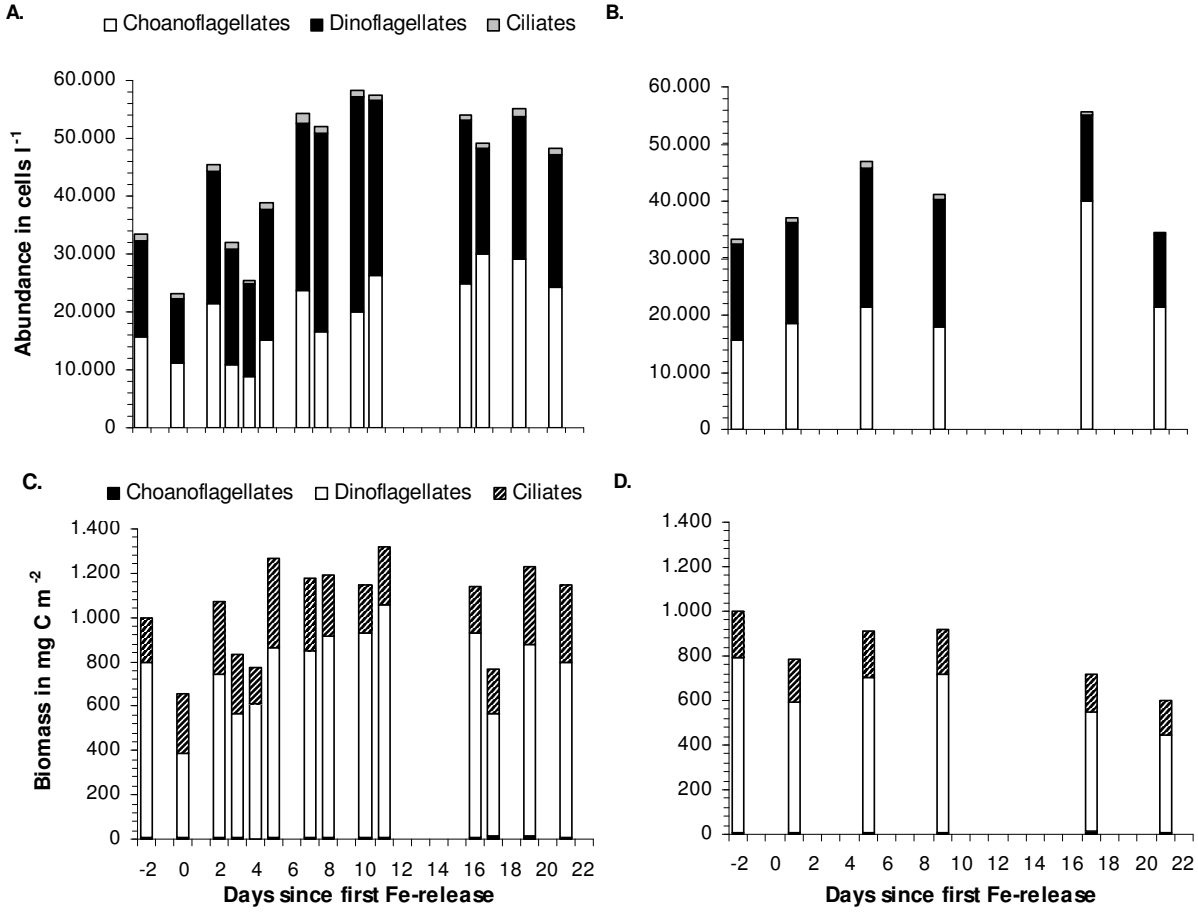

A.
Choanoflagellates
Dinoflagellates
Ciliates
Abundance in cells l-1
60.000
50.000
40.000
30.000
20.000
10.000
0
B.
C.
Choanoflagellates
Dinoflagellates
Ciliates
Biomass in mg C m-2
1.400
1.200
1.000
800
600
400
200
0
-2 0 2 4 6 8 10 12 14 16 18 20 22
Days since first Fe-release
D.
-2 0 2 4 6 8 10 12 14 16 18 20 22
Days since first Fe-release

Fig. 2

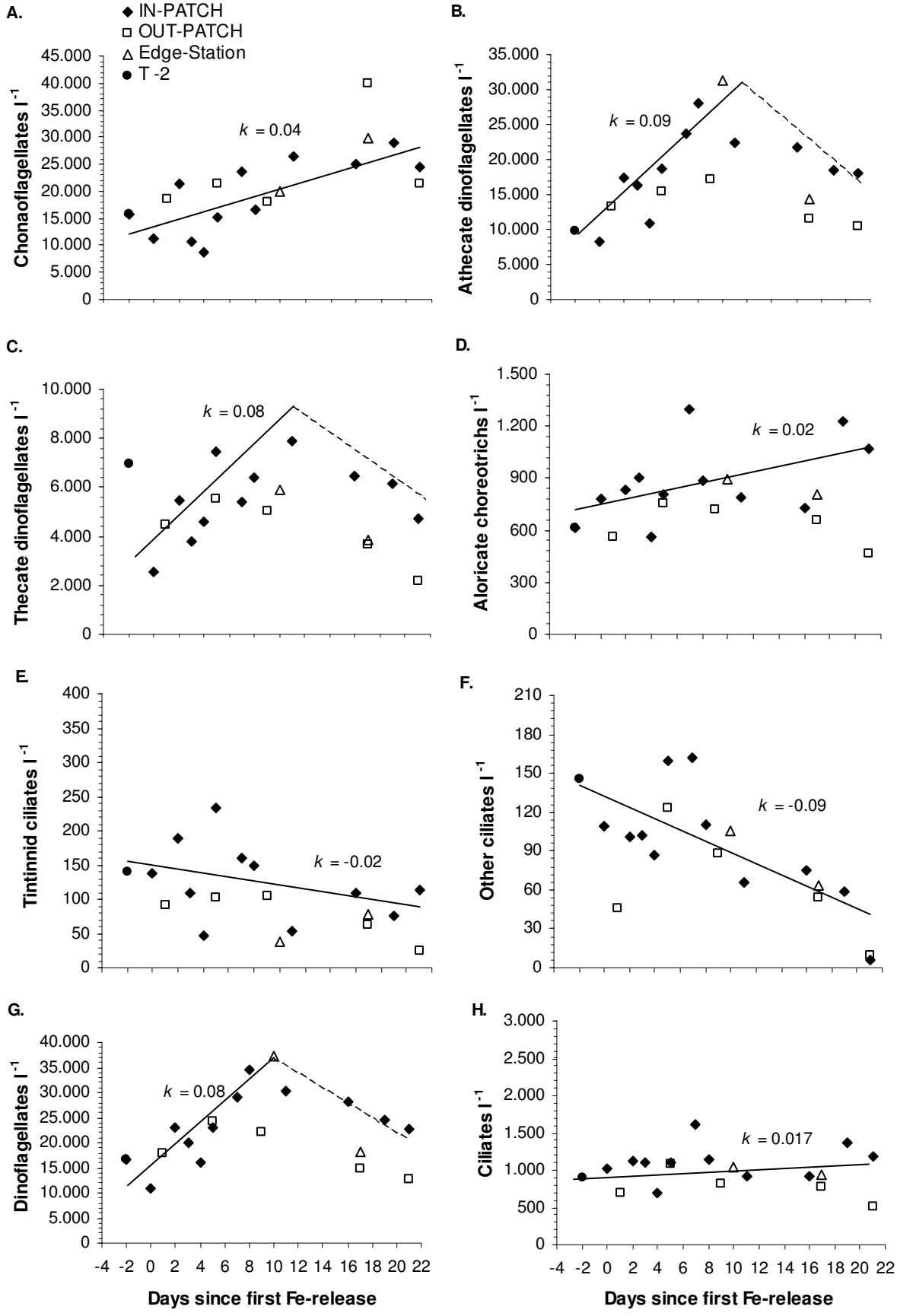

A.
IN-PATCH
OUT-PATCH
Edge-Station
T -2
Chonaoflagellates l-1
k = 0.04
B.
Athecate dinoflagellates l-1
k = 0.09
C.
Thecate dinoflagellates l-1
k = 0.08
D.
Aloricate choreotrichs l-1
k = 0.02
E
Tintinnid ciliates l-1
k = -0.02
F.
Other ciliates l-1
k = -0.09
G.
Dinoflagellates l-1
k = 0.08
H.
Ciliates l-1
k = 0.017
Days since first Fe-release
Days since first Fe-release

Fig. 3

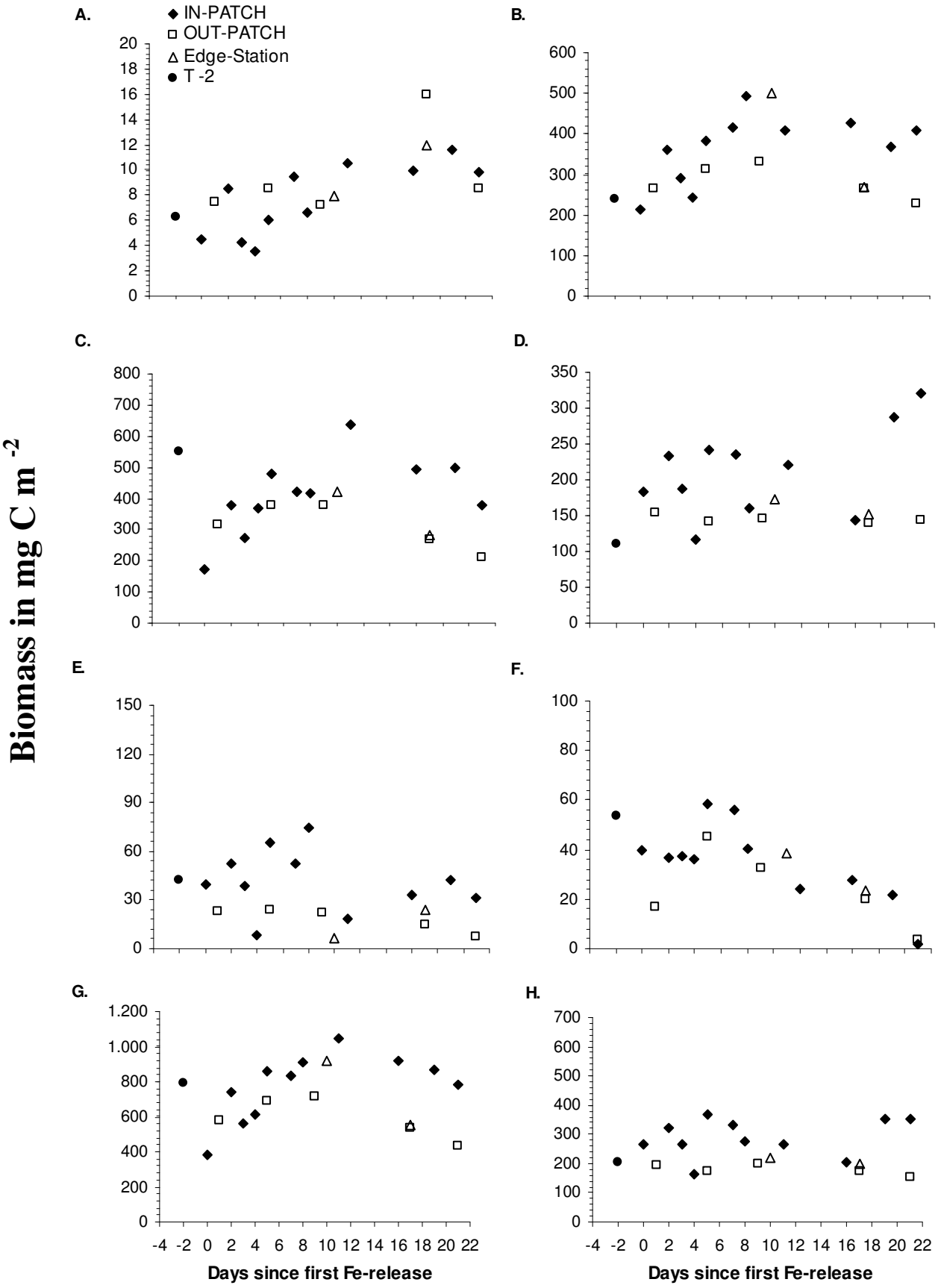

A.
IN-PATCH
OUT-PATCH
Edge-Station
T -2
B.
C.
D.
E.
F.
G.
H.
Biomass in mg C m -2
Days since first Fe-release
Days since first Fe-release

Fig. 4

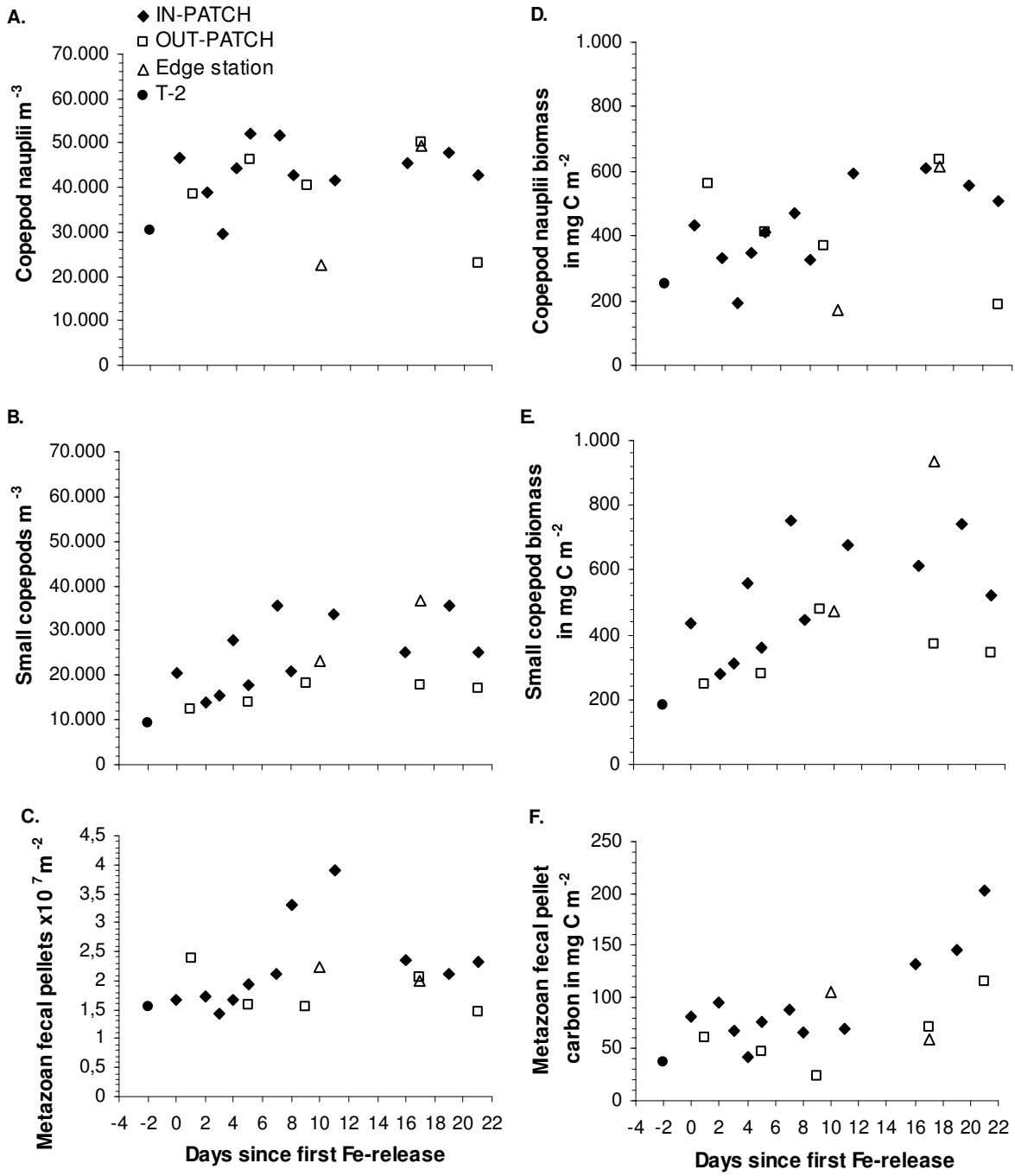

A.
IN-PATCH
OUT-PATCH
Edge station
T-2
Copepod nauplii m -3
70.000
60.000
50.000
40.000
30.000
20.000
10.000
0
D.
Copepod nauplii biomass in mg C m -2
1.000
800
600
400
200
0
B.
Small copepods m -3
70.000
60.000
50.000
40.000
30.000
20.000
10.000
0
E.
Small copepod biomass in mg C m -2
1.000
800
600
400
200
0
C.
Metazoan fecal pellets x10 -7 m -2
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
-4 -2 0 2 4 6 8 10 12 14 16 18 20 22
Days since first Fe-release
F.
Metazoan fecal pellet carbon in mg C m -2
250
200
150
100
50
0
-4 -2 0 2 4 6 8 10 12 14 16 18 20 22
Days since first Fe-release

Fig. 5

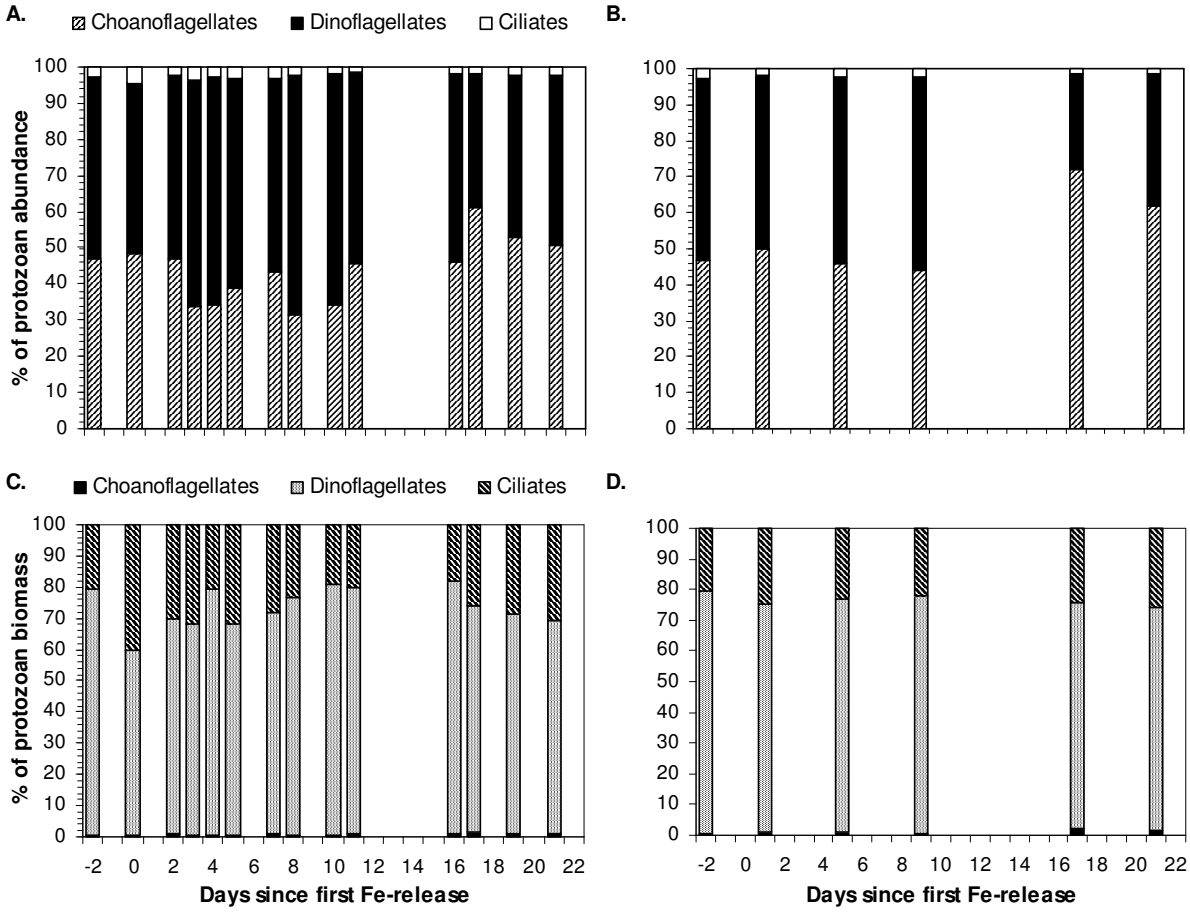

A.
Choanoflagellates
Dinoflagellates
Ciliates
% of protozoan abundance
B.
C.
% of protozoan biomass
D.
Days since first Fe-release
Days since first Fe-release

Fig. 6

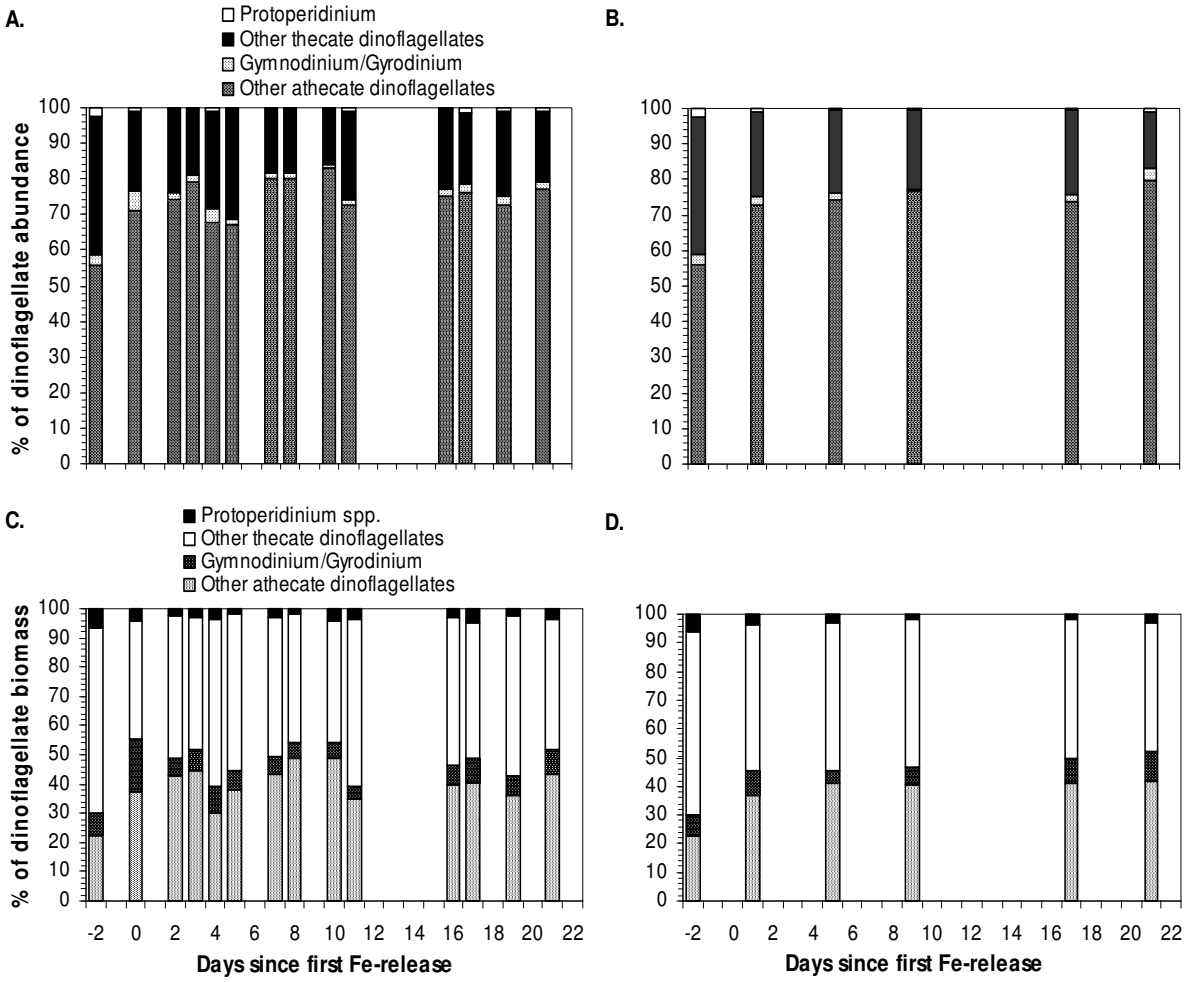

A.
Protoperidinium
Other thecate dinoflagellates
Gymnodinium/Gyrodinium
Other athecate dinoflagellates
% of dinoflagellate abundance
B.
C.
Protoperidinium spp.
Other thecate dinoflagellates
Gymnodinium/Gyrodinium
Other athecate dinoflagellates
% of dinoflagellate biomass
D.
Days since first Fe-release
Days since first Fe-release

Fig. 7

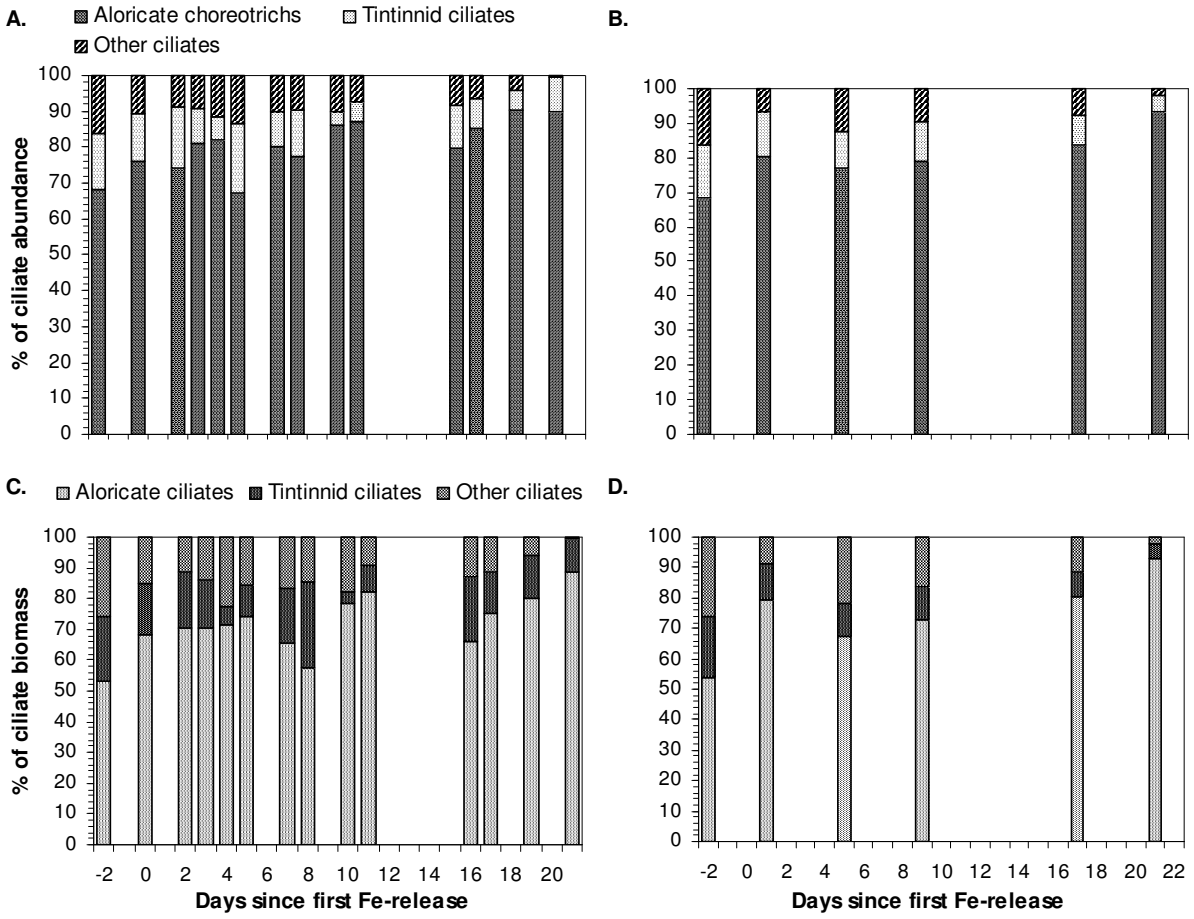

A.
Aloricate choreotrichs
Tintinnid ciliates
Other ciliates
% of ciliate abundance
B.
C.
Aloricate ciliates
Tintinnid ciliates
Other ciliates
% of ciliate biomass
Days since first Fe-release
D.
Days since first Fe-release

Fig. 8

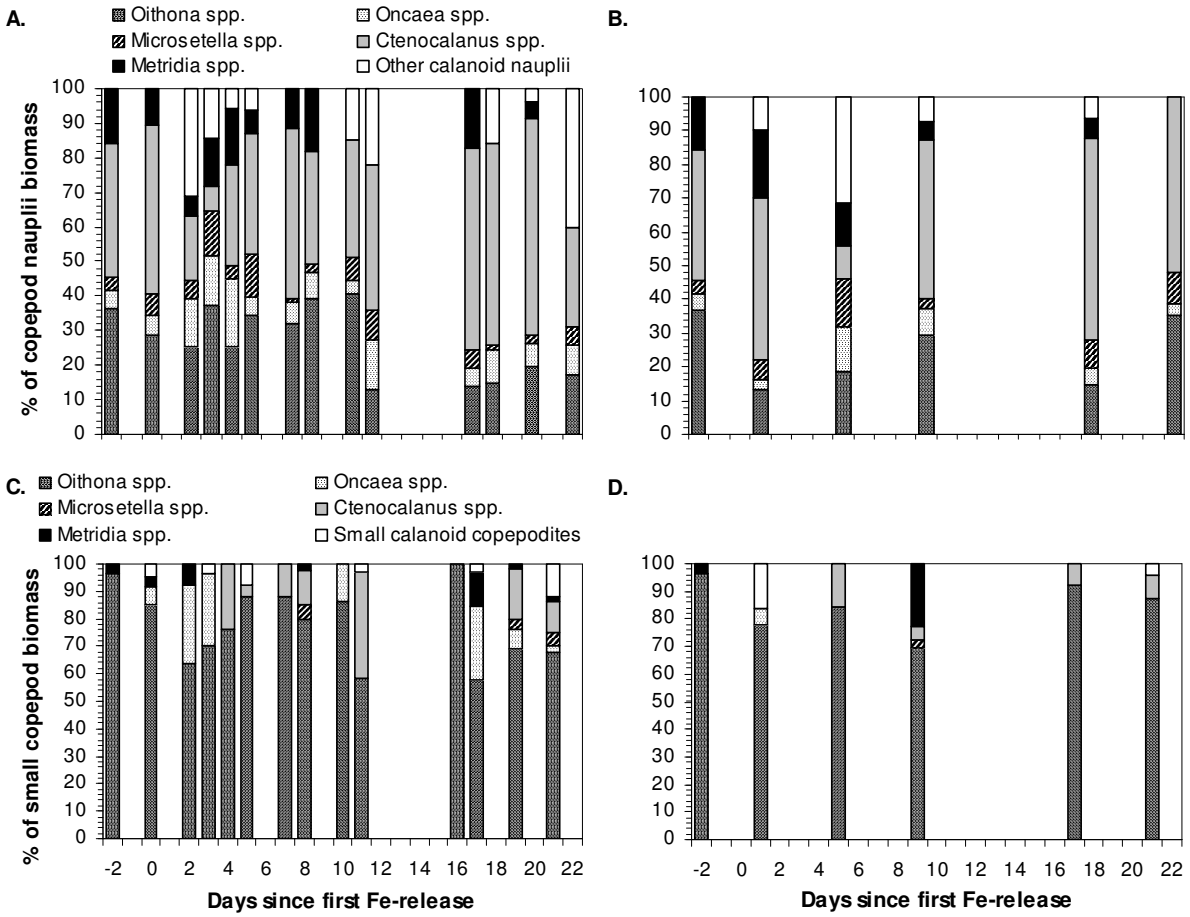

A.
Oithona spp.
Oncaea spp.
Microsetella spp.
Ctenocalanus spp.
Metridia spp.
Other calanoid nauplii
% of copepod nauplii biomass
B.
C.
Oithona spp.
Oncaea spp.
Microsetella spp.
Ctenocalanus spp.
Metridia spp.
Small calanoid copepodites
% of small copepod biomass
Days since first Fe-release
D.
Days since first Fe-release

Fig. 9

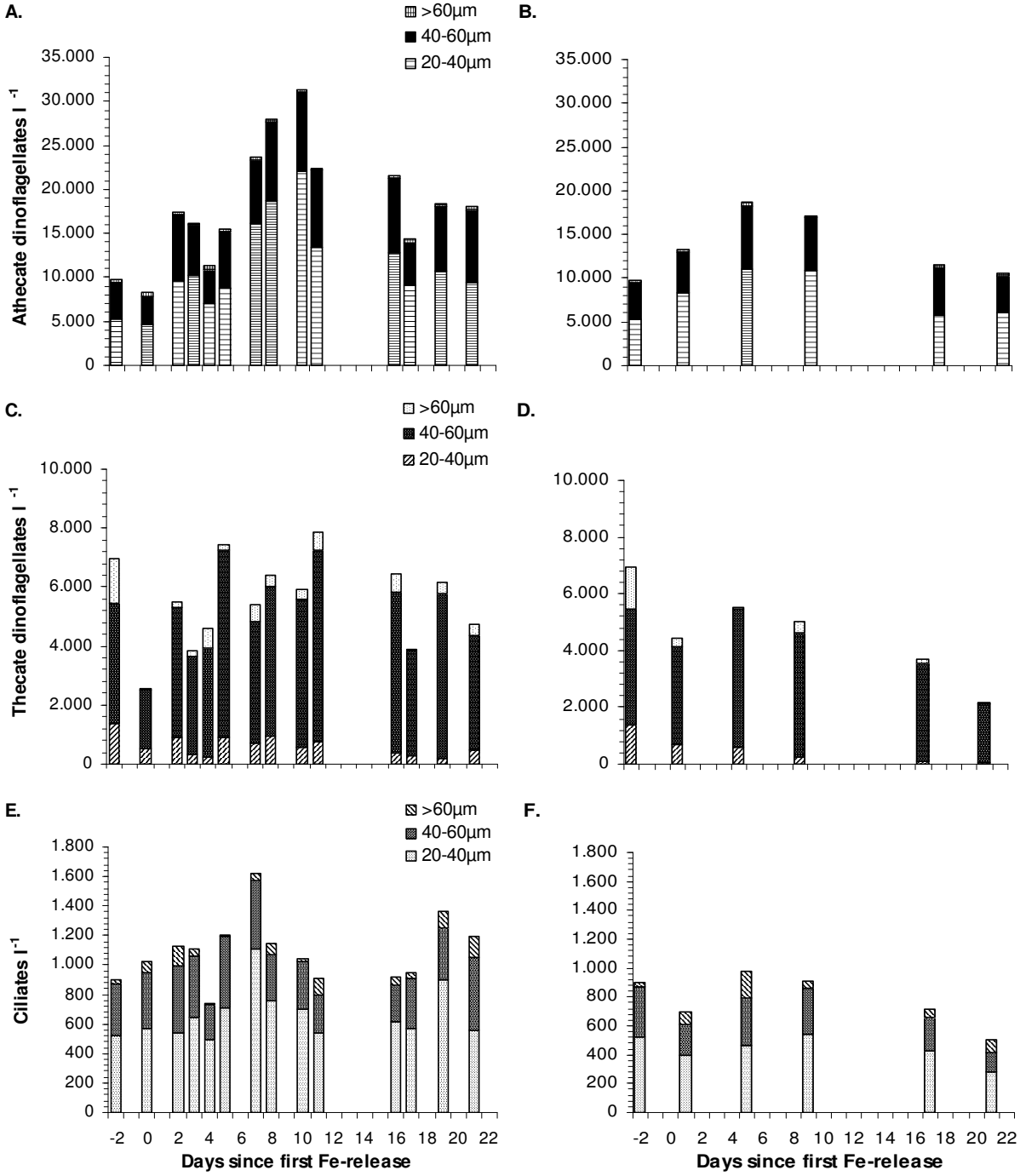

A.
B.
C.
D.
E.
F.
>60µm
40-60µm
20-40µm
Athecate dinoflagellates l -1
Thecate dinoflagellates l -1
Ciliates l-1
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0
8.000
6.000
4.000
2.000
1.800
1.600
1.400
1.200
1.000
800
600
400
200
-2 0 2 4 6 8 10 12 14 16 18 20 22
Days since first Fe-release

Fig. 10

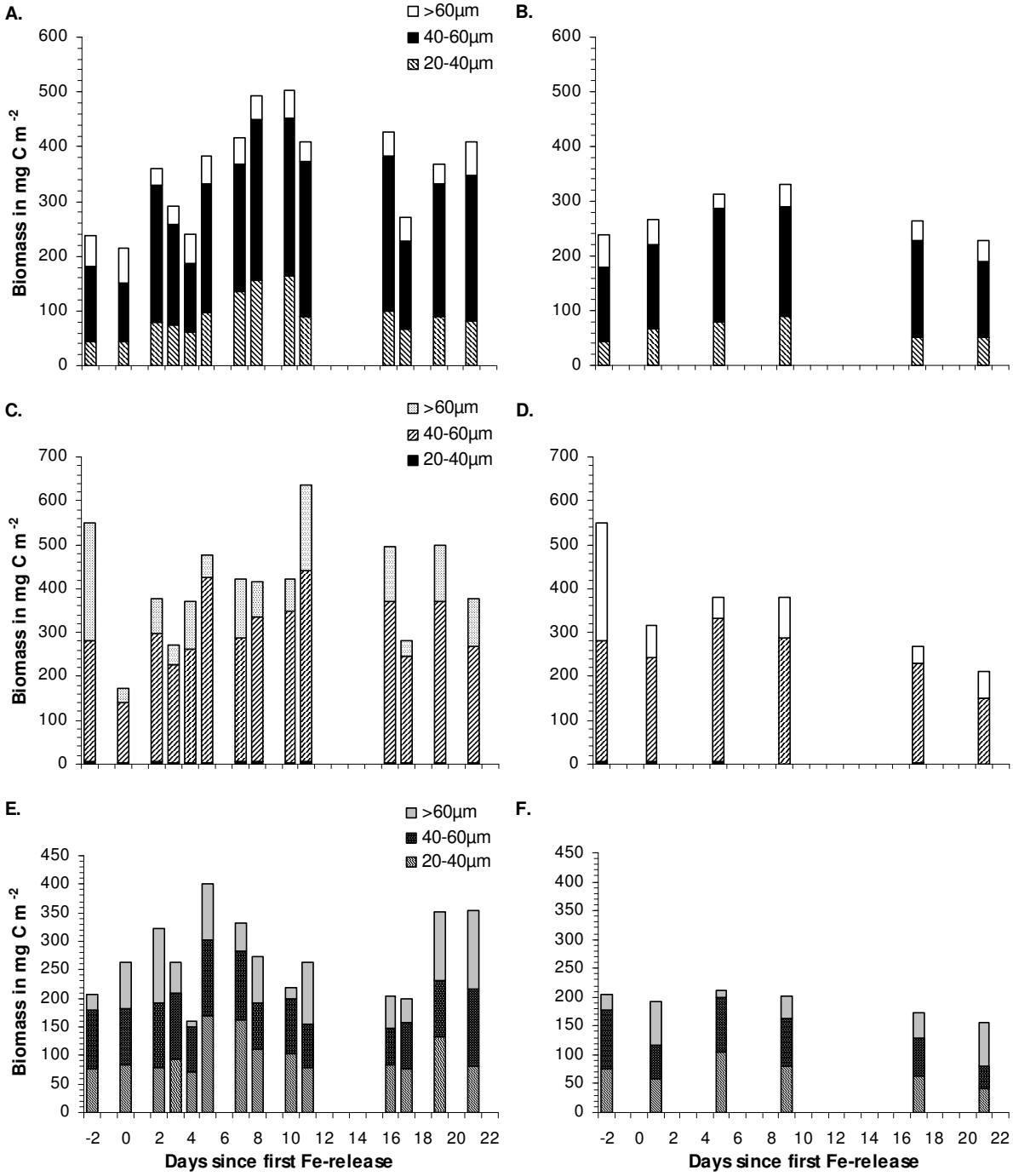

A.
B.
C.
D.
E.
F.
>60µm
40-60µm
20-40µm
Biomass in mg C m -2
Days since first Fe-release

Fig. 11

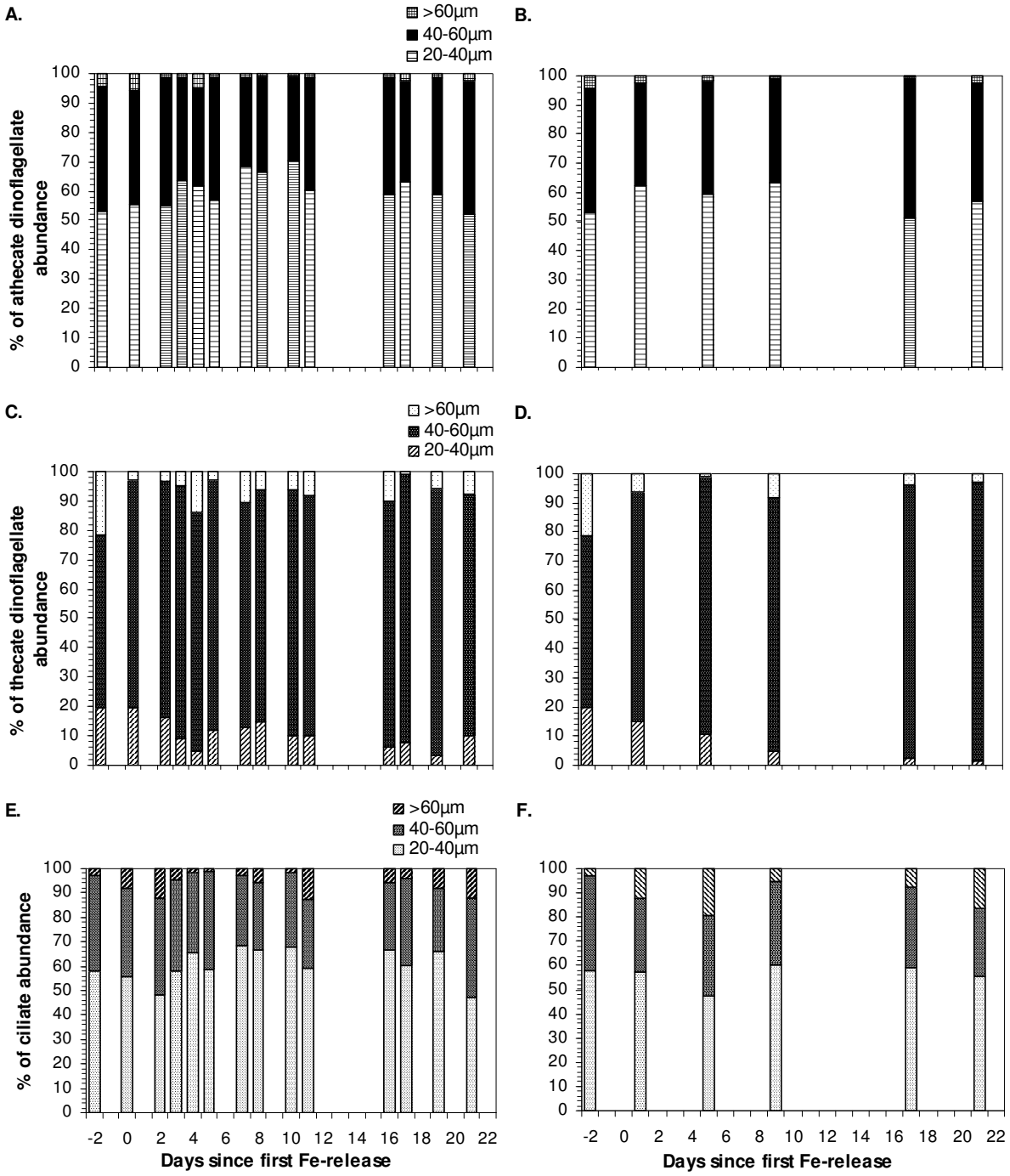

A.
B.
C.
D.
E.
F.
>60µm
40-60µm
20-40µm
% of athecate dinoflagellate abundance
% of thecate dinoflagellate abundance
% of ciliate abundance
Days since first Fe-release

Fig. 12

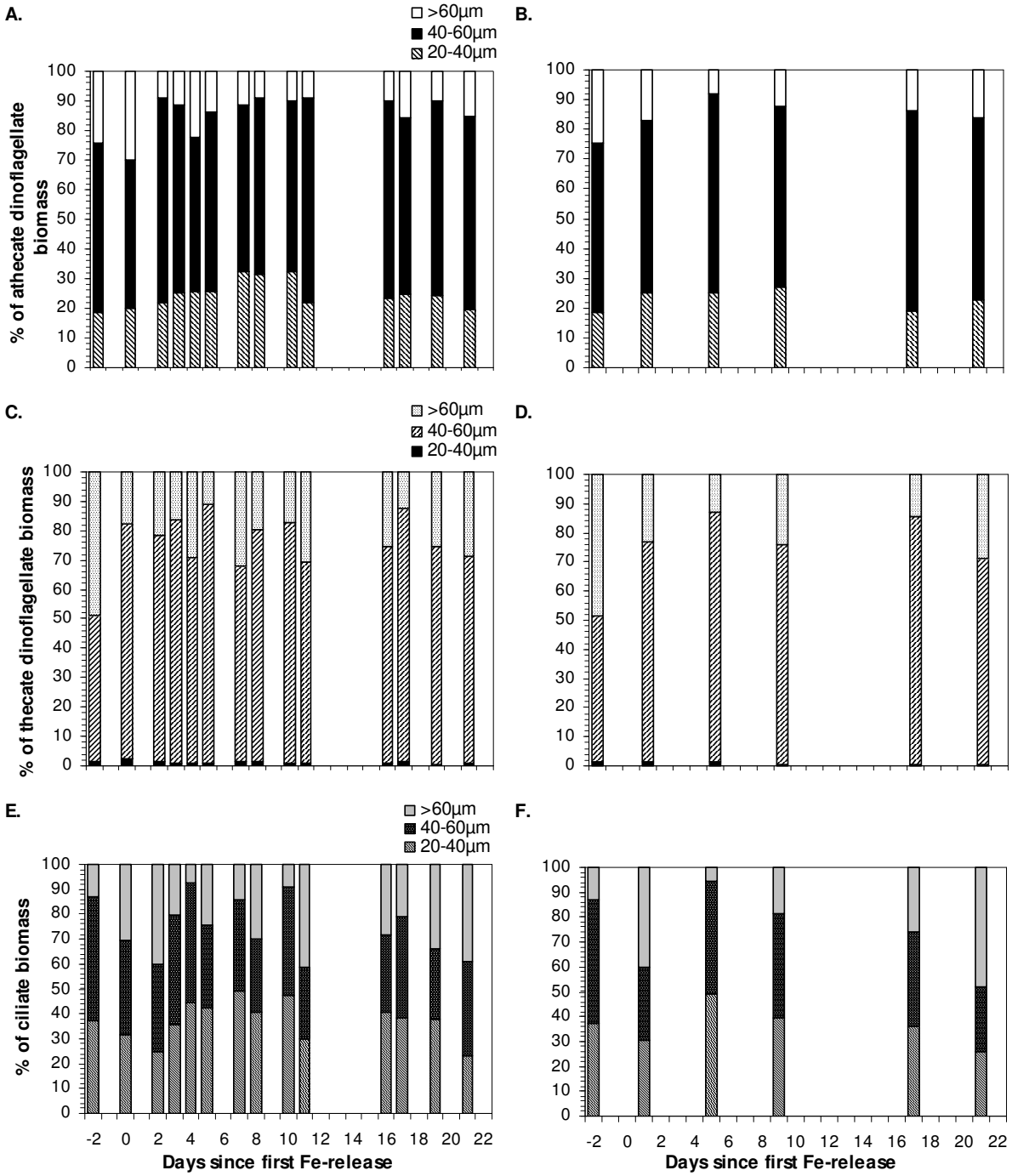
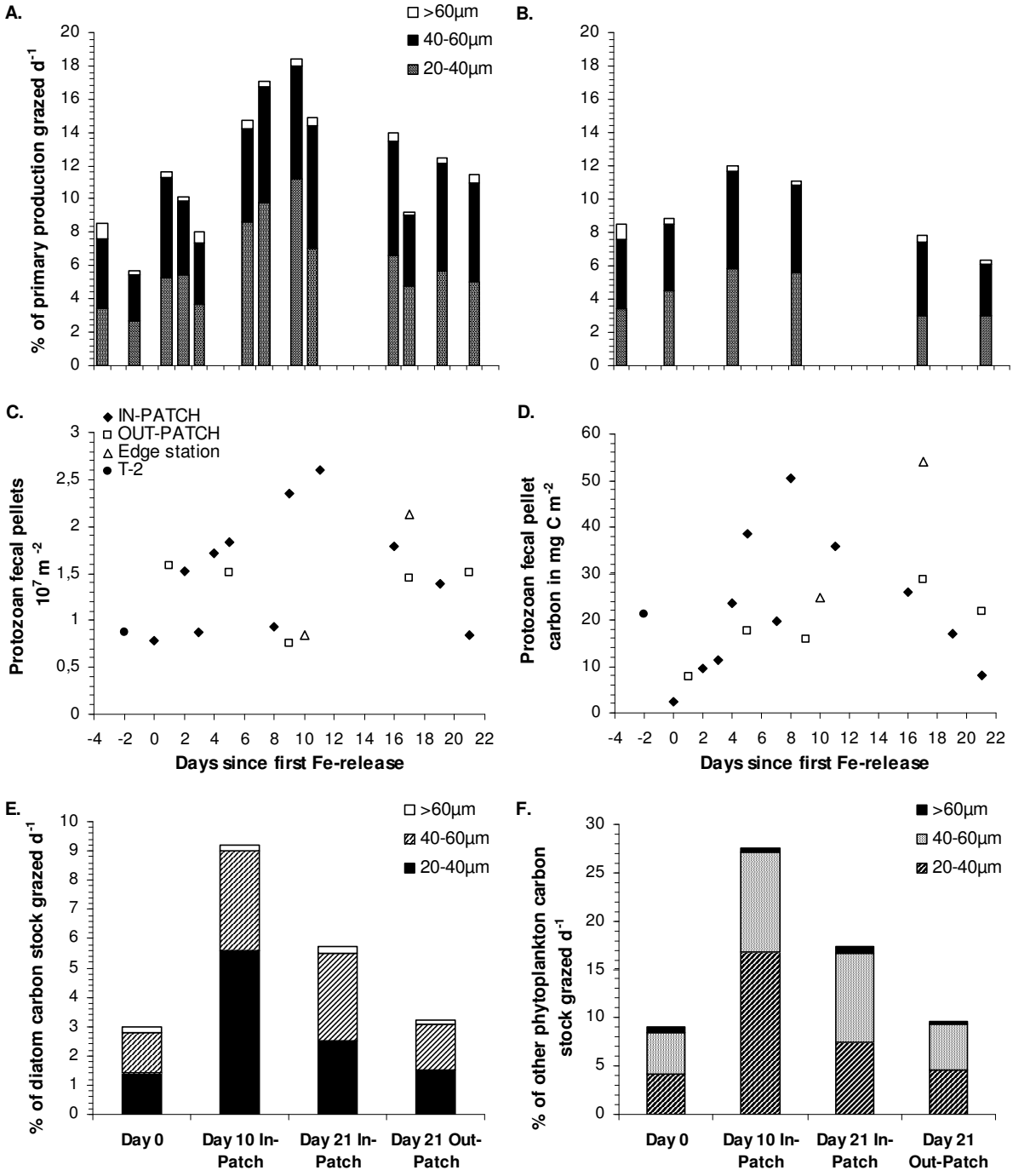

A.
B.
C.
D.
E.
F.
>60µm
40-60µm
20-40µm
% of athecate dinoflagellate biomass
% of thecate dinoflagellate biomass
% of ciliate biomass
Days since first Fe-release

Fig. 13


A.
>60µm
40-60µm
20-40µm
% of primary production grazed d-1
B.
C.
IN-PATCH
OUT-PATCH
Edge station
T-2
Protozoan fecal pellets 10^7 m^-2
Days since first Fe-release
D.
Protozoan fecal pellet carbon in mg C m^-2
Days since first Fe-release
E.
>60µm
40-60µm
20-40µm
% of diatom carbon stock grazed d-1
Day 0
Day 10 In-Patch
Day 21 In-Patch
Day 21 Out-Patch
F.
>60µm
40-60µm
20-40µm
% of other phytoplankton carbon stock grazed d-1
Day 0
Day 10 In-Patch
Day 21 In-Patch
Day 21 Out-Patch

Manuscript 4

Response of larger protozooplankton to an iron-induced phytoplankton bloom in the Polar Frontal Zone of the Southern Ocean (EisenEx)

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Abstract

During the *in situ* iron fertilization experiment (EisenEx) conducted in the Polar Frontal Zone of the Southern Ocean in austral spring (November) 2000, the responses of larger (>50µm in diameter) protozooplankton groups to an induced phytoplankton bloom were studied for the first time. In the 21 d experiment, samples were collected from 7 discrete depths in the upper 150m in inside and outside the patch for the enumeration of acantharia, foraminifera, radiolaria, heliozoa, tintinnid ciliates and heterotrophic thecate dinoflagellates. Inside the patch, acantharian numbers increased three-fold, but only negligibly in surrounding waters. This is of major interest, since acantharia are suggested to be responsible for the formation of barite found in sediments and which is a paleo-indicator of high productivity regimes. Foraminiferans also increased significantly in abundance, however the marked increase of juvenile individuals after a full moon event suggests a lunar periodicity in the reproduction cycle of some foraminiferan species rather than a reproductive response to enhanced food availability. Larger thecate dinoflagellates almost doubled in numbers and biomass, but also showed an increase outside the patch. We found indications, however, that loss terms such as grazing pressure had a significant influence on dinoflagellate population growth during EisenEx. In contrast, adult radiolaria showed no clear trend during the experiment, but juveniles increasing three-fold indicating elevated reproduction. changed of Tintinnids decreased two-fold, whereas their carbon stocks remained more or less constant caused by a change in the relative importance of species. Empty tintinnid loricae, however, increased by a factor of two indicating that grazing pressure on this group intensified during EisenEx. The results of this experiment clearly show that iron-fertilization experiments can shed light on the biology and the role of these larger rarer organisms in pelagic ecosystem which will improve their use as proxies in paleoceanography.

Introduction

The trophic diversity and the function of larger protozoans (sarcodines, larger dinoflagellates and larger tintinnid ciliates) in pelagic food webs has always lagged behind their role as proxy for paleoceanographic reconstruction, and is just started to be described (Gowing, 1989; Caron and Swanberg, 1990; Gowing and Garrison, 1991; Nöthig and Gowing, 1991; Gowing and Garrison, 1992; Gowing *et al*., 2001; Klaas, 2001). Concerning biogeochemical fluxes, oceanic distribution and cycling of specific elements by planktonic sarcodines as the silicious ($Si(OH)_4$) radiolarians and the carbonate ($CaCO_3$) foraminiferans, studies have suggested that acantharians, sarcodine protists secreting celestites ($SrSO_4$) for their skeletons, may play an important role in the marine chemistry of strontium (Sr) and barium (Ba) which are both components of the acantharian celestite (Bernstein *et al*, 1987, 1992, 1998; Dymond and Collier, 1996). The importance of acantharians in the biogeochemical cycling especially of Ba has been stressed by the relationship between the marine chemistry of this element and ocean productivity which has led to the use of solid-phase Ba compound (barite) as a proxy for both modern and ancient biological processes (Dehairs *et al*. 1991, 1992, 1997; Dymond *et al*., 1992; Francois *et al*., 1995; Dymond and Collier, 1996).

Despite their underestimated role of in the biological processes of Southern Ocean ecosystem and the major interest in their function as a tool for paleoceanographic reconstruction, studies of larger protozoan abundances, vertical distribution patterns and food web interrelationships have focused primarily on limited regions of the Southern Ocean (Weddell Gyre, Ross Sea and Weddell-Scotia Confluence) and on a limited number of taxa (forminiferans and radiolarians). Only recently, spatial and temporal coverage of abundances, vertical zonation patterns and community composition of all important taxa in the Polar Frontal Zone of the Southern Ocean have started to be examined (Abelmann and Gowing 1996; 1997; Klaas 2001). Furthermore, there have been no studies carried out so far in the Southern Ocean investigating the mentioned patterns and assemblage composition under *in situ* experimental conditions. In iron fertilization experiments, conducted in the Pacific sector of the Southern Ocean (SOIREE) as yet, the pelagic system is relieved from its limiting resource, which in the Southern Ocean is iron. As a consequence, since all other dissolved nutrients are in surplus, primary productivity and mostly population growth of other compounds of the trophic cascade is enhanced (Gall *et al*., 2001; Gervais *et al*., 2002).

Thus, the aim of this study was to follow the responses of the important taxa of larger (> 50µm) protozoans (i.e. acantharians, foraminiferans, radiolarianns, heliozoans, tintinnid ciliates and larger thecate dinoflagellates) during an iron fertilization experiment in the Polar

Frontal Zone of the Southern Ocean (EisenEx) and to give indications for the possible reason why these responses occur. Protozooplankton larger than 50µm in one dimension were examined in this study for two reasons: (1) to study the role of these organisms as a trophic link between smaller protozooplankton and larger metazooplankton (Gowing, 1989) and (2) to study a size fraction and a group of taxa that are important for paleoceanographic studies (Boltovskoy *et al.*, 1996; Abelmann and Gowing, 1996, 1997), but whose relatively low abundances cannot be reliably assessed in water sample volumes used for the enumeration of smaller or skeletonless and athecate protozooplankton (Henjes and Assmy, to be submitted).
Beyond this, we want to discuss the importance of iron fertilization experiments to study the role and biological significance of larger protozooplankton in the pelagic ecosystem and their implications to paleoceanographical reconstruction.

Material and methods

Abundance

For quantitative assessment of sarcodines (acantharia, radiolaria and foraminifera) and other protozooplankton (tintinnid ciliates and thecate dinoflagellates) larger than 50µm, water samples were taken at seven discrete depths (10, 20, 40, 60, 80, 100 and 150m) at 19 in and out patch CTD (Conductivity Temperature Depth) stations using Niskin bottles. The entire contents of the Niskin bottle (~12l) were passed through a 10µm mesh plankton net and concentrated to a fixed volume of approx. 50ml. The concentrating procedure of the samples is particularly gentle to delicate organisms like sarcodines.
The concentrated samples were preserved with hexamine buffered formaline solution at a final concentration of 0.5% and stored at 4°C in the dark for subsequent counting in the home laboratory. Since the concentration method is inappropriate for quantitative estimates of abundances of skeletonless or athecate protozooplankton, which are easily deformed and therefore can squeeze through the net or be destroyed by net damage (Nöthig and Gowing, 1991), only tintinnids and thecate species were counted among the ciliates and dinoflagellates respectively.
In case of the Acantharians, organisms were counted mainly directly on board and although strontium sulfate was not added to the samples to preserve the celestite skeletons of acantharians (Beers and Stewart, 1970a; Michaels, 1988a), they were recognizable by their myonemeres and of their soft parts even after dissolution of the skeleton.. To ensure that

dissolution of acantharian celestites still did not influence their abundances, stations were counted in a random order.

Organisms were identified and enumerated using inverted light and epifluorescence microscopy (Axiovert 25, Axiovert 135 and IM 35) according to the method of Utermöhl (1958). Prior to counting 70µl of stock solution of the nuclear fluorochrome 4', 6-diamidino-2-phenylindole (DAPI) (Coleman, 1980; Porter and Feig, 1980) were added in random order to the samples to stain the nucleus. Cells with stained nuclei were considered alive at the time of capture. The nucleus of foraminifera was difficult to see, because of their tests. As long as the tests were not translucent, meaning without cytoplasm, individuals were regarded as alive (Nöthig and Gowing, 1991). Except for the very abundant tintinnid ciliates, organisms were counted in the whole chamber (~3 ml) at magnifications of 200x. If possible aliquots were counted up to a minimum of 50 cells of the most abundant taxa or a max. total of 300 organisms. All water samples were equally treated and gently mixed prior to counting.

Larger thecate dinoflagellates, tintinnid ciliates and foraminiferans were identified to genus or species, polycystine radiolarians were grouped according to taxonomy into nassellarians and spumellarians and identified to genus or species; phaeodarians, heliozoans and acantharians were not identified further. Juvenile individuals were only identified on the level of class and order, respectively. Some of the juvenile foraminiferans and radiolarians were smaller than 50µm, and were therefore discussed separately.

Concerning tintinnid species identification, we have referred to the tintinnid ciliate *Codonolopsis pusilla* (Cleve, 1900b) as such because, although our specimens fit the description of the genus (Balech, 1971a) it is still possible that the specimens found in our samples belong to the genera *Stenosomella* spp. Although there have only been little evidence of *Stenosomella* spp being present in subantartic waters and/or Antartic waters (Hada, 1970), whereas *Cd. pusilla*. is a common species in the Polar Frontal Zone (Thompson, 1999). Moreover our specimen are larger in size (56µm long, 32µm wide lorica) compared to specimen found by Hada (1970) and Gowing and Garrison (1992) in subantartic and Antartic waters, respectively, and their collar was higher with at least three spiral turns (Fig. 9B).

Volume measurements and biomass conversion

Biovolume was determined for all sarcodines and other larger protozoans observed in the bottle-collected samples and estimated from dimensions of at least 10-20 randomly chosen individuals of each taxon. The biovolume of the foraminifera was determined by assuming a

spherical shape and using the longest dimension across the calcite test as the diameter (Bè *et al*., 1977). For acantharia, the radius of spherical individuals and multiple radii for oblong individuals were recorded. Biovolumes were calculated from these dimensions assuming a sphere, or a spheroid shape (Michaels, 1995). For adult radiolarians, bio volume was measured as the diameter of the spherical central capsule (Michaels, 1995).

Cell volume was converted to cellular carbon content through recommended carbon conversion equations using the following carbon to volume relationships: for dinoflagellates, C $cell^{-1}$ = 0,444*$V^{0.864}$; and for tintinnid ciliates, C $cell^{-1}$ = 0.679*$V^{0.841}$, with *V* representing total cell volume (μm^3) and C describing cellular carbon content (pg) (Menden-Deuer and Lessard, 2000). It has to be mentioned that tintinnid ciliates contain carbon in their loricae as well as in their cytoplasm. As mentioned by Gowing and Garrison (1992) loricae of *Cymatocylis* spp. would also be expected to contain the most organic carbon of the loricae observed in this study because of their large size and non-agglutinated nature. We did not include carbon from tintinnid loricae in our calculations because we observed on several occasion completely intact loricae inside copepod fecal pellets (pers. observation) Similar observations have been stated by Gowing and Garrison (1992) with the conclusion that carbon from tintinnid loricae cannot considered to be available as a carbon source for the next trophic level.

Sarcodine biovolumes were converted to biomass (carbon) estimates using measured carbon:volume ratios by Michaels (1995). This is a carbon:volume ratio of 0.0026 pg μm^{-3} for acantharians, 0.089 pg μm^{-3} for foraminifera and 0.01 pg μm^{-3} for radiolarians. As noted by other authors these carbon estimates are conservative, because amount of cytoplasm shrank during fixation (Gowing and Garrison, 1991, 1992; Michaels, 1995).

Findings by Beers et al. (1982), Bishop *et al*. (1977, 1978, 1980), Caron and Swanberg (1990), Gowing and Garrison (1991, 1992), Klaas (2001) and Michaels (1988a, 1991, 1995) showed that sarcodines and other large protozooplankton have pronounced vertical distribution features. Therefore partial correlation analyses between standing stocks of the different protozoan taxa in relation to biological and physico-chemical parameters in the water column were carried out by comparing discrete depth values from each station sampled.

Environmental setting

Results of an *in situ* mesoscale iron fertilization experiment (EisenEx) conducted in the Atlantic Sector of the Southern Ocean (~21°E, 48°S) in austral spring (6-29 November 2000)

during the cruise ANT XXVIII/2 on the R/V *Polarstern* are presented here. A cyclonic eddy (approximately 120 km wide) shed by the Antarctic Polar Front (APF) was chosen as the "container" for the experiment and its centre marked with a drifting buoy. An area of about 40 km^2 around the buoy was fertilized with 4 tonnes of acidified iron sulfate solution ($FeSO_4$) on three occasions at eight day intervals (Strass *et al*. 2001). Sulphur hexafluoride (SF_6) was added as an inert tracer at the first iron infusion in order to relocate the iron fertilized "patch" (Watson *et al*. 2001). Since SF_6 can be rapidly measured on board, measurements of the fertilized (inside patch) and reference (outside patch) waters can be made online. Inside and outside stations were chosen according to SF_6 concentration measured along horizontal surface surveys. So called "in-stations" were situated at the highest observed SF_6 concentrations, whereas "out-stations" were within adjacent waters of the fertilized patch with background SF_6 concentrations. Two of the in-stations were, in fact, situated at the edge of the iron-enriched patch ("edge stations") as indicated by intermediate SF_6 concentrations. The station "T-2", which occurred 2 days prior to the first fertilization and/or day 0 were referred to as an reference station. The mixed layer depth varied considerably over the course of the experiment. At the beginning of the experiment the seasonal mixed layer depth of 10-50 m was relatively shallow for spring both inside and outside the patch providing favourable light climate for phytoplankton growth (Strass *et al*., 2001). Three times, on day 5 and on two occasions in the second half of the experiment, wind speeds exceeded 20m s^{-1} (gale force winds) that deepened the mixed layer down to occasionally more than 80m.

Surface temperatures increased from initially 3.5 °C to 4.0 °C by day 21. Surface salinity stayed more or less stable at 33,8 ‰ during EisenEx (Strass *et al*., 2001). The daily mean irradiance averaged over depth was between 100 and 150 µmol m^{-2} s^{-1}. The areal daily primary production increased inside the patch and reached the maximum of 790 mg C m^{-2} d^{-1} on day 16, and decreased thereafter to almost initial values. Chlorophyll-*a* concentrations increased from initially 0.5 mg m^{-3} to maximal values of 2.5 mg m^{-3} in the upper 80 m inside the patch by the end of the experiment, but stayed more or less constant in adjacent waters (Gervais *et al*., 2002). Diatoms dominated phytoplankton standing stocks in the second half of the experiment increasing to a standing stock of ~3.5 g C m^{-2} (integrated over 80m depth) on day 21 with *Pseudonitzschia* spp. being the most dominant genus (Assmy and Henjes, to be submitted).

Horizontal dispersion of the patch had doubled its size every four to five days on average and caused the increase from initially 40 km^2 to 950 km^2 by day 21 (Watson *et al*., 2001). Transmission values co-varied with chlorophyll-*a* fluorescence both indicating the increase in

phytoplankton particle abundance inside the patch. The 80 m depth integrated POC, PON and BSi standing stocks had increased from 439 mmol m^{-2}, 82 mmol m^{-2} and 61 mmol m^{-2} at the beginning of the experiment to 679 mmol m^{-2}, 126 mmol m^{-2} and 227 mmol m^{-2} respectively by day 21 inside the patch (Riebesell *et al*., submitted). Bacterial abundance almost doubled inside the patch and remained constant outside with no changes in bacterial community composition (Arrieta *et al*., submitted). Choanoflagellates and aloricate ciliates >20 µm showed a slight increase in abundance as well as in biomass. Heterotrophic flagellates, heterotrophic thecate and athecate dinoflagellates initially showed a strong increase until day 11 and 10, respectively, and declined to values to-fold higher than initial values (Henjes and Assmy, to be submitted). The trend in dinoflagellate faecal pellet carbon, with an initial increase and a decline after day 8, resembles the distribution of heterotrophic thecate and athecate dinoflagellates (Henjes and Assmy, to be submitted). Small metazoa, including nauplii, copepodites and small adult copepods were very abundant at the start of the experiment and further increased towards the end of the experiment. This increase is reflected by the accumulation of copepod faecal pellet carbon during the experiment (Henjes and Assmy, to be submitted).

Results

All EisenEx data presented are 0-150m depth integrated abundances and biomasses, but quoted in ind. m^{-3} and µg C m^{-3}, respectively, by dividing through the sampling depth for better comparison. For vertical distribution of genera or groups see Section 4.2. The temporal development of the sarcodine (acantharians, foraminiferans, radiolarians, heliozoans) and other larger protozooplankton (tintinnid ciliates and thecate dinoflagellates >50µm) assemblage inside and outside the fertilized patch is described for all groups identified. The two “edge st.” are discussed as “in patch” long as they do not considerably deviate from other inside values.

Abundance and biomass of living sarcodines and other larger protozooplankton

Total abundance of larger protozooplankton (>50µm) increased from 161.8×10^3 ind. m^{-3} two days prior to fertilisation (T-2) to 2.0×10^5 ind. m^{-3} at the end of the experiment (after 21 days) with the highest value of 204.1×10^3 ind. m^{-3} on day 5 (Fig. 1A; Tab. 1). Outside the fertilized patch total abundances consistently decreased to a value of 117.8×10^3 ind. m^{-3} on day 21 (Fig,

1B; Tab. 1). Total abundance of larger protozooplankton were significantly different between in- and out-patch station from day 11 to day 21 ($P < 0.05$, unpaired t-test).

Relative abundances inside the fertilized patch show that tintinnid ciliates dominated abundance of larger protozooplankton in the beginning of the experiment (34-68% until day 8), but decreased thereafter to a relative amount of 42% (lowest value of 27% on day 11) after 3 weeks (Fig. 2A). Large thecate dinoflagellates contributed 25% to total larger protozooplankton on day T-2 increasing up to 47% on day 19. The sarcodines (acantharians, foraminiferans, radiolarians and heliozoans) made up for the smallest fraction of larger protozooplankton abundance ranging from 14% on day T-2 up to 22% (highest value of 30% on day 10) at the end of the experiment. Within the sarcodines, acantharians (20-54% of numbers) contributed most to abundances followed by foraminiferans and radiolarians (9-54% and 12-48% of abundance, respectively; Fig. 3A). Heliozoans made up for the smallest fraction of total sarcodine abundance (0-28%). Acantharian and foraminiferan relative numbers increased in the course of the experiment from 27 (T-2) to 56% (day 19) and from 16 (T-2) to 39% (day 21) of total sarcodine abundance, respectively. In contrast, radiolarian (T-2: 32%; day 21: 21%) and heliozoan (T-2: 26%; day 21: 10%) importance declined during the 3 week experiment (Fig. 3A).

Outside the fertilized patch tintinnid ciliates also dominated larger protozooplankton on day T-2 (60% of abundance), but decreased continously until day 21 (20% of abundance; Fig. 2B). Larger thecate dinoflagellates increased their relative abundance during the course of the experiment (from 25% on day T-2 to 54% after 3 weeks). Sarcodines accounted for 14% of total larger protozooplankton at the beginning of the experiment, increasing to 23% on day 21. Within the sarcodines, foraminiferans (7-49% of abundance) contributed significantly to total sarcodine abundance followed by acantharians (26-38%), radiolarians (17-36%) and heliozoans (2-26%; Fig. 3B). Relative numbers of foraminiferans increased to a maximum of 49% of total sarcodine abundance, whereas radiolarian and heliozoan importance declined in course of the experiment (20 and 5% of abundance, respectively). Relative abundances of acantharians remained rather constant during the 3 weeks of the fertilization experiment (Fig. 3B).

Within the fertilized patch, carbon standing stock of larger protozooplankton increased from 875 µg C m^{-3} on day T-2 to 1,437 µg C m^{-3} after 3 weeks with the highest value (1,509 µg C m^{-3}) on day 11 (Fig. 1C; Tab. 1). The biomass of total organisms outside the fertilized patch ranged from 875 µg C m^{-3} to 1057 µg C m^{-3} (Fig. 1D; Tab. 1). There was no significant

difference between inside and outside the fertilized patch in total larger protozooplankton biomass.

Concerning relative biomass inside the patch, larger thecate dinoflagellates dominated total organism biomass (33-65%), but showed no increase in the course of the experiment (Fig. 2C). Tintinnid ciliates followed large thecate dinoflagellates in relative standing stock (9-54%), but decreased from 33% on day T-2 to 18 % after 3 weeks. Relative biomass of sarcodines was the smallest fraction increasing from 17 to 29% (highest value: 39% of biomass on day 4) over the 3 weeks of the experiment. Among the sarcodines, forminiferans contributed significantly to total sarcodine carbon standing stock at all stations (20-87% of biomass), followed by radiolarians (8-49%), heliozoans (0-31%) and acantharians (5-22%; Fig. 3C). Foraminiferans and acantharians showed an increase in relative importance (T-2: 41%; day 21: 74% and T-2: 9%; day19: 18%), while radiolarians and heliozoans decreased from day T-2 (28 and 22%, respectively) to day 21 (12 and 6%, respectively; Fig. 3C).

Outside the fertilized patch organism carbon standing stock was also dominated by large thecate dinoflagellates increasing from 50 to 64% during the 3 weeks of the experiment, followed by tintinnid ciliates decreasing from 33 (day T-2) to 9% (day 21), and sarcodines increasing from 17 (day T-2) to 27% (day 21) of total biomass with highest value of 39% on day 17 (Fig. 2D). Foraminifera contributed significantly to sarcodine biomass (13-86%), increasing over the 3 week experiment (80% on day 21; Fig. 3D). Acantharians made up for 6-18% of total sarcodine biomass at all stations, showing no trend in the course of the experiment (Fig. 3D). Radiolarians and heliozoans also contributed significantly to biomass during the fertilization experiment (7-44% and 1-24%, respectively), but declined from day T-2 (28 and 26%, respectively) to day 21 (11 and 3%, respectively; Fig. 3D).

Sarcodines

Acantharia. Inside the fertilized patch abundances of acantharians increased markedly (three-fold) over the 3 weeks of the experiment, starting with 6,171 ind. m^{-3} on day T-2 and showing the highest value of 19,696 ind. m^{-3} on day 19 (Fig. 4A; Tab. 1). The increase occurred in 3 steps over the 3 weeks of the experiment. In the first 5 days abundances ranged from 4,048 to 7,715 ind. m^{-3}; from day 7 to 11 abundances ranged from 11,065 to 14,450 ind. m^{-3} and on the last 4 days of the experiment they ranged from 13,731 to 20,947 ind. m^{-3} (Fig. 4A). Outside the fertilized patch, acantharian abundance increased only slightly (8,016 ind. m^{-3} on day 21; Fig. 4A).

Biomass of acantharians increased from 13.6 µgCm^{-3} on day T-2 to 43.4 µg C m^{-3} after 19 days inside the fertilized water, compared to out-patch values that showed a small increase (17.7 µg C m^{-3} on day 21; Figs. 5A, B). From day 7 on, the difference between in- and out-patch stations was significant ($P< 0.05$, unpaired t-test).

Foraminifera. Total foraminiferan abundance inside the patch showed a strong increase (7-fold) during EisenEx starting with abundances of 3,971 ind. m^{-3} on day T-2, ending with 27,900 ind. m^{-3} on day 21 (Fig. 4C, Tab. 1). Adult (mature) individuals made up for 91% (3,601 ind. m^{-3}) of total foraminiferans on day T-2, but decreased in relative importance until day 21 (62% of total abundance; 17,298 ind. m^{-3}). In juvenile foraminiferans very low abundances were observed until day 3 (0 to 1,012 ind. m^{-3}), but numbers increased strongly afterwards to a maximum of 21,310 ind. m^{-3} on day 7 (Fig. 4C). Until day 21, abundances decreased again to 10,602 ind. m^{-3}. Outside values of total, adult and juvenile foraminferan abundances were not significantly different from inside values (Fig. 4D). Relative abundance from adult organisms also decreased to 62% at the end of the experiment. The amount of empty foraminiferan tests was negligible during EisenEx.

Total foraminiferan biomass increased from 60.7 to 334.3 µg C m^{-3} inside the patch over the 21 days of the experiment (Tab. 1). Control waters showed no significant difference to fertilized waters with highest biomass of 244.3 µg C m^{-3} on day 21 (Tab. 1). Adult foraminiferans clearly dominated relative foraminiferan biomass during the course of the experiment, making up between 60 and 100% of total forminiferan carbon standing stock inside and outside the Fe-enriched patch.

Radiolaria. Inside the patch total abundances of living radiolarians ranged from 3,117 to 19,173 ind. m^{-3} and showed no constant trend in the course of the experiment. In adult polycystine radolarians, nassellarians were more abundant than spumellarians ranging very broadly from 711 to 6,347 ind. m^{-3} and showing no change over the 3 weeks of the experiment (T-2: 4,479 ind. m^{-3}; day 21: 5,913 ind. m^{-3}; Fig. 4E), which was not significant to out-patch stations. Spumellarian abundances increased during the 3 week experiment with values of 1,442 two days prior to fertilization and the highest value of 4,026 ind. m^{-3} on day 10. Compared to outside stations, there was a significant difference in numbers from day 16 ($P < 0.05$, unpaired t-test; Fig. 4F). Adult living phaeodarian radiolarians had the lowest abundances of all radiolarian classes, ranging from 0 to 2,981 ind. m^{-3} inside the patch with no trend being observed (Fig. 4E). Distribution of some taxa were patchy, with no specimens present at all in water sampled at some depths at some stations. Still numbers of living

phaeodarians were significantly different between in- and out-patch stations until day 16, caused by high abundances (2,054 to 3,752 ind. m^{-3}) outside the fertilized patch (Fig. 4F).

Juvenile radiolarian (polycystine and phaeodarian radiolarians) abundances increased from 3,357 (T-2) to a maximum of 11,178 ind. m^{-3} (day 16) inside the Fe-enriched patch, but dropped markedly at the last three stations (Fig. 4G). Outside the fertilized patch abundances of juvenile radiolarians stayed constant. Relative abundances of juvenile polycystine and phaeodarian radiolarians contributed significantly to total radiolarian numbers inside and outside the Fe-enriched patch (10-68% of abundance) showing an increase in relative importance in the course of the experiment in both waters.

Biomass of total radiolarians ranged between 9.2 and 69.2 µg C m^{-3} showing also no trend during EisenEx. Adult nassellarians made up for the largest fraction of adult polycystine biomass, ranging from 3.4 to 35.7 µg C m^{-3} and increasing very little within the 3 weeks of the experiment (T-2: 25.2 µg C m^{-3}; day 21: 33.3 µg C m^{-3}; Fig. 5C). The difference between in- and out-patch stations was not significant (Fig. 5D). Biomass of adult spumellarians increased from 8.0 (day T-2) to 20.9 µg C m^{-3} (day 16) showing a significant difference to out-patch stations at the end of the experiment (day 11 to 21; $P < 0.05$, unpaired t-test). Adult phaeodarian carbon standing stocks ranged from 0 to 12,1 µg C m^{-3} inside the Fe-enriched patch showing a slight increase from day T-2 (7.5 µg C m^{-3}) to day 21 (9.0 µg C m^{-3}; Fig. 5C). Outside the patch adult phaeodarian biomass declined during the 21 days of the experiment, but no significant difference to in patch stations could be observed (Fig. 5D).

Inside the fertilized patch juvenile radiolarian biomass went up from 1.2 (day T-2) to 4.1 µg C m^{-3} (day 21), compared to out-patch waters, where biomass remained stable (Tab. 1). Relative biomass of juvenile radiolarians doubled inside and outside the Fe-enriched patch in the course of the experiment, but made up for only a small fraction of total radiolarian biomass (1-15% of biomass).

Heliozoa. Abundances of heliozoans ranged from 0 to 6,744 ind. m^{-3} inside the patch showing a strong decline over the course of the experiment (T-2: 5,977 ind. m^{-3}; day 19:1,406 ind. m^{-3}; Fig. 4B; Tab 1). A decrease in abundances could also be observed outside the patch (1,481 ind. m^{-3} on day 21) resulting in no significant difference among in- and out-patch stations. Generally distributions of these organisms were also patchy (Fig. 4B; Tab. 1).

Heliozoan carbon standing stocks showed the same pattern as the abundances ranging from 0 to 37.3 µg C m^{-3} decreasing inside and outside the Fe-enriched patch during the 21 of the experiment. Biomass of heliozoans were not significantly different in fertilized and unfertilized waters (Figs. 5A and 5B).

Tintinnid ciliates

Abundances of larger tintinnid ciliates inside the patch declined ~two-fold from 97,743 two days prior to fertilization to 52,976 ind. m^{-3} after 19 days (Fig. 1A; Tab 1). Out-patch stations also evince a decrease with the lowest value of 26,200 ind. m^{-3} on day 21 and showed no significant difference to in-patch stations (Fig. 1B; Tab. 1). Biomass of larger tintinnid ciliates stayed more or less constant, starting with 288.2 µg C m^{-3} on day T-2 ending with 262.8 µg C m^{-3} after 21 days (Fig. 1C; Tab 1). In control waters, however, carbon standing stocks of larger tintinnids markedly decreased to a value of 97.8 µg C m^{-3} resulting in a significant difference inside and outside the Fe-enriched patch at the end of the experiment (from day 16 to day 21; Fig. 1D; Tab. 1).

Large thecate dinoflagellates

During EisenEx, larger thecate dinoflagellates almost doubled in numbers increasing from 40,926 (day T-2) to 74,017 ind. m^{-3} on day 21 (Fig. 1A; Tab. 1). Carbon standing stock of larger thecate dinoflagellates also increased in course of the experiment starting with 440.1 µg C m^{-3} on day T-2 and ending with 753.3 µg C m^{-3} after 3 weeks (Fig. 1C; Tab. 1). Outside the Fe-enriched patch abundances and biomass showed also an increase in numbers and standing stocks with 64,192 ind. m^{-3} and 674.8 µg C m^{-3} on day 17, respectively (Fig. 1B, 1D; Tab. 1). Nevertheless, both in abundances and biomass only a marginally significant difference between inside and outside station could be observed.

Vertical distribution

Abundances of several taxa differed between the upper 80m and from 80 to 150m. Total large protozoans were most abundant in the upper 80m and ranged from 44,870 to 489,760 ind. m^{-3} inside and from 48,180 to 450,942 ind. m^{-3} outside the fertilized patch ($P < 0.05$, Mann-Whitney U-test). Abundances decreased with depth and ranged from 4,222 to 139,711 ind. m^{-3} in-patch and 19,563 to 136,965 m^{-3} out-patch from 80 to 150m. Acantharians, large thecate dinoflagellates and tintinnid ciliates were more abundant in the upper 80m than below in- and outside the Fe-enriched patch ($P < 0.05$, Mann-Whitney U-test). Adult foraminiferans were more abundant in the upper 80m than below inside the Fe-enriched patch ($P < 0.05$, Mann-Whitney U-test), with no significant difference outside the patch. Heliozoans were more abundant from 80 to 150m than in the upper 80m in both in- and out-patch stations ($P < 0.05$,

Mann-Whitney U-test). Within the adult radiolarians, nassellarians, spumellarians and phaeodarians showed no significant difference within the two depth zones in the fertilized patch. In control waters, only nassellarians were more abundant from 80 to 150m than in the upper 80m ($P < 0.05$, Mann-Whitney U-test), spumellarians and phaeodarians again showed no significant difference between the two depth zones. Juvenile radiolarians were more abundant from 80 to 150m than in the upper 80m only in fertilized waters ($P < 0.05$, Mann-Whitney U-tests).

Tintinnid ciliates dominated large protozooplankton in the upper 80m (39-61% of abundance), followed by large thecate dinoflagellates (17-49%) and acantharians (6-8%). From 80 to 150m, tintinnids were still the largest fraction (27-59%); heliozoans accounted for 1-20%, followed by radiolarians (10-17%). Juvenile radiolarians accounted for 33-59% of total radiolarian abundance (Fig. 6A). In the upper 80m of control waters, 38-57% of the organisms were tintinnid ciliates. Large thecate dinoflagellates made up for 20-52% of relative abundance, followed by foraminiferans (3-9%). Juvenile radiolarians made up for 29-64% of total radiolarians. Tintinnids (17-47%) contributed significantly to larger protozoans from 80 to 150m with large thecate dinoflagellates (22-23%) and radiolarians (10-22%) making up for another important fraction (Fig. 6B). Juvenile foraminiferans numerically dominated total foraminiferans below 80m inside and outside the fertilized patch (64-73% and 51-60%, respectively).

Abundances of several of the groups were significantly correlated with depth, salinitiy and temperature and other biological parameters (Tab. 2). In the study area, temperature decreased with depth, whereas salinity increased with depth (Strass et al., 2001). Chl *a*, primary production and diatom biomass decreased significantly with depth (partial correlation, $P < 0.001$) inside and outside the study area, whereas phaeopigments decreased only inside the patch with depth ($P < 0.001$) and showed no significant correlation in outside waters. Inside the patch abundances of tintinnid ciliates, large thecate dinoflagellates, acantharians and foraminiferans were negatively correlated with depth, whereas heliozoans were positively correlated (Tab. 2). Tintinnid ciliates were also negatively correlated with temperature, whereas large thecate dinoflagellates, acantharians and foraminiferans showed a positive correlation. Radiolarians were negatively correlated with temperature at the beginning of the experiment, but in the course of the experiment a positive correlation with temperature could be observed (Fig. 7D). Large thecate dinoflagellates, acantharians and foraminiferans were positively correlated with chlorophyll *a*, diatoms, bacteria and small copepods; acantharians and foraminiferans were also positively correlated with heterotrophic dinoflagellates <50µm.

Tintinnid ciliate abundances showed a trend over the 3 weeks of the experiment with positive correlation to chlorophyll *a* and diatoms in the beginning and in the first half of the experiment, respectively, changing to a negative correlation in the course of the experiment (Figs. 7A and 7B). Whereas in heliozoans an increasing positive correlation with primary production could be observed (Fig. 7C). Outside fertilized waters, group abundances showed correlations with physical and chemical parameters, but were hardly correlated with biological parameters (Tab. 2).

Biomasses of total organisms ranged inside the patch from 155 to 4,584 µg C m^{-3} and in control waters from 192 to 3,013 µg C m^{-3} in the upper 80m, respectively, and were significantly higher than from 80m to 150m ($P < 0.05$, Mann-Whitney U-test). Below 80m, biomasses decreased and ranged from 19 to 725 µg C m^{-3} in-patch and from 93 to 1,070 µg C m^{-3} out-patch, respectively. Biomasses above and below 80m showed the same pattern as abundances for the different taxa as assessed with Mann-Whitney U test. Inside the fertilized patch, total organism biomass was dominated by larger thecate dinoflagellates and tintinnid ciliates making up for 71 to 89% in the upper 80m, followed by foraminiferans (8-19% of biomass) and than by radiolarians (1-5%). Both juvenile foraminiferans and radiolarians ranged from 8-14% and 6-22% of total foraminiferan and radiolarian carbon standing stock, respectively. From 80 to 150m foraminiferans accounted for approx. 40% of larger protozoan biomass, followed by larger thecate dinoflagellates (16-18%) and by radiolarians (12-19%). Below 80m, juvenile radiolarians accounted for only 4-7% of total radiolarian biomass (Fig. 6C).

Biomass in the upper 80m was also dominated by tintinnids and larger thecate dinoflagellates outside the Fe-enriched patch (74 to 91% of biomass), followed by foraminiferans (7-29%) and than by radiolarians (1-6%). Juveniles of foraminiferans and radiolarians contributed only little to total taxa biomass (5-12% and 4-20%, respectively). Biomass from 80 to 150m was dominated by larger thecate dinoflagellates and foraminiferans (31-37% and 25-37%, respectively), followed by radiolarians (10-15%). Again juvenile foraminiferans and radiolarians contributed only marginally to total foraminiferan and radiolarian carbon (Fig. 6D).

Taxonomic groups

Sarcodines. Within the foraminifera, *Turborotalita quinqueloba* was the most abundant species making up between 21 and 88% of the adult foraminiferans at all stations and

increasing in importance inside the patch over the 3 weeks of the experiment (T-2: 41%; day 21: 59%). *Globigerina bulloides* was also numerous and accounted for maximal 60% of adult forminiferans at all stations.

Among the radiolarians, nassellarians made up for max. 90% of the adult radiolarians at all stations with *Cycladophora bicornis* being the most numerous species (max. 60% of abundance). *Spongotrochus glacialis* and *Protocystis swirei* were the dominant species of spumellarians and phaeodarians respectively, making up for a maximum 100% of total numbers of these taxonomic groups.

Sticholonche zanclea was the predominant heliozoan specimens, but we have described them as *Sticholonche* spp. because some of the individuals may have been another species of *Sticholonche* found by Takahashi and Ling (1980). These authors have questioned whether other reports have included data on species other than *Sticholonche zanclea*.

Tintinnid ciliates. Within the larger tintinnid ciliates, *Codonellopsis pusilla* and *Cymatocylis* spp. (including *C. antarctica*, *C. calyciformis* and *C. vanhoffeni*) contributed significantly to tintinnid numbers at all stations (32-83%), followed by *Amphorides* spp (5-28%; Fig. 8A). Other tintinnid ciliates observed were *Salpingella* spp., *Acanthostomella norvegica*, *Protorhabdonella* spp. and a few other specimens or genera that were very rare in the samples (e.g. *Steenstrupiella* spp. and *Cymatocylis drygalskii*). They contributed up to 56% to total larger tintinnid ciliate at all stations (Fig. 8A). Especially the relative amount of *Amphorides* spp. and *Cymatocylis* spp. changed in the course of the experiment showing in *Amphorides* spp. a decrease inside the Fe-enriched patch (T-2: 13%; day 21: 8%) and an increase in control waters (26% after 3 weeks); in *Cymatocylis* spp. an increase in- and outside the fertilized patch (T-2:13%; day 19: 30% and day 21: 31%, respectively; Figs. 8A and B).

Dinoflagellates. Large thecate dinoflagellates were predominantly *Protoperidinium* sp(p). and *Dinophysis spp.* (23-78% and 20-70%, respectively). Both increased in relative importance within the 3 weeks of experiment inside the patch and stayed fairly constant in unfertilized waters (Figs. 8C and D). Other large thecate dinoflagellates including *Achradina* spp. and *Podolampas* spp. (most likely *Podolampas antartica*) contributed only little to total larger thecate dinoflagellate composition (2-26%).

Agglutination

Codonellopsis pusilla had agglutinated predominantly coccoliths of *Emiliania huxleyi*, which were found very often in the samples. Very occasionally centric diatoms of the genus *Thalassiosira* sp(p). and *Thalassionema nitzschoides* were also present on loricae below the collar (Figs. 9A and B). *Codonellopsis gaussi* had agglutinated predominantly diatom fragments of these two species.

Abundances of empty tintinnid loricae and radiolarian skeletons

Integrated standing stocks of empty tintinnid loricae ranged between 1.9 $\times 10^6$ and 1.1 $\times 10^8$ ind. m^{-2} and increased from 46 $\times 10^6$ (T-2) to 100 $\times 10^6$ ind. m^{-2} (day 21) inside the Fe-enriched patch. Outside the fertilized patch empty loricae first increased in abundance, but declined from day 9 with a value of 45 $\times 10^6$ ind. m^{-2} after 3 weeks (Figs. 10A and B). There was a significant difference between in- and out-patch stations from day 7 ($P < 0.05$; unpaired t-test). Percentage of empty tintinnid loricae ranged 53 to 95% of full plus empty loricae abundances depending on depth and species and increased in total from 76% on day T-2 to 89% after 21 days during EisenEx (Figs. 11A and B). Inside the patch *Acanthostomella norvegica* and *Cymatocylis* spp. accounted numerically for 75% (84% out-patch) of empty loricae after 3 weeks, with *A. norvegica* showing an increase from day T-2 (35% of abundance) to day 21 (46%) and *Cymatocylis* spp. a decline (T-2: 34%; day 21: 19%) in relative numbers (Figs. 10C and D). Abundances of empty tintinnid loricae from individual depth were significantly correlated with abundances of live tintinnids, small copepods and total sarcodines (partial correlation, $P < 0.05$) inside the patch. In some species a significant difference was found in the relative proportion of empty loricae between in- and out-patch stations over the course of the experiment (Figs. 10C and D). Vertical distribution of empty loricae showed a distinct trend in the course of the experiment with abundance maxima in upper 20-40m of the mixed layer to a homogenous vertical distribution throughout the mixed layer after day 11.

Total integrated standing stocks of dead radiolarian skeletons ranged between 1.8 $\times 10^5$ and 4.1 $\times 10^5$ ind. m^{-2} and constituted on average 18% of respective totals (dead plus alive individuals). At individual depths, abundances ranged from 0 to 5,651 empty radiolarian skeletons m^{-3}. They belonged mainly to juvenile nassellarians and spumellarians which could

not be identified further. Other skeletons observed were from the species *Antarctissa strelkovi* and *Spongotrochus glacialis*.

Discussion

The addition of Fe created an almost 950 km² patch in the Polar Frontal Zone of the Southern Ocean in austral spring had a considerable impact on some important taxa of larger protozoans, which showed a clear response to the iron-induced diatom bloom. There is evidence that some sarcodines are able to profit from elevated phytoplankton biomass by an increase in abundances, despite their expected longer generation times in Southern Ocean waters. The lack of significant increases in biomass of the microbial components of the food web (Arrieta *et al*., submitted; Veldhuis, unpubl. data; Henjes and Assmy, to be submitted) suggest that a tight coupling between prey and predators was maintained during this iron fertilization experiment.

Larger protozooplankton abundances and biomass

Larger protozooplankton are a common component of the microzooplankton in many marine environments (Beers *et al*., 1975, 1982; Bishop *et al*, 1977, 1978, 1980). However, their abundance and biomass has generally been underestimated in many studies. Understanding the patterns of larger protozoan abundance is a necessary initial step in determining the ecological role of these protozoa.

Maximal initial values of abundance and biomass of larger protozooplankton found during EisenEx (286,181 ind. m^{-3} and 1,890 µg C m^{-3}, respectively, in 40 m depth) are significantly higher than results from other studies in the Southern Ocean and are one of the highest yet reported for larger protozooplankton (>50µm) in the upper 150m of the Southern Ocean. Klaas (2001) found maximal values from 10,930 ind. m^{-3} (452 µg C m^{-3}) of larger (>64µm) protozoans in the upper 100m in the Polar Frontal region during austral spring. During austral winter, Gowing and Garrison (1992) observed abundance maximum of 12,091 ind. m^{-3} (217 µg C m^{-3}) in the upper 125m of the Weddell/Scotia Seas whereas Nöthig and Gowing (1991) found very low abundances (max. 374 ind. m^{-3}) and carbon standing stocks (max. 3 µg C m^{-3}) in Weddell Sea during winter. Abundances and biomass of larger protozooplankton found during late austral summer in the Weddell-Scotia Confluence by Alder and Boltovskoy (1993) are within the range of values found within this study. Besides temporal and spatial

variability, differences in methods of sampling and processing seem to have a major influence in determining abundances and biomass of larger protozooplankton. Vertical and horizontal variability in the five studies ranged over a few orders of magnitude. Furthermore, counts of living sarcodines taken mainly from non-metered net tows over the upper 200m of the water column show similar seasonal and vertical variability in Southern Ocean waters (Peters, 1929). If larger protozoans are not always adequately sampled by nets, as has been shown for sarcodines and larger dinoflagellates by Michaels (1988) and Stoecker *et al*. (1996), the significantly higher abundances found in the present study may be due in part to the use of water bottles for collecting and the gentle concentration method. The methological differences in sampling can cause these organisms to be underestimated by plankton nets in same taxa an order of magnitude to the Niskin-bottle-calculated densities (Michaels, 1988). Therefore results of this study support the assumption that larger protozooplankton can be seasonally and spatially abundant and thus, might have a larger impact on biological processes in the upper layers of the Southern Ocean than previously thought.

The larger protozoan taxa accounted for a minor fraction (maximum 2%) of total protozooplankton and small zooplankton abundance in the upper 80m and below 80m during EisenEx (Henjes and Assmy, to be submitted; Krägefsky *et al*., to be submitted). However inside the patch, large protozoan biomass was relatively high in the upper 80m and in the layers below 80m, representing up to 24 and 54% of large protozoan and small metazoan [of the same size range as larger protozoans] biomass, respectively, showing that large protozoans comprised a significant fraction during the experiment. At all in patch station foraminiferans contributed significantly to larger protozoan carbon standing stocks below 80m (Figs. 6C and D). Despite the fact that feeding and metabolic rates of these organisms have not been measured during the experiment, these results suggest that foraminiferans might had an important influence on biological processes occurring below the mixed layer during EisenEx.

The study on different size groups of microprotozooplankton counted in small volumes of water samples for the upper 80m of the water column during EisenEx (Henjes and Assmy, to be submitted) showed that biomass of athecate dinoflagellates and aloricate ciliates (>50µm) contributed significantly to protozoan standing stock during the experiment. At the end of EisenEx, biomass of athecate dinoflagellates and aloricate ciliates (>50µm) were comparable to the values of larger protozoans observed during this study. Gowing and Garrison (1992) found in their study on the larger protozoan community including athecate dinoflagellates and aloricate ciliates in the Weddell-Scotia Confluence during austral winter, that aloricate ciliates

accounted for a mean of 9-32% of total larger (>50µm) protozoan and 4-40% of biomass. These results suggest athecate dinoflagellates and aloricate ciliates should have accounted significantly to total larger (>50µm) protozoan community in the upper 80m of the water column during EisenEx.

Response of the larger protozooplankton assemblage

The larger protozoan assemblage composition showed marked differences in response to the fertilized plankton patch both in temporal development and between depth intervals. In the following discussion, possible factors influencing seasonality and response pattern to the induced phytoplankton bloom of the different taxa studied and their impact on biological and biogeochemical processes will be discussed in the light of results from this and previous studies on abundance, biomass and vertical zonation of larger protozoans in the Southern Ocean.

Sarcodines

Acantharians. The max. initial abundances of acantharians (10,860 ind. m^{-3} in 10m depth) found during this study were one of the highest ever observed in Southern Ocean waters and the first documented in the Polar Frontal Zone during austral spring. The maximum fall abundance in the Weddell Sea was 2,758 m^{-3} (Gowing, 1989) and winter abundances did not exceed 342 m^{-3} in the upper 85 m (Gowing and Garrison, 1992). In the southeast Atlantic, Bishop *et al*. (1978) observed max. concentration of ~33,000 ind. m^{-3} in the surface layer during summer. Acantharians abundances in the upper 200 m in lower latitude waters range from <1 to max. 30,000 m^{-3} (reviewed by Caron and Swanberg, 1990; Bishop *et al*., 1978) and Beers *et al*. (1975, 1982) reported consistently high values of 10,000-40,000 acantharians m^{-3} in the upper 200 m of the North Pacific Central Gyre.

Furthermore, acantharians increased in abundance and biomass by a factor of three in fertilized waters, whereas in unfertilized waters abundance and biomass changed only slightly. There are two possible explanations for this marked increase inside the patch. First, acantharians responded to favourable food concentrations during EisenEx by enhanced reproduction and thus growth of population size. The positive correlations between acantharian abundance and various food items (Tab. 2 and empty tintinnid loricae) indicate that acantharians were actively feeding during EisenEx. Studies by Trégouboff (1957), Caron and Swanberg (1990) and Swanberg and Caron (1991) support these findings showing that

acantharians feed predominantly on tintinnids and other ciliates, but also on bacteria, diatoms, dinoflagellates, and even small metazoans in lower-latitude waters. However, diets of Antarctic species have yet to be determined.

Secondly, acantharians contained intracellular symbiotic eukaryotic algae, which could have enhanced productivity due to iron addition and thus contributed significantly to the nutrition and growth of their host via the translocation of symbiont-derived organic compounds or via direct symbiont digestion as proposed by Caron *et al*. (1995). During EisenEx, symbiotic algae of unknown phylogeny were observed in several by epifluorescence microscopy examined specimens. This is a common phenomenon in many species of planktonic acantharians found in lower-latitude oligotrophic waters (Michaels, 1988a; Michaels, 1991; Caron *et al*., 1995) and in the Southern Ocean (Gowing and Garrison, 1992). In a seasonal study in the North Pacific, Michaels (1991) found that approximately half of the acantharian specimens in the upper 150 m investigated with epifluorescence microscopy contained symbionts in their cytoplasmic vacuoles and that these symbionts could meet 50-100% of their host`s respiratory requirements. Michaels (1988a) also proposed that the energy substrate provided by the symbiont might allow the host to use prey biomass more efficiently, indicating that a combination of active feeding and contribution of organic nutrients via symbiont photosynthesis could have applied during EisenEx. Estimations on the carbon fixation ability of acantharian symbionts show that they are able to account for up to 20% or more of the total carbon fixed in the upper euphotic zone of lower latitude oligotrophic waters when acantharian abundances are high (30,000 m^{-3}; Michaels, 1988a). During EisenEx, acantharians were very abundant (up to 35,000 m^{-3} depending on station and depth) indicating that the symbionts of these organisms could have contributed to some extent to total primary production. Acantharians peaked in the upper 20m of the water column at most of the stations. Deeper maxima (60m) were only found after storm events, supporting the findings of Michaels (1988a) that the need for light for symbionts might control their position in the water column and imply a preference for surface waters during calmer weather conditions. However, precise data on the percentage of acantharians containing symbiotic algae and measurements of primary production rates of these symbionts are lacking from this study to clearly validate the effect of iron addition on the symbionts and thus the impact on acantharian population size and on total primary productivity. Furthermore, life-spans of sarcodines (incl. acantharians) are reported to be in the order of several days to several weeks (reviewed by Caron and Swanberg, 1990), which would be too long for their numbers to grow on such short time scales (3 weeks), in response to enhanced availability of adequately-sized

food and/or nutrients provided by highly productive symbionts. In contrast, nutrition appears to affect growth rates in many sarcodines (Be *et al*., 1981; Bjørklund and Swanberg, 1987) and the high flux rates of skeletal structures that have been observed indicate that sarcodines must be highly productive and that a relatively rapid turn over of the living populations must be taking place (Caron and Swanberg, 1990). Furthermore, the life cycle of acantharians is far from being completely understood at present.

Even if the reasons behind the observed response of acantharians to an iron-enriched water mass still needs to be further investigated, the potential and importance to study the biology of these organisms in the context of iron-fertilization experiments is without doubt. This might be particularly interesting for biogeochemistry since acantharians are discussed to be involved in the distribution patterns and fluxes of Sr and Ba (Bernstein *et al*, 1987, 1992, 1998; Dymond and Collier, 1996) being components of their celestite tests. Biogeochemical cycling especially of Ba has been stressed by the relationship between the marine chemistry of this element and ocean productivity which has led to the use of solid-phase Ba compound (barite) as a proxy for both modern and ancient high productivity regimes (Dehairs *et al*., 1991, 1992, 1997; Dymond *et al*., 1992; Francois *et al*., 1995; Dymond and Collier, 1996). However, Ba removal mechanism from surface seawater is poorly understood, but barite ($BaSO_4$) is thought to be the primary Ba carrier phase (Bishop, 1988; Dehairs *et al*., 1980; Stroobants *et al*., 1991). One process driving the biological removal and transport of Ba from surface to deep water has been proposed as the "celestite model" (Esser and Volpe, 2002). Here barite, which is undersaturated in surface ocean water, precipitates in microenvironments requiring an additional source of Ba and sulphate which is provided by the dissolution of acantharian celestite tests (Bernstein *et al*., 1998; Dymond and Collier, 1996). The primary findings that support this model are the observed correlation between surface water Sr depletion and elevated sediment-trap acantharian abundance in the North Pacific (Bernstein *et al*., 1987) and the preferential uptake of Ba (in relation to Sr) by acantharians (Bernstein *et al*., 1998). Further iron-fertilization experiments are intended to test these findings in Southern Ocean waters.

Foraminiferans. Maximal initial abundances of foraminiferans during this study (8,406 ind. m^{-3} in 80m depth) are comparable with values found by Klaas (2001) during austral spring north of the Polar Front (max. 6,118 ind. m^{-3} in 50-100 depth interval). Our findings support the thesis of Klaas (2001) that foraminiferan abundances and seasonality in the Southern Ocean appear to be primarily determined by productivity with lowest values during the winter

and an increase in abundance of both smaller (>20µm) and larger individuals (>100µm) during spring, summer and fall. The species *Turborotalita quinqueloba*, which was the most abundant specie during EisenEx, is known to have their highest abundances even during spring bloom conditions north of the Polar Front (reviewed by Kemle-von Mücke and Hemleben, 1999). High abundances can also be found during winter (max. 13,000 ind. m^{-3}; Dieckmann *et al*., 1991) in the upper 60m of the water column underlying new ice, and are also always associated with high phytoplankton stocks. Significantly higher densities of foraminiferans in the same size range as considered during this study were observed in the upper 100 m of the southeast Atlantic Ocean, reaching up to 44,000 ind. m^{-3} (Bishop *et al*., 1978).

Foraminiferan abundances and standing stocks increased significantly during EisenEx and were positively correlated with phytoplankton, bacteria and small dinoflagellates and negatively correlated with depth. Studies by Gowing (1989) and Gowing and Garrison (1992) during austral winter and fall in the Weddell Sea show that foraminiferans tend to feed predominantly on diatoms and other algal cells, but particularly spinose species are known to consume mainly heterotrophic prey (Hemleben *et al.*, 1989). Foraminiferans were also positively correlated to phaeopigments (an indicator of algal detritus) suggesting that they could be feeding on sinking detritus with associated plant and animal cells. During EisenEx, foraminiferans were significantly more abundant above 80m than below inside the patch and showed clear peak abundances in the chlorophyll maximum in the course of the experiment. Thus, results during this study support the findings from lower latitude waters where foraminiferans tend to concentrate in the chlorophyll maximum developed mostly at the bottom of the euphotic zone. Moreover, our data could be an indication that growth rates and productivity might be positively influenced by feeding frequency as shown by Bè *et al*. (1981) in culture experiments. The positive effect on temporal development of foraminferan numbers by symbionts could only be applied for one specie (*T. quinqueloba*) since the other species found during this study where either non-spinose or known to be devoid of symbionts (*G. bulloides*; Kemle-von Mücke and Hemleben, 1999). However, the same implication concerning the length of generation times (between 4 days and 4 weeks) as discussed for the acantharians apply to foraminiferans (Bè *et al*., 1977; Hemleben *et al*., 1989). Furthermore, abundances and standing stocks of foraminiferans also showed an increase outside the patch, although phytoplankton stocks increased only slightly in unfertilized waters.

At the first week of the experiment, we found indications that the abundance of some species of foraminiferans might be influenced by the phase of the moon and thus might be the driving

cause for the forminiferal development during EisenEx. To analyze the trends of the juvenile fraction, residuals were calculated as recommended by Bijma *et al.* (1990). Plotting these data, we found that about three to five days after the full moon (on day 3 after fertilization) inside and outside the fertilized patch, a significant increase in juvenile specimen were occurring with maximum abundances on the fourth day after the full moon event, but decreasing in numbers thereafter (Fig. 4C and D). Moreover, abundances of adult (mature) foraminiferans showed a clear maxima in deeper waters (100m) in the station after full moon. Previous observations of several authors has clearly demonstrated that at least some species have a synodic lunar periodicity in their reproduction cycle with gametogenesis occurring mainly within a time span of three to seven days deeper in the water column (> 60m) after full moon (Spindler, *et al*., 1979; Hemleben *et al*., 1989; Bijma, *et al.*, 1990). However, there are two constraints making it difficult to explain the trend during EisenEx as a lunar reproduction cycle. It has only been demonstrated in one spinose specie found during this study (*G. bulloides*) and which is suggested to reproduce above 60m depth in the first week after new moon as found in the eastern North Atlantic (Schiebel *et al*., 1997). Marchant (1995), however, suggested from his sediment trap investigations off Chile that *G. bulloides* reproduces twice a month, which could indicate that *G. bulloides* did undergo an additional occurrence of mass reproduction around full moon during this study.

Within the first seven days after full moon adult (mature) individuals of investigated species usually reach their minimum abundances with complete absence on the fifth day after full moon as found by Bijma *et al*. (1990). This is to some extend in contradiction with findings during EisenEx where abundances of adult (mature) individuals were still present in the mixed layer after full moon. However, they decreased in the week following full moon and had their minimum on day five after the full moon event. Furthermore, Bijma *et al*. (1990) reported from his species that not necessarily all mature individuals go into reproduction phase after full moon. In addition it could be possible that some of the species found during EisenEx did not undergo lunar dependent reproduction at all since juvenile specimen were only identified on the level of class. Hence the detailed study of mature as well as juvenile forms on species level is necessary during future fertilization experiments lasting over more than one lunar cycle to get further insights on the possible lunar periodicity in Southern Ocean foraminifera.

Radiolarians. It is difficult to form generalizations concerning the abundance and biomass patterns of radiolarian since they had the smallest total number of individuals enumerated in

this study. Undoubtedly, the high variability in the course of the experiment associated with these estimates is partly due to the low total number of radiolarians observed. Nevertheless the max initial abundances (11,116 ind. m^{-3} in 80m depth) that we found of adult radiolarians are greater than those reported in most studies from the Southern Ocean, although they are within the overall range. In winter, Gowing and Garrison (1992) reported max. values of 2,663 ind. m^{-3} in the Weddell and Scotia Sea. At the Polar Front in spring, Klaas (2001) found max. abundances of 3,803 m^{-3} in the 50-100m depth range and in austral fall Gowing (1989) observed max. densities of 4,647 m^{-3} in 150m depth. In the South Eastern Atlantic, however, abundances of radiolarians can reach max. values of >10,000 m^{-3} (Bishop *et al*., 1978). During EisenEx adult polycystine and phaeodarian radiolarians showed no clear trend in abundances or standing stocks in the course of the experiment. However the increase in abundances of juvenile individuals inside the patch could be an indication that reproduction was enhanced. No relationship was found between both classes and abiotic parameters inside the patch. The positive coherence between radiolarians and empty tintinnid loricae and small dinoflagellates (<50µm), respectively, gives indication that they were consuming tintinnids and small dinoflagellates during this study. Tintinnids are known to be the major nutrition of radiolarians in lower latitude waters (Swanberg and Caron, 1991), but both empty tintinnid loricae and dinoflagellates were also found in food vacuoles of radiolarians in the Southern Ocean (Gowing, 1989; Nöthig and Gowing, 1991). Besides food availability, it has been suggested that temperature is more important in determining polycystine but also phaeodarian vertical distribution patterns in the Southern Ocean with higher abundances generally associated with warmer water masses (Gowing and Garrison, 1991; Boltovskoy and Alder, 1992a). A study by Abelmann and Gowing (1996), however, do not support these conclusions for the larger (>55µm) size fraction of polycystine radiolarians. During EisenEx, neither polycystine nor phaeodarian radiolarians showed a relationship with temperature or were even negatively correlated in unfertilized waters. However, prey abundance cannot be the only factor defining radiolarian vertical distribution since they were not significantly more abundant in the upper 80m of the water column where large phytoplankton and heterotroph standing stocks were highest. Living phaeodarians, nassellarians and spumellarians predominated at most depths with no clear vertical distribution of empty radiolarian skeletons in the 150m sampled. This is in good agreement with findings from other studies in the Polar Front during summer (Abelmann and Gowing, 1996) and spring (Klaas, 2001) showing significantly higher values at depths below 100-200m.

Heliozoans. The heliozoan genera *Sticholonche* spp. have rarely been investigated in the Southern Ocean. Only three studies mention their distribution and abundances in the western Weddell Sea, Weddell-Scotia-Confluence (WSC) and west of the Antartic Pensinsula, in autumn (max. 4,215 ind. m^{-3} in 100m depth; Gowing, 1989), winter (max. 2,779 ind. m^{-3} in the 115/125 depth range; Gowing and Garrison, 1992) and north of the Polar Front in spring (max. 1,512 ind. m^{-3} in the 50-100m depth interval; Klaas, 2001) with relatively low abundances [which probably is an underestimation due to the use of plankton nets] of *Sticholonche* spp. found below 80m depth. Max. initial abundances found during EisenEx (16,673 in. m^{-3} in 80m) were higher than values found by Klaas (2001) giving indications that abundances of *Sticholonche* spp. show seasonality with lower values during winter and an increase in abundances from early spring to fall. Furthermore, there is some evidence during EisenEx that distribution of *Sticholonche* spp. could be linked to elevated primary production as indicated by Klaas (2001). No correlation, however, was found between *Sticholonche* spp. abundance and sarcodine and metazooplankton standing stocks, respectively. During this study abundances were also significantly more abundant below the pycnocline and showing a significant correlation with depth inside the patch. In contrast to these findings, abundances of *Sticholonche* spp. in lower latitudes, e.g. in the equatorial Pacific, were markedly higher and peaked in the pycnocline where total and nanoplankton chlorophyll *a* concentrations also peaked at the three sites sampled (Takahashi and Ling, 1980). In the East China Sea abundances peaked in summer more than an order of magnitude higher than found during this study, and were most abundant in the upper 20m correlated with high water temperatures and high phytoplankton concentrations (Tan *et al*., 1978). At present it is not clear why Subantarctic populations of *Sticholonche* spp. in spring (this study; Klaas 2001) as well as Antarctic populations in autumn and winter (Gowing, 1989; Gowing and Garrison, 1991) are found deeper in the water column than populations from lower latitudes. Moreover, there is no straight-forward explanation for the four-fold decline of *Sticholonche* spp. abundance and biomass in the course of the experiment. In the Weddell Sea, Gowing (1989) found that *Sticholonche* spp. was consumed by *Phaeodina antarctica* (Phaeodaria) and Hopkins and Torres (1989) observed *Salpa thompsoni* and calanoid copepods feeding on *Sticholonche* spp. suggesting that grazing pressure of other larger sarcodines and metazoans could be a possible explanation for the strong decrease.

Tintinnid ciliates

During EisenEx, abundances of larger tintinnid ciliates declined by a factor of two; however tintinnid carbon standing stocks remained stable due to changes in species relative importance. Only the largest species predominantly *Cymatocylis vanhoffeni* and *Cy. calyciformis* showed an increase in numbers during the experiment. Empty tintinnid loricae, however, increased two-fold in the course of the experiment making up for 89% of empty + live tintinnid ciliates. Two possible explanation can be taken into account for this development: a) The high percentage of empty tintinnid loricae found during this study are comparable with values observed by Nöthig and Gowing (1991) and Klaas (2001) who both counted multi net samples. Klaas (2001) concluded that her high percentage of empty loricae was possibly due to the use of nets for sampling, causing the tintinnids to detach from the loricae by mechanical disturbance. This assumption was supported by studies of Gowing and Garrison (1992) finding significantly lower percentage of empty loricae in the WSC during winter by counting reverse filtered water samples (23-85% depending on depth and species) and by the positive correlation between tintinnid ciliates and empty tintinnid loricae found during this study. Thus, the high percentage of empty tintinnid loricae in this study could be an artifact caused by the concentration procedure. Furthermore, it could be likely that abundances, biomasses and vertical distribution patterns of live tintinnid ciliates are not properly described with the concentration method used during this study. As a consequence, living tintinnids possibly dominated larger protozooplankton standing stocks in the upper layers (0-150m) during EisenEx and at the Polar Front in general. However, this is in contrast to observations of live tintinnids and empty tintinnid loricae counted from sedimented water samples with no mechanical disturbance occurring. Comparison of the temporal trend of both data sets showed no significant difference (linear regression analysis, $P > 0.05$, $R^2 = 0.921$) in the course of the experiment suggesting that the concentration procedure did not seem to affect the trends of live tintinnids and empty tintinnid loricae, respectively. To what extend preservation of samples, which can provoke detachment of the cell from the lorica as indicated by Paranjape and Gold (1982), had an influence on abundances during this study cannot be quantified.

b) The presence of abundant empty tintinnid loricae during EisenEx raises the question of whether this can be interpreted as non-destructive mortality. In this context we looked at the morphology of naked ciliates in concentrated samples to see if they looked identical to tintinnids with a lorica, and did not see convincing evidence of this. Thus, empty tintinnid

loricae could be an argument of mortality. Furthermore, the positive correlation between empty tintinnid loricae and small copepods (mainly *Oithona*) and frequent observations of empty tintinnid loricae of *Cymatocylis antarctica* and *Acanthastomella norvegica* in copepod feces support findings by Atkinson (1996) that copepod predation on ciliates including tintinnids can account for 57% of their daily production under low phytoplankton conditions (~0.7 mg m^{-3}) in the Polar Front. Especially the abundant cyclopoid copepod *Oithona* spp. (nauplii, late copepodite stages and adults) is known to feed frequently on tintinnid ciliates, but its grazing impact on tintinnids ciliates appears to be modest in the Polar Frontal Zone (up to 1.7% d^{-1} of tintinnid standing stocks; Lonsdale *et al*., 2000). However, Lonsdale *et al*. (2000) used concentrations of *Oithona* spp. averaging 1,864 m^{-3} which are more than an order of magnitude below *Oithona* spp. abundances found during this study (average over the experiment inside the patch of 24,688 m^{-3} of copepod nauplii, copepodites and small adults; Henjes and Assmy, to be submitted). Taking the clearance rates observed by Lonsdale *et al*. (2000) into account for copepodites and adults and nauplii, respectively, we would estimate a predation impact of *Oithona* spp. up to 22.1% d^{-1} on tintinnid ciliates stocks. This would suggest that *Oithona* spp. could have been a pivotal factor responsible for the decline of tintinnid standing stocks during EisenEx. Other factors could be grazing pressure by large sarcodines (particularly acantharians) found by Caron and Swanberg (1991) in the lower-latitude waters and by Gowing (1989), Nöthig and Gowing (1991) in the Weddell Sea during autumn and winter, respectively. Parasitism by dinoflagellates is also known to have a significant impact on population dynamics of some tintinnid species leaving unharmed empty loricae after the ciliate has died or has been forced from its lorica (Coats, 1988; Coats *et al*., 1994). But to our knowledge this has not been observed in Southern Ocean waters so far. To what extend these two factors had an impact on tintinnid population decline, during this study, however, remains unknown. The fact that *Acanthostomella norvegica* and *Cymatocylis* spp. accounted for the highest relative amount of empty tintinnid loricae (Fig. 10B) indicates that they were preferentially grazed due to their optimal size. However, relative numbers of *Cymatocylis* spp. empty loricae decreased suggesting that grazing pressure on this genera decreased in the course of EisenEx. In general, the decline of live tintinnids by a factor of two and the doubling of empty tintinnid loricae abundance suggests that grazing pressure entirely compensated tintinnid population growth during the experiment.

During this study, tintinnid abundances were significantly higher in the upper 80m of the water column and negatively correlated with depth. Peak abundances often occurred between 10 and 60 m depth. Below 80 m depth tintinnid concentrations decreased significantly and

only a few individuals m^{-3} were found in 150 m depth. This distribution pattern is consistent with the spring distribution patterns of Klaas (2001), who also found highest abundances of tintinnid ciliates between 50 and 100 m of the water column in the Polar Frontal Zone. Our results therefore, support the assumption of Klaas (2001) that larger tintinnids (>50µm) are primarily surface-dwelling organisms. However, their distribution within the mixed layer inside the patch seemed to be mainly dependent on food concentrations which appeared to be particularly nanophytoplankton and partly diatoms at least in the first half of the experiment (Fig. 7B). These findings are in good agreement with observations of Gowing (1989) and Gowing and Garrison (1992) who found that food vacuoles of autumn and winter tintinnids also contained diatoms. Froneman and Perissinotto (1996) found in their study that ciliates including tintinnids mainly feed on pico- and nanophytoplankton size fractions. The fact that dinoflagellates are also a frequent food source of tintinnid ciliates and can thus regulate their distribution patterns, as suggested by Stoecker *et al*. (1984) and Alder and Boltovskoy (1991), who reported very high coherence between spatial distribution of these two groups, can be supported by the significant correlation between tintinnids and dinoflagellates <50µm outside the patch during this study. Inside the patch, however, these two groups were negatively correlated which is additional support that phytoplankton might have been the preferred food source in fertilized waters.

Both *Codonellopsis pusilla* and *Cd. gaussi* had agglutinated particles to their loricae (Figs. 9A and B). Takahashi and Ling (1984) and Winter *et al*. (1986) have indicated that tintinnids would be more likely to agglutinate specific particles such as single types of coccoliths after ingesting them as food. Wasik *et al*. (1994) and Wasik (1998), however, suggest that certain diatoms are actively selected and agglutinated by particular tintinnid species including *Cd. gaussi*. Since the abundant specie *Cd. pusilla* exclusively agglutinated coccoliths of the species *E. huxleyi* during EisenEx despite a high concentration of diatom fragments in the water column, it is most likely that *Cd. pusilla* was actively feeding on *E. huxleyi* from its immediate vicinity and incorporated the coccoliths into its loricae. To what extend feeding of *Cd. pusilla* had an impact on *E. huxleyi* population size and whether the agglutinated tintinnid population may play a major role in the transportation of coccoliths to deep ocean waters and sediments due to fast sinking rates of empty loricae as indicated by Winter *et al*. (1986), still needs to be investigated. The agglutination of diatom fragments of two relatively rare diatom species by *Cd. gaussi* suggests that this organism rather selects specific particles for lorica building than taking them randomly out of the water column.

However, it can be ruled out that *Cd. pusilla* and *Cd. gaussi* built their lorica near the sediment-water interface and than moved up into surface waters as hypothesized by Gold and Morales (1976) for species of the family *Codonellopsidae*, because mineral particles were completely lacking in the loricae observed and the sea floor depth exceeded 3500 m at most stations.

Larger thecate dinoflagellates

Results from this and previous studies indicate that larger thecate dinoflagellate abundance show strong seasonality with low values during winter (max. 160 m^{-3}, Nöthig and Gowing, 1991; max. 599 m^{-3} Gowing and Garrison, 1992) and an increase in abundances from spring to summer and fall (average 40,926 m^{-3}, this study; average 109,000 m^{-3}, Alder and Boltovskoy, 1991) in Southern Ocean waters. Moreover, larger thecate dinoflagellate abundance nearly doubled during Eisenex and were significantly correlated with chlorophyll *a*, primary productivity and diatom abundance indicating that these organisms were able to respond to increasing productivity and diatom standing stocks during the Fe-induced bloom and suggesting that overall distribution and seasonal patterns are possibly related to productivity and prey concentration. Depth distribution with significantly higher abundances of larger thecate dinoflagellates in the upper 80m also support these conclusions. Net thecate dinoflagellate assemblage appears mainly be dominated by species of the genus *Protoperidinium* (Nöthig and Gowing, 1991; Gowing and Garrison, 1992; Klaas, 2001), which is also in good agreement with findings during this study. This genus feeds by extracellularly digesting algal cells predominantly diatoms (Gaines and Taylor, 1984; Jacobson and Anderson, 1986; Buskey, 1997). Generation times of heterotrophic dinoflagellates are on the order of days (Archer *et al*., 1996) which allows them to promptly react to favourable food concentrations. However, Klaas (2001) found during her study in austral spring that larger thecate dinoflagellates did not show a very significant response to an increase in phytoplankton standing stocks (~2mg chlorophyll *a* m^{-3}) at the Polar Front. As concluded by Klaas (2001) loss terms such as grazing pressure by metazooplankton might influence heterotrophic dinoflagellate distribution in addition to productivity during spring. Findings by Jeong, (1994b) and Merrell and Stoecker (1998) indicate that even nauplii stages and smaller copepods actively feed on heterotrophic dinoflagellates >40µm. Since abundances of metazoans, especially copepod nauplii, copepodites and small adults, with very high initial values, were mostly increasing during the experiment (average of 40,378 ind. m^{-3}) and were positively correlated with larger thecate dinoflagellate abundances, it is more than

likely that grazing pressure also exerted an impact on population growth of larger thecate dinoflagellates during EisenEx. The accumulation of cell numbers in outside waters could be explained with the elevated out-patch diatom abundances as the seasonal biomass increase. However, no positive correlation was found between larger thecate dinoflagellates and biological parameters in unfertilized waters.

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Figure legends

Fig. 1. Temporal development of total large (> 50µm) protozooplankton abundance and biomass. (A, C) in patch stations; (B, D) out patch stations.

Fig. 2. Large (> 50µm) protozooplankton community composition given as percentage of large protozoan abundance (ind. m^{-3}) and biomass (µg C m^{-3}), respectively. (A, C) in patch stations; (B, D) out patch stations.

Fig. 3. Adult sarcodine community composition given as percentage of sarcodine abundance (ind. m^{-3}) and biomass (µg C m^{-3}), respectively. (A, C) in patch stations; (B, D) out patch stations.

Fig. 4. Temporal development of large (> 50µm) protozooplankton abundance. (A) acantharians; (B) heliozoans; (C) total foraminiferans in patch, FM = full moon event; (D) total foraminiferans out patch, FM = full moon event; (E) adult radiolarians in patch; (F) adult radiolarians out patch; (G) juvenile radiolarians.

Fig. 5. Temporal development of adult sarcodines and adult radiolarian biomass. (A, C) in patch stations; (B, D) out patch stations.

Fig. 6. Mean relative abundance (A, B) and biomass (C, D) of larger (> 50µm) protozooplankton taxa over the sample depths (10-150m). (A, C) in patch stations; (B, D) out patch stations.

Fig. 7. Temporal development of partial correlation coefficients. (A) tintinnid ciliates versus chlorophyll *a* and (B) versus diatoms; (C) heliozoans versus primary production; (D) adult radiolarians versus temperature.

Fig. 8. Tintinnid ciliate and larger dinoflagellate community composition given as percentage of tintinnid and larger dinoflagellate abundance (ind. m^{-3}) respectively. (A, C) in patch stations; (B, D) out patch stations.

Fig. 9. (A) lorica of *Codonellopsis pusilla* from station 61 (50m). Scale bar = 20µm. (B and C) coccoliths and diatom frustule agglutinated to the lorica of *Codonellopsis pusilla* from station 61 (50m). Scale bar = 5µm.

Fig. 10. Abundance of empty tintinnid loricae. (A) temporal development. (B) taxonomic composition two days prior to first fertilization and on day 21 inside and outside the patch. (C) community composition given as percentage of empty tintinnid loricae abundance ($\times 10^6$ ind. m^{-2}).

Fig. 11. Relative abundance of empty and full tintinnid loricae (A) inside and (B) outside the fertilized patch.

Table 1

Abundances and biomass of larger protozooplankton from counts of concentrated samples 2 days prior (T-2) to first fertilization and after 3 weeks inside and outside the fertilized patch. Values are depth-integrated samples from 0-150m and recalculated in ind. m^{-3} and µg C m^{-3} respectively.

Taxon	Abundance (x10^3 ind. m^{-3})			Biomass (µg C m^{-3})		
	day T-2	day 21 in patch	day 21 out patch	day T-2	day 21 in patch	day 21 out patch
Acantharia	6.2	19.7*	8.0	13.6	43.4*	17.7
Adult foraminifera	3.6	17.3	12.5	60.0	312.9	228.5
Juvenile foraminifera	0.4	16.1*	7.8	0.7	32.4*	15.8
Total foraminifera	4.0	27.9	20.3	60.7	334.3	244.3
Nassellaria	4.5	5.9	4.1	25.2	33.3	23.1
Spumellaria	1.4	1.8	0.7	8.0	9.7	4.0
Phaeodaria	1.5	1.6	0.6	7.6	9.0	3.6
Juvenile radiolaria	3.4	4.9	4.0	1.2	4.1	1.9
Total radiolaria	10.8	14.2	9.4	41.9	56.1	32.6
Heliozoa	6.0	1.4*	1.5	32.0	7.8*	8.2
Large tintinnid ciliates	97.7	53.0*	26.2	288.2	262.8	97.9
Large thecate dinoflagellate	40.9	74.0	44.0*	440.1	753.3	419.8*
Total larger protozooplanktc	161.8	204.1	117.8	874.7	1437.1	1056.8

* highligths species with abundance and biomass values taken on days 19 and 17 for inside and outside the patch respectively.

Table 2

Partial correlation analysis for larger protozooplankton abundances. Values of correlation coefficients for significant correlations are shown. (N.S.) = not significant (P > 0.05); N = 96 (in patch); N = 42 (out-patch). Comparison between discrete depth samples were done with following parameters: Depth (m); salinity (ppt); Chl. *a* (chlorophyll *a* in µg chl. *a* l^{-1}); PP (primary production in µg C l^{-1} d^{-1}); Diatoms (cells l^{-1}); small copepod, other ciliates and dinoflagellates <50µm (ind. m^{-3}); Bacteria (cells ml^{-1})

Parameter	Protozooplankton group											
	Tintinnid ciliates		Thecate dinoflagellates		Acantharia		Foraminifera		Radiolaria		Heliozoa	
	In-patch	Out-patch	In-patch	Out-patch	In-patch	Out-patch	In-patch	Out-patch	In-patch	Out-patch	In-patch	Out-patch
Depth	-0.612	-0.527	-0.713	-0,661	-0.437	-0.524	-0.213	N.S.	N.S.	0.392	0.483	N.S.
Temp.[a]	-0.208	-0.432	0.290	0,332	0.503	0.346	0.486	0.453	Fig. 7D	-0.325	N.S.	-0.415
Salinitya	N.S.	N.S.	N.S.	N.S.	-0.312	N.S.	-0.466	-0.313	N.S.	N.S.	N.S.	-0.354
Chl. *a*[b]	Fig. 7A	N.S.	0.380	N.S.	0.373	N.S.	0.548	0.368	N.S.	N.S.	N.S.	N.S.
Phaeopigments[c]	N.S.	N.S.	N.S.	N.S.	0.282	N.S.	0.439	N.S.	N.S.	N.S.	N.S.	N.S.
PP[d]	N.S.	N.S.	0.361	N.S.	N.S.	-0.370	N.S.	N.S.	N.S.	N.S.	Fig. 7C	N.S.
Diatoms[e]	Fig. 7B	N.S.	0.353	N.S.	0.431	0.462	0.524	0.629	N.S.	N.S.	N.S.	N.S.
Small copepods	N.S.	-0.327	0.388	N.S.	0.313	N.S.	0.362	N.S.	N.S.	N.S.	N.S.	N.S.
Other ciliates	-	-	N.S.	N.S.	0.210	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Bacteria[f]	N.S.	-0.550	0.470	N.S.	0.302	N.S.	0.498	0.682	N.S.	N.S.	N.S.	-0.559
Dinoflagellates <50µm	-0.258	0.392	-	-	0.269	N.S.	0.272	N.S.	0.221	N.S.	N.S.	N.S.

[a] Strass et al. (2001)
[b] Riebesell et al. (to be submitted)
[c] Peeken (unpubl. data)
[d] Gervais et al. (2002)
[e] Assmy and Henjes (to be submitted)
[f] Arietta et al. (2003)

Fig. 1

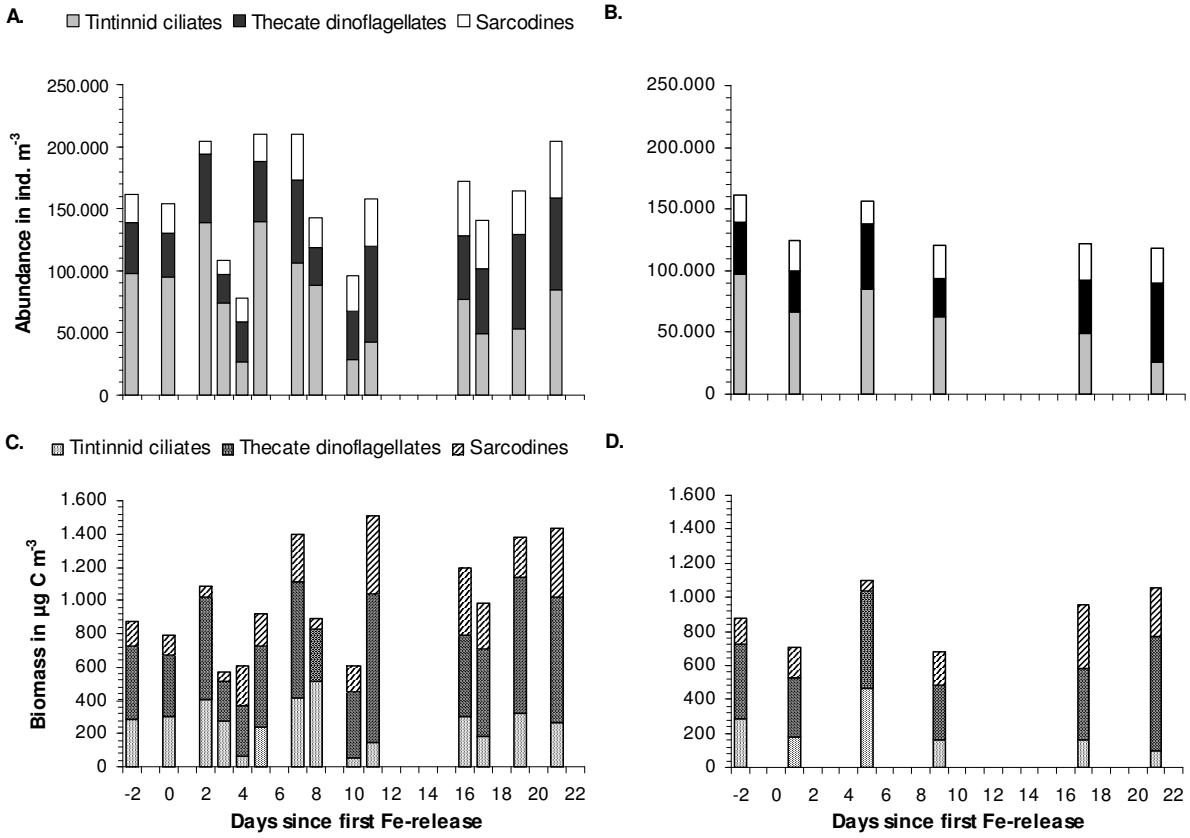

A.
Tintinnid ciliates
Thecate dinoflagellates
Sarcodines
Abundance in ind. m-3
250.000
200.000
150.000
100.000
50.000
0
B.
C.
Tintinnid ciliates
Thecate dinoflagellates
Sarcodines
Biomass in µg C m-3
1.600
1.400
1.200
1.000
800
600
400
200
0
-2 0 2 4 6 8 10 12 14 16 18 20 22
Days since first Fe-release
D.
Days since first Fe-release

Fig. 2

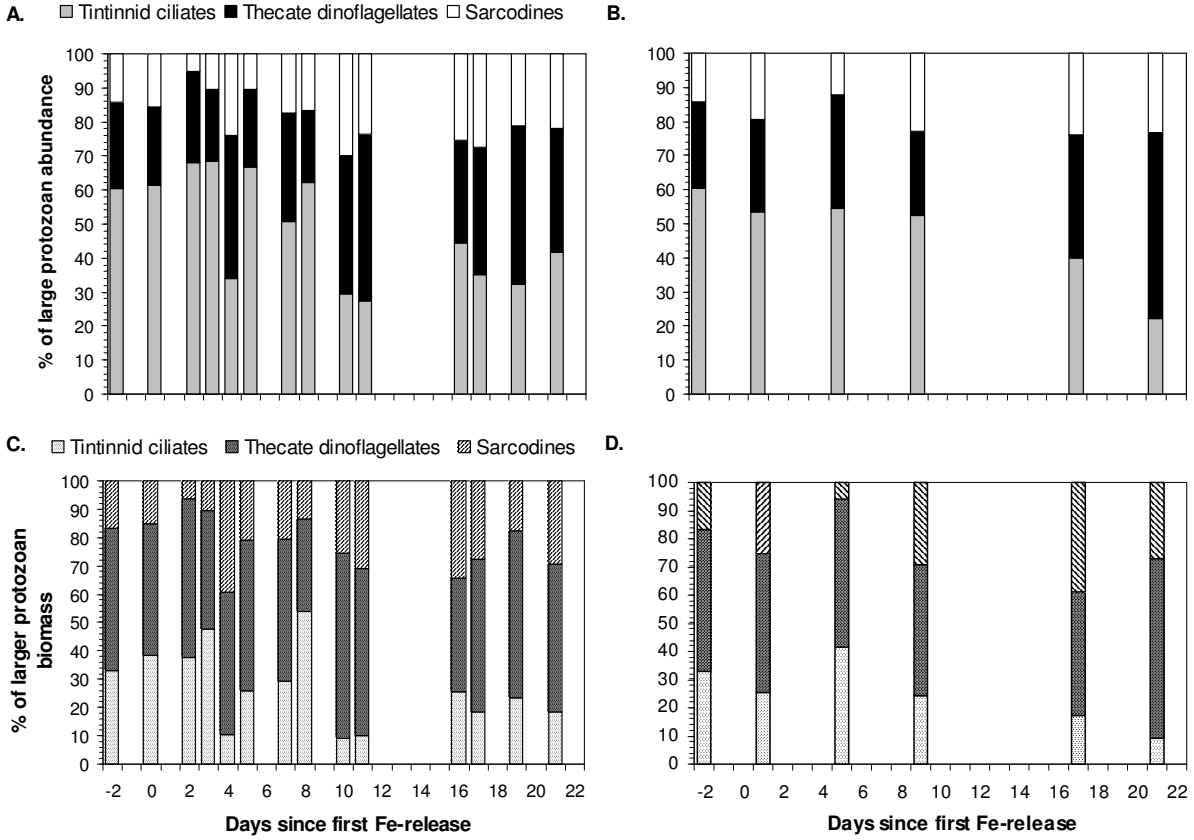

A.
Tintinnid ciliates
Thecate dinoflagellates
Sarcodines
B.
% of large protozoan abundance
C.
D.
% of larger protozoan biomass
Days since first Fe-release
Days since first Fe-release

Fig. 3

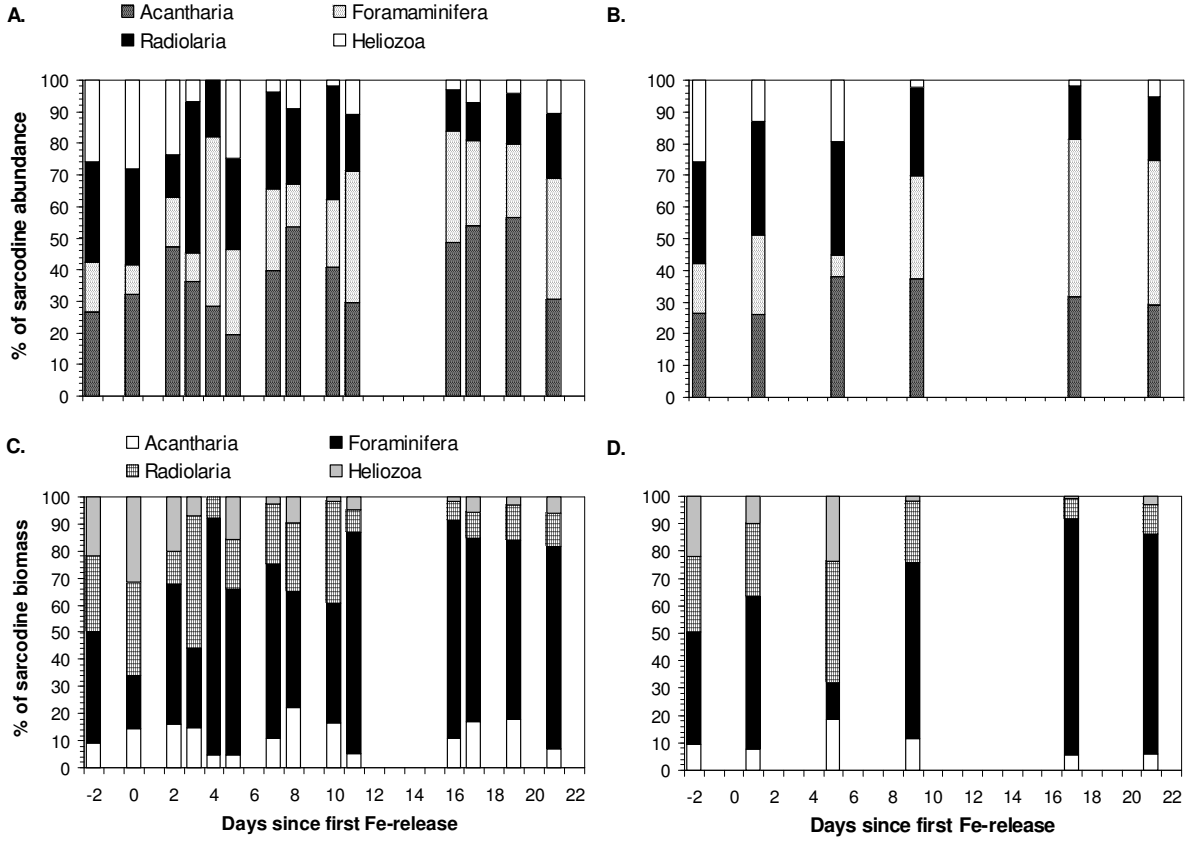

A.
Acantharia
Foramaminifera
Radiolaria
Heliozoa
% of sarcodine abundance
B.
C.
Acantharia
Foraminifera
Radiolaria
Heliozoa
% of sarcodine biomass
D.
Days since first Fe-release
Days since first Fe-release

Fig. 4

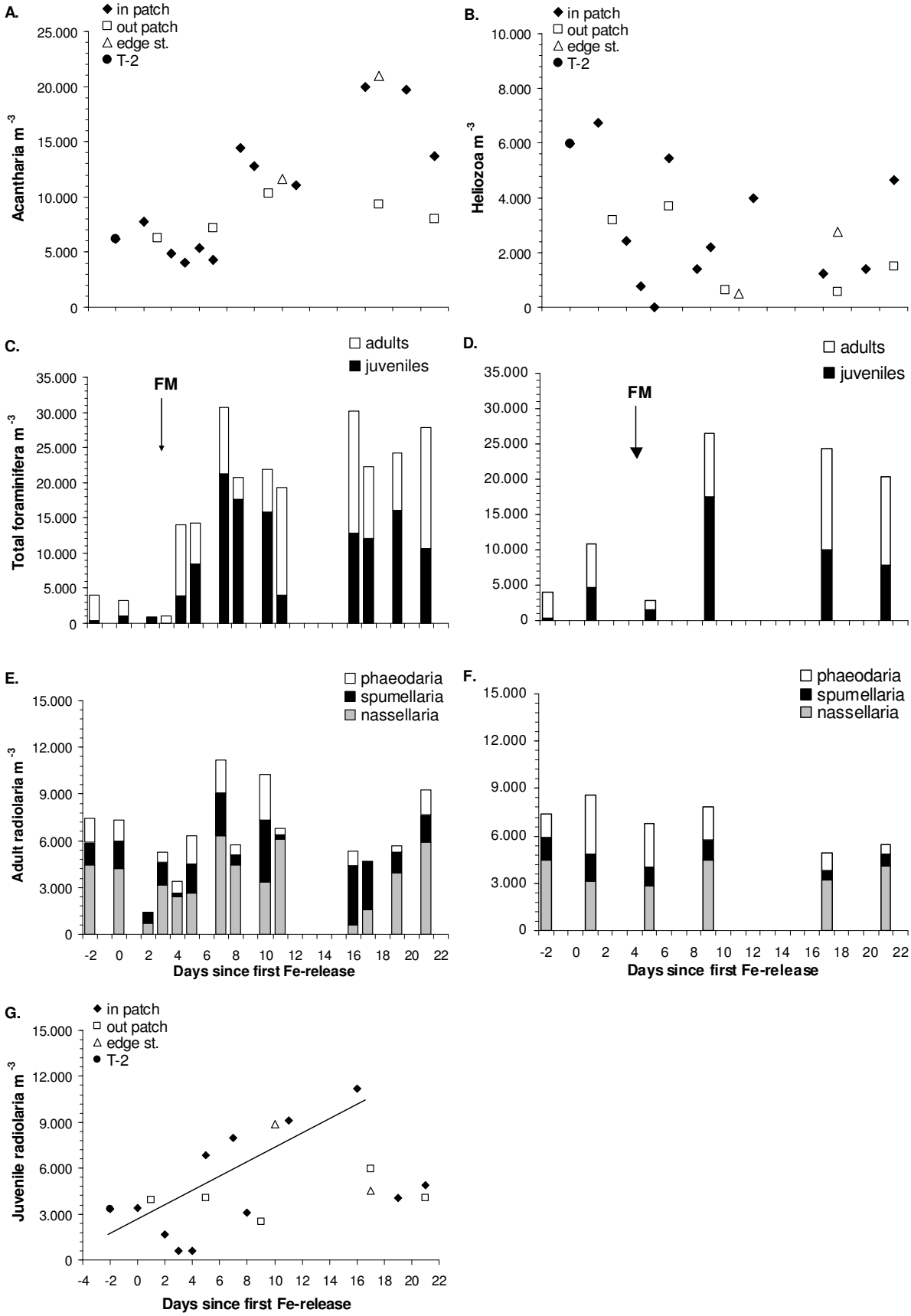

A.
in patch
out patch
edge st.
T-2
Acantharia m -3
25.000
20.000
15.000
10.000
5.000
0
B.
in patch
out patch
edge st.
T-2
Heliozoa m -3
10.000
8.000
6.000
4.000
2.000
0
C.
adults
juveniles
FM
Total foraminifera m -3
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0
D.
adults
juveniles
FM
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0
E.
phaeodaria
spumellaria
nassellaria
Adult radiolaria m -3
15.000
12.000
9.000
6.000
3.000
0
-2 0 2 4 6 8 10 12 14 16 18 20 22
Days since first Fe-release
F.
phaeodaria
spumellaria
nassellaria
15.000
12.000
9.000
6.000
3.000
0
-2 0 2 4 6 8 10 12 14 16 18 20 22
Days since first Fe-release
G.
in patch
out patch
edge st.
T-2
Juvenile radiolaria m -3
15.000
12.000
9.000
6.000
3.000
0
-4 -2 0 2 4 6 8 10 12 14 16 18 20 22
Days since first Fe-release

Fig. 5

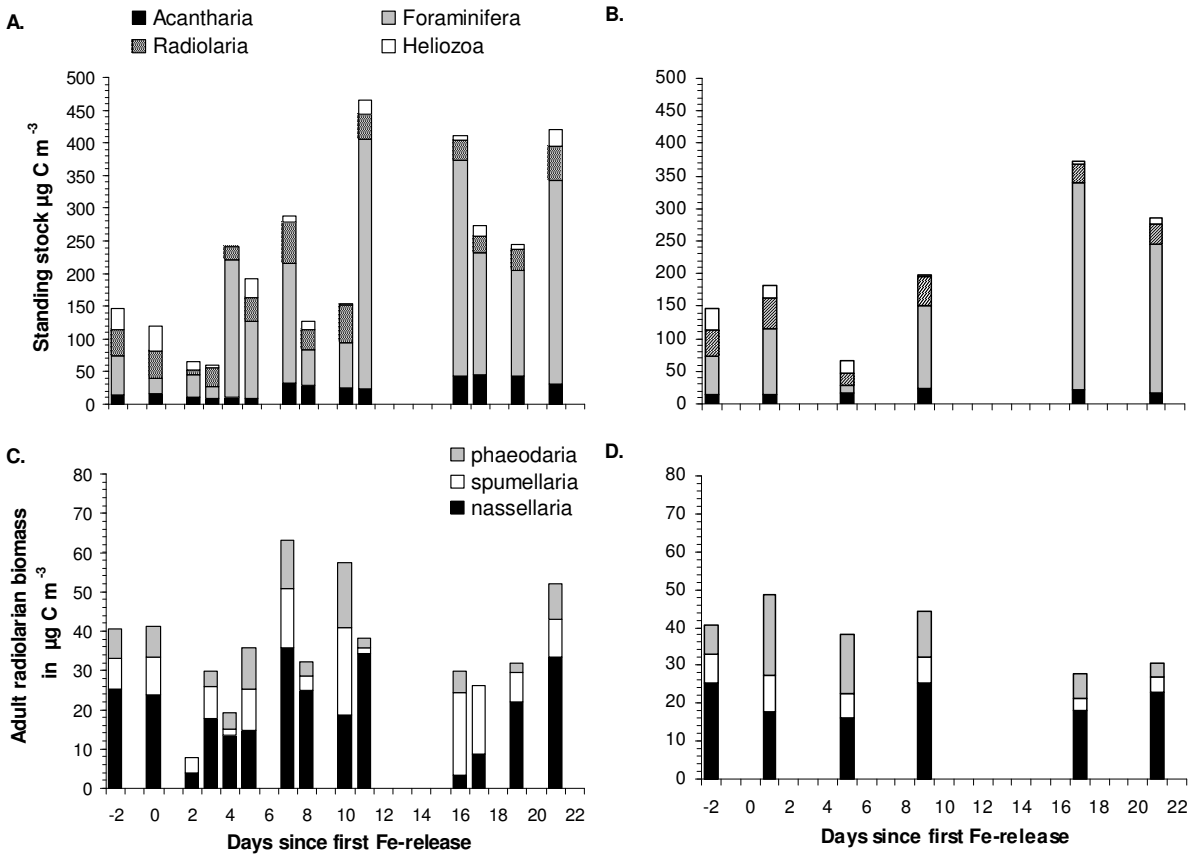

A.
Acantharia
Foraminifera
Radiolaria
Heliozoa
Standing stock µg C m-3
B.
C.
phaeodaria
spumellaria
nassellaria
Adult radiolarian biomass in µg C m-3
Days since first Fe-release
D.
Days since first Fe-release

Fig. 6

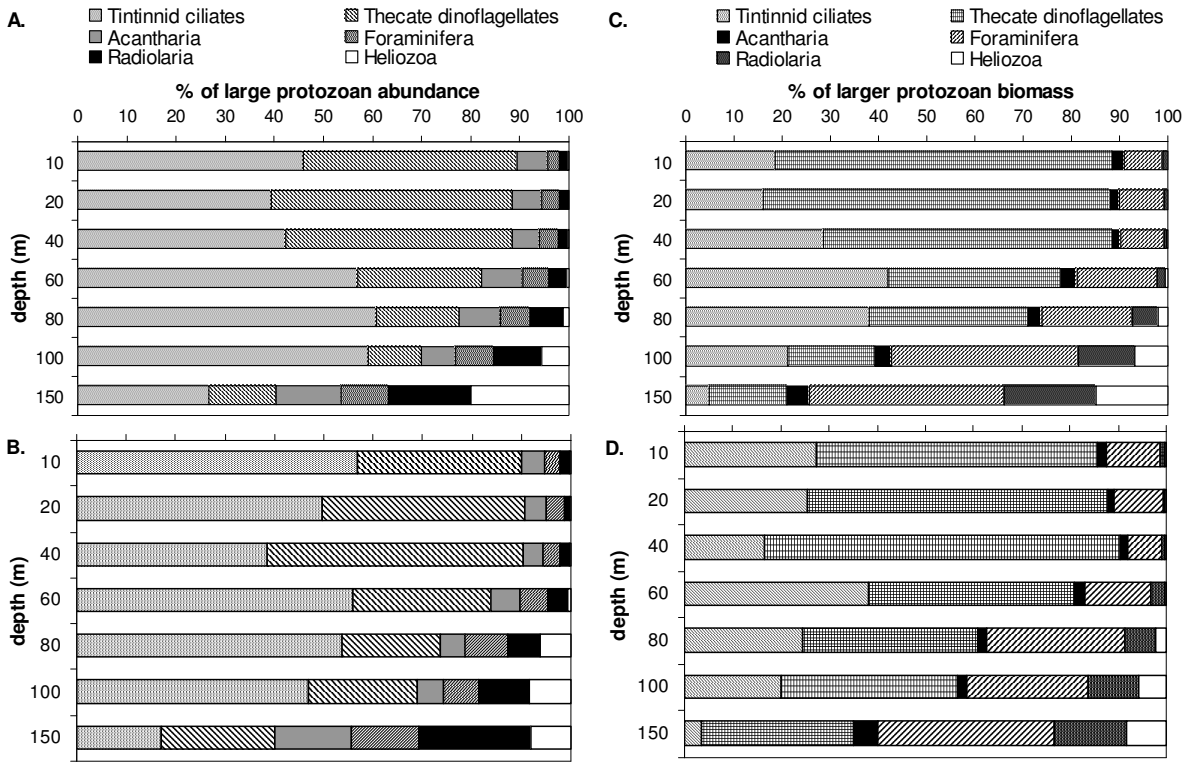

A.
Tintinnid ciliates
Thecate dinoflagellates
Acantharia
Foraminifera
Radiolaria
Heliozoa
% of large protozoan abundance
0 10 20 30 40 50 60 70 80 90 100
depth (m)
10 20 40 60 80 100 150
B.
C.
% of larger protozoan biomass
D.

Fig. 7

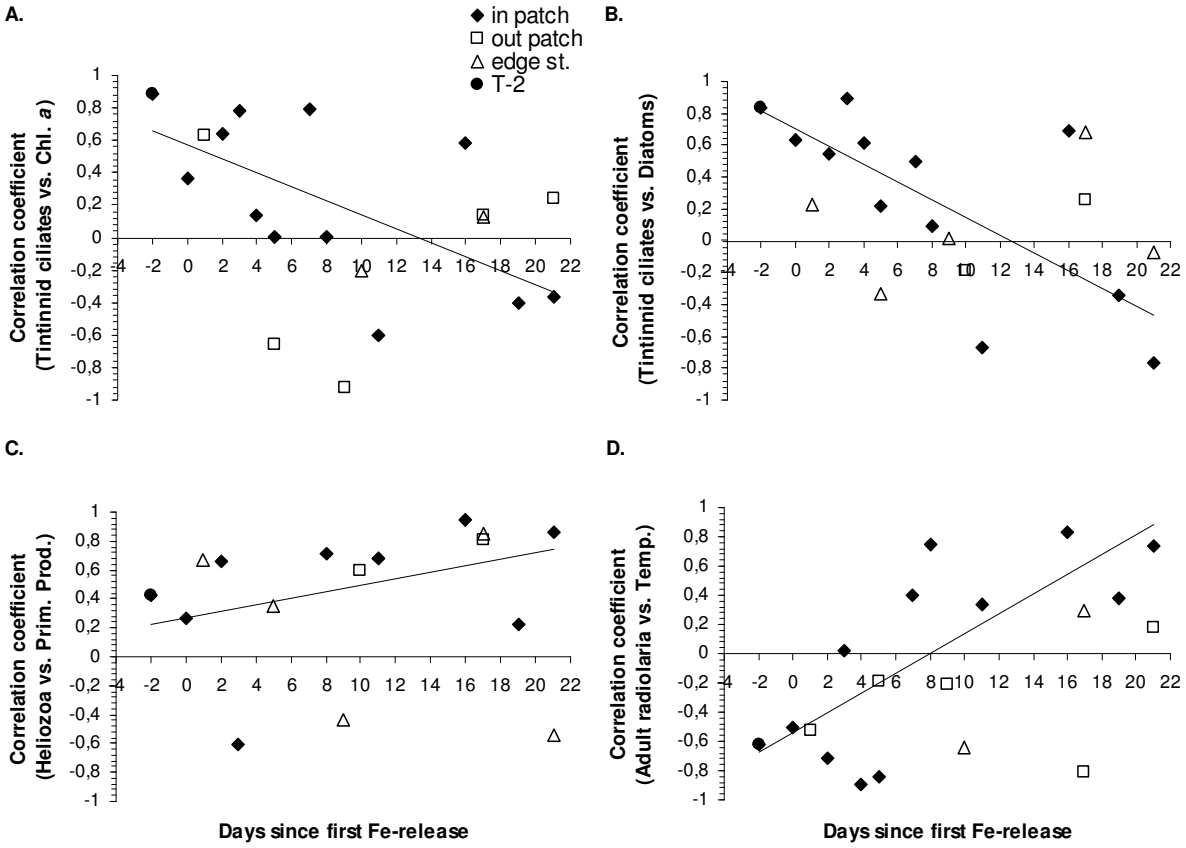

A.
in patch
out patch
edge st.
T-2
Correlation coefficient (Tintinnid ciliates vs. Chl. a)
B.
Correlation coefficient (Tintinnid ciliates vs. Diatoms)
C.
Correlation coefficient (Heliozoa vs. Prim. Prod.)
D.
Correlation coefficient (Adult radiolaria vs. Temp.)
Days since first Fe-release
Days since first Fe-release

Fig. 8

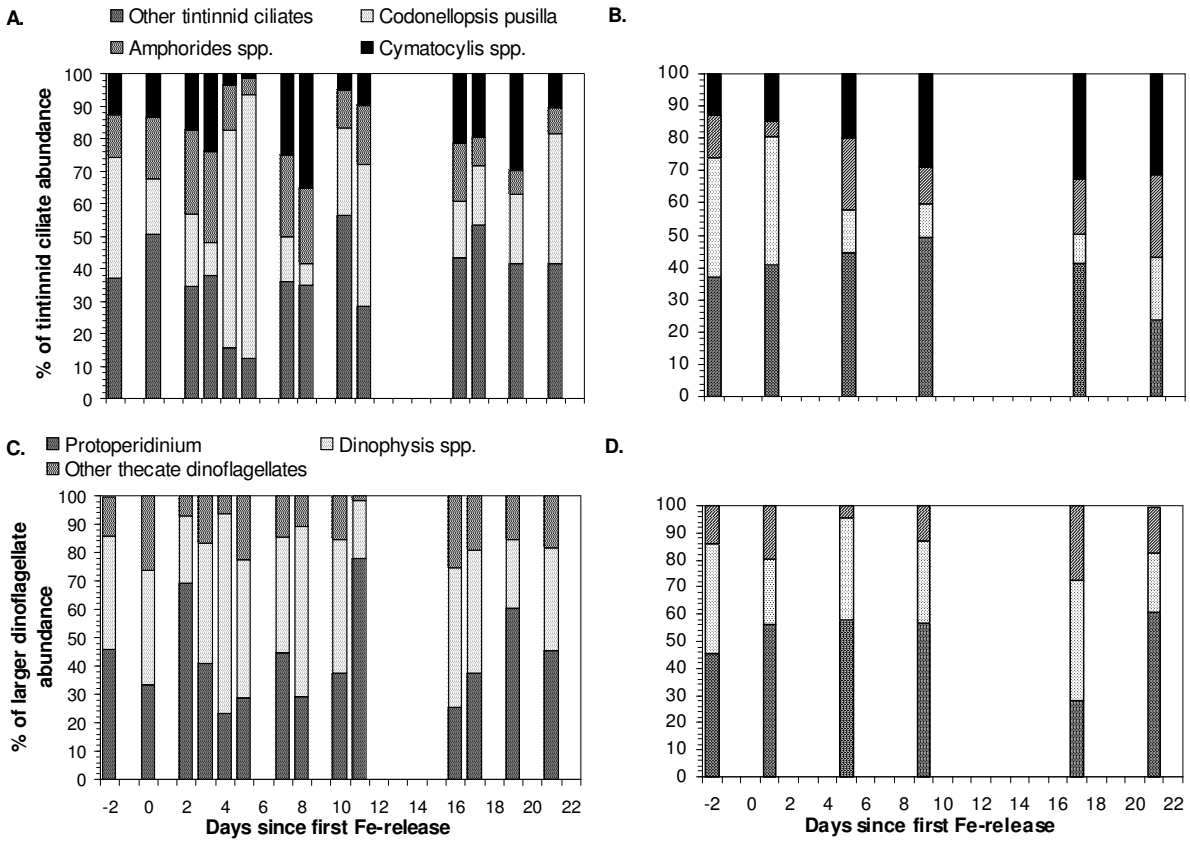
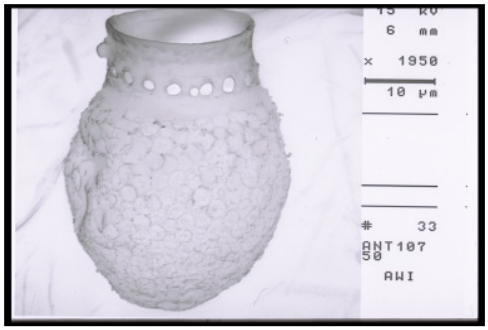
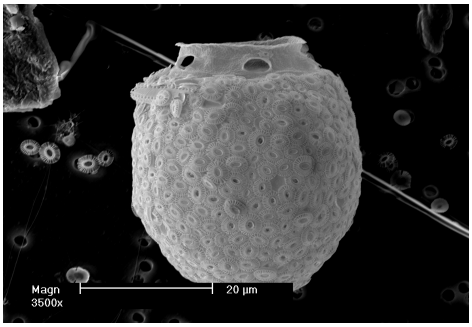

A.
Other tintinnid ciliates
Codonellopsis pusilla
Amphorides spp.
Cymatocylis spp.
% of tintinnid ciliate abundance
B.
C.
Protoperidinium
Dinophysis spp.
Other thecate dinoflagellates
% of larger dinoflagellate abundance
D.
Days since first Fe-release
Days since first Fe-release

Fig. 9

A.


15 kV
6 mm
× 1950
10 µm
33
ANT107
50
AWI

B.


Magn
3500x
20 µm

C.

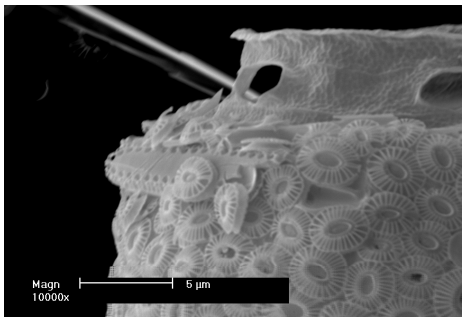

Magn
10000x
5 µm

Fig. 10

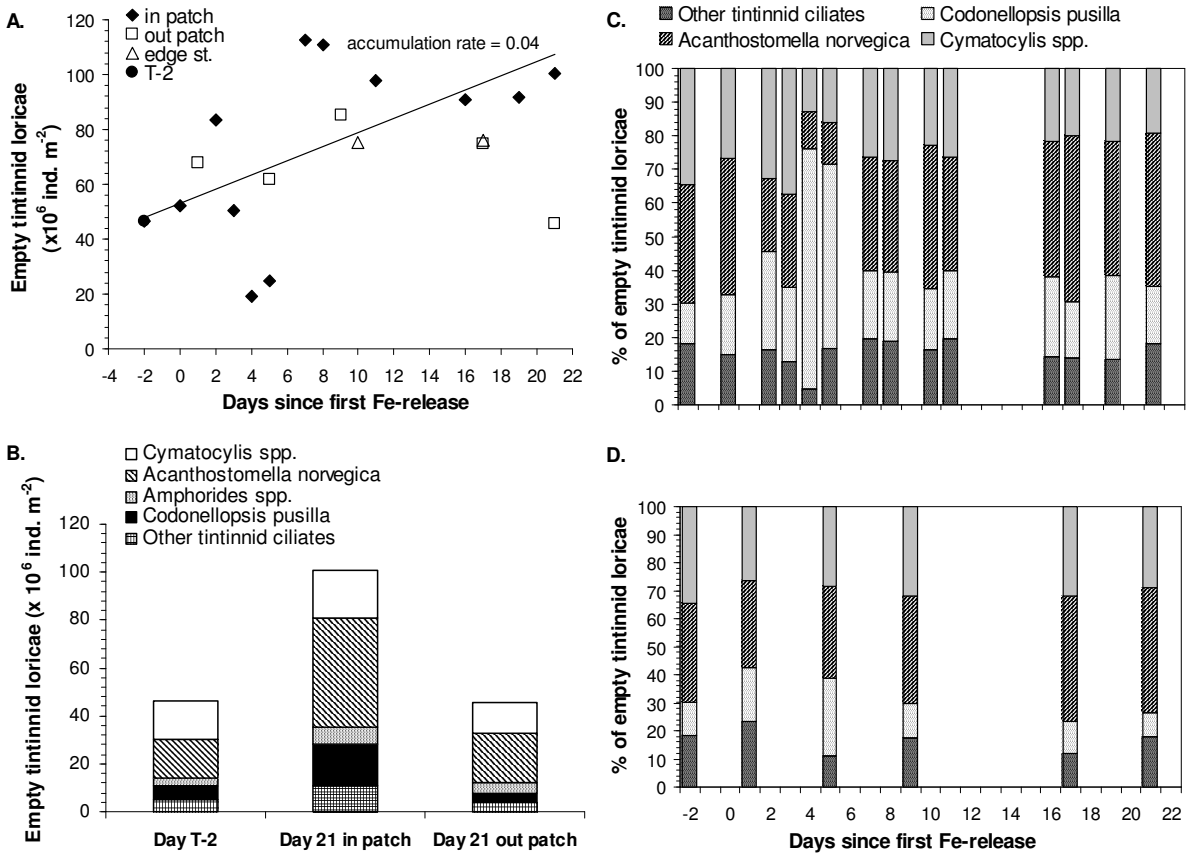
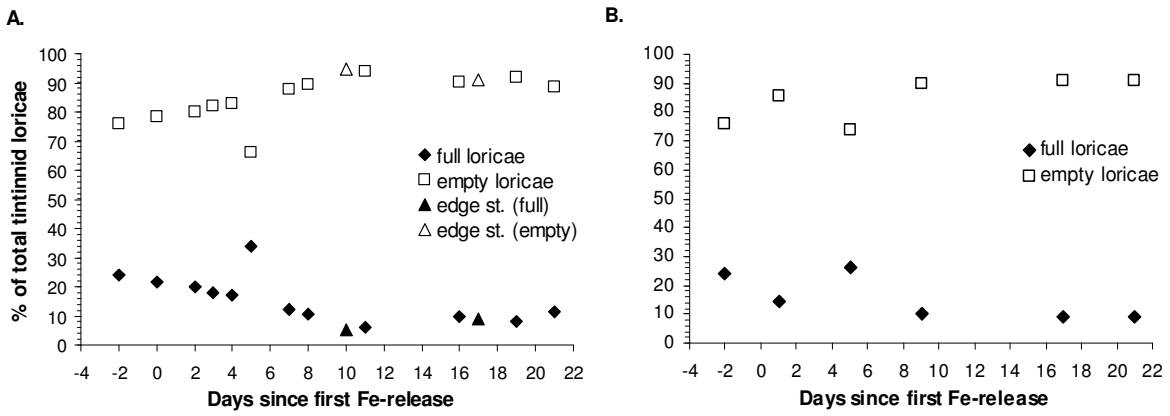

A.
in patch
out patch
edge st.
T-2
accumulation rate = 0.04
Empty tintinnid loricae (x10^6 ind. m^-2)
Days since first Fe-release
B.
Cymatocylis spp.
Acanthostomella norvegica
Amphorides spp.
Codonellopsis pusilla
Other tintinnid ciliates
Empty tintinnid loricae (x 10^6 ind. m^-2)
Day T-2
Day 21 in patch
Day 21 out patch
C.
Other tintinnid ciliates
Codonellopsis pusilla
Acanthostomella norvegica
Cymatocylis spp.
% of empty tintinnid loricae
D.
% of empty tintinnid loricae
Days since first Fe-release

Fig. 11


A.
% of total tintinnid loricae
Days since first Fe-release
full loricae
empty loricae
edge st. (full)
edge st. (empty)
B.
Days since first Fe-release
full loricae
empty loricae

Mansucript 5

The role of grazing in structuring Southern Ocean pelagic ecosystems and biogeochemical cycles.

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submitted to Antarctic Science

Abstract: This review examines the links between pelagic ecology and ocean biogeochemistry with emphasis on the role of the Southern Ocean in global cycling of carbon and silica. The structure and functioning of pelagic ecosystems is determined by the relationship between growth and mortality of its species populations. Whereas the key role of iron supply in conditioning the growth environment of land-remote oceans is now emerging, the factors shaping the mortality environment are still poorly understood. We address the role of grazing as a selective force operating on the structure and functioning of pelagic ecosytems within the larger conceptual framework of evolutionary ecology. That grazing pressure decreases with increasing cell size is widely taken for granted. We examine the impact of this principle across the range of size classes occupied by Southern Ocean plankton and show that relatively few species play crucial roles in the trophic structure and biogeochemical cycles of the Southern Ocean. High grazing pressure of small copepods and salps within the regenerating communities characteristitc of the iron-limited Southern Ocean results in accumulation of large, heavily silicified diatoms that drive the silica pump. In contrast high growth rates of weakly silicified diatoms result in build-up of blooms under iron-sufficient conditions that fuel "the food chain of the giants" and drive the carbon pump. The hypotheses we derive from field observations can be tested with *in situ* iron fertilization experiments.

Key words: Evolutionary ecology, silica cycle, biological carbon pump, iron-limited systems, iron-replete systems, ocean fertilization

Introduction

The Southern Ocean (SO) is the largest upwelling region in the world ocean but it is also the only one where the bulk of the upwelling macronutrients nitrate and phosphate is returned with downwelling surface water to the ocean interior. This implies that the ecology of the surface layer of the Antarctic Circumpolar Current (ACC) has little effect on global nitrate and phosphate cycles, and hence also that of carbon, in the course of thermohaline ventilation of the deep ocean. In striking contrast, the concentrations of dissolved silica (silicic acid) in the surface mixed layer decline drastically across the ACC with Si:N ratios ranging from 3 in the Antarctic Zone in the south, to 0.3 in the sub-Antarctic Zone prior to subduction. The bulk of silica, but not nitrogen and phosphate, is extracted from the surface mixed layer and transferred to the Circumpolar Deep Water (CDW) where it is retained within a SO "silica loop", and unlike N and P, sequestered from the rest of the world ocean. This SO loop is reflected in nitrate concentrations of ~35 mmol m^{-3} (Sigman & Boyle 2000) and Si:N ratios of 4:1 in CDW as compared to 30 mmol m^{-3} and 1:1 in outflowing Antarctic Intermediate Water (AAIW) (Falkowski *et al*. 1998). Hence the modern ACC is a weak carbon but strong Si sink in the world ocean (Brzezinski *et al*. 2003, Sarmiento *et al*. 2004).

If all the nitrate and phosphate present in the surface mixed layer (80m depth) across the entire ACC were converted into organic carbon by phytoplankton, the resulting carbon dioxide deficit which could be compensated by uptake from the atmosphere would amount to about 6 Gigatonnes of carbon (GTC). This is equivalent to the annual global emmission of fossil fuel carbon. The rate of replenishment of N and P in ACC surface water by upwelling CDW determines the annual potential uptake of atmospheric CO_2 which ranges from 0.8–1.4 GTC y^{-1} (Schiermeier 2003). These figures represent the maximum capacity of the Southern Ocean to draw-down atmospheric CO_2. However, the long-term impact of such a drastic increase in productivity, assuming it occurs every year, depends on various independent factors pertaining to hydrography and ecology of the ACC. Crucial to the calculations are the residence time of water in the Winter Mixed Layer (ca. 200 m deep compared to the ca. 80 m annual, mixed surface layer) across the ACC and the magnitude and transport depth of vertical carbon flux from it. The latter is dependent on ecological processes determining the fate of suspended organic matter, both living and detrital.

The potential role of Southern Ocean productivity in regulating atmospheric carbon dioxide levels over glacial/interglacial cycles was formulated by the late John Martin (1990) in his iron hypothesis. Since then evidence has been mounting that phytoplankton growth rates in the land-remote SO are limited by iron availability which is the reason why

chlorophyll biomass is continuously low and macronutrients are in surplus. As a result the iron-limited SO plays only a minor role in the global carbon budget compared to the North Atlantic (Falkowski *et al*. 1998; Treguer & Pondaven 2002). The latter receives much more iron from its extensive shelves, rivers and from North African dust which relieves iron limitation and results in a spring phytoplankton bloom terminated by exhaustion of macronutrients (Falkowski *et al*. 1998). However, annual levels of new production in both oceans, based on nitrate uptake within the mixed surface layer are similar (Jennings *et al*. 1984), indicating that it is not the magnitude of production but presence or absence of a distinct bloom which determines how much carbon dioxide is sequestered in the ocean. Apparently blooms developing under iron-replete conditions activate the biological carbon pump whereas iron-limited production has little effect on it. In contrast the effect on the silica pump appears to be the reverse. It follows that the species characteristic of these two types of system – the carbon and silica sinking systems - have evolved under differing suites of selective pressures resulting in major differences in functioning of the two system types.

Two questions arise from this ocean comparison: 1) Did iron fertilization of the Southern Ocean by elevated levels of glacial dust input (Petit *et al*. 1999, Mahowald *et al*.1999) induce North Atlantic conditions similar to those reported from the North Pacific (Sancetta 1992) and, if so, will artificial fertilization be an effective CO_2 mitigation mechanism? 2) What was the effect of iron-replete conditions on the structure of glacial Southern Ocean food webs and what could be the effect of artificial iron fertilization on the current ecosystems? Would the whale populations increase in size? Whereas the lively debate on the first question has received much publicity (Schiermeyer 2003), the second has not yet been given serious consideration. Yet the answers to both questions ultimately converge on the fate of the organic matter produced by iron-replete versus iron-limited systems. There is no ecological reason to assume a linear relationship between productivity and the amount exported to the deep sea. Nor should one expect a linear response of the food chain to increasing productivity with all trophic levels increasing proportionately. The point is that we do not yet know the answers to these fundamental questions.

Pelagic ecology and biogeochemistry

Much research still needs to be done to bolster our information base on pelagic ecosystem functioning, and the intention of this review is to help focus future efforts on what we believe are crucial questions. We argue that the thrust of these investigations should be directed

toward testing quantitative hypotheses of organism interactions rather than piecing together, within hypothetical views of how pelagic ecosystems function, the information from snap-shot, observational surveys. The broad range of new techniques for assessing ocean productivity, ranging from submicron to global scales, has provided a fairly robust estimate of ocean primary production. However, our understanding of the ecological underpinnings of biogeochemical processes has not advanced, since we are still far from answering the basic questions posed above. It should go without saying that ecosystem processes ultimately drive global biogeochemical cycles which can hence only be understood in the light of evolving organism interactions. These are structured according to ecological principles.

Ecological principles emerge from the evolution of species interacting with one another within physico-chemical constraints of the ecosystem. Little is known about the evolutionary ecology of plankton because the fossil record of pelagic organisms is highly selective, quite unlike the rich legacy left behind by terrestrial plant assemblages. The morphological evolution of the latter – from algal mat to moss carpet and eventually forest - is clearly shaped by resource competition, whereas speciation, demonstrated impressively by angiosperms, is also shaped by animal vectors. Land plants are susceptible to various pathogens and grazed by animals ranging from nematodes and insects to ungulates and elephants against whose depredations a variety of defences from mechanical to chemical have evolved. In contrast to the detailed, species-specific information available on the roles of resource competition versus mortality factors (bottom-up vs. top-down) in shaping the structure and functioning of terrestrial systems, comparable knowledge from pelagic systems is scant (Verity & Smetacek 1996).

The much greater research effort focussed on factors controlling growth rates of phytoplankton has resulted in neglect of the other side of the coin – the factors regulating mortality rates. The disparity in treatment is reflected in the common usage of the term „growth environment“ and the unfamiliarity of its equivalent „mortality environment“. Yet it is the balance between growth and mortality that determines the survivorship of species populations, the accumulation rate of biomass and eventually its fate. The causes of mortality range from internal programming (death at old age) to death by external agents: pathogens (viruses and bacteria), parasitoids (parasites that kill their hosts) and predators (proto- and metazooplankton). Each of these groups plays a significant but very different role in structuring the mortality environment, yet their relative roles are poorly known. But these ecological factors determine biomass accumulation by phytoplankton and the fate of the produced material, so it is they that determine food chains and biogeochemical cycles (Banse

1995). In this review we discuss current thinking in Southern Ocean pelagic ecology and biogeochemistry with an aim to develop a common conceptual framework based on logical inference within which hypotheses can be formulated and tested.

We approach the current state of our knowledge from an historic perspective. We review the pioneer views on Southern Ocean plankton ecology to assess how much, or how little, they have changed as a result of the vast body of data that has accumulated since then. We proceed to analyse the new information with a view to establishing basic ecological principles that govern the structure and functioning of pelagic ecosystems with particular attention to the mortality environment. We identify unique features of Southern Ocean ecology and biogeochemistry and attempt to relate these with each other. The causal links are traced within the frameworks of ocean biogeochemistry and evolutionary trends within pelagic organisms. These hypotheses can now be tested with a range of new methods ranging from molecular tools to large-scale *in situ* fertilization experiments. New insights into adaptive strategies followed by the various organisms will emerge that will enable the quantification of ecosystem functioning at the genome level. This information can be used to formulate new kinds of models linking biodiversity and ecology with biogeochemistry. The intention of this review is to stimulate thinking at the broad-brush, big-picture scale where exceptions define the rules.

History of views on Antarctic productivity

The history of plankton ecology, ever since its inception about 150 years ago, has been dictated by its methods. Their refinement over the decades has successively revealed fundamentally new aspects of the structure of pelagic ecosystems, but we are still groping in the dark trying to put the pieces revealed by the methodological advances into a coherent, consistent big picture. Phytoplankton was initially sampled by vertically hauled fine-meshed nets and hence the diatom-copepod-fish food chain dominated thinking well into the seventies of the last century. The enormous catches of diatoms in net tows from Antarctic waters as compared to the veritable absence of phytoplankton in net tows from subtropical and sub-Antarctic waters was the basis for the earlier view of a highly productive Southern Ocean (Hardy 1967). The productivity was reflected by the enormous populations of conspicuous, warm-blooded top predators and verified by their phenomenal growth rates. Blue whales were found to reach sexual maturity within 3-4 years of egg fertilization (Hardy 1967).

The findings of the RRS Discovery cruises from 1925-1939 were summarized by Hart (1934, 1942) who concluded that "the physical factors of the environment play the most important part in determining the course of phytoplankton production" over the seasonal cycle. The factors he considered were the influence of mixing depth, intermittent cloud cover and pack ice on light climate. However, "the main reason for the extremely rich production, which will probably be found to exceed that of any other large area in the world" was due to the fact that "nutrient salts are not at any time limiting for phytoplankton growth within the Antarctic surface water" (Hart 1934). He mentioned that silicate was a possible exception in the northern ACC implying that he was aware of selective removal of this nutrient relative to N and P. But "none of the factors so far examined adequately account for the vastly greater richness of the neritic as compared with the oceanic regions. We are left with the hypothesis that extremely small amounts of organic compounds, iron and manganese derived from the land, exert a strongly favourable influence on phytoplankton production" (Hart 1942). He also considered the fate of phytoplankton biomass: "Observed reduction of phosphate and observed average standing crop are alone sufficient to show that there must be a huge loss in our southern localities (...) it seems probable that this is mainly due to grazing (...) but it must not be overlooked that actual death and sinking of diatoms may account for some of it" (Hart 1942).

Considering the poor methodology of the time, Hart´s insights regarding the role of bottom-up factors in determining seasonal and regional productivity are remarkably close to the modern view. This thoughtful treatment contrasts strikingly with his rather perfunctory attitude to the role of grazing, despite the fact that zooplankton populations were invariably sampled simultaneously. But in his view one of the missions of RRS Discovery, to assess the food supply of the whales, appeared to be have been answered: The food of the whales consisted almost entirely of krill. "The food of these Euphausiids consists very largely, if not entirely of diatoms and other phytoplankton organisms. Thus we have here one of the simplest food chains possible, the building up of the vast body of the whale being only one stage removed from the organic fixation of the radiant energy of the sun by the diatoms" (Hart 1934). Hart (1942) was well aware that this "food chain of the giants" was restricted to a few productive regions, particularly the SW Atlantic Sector. After decades of dedicated research we now know that this view is overly simplistic and does not explain why this system prevails in the Antarctic. So the role of grazing remains as obscure as in his time. The questions remain although the motivation for carrying out productivity studies has radically changed:

from a food-chain, hence grazer-oriented approach to one where biogeochemistry occupies centre stage.

The current view

The introduction of new methods in the latter third of the last century revealed that phytoplankton stocks, as reflected in chlorophyll concentrations, were low over much of the land-remote Southern Ocean and more similar to the nitrate-limited, warmer waters of lower latitudes, the so-called "deserts of the ocean"(Karl 2002). The structure of the communities was also found to be similar and dominated by pico- and nanoplankton with autotrophs, bacteria and heterotrophic protists (protozoa) each contributing roughly a third of the total biomass. Investigation of this thriving, previously over-looked community, dubbed the microbial loop initially (Azam et al 1983), was focussed on the measurement of rates. The new microbial methods diverted the attention of plankton ecologists from the large-celled or net phytoplankton to the microbial assemblages accessed by the range of sophisticated new sampling devices. Methodological advances enabled rapid assessment of the concentration and production rates of bacteria and phytoplankton. The new insights on functioning of the microbial system were based primarily on these two components. The protozoan component received the least attention and tended to be treated simply as a mortality term (Landry & Hassett 1982).

The early years of microbial-loop research were accompanied by a vigorous debate on whether it represented a sink or a link to higher trophic levels. The issue of how much organic matter produced by pico- and nanoplankton is indeed channelled by protozoa to the familiar top predators (big fish, mammals etc.) has yet to be resolved but the debate faded out with the shift of focus from the food chain to the biogeochemical context of plankton ecology which occurred in the course of the nineties. Attention was now focussed on the role of the microbial community in the global cycles of carbon and other biogenic elements. In a sense, this was the link-sink debate revisited, albeit in the context of atmosphere, surface and deep oceans. In contrast to the trophic link-sink debate, this one now appears to be resolved. The contemporary Southern Ocean is believed to play a minor role in the carbon cycle (Treguer & Pondaven 2002), implying that its characteristic microbial community funnels only a small fraction of total production to the ocean interior. That it plays but a minor role in the carbon pump is reflected in the fact mentioned in the introduction that upwelling nitrate is returned more or less unused by downwelling to the ocean interior.

As also mentioned above and in striking contrast to N and P, almost all the silicic acid is removed from surface water and sinks to the deep ocean as biogenic silica where most of it dissolves and is retained in Circumpolar Deep Water (CDW). However, the fraction buried in the sediment underlying the ACC represents a major sink in the ocean´s silica cycle. So we are left with a paradox: an ocean dominated by the microbial community which happens to be a major global silica sink (Treguer 2002; Pondaven *et al*. 2000). Clearly diatoms are responsible for the silica flux but the species in question apparently do not occur in blooms of the N. Atlantic type. So the silica paradox cannot be resolved within the framework of the now firmly established two-system view of the pelagial: the distinction between the ubiquitous, recycling microbial communities and the regionally/seasonally restricted, new-production-based blooms of large phytoplankton, generally diatoms (Smetacek *et al*. 1990, Waters *et al*. 2000, Boyd *et al*. 2000).

Clearly, there are organism groups in the ocean that have been accorded a relatively minor role in the ecosystem, i.e. the organic carbon economy, but whose global significance in biogeochemical cycles is documented by their mineral skeletons in the sediments. Modellers categorise them into functional groups based on their impact on local and global biogeochemical fluxes. A well-known example are the carbonate-shelled foraminifera of the nitrate-depleted, warm-water sphere. We will argue that the heavily silicified diatoms of the iron-limited Southern Ocean and the Subarctic Pacific belong to a comparable ecological category that is part of the recycling microbial community and hence distinct from the diatom species that comprise highly productive, carbon-sinking blooms. In the following we present brief overviews of the structure and functioning of pelagic ecosystems and derive ecological principles from them by logical inference. We apply these principles to deduce possible scenarios of organism interactions for further differentiation of the current, two-system view. These hypotheses can now be tested in large-scale, *in situ* experiments.

Regenerating systems

The microbial network (Smetacek *et al*. 1990) is a biomass-balanced system maintained by recycling of essential elements between auto- and heterotrophs in the surface mixed layer which implies that the rate of primary production is regulated by the rate of nutrient remineralisation. Since autotrophic biomass stays more or less constant over months, it follows that cell division is balanced by cell mortality. Satellite images clearly show that this balanced system occupies most of the ocean surface and *in situ* measurements indicate that

the amount of biomass present in the mixed surface layer is constrained by the amount of the essential nutrient in shortest supply. Throughout the warm water sphere, nitrogen and phosphate clearly limit biomass accumulation but in the oceanic upwelling regions, where these macronutrients are supplied from deep water, the role of iron as the limiting nutrient is currently gaining acceptance. These are the high-nutrient, low-chlorophyll (HNLC) regions of the world ocean, of which the Southern Ocean is the largest. The other HNLC regions are located in the Subarctic and Equatorial Pacific (Falkowski *et al*. 1998). In all 3 regions iron fertilization experiments have shown that conversion of the available macronutrients into biomass is limited by iron availability (Coale *et al*. 1996, Boyd *et al*. 2000, Tsuda *et al*. 2003). It took biological oceanographers a long time to accept that the supply of a trace element present in nanomolar concentrations could have the same effect in structuring pelagic ecosystems as the macronutrients present at concentrations 3 orders of magnitude higher.

Macronutrients provide the building blocks of biomass and the C:N:P ratios of particulate organic matter in the surface layer correspond closely with those of the respective dissolved nutrients in deep water (the Redfield ratio) over a wide range of concentrations. This indicates that deep water chemistry is conditioned by the supply and eventual breakdown of sinking particulate matter. However, iron does not follow the Redfield rule. Its insolubility in oxygenated, alkaline ocean water implies a short residence time hence selective loss relative to the dissolved macronutrients, reflected in its low concentrations in deep water.

Although only trace amounts of iron are required by plankton, its involvement and indispensibility in many key enzyme systems (chlorophyll synthesis, nitrate utilisation, redox systems, etc.) results in its playing as much a „building block“ role as the macronutrients (Morel & Price 2003). The fact that microbial systems limited by nitrogen or iron have similar structures, down to the species level, and function at much the same temperature-dependent rates, suggests that organism interactions rather than the chemistries and physiological functions of the respective limiting nutrients govern the ecology of balanced systems. Microbial systems are balanced by the interaction of three components with distinct yet overlapping functions: plants (phytoplankton), bacteria (Eubacteria and Archaea) and protozoa. Phytoplankton and bacteria convert dissolved substances into particles which are ingested by protozoa, and bacteria and protozoa are heterotrophic and depend on primary production of phytoplankton (all chlorophyll-bearing organisms) for sustenance.

Pico- and nanophytoplankton

Representatives of all phytoplankton lineages - more than 10 - are present in the pico- and nanophytoplankton (Fig. 1). In contrast, land plants stem from a single lineage, and the metazoa, together with the fungi and choanoflagellates, from another one. Picoplankton (cells <2 µm) dominate the abundance spectrum although biomass is often dominated by nanoplankton (cells in the 2 - 10 µm range). Various types of phytoflagellates, most of which also ingest particles, dominate the nanoplankton. These comprise haptophytes (coccolithophorids, *Phaeocystis* and several other genera), various dinoflagellates, chrysophytes and cryptophytes. Interestingly, solitary centric and pennate diatoms are also a prominent member of nanoplankton oceanwide but their frustules apparently dissolve rapidly after death and are recycled in the surface layer.

A distinguishing feature of the microbial system south of the Antarctic Polar Front (APF) is the absence of the ubiquitous cyanobacteria genera *Synechococcus* and *Prochlorococcus*, the low diversity and minor role of autotrophic dinoflagellates and the paucity of coccolithophorids. All these groups play prominent roles in the Sub-Antarctic Zone (SAZ) between the Subtropical and Sub-Antarctic Fronts (SAF). Deterioration of the populations of these groups advected southward across the Polar Frontal Zone (PFZ) between the SAF and the APF suggest that lower temperatures pose a physiological barrier. Since cyanobacteria are abundant in ice-covered lakes on the continent, temperature cannot be a constraint for the lineage per se but has to be regarded in combination with salinity (or a specific ion) and physiological properties of just these two genera. Similarly a gymnodinoid dinoflagellate has been found to be abundant in association with Antarctic sea ice (Smetacek *et al*. 1992; Okolodkov 1999). Besides, autotrophic dinoflagellates are prominent and diverse in the Arctic, (Okolodov 1996) suggesting that temperature per se is not the only constraining factor for this group.

It has been suggested that deep mixed layers and higher turbulence are unfavourable for dinoflagellates (Margalef 1978). Since low light and high turbulence levels are characteristic of the ACC, one would expect these groups to be more abundant in the protected, shallow mixed layers around the continent. Since this is not the case, these are unlikely to be the constraining factors for their scarcity in the ACC. Interestingly, unexpected phylogenetic diversity in the picoplankton, including dinoflagellates, of the Southern Ocean has been recently described (Moon-Van Der Staay *et al*. 2001). This finding implies that adaptation of picoplanktonic, eucaryote lineages to the environmental conditions of this geologically young environment has been more successful than in the nanoplankton. Whether

the absence of small cyanobacteria has enabled other, albeit eucaryotic, groups to speciate within the picoplankton can be ascertained on the basis of detailed, comparative studies of the roles of picoplankton in different oceans.

The solitary flagellate stage of *Phaeocystis antarctica*, also a haptophyte like the coccolithophores, is a dominant member of the nanoplankton throughout the Southern Ocean. Haptophytes outgas DMS in large quantities to the atmosphere, so its precursor molecule DMSP must play a similar role in their physiologies. In terms of its role in the regional sulphur cycle, *Phaeocystis* might be regarded as a "replacement" for coccolithophores ubiquitous in other oceans, implying that the haptophytes represent a functional group. Alternatively, the haptophytes have developed defence mechanisms against certain classes of predators that enable their better survivorship relative to other groups. DMSP has been reported to function as an antioxidant (Sunda 2003) but it has also been implicated as an herbivore deterrent (Wolfe *et al.* 1997). So the molecule might play a dual role in bottom-up and top-down selection. Interestingly, coccolithophores and *Phaeocystis* are the only groups other than diatoms that have been reported to dominate open ocean blooms (Verity & Smetacek 1996). We return to this point later.

Mortality apparently constrains pico- and nanophytoplankton stocks at average chlorophyll *a* concentrations around 0.2 mg Chl *a* m^{-3} during the growth season. Typical primary production rates are in the order of 200 mg C m^{-2} d^{-1} which, depending on the mixing depth, is equivalent to cell division rates from 0.3 to 1 per day. It follows that mortality rates must be equivalent. In the presence of adequate resources (light and nutrients) primary production rates increase significantly but biomass of this size class does not. So the growth rates of grazer populations must keep pace with that of their food. Not all phytoplankton cells represent equally attractive food sources; besides, some defend themselves, others escape attack by actively swimming away (Tillmann & John 2002). So severe grazing pressure will select for less palatable and less defended species, which in turn will accumulate till their specialized predators have built up their respective population size and so on. Perhaps this type of species specific predator-prey interaction is a powerful force in driving annual species succession, an ubiquitous, albeit poorly explained phenomenon characteristic of all pelagic systems (Margalef 1978). Possibly, pathogens also play a role in constraining the biomass of small-celled phytoplankton and driving species succession, although there is as yet little evidence for this. We return to this evolutionary „arms race" (Smetacek 2001) later.

Bacteria

Bacteria differ from phytoplankton in their energy source which is organic matter instead of light, but share two crucial properties with them: both take up dissolved substances and are capable of synthesizing organic building blocks such as amino-acids from inorganic nutrients. As a result, growth rates of both groups can be limited by the element in shortest supply, i.e. nitrogen or iron. Bacteria take up dissolved amino-acids and sugars (monomeres) which they cleave from complex proteins and carbohydrates (polymeres) respectively, by means of exoenzymes. The concentrations of amino-acids are always vanishingly small which indicates that uptake of desirable molecules by the bacterial stock is extremely rapid. It follows that resource competition within the bacterial assemblage for labile, dissolved organic matter will be intense. But this is not true for particulate substrates which accumulate in the form of detritus (dead cells, faeces, slime flakes etc.) in microbial dominated systems indicating that its production rate is greater than its utilisation rate. The organic carbon in this particulate detrital pool generally exceeds that in the living organism pool by far. Evidently, competition for polymeres in detritus, mediated by the efficiency and quantity of exoenzymes deployed, seems to be much less intense than for monomeres, mediated by molecular diffusion and bacterial uptake surface. Unlike the recalcitrant molecules comprising the dissolved organic carbon (DOC) pool, which persist in sea water for hundreds of years, the particulate detritus is broken down within days or weeks, implying that it could also be broken down in hours if more exoenzymes were at work. Since organic particles are heavier than their medium, detritus will be sinking in accordance with Stoke´s law. The higher the breakdown rates the lower the sinking losses. So what factors control the breakdown rates of particulate detritus in sinking matter?

As mentioned above, bacterial growth rates can be limited by iron resulting in accumulation of iron-poor, albeit energy-rich organic matter in surface water. But alleviation of limitation does not result in bacterial biomass build-up, i.e. bacteria do not „bloom“ the way phytoplankton do. Bacterial numbers in the surface mixed layer during the growth season are remarkably constant oceanwide, ranging around one million cells/ml (Fenchel 2002). Their division rates are a function of resource supply but, as in the case of the microbial phytoplankton, higher growth rates are marginally reflected in abundance, so growth is balanced by mortality. As we shall see below, bacterial numbers are kept in check by grazing of minute flagellates that in turn are eaten by larger ciliates and flagellates (Fenchel 2003). Recently, the role of bacteriophages (viruses) is emerging as an equal if not more effective

control on bacterial numbers, also in the Southern Ocean (Pearce & Wilson 2003). The relative roles of pathogens and predators in constraining bacterial numbers are yet to be established but there is little doubt that both will be working in tandem (Pearce & Wilson 2003).

The taxonomical composition of bacterial assemblages is currently coming to light by application of molecular genetic methods. Preliminary results indicate that Southern Ocean pelagic bacteria have much in common with the bacteria from warmer oceans implying adaptation of extant taxa to lower temperatures. Thus the cosmopolitan bacterial clade SAR11 is also dominant in the ACC, indicating successful adaptation to a temperature range of ~25°C (Morris *et al*. 2002). This ubiquity of the same clade across such a broad range of growth environments suggests that variation in bottom-up factors (growth rates) plays a lesser role in determining dominance status than top-down factors (longer survivorship). The results of the iron fertilization experiment EisenEx carried out in the ACC support this hypothesis. The composition of the bacterial assemblage did not change during the three weeks of bloom development despite a significant change in the growth environment to which they responded by increasing exoenzyme production and growth rates (J.M. Arietta unpubl. data). Apparently the mortality environment is less variable, as much the same types of pathogens and predators are present throughout the ocean.

If bacterial mortality were lower, more bacteria would mean more exoenzymes, hence higher breakdown rates of detritus and more biomass in the microbial network which could be channelled to higher trophic levels. The end effect would be a weaker carbon pump, lower food supply to the deep sea and the benthos and less sequestration of CO_2 in the ocean interior. But bacterial numbers are at near constant values throughout the ocean which must represent a threshold maintained by density-dependent grazing pressure and infection by pathogens. The interesting point is that the overall slowdown in physiological rates as also increase in viscosity with declining temperature down to the freezing point is not reflected in a corresponding trend in the relative proportions of the components of the microbial community (Lochte *et al*. 1997). So the efficiency of the biological carbon pump, integrated over the entire growth season, is independent of temperature because density dependent mortality is constant.

Protozooplankton

Small, heterotrophic nanoflagellates (HNF, < 5 µm), dominated worldwide by a few tens of species belonging to two widely separated lineages (Fig. 2), are believed to be the main

agents keeping picoplankton populations in check (Fenchel 1987). The kinetoplastids (better known as bodonids) represent an ancient lineage widely separated from the other group, the choanoflagellates which belong to the comparatively recent metazoan lineage. Since picoplankton belong to the earliest life forms and bodonids to the earliest eucaryote lineages it is reasonable to assume that co-evolution has occurred throughout an immense time span. However, the comparatively late arrivals, the choanoflagellates, have evolved into the bodonid niche successfully. They differ from all other HNF in the umbrella or parachute like structures (costa) within which they live. The stiff rods of these "houses" are made of silica, which, like that of the small pennate diatoms, will be recycled rapidly. This house can be interpreted as a mechanism improving the efficiency of food capture. On the other hand, the house is much larger than the cell suspended inside it, so could serve as a deterrent to other small ingesting predators. The absence of cyanobacteria seems to have little effect on the composition and function of the HNF of the Southern Ocean which are very similar to those of other oceans. Apparently both HNF lineages have adapted equally well to the cooling of the Southern Ocean.

The major grazers of the HNF and nanophytoplankton comprise small ciliates and dinoflagellates ranging around 5 – 10 µm in size. However, not much is known about interaction within the small flagellates, and a sizeable proportion of primary production could well be burnt up within the HNF. Clearly this would be the most efficient way of recycling elements within the surface layer. But the next size class of grazers, the afore mentioned ciliates and dinoflagellates, also build up substantial biomass when productivity of the microbial food web is boosted. They in turn are grazed by still larger protozoa of the microzooplankton (20 – 200 µm) but also by the larvae of copepods and the adults of the ubiquitous small species which also feed directly on HNF (Turner *et al*. 2001, Juergens *et al*. 1996). So while much of the bacterial biomass production is remineralised within the small size classes, some of it is indeed passed up to higher trophic levels.

Ciliates are ubiquitous grazers of small flagellates in all aquatic environments, from puddles to open ocean yet only about 2000 species are reported to occur world-wide (Fenchel & Finlay 2002). This indicates remarkably effective adaptation to a broad range of environmental factors. A case in point is the autotrophic ciliate *Mesodinium rubrum* which forms dense blooms across a range of habitats from the poles to the tropics and from brackish water to open ocean (Lindholm 1985). Most pelagic ciliates belong to a single group, the choreotrichs (Fig. 3). They are bullet shaped, rapid swimmers ranging in size from 5–100 µm and tend to have to some degree mechanical protection. One subgroup, the tintinnids, live in

vase-shaped houses. Ciliates feed by collecting and ingesting food items whole so there is an upper limit to prey size. Besides, they are deterred by spines and bristles (Verity & Villareal 1986).

In contrast heterotrophic dinoflagellates have evolved an array of formidable techniques to capture and feed on prey of similar size and even much larger than themselves (Fig. 4). Dinoflagellates contribute significantly to diversifying the mortality environment (Jacobsen 1999). They are slow-moving but can capture much faster ciliates by stunning them. A range of species have developed feeding-tubes (peduncles) with which they pierce their prey and suck out the cell contents. This strategy enables feeding on much larger cells but appears to be only effective with soft-bodied prey. Peduncle feeding on diatoms has only been reported from *Paulsenella chaetoceratis* feeding on one genus of diatoms (Schnepf *et al.* 1988). The diatom-feeding dinoflagellates do so by enveloping cells or even long, spiney chains much larger than themselves with a feeding veil (pallium) within which the prey item is digested. The empty frustules are discarded when the feeding veil is retracted. These dinoflagellates are protected by an armour of thick cellulose-like plates but the extruded feeding veil will be vulnerable to attack. Dinoflagellates without rigid armour are highly deformable and ingest prey of equal or even larger size, taking on the shape of the prey item in the process. Unlike the other feeding modes, there will be an upper limit to particles that can be ingested.

The two most common groups of larger protozoa – the ciliates and dinoflagellates – differ fundamentally in their feeding strategies, hence organisms of the same size class will not be competing for food, indeed they will also be preying on each other. Ciliates use feeding currents to capture prey items much smaller than themselves. Dinoflagellates are powerful predators that tackle large prey items which they capture individually. Ciliates multiply by a form of budding and tend to have higher growth rates. Both groups can equal or even exceed the growth rates of phytoplankton. However, both groups are preyed upon by the same predators dealt with further below.

Large phytoplankton of regenerating systems

Whereas pico- and nanoflagellates contribute the bulk of phytoplankton biomass in the iron-limited Southern Ocean, microplankton diatoms (> 20 µm) are a universal feature. They are highly diverse in shape and size and comprise over a 100 species from a broad range of the common diatom genera. Some of the species are cosmopolitan but the Southern Ocean has the

largest percentage of endemic species of any ocean region (Priddle & Fryxell 1985) suggesting that specific adaptation to the environment has occurred. It should be mentioned that we are referring to species differentiated under the microscope, i.e. on the basis of their morphology. Genetic analyses are sure to yield large numbers of „cryptic“ species within morphologically identical frustules. These will represent environmental adaptation in various forms, e.g. to temperature or low light levels, at the physiological but not the morphological level. A reason why morphology is conserved in some cases but not in others can be sought in adaptation to the mortality environment.

Prior to discovery of the microbial system it was believed that the zooplankton stocks present in impoverished waters depended on larger phytoplankton (diatoms) for sustenance (Hart 1932). That zooplankton stocks of similar magnitude were also present in waters more or less devoid of net-caught plankton such as in the Sub-Antarctic Zone (Hardy 1967) should have indicated that they had other sources of food. Indeed, some large zooplankton such as salps are able to thrive on the microbial community by virtue of their specialised feeding mechanisms. The small copepods characteristic of recycling systems on the other hand preferentially prey on the larger protozoa (Stoecker & Capuzzo 1990; Sander & Wickham 1993), particularly ciliates, based on the microbial community (Atkinson 1996; Lonsdale *et al*. 2000). Clearly, smaller diatoms will be a welcome addition to their diet. So, grazing pressure on diatoms will potentially be high both by protozoa and metazooplankton based on the microbial communities.

Because of their low concentrations over vast regions of the Southern Ocean, diatoms have tended to be overlooked in the small-volume water samples of recent studies. But in restricted regions along fronts where iron, albeit at low concentrations, is introduced to the surface in upwelling water, diatom biomass can exceed that of the microbial phytoplankton by a factor of 2 or more (Klaas 1997, Smetacek *et al*. 2002a). About 6 species from disparate genera tend to contribute the bulk of biomass. They are all giants of their respective genera and of eye-catching appearance due to their extravagant use of silica (Si:N >2) (Fig. 5). Since it appears that these species are responsible for the bulk of silica depletion across the ACC, albeit in different ways, we shall describe their distinguishing features separately.

Fragilariopsis kerguelensis. Some 13 species of *Fragilariopsis* occur in the Southern Ocean but this species is conspicuouly different by virtue of its thickly ribbed frustules and its curved, ribbon-like chains that can be over 100 cells long under favourable growth conditions. It is most common in the Antarctic Zone (AZ) and along the APF with peak occurrence during spring and autumn (Hart 1934). At other times (winter and mid-summer) chains are

shorter and up to 50% or more of randomly distributed frustules in a chain can be empty (El-Sayed & Fryxell 1993, P. Assmy & J. Henjes unpubl. data). The fate of the missing cell contents is unknown. Despite the extra ballast due to thick frustules and empties in the chains, chains with live cells manage to remain in suspension in the mixed layer. Observations of the same water mass during the iron fertilization experiment SOIREE even indicated positive buoyancy of the population (Waite & Nodder 2001). This is truly an enigmatic species warranting further dedicated research. The frustules have been shown to be remarkably strong and can withstand five times higher pressure loads than those of a centric diatom of similar size (Hamm et al. 2003). The species can dominate diatom biomass in the PFZ at silica concentrations <5 mmol m-3 indicating that high ambient concentrations are not a prerequisite for making thick frustules (Freier *et al*. 2000). This suggests that mechanical protection afforded against smaller hence weaker crustacean zooplankton by the thick frustules is part of the survival strategy of this species. However, its absence from the seasonal sea-ice zone (SIZ) has been attributed to its inability to survive enclosure within the confines of the brine channels in sea ice (Scharek 1991). *F. kerguelensis* frustules contribute by far the bulk of the silica ooze accumulating under the ACC (Zielinski & Gersonde 1997).

Thalassiothrix antarctica is a needle-shaped pennate that can be 5 mm long (the longest of all diatoms) but only 4 µm wide. It occurs singly or in bunches joined together at one end when growth conditions are favourable. It also has exceptionally thick frustules (1 µm wide) ornamented with prominent sharp barbs 1 µm long. The overall appearance reflects defence against grazers, although the function of the barbs is unknown but speculated on below. The frustules of this species are also prominent in surface sediments of the ACC but most are broken. In several regions of the ocean, layers of densely packed mats of intact *Thalassiothrix* spp. frustules have been found, identifying this genus as a major silica sinker (Kemp *et al*. 2000). The vast deposits of dense mats from the Neogene of the eastern Equatorial Pacific suggests that this region was the major ocean sink of silica before opening of the Drake Passage resulted in its shift to the ACC (Kemp & Baldauf 1993).

Thalassionema nitzschioides is perhaps the most widely distributed diatom (Raymont) in the ocean but the form common in the ACC has more robust frustules than those of the subtropical gyres. The rod-shaped cells are joined at the ends at perfect right angles giving the chains their characteristic zig-zag appearance. Its frustules are also common in the ooze belt (Fischer *et al*. 2002*)*.

Corethron pennatum (formerly *criophilum*) is perhaps the most eye-catching diatom of the entire Southern Ocean. In striking contrast, its N. Atlantic counterpart *C. hystrix*, albeit of

similar morphology, is fragile and easily overlooked in plankton samples. The cells of *C. pennatum* range in diameter and length from 60 – 80 and 20 -200 µm respectively. The cells carry rings of long, stout, barbed spines that sweep back like the ribs of an half-opened umbrella. The spines are hinged at the base and the function of the auxiliary ring of short, hooked spines at one end of the cell is to open the long spines following cell division so that they click into the desired angle of 45° set by the hinges (Crawford *et al*. 1997). This complex engineering demands a specialised function and deterrence of small ingestors appears to be the most likely purpose. Because the spines are laid down inside the frustule of the parent cell, reduction of cell diameter proceeds more rapidly in the course of cell division than in other diatoms. The original size is restored by auxospore formation following a sexual phase where the cell contents are converted into gametes and the frustules discarded. Mass sinking of empty frustules following a sexual phase was observed by Crawford (1995) who suggested that such events could explain the monospecific layers of this species found in the sediments of some regions of the Antarctic. Apart from such restricted layers, this species is generally absent from the ooze. Since this species occurs throughout the Southern Ocean, including the SIZ, and frequently dominates diatom biomass, it must play a major role in transporting silica out of the surface layer.

Chaetoceros spp. of the subgenus *Phaeoceros*. The species of this subgenus differ from those of the other subgenus *Hyalochaetae* by their larger size and the presence of chloroplasts inside the long spines that extend outward from the 4 corners of the rectangular cells. Medium-sized species of the *Hyalochaetae* are prominent in „regular“ blooms and dealt with separately below. Several, morphologically similar species of *Phaeoceras* prominent in the ACC are again the largest of the genus with formidable spines up to 1 mm (Hart 1942) long that tend to be covered with minute barbs. These species do not make resting spores and presumably survive the winter as vegetative cells. Because of their large size and long spines they will deter protozoan grazers and also smaller copepods. Their frustules are absent from the sediments but they are likely to be a significant component of vertical flux from the surface layer because the morphology lends itself to entanglement in sinking aggregates.

Pseudonitzschia spp. This cosmopolitan genus comprises many species that are difficult to differentiate under the light microscope. The cells are needle-shaped and joined at their tips to form long chains that can be over 1 mm long. This genus is prominent in the entire Southern Ocean and the largest species occur in the ACC (Tomas 1996). The frustules are not thick but their high surface to volume (s/v) ratio will confer a Si:N ratio >1. This genus is absent from the sediments but is likely to contribute significantly to silica flux from

the surface layer because of its widespread dominance along frontal regions (Smetacek et al. 2002a). *P. lineola* contributed ca. 25 % (50% of abundance) of the total biomass at the end of the iron fertilization experiment EisenEx (P. Assmy & J. Henjes unpubl. data).

Thalassiosira lentiginosa is a large, heavily silicified species of this cosmopolitan genus. It is widespread in the ACC but has, to our knowledge, not been reported to contribute significantly to diatom biomass. However, its frustules are prominent in the ooze belt, indicating that they are exceptionally resistant to dissolution.

The diverse assemblage of species comprising the background diatoms consist of smaller and more fragile forms than the giants described above. Because of their more favourable surface to volume ratio they should be more efficient than the giants at taking up the limiting nutrient, iron, from the recycling pool. But the range of morphologies represented, also by the giants, does not indicate competitive advantage of a particular form, such as the needle shape, to maximise the surface/volume (s/v) ratio. So the rules of uptake efficiency do not explain why the largest and most robust cells accumulate the most biomass. Invoking selective grazing as the decisive ecological principle is more logical. In this scenario, the smaller diatoms will be subject to much heavier grazing pressure than the giants, which, because of lower mortality, will be sequestering nutrients from the recycling pool in the course of time. This scenario was postulated to explain stable patterns of dominance of three of the giant species maintained in the face of heavy grazing pressure of small copepods along the APF over a 4 week period of austral summer (Smetacek *et al*. 2002b). Although each of the giant dominants contributed >30% of total phytoplankton biomass in their respective zones, it was apparently the smaller size classes, including the background or recycling diatoms, that bore the brunt of the grazing pressure. This would be an extension of the principles balancing microbial food webs into the size class of microplankton and zooplankton, suggesting that the bulk of the ACC diatom species are part of the recycling system.

Interestingly, the same or morphologically similar species are prominent in the HNLC region of the North Pacific. The role of *Fragilariopsis kerguelensis* is played by *Denticulopsis seminae* which, together with *Thalassiosira lentiginosa*, also contributes substantially to the diatom ooze accumulating under this region (Sancetta 1982). The genera *Corethron* and *Pseudonitzschia* are also prominent in the surface layer. The striking similarity in ecosystem structure of the two high-latitude HNLC regions is further evidence that nutrient uptake efficiency, hence growth rate, is not the decisive factor determining the contribution of the various genera to the microplankton component of recycling systems. That the frustules of

these species are indeed the major contributors to vertical flux was demonstrated by sediment trap catches (Takahashi 1997).

It should be pointed out that in the nitrate and silica-limited systems of warmer waters, large, armoured autotrophic dinoflagellates (e.g. *Ceratium* spp.) seem to play a similar role as the giant diatoms in HNLC waters. Their armour is of thick cellulose plates and energetically more expensive to make than silica shells, but their presence too can well be explained by the mortality environment. Large diatoms of various genera also occur in the warm water sphere and contribute significantly to both carbon (Scharek *et al*. 1999) and silica flux (Kemp *et al*. 2000). It has been suggested that these large diatoms are part of an opportunistic shade flora that build up biomass when nutrients from deeper water are mixed upwards following storm events (Goldman 1993, Kemp *et al*. 2000). However, smaller phytoplankton would have a competitive advantage in such a scenario. So it is likely that lower mortality rates due to effective defences rather than higher growth rates is the main factor determining the size of the giant diatom populations (Smetacek 2000). An exception would apply where giant diatoms carry out vertical migration to take up nutrients from subsurface layers because they would sink and rise faster than smaller cells if they could regulate buoyancy (Villareal 1999). Little is known about buoyancy regulation in diatoms other than that they are capable of it. The quantitative significance of this strategy is currently not established.

Metazooplankton grazers

Up to the early nineties it was believed that phytoplankton biomass accumulation in HNLC regions was controlled by grazing pressure, implying that this factor alone could suppress bloom development (Parsons & Lalli 1988). Differences in the life cycles of the dominant copepods in the North Pacific and North Atlantic were suggested to be the prime cause for a recurrent spring bloom in one ocean and its absence in the other (Parsons & Lalli 1988). The differences in overwintering strategies of the copepods were related to differences in the depth of winter mixing (Evans & Parslow 1985). In a later version, Frost (1991) invoked protozooplankton as an added factor in the grazer-control hypothesis. Interestingly the annual cycles of zooplankton biomass and the relative contribution of the different groups in the two oceans vary by less than a factor of two whereby biomass of the N. Pacific zooplankton is higher than in the N. Atlantic (Parsons & Lalli 1988). This indicates that oceanic zooplankton are geared to the recycling system and that the diatom bloom does not have a lasting effect on them.

All major zooplankton groups are represented in the Southern Ocean implying that the grazer populations have adapted wholesale to the cooling process. As in other ocean regions, copepods are by far the most abundant zooplankton and generally contribute >50 % to total metazoan biomass, followed by euphausiids and salps (Voronina 1998). Pteropods and appendicularians can be regionally and seasonally important but are not considered here. All these groups feed on phytoplankton and protozoa directly, albeit with widely differing feeding modes. Copepods are the most selective as they capture, examine and ingest prey items individually at rates faster than can be followed by the human eye (Strickler 1982). The other herbivores are much larger and collect suspended particles indiscriminately by using different techniques to concentrate them from the water.

Pelagic copepods span an order of magnitude in size and, despite the many species that occupy different ecological niches, they are of strikingly similar shape (Huys & Boxshall 1991). It has been argued that the evolutionary success of the copepod shape is due to its superior escape ability (Verity & Smetacek 1996). Approaching predators are sensed by the long antennae and powerful paddle-shaped thoracic appendages enable the copepods to jump out of the way (Yen 2000). Copepods crush their food prior to ingestion with powerful mandibles edged with silica that are suggested to have co-evolved with diatoms. Before the discovery of the important role of protozoa, it was believed that copepods were the main grazers of diatoms. But it has since been established that copepods tend to prefer large protozoa, particularly ciliates, to diatoms (Stoecker & Capuzza 1990, Gifford 1991). The fact that copepod faeces are generally packed with crushed but also whole diatom frustules does not mean that diatoms are the sole diet as most protozoa, with the exception of tintinnids and armoured dinoflagellates, do not leave identifiable remains in the faeces.

The cosmopolitan genus *Oithona* is the smallest and most ubiquitous. Its feeding apparatus indicates that it selects food items in the microplankton range, although its small size will set an upper limit to the particles it can handle and the frustules it can crack (Hamm *et al*. 2003). Although *Oithona* feed on a wide range of particles, they have been shown to also feed on faecal pellets of larger copepods (Gonzales & Smetacek 1994). This behaviour will retard vertical flux from the mixed layer and contribute to the recycling system. Three medium-sized species (*Rhincalanus gigas*, *Calanoides acutus* and *Calanus propinquus*) contribute the bulk of copepod biomass south of the APF, although several other smaller species can be much more abundant. Interestingly, only one of these species (*C. acutus*) has a life cycle in which the final copepodite (subadult) stage obligately overwinters at great depth. Overwintering in the other species seems to be more flexible with parts of the population

present in an active stage in the surface layer throughout the winter (Schnack-Schiel & Hagen 1994).

Copepod biomass appears to vary widely and generalisations are difficult to draw. Foxton (1956) reported that average biomass was relatively high throughout the year suggesting that recruitment more or less balanced mortality. However, larval stages of most species do not exhibit marked seasonality indicating that recruitment tends to be diffuse and not by distinct, age-class cohorts. Grazing pressure as a function of available food also appears to vary widely. By and large grazing pressure decreases with increasing food supply, implying that grazing pressure is high and about equivalent to primary production during HNLC conditions. The fact that the biomass of large, tough diatoms increases when iron limitation is alleviated, indicates that selective grazing pressure of copepods is a major factor shaping species dominance of the phytoplankton assemblage.

It should be pointed out that the published estimates of copepod biomass are unreliable for methodological reasons. Copepods and euphausiids are capable of escaping from nets and estimating biomass from acoustic data is highly dependent on the calibration factor used. Thus signals from the same copepod population can vary by a factor 10 depending on whether the animals are positioned vertically or horizontally. A survey carried out with an optical counter mounted on an undulating instrument (SeaSoar) towed at high speed (8 kn = 4 m sec^{-1}) compared to the speed of vertical net tows (1 m sec^{-1}) revealed copepod biomass of 7 gCm^{-2} along the APF (Pollard *et al*. 2002). This was in the same range as phytoplankton biomass of which 75% was contributed by giant diatoms (Smetacek *et al*. 2002a) and provides an impression of the important role of zooplankton in recycling nutrients and selecting species composition of the plankton.

Different euphausiid species are characteristic of the different water masses with *Euphausia crystallarophias*, *E. superba*, *E. frigida*, *E. longirostris and E. vallentini*, occupying the Coastal Current, the oceanic SIZ, the AZ and the PFZ respectively. Only *E. superba* appears to play a major role as a grazer of phytoplankton, although all the above species feed in a similar fashion and are morphologically similar to it, albeit smaller. Euphausiids capture water parcels and filter them through sieve-like maxillipedes which can retain particles >6 µm efficiently. The ingested food is crushed in a muscular gizzard lined with teeth reminescent of copepod mandibles (Suh & Toda 1992). Most of our knowledge of euphausiid ecology is based on *E. superba* which is reported to feed on a wide range of food items including microplankton, copepods and other euphausiids. Swarms of euphausiids are often encountered but the percentage of the population present as solitary individuals or in

swarms is not known. Clearly, swarms will exert considerable local grazing pressure but, because of their patchy distribution, their effect on microplankton is also likely to be patchy. In any case, adult euphausiids are not likely to be deterred by the giant diatoms, with the possible exception of dense aggregations of *Thalassiothrix*.

Salps are very different to the crustacean zooplankton because, for their size, they are slow-moving and have high reproductive rates achieved by vegetative budding of adults. Although this ability should have its greatest advantage in developing blooms, salps are most abundant under the impoverished conditions prevailing in the AZ. They maintain their barrel-like shape by means of a hydroskelett and external cuticle made of cellulose and are wrongly regarded as gelatinous (Godeaux *et al.* 1998). They swim, breathe and feed by pumping water through their body by rhythmic contraction of transversal bands of muscles lining the cuticle (Madin 1974). Particles down to the picoplankton size class are collected by pressing the water though slits in their pharynx. This feeding mode is highly effective at collecting small particles indiscriminately (Le Fèvre *et al*. 1998) but its major drawback is that increasing particle loads result in clogging of the fine meshed sieve. Salps can reverse flow and flush out the unwanted particles but long cells with barbs might well be difficult to dislodge. High concentrations of large diatoms (e.g. *Corethron* spp. and *Thalassiothrix* spp.) are likely to deter salps as clogging of the pharynx will eventually result in asphyxiation (Harbison *et al*. 1986). The relationship between salps and krill in the Southern Ocean has been discussed by Pakhomov *et al*. (2002).

Zooplankton carnivores

All the major invertebrate carnivore groups – coelenterates, ctenophores, chaetognaths, polychaetes, amphipods and gastropods – are present throughout the Southern Ocean, each group represented by a few species. Hence, the overall invertebrate zooplankton assemblage is similar to that of other oceans, although the adults tend to be larger than elsewhere. However, it is the vertebrate carnivores that are very different: Surface-living planktivorous fish such as clupeids and capelin are absent and their potential food – crustacean zooplankton - is eaten by baleen whales and squid. Since all the phyla are represented, also in the coldest water around the continent, temperature per se does not appear to be the limiting factor. Besides, planktivorous fish play a prominent role in comparable regions of the Arctic: capelin and polar cod in the SIZ and herring and sprat in adjacent waters. Further, mesopelagic myctophid fish seem to thrive in the ACC. The fact that salinity is higher, hence freezing point lower, in the Antarctic is not relevant, because during glacials, when sea levels were

much lower, average salinity in the Arctic would have been significantly higher than today. Perhaps the absence of extensive shelves and estuaries, characteristic of today´s Arctic, which provide favourable breeding grounds to pelagic fish, could be a reason. But then, during glacials, the high Arctic shelves will have been much narrower and not as different to the contemporary Antarctic.

What effect a different suite of top predators has on quantitative relationships within the rest of the zooplankton is worthy of closer investigation as it will shed light on interactions shaping the structure of pelagic ecosystems in general. A recent study, carried out on terrestrial ecosystems with intact megafauna (the Serengeti), derived quantitative relationships between size classes of herbivores in relation to the major predator (lions) (Sinclair *et al*. 2003). Similar quantitative rules are likely to structure pelagic ecosystems of comparable environments. However, the reasons for absence of a given group needs to be established as they could be due to evolutionary constraints impinging on the lineage, whether physiological, behavioural or life cycle dependent as discussed in the case of bony fish. On the other hand, the reasons may be due to the food supply or the mode of food capture. For instance, the minor role of fish and their larvae in the Southern Ocean could be the reason why siphonophores, many of which feed largely on small fish, are less prominent than in other oceans (Rodhouse & White 1995). Alternatively, the high energy waters of the ACC could be detrimental to the apparently delicate tentacles of the coelenterates which seem to be more common in quieter waters further south.

Another as yet unexplored trophic link is based on the hyperiid amphipod (*Themisto gaudichaudii*) which is common in the ACC (Kane 1966). It is strikingly robust for a pelagic organism and its large eyes and grappling appendages clearly indicate it to be a visual predator of large, tough organisms. We speculate that it feeds on salps and the fact that salps are remarkably transparent suggests that they are camouflaged against highly effective visual predators. Indeed only the salp guts are rendered visible by their pigmented food, suggesting that they are vulnerable during feeding. Salps are too voluminous to be ingested by predators of similar size. This form of defence is also adopted by the better known jellies, albeit by means of firm gelatinous tissue. Salps are more like balloons of water that, once shredded, can be ingested by a predator of the same size range. The muscles and gut will be valuable food but not the cellulose cuticle. We speculate further that the main predators of the abundant amphipods are squid which, with their beaks and tentacles, are well equipped for coping with the robust amphipods. The role of squid in the Southern Ocean is enigmatic but there is no question that this group is much more abundant than in the Arctic. Is this related to the

absence or presence of large fish stocks respectively (Rodhouse & White 1995) Be that as it may, such a trophic link from microbes, salps, to amphipods and to squid has not previously been suspected. Such a food chain would be of low efficiency but could explain the presence of top predators in the iron-limited ACC.

Concluding remarks

We have seen that the microbial network in the iron-limited regions of the Southern Ocean operates in a similar manner to those of nitrogen-limited waters, despite the absence of some major groups of pico- and nanophytoplankton. The major difference lies in the accumulation of large-celled, grazer-defended diatoms with high silica demand in the silica-rich HNLC Southern and North Pacific Oceans. These diatoms sink silica but not carbon from the surface layer, although the relative roles of grazing, pathogens or natural mortality in fuelling vertical flux of frustules is yet unknown. The mode of flux, whether packed in pellets, as aggregates of living cells and detritus or as individual frustules will determine its depth and intensity. This will depend on the species composition of the assemblage and the mortality environment. The extent to which iron is recycled with the macronutrents by the surface community and eventually lost to deeper layers via the route followed by macronutrients, i.e. sinking particles, is currently not well known. Protozoan grazing has been shown to recycle iron together with macronutrients (Barbeau *et al*. 1996) but there are no studies yet on the role of metazooplankton. Indirect evidence discussed below, indicate that their grazing also contributes to the regenerating system. The fact that deep waters are impoverished in iron relative to nitrate and phosphate indicates selective loss from the water column but how this loss takes place is a key question for balancing ocean biogeochemical budgets.

Phytoplankton blooms

Along the continental margins and in island plumes, iron concentrations are much higher than in land-remote oceanic water (Martin *et al.* 1990). Under these iron-replete conditions, phytoplankton biomass is much higher than in balanced systems. With a few exceptions which prove the rule, the bulk of excess biomass is contributed by large-celled phytoplankton – medium-sized diatoms and the colonial stage of *Phaeocystis*. The secular hypothesis of (Cullen 1991) explains this ecological principle with the rule of decreasing mortality rate with increasing size: only larger phytoplankton escape the continuous heavy grazing pressure exerted on the microbial assemblages and are able to convert all macronutrients into their

biomass. As we have seen above, this general rule based on the mortality environment does seem to apply but it does not explain why only relatively few species tend to dominate biomass in their respective ecological groups and size classes. The biomass of blooms around Antarctica, as also in other ocean margins, tends to be dominated by relatively few species from disparate genera, comprising <10% of the total species assemblage. These species are medium-sized (10 – 50 µm), many have long chitin bristles or silica spines and grow in long chains. Many of the background species belong to the same genera as the dominants and occur in reasonably predictable seasonal and regional patterns. So evolution of the genera contributing bloom-forming species cannot be regarded as having been driven by selection for fast growth rates. Indeed, there is no obvious reason why episodic, dramatic increases in population size of any species should confer greater evolutionary fitness or be regarded as ecologically more „successful". So the widespread belief that bloom-forming species out-compete the background ones is meaningless in an evolutionary context because both survive equally well.

Bloom-forming species

The following diatom species (Fig**.** 6) contribute the bulk of biomass in iron-replete SO blooms, although their relative proportions vary considerably: *Thalassiosira antarctica*, *T. gravida*, *Chaetoceros socialis*, *C. curvisetus*, *C. neglectus*, *Rhizosolenia hebetata*, *Proboscia alata*, *Fragilariopsis cylindrus* and *F. curta*. *Corethron pennatum* is the only giant species observed to also dominate iron-replete, coastal blooms. With the exception of the two *Fragilariopsis* species and *Phaeocystis antarctica* none of the others contribute substantially to the assemblage growing within the ice habitat, although many of them have stages capable of overwintering in it. The bloom-forming *Chaetoceros* species belong to the subgenus *Hyalochaetae* and are much smaller than the giant *Phaeoceros* species dominating iron-limited assemblages. Several of the bloom-forming species are bipolar and also contribute to blooms in temperate shelf areas. *C. socialis* and *C. curvisetus* are cosmopolitan. *C. socialis* grows in spherical colonies that bear a superficial resemblance to those of *Phaeocystis* and can even be confused with them under low magnification. Interestingly, the two species dominate blooms in the Arctic and Hart (1934) classifies them together as indicative of open ocean, melt-water associated blooms. All bloom-forming *Chaetoceros* species are capable of converting vegetative cells into thick-walled resting spores that overwinter on the sediment surface in shallow environments, inside the sea-ice in the SIZ (Ligowski *et al*. 1992) and in

the pycnocline in the open ocean (McQuoid & Hobson 1996). *Chaetoceros debilis*, a species closely related to *C. curvisetus* contributed 90% of the cell densities induced in the iron fertilization experiment SEEDS carried out in the N. Pacific HNLC region (Tsuda *et al*. 2003).

The factors responsible for the dominance of the above species in Antarctic waters have barely been addressed because biological oceanographers tend to search for ecological principles governing biogeochemical cycles or food-web relationships above the species level of the primary producers (Verity & Smetacek 1996). Indeed the fact that blooms world-wide are dominated by relatively few species, of which many are cosmopolitan, is not widely appreciated. A corollary to this principle is that the much lower abundance of all the other species is still well above that necessary to maintain a viable population in the pelagial. It follows that only some species have evolved „boom-and-bust" life cycles defined by the annual range in population size. According to chaos theory species with widely fluctuating population size (the *r* regulated species of the logistic equation) are more prone to extinction than species with moderate fluctuation (the *k* regulated species).

The question that arises is why only some species express high growth rates when resources are replete whereas most other species do not react in the same way. Possibly, internal controls restraining high growth rates operate in the „background" species. The presence of such internal controls geared, for instance to seasonality, can be inferred from the predictable annual cycles of species succession. Since bloom-forming species belong to the same genera as many background species it would appear unlikely that the latter lack the physiological potential to achieve high growth rates. Lower growth rates could also be due to investment in defences, i.e. adaptation to the mortality rather than the growth environment. An analogy can be drawn with terrestrial plants of which only a very small percentage of the total species can be classed as agricultural weeds. Also in analogy to land plants, some species have the ability to flood the newly established, favourable growth environment with large numbers of seeding cells which leads to dominance of blooms even with mediocre growth rates. This seems to apply to *Fragilariopsis cylindrus* and *F. curta* and possibly also *Phaeocystis* (see below) which build up large stocks in the ice habitat which are then released to the water column following large-scale melting where they continue to grow (Scharek *et al*. 1994, Gleitz *et al*. 1996).

The case of *Phaeocystis* can shed light on the relationship between growth and mortality environments in speciation. At least 6 species have been identified worldwide based on morphological and genetic characteristics but only 3 species – *P. pouchetii* (Arctic), *P.*

globosa (warm waters), *P. antarctica* (Southern Ocean) – dominate blooms (Medlin *et al.* 1994). These are also the only species thus far observed to form colonial stages comprising many hundreds of cells. The others occur only as the solitary nanoflagellate stage. Clearly, the flagellates have much higher mortality rates than the cells in large colonies simply because the former are susceptible to a much larger range of potential grazers and pathogens than the latter. Although *Phaeocystis* colonies are a widespread feature of iron-replete blooms, their contribution to bloom biomass varies widely and has yet to be explained. In the North Sea, *P. globosa* tends to attain dominance in the later spring, in the aftermath of the diatom bloom following silica exhaustion. So it has been argued that as long as silica is available, diatoms out-compete *Phaeocystis* for nitrate and phosphate but following silica exhaustion, the latter utilises the remaining nutrients (Lancelot 1998). However, field observations do not support this scenario as *Phaeocystis* often dominates also in the presence of high silica concentrations, particularly in the Southern Ocean. Indeed, given the wide range of growth conditions under which *Phaeocystis* blooms have been observed, it is not evident that a specific attribute of the growth environment, such as nutrient concentrations and ratios, or depth of the mixed layer, is a prerequisite for bloom development. Besides, solitary *Phaeocystis* and various stages of colony formation including individual large colonies are ubiquitous from the ACC to the Coastal Current even though blooms dominated regularly by *Phaeocystis* occur only in the Ross Sea (Arrigo *et al*. 1999). Bottom-up control on the size of the *Phaeocystis* population would not explain why some colonies do manage to develop but the majority not. We sketch a scenario in which variation in the mortality, rather than the growth environment, in the early stages of colony growth determines the build-up of blooms.

Solitary *Phaeocystis* flagellates initiate colony formation by shedding their flagellae and secreting a capsule-like structure around themselves. The cells divide within this capsule which grows simultaneously such that the individual cells maintain much the same distance from each other in a single peripheral layer regardless of the colony size achieved. It is widely believed that this „capsule“, and the colony matrix, is gelatinous, i.e. colonies are balls of jellies that grow outward. Hamm *et al*. (1999) have shown that this is not the case in *P. globosa* and that the colonies are more analogous to distended balloons of water in which the cells are suspended in an endocolonial, peripheral network attached to the internal colony surface. The colony surface is in reality a skin which is extremely tough and plastic but, unlike a balloon, it is not elastic, i.e. it cannot be distended but is malleable. Considerable force is required to break the skin but once pierced the colony loses its organisation and disintegrates. These mechanical properties require that the skin grow over the entire surface

area and from a chemical standpoint this is entirely feasible given that the building blocks of the skin are protein-carbohydrate complexes (Hamm *et al*. 1999) that can be inserted into the matrix of the skin anywhere over the surface. The skin is highly permeable to dissolved molecules and ions up to the size of large carbohydrates (<2 nm diameter) and possibly digestive enzymes. The fact that colony cells have similar or even higher growth rates than solitary cells indicates that the nutrient supply to colony cells, despite their crowdedness, is not lower than in the case of solitaries. This rule should also apply to diatom cells in chains.

Apparently, the colony skin is an effective defence against a range of protozoa (small ingestors, peduncle and pallium feeders). However, smaller colonies will be susceptible to zooplankton grazing but the larger the colony, the smaller the number of potential grazers that can „get a grip“ on them (Verity 2000). This „grazer gauntlet“ through which the growing colonies have to pass determines how many „get through“ and attain the relative safety of large colony size (>500 µm diameter). The fact that colonies in their early stages are often found attached to the spines of large diatoms (e.g. *Corethron pennatu*m) also suggests that small colonies are vulnerable and find protection on these large diatoms. Constraints exerted by the mortality environment hence determine the occurrence of *Phaeocystis* blooms. This hypothesis can be tested by comparing the grazer communities of parts of the Ross Sea, where blooms are recurrent features, with the Weddell Sea, where *Phaeocystis* blooms tend to be sporadic (Arrigo *et al*. 1999).

Phaeocystis colonies bear superficial resemblance to the cells of large, rounded diatoms. Both are encased in tough outer coatings, that provide protection against smaller grazers. These are lined on the inside with the cells and, in the case of diatoms, chloroplasts. One is tempted to suggest that *Phaeocystis* colonies are a flagellate´s way of copying the diatom strategy. Interestingly, *Phaeocystis* colony cells are immune to viral attack in contrast to the solitary cells (Brussaard *et al*. 1999); diatom cells infected by viruses have not yet been reported although intensively looked for (G. Herndl pers. comm). Given this similarity, the question arises why there are so many more diatom species but only 3 species of *Phaeocystis*, although differentiation is estimated to have occurred 30 Mill years ago together with planetary cooling, which is ample time for speciation (Medlin *et al*. 1994). Hamm *et al*. (2003) suggest that this is due to the greater malleability of silica as compared to a spherical colony skin. The latter represents the single optimum solution given the properties required of the skin, i.e. growth across the entire surface coupled with toughness. In contrast, the cellulose armour of dinoflagellates is more like silica in its material properties and there are many more species of armoured than „naked“dinoflagellates described. The relationship

between form and function has long been ignored by planktologists (Smetacek *et al*. 2002b). Smetacek (2001) interprets the evolution of this relationship in the light of the arms race, i.e. in the context of the mortality rather than the growth environment. The example of *Phaeocystis* and the numerous cosmopolitan bloom-forming species indicates that a single species can adapt to wide range of growth environments ranging from shallow coastal to open ocean and even including sea ice. So the mortality environment is likely to play a greater role in governing abundance.

Grazing on blooms

In most regions of the world ocean grazing pressure on diatom blooms is low, so the nutritious adequacy of an exclusive diatom diet for zooplankton has been under debate since the days of Victor Hensen (1887) who coined the term plankton (Smetacek *et al*. 2002b). An alternate explanation is that teratogens present in diatoms impair copepod reproduction without affecting the feeding adults (Ianora *et al.* 2003). Aldehydes cleaved from structural fatty acids after crushing of the cells have been implicated as the relevant toxins (Pohnert *et al*. 2002). Such wound-induced chemical defences are common place elsewhere but in the plankton the only other report is that of DMSP from haptophytes (Wolfe 2000), high-lighting the orphan status of this research field in pelagic ecology in general and in the Southern Ocean in particular.

As part of the ongoing evolutionary arms race, there will be species-specific differences in the amount of toxin produced and the abilities of grazers to cope with them. Since diatoms constitute a greater proportion of the diet of *Euphausia superba* than most other zooplankton, one would expect some degree of specific adaptation. Indeed the proteases (enzymes that break-down proteins) of Antarctic Krill are exceptionally aggressive and are even used in human medicine to clean burn-wounds (Hellgren *et al.* 1991). It is these proteases that cause the rapid degeneration of krill catches squeezed in the cod end of nets – a serious problem for the krill fishery. Clearly, such efficient proteases – not present in other euphausiids - will increase the efficiency of food assimilation but it is tempting to suggest that another function could well be to neutralise the enzyme (a protein) that produces the teratogenic aldehydes in crushed diatoms as rapidly as possible.

The regions where blooms occur are the ones that support the proverbially large stocks of mammals, birds and squid based on the „food chain of the giants“. However, even if we assume a very high transfer efficiency of 10:1 from phytoplankton to krill, the annual food

demand is enormous as this simple calculation based on conservative estimates will show. Thus the food requirements of the whale stock prior to whaling (200 Mill tonnes of krill year^{-1}) indicate a total krill stock size of at least 600 Mill. tonnes, equivalent to 12 g C m^{-2} for the high productive areas (5 Mill km^2, the extent of the SIZ is 20 Mill km^2). This krill stock will require for its maintenance and recruitment an annual production of ~1.5 g C m^{-3} (ecological efficiency 10:1) over the 80m mixed surface layer, equivalent to nitrate consumption of ~19 mmol m^{-3}. But the nitrate inventory in the productive regions of today´s ocean rarely sink below 10 mmol m^{-3} indicating that maximum new production averaged over the regions is ca. 20 mmol m^{-3} nitrate. Clearly much of the food of krill will be based on regenerated production implying that much of the nitrate taken up is passed on in the surface layer as ammonia to the regenerating community.

The percentage of nitrate present in krill standing stock relative to that present in phytoplankton biomass within the water column amounts to about 400%, assuming chlorophyll concentrations of 1 mg Chl m^{-3} and a C:Chl ratio of 40. If we consider also the biomass of copepods, the total zooplankton stock turns out to be higher than expected. However, as mentioned above, current estimates of zooplankton standing stocks need to be treated with caution. Thus krill stock size estimated from net catches and acoustic surveys and extrapolated over the known krill areas, range between 1000 Mill. tonnes and 80 Mill tonnes (Everson *et al*. 1990; Nicol *et al*. 2000). Could it be that krill stock size has declined with that of the whales?

Interestingly, winter nitrate levels throughout the Southern Ocean south of the Polar Front are near constant (30 mmol m^{-3} ± 5%) implying that annual vertical flux is balanced by input of new nutrients through winter mixing. So, much of the nitrate, but not the silica, is retained within the winter mixed layer both in the productive and HNLC areas of the Southern Ocean because of sustained heavy grazing pressure and efficient recycling of essential nutrients in the surface and subsurface layers.

This above conclusion does not necessarily imply that the fate of Southern Ocean blooms is to be recycled in the surface layer, in contrast to the situation in the N. Atlantic and along continental margins with their high carbon export production. Mass sinking of living cells, spores and phytodetritus is the common fate of iron-replete blooms in these regions (Smetacek 1985) and similar sinking events are reported from the Southern Ocean (Bodungen *et al*. 1986, Wefer 1989). However, blooms in other oceans are generally terminated by nitrate or silicate exhaustion which is the exception in the Southern Ocean and has only been reported from situations where shallow surface layers promoted high accumulation rates

(Smetacek *et al*. 1992). So the effect of deep mixed layers in iron-replete, unprotected waters would be to lower accumulation rates and prolong the residence time of Antarctic blooms which might be one of the reasons why a greater percentage of bloom biomass is eventually channelled up to higher trophic levels.

Another reason discussed by Verity and Smetacek (1996), can be sought in the biology of krill which enables it to gear such exceptionally high standing stocks to the regions of high productivity. The bulk of krill faeces tend to be recycled in the surface layer despite their potentially high sinking rates (Gonzales & Smetacek 1994); were this not the case, the surface layer would soon be depleted of essential nutrients as indicated by the above calculations. However, substantial amounts of krill faeces have been collected in sediment traps below the mixed layer suggesting that their faeces can contribute to flux, possibly when the capacity of faeces utilizers is saturated (Bodungen *et al*. 1987, Gersonde & Wefer 1987). Since krill is capable of easily handling all diatoms, including the giants and large colonies of *Phaeocystis*, accumulation of the latter will reflect the extent of krill grazing pressure. This is probably the reason why the giant diatoms do not accumulate in the surface layers of productive regions where krill are the dominant grazers. So perhaps Hart (1942) was right, the reason why summer phytoplankton stocks are low in most of the coastal waters is because their stocks are grazed down. An iron fertilization experiment in a summer mesoscale eddy of the Bransfield Strait (Heywood & Priddle 1987) could help resolve this age-old question.

In striking contrast to the diatom ooze underlying the HNLC ACC, which is dominated by the frustules of giants, the sediments underlying the productive waters of the continental margin have higher carbon contents and are dominated by the small resting spores of the bloom-forming *Chaetoceros* species (Crosta 1997). These spores are also prominent in sediments throughout the productive regions of the ocean. Clearly by virtue of their resistence, these spores are by and large the only microfossils that document the presence of iron-replete blooms in overlying waters and are hence of value in palaeoceanographic research (A. Abelmann *et al*. unpubl. data). The association between *Phaeocystis* and *Chaetoceros socialis* mentioned above would suggest that spores of the latter are also a proxy for *Phaeocystis* blooms although the relationship is not obligatory.

Concluding remarks

Phytoplankton blooms are restricted in the modern Southern Ocean to waters receiving an adequate iron supply from landmasses and sediments. Evidence is mounting that the main supply route of iron to the land-remote ocean is via wind-blown dust from the continents.

How much of this mineral iron enters the pelagic ecosystem and how plankton access it is under debate. The chemistry of iron is complex and plankton organisms will have developed mechanisms to deal with it. Since much of the atmospheric dust is settled out by precipitation, the aggressive chemistry of cloud droplets should render the iron bound in mineral form more accessible to plankton uptake. Indeed, Edwards & Sedwick (2001) have shown that 10-90 % (average of 32%) of the iron in dust settled out by snow is taken up by plankton following melting. It follows that snow-deposited dust released by icebergs melting in HNLC waters is another significant source of iron, depending on the dust content and volume of the icebergs (Fig.7). Snow deposited during glacial times carried ten-fold or more dust than contemporary snow (Petit *et al*. 1999).

The development of distinct blooms with different dominant species (*Corethron pennatum*, *C. inerme* and *Fragilariopsis kerguelensis*) was recorded in the land-remote PFZ of the Atlantic Sector (10° W) over a period of several weeks in 1992 (Smetacek *et al*. 1997). Throughout this period, iceberg concentrations ranged between 5-60 icebergs in a 22 km range (van Franeker 1994) and iron concentrations throughout the mixed layer were consistently >1 nmol l^{-1} hence well above limiting concentrations (de Baar *et al*. 1995). This year was clearly exceptional as icebergs are normally rare to absent in the ACC; however a systematic record of iceberg frequency does not seem to be available. Interestingly, the first voyage of RRS Discovery in 1925-27 happened to be an iceberg year, graphically described by Hardy (1967) in „Great waters“. The ship encountered phytoplankton blooms also in the land-remote ACC including one dominated by *Phaeocystis*, the first and only such report from the land-remote ACC. Could Hardy´s impression of the great productivity of Antarctic waters have been shaped by this first encounter in an exceptional year?

The biomass produced by blooms drives the ocean´s biogeochemical pumps, hence a quantitative understanding of the mechanisms selecting species composition of bloom biomass is a prerequisite to modelling the role of productivity in regulating atmospheric carbon dioxide levels. The species composition of the bloom is bound to have an effect on the amounts and ratios of biogenic elements exported from the surface layer. For instance, species that form resting spores following nutrient exhaustion that sink out of the surface layer will export much more carbon than species that maintain their presence in the mixed layer throughout the year. The better defended of the latter will maintain higher stocks than the less defended that are vulnerable to attack by a greater range of grazers. The persistence of the bloom induced by the iron-fertilization experiment SOIREE for over a month (Boyd *et al*. 2000) could well be due to the *F. kerguelensis* population. Cell shape, size and other

properties such as presence or absence of spines affects the composition and nature of vertical flux (e.g. algal aggregates or faecal pellets). Species properties also bear on the food quality. Thus, the regions where blooms recur annually support large populations of vertebrate and cephalopod top predators. Obviously grazing must play a decisive role in determining the fate of biomass and its role in biogeochemical cycles.

Conclusions and Perspectives

The overall conclusion that can be drawn from this broad overview of the structure and functioning of pelagic ecosystems is that organism interactions played out in the mortality environment play a major role in shaping the evolution of plankton and hence biogeochemical cycles. Under conditions of iron-limitation that prevail over most of the Southern Ocean, grazing pressure is intense and the evolutionary arms race (Smetacek 2001) a driving force. Defences that range from mechanical, chemical, behavioural (escape reactions), or expressed in population life cycles are selected for. The fact that only a few phytoplankton species of widely differing shape and size and from widely disparate genera express the growth rates necessary to break out of the grazer gauntlet and form blooms indicates that this type of boom-and-bust life cycle is one of several, if not many, life cycle types prevalent in the surface layer. In the bloom-forming life cycle, population size fluctuates annually over three or more orders of magnitude. In all the other plankton species, annual fluctuation in population abundance is constrained within two or less orders of magnitude. These life cycles can be differentiated along a gradient extending from fast-growing, heavily grazed species to slow-growing, because heavily defended ones. The bulk of the species fall somewhere in between, so it is the properties of the extreme species that accumulate biomass by virtue of fast growth rates or effective defences that need to be examined more closely if we are to gain an understanding of their mechanistic role in trophic transfer and biogeochemical cycles.

Picoplankton represent the plankton populations most tightly constrained by mortality at levels below the biomass carrying capacity of the system. Under HNLC conditions, this microbial system is balanced, but an increase in resource supply in the form of iron or organic carbon is utilised by larger size classes. The stability of this system suggests that similar constraints apply to the biomass of their grazer populations. Small copepods and salps are the top grazers of the microbial food web that, in view of their high biomass in the HNLC ACC, must in turn support as yet unknown higher trophic levels. We postulate a food chain leading via amphipods to myctophid fish and squid. Studies carried out in lakes indicate that the length of the food chain is a function of the area of the lake (Post *et al*. 2000). It follows that

the vast areal extent of microbial communities of the Southern Ocean should be capable of supporting food chains extending to top predators such as squid, albeit at population sizes much lower than those in productive, iron-replete regions.

Giant, heavily silicified diatoms dominate biomass along fronts where upwelling deep water provides iron albeit in low concentrations (<0.5 nmol Fe l^{-1}). Heavy grazing pressure within the microbial community and on small and medium-sized, weakly silicified diatoms results in sequestration of the limiting nutrient in the largest and best defended species. The biomass of these diatoms is eventually recycled in the surface layer, presumably by larger copepods and euphausiids, but their robust frustules sink out of the surface layer, resulting in retention of N and P but loss of Si. These species are the main silica sinkers. The mode of loss of biogenic silica can range from individually sinking frustules, aggregates bound together by mucus (Alldredge *et al*. 1995) or compact faeces. It remains to be ascertained how the relative roles of these transport mechanisms vary with the species and what effect they have on the biogeochemistry of the water column and underlying sediments (Fig.8). It should now be clear that the differences in the Si:C ratio between iron-limited HNLC diatoms and their iron-replete counterparts in blooms is due to concomitant differences in the species composition and not necessarily an intrinsic property of individual diatom species.

A powerful new methodology to test the hypotheses outlined above are *in situ* iron fertilization experiments that enable the study of interactions within ecosystems with their full complement of grazers and pathogens. The SOIREE and EisenEx experiments carried out in the HNLC ACC indicate that the giant diatoms are the first to respond by virtue of their large stock size but none of the experiments lasted long enough to enable the fast-growing species to build-up significant biomass. In the EisenEx experiment, a single fast-growing species, *Pseudo-nitzschia lineola* contributed >25% of bloom biomass by the end of 3 weeks. This species and another fast-growing, albeit weakly silicified one – the resting spore forming *Chaetoceros curvisetus* – had accumulation rates double those of all other species present (Assmy and Henjes, ms.). Interestingly, the sibling species *C. debilis* grew at exceptionally high growth rates during the SEEDS fertilization experiment in the N. Pacific and contributed >90% of bloom biomass. The experiment ended before the fate of the bloom could be ascertained, but it is very likely that it was followed by mass sinking (Tsuda *et al*. 2003).

It is too early to pass judgement on the fate of iron-induced biomass. Indeed there is no reason to believe that an artificially induced bloom, if nurtured long enough and over a large enough area will behave differently to the blooms that occur around the continental margins. The results of EisenEx indicated a significant response by copepods within 3 weeks

indicating that the zooplankton were food limited (J. Henjes & P. Assmy unpubl. data). The effect of iron fertilization on higher trophic levels will depend on the locality and duration of the experiment. Thus iron fertilization augmenting the natural iron supply around continental margins or along iron-rich island plumes is likely to increase phytoplankton biomass and attract krill. Clearly much can be learned from experiments carried out in various regions around the Southern Ocean. There is legitimate concern over plans by geo-engineering companies to exploit the HNLC ACC as a CO_2 sink (Chisholm *et al*. 2001). The international community will have to decide how best to deal with such issues. It is the task of scientists to ascertain the long-term effects of any such climate management scheme. For instance an areal extension of the productive Peninsula plume could well have a beneficial effect on populations of top predators (mammals and birds) if they are food-limited.

The current debate on productivity of the Southern Ocean during glacial times is based on interpretations of a range of geochemical proxies that provide conflicting evidence (Sigman & Boyle 2000, Anderson *et al*. 2002). From an ecological standpoint and in view of the well established higher iron supply during glacials, it is hard to imagine that productivity will not have been correspondingly higher. The current impasse between ecologists and geochemists is most likely to be broken by introduction of new and more reliable biological proxies that can best be validated in the context of iron fertilization experiments. An added incentive to carrying out more such experiments is the ideal training ground they offer for the kind of international, interdisciplinary research demanded by earth system science investigating global change (Falkowski *et al*. 1998).

The Southern Ocean lends itself to the experimental study of the links between evolutionary ecology and biogeochemistry. Antarctica is the only continent whose entire margin lies more or less within the same climate zone, surrounded by an ocean consisting of broad, concentric bands of water rotating within their respective climate regimes. The “closed circle” nature of Antarctic ecosystems guarantees the biota evolutionary continuity within a narrow range of environmental properties. Continuity coupled with predictability will result in tighter gearing between ecosystem components and the role of top-down factors will accordingly be greater in shaping form and function of individual organisms, the life cycles of species and the patterns of community interaction than in the one-way-street regimes such as the North Atlantic. These “merry-go-round ecosystems” of the Southern Ocean are temporally more stable (in terms of carry-over of one year's success to the next), and their biota less prone to expatriation than other marine systems with the possible exception of the subtropical gyres. This situation has prevailed for millions of years and resulted in evolution of unique

adaptations, most evident amongst higher animals. In contrast the unicellular and zooplankton components of the pelagic ecosystems do not appear to differ significantly from those of comparable ocean regions elsewhere. Inter-ocean comparisons of the structure and functioning of the respective pelagic ecosystems will do much to further our ability to predict trophic cascades, assess paleoceanographic conditions and forecast future climate scenarios, with or without recourse to large-scale iron fertilization as a CO_2 mitigation measure.

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Figure captions

Fig. 1 Common representatives of the nanophytoplankton: A) pennate diatoms, B) athecate dinoflagellate, C) prasinophyte, D) chlorophyte, E) *Phaeocystis* (solitary cell), F) *Chromulina*, G) *Cryptomonas*, H) *Prorocentrum* (thecate dinoflagellate), I) *Dictyocha speculum* (silicoflagellate). Redrawn from Tomas (1993).
Scale bar = 10 µm

Fig. 2 Heterotrophic nanoflagellates (A-D) and their major predators: A), B) bodonids, C), D) choanoflagellates, E) aloricate ciliate, F) thecate dinoflagellate), G) athecate dinoflagellate. Redrawn from Tomas (1993).
Scale bar = 10 µm

Fig. 3 Ciliates: A) *Lohmanniella*, B) *Strombidium*, C) *Acanthostomella* (tintinnid), D) *Codonellopsis* (tintinnid with agglutinated lorica), E) *Cymatocylis* (tintinnid).
Redrawn from Alder (1999).
Scale bar = 10 µm

Fig. 4 Dinoflagellate feeding modes: A+B) gulp feeding showing an ingested dinoflagellate and diatom chain respectively, C+D) peduncular feeding on a tintinnid and an aloricate ciliate respectively, E+F) pallium feeding on a diatom chain and a dinoflagellate respecively. Redrawn from Jacobson (1999).
Scale bar = 10 µm

Fig. 5 Giant diatoms: A) *Thalassiothrix antarctica* (colony and the tips of a single cell showing barbs). B) *Fragilariopsis kerguelensis* (single cell in valve view and a chain of 5 cells). C) *Thalassiosira lentiginosa*. D) *Chaetoceros atlanticus*. E) *Corethron pennatum*. Redrawn from Tomas (1996).
Scale bars = 10 µm

Fig. 6. Bloom-forming phytoplankton: A*) Chaetoceros neglectus*, B) *Chaetoceros curvisetus*, C) *Pseudo-nitzschia lineola*, D) *Thalassiosira* sp., E) *Phaeocystis antarctica* (close-up of cells in colony periphery and entire colony), F) *Fragilariopsis cylindrus* (single cell and short

chain). Redrawn from Tomas (1996). Scale bars = 10 µm, except for *Phaeocystis* colony = 50 µm. Redrawn from Tomas (1996).

Fig. 7. Schematic representation of an iron-replete, carbon-sinking phytoplankton bloom system. Iron is supplied from land run-off and shelf sources and in land-remote areas from aeolian dust or melting icebergs. Vertical flux comprises diatom cells, phytodetritus or faeces of crustacean zooplankton with lower Si:C ratios than in iron-limited systems.

Fig. 8. Schematic representation of an iron-limited, silica-sinking, open ocean system. The regenerating system comprises bacteria (not shown) and pico- and nanophytoplankton together with their protozoan grazers. Salps graze on this system. The weakly-silicified "background diatoms" are grazed by salps and crustacean zooplankton (in addition to protozoa), whereas the "giant diatoms" are only grazed by large copepods and euphausiids. Vertical flux consists mainly of silica. The composition and quantitative role of salp faeces is unknown, as also the fate of salp biomass. The arrows leading from salps to amphipods and squid are hypothetical.

Fig. 1

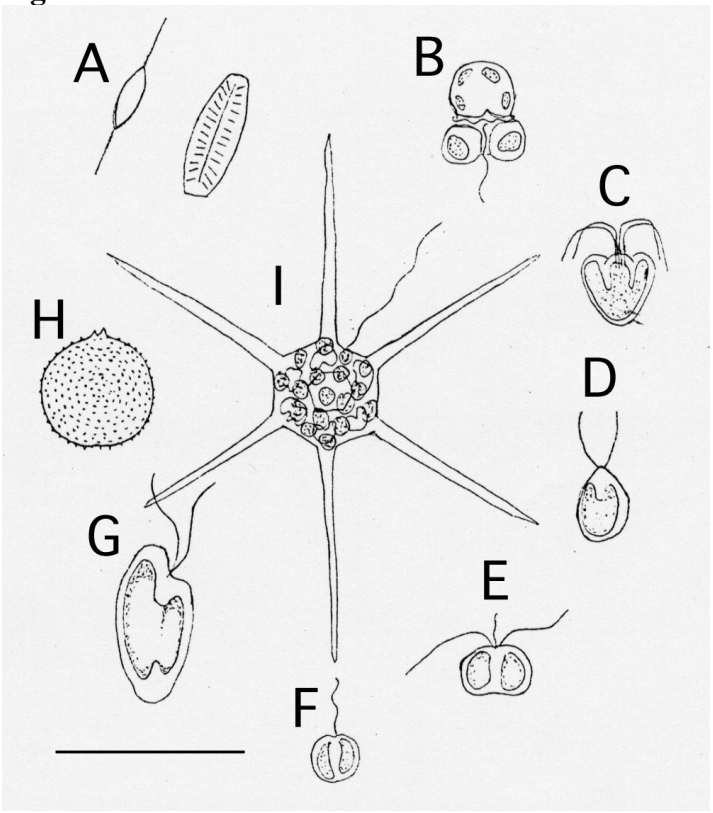


Fig. 2

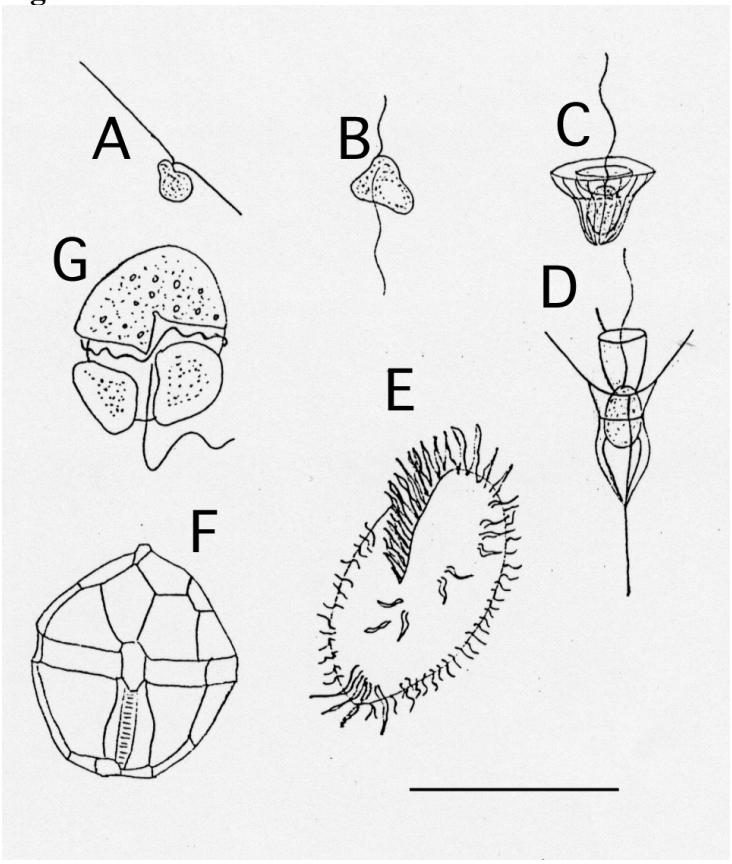


Fig. 3

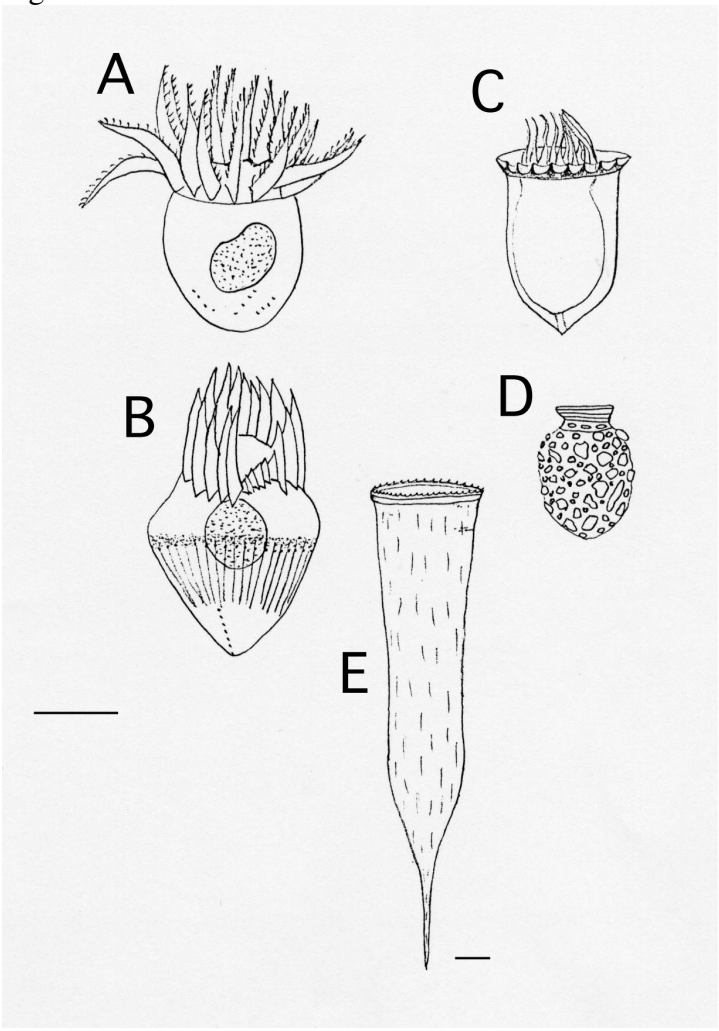


Fig. 4

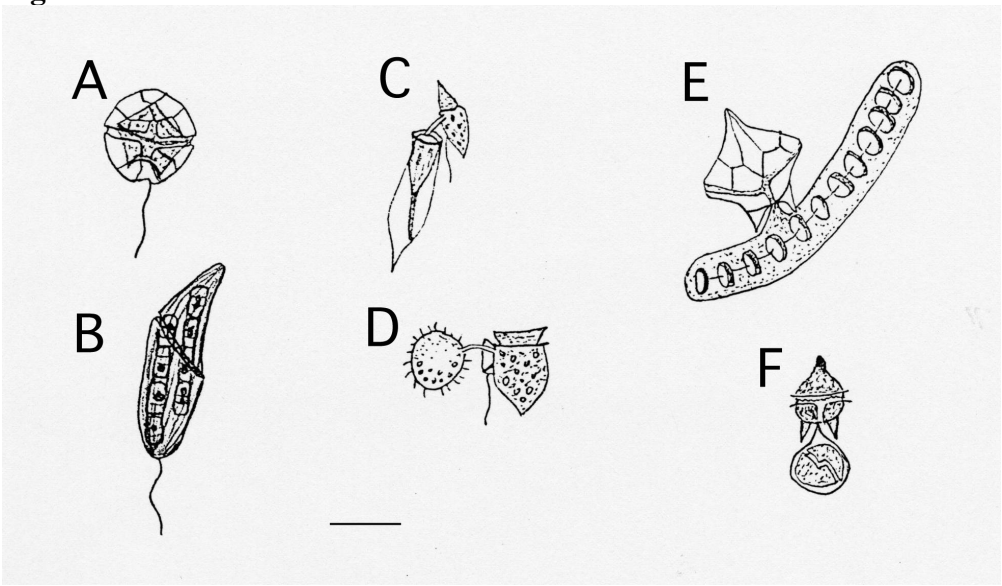


Fig. 5

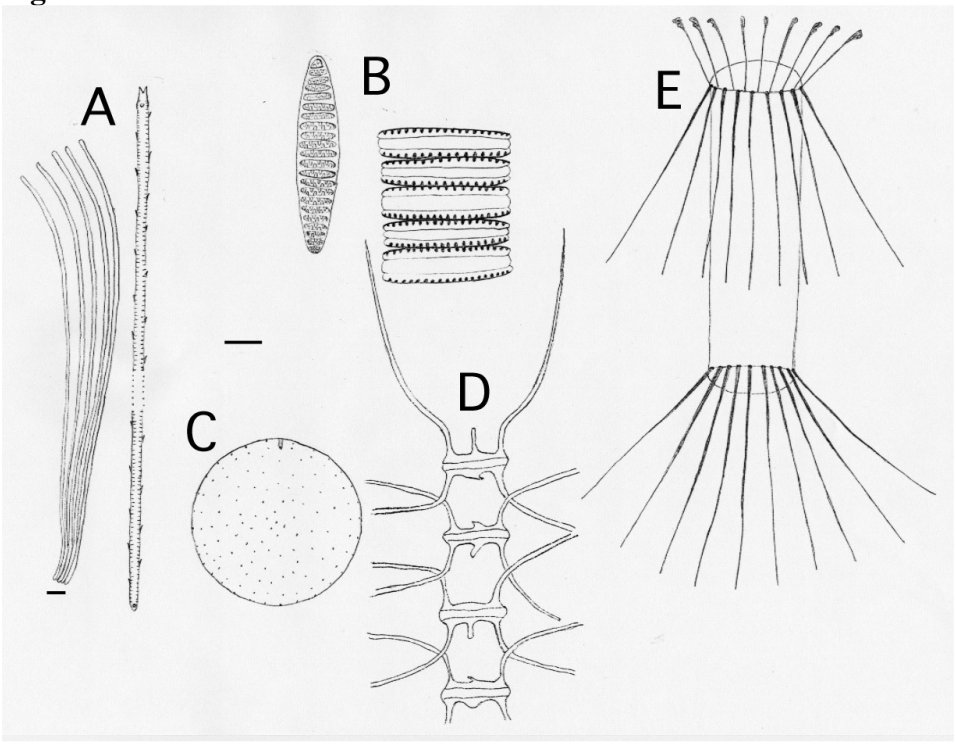


Fig. 6

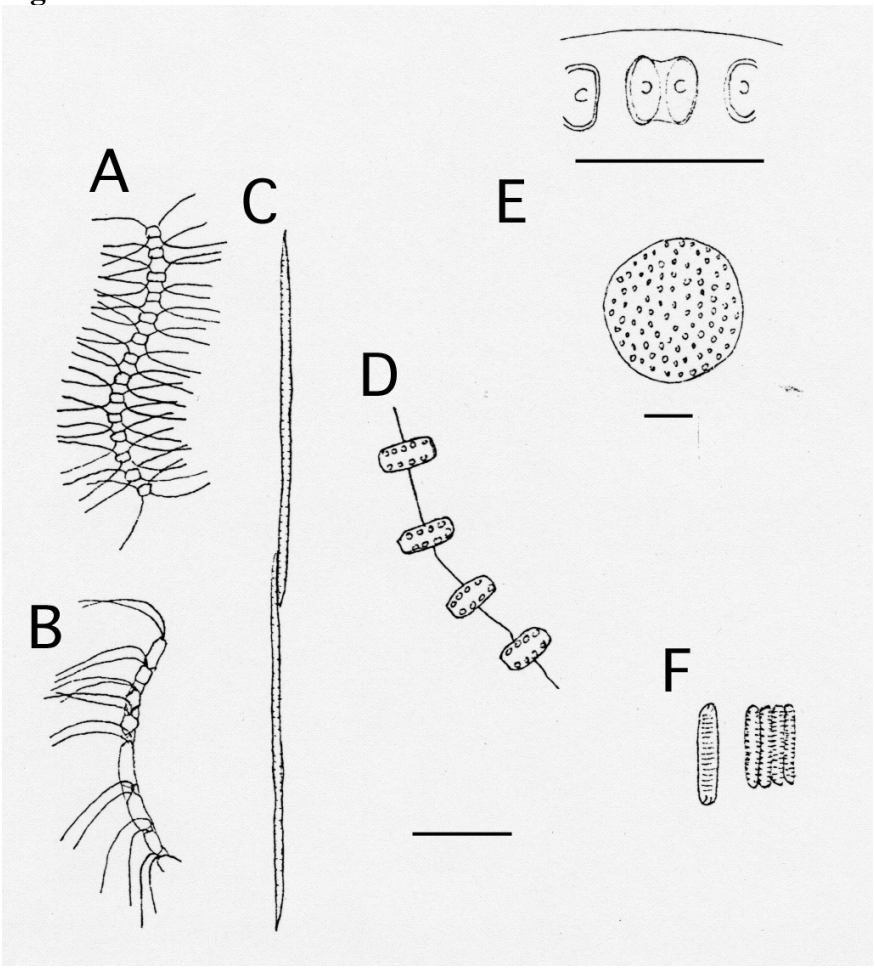


Fig. 7

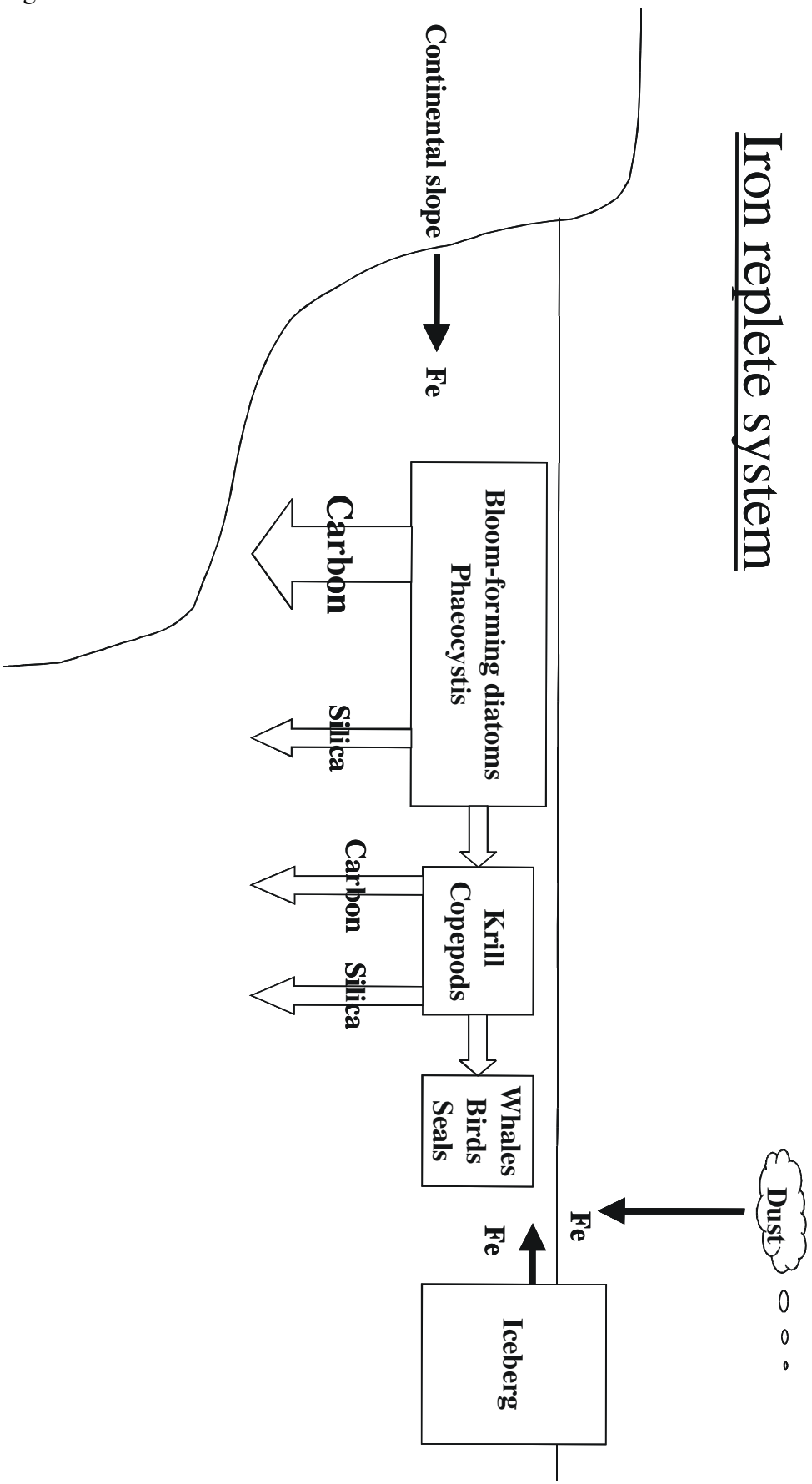
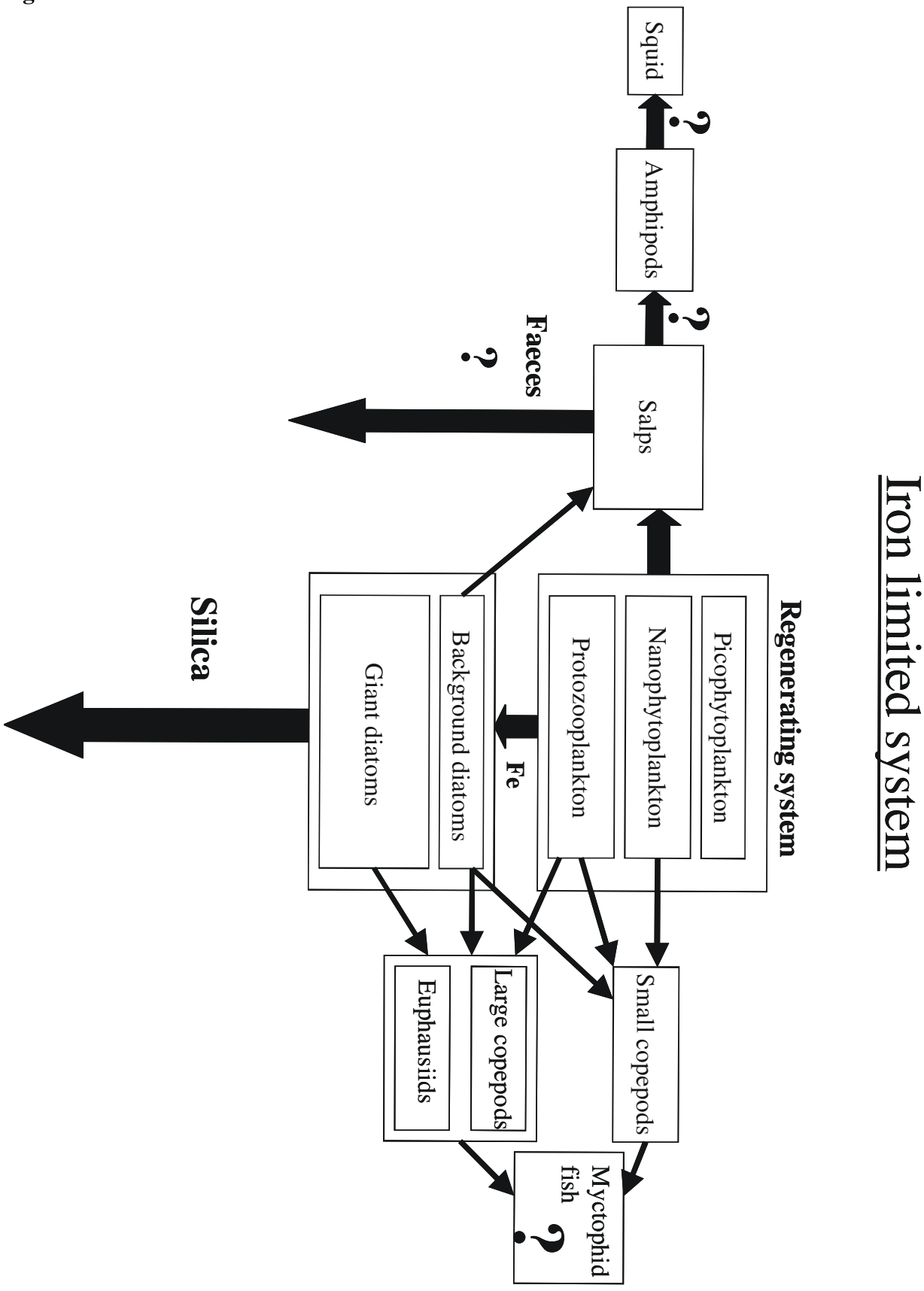

Iron replete system
Continental slope
Fe
Bloom-forming diatoms
Phaeocystis
Carbon
Silica
Krill
Copepods
Carbon
Silica
Whales
Birds
Seals
Fe
Fe
Dust
Iceberg

Fig. 8


Iron limited system
Squid
?
Amphipods
?
Salps
Faeces
?
Regenerating system
Picophytoplankton
Nanophytoplankton
Protozooplankton
Fe
Silica
Giant diatoms
Background diatoms
Small copepods
Large copepods
Euphausiids
Myctophid fish
?

5. Synthesis

5.1 Response of a phytoplankton assemblage to iron addition and its implication for plankton ecology

All three iron-fertilization experiments carried out in the ACC so far (Boyd et al. 2000, Gervais et al. 2002, Dalton 2002) induced phytoplankton blooms. These results clearly demonstrate that iron availability is the key factor controlling phytoplankton growth in HNLC regimes like the Southern Ocean. Furthermore the response of the plankton community to mesoscale *in situ* perturbation experiments sheds new light on the functioning of pelagic ecosystems and how these influence the biogeochemical cycles.

During the SOIREE experiment *Fragilariopsis kerguelensis* had by far the highest initial concentrations and dominated bloom biomass by the end of the experiment (Gall et al. 2001). During SOFEX two fertilization experiments were carried out north and south of the APF respectively inducing dominance of different floristic communities. The patch north of the APF was dominated by *Pseudo-nitzschia* spp. whereas the southern patch was characterised by a preponderance of various large diatom species (M. Gorbunov, personal communication). Detailed floristic analyses of major components of the phytoplankton assemblage from the EisenEx experiment revealed changes in species dominance that shed light on the ecology of these key species. The majority of species within the diatom assemblage could be assigned to four major response types. Diatom species that were not characteristic of any of the response types showed either no response to iron addition or even declined in abundance and constituted a background flora. The persistent species dominated the phytoplankton initially and responded to fertilization by increasing their growth rates albeit only after one week of stagnated growth. Characteristic of this response type were the heavily silicified diatoms *F. kerguelensis* and *Thalassionema nitzschioides*. Large-celled diatoms including *Corethron pennatum* and *Haslea* sp. exhibited stable medium growth rates and accounted for a considerable fraction of the total diatom standing stocks despite relatively low abundances. Another response type consisted of species that showed an initial increase and a decline thereafter. Rather small and delicate diatoms comprising *Cylindrotheca closterium* and *Nitzschia* sp. belonged to this category. In contrast, two less silicified species exhibited higher growth rates than all other diatoms that were sustained throughout the 3 weeks of the experiment. One of these species *Pseudonitzschia lineola* contributed more than 50% of the total diatom abundance and 25% of total diatom biomass by the end of the experiment. The

other fast-growing species *Chaetoceros curvisetus* – a known carbon sinker from shelf regions – built up less biomass because of its low initial population size. Other major components of the non-diatom phytoplankton assemblage, including *Phaeocystis antarctica*, phototrophic dinoflagellates, *Emiliania huxleyi* and *Dictyocha speculum*, were only a minor contribution to the iron induced bloom. Although both *P. antarctica* and phototrophic dinoflagellates exhibited an initial increase in response to iron addition they could not sustain these net growth rates and declined during the second half of the experiment. No significant difference between inside and outside waters could be detected for *D. speculum* and the enhanced iron availability in the fertilized patch seemed to have little or no effect on its growth rates. In the case of *E. huxleyi* a dramatic decrease was observed over the course of the experiment indicating that factors other than iron were controlling the growth rate of this species.

Some of the diatom species mentioned above have also dominated natural blooms along the APF growing at high ambient iron concentrations. Such a natural fertilization event was encountered during the Southern Ocean JGOFS cruise ANT X/6 of R.V. "*Polarstern*" in austral spring 1992 when large numbers of icebergs (about 10/200 km^2) coincided with order of magnitude higher iron concentrations than usual in the ACC (Smetacek et al. 1997). At least two distinct blooms developed along latitudinal zones north of the Antarctic Polar Front (APF) which extended down to about 100 m water depth (Bathmann et al. 1997). One of the blooms was dominated by *Corethron pennatum* and culminated in a mass sexual event with massive sinking out of empty gametangial cell walls (Crawford 1995) which will have resulted in decoupling of Si from C, N and P inventories in the surface layer. The other area of high chlorophyll concentrations consisted mainly of *Fragilariopsis kerguelensis* and viable cells were found down to depths of 300 m (Bathmann 1998). The preponderance of this robust species is reflected in the maximum molar ratios of biogenic silica (BSi) to particulate organic carbon (POC) of 1.75 (Quéguiner et al. 1997). During a later Southern Ocean JGOFS cruise (ANT XIII/2), conducted in austral summer 1995/96, three diatom species complexes (*Thalassiothrix antarctica*, *Chaetoceros atlanticus/dichaeta*, *Pseudo-nitzschia* cf. *lineola*) dominated biomass along the APF. These populations persisted relatively unchanged for several weeks in the surface layer (Smetacek et al. 2002). The same assemblage was encountered in austral autumn 1999 (ANT XVI/3) south of the APF and *F. kerguelensis* accounted for most of the biomass along the front (unpublished data). During this cruise two mass sinking events triggered by subduction of the surface layer were observed. The first event took place in part of the *F. kerguelensis* population and led to mass sinking out of

empty frustules, presumably following cell death. The C:Si ratio of the sinking material was 1:2 and can therefore explain the decoupling of the carbon from the silica cycle that is evident from nutrient inventories of the ACC but could not be confirmed from C:Si ratios of suspended matter which tend to range around 6:1. The other event occurred south of the APF but the composition of the sinking aggregates, prominent in vertical profiles, could not be measured. The above are the only reports of mass sinking events from the ACC and demonstrate the over-riding role of the key species involved in shaping nutrient inventories. Laubscher et al. (1993) found distinct phytoplankton blooms at three oceanic fronts (the Subtropical Front (STF), Subantarctic Front (SAF) and APF) during austral summer 1990/91 with diatoms constituting a major component of the standing stocks in the latter two regions. In the STF diatoms were only a minor component of total chlorophyll *a* and mainly consisted of chain-forming *Nitzschia* spp. (probably *Pseudo-nitzschia* spp.). In contrast the SAF and the APF were dominated by chain-forming *Chaetoceros* spp. and *F. kerguelensis* and chain-forming *Nitzschia* spp. (again probably *Pseudo-nitzschia* spp.) respectively in early summer. Later during the season nanophytoplankton became more important and only phytoplankton biomass at the APF was dominated by the diatom species *Corethron pennatum*.

Data from iron fertilization experiments as well as from observations of natural blooms in the Southern Ocean suggest that the species contributing most of the biomass are those represented by sizeable seed populations prior to the iron addition or by high growth rates. Large seed populations are a result of rapid increase of population size as a response to favourable growth conditions and/or lower mortality of the population during periods of unfavourable conditions. Large and robust species like *F. kerguelensis* belong to the latter category whereas species like *Chaetoceros curvisetus* can compensate low initial abundances by fast growth rates. It appears that in the presence of adequate resources fast growth rates are attained and sustained by only some species or even strains and that all the other species maintain much lower growth rates or do not respond to radical improvement in growth conditions at all. These results cast doubt on evolutionary selection for fast growth rates as a universal principle and hence on the theory of competitive exclusion as a shaping force in the plankton. Furthermore the iron induced shift in dominance from persistent, heavily silicified diatoms to weakly silicified, “boom-and-bust” species encountered during EisenEx will have major consequences for the nutrient inventories of the Southern Ocean. The “giant diatoms” of the iron deplete ACC drive the silica pump while their carbon is recycled at the surface. Fast growing species like *C. curvisetus* on the other hand might induce oceanic blooms of a similar scale to those of coastal blooms under iron-replete conditions and hence increase the

strength of the BCP. The prevalence of either one of these systems will ultimately shift the biogeochemical cycles of the Southern Ocean towards a silica dominated flux during iron limited conditions and towards a carbon dominated flux during iron replete conditions.

5.2 Factors influencing the vertical distribution of non-motile particles and planktonic organisms

Various factors affect the vertical distribution of dead particles and life organisms in the water column. The pelagic realm of the Southern Ocean is exposed to large fluctuations in the physical environment be it by intense turbulent mixing induced by heavy winds, the intensity of radiant energy, advection, deep convection during the winter or up-welling of CDW. Gradients in chemical properties, e.g. nutrient concentrations and messenger substances, might influence the orientation of planktonic organisms in the water column. Furthermore biological interactions between organisms and organisms and particles could considerably impact on the vertical heterogeneities of the respective groups.

During EisenEx the vertical distribution of both non-motile particles and major components of the heterotrophic community was mainly influenced by physical and biological factors. Among the physical factors wind induced vertical mixing seemed to have played the major role. Recurring gale force winds caused the variability in mixed layer depth (MLD) over the course of the experiment and deepened the MLD down to occasionally over 80 m. In such a turbulent environment planktonic organisms have to adapt strategies to maintain their position in the water column. These range from actively swimming in the case of flagellates, ciliates or copepods to buoyancy regulation in the case of diatoms or cyanobacteria. Non-motile particles on the other hand are not capable of active movement against velocity fluctuations in the MLD and the gravitational pull exerted on any particle denser than the surrounding medium. The settling velocity of a given particle is largely dedicated by Stoke´s law, the larger and denser a particle the faster it sinks. Hence it will inevitably sink out of the water column and be dislodged from the productive surface layer if it were not for other factors retarding or accelerating its flux or redistributing its position.

Many of the heterotrophic organisms encountered during EisenEx exhibit swimming speeds exceeding the velocity fluctuations in the water column (Smayda and Bienfang 1983, Yamazaki and Squires 1996) and are therefore able to actively choose their position in the water column. The preponderance of both dinoflagellates and ciliates at relatively shallow depths despite deep mixing events point to their ability to maintain at certain depth horizons

where favourable food concentrations prevail or potential grazers are scarce. Copepods are even more obvious active swimmers as documented by the diurnal and seasonal migrations of many species. However juvenile and small adult copepods seemed to have largely stayed at the surface during EisenEx. Species of the smaller copepod genera, e.g. *Oithona*, have been reported to stay at shallower depths and feed throughout the year (Ashjian et al. 2003, Dubischar et al. 2002). The iron-induced phytoplankton bloom provided ample food for the juvenile and small adult copepod assemblage at the surface even supporting an increase in copepodite abundances (see Manuscript 3).

The non-motile particles dealt herein all of biological origin and were produced by various processes. A whole array of fecal materials was encountered during the experiment ranging from small ellipsoid to spherical pellets mainly of protozoan origin to large cylindrical pellets of crustacean grazers. The packaging of single food items into compact fecal pellets enhances the settling velocity with up to several 100 m d^{-1} in the case of larger crustacean pellets (Komar et al. 1981). Hence the biological activity of these grazers will eventually boost the BCP. The distribution of smaller particles such as intact empty and broken diatom frustules and empty tintinnid loricae is also a product of various biological processes. During our study grazing seemed the major factor regulating their vertical distribution. Broken frustules could only derive from crustacean grazing and are mainly re-suspended after disintegration of fecal pellets. This is supported by the close correlation of broken frustules with crustacean fecal pellets and juvenile and small adult copepods. Intact empty frustules on the other hand might stem from various uni- and multicellular grazers as well as pathogens and parasitoids. However the major source of intact empty frustules during the fertilization experiment was probably protozoan grazing. Protozoans are incapable of braking diatom frustules and thus release them intact into the water column after feeding. Empty tintinnid loricae may originate from both proto- and metazoan grazing but the frequent occurrence of empty loricae in crustacean fecal pellets (personnel observation) suggests that metazoan grazing dominated tintinnid mortality. In contrast to large fecal pellets these smaller particles exhibit considerably lower settling velocities and hence have a longer resistance time in the water column. The resistance time in the water column may actually exceed the lifetime of many of these particles because they dissolved easily or are rapidly degraded. These particles thus have a negligible contribution to the vertical flux. An exception are large heavily silicified diatoms like *Fragilariopsis kerguelensis* that settle out rapidly and dissolve slowly and thus contribute to the high silica concentrations in CDW and to the accumulation of the opal belt surrounding the Antarctic continent. Another interesting aspect of vertical heterogeneities

observed during EisenEx was the accumulation of fecal pellets and broken diatom frustules at the bottom of the mixed layer especially towards the end of the experiment. Feeding activities of coprophagic copepods at this depth horizon seemed to have fragmented fecal material and supported release of broken frustules back into the water column. This recycling community may thus have considerably contributed to the retardation of vertical flux and may therefore retained essential nutrients within the euphotic zone for further phytoplankton growth.

5.3 The mortality environment

The focus of marine ecologists has been on the "growth environment", e.g. the determination of growth rates, while the other side of the coin, namely the "mortality environment", has largely been neglected. However grazing plays a decisive role in structuring pelagic ecosystems as already indicated in the previous section. As the general role of grazing in structuring Southern Ocean pelagic ecosystems and biogeochemical cycles has already been discussed at length in Manuscript 5 I will herein confine myself to its contribution on diatom community composition and succession.

Mortality as a prime ecological factors determining ecosystem structure becomes especially obvious in the iron limited diatom assemblage of the land remote ACC. This assemblage is dominated by the "giant diatoms" that have evolved specialised defence morphologies to deter grazers. These morphological features range from remarkably robust silica frustules in the case *of Fragilariopsis kerguelensis* to long barbed spines in the case of *Corethron pennatum*. Frustules of *F. kerguelensis* have been shown to resist five times higher pressure loads than those of a centric diatom of similar size (Hamm et al. 2003). This species is therefore an extremely hard "nut" to crack especially for smaller crustacean zooplankton. *Thalassiothrix antarctica* resembles a stiff silica rod equipped with minute barbs that can on the one hand withstand pressures imposed by copepod mandibles and on the other hand severely clog the feeding apparatus of filter feeders like salps. The long spines of *Chaetoceros* species of the subgenus *Phaeoceros* as well as of *C. pennatum* may also serve the latter purpose. Large size, long vindicated appendages as well as chain formation as in the case of *Pseudo-nitzschia* spp. are especially effective defence strategies against protist grazers that are restricted to an upper size limit. The combined array of defence morphologies minimises the number of potential grazers and thus grazing induced mortality. If it were not for these defence structures smaller and less defended diatoms of the background community with a more efficient nutrient uptake due to their favourable surface to volume ratio should be

dominant. Therefore selective grazing as the ruling ecological principle seems to be the logic explanation for the dominance of the "giants" in the iron limited system.

Coastal blooms thriving under iron replete conditions are dominated by relatively few species that often occur world wide. Typical representatives of these bloom-forming species are smaller, weakly silicified *Thalassiosira* spp. and *Chaetoceros* spp. of the subgenus *Hyalochatae*. The questions therefore arises why these more delicate species are able to build up considerable biomass while other species from the same genera remain at background concentrations. A possible explanation for the dominance of these species under bloom conditions is their exceptionally high growth rates. This "boom-and-bust" life cycle explains the annual variations in population size over several orders of magnitude. During EisenEx two species characterised by exponential net growth rates could be categorised to such a life cycle. *Chaetoceros curvisetus* is one example of such a life strategy. This species emerged from negligible concentrations prior to fertilization to a sizeable fraction of the diatom assemblage at the end of the experiment, at least in cell numbers, due to its high net growth rates. *Pseudo-nitzschia lineola*, the dominant diatom by day 21, also belongs to a cosmopolitan genus and may represent an intermediate type with high growth rates and an effective grazer defence due to their long, needle shaped chains. Both fast-growing species were eventually able to maintain their growth rates well above grazer induced mortality and therefore showed the highest accumulation inside the fertilized patch.

5.4 Perspectives for future research

Mesoscale *in situ* perturbation experiments like EisenEx now enable to follow the waxing and waning of plankton blooms in space and time and derive ecological principles underpinning the biogeochemical cycles that significantly influence the global climate. The Southern Ocean ecosystems especially lend themselves to ecosystem studies like mesoscale fertilization experiments due to their confined extent within a distinct climate zone. Owing to the circumpolar character of the Southern Ocean very few warm water species ever reach far south and become established (Hendey 1937). Therefore the ecosystems of the Southern Ocean are more stable and less prone to expatriation than other marine systems (Verity and Smetacek 1996) and may provide ecological principles also applicable to other marine systems. The detailed floristic analysis from the EisenEx experiment revealed challenging aspects on species interactions in a pelagic ecosystem from which more general ecological principles could be derived that would help improve ecosystem models. However many open

questions remain that could be tackled with further large-scale perturbation experiments. Is for example evolution in the plankton really selecting for fast growth rates and hence competitive exclusion the shaping force in the plankton? Or is the "mortality environment" actually the driving force in pelagic environments as indicated in Manuscript 5? Furthermore it would be interesting to investigate the genetic variability of species populations to see whether the strains within a population react differently to iron fertilization. This might answer the question to what extent will the degree of genetic variability of a defined species reflect in greater ecological fitness. Will a species with a large set of strains tuned to various environmental conditions be more successful in ensuring viable populations throughout the year? We intend testing these hypotheses in the next iron fertilization experiment scheduled for 2004 (EIFEX).

Another major issue is whether geoengineering plans like iron fertilization to enhance the biological pump will eventually enhance oceanic sinks and lead to a greater sequestration of anthropogenic CO_2. Could iron fertilization actually induce North Atlantic conditions in the ACC? Although all iron fertilization experiments stimulated surface productivity, it still, remains to be shown whether the increased productivity will eventually result in an enhanced strength of the BCP and a subsequent greater oceanic sequestration of atmospheric CO_2. In future iron fertilization experiments, it will therefore be essential to follow the fate of carbon export to mesopelagic layers and the deep sea.

Iron availability limits plant growth over large areas of the open ocean whereas the availability of water constrains plant growth in many terrestrial ecosystems. Most water precipitating over the continents originates from evaporation over the oceans while iron reaches the oceans via wind blown dust or direct leaching from the continental shelves. Both cycles affect each other and the productivity of the respective ecosystems geared to them. During glacials the overall global climate was drier and the wind velocities higher. Next to the fact that much of the continental shelves were exposed to the atmosphere the areal extent of terrestrial vegetation was reduced due to increased water limitation on land. Thus more bare land was exposed to the erosive winds that blew dust over the wide expanses of the open ocean. This dust carried the iron suggested to have stimulated surface productivity in the HNLC regimes of today´s world ocean during glacial periods and enhanced the strength of the BCP resulting in a more effective sequestration of atmospheric CO_2. Today the continental shelves are submerged, wind velocities have decreased and land vegetation has reoccupied many habitats that were once deserted. Hence much less iron is transported to the ocean and large areas of open ocean that might have experienced North Atlantic conditions during

glacial periods are now “oceanic deserts”. This sketch illustrates how elemental cycles and ecosystem processes are interwoven and how they may affect global climate change. It further demonstrates the tight gearing of terrestrial and marine biomes.

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Erklärung
gem. § 6 (5) Nr. 1-3 PromO

Ich erkläre, dass ich

1. die Arbeit ohne unerlaubte fremde Hilfe angefertigt habe,

2. keine anderen, als die von mir angegebenen Quellen und Hilfsmittel benutzt habe

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3. die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

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