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The role of mesozooplankton grazing in the biogeochemical cycle of silicon in the Southern Ocean

DISSERTATION

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Auch denen ist’s wohl,
die ihren Lumpenbeschäftigungen oder wohl gar ihren Leidenschaften prächtige Titel geben,
und sie dem Menschengeschlechte als Riesenoperationen
zu dessen Heil und Wohlfahrt anschreiben.

(Johann Wolfgang v. Goethe)

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ZUSAMMENFASSUNG

Thema dieser Doktorarbeit ist die Rolle des Zooplanktons, insbesondere der Copepoden, in den biogeochemischen Kreisläufen von Silizium (Si) und Kohlenstoff (C) des Südozeans. Das Fraßverhalten zweier dominanter calanoider Copepodenarten des Antarktischen Zirkumpolarstroms (ACC), *Calanus simillimus* und *Rhincalanus gigas*, wurde während einer Diatomeenblüte in den Gewässern nördlich der Antarktischen Polarfront (APF) untersucht. Die Blüte war mit Hilfe einer *in situ* Eisendüngung induziert worden. Solche Düngungsexperimente erzeugen eine natürliche Störung im pelagischen Ökosystem, die es ermöglicht, die funktionalen Beziehungen pelagischer Vergesellschaftungen genauer zu studieren.

Ein wichtiges Ergebnis dieser Arbeit ist der Unterschied im Fraßverhalten von *C. simillimus* und *R. gigas* vor der Blüte, und wie die beiden Copepodenarten es im Laufe der Blüte ändern. Wie die Ergebnisse zeigen bestimmt eben dieser Unterschied die jeweilige Rolle und Wichtigkeit eines Fraßorganismus' in der pelagischen Biogeochemie. *C. simillimus* zeigt eine kontinuierlich hohe Fraßaktivität auf Diatomeen und verstärkt somit möglicherweise den Export von primärproduziertem C und Si. Dies gilt sowohl für den eisenlimitierten „High-Nutrient-Low-Chlorophyll"-Status (HNLC) des Südozeans als auch für eisenreiche Blütensituationen. Der Fraßdruck dieser einzelnen Copepodenart auf die Vergesellschaftung des Mikroplanktons im nördlichen ACC kann so hoch werden, dass dies die Populationsdynamik gewisser Diatomeenarten beeinflusst. In wiederholtem Maße wird auch in der Literatur auf die Wichtigkeit dieser Copodenart hingewiesen. Dies macht *C. simillimus* zu einer Schlüsselart für weitere wissenschaftliche Untersuchungen.

Im HNLC-Status des pelagischen Ökosystems stellt *R. gigas*, was den Partikelexport aus der Deckschicht angeht, einen ökologischen Gegenpol zu *C. simillimus* dar. Bei niedrigen Phytoplanktonkonzentrationen ernährt sich *R. gigas* von heterotrophen Beuteorganismen und Phytodetritus, in diesem Falle Kotballen. Durch seine Fraßaktivität wird der von *C. simillimus* produzierte Kotballenfluss stark reduziert. Diese Schlussfolgerungen beruhen fast ausschließlich auf Quervergleichen. Die Ergebnisse dreier klassischer Methoden in der Zooplanktonforschung, siehe unten, wurden miteinander und mit Ergebnissen für die Gattung *Neocalanus* sp. verglichen. Diese ist im subarktischen Pazifischen Ozean beheimatet, der ein weiteres wichtiges HNLC-Gebiet darstellt. Auf Grundlage dieses Vergleichs werden *C. simillimus* und *R. gigas* als ökologische Gegenspieler im ACC gesehen, was unter HNLC-Situationen zur Ausbildung eines „Copepoden-Retentions-Systems" für organisches Material

in der Deckschicht führt. Das Analog dieses Systems im subarktisch Pazifischen Ozeanwirbel verkörpern möglicherweise *Neocalanus plumchrus* und *N. cristatus*.

In Folge der Diatomeenblüte erhöhen alle untersuchten Copepoden ihre Fraßaktivität auf Diatomeen. Damit einher geht zum einen ein reduzierter Fraßdruck auf heterotrophe Beuteorganismen. Zum anderen lockert sich auch die Fraßkontrolle über den Detritusfluss aus der Deckschicht. Dieser schon bekannte Nahrungswechsel der Copepoden, engl. prey switching, hat somit möglicherweise einen großen Einfluss auf die Bedeutung des Zooplanktons als treibende Kraft im Vertikalfluss von Partikeln. Der Nahrungswechsel wandelt das „Copepoden-Retentions-System“ in ein „Copepoden-Export-System“ um, in welchem wahrscheinlich der Großteil der epi-pelagischen Fraßorganismen durch Kotballenproduktion zum Fluss von C und Si aus der Deckschicht beiträgt. Die Rolle von Copepoden in der Biogeochemie dieser Elemente ist demnach vom Status des pelagischen Ökosystems abhängig. Zieht man den Gesamtfraßdruck der Copepodengemeinschaft während des Eisendüngungsexperimentes in Betracht, dann kann auch Copepodenfraß für einen beachtlichen Export von partikulärem Si im ACC verantwortlich sein.

An sich führt die Verdauung von C jedoch nicht von Si zu einer Entkopplung der Kreisläufe beider Elemente durch den Fraßvorgang. Die Retention von biogenem Kohlenstoff in der Deckschicht durch die aufeinanderfolgende Verdauung von Diatomeen und Kotballen mit Diatomeeninhalt wird diese Entkopplung theoretisch noch weiter verstärken. Daher wurde in einer Reihe von Fraß- und Lösungsexperimenten auch der Aspekt der Silikatlösung durch den Zooplanktonfraß untersucht. Generell hat der Fraß von Copepoden und Krill auf Diatomeen dabei die spezifische Lösungsrate einer Diatomeenvergesellschaftung verlangsamt, was auf den Einschluss der Silikatschalen in das Kotmaterial zurückzuführen ist. Eine leicht reduzierte Effizienz Opal zu konservieren konnte für die zerbrechlichen Kotschnüre des Krills beobachtet werden. Dies wird jedoch sicher durch eine höhere Sinkrate der Krillkotschnüre im Vergleich mit den Copepodenkotballen kompensiert, so dass der relative Anteil von Krill- und Copepodenkot am Export von Material bis in eine gewisse Wassertiefe nicht so sehr eine Frage der Qualität ist. Von großer Bedeutung ist erneut die Struktur des pelagischen Ökosystems. In den Experimenten führte das coprophage Fraßverhalten von *Oithona* sp. auf Kotballen von *Calanus propinquus* offensichtlich zu einer Beschleunigung der Silikatlösung. Demnach wird im „Copepoden-Retentions-System“ sowohl C als auch Si in der oberen Wassersäule zurückgehalten.

Abschließend wurden die Ergebnisse dreier Standardmethoden die im Rahmen dieser Studie verwendet wurden – *in vitro* Inkubationsversuche, Darmfluoreszenz- und Respirations-

messungen – für die untersuchten Copepodenarten verglichen. Dies sollte Aufschluss darüber geben, woher die in der Literatur berichtete Variabilität und vermutete Unzulänglichkeit rührt. Aus dem Vergleich wird geschlussfolgert, dass unterschiedliches Fraßverhalten der Copepoden einer der Gründe ist, warum die experimentelle Bestimmung von Tagesrationen, engl. daily ration, manchmal unter dem gemessenen Respirationsbedarf liegt. Für ein genaues Bild der Fraßaktivität ist es nötig die derzeit existierenden Methoden zu kombinieren. Ganz generell jedoch können die experimentellen Abschätzungen der Kohlenstoffaufnahme als realistisch angesehen werden, da sie mit publizierten Eiproduktionsraten, *in situ* Wachstumsraten und der abgeschätzten Dauer des Lebenszyklus größtenteils übereinstimmen.

SUMMARY

This dissertation addresses the role of zooplankton grazers, especially copepods, in the biogeochemical cycles of silicon (Si) and carbon (C) in the Southern Ocean. Feeding behavior of two dominant calanoid copepods of the Antarctic Circumpolar Current (ACC), *Calanus simillimus* and *Rhincalanus gigas*, was studied during a diatom bloom north of the Antarctic Polar Front (APF). The bloom was induced via *in situ* iron fertilization, a new method to simulate a natural perturbation in the pelagic ecosystem. It allows investigating the structure and functioning of pelagic communities by studying their response.

A major finding is a difference in feeding behavior between *C. simillimus* and *R. gigas* before the bloom and in response to it. This difference determines their respective role and importance in pelagic biogeochemistry. The continuously high feeding activity of *C. simillimus* on diatoms is conducive to enhance the export of primary produced C and Si, both in the iron-limited High-Nutrient-Low-Chlorophyll-state (HNLC) of the Southern Ocean as well as in the iron-replete bloom situation. Furthermore, the grazing impact of this single species on microplankton communities in the northern ACC can be high enough to influence population dynamics of some diatom species. Repeated accounts on the importance of this grazer in the literature clearly identify it as a key species for further investigation.

In the HNLC-state of the pelagic ecosystem, *R. gigas* has the opposite effect to *C. simillimus* in terms of particle export from the surface layer. At low phytoplankton concentrations it feeds on heterotrophic prey and pigmented detritus, i.e. on fecal pellets, thereby reducing the vertical fecal flux produced by *C. simillimus*. The conclusions drawn for *R. gigas* rely to a large part on the inter-comparison of results: among three different methods commonly used in zooplankton research and with results for the genus *Neocalanus* from the sub-arctic Pacific Ocean, another important HNLC-system. This comparison leads to the proposal of a "Copepod-Retention-System" for organic material at work under HNLC conditions, with *C. simillimus* and *R. gigas* representing ecological counterparts in the ACC analogue to *Neocalanus plumchrus* and *N. cristatus* in the sub-arctic Pacific gyre.

In response to the diatom bloom, the studied copepods increase their feeding activity on diatoms, thereby sparing not only heterotrophic prey organisms but also lifting the grazing check on the detritus flux out of the surface layer. This well known change in feeding preferences, i.e. prey switching, has thus a potentially large impact on the importance of zooplankton grazers in driving particle flux. It transforms the "Copepod-Retention-System" into a "Copepod-Export-System" in which probably most of the epi-pelagic grazer

community contributes to the flux of C and Si out of the surface layer. The biogeochemical role of copepods depends, therefore, on the state of the pelagic ecosystem. Considering the overall grazing impact of copepods on diatoms during the iron-fertilization experiment, copepod grazing can be responsible for substantial export of particulate Si in the ACC.

Per se, the digestion of C but not Si during grazing decouples the cycles of both elements. The retention of biogenic carbon via subsequent digestion of diatoms and fecal pellets with diatom content under HNLC conditions should further amplify the decoupling. In a series of grazing and dissolution experiments, this dissertation also addresses the aspect of Si recycling via zooplankton grazing. In general, copepod and krill grazing reduced the specific dissolution rate of diatom assemblages. This was due to the enclosure of the frustules into fecal material. Slightly reduced efficiency to preserve opal by fragile krill fecal strings is most certainly compensated by higher sinking rates of krill feces compared to copepod fecal pellets. The relative importance of krill or copepod feces in export of material to a certain depth is thereby not so much a question of quality. Of central importance is again the structure of the pelagic ecosystem. In the experiments, coprophagy by *Oithona* sp. on fecal pellets of *Calanus propinquus* apparently led to enhanced recycling of Si, indicating that the "Copepod-Retention-System" retains both C and Si in the upper water column.

Finally, results from three standard methods applied in the framework of this study – *in vitro* incubations, gut fluorescence and respiration measurements – were compared among grazers to investigate reasons of variability and shortcomings apparent from the current literature. It is concluded that differences in feeding behavior of copepods can be a reason why experimental determination of daily rations sometimes remains below estimated respiratory needs. A precise picture of grazing activity was only obtained through a combination of presently available methods. In general, however, experimentally derived estimates of carbon ingestion are realistic considering published *in situ* growth rates, egg production and life cycle duration.

GENERAL INTRODUCTION

In nature, vital elements like carbon (C), oxygen (O) and nitrogen (N) but also iron (Fe), phosphorus (P), silicon (Si) or sulfur (S) are under constant biological, chemical and physical transformation. Through complex, so-called "biogeochemical cycles" and frequently changing chemical and physical states, the elements enter and exit the atmosphere, biosphere, hydrosphere, and lithosphere on variable temporal and spatial scales. Seventy-one percent of our planet's surface are covered by oceans and the marine ecosystem is the world's largest. Thus it is not surprising but nevertheless fascinating that processes driven by plankton organisms on micrometer scales can be of global biogeochemical significance.

A group of organisms with fundamental impact on both the carbon and silicon cycle in today's ocean are diatoms, a particularly successful class of phytoplankton. Each diatom cell builds a frustule of biogenic silica (BSi; opal). This transfers silicon, taken up as silicic acid ($Si(OH)_4$), from the hydrosphere to the biosphere. Most global biogenic silica production in the modern ocean is accomplished by diatoms (Nelson et al. 1995, Tréguer et al. 1995). As long as $Si(OH)_4$ is available, phytoplankton communities of nutrient rich and turbulent systems are dominated by diatoms (Dugdale et al. 1995). Si-limitation in coastal areas for example leads to proliferation of harmful algal blooms (Smayda 1990). Diatoms also drive the biological carbon pump (BCP; Falkowski et al. 1998). Thereby, primary production converts inorganic CO_2 to organic carbon, which is exported out of the photic zone via sedimentation of particulate material. This "export production" is based to a large extent on diatoms (Goldmann 1993, Dugdale et al. 1995, Kemp et al. 2000).

Sedimentary deposits of BSi are found at all depths and in all climate zones, and have the strong potential to serve as proxy for the functioning of the BCP on temporal and spatial scales. For a successful reconstruction of paleo-productivity from the opal sediment record it is necessary to calibrate the proxy in the modern ocean for mechanisms that control the silicon cycle in close relation to the carbon cycle. Especially the strong spatial variation observed for the percentage of BSi produced in the surface layer that is eventually deposited, i.e. BSi preservation, needs to be explained. Furthermore, processes that modify the Si:C production rate or that decouple both cycles are of central interest in the current research effort (Ragueneau et al. 2000).

The role of the Southern Ocean in the biogeochemical cycles of Si and C

The modern Southern Ocean (SO) plays a crucial role in the global Si cycle but only a minor role in the global C cycle (Tréguer & Pondaven 2002 a/b). Primary production in this region is limited by iron availability. Therefore, macronutrients – especially nitrate and phosphate – remain largely unused, a condition known as the high-nutrient-low-chlorophyll (HNLC) status. Artificial iron fertilization of HNLC waters induces phytoplankton blooms (e.g. Boyd et al. 2000). Zones of naturally increased primary production in the SO such as the Polar Frontal Zone (PFZ) are also related to enhanced iron availability (Laubscher et al. 1993). On geological timescales, an iron-stimulated increase in SO productivity and in the effectiveness of the BCP can potentially influence glacial/interglacial variations in atmospheric CO_2 (Martin 1990, Francois et al. 1997, Sigman & Boyle 2000).

The Southern Ocean ecosystem, especially the remote and iron-limited waters of the Antarctic Circumpolar Current (ACC), is characterized by many heavily silicified diatom species, e.g. *Fragilariopsis kerguelensis*, *Thalassionema nitzschioides*, *Thalassiothrix* sp. These diatoms are well preserved in the sediments below the ACC (Zielinski & Gersonde 1997). Important contributors to diatom assemblages are also large-celled and/or chain forming species such as *Corethron pennatum*, *Pseudonitzschia* spp. and the *Chaetoceros* species *Ch. atlanticus* and *Ch. dichaeta* (Smetacek et al. 1997, Smetacek et al. 2002). SO diatom communities have a Si:C ratio up to an order of magnitude higher than cultured diatoms (Brzezinski 1985, Quéguiner et al. 1997) which is also linked to iron limitation (Hutchins & Bruland 1998, Takeda 1998; but see also Martin-Jézéquel et al. 2000, Claquin et al. 2002). In the iron-replete waters closer to the continent and in the sea ice, weakly silcified diatoms of the genera *Chaetoceros* (*Ch. curvisetus*, *Ch. neglectus*) and *Fragilariopsis* (*F. cylindrus*, *F. curta*) dominate (Garrison & Buck 1985, Légendre et al. 1992).

BSi production estimates for the SO range from 50 to 80 x 10^{12} mol Si yr^{-1}, which is up to a third of the 230 x 10^{12} mol Si yr^{-1} produced in the global ocean (DeMaster 2002 and references therein). Opal preservation efficiency is presently determined between 1 and 6 % (Pondaven et al. 2000, Nelson et al. 2002) and is similar to the global average of 3 %. Southern Ocean sediments hence represent one of the major repositories of opal in the world (Pondaven et al. 2000, DeMaster 2002). Antarctic surface waters are preferentially depleted in silicate relative to nitrate. This unique nutrient signature influences productivity at low latitudes (Sarmiento et al. 2004). Without doubt the modern Southern Ocean has a major impact on the global Si budget and this effect is linked to the growth and export of diatoms.

Mesozooplankton grazing effects on ocean biogeochemistry

Under HNLC conditions, grazing can regulate biomass build-up and influence the population dynamics of microplankton communities (Banse 1995). In productive regimes, the most important effect of grazing is modification of the quality and quantity of the particle flux (Kiørboe 1997). Wassmann (1998) points out that the balance between retention or export of organic matter and nutrients depends on the structure and function of the prevailing pelagic food web. Simultaneous investigations on the structure of the food chain and how it regulates vertical flux are scarce. In a modeling approach, Wassmann demonstrates how the abundance of mesozooplankton grazers and their feeding preferences, i.e. herbivory, omnivory or carnivory, regulate the magnitude of vertical flux. Grazer abundance and feeding pressure in relation to available phytoplankton production depends also on the life cycle strategies of organisms. To investigate the relationship between plankton ecology and vertical flux, Wassmann proposes to derive the knowledge from "'old' open ocean ecosystems with circular circulation pattern, such as the Pacific Ocean and Antarctica". *In situ* iron-fertilization experiments provide a useful framework for an investigation in this respect. They initiate the transition between the iron-limited HNLC state to the iron-replete and potentially high productive state of the Southern Ocean. The reaction to the shift of certain components of the pelagic food web in general, and changes in grazing activity and feeding behavior in particular, potentially yield information on the ecological processes governing elemental cycles in the one state or in the other.

Priddle et al. (2003) recently demonstrated that the variation in biogeochemical carbon and nitrogen cycles in the South Georgia pelagic ecosystem is determined largely by changes in zooplankton community composition and its impact on phytoplankton dynamics. No study has addressed the role of mesozooplankton grazing in the Si cycle in connection with the C cycle in greater detail so far. Central questions that are open to investigation are:

- By which mechanisms can mesozooplankton grazing alter the build-up and flux of BSi, i.e. diatom growth and export?
- Is grazing responsible for increased recycling or preservation of BSi?
- With respect to the cycling of Si and C, can key organisms be identified within the prevailing zooplankton assemblage?
- Do different members of the grazer community have dissimilar effects on the Si and C cycle?

Zooplankton communities of the Southern Ocean

Copepods dominate zooplankton communities of the open Southern Ocean in numbers and biomass (Smith & Schnack-Schiel 1990, Pakhomov et al. 2000). Highest abundances are associated with the productive waters North and South of the Polar Front (Pakhomov et al. 2000). Most common calanoid copepod species with a circumpolar distribution are *Calanoides acutus*, *Calanus propinquus*, *Calanus simillimus*, *Metridia gerlachei* and *Rhincalanus gigas* (see the review by Razouls et al. 2000). Aside from these "large" copepods, *R. gigas* for example reaches an adult body size of up to 9 mm (Ommaney 1936), the importance of "small" copepods like *Ctenocalanus citer* or the cyclopoid *Oithona* sp., the latter with adult body size an order of magnitude smaller than *R. gigas*, has been increasingly recognized in recent years. Abundances of almost 50 ind^{-1} l^{-1} were recorded for *Oithona* sp. in waters of the PFZ (Dubischar et al. 2002) whereas characteristic abundances of the larger copepods are an order of magnitude lower (e.g. Fransz & González 1997, Pakhomov et al. 2000). Salps are another group of organisms with a high grazing potential (Dubischar & Bathmann 1997) and importance for vertical flux (Bathmann 1988). These tunicates can react to increased food abundance with rapid proliferation via asexual budding (Hagen 1999). Last but not least needs to be mentioned *Euphausia superba*, the Antarctic krill, which builds-up impressive biomass in the waters closer to the continent and associated with the sea-ice. It can exert substantial grazing pressure on the phytoplankton standing stock by ingesting more than 100 % of daily primary production (e.g. Mayzaud et al. 2002a).

Investigations in the framework of this dissertation dealt with several representatives of the large copepods, in particular *C. simillimus* and *R. gigas,* a taxonomic mixture of copepods < 2 mm cephalothorax length and, to a lesser extent, *E. superba*. Both, *C. simillimus* and *R. gigas* are primarily inhabitants of the ACC. *C. simillimus* is considered a sub-Antarctic form and is abundant in waters north of the PF (Atkinson 1991). *R. gigas*, a true Antarctic copepod, also occurs in greatest abundance in the vicinity of the PF (Ommanney 1936, Ward et al. 1997, Pahkomov et al. 2000) but is present in water masses ranging from the sub-Antarctic to the Weddell Sea (Atkinson 1991). During the "Discovery" cruises *R. gigas* dominated the catches throughout the study area which made Ommanney (1936) assume that "it may be said with some safety (…) that the life history of this species typifies that of the Antarctic macroplanktonic Copepoda". But as it seems, life cycles of Antarctic copepods are diverse and especially the strategy of *R. gigas* is still under debate (Atkinson 1998). Time to complete a life cycle is generally one year, in the case of *R. gigas* potentially longer, especially in the colder water masses of the Weddell Sea (Bathmann et al. 1993, Ward et al.

1997). A true state of diapause with concurrent reduction of metabolism appears to be the exception for Antarctic copepods. It has only been confirmed for *C. acutus* (Drits et al. 1994). Copepodite stages as well as adults of several species, *C. simillimus*, *R. gigas*, *M. gerlachei* and *C. propinquus* for example, continue to feed throughout the winter with a part of the population remaining in the surface layer (Atkinson 1991, Pasternak & Schnack-Schiel 2001). Grazers that are present in large numbers before the onset of the spring bloom can potentially regulate diatom production. Such a scenario has been proposed for copepods in the Norwegian Sea (Bathmann et al. 1990a). Lipid storage patterns follow the life cycle trends, with highest levels found in the diapausing species *C. acutus* (Atkinson 1998). In addition to wax esters (WE), the common storage lipid of Arctic zooplankton, triacylglycerol (TAG) is synthesized by some Antarctic species, e.g. *C. simillimus* or *C. propinquus*. The latter compound is considered to be a short-term lipid store for animals that do not experience a prolonged shortage of food (see Ward et al. 1996 and references therein). To survive periods of low phytoplankton availability in a system with such strong seasonality as the Southern Ocean, omnivory and carnivory is common and metabolically important among "herbivorous" Antarctic copepods (Froneman et al. 1996, Pasternak & Schnack-Schiel 2001). Feeding on detritus (fecal pellets, aggregates) also appears to be of significance in the nutrition of Antarctic copepods (Arashkevich 1978 cited in Bathmann et al. 1993, Atkinson 1998) and plays an important role in retarding vertical flux of particulate material (González and Smetacek 1994). Feeding preferences determine the ecological and biogeochemical significance of a grazer. For dominant grazer species in a given ecosystem it needs to be investigated how feeding changes throughout the life cycle of the grazer, as a function of food abundance and the microplankton community composition.

Selective grazing

On evolutionary terms, grazing, or more generally spoken top-down control, is hypothesized to be a structuring force in pelagic ecosystems leading to the development of key organisms which in turn have a major impact on biogenic fluxes (Verity & Smetacek 1996). Experimentally, zooplankton grazing has been shown to control the species composition and size distribution of a phytoplankton assemblage (Ryther & Sanders 1980, Granéli et al. 1993). Only selective grazing can influence the composition of a phytoplankton community. Organisms can be selected or avoided, i.e. eaten in greater or smaller proportion than their contribution to the available food spectrum, and deviation expressed mathematically with so-called "selectivity indices" (e.g. Chesson 1978, Vanderploeg &

Scavia 1979). Reasons for selectivity are suitable size (Frost 1972, Wilson 1973), high nutritional quality (Paffenhöfer & Van Sant 1985, DeMott 1989) or easy perceptibility, for example motility (DeMott & Watson 1991). In temperate waters, Meyer-Harms et al. (1999) have shown that copepod grazers selectively prey on microzooplankton before and after the diatom spring bloom and change to preferential ingestion of diatoms during the bloom. This type of selectivity is called "prey-switching" and not only influences the quality of the zooplankton driven particle flux, i.e. the content of fecal pellets, but also the population dynamics of plankton communities (Kiørboe et al. 1996, Gismervik & Andersen 1997). Certain types of prey may be avoided by grazers for reasons similar to the ones just mentioned: the size might be unsuitable or the cell of poor nutritional quality. The strong silicification of diatoms such as *F. kerguelensis* greatly increases the mechanical resistance of the cell (Hamm et al. 2003) which has since some time been speculated to protect it from grazing (Verity & Smetacek 1996). Smetacek et al. (submitted) argue that the dominance of many large or heavily silicified diatom species in today's Southern Ocean (see above) and their preservation in underlying sediments is due to reduced grazing pressure on these phytoplankters. Several grazing studies in the Southern Ocean have addressed selectivity so far and results are variable and at times contradicting. Large copepods graze preferentially on the most abundant size class (e.g. Schnack 1983, Schnack 1985) and Atkinson concludes in his three studies (Atkinson 1994, Atkinson 1995, Atkinson 1996) that large diatoms are cleared most efficiently from the water column. This is in apparent contradiction to the findings of Perissinotto (1992) who demonstrated selective feeding on the size class < 20 µm and even 1-5 µm by *C. simillimus* for example. Motile prey is especially important in the diet of small copepods that clear large diatoms > 100µm only with reduced efficiency (Atkinson 1996). In order to evaluate whether the observed feeding preferences have an influence on population dynamics in the plankton community, grazing mortality of species and taxa has to be compared to growth or accumulation rates of these in the same environment, which in the open ocean can only be accomplished in the framework of a Lagrangian type study such as the *in situ* iron-fertilization experiment for example.

Grazing modification of the particle flux

In general, mesozooplankton grazing activity is not able to suppress the occurrence of diatom blooms because of the large disparity in growth rates of the predator and its prey, and reproductive impairment of copepod populations recruited on a diatom diet (Miralto et al. 1999, Ianora et al. 2004). A mean of 60 % of the opal produced by diatom populations already

dissolves in the upper 50 to 100 m (Nelson et al. 1995). Assuming diatom dissolution rates of 10 yr^{-1} (O. Ragueneau pers. comm.) and sinking rates of solitary cells of 1 m d^{-1} (Smayda 1971) a single cell will dissolve before it leaves the surface layer. Only BSi that is subjected to enhanced sinking or slowed dissolution will be able to export silicon and associated elements (C, N, P, Fe) to significant depth.

Sedimentation of particulate material is mainly achieved with phytoplankton aggregates or feces produced by herbivorous zooplankton grazers (Wassmann 1998). Aggregates, still bearing considerable amounts of chl *a*, are often found at great depth (e.g. Smayda 1971) and their rapid sedimentation due to the production of transparent exopolymer particles (TEP) by certain diatom species (Crocker & Passow 1995, Passow et al. 2001). Copepod fecal pellets sink with rates of > 100 m d^{-1} (Honjo & Roman 1978), fecal strings of krill up to 800 m d^{-1} (Cadée et al. 1992) and the dense feces of salps up to 2700 m d^{-1} (Bruland & Silver 1981). Physical or food web mediated aggregation therefore in principal greatly enhances the downward flux of particulate matter if the material is not intercepted by other members of the pelagic and the mesopelagic food web. In impoverished waters, so called “flux feeders” exploit the settling particles as food source (Jackson 1993, Kiørboe 1997). Especially the relatively small and slow sinking fecal pellets of copepods are intercepted by other copepod grazers in or slightly below the surface layer (Lampitt et al. 1990, González & Smetacek 1994). They either completely ingest fecal pellets (coprophagy) or fragment them (coprorhexy, Noji et al. 1991). The reworked material is subject to slowed sedimentation and therefore enhanced recycling. Nevertheless, repackaging of detritus via repeated ingestion in deeper layers, potentially by amphipods, can also lead to new formation of solid and fast sinking material (Bathmann et al. 1990b). These processes apparently produce a strong spatial and temporal variability in the fecal pellet flux (Bathmann et al. 1991, Dubischar & Bathmann 2002) but presently it is impossible to predict variations in ecosystem structure that lead either to export or retention and recycling of fecal/detrital material. Again, key grazers and their feeding behavior, i.e. herbivory, omnivory or detritivory, need to be identified in the surface layer but also in the meso- and bathypelagic zone.

The effect of grazing on opal dissolution

Given that fecal pellets are able to escape further grazing activity, it is of interest how the quality of the feces originating from different grazers changes the rate of dissolution of BSi. From the physico-chemical aspect, water column dissolution of opal is influenced by temperature, pH and most importantly the concentration of silicic acid in the medium as well

as the available surface area (Lewin 1961, Lawson et al. 1978, Kamatani & Riley 1979, Kamatani 1982, Greenwood 2001). Seawater is undersaturated in silicic acid with respect to opal dissolution and an organic coating protects the frustule of a live diatom cell from dissolving (Lewin 1961). Removal of this coating by bacteria greatly accelerates the dissolution of diatom silica (Bidle & Azam 1999). No comprehensive study on the dissolution rate of opal enclosed in feces or phytoplankton aggregates is available in the current literature.

Krill produces fast sinking but loose fecal strings, and also salps have been shown to egest fluffy pseudo-feces, both of which potentially disintegrate rapidly (González 1992). The compact and solid fecal pellets of copepods are generally assumed to protect diatoms from dissolution (e.g. Schrader 1971, 1972, Ferrante & Parker 1977, Honjo & Roman 1978). Copepod fecal pellets have been shown to remain intact for up to 20 days at 5 °C, therefore, fast sinking pellets of large copepods are potentially able to transfer BSi to significant depth in deep ocean environments such as the Southern Ocean. Degradation is enhanced by bacterial colonization of the peritrophic membrane that encases the digestive leftovers (Honjo & Roman 1978). Presence of enteric bacteria in the guts of copepods accelerates colonization of fecal pellets (Bianchi et al. 1992, Nagasawa 1992). However, not all grazers appear to possess such an intestinal flora (Nagasawa 1992). If the enteric bacteria are present on the outside of the fecal pellet it seems reasonable to assume that they will also be present on the inside associated with the diatom debris. This could be the reason for first signs of dissolution observed for diatoms frustules in freshly produced fecal pellets (Jansen 2002) since bacteria have been shown to enhance dissolution of BSi. Gut pH of copepods can have a substantial effect on calcite dissolution (Jansen & Wolf-Gladrow 2001) but BSi appears to be relatively inert during gut passage (Tande & Slagstad 1985, Cowie & Hedges 1996). This preferential recycling of carbon over silicon through zooplankton grazing possibly contributes to the globally observed increase in Si:C ratio with depth and the high variability observed in the decoupling of carbon and silicon experimental cycles (Ragueneau et al. 2002).

Current methodology for studies on zooplankton grazing

The two most widespread methods to study zooplankton feeding behavior and to quantify ingestion are the gut fluorescence (Mackas & Bohrer 1976) and the incubation method (Frost 1972), both introduced more or less three decades ago. Gut fluorescence was initially proposed for a rapid assessment of *in situ* grazing of copepods on phytoplankton, when the view of the pelagic ecosystem was still that of a straight food chain from diatoms to copepods to fish. In principle, the gut pigment content, i.e. chl *a* and phaeopigments, of

freshly caught grazers is determined with simple fluorometric measurements and converted to ingestion rates taking into account the rate at which grazers empty their guts, assuming that ingestion and egestion rate are in equilibrium (Dam &. Peterson 1988). Although controlled laboratory studies support the feasibility of the approach over a wide range of food concentrations its application in the field is associated with high variability of gut content, gut clearance rate and pigment destruction (see the review by Pasternak 1994). Gut fluorescence measurements have the advantage to provide information on finer temporal and spatial scales at which grazers feed, for example over a diel cycle (Atkinson et al. 1992 a). The quality of the food, other than that it is pigmented, cannot be described in greater detail, however, and neither kind of feeding selectivity can be inferred. The qualitative pendant to gut fluorescence is a microscopic gut content analysis, which reaches its limitations in that it only reflects prey organisms that are not completely digested, e.g. diatoms or armored dinoflagellates, but not naked ciliates for instance. The apparent solution to the problem "quantitative-or-qualitative" should be provided by controlled incubations of grazers in a suspension of defined prey organisms. The contribution of various food items to the grazer's diet is inferred from a count before and after the incubation and the "missing" particles assumed to have been ingested by the added grazer organism. An approach that works fine in simple food media such as cultures of single species (e.g. Frost 1972) but that suffers greatly from trophic interactions in natural communities. Major problems are selective stimulation of growth for some prey organisms due to ammonia excretion of grazers (Roman & Rublee 1980) or trophic cascading leading to underestimation of ingestion rates (Nejstgaard et al. 2001). Nevertheless, especially the comparative use of the incubation method among different grazers and/or over extended temporal scales, i.e. the development of a bloom, has already yielded valuable information on feeding behavior and selectivity of grazers in the Southern Ocean (Atkinson 1994, 1995, 1996). Especially the role of microzooplankton as crucial food supply to copepods (Gifford 1993a, Froneman et al. 1996) could only be evidenced via the incubation method.

Feeding on detritus cannot be estimated based on a single approach. Qualitative evidence potentially comes from gut content analyses (e.g. Dagg 1993b) but the quantitative component that is of central importance in export flux calculations needs to be inferred from additional measurements. A simple and robust method to estimate the minimum carbon requirement of different grazers is the determination of their oxygen consumption which is translated into carbon based on stochiometric calculations (Ikeda et al. 2000). The assumption is that grazers ingest at least the amount of carbon necessary for basal metabolism. Care in the use of this method has to be given to the fact whether the grazer is in effect actively feeding

on the available food supply or relying on accumulated lipid reserves (Hagen 1988). With similar caution has to be acted when carbon requirements shall be inferred from egg production rates of adult females (e.g. Mayzaud et al. 2002b). Rey-Rassat et al. (2002) have shown that egg production relies in part on the utilization of internal resources (lipid, protein) and is rather related to the feeding history than the *in situ* food conditions. Ingestion rates of copepod grazers in the Southern Ocean are variable which is reflected in the grazing impact on the phytoplankton community ranging from < 1 % (e.g. Zeldis 2001) up to 36 % of phytoplankton standing stock per day (Bernard & Froneman 2003). Ingestion often remains far below the estimated respiratory requirements (Atkinson 1996, Zeldis 2001, Mayzaud et al. 2002a). Light needs to be shed on the reasons for this variability, whether it is a methodological problem or actually represents situations of severe food limitation. Only a precise and meaningful estimate of carbon ingestion will enable us to assess the importance of grazing in the biogeochemical cycling of elements.

AIMS AND OUTLINE OF THE THESIS

OBJECTIVES

CONSIDERING the current appreciation of the role of mesozooplankton grazing in pelagic ecosystems, it seems of utmost interest to identify key grazers and their feeding preferences in the iron-deplete and iron-replete state of the Southern Ocean.

RECOGNIZING that a precise and physiologically meaningful estimate of *in situ* feeding activity is fundamental to the appreciation of the ecological importance of a grazer, a continued calibration of methods in a given environmental context is necessary.

RECALLING that modification of particle flux through grazing can be of major importance in the export or retention of biogenic material, further investigation is required on the nature of this modification and its potential to enhance or reduce flux out of the surface layer.

Therefore, this dissertation is based on the following working hypotheses:

1. The feeding behavior of dominant copepod grazers in the ACC changes as a function of food abundance and composition of the microplankton community.
2. The contribution of diatoms in the diet of grazers varies with food abundance and composition of the microplankton community.
3. Certain diatom species or genera are grazed preferentially. This can shape the composition and population dynamics of a diatom community.
4. Methods, notably gut fluorescence measurements and bottle incubations, are appropriate to detect variations in feeding preferences and yield comparable estimates of ingestion rates.
5. Experimental determination of carbon ingestion is representative of minimum carbon requirements of copepod grazers in the Southern Ocean.
6. Grazing of copepods and krill influences the dissolution rate of Antarctic diatom assemblages.

The hypotheses are addressed in the framework of three individual manuscripts that have either already been submitted or that will be submitted to the appropriate journals shortly. **Manuscript 1** explores hypotheses 1-3 and research was carried out in the framework of

EisenEx, the second *in situ* iron-fertilization experiment in the Southern Ocean. A mesoscale eddy shed from the Antarctic Polar Front was enriched with iron sulphate, which stimulated a marked diatom bloom (Bathmann & Smetacek 2001). In comparison to previous fertilization experiments in the equatorial Pacific (IronEx I and II) and the Southern Ocean (SOIREE), EisenEx put strong emphasis on the detailed assessment of changes in the species composition and trophic relationships within the pelagic ecosystem. Assmy (2004) describes an impressive floristic shift within the diatom community with diatom carbon increasing by a factor four in 21 days. Dinoflagellate and ciliate populations remained remarkably stable but increased in biomass by a factor two (Henjes 2004). The general objective for research on mesozooplankton was to investigate changes in the spatial distribution as well as the composition of the grazer community and its grazing impact on the developing phytoplankton bloom. The specific objective of the study presented in **manuscript 1** was to analyze feeding behavior and diet composition of dominant copepod grazers before and during the bloom with *in vitro* incubations. Experiments run before the iron fertilization should give insight into feeding patterns representative for grazers facing the impoverished and strongly regenerating community that dominate the open Southern Ocean most of the year (Smetacek et al. 1990). The iron-induced bloom then yields the possibility to monitor the grazers' feeding response to the enhanced food supply and to the shift in the composition of its prey field, presumably a reflection of the iron-replete ecosystem of the Southern Ocean. Results are interpreted in terms of differences in foraging behavior of the grazers. Furthermore, the grazing impact of dominant members of the copepod community on the microplankton standing stock is estimated. For selected diatom species, e.g. *F. kerguelensis*, grazing mortality is evaluated and related to *in situ* accumulation patterns in the mixed layer. This leads to an assessment of how important the studied grazers are in shaping microplankton communities of the area.

Research for **manuscript 2** was also carried out in the framework of the iron-fertilization experiment EisenEx. Results from SOIREE, the first iron-enrichment study in the Southern Ocean, had highlighted once more the "enigma of copepod nutrition" (Zeldis 2001). Ingestion rates based on classical methods (gut fluorescence and incubations) were extremely low and grazers presumably acquired at most 15 % of their daily respiratory carbon needs. Earlier investigations (e.g. Atkinson 1996, Pakhomov et al. 1997) had already reported this discrepancy but at present no explanation is at hand. If food limitation of copepod grazers in the Southern Ocean was indeed so severe even under bloom conditions, it would be surprising that copepods maintain themselves so successfully and in high numbers in this ecosystem. Therefore, hypotheses 4 and 5 were investigated based on the grazing and respiration data

collected during EisenEx. For two large copepods, *C. simillimus* and *R. gigas*, and the copepod size class < 2 mm, ingestion rates determined with gut fluorescence and *in vitro* incubations, as well as respiratory requirements estimated from O_2 uptake experiments, are compared with previously published values from the Southern Ocean. Results are discussed in relation to theoretical models that were proposed by Dagg et al. (1982), Dam & Peterson (1988) and Morales et al. (1990) in order to estimate ingestion and respiration from grazer size or *in situ* temperature. The equations used in these models were established with temperate and boreal zooplankton but investigations in the Southern Ocean frequently take recourse to them when a particular parameter, for example the gut clearance coefficient or basic respiratory needs, could not be determined experimentally. **Manuscript 2** provides a brief appreciation of currently available methods to infer grazing pressure, highlights the strong variability that can be observed in the data collected during the last decade from the Southern Ocean, and explores reasons that are at the origin of this variability. Furthermore, the comparison of methods allows reconfirming differences in feeding behavior between copepod grazers.

A correct estimate of diatom ingestion is a prerequisite to describing the importance of zooplankton grazing in the cycle of Si. In this context, **manuscript 3** investigates the net effect of grazing on the dissolution rate of diatom assemblages. Diatoms that have passed through the gut of a grazer have experienced a series of transformations, of which mechanical destruction, digestion of carbon and packaging into condensed fecal matter are only the most obvious. More generally, grazing activity also increases the number of broken diatom frustules in the water column (e.g. Roman & Rublee 1980, Assmy 2004) and recycling of N in form of $NH4^+$ excretion stimulates the microbial loop and bacterial activity. Of these phenomena associated with grazing activity some may enhance opal dissolution, e.g. breakage of frustules, and some slow down or prevent its remineralization, for example the packaging in fecal pellets. Hypothesis 6 was tested experimentally during an expedition of FS *Polarstern* to the Bellingshausen Sea in austral autumn 2001. A natural diatom assemblage was collected from a dense bloom dominated by *Pseudonitzschia* spp., *Chaetoceros* spp. and *Corethon pennatum*, and a second assemblage with similar species composition melted from first year sea-ice. Both were submitted to grazing pressure of *Metridia gerlachei*, *Calanus propinquus* and furcilia of *Euphausia superba*. Subsequently, dissolution of control assemblages and grazed assemblages – the latter containing un-eaten cells, broken cells and fecal pellets of the grazers – was followed in batch incubations during 3 months in the dark. Similarly, cultures of *Fragilariopsis kerguelensis* and *Thalassiosira* sp., isolated from the Polar Front during the

iron-fertilization experiment, were submitted to grazing pressure and dissolution measured. **Manuscript 3** presents the results of this comprehensive experimental study, which in this form has not yet been carried out. Specific dissolution rates of grazed and un-grazed diatoms are discussed with respect to the phytoplankton species composition and the type of grazer, i.e. copepod or krill, which fed on them. In addition, the feeding interaction of *Oithona* sp. was explored to estimate the importance of coprophagy on the recycling of Si in the upper ocean.

MANUSCRIPTS

This dissertation is based on three manuscripts prepared for submission or already in review. The contribution of the authors is specified.

Manuscript 1

S. Schultes, P. Verity & U. Bathmann; to be submitted to Journal of Experimental Marine Biology and Ecology

"Feeding patterns of Calanus simillimus and other copepods in an iron-induced phytoplankton bloom in the Polar Frontal Zone of the Southern Ocean and their potential to shape diatom populations."

The concept was developed by S. Schultes. Experiments were carried out and data was analyzed by S. Schultes. P.Verity supplied the microzooplankton count. The manuscript was prepared by S. Schultes in cooperation with the co-authors.

Manuscript 2

S. Schultes & U. Bathmann; to be submitted to Journal of Plankton Research

"Grazing and metabolic activity of copepods at the Antarctic Polar Front – How well agree ingestion estimates from gut fluorescence and in vitro incubations with respiratory carbon demand?"

Data was collected and analyzed by S. Schultes and U. Bathmann who provided the gut fluorescence measurements. The manuscript was prepared by S. Schultes in cooperation with U. Bathmann.

Manuscript 3

S. Schultes, S. Jansen & U. Bathmann; in review with Marine Ecology Progress Series

"Influence of mesozooplankton grazing on the dissolution rate of Antarctic diatom silica"

The concept was developed by S. Schultes. Experiments were carried out by S. Schultes and S. Jansen. The data was analyzed by S. Schultes. The manuscript was prepared by S. Schultes in cooperation with the co-authors.

MANUSCRIPT 1

Feeding patterns of *Calanus simillimus* and other copepods in an iron-induced phytoplankton bloom in the Polar Frontal Zone of the Southern Ocean and their potential to shape diatom populations

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Abstract

Feeding activity, selective grazing and the potential grazing impact of two dominant grazers of the Polar Frontal Zone, *Calanus simillimus* and *Rhincalanus gigas*, and of the copepods < 2 mm were investigated with incubation experiments in the course of an iron fertilized diatom bloom in November 2000. All grazers were already actively feeding in the low chlorophyll waters prior to the onset of the bloom. *C. simillimus* maintained constant clearance rates, fed predominantly on diatoms and increased ingestion with the developing bloom. *R. gigas* and the small copepods strongly increased their clearance and ingestion of diatoms in response to their enhanced availability in the environment. All grazers preyed on microzooplankton, most steadily on ciliates, confirming the view that pure herbivory appears to be the exception rather than the rule in copepod feeding. The grazers exhibited differences in feeding behavior based on selectivity indices. *C. simillimus* and *R. gigas* showed prey switching from dinoflagellates to diatoms in response to the phytoplankton bloom. All grazers preferentially grazed on large diatoms leading to differences in daily loss of standing stock for large and small species, e.g. *Corethron* sp. or *Thalassionema nitzschioides*. Species-specific diatom mortality rates due to grazing suggest that the high feeding activity of *C. simillimus* prior to and during the bloom played a role in shaping diatom population dynamics.

Introduction

The high nutrient – low chlorophyll (HNLC) ecosystem of the Southern Ocean is characterized by a strong temporal and spatial variability of food resources for zooplankton grazers. Primary production is severely limited by the strong seasonal oscillations of daylight in high latitudes, by deep and unstable mixed layers, and by the scarcity of essential micronutrients. Strongly enhanced phytoplankton growth following artificial infusion of iron has demonstrated the crucial role of iron in limiting primary production in this area (Boyd et al. 2000). Therefore, zooplankton grazers face a dilute, rapidly regenerating background community dominated by nanophytoplankton (Smetacek et al. 1990) most of the year. To cope with the shortage of phytoplankton, Southern Ocean copepods are generally omnivorous and fulfill a large percentage of their carbon need by preying on microzooplankton (Froneman et al. 1996) or even resort to carnivorous feeding on other crustaceans during winter (Pasternak & Schnack-Schiel 2001). Life cycle strategies are adapted to the strong seasonal variability in quality and quantity of food. A true state of diapause, however, has only been shown for *Calanoides acutus* (Atkinson 1998). Most copepods undergo seasonal vertical migrations to deeper water layers but several species seem to adopt a dual over-wintering strategy and maintain a part of the population in the surface waters that continues to feed (Atkinson 1991) which is also reflected in the accumulation of typical short term storage lipids by some species (Ward et al. 1996).

A rather productive area within the Southern Ocean is associated with the Antarctic Polar Front (APF) for which blooms are frequently reported (Laubscher et al. 1993, Bathmann et al. 1997). These are linked to increased iron concentrations following iceberg melting (Smetacek et al. 1997) or local upwelling events of deep water in connection with meandering of the APF producing areas of enhanced food availability for grazers (Strass et al. 2002). The transient bloom events are usually dominated by large micro-phytoplankton, on which copepod grazers have been shown to feed efficiently (Atkinson 1994, 1995, 1996). Apparently, these pulses of productivity are sufficient to sustain the increased copepod populations of the area compared to most other sectors of the Southern Ocean (Pakhomov et al. 2000).

Considering the important role of the modern Southern Ocean in the global silicon cycle (Tréguer et al. 1995, Sarmiento et al. 2004) and in modulating atmospheric CO_2 concentrations on geological timescales (Martin 1990), key processes that influence the biogeochemical cycles need to be identified. *In situ* iron fertilization experiments provide the

possibility to study the functioning of the pelagic ecosystem in the iron-deplete and iron-replete state of the Southern Ocean. The current study was carried out with the goal to shed light on the structuring potential of copepod feeding on the development of an iron-induced diatom bloom at the APF in austral spring 2000. Feeding of dominant copepod grazers on diatoms and microzooplankton was investigated in incubation experiments before and during the artificially stimulated bloom. Special attention was given to feeding selectivity as it influences prey population dynamics and determines the ecological and biogeochemical efficiency of the grazer.

Material and Methods

Study area and iron fertilization

The *in situ* iron fertilization experiment „EisenEx“ was carried out in the Atlantic Sector of the Southern Ocean (~21°E, 48°S) during the cruise ANT XVIII/2 of R.V. *Polarstern* in austral spring (6-29 November 2000). 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 tons 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”. “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 the patch. Station 9, occupied two days prior to fertilization within the centre of the eddy, was chosen as the initial reference station. Sampling was performed inside and outside the patch throughout the experiment.

Mesozooplankton sampling and analyses

Abundance and composition of the copepod community were determined from samples collected with vertical hauls of a multiple opening and closing net (MN; Weikert and John 1981) equipped with five 100 µm mesh nets. Depth strata down to 1500 m were chosen variably before each haul in order to resolve distinct bands of organisms observed with acoustic methods. However, in all but one of the hauls (cast 38-09) at least two nets were closed in the upper 150 m of the water column. Details on the time, position and depth strata of the MN casts are presented in Table 1. Zooplankton samples were preserved in

hexamethyltetramin buffered formalin (final concentration of 4 %) and counted following the JGOFS protocol No. 9. Copepod species > 2 mm were identified according to Razouls (1994). Sample volumes were estimated by multiplying the net opening area (0.25 m^2) by the length of the corresponding depth layer. Copepod standing stock (individuals m^{-2}) integrated over 150 m is based on the mean abundance estimated for the according depth strata assuming a homogeneous distribution of individuals. Continuous MN tows are only available up to day 10 of the 3 week study. Due to time constraints sampling then carried on with a 300 µm Bongo net only towed through the upper 100 to 350 m of the water column. Samples from the Bongo net hauls were analyzed according to those from the MN. They are not included in estimating absolute abundance and standing stock but serve to confirm the stable composition of the copepod community and tendencies in mesozooplankton accumulation inside the patch as evidenced from acoustic measurements. The latter results will be presented elsewhere (Krägefsky et al. in prep.). Mesozooplankton for feeding experiments were caught with the 300 µm Bongo net towed vertically at 0.3 m sec^{-1}. Immediately after the catch, the content of the cod-end was diluted with natural seawater. Actively swimming and apparently undamaged individuals were sorted with a large pipette under a stereomicroscope and maintained in 0.2 µm filtered seawater until the start of the experiment 2 to 5 hours after the catch. All manipulations were performed in a cooled laboratory container.

Feeding experiments

In regular intervals at stations inside and outside the Fe-fertilized patch, mesozooplankton grazing activity was investigated with bottle experiments according to the method of Frost (1972). Natural seawater was used as a prey assemblage and was sampled with a CTD from the chl *a* maximum depth (18-50 m). Water from two Niskin bottles of the same cast and closed at the same depth were mixed in a plastic carboy and well homogenized before the experimental bottles were filled. The incubation medium was not pre-screened to remove other grazers (nauplii and copepodite stages of *Oithona* sp.; see also Henjes 2004) as this procedure would have taken out large and chain-forming diatoms at the same time. Furthermore, mesozooplankton was not acclimatized to feeding conditions before the start of the incubation assuming that the grazers were already acquainted with the food organisms *in situ*.

The choice of grazer used in the various experiments depended on the visual dominance of the organism in the Bongo net catch and the availability of sufficient numbers of healthy, and in the case of *Calanus simillimus* and *Rhincalanus gigas* adult (C6♀)

individuals. Three feeding experiments were carried out for each of the three grazer categories: *C. simillimus*, *R. gigas*, and an arbitrarily chosen mixture of copepods < 2 mm. A first experiment for each grazer was completed during the initial stage of the iron fertilization (experiments 1 and 2, Table 2). Subsequently, experiments for each grazer were run at stations inside the fertilized patch and one outside the patch. In the following these experiments will be referred to as "initial", "in-patch" and "out-patch". Grazers were incubated in 1 liter bottles and grazer densities ranged from 4 to 20 individuals per liter, depending on grazer size and prior experience of their feeding capabilities. Details on experimental conditions are presented in Table 2. Bottles were kept on a plankton wheel, in the dark, at 4°C. In every experiment, two control bottles and three replicates for every grazing treatment were incubated. Following the incubation, grazers, except the copepods < 2 mm, were recovered and checked for mortality which was negligible. All incubation bottles were well mixed by rotating them end over end before any sub-sampling was performed. On board, initial and final concentrations of chl *a* were determined from a 500 ml sub-sample following standard JGOFS procedures. The decrease of chl *a* in the grazing bottles compared to the control was 38% at most. The initially chosen duration of 24 h was extended to up to 43 h as the first experiment had failed to give a clear reduction in chl *a* concentration through the feeding activity of the added grazers.

Diatom and microzooplankton analyses

One 200 ml sub-sample of incubation water from each control and grazing bottle was fixed with acidic Lugol's solution for a microscopic analysis of diatom community composition. At the institute, cells in a 20-50 ml aliquot from every treatment were allowed to settle for 24 h in an Utermöhl chamber and diatoms counted if possible to the species level under an inverted microscope (Utermöhl 1958). Abundant organisms were counted on single or several transects across the chamber, for less abundant ones half of the chamber or the whole chamber was counted. This resulted in a mean of 956 cells in total being counted in the control (minimum 326 cells, maximum 2086 cells). For a specific prey organism the same chamber surface was counted in the control and in the sub-samples from the grazing treatments. A detailed list of the diatom species and genera identified in the phytoplankton community of the fertilization experiment is presented by Assmy (2004). For the purpose of this grazing study more than 30 species were routinely identified and counted as such. However, species had to be regrouped into genera later on as clearance could only be calculated for diatoms or groups (see Table 3) of which at least 30 cells had been counted in

the control (Atkinson 1995). Diatoms were also cumulated into four size classes according to the mean length of the cell: < 20 µm, 20-50 µm, 50-100 µm and > 100 µm. However, these categories tend to cut across species or genera and their interpretation is of limited value considering that one aim of the present study was to gain insight in the interactions between key grazers and key diatoms in the Polar Frontal ecosystem. Diatom carbon was calculated using the geometric formulas according to Edler (1979) and the volume to carbon conversion factors proposed by Menden-Deuer et al. (2000).

Ciliates, heterotrophic dinoflagellates (hdinos), phototrophic nanoflagellates (pnanos) and heterotrophic nanoflagellates (hnanos) were enumerated from sub-samples preserved in glutaraldehyde (final concentration = 0.3 %), stained with 3-6-diaminoacridine hemisulfate (proflavin) for 1 minute (5 µg ml^{-1} final concentration) and 4'6-diamindino-2-phenylindole (DAPI) for 4 minutes (5 µg ml^{-1} final concentration). Discrete volumes were filtered onto 0.8 µm black Nuclepore filters. To achieve an even distribution for counting and measurement purposes, black filters were placed on top of pre-wetted Whatman GF/F backing filters. After filtration, the damp filter was placed on a slide. A drop of low fluorescence immersion oil was placed on top of the filter, which was covered with a cover slip and frozen at -20 °C. Samples were analyzed upon return to the laboratory. An Olympus BX-60 microscope equipped with 100-watt epifluorescence illuminators was employed, with appropriate exciter/barrier filter sets for UV (335-365 nm), blue (435-490 nm), and green (510-560 nm) excitation. Dinoflagellates were distinguished from other flagellates based upon cell morphology and structure of the nucleus, especially the unique condensed chromosomes visible by DAPI staining. Heterotrophic and autotrophic cells were discriminated by the absence and presence of autofluorescent chloroplasts, respectively. The autotrophic category also includes mixotrophic cells. Cell abundance, dimensions, and biovolumes were determined via quasi-automated color image analysis (Verity & Sieracki 1993). A minimum of 200 plankton cells of each type was measured. The average coefficient of variation of triplicate counts of nanoplankton was 11 %. Cell biovolume measurements were converted to carbon biomass using conversion factors based on literature values of carbon density of microplankton (Verity et al. 1992 and references therein). This method of enumeration precludes knowledge on the species level. Therefore, results on the clearance and ingestion have to been seen as an average for a large and very variable group of organisms. Nevertheless, they yield important information on trophic pathways and interactions. A detailed description of the microprotozooplankton community is given by Henjes (2004).

Calculation of clearance and ingestion rates

All clearance and ingestion rates are presented as a mean of the three grazing replicates with the calculated standard deviation of the mean in brackets.

Clearance rates were calculated following the equation of Frost (1972) modified to:

$$F = \ln(C_c/C_g)*V/(n*t)$$

(Atkinson 1996) where F is the clearance rate (ml ind^{-1} h^{-1}), C_c the final concentration in the control, C_g the final concentration in the grazing treatment, V the experimental volume (ml), n the number of grazers (l^{-1}) and t the duration (h) of the experiment. Differences between clearance rates were tested with a homoscedastic, two-tailed t-Test.

Total ingestion I_{total} (µg C ind^{-1} d^{-1}) was obtained according to the following. Single ingestion rates (I_i; ng C ind^{-1} h^{-1}) were calculated by multiplying positive single clearance rates F_i of a given organism (i) with its final abundance in the control bottle $C_{c,i}$. This was done for every experimental bottle separately and to the greatest detail possible, i.e. in the case of diatoms based on clearance rates for species and genera plus a category "other diatoms" regrouping leftover diatom counts. Total diatom ingestion for each replicate bottle was achieved by summation of all I_i. This approach was used assuming that a grazer is not tightly geared to one food organism but fulfills its carbon needs from a variety of suitable prey. Furthermore, prey organisms may not be 100 % homogeneously distributed in every replicate bottle. Ingestion based on an average clearance rate between replicate bottles may underestimate carbon intake by the grazer in the same way as ingestion based on an average clearance calculated for a large variety of food items. An additional clearance of diatoms in general ("diatoms average") was estimated from total diatom ingestion divided by the diatom carbon concentration in the control bottles.

Estimation of selective feeding behavior

Selective feeding of copepods was characterized using the chi-square (χ^2) goodness-of-fit test (Cowles 1979), as described by Sokal & Rohlf (1969). The frequency distribution of food taxa in the copepod diet was compared with that in the environment. Selective feeding was indicated by a significant divergence of the distribution (Kleppel et al. 1996). Food selection on specific food taxa was quantified using the selectivity index (SI) α, according to Chesson (1978). The calculation of α is based on the relative contribution of the taxon abundance to total abundance in the control and the relative contribution of the ingested prey organism to total ingestion. According to Chesson (1978), $\alpha = 0.5$ indicates non-selective feeding, $\alpha > 0.5$ a preference for a prey organism, and $\alpha < 0.5$ discrimination against a taxon.

Results

Copepod abundance and community composition

The dominant copepod grazer in the > 2 mm size class was *Calanus simillimus* with a mean relative contribution to total copepod abundance of 57.5 ± 26.0 % in-patch and 69.2 ± 17.5 % out-patch (Figure 1). On average, *Calanus propinquus*, *Calanoides acutus*, *Metridia* spp., *Pleuromamma robusta* and *Rhincalanus gigas* each contributed an order of magnitude less to total copepod abundance than *C. simillimus*. As an exception, *Metridia* spp. accounted for 58 % of the copepod community in cast 38-09 (in-patch day 3). *Lubbockia aculeata*, *Heterorhabdus* spp., *Paraeuchaeta* spp. and undetermined Calanoida – all regrouped in the category "other copepods" – were frequently observed in the samples but always of minor numerical importance. Analysis of the Bongo net catches confirmed the stable composition of the copepod community and the dominance of *C. simillimus* throughout the fertilization experiment (data not shown). A difference though was noted for the relative abundance of *R. gigas*, which contributed on average 4 % to the in-patch copepod standing stock (Figure 1) based on the MN samples. In the Bongo net hauls, *R. gigas* contributed a mean of 22 ± 4.2% to the copepod standing stock and consistently represented the second most abundant grazer following *C. simillimus*. The Bongo net was usually hauled vertically through the upper 200 to 350 m of the water column and the higher relative abundance reflects the more even distribution of *R. gigas* down to 500 m compared to *C. simillimus* that concentrated the bulk of its population in the upper 100 to 200 m (MN data not shown).

The overall standing stock of copepods > 2 mm, integrated over 150 m, was generally higher in-patch than out-patch. Due to the restricted amount of net hauls a temporal trend is not obvious. Taking into account the standing stock of copepods > 2 mm from the initial and the in-patch hauls, a gradual increase to a final factor of 2.4 is indicated over the twelve day sampling period from 21380 ind m^{-2} (cast 09-02) to 51490 ind m^{-2} (cast 49-06). Acoustic measurements of zooplankton abundance over the entire cruise confirmed this trend and allow a plausible extrapolation to an overall increase of the copepod standing stock by a factor of 3.6 on day 21, i.e. the last day of sampling three weeks after the first Fe infusion (S. Krägefsky unpubl. data). Maximum estimates of copepod abundance mentioned further below were calculated from the mean standing stock of casts 9-02 and 11-04 multiplied by a factor of 3.6. A detailed presentation of the temporal development of the zooplankton community during EisenEx based on acoustic methods is in preparation (Krägesfky et al. in prep.).

The abundance of copepods between 1-2 mm (Table 1) followed the pattern of the larger copepods, with the exception of cast 38-09. Taxonomic identification of the small copepod fraction is not available to date. Furthermore, the standing stock estimates have to be heightened by substantial counts of the smallest fraction, mostly copepodite and adult stages of *Oithona* sp., estimated from Niskin bottle samples and presented elsewhere (Henjes 2004).

Temporal development of the prey field

The composition of the microplankton community on which copepod grazers could prey was estimated from the abundance of diatoms, heterotrophic dinoflagellates (hdinos) and ciliates in the control bottles. Before the first iron fertilization (day -2), in the initial stage of the bloom (day 2) and outside the fertilized patch (days 5 and 17), diatoms accounted on average for 57 %, hdinos for 26 % and ciliates for 14 % of total abundance. In-patch, the contribution of diatoms gradually increased to 81 %, whereas for hdinos and ciliates it decreased to 9 % and 8 % respectively (Figure 2). Contribution of the silicoflagellate *D. speculum* varied from 0.1 % to 6.2 % but was usually around 3 % and is not included in Figure 2. Phototrophic and heterotrophic nanoflagellates (pnanos, hnanos) theoretically dominated abundance with > 90 % but are not readily available to the all the larger grazers and therefore not included in the graph. Prey availability in this study was estimated from counts on sub-samples from the control bottles. Frequency distribution of the major prey taxa diatoms, hdinos and ciliates over the series of incubation experiments (Figure 2) mirrors well the *in situ* development and can therefore be regarded as being representative for the actual situation inside and outside the fertilized patch.

Initially, small and delicate species like *Cylindrotheca closterium*, as well as the heavily silicified pennates *Fragilariopsis kerguelensis* and *Thalassionema nitzschioides* were the most important diatoms in terms of abundance. Biomass was at this stage dominated by *F. kerguelensis* and several large cylindrical diatoms, such as *Dactyliosolen antarcticus*, *Corethron pennatum* and *Guinardia* spp. In response to the iron addition *Pseudonitzschia lineola* and *Chaetoceros curvisetus*, both weakly silicified and chain-forming diatoms, showed the highest accumulation rates and dominated in-patch abundance at the end of the three week study. Large *Corethron pennatum* still contributed significantly to in-patch biomass on day 21. Out-patch, diatom biomass doubled due to an increase of the large cylindrical species *R. chunii*, *Proboscia alata*, *G. cylindrus*, *C. pennatum* and *D. antarcticus* (Assmy 2004). Concerning ciliates and heterotrophic dinoflagellates, changes due to iron fertilization were not as drastic as for the diatom community. Overall abundance and biomass

of microprotozooplankton doubled and was dominated by the size class 20 to 60 µm for both taxa. Species composition remained stable (Henjes 2004).

Mesozooplankton feeding activity in response to the bloom

C. simillimus

Compared to the initial value of 3.67 ± 0.86 µg ind^{-1} d^{-1}, total ingestion of *C. simillimus* increased in the in-patch experiment on day 8 by a factor of 1.4 to 5.02 ± 0.80 µg ind^{-1} d^{-1}, whereas in the out-patch experiment on day 17 it decreased to 2.67 ± 0.49 µg ind^{-1} d^{-1}. In all experiments, *C. simillimus* predominantly fed on diatoms (Figure 3a). The contribution of diatoms to total carbon ingestion varied between 71 and 88 %. Ciliates were the second most important carbon source with a contribution of 9 to 13 %. Taken together, carbon drawn from heterotrophic dinoflagellates and the silicoflagellate *Dictyocha speculum* supplied another 3 to 15 %. Overall, diatom ingestion inside the patch increased by a factor of 1.4 and remained unchanged in the out-patch experiment. Ciliate and dinoflagellate ingestion remained unchanged in the in-patch experiment but decreased substantially in the out-patch experiment: for ciliates by a factor of two, for dinoflagellates by one order of magnitude. Figure 3b presents the contribution of various diatom species and genera to overall diatom ingestion by *C. simillimus*. Consistently, *Corethron* spp., *Chaetoceros* spp., *Guinardia* spp. and *R. chunii* supplied the bulk of diatom carbon taken up by the grazer.

R. gigas

From the initial and out-patch experiments, total ingestion of *R. gigas* was estimated at similar values of 0.94 ± 0.13 µg C ind^{-1} d^{-1} and 0.95 ± 0.03 µg C ind^{-1} d^{-1}. Inside the fertilized patch, ingestion increased by more than a factor of five to 5.38 µg C ind^{-1} d^{-1} (Figure 4a). This increase is reflected in all major food sources. Diatom ingestion increased by more than one order of magnitude and feeding pressure on ciliates and hdinos doubled. In the initial and out-patch experiments, carbon ingestion of *R. gigas* was dominated by ciliates that contributed 52 % and 54 % to total ingestion respectively. In-patch, diatoms were the dominant food source and accounted for 65 % of the daily carbon intake. Hdinos generally supplied between 11 % and 27 % to the budget. Figure 4b presents the composition of diatom ingestion for *R. gigas*. Solely *Corethron* spp. supplied more than 50 % of diatom carbon to *R. gigas* in the initial and out-patch experiments. In-patch, *P. lineola* and *P. turgidula* accounted for 1.7 µg C ingested per copepod per day, i.e. 50 % of the total diatom ingestion, *Corethron* spp. and *Chaetoceros* spp. combined for another 25 %.

Copepods < 2mm

Total ingestion of the copepod fraction < 2 mm was initially estimated at 177 ± 85 ng C ind^{-1} d^{-1} and remained constant with 174 ± 65 ng C ind^{-1} d^{-1} in the in-patch experiment on day 7 although diatom ingestion increased by a factor of 1.3. Outside the patch on day 17, total ingestion increased to 241 ± 76 ng C ind^{-1} d^{-1} (Figure 5a) with diatom ingestion increasing by a factor of 2.5. Note the change in order of magnitude compared to *C. simillimus* and *R. gigas*. Feeding of the small copepods was not clearly dominated by either type of prey; diatoms contributed between 22 % and 49 %, ciliates 17 % to 40 %. All four groups of flagellates, i.e. hdinos, silicoflagellates, pnanos and hnanos, supplied carbon to these grazers, the cumulated value ranging from 35 to 61 %. Diatom carbon was initially only provided by *Corethron* spp. and *Chaetoceros* spp (Figure 5b). In-patch, *F. kerguelensis*, *P. turgidula* and other diatoms of the size fraction 20-50 µm were ingested additionally. In the out-patch experiment, the copepods < 2 mm fed on all investigated diatom groups and species, the bulk of carbon originating from *R. chunii*, *Corethro*n spp. and *Guinardia* spp.

Clearance rates and grazing selectivity

C. simillimus

Clearance rates estimated for various prey organisms are presented in Table 4. When compared between diverse types of prey, clearance rates vary over one order of magnitude. No consistent relationship of the clearance rate with cell volume, cell carbon content and cell abundance could be discerned. For diatoms, however, the observed differences were related to cell size. In Figure 6a clearance rates for various diatom species or genera estimated in all three experiments are plotted against their respective cell size. The horizontal lines indicate the average clearance rates calculated for the four diatom size classes (see above). Both, the detailed rates and the size class rate showed an increase with increasing size of the diatom cell. The average clearance for cells > 100 µm was not higher than the rates determined for *R. chunii* (80 µm), *Corethron* spp. (90 µm) and *P. lineola* (95 µm), indicating that feeding efficiency might have reached a maximum at a cell size of approximately 80 µm. Furthermore, chain formation did not seem to influence the grazing efficiency on species like *P. turgidula* (usually in chains of 3-5 cells) and *P. lineola* (chains of 5-10 cells) but must have played a role in the high clearance rates observed for *Chaetoceros* spp. despite the small size of the actual cell. Average clearance for *C. simillimus* on diatoms remained stable; the slight increase from 12 to 14 ml ind^{-1} h^{-1} is not significant ($p < 0.05$). Clearance calculated on the species or genus level was more variable when compared between experiments but changes

showed no consistent trend. Clearance rates for pnanos and hnanos were consistently negative. Rates for hdinos and ciliates showed a stepwise decrease over the course of the three experiments; however, this variability was not related to the absolute abundance of the prey taxon.

R. gigas

Clearance rates determined in the experiments with *R. gigas* warrant a more detailed analysis. For diatoms, the overall and detailed rates were similar in the initial and out-patch experiment but showed a strong increase in the in-patch experiment on day 16. In the latter incubation, clearance rates for different diatoms were again related to size, albeit this trend is only resolved with the average clearance rates for diatom size classes (Figure 6b). Plotting single diatom species rates did not yield the clear and informative picture obtained for *C. simillimus*. However, as was the case for *C. simillimus*, rates for pnanos and hnanos remained below zero and clearance for hdinos and ciliates decreased with time. In Figure 7, overall clearance rates on diatoms, hdinos and ciliates are plotted against the respective abundance of the prey taxon and for every experiment separately. The decreasing clearance on ciliates and hdinos over the course of all three experiments showed an inverse relationship with abundance. In the initial and out-patch experiments, variation of clearance between the three different taxa also displayed this correlation. However, differences of the clearance rates between diatoms, hdinos and ciliates from the in-patch experiment showed no relationship with absolute abundance of the three groups. These results possibly indicate selective feeding and a change in foraging behavior and will be dealt with below.

Copepods < 2 mm

Clearance rates estimated for the small fraction of grazers either remained constant over the course of the three experiments or increased (see Table 4). Clearance rates for ciliates, hdinos, pnanos, hnanos and the silicoflagellate *D. speculum* were always positive and remained constant. This is also true for the diatom genera *Chaetoceros* spp. and *Corethron* spp. All other rates determined for diatoms, except diatoms > 100 µm, gradually increased from initially negative values to all positive values in the out-patch experiment. Large standard deviations, however, rule out a statistical significance of this increase for 60 % of the rates (Student's t-Test, $p < 0.05$). Variability in clearance rates for different taxa or between experiments showed no consistent relationship with abundance, size or carbon content of the prey. Only in the out-patch experiment, where grazing on diatoms was strongest, could a

tendency be resolved between cell size and clearance rate (Figure 6c). However, this was only evident in the average values, i.e. the horizontal bars, for the cumulative size classes.

Selective grazing

In a first step, frequency distribution of diatoms, silicoflagellates, hdinos and ciliates in the environment and the diet were compared. The contribution of pnanos and hnanos was omitted as the small flagellates were by one to two orders of magnitude more abundant than the other prey taxa which would have biased the SI toward exceptionally high positive selectivity of the less abundant taxa. In fact, the explanatory power of SIs in general relies on the ability not to compare apples with pears. The χ^2 test indicated selective grazing in all experiments except the initial incubation with *C. simillimus* on day 2. Values calculated for χ^2 and the level of significance are given in Table 5. Figure 8 presents the results of the SI α estimated for diatoms, hdinos and ciliates over the course of the experiments. Error bars indicate the standard deviation of the mean calculated from three replicate grazing bottles. *C. simillimus* (Figure 8a) was not grazing selectively in the initial experiment as indicated by the result of the χ^2 test, and the SI for the three prey taxa is not significantly different from 0.5. In-patch, a drift could be observed towards a positive selection for diatoms and a negative selection against hdinos. The last experiment, in which copepods accidentally caught from the edge of the patch were incubated in sample water with low microplankton abundance from outside the patch, shows a further specialization on diatoms and selection against hdinos with development of the bloom. In both experiments, in-patch and edge-patch, ciliates continued to be ingested invariantly according to their contribution to abundance in the environment.

Grazing of *R. gigas* (Figure 8b) was selective in all three experiments according to the results of the χ^2 test. The inverse relationship of clearance rate and abundance (see again Figure 7) was reflected in a positive selection of ciliates and hdinos and overall avoidance of diatoms in the initial and out-patch experiment. In-patch, *R. gigas* displayed a very different grazing behavior. Diatoms were ingested proportionally to their presence in the environment and hdinos apparently avoided. Ciliates were still positively selected.

Figure 8c presents the results for the small copepod fraction. Although the values estimated for χ^2 indicate selective grazing in all three experiments, large standard deviations preclude almost entirely identification of the taxon responsible for this selectivity. In these incubations, a mixture of species and stages was incubated and among replicate bottles variability in the type of prey ingested - but not the total amount of carbon ingested - was strong. The overall trend for the initial and in-patch experiment, one week after the first Fe

infusion, indicated selection for ciliates, invariant ingestion of hdinos, and avoidance of diatoms. As was the case for *C. simillimus*, small copepods in the last experiment originated from the edge of the patch. In this incubation, feeding seemed to be proportional to the contribution of the groups in the environment. A slight avoidance was indicated for hdinos, as was already observed for the larger grazers.

Differential mortality of prey populations

Based on available ingestion rates, on *in situ* diatom standing stock, and on abundance estimates of copepod grazers, mortality rates for diatoms in % of the in-patch standing stock per day (% s.s. d^{-1}) have been calculated. For *C. simillimus* a detailed calculation at three points in time is presented in Table 6. For day 2 and day 8, mortality is based on ingestion rates determined for several diatoms in incubation experiments 2 and 5, and *C. simillimus* standing stock estimates from cast 14-03 and 45-06 respectively. Cast 45-06 on day 7 was chosen as no MN data is available for day 8. For day 21, maximum ingestion rates determined in the three experiments and an extrapolated abundance value of 52 400 ind m^{-2} (see explanation above and Krägefsky et al. in prep.) is assessed. Copepod standing stock was integrated over 150 m as the larger grazers showed strong DVM behavior. Note that the abundance of *C. simillimus* at station 14 is exceptionally high but according to acoustic measurements a recurrent phenomenon on small spatial and temporal scales (S. Krägefsky unpubl. data). Mortality of diatoms ranged from 0.3 % s.s. d^{-1} for *T. nitzschioides* to 44 % s.s. d^{-1} for *Corethron* spp. Overall mortality of diatoms was highest on day 2, with 15.2 % s.s. d^{-1} and gradually decreased to 9.3 % s.s. d^{-1} on day 8 and 5.6 % s.s. d^{-1} on day 21. For comparison, the maximum ingestion rates of *R. gigas* and the fraction of copepods 1-2 mm measured for total diatoms were combined with their respective standing stock estimated from cast 45-06 (Figure 1) and diatom standing stock at station 46 (Table 6), resulting in an additional diatom mortality of 1.4 % s.s. d^{-1} due to grazing of *R. gigas* and 1.7 % s.s. d^{-1} due to the small copepods. The latter two estimates have to be viewed with caution. Gut fluorescence measurements indicated a substantially higher ingestion of autotrophic prey for *R. gigas* and the small copepod fraction than determined by the incubation experiments. Ingestion rates for *C. simillimus* were in accordance for both methods (Schultes & Bathmann in prep.).

Standing stock estimates for hdinos and ciliates and the silicoflagellate *D. speculum* are not available to date. However, based on counts of tintinnids and dinoflagellates at station 46 (Henjes 2004) mortality for these two prey taxa amounted to 12.8 % and 1.4 % s.s. d^{-1}

respectively, due to grazing activity of *C. simillimus*, *R. gigas* and copepods 1-2 mm combined (data not shown).

Discussion

Composition and development of the copepod grazer community

The iron fertilization experiment EisenEx (Strass et al. 2001) induced a diatom bloom in a stable cyclonic eddy north of the Polar Front (PF), with chl *a* concentrations and diatom biomass inside the fertilized patch increasing about fourfold (Gervais et al. 2002, Assmy 2004). 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). At the start of the fertilization experiment, copepod abundance in the center of the eddy was low compared to the surrounding waters. Measurements with multi-frequency acoustics indicate an increase in abundance by a factor of 3 over the 21 days of the study, which seems to be due to changes in diurnal vertical migration behavior (DVM) embedded in a vertically sheared flow field, leading to accumulation of copepods in the blooming patch (Krägefsky et al. in prep.).

The grazer community during EisenEx was a typical sub-Antarctic assemblage characteristic for waters north of the PF (e.g. Perissinotto 1992, Atkinson 1996, Bernard & Froneman 2003). In general, abundance and biomass of mesozooplankton are enhanced in the PFZ compared to the Permanently Open Ocean Zone (POOZ) and dominated by copepods (Pakhomov et al. 2000). Euphausids and salps were of minor importance in this study (Krägefsky et al. in prep.) and the composition of the community remained stable. Large numbers of the small and ubiquitous cyclopoid copepod *Oithona* sp. were present in the water column with an average of 18 000 individuals m^{-3} in the upper 150 m (Henjes 2004). Details on the composition, distribution and grazing impact of this smallest mesozooplankton fraction could not be investigated with the large-meshed sampling gear used in this study. Results from Niskin bottle sampling are presented in Henjes (2004). However, a series of experiments was run with the smallest copepods retained in the 300 µm Bongo net, i.e. a mixture of individuals with 1-2 mm body length. This fraction is probably dominated by *Ctenocalanus* sp. or *Clausocalanus* sp., both widespread and very abundant in the PFZ in spring (Pahkomov et al. 2000, Dubischar et al. 2002), but positive identification is not available. With a standing stock up to a factor of three higher than the copepods > 2 mm (Table 1) these small grazers are likely to contribute substantially to community grazing pressure.

In the size class > 2 mm late copepodite and adult stages of *Calanus simillimus* represented the major fraction in terms of abundance (Figure 1) and biomass (data not shown), making *C. simillimus* a key grazer inside and outside the iron fertilized patch. Abundance of *C. simillimus* is at the higher end of the published range and comparable to concentrations of 273 to 1055 individuals m^{-3} estimated by Perissinotto (1992) in the vicinity of the Prince Edward Archipelago. Compared to *C. simillimus, Rhincalanus gigas* was of minor numerical importance in the upper 150 m with maximum concentrations reaching 50 individuals m^{-3}. This peculiar giant among copepods, with an adult body size of almost 9 mm (Ommaney 1936), is found throughout the Southern Ocean, with highest abundances near the PF (e.g. Froneman et al. 2000). The duration of its life cycle is still under debate and a regional variation between one year in the northern waters including the PFZ and two years in the Weddell Sea is discussed (Ward et al. 1997). According to Ward et al. (1997) the northern population of *R. gigas* reaches the surface waters in November so the timing of EisenEx probably coincided with the arrival of this grazer in the upper water column. In comparison, Atkinson (1991 and 1998) proposes a one year life cycle for *C. simillimus* with mating in the top 250 m in early spring followed by a rapid development of the population and possibly a second generation in the same year. Neither *C. simillimus* nor *R. gigas* is assumed to undergo seasonal diapause (Atkinson 1998).

Feeding response to the iron induced diatom bloom

Already before the onset of the iron fertilization experiment, the key grazer *C. simillimus* was actively feeding in the surface layer and on all four taxa available to it: diatoms, silicoflagellates, hdinos and ciliates, albeit diatoms represented the most important carbon source. Feeding activity and diet composition of the grazer changed little in response to the iron induced diatom bloom. Mayzaud et al. (2002) present the only grazing estimate available for *C. simillimus* in spring, measured in the Indian Sector of the Southern Ocean in October at chl *a* concentrations of 0.5 µg l^{-1}. Their rate of 4.2 µg C ind^{-1} d^{-1} is based on gut fluorescence and comparable to the estimates from EisenEx. Diatom clearance of *C. simillimus* remained constant and ingestion inside the fertilized patch increased with diatom abundance by a factor of 1.4. Similar observations are reported by Atkinson & Shreeve (1995) studying the response of a copepod community to the spring bloom in the Bellingshausen Sea. *Calanus propinquus* – a congener of *C. simillimus* –, *Metridia gerlachei* and *Oithona* sp. were already actively feeding in ice covered waters with ~ 0.1 µg chl *a* l^{-1}. Furthermore, copepods maintained similar clearance rates over a fourfold increase of chl *a*

from 0.8 to 3.2 µg l^{-1}. The authors speculate that feeding must at times be severely food limited given that the same feeding effort resulted in approximately half the carbon intake.

C. simillimus has been termed an omnivorous copepod (Atkinson 1998) but detailed information on the composition of its diet with changing environmental conditions is scare. The only study that investigated feeding of *C. simillimus* in greater detail was conducted at a sub-Antarctic site near South Georgia in summer with concentrations of 0.8 µg chl *a* l^{-1} (Atkinson 1996). Algal carbon and heterotrophic food roughly contributed equal amounts to the grazer's diet. During a study at the PF in the Pacific Sector of the Southern Ocean contribution of phytoplankton to daily dietary carbon intake has been reported to be as low as 3 % in late summer with chl *a* concentrations in the environment of 0.6 µg l^{-1} (Urban-Rich et al. 2001). Further evidence for omnivorous feeding and variable diet composition in response to availability is presented by Ward et al. 1996 who analyzed lipid content, hydrocarbons, lipid class and fatty acid composition in PFZ copepods from an intense diatom bloom (up to 20 µg chl *a* l^{-1}) and a post bloom situation (< 1 µg chl *a* l^{-1}) four weeks later. High amounts of pristane, a marker for recent feeding, were found in *C. simillimus* from both samplings, indicating that it was actively feeding. However, contribution of the diatom alkene $C_{21:6}$ was substantially lower in the post-bloom situation than in the diatom bloom, so diatoms must have not been the major food source after the bloom. Additionally, ratios of polyunsaturated fatty acids (PUFA) suggested that microheterotrophs were important carbon suppliers (Ward et al. 1996). Results from this study solidify the perception of *C. simillimus* as an omnivorous grazer and high feeding activity even at low food availability. The clear dominance of siliceous organisms in the grazer's food spectrum observed during EisenEx has not been reported so far.

In contrast, *R. gigas* responded to the iron induced bloom, not only with greatly increased feeding activity but also with a switch in diet composition from mainly heterotrophic prey to diatoms. In-patch, clearance of diatoms increased 3-fold thereby increasing diatom ingestion by an order of magnitude. Despite a decrease in clearance on hdinos and ciliates the latter continued to be ingested at similar or even higher rates, which is due to an increasing biomass for these two taxonomic groups in the field (Henjes 2004). Atkinson (1998) reviewed the diet and seasonal feeding activity of *R. gigas*. In summer/bloom situations phytoplankton is the major food source. In spring and autumn, or pre- and post-bloom situations, diatoms continue to be present in the diet but protozoans, crustaceans and amorphous debris are additionally ingested. The results obtained before and during the EisenEx bloom confirm the existing knowledge.

The initial, predominantly heterotrophic feeding activity of *R. gigas* resulted in low daily carbon rations of 0.5 % body carbon (Schultes & Bathmann in prep.). Several reasons for these low ingestion rates can be envisaged. Similarly low rations of 0.1 to 0.4 % were determined for *R. gigas* in late summer by Pakhomov et al. (1997) and are interpreted as possible seasonal feeding arrest. However, respiration rates determined in parallel with the feeding experiments during EisenEx remained stable, with 1.37 ± 0.19 µl O_2 $ind^{-1}h^{-1}$ (out patch) and 1.24 ± 0.05 µl O_2 $ind^{-1}h^{-1}$ (in patch), equivalent to ~ 7 % body carbon day^{-1} (Schultes & Bathmann in prep.). This suggests high and overall unchanged metabolic activity over the development of the bloom. Secondly, the low daily ration could also reflect food limitation. Froneman et al. (1996) found *R. gigas* clearing microzooplankton at a rate of 12 ml ind^{-1} h^{-1}, similar to rates of 10 to 12 ml ind^{-1} h^{-1} in this investigation. Considerably higher abundance of microzooplankton during the investigation of Froneman et al., however, resulted in ingestion rates of 8.5 µg C ind^{-1} d^{-1}, sufficient for the grazer to meet its minimum metabolic requirements. Finally, trophic cascading inside the bottle due to predation of the copepod on microzooplankton can lead to an underestimation of ingestion (Nejstgaard et al. 2001). Schultes & Bathmann (in prep.) present a detailed discussion on methodological problems when assessing ingestion rates. They conclude that feeding on detritus, which cannot be estimated from incubation experiments, has to be considered as an additional carbon source for *R. gigas*.

Evidence for a response of the fraction of copepods < 2 mm to the iron fertilized bloom is ambiguous. In the in-patch experiment, diatom ingestion appeared to be slightly enhanced but the difference is not significant due to large standard deviations. However, ingestion rates estimated from gut fluorescence increased inside the patch by a factor of two (Schultes & Bathmann in prep.). A strong increase in diatom clearance and ingestion was also noted in the out-patch incubation experiment. Grazers for this experiment originated from a Bongo net haul on the edge of the patch. Regardless whether the increase in diatom clearance in the third experiment resulted from the preconditioning of the copepods on the edge of the bloom or in response to the seasonal increase of diatoms taking place also outside the fertilized patch (Assmy 2004), we conclude that also the small fraction of copepods increased its feeding activity on diatoms with their greater availability in the mixed layer.

Diet composition of the small grazers encompasses all prey organisms available in the environment including pnanos and hnanos. Despite similar clearance values and about equal abundance, hnanos supply a greater amount of carbon than pnanos to the grazers due to their higher carbon content per cell, 10 pg C $cell^{-1}$ compared to 8 pg C $cell^{-1}$ for pnanos. Small

grazers, like *Oithona* sp. for example, can feed with high rates on flagellates (e.g. Atkinson 1994). The nanoflagellates represent an important carbon pool that is apparently not available to the larger *C. simillimus* and *R. gigas*. Clearance rates on pnanos and hnanos for those two copepods were consistently negative. This net growth of prey is probably due to the copepods' predation on ciliates and dinoflagellates, which relieved grazing pressure from the nanoflagellates. Although this does not rule out that *C. simillimus* and *R. gigas* also ingested nanoflagellates, the small size of less than 5 µm presumably made feeding on them inefficient in general (Nival & Nival 1976). Berggreen et al. (1988) demonstrated a shift in optimum particle size with prosome length for the developmental stages of *Acartia*. During EisenEx, younger copepodite stages *R. gigas* actually could feed on the nano size fraction. The diet composition of C V was not different from the adult stages. C III/IV, however, also grazed on hnanos that supplied 3 to 8 % of total carbon ingested (data not shown). These dietary differences among developmental stages and species lead to an effective partitioning of food between copepods (Atkinson 1994).

Selective feeding

Two basic types of foraging behavior of copepods have been observed with high speed cinematography: a relatively passive mode of suspension feeding with continuous low amplitude flapping of the second maxillae, and ambush feeding, where cells are detected individually with an active, oriented capture response. Whereas small cells are accumulated in the passive mode, grazers also show ambush feeding on large cells. Copepods are able to switch back and forth between both types of feeding behavior in a mixture of particles with different quality and quantity in order to maximize carbon intake with minimum feeding activity (Price and Paffenhöfer 1986 and references therein; see also the review by Price 1988). The iron induced diatom bloom had a major impact on the particle composition of the prey field. In response copepods showed variable changes to their feeding behavior. According to the calculated selectivity indices *C. simillimus* became a more specialized feeder, *R. gigas* and the small copepods more invariant. As the results for small copepods are associated with a lot of scatter and represent the grazing activity of a variety of organisms, the discussion of changes in grazing selectivity will be largely restricted to *C. simillimus* and *R. gigas*.

The unselective feeding of *C. simillimus* before the fertilization develops into positive selection for diatoms compared to dinoflagellates and ciliates. This selectivity, however, is most probably a reflection of the overall increase in size of the particles in the water column.

The diatom assemblage shifted from a community initially dominated by small diatoms to one that was dominated by large and chainforming diatoms at the end of the experiment. The size of the particle has great influence on its clearance rate which biases the selectivity coefficient towards positive selection of the large compared to small prey (Boyd 1976). In general, *C. simillimus* appears to be a specialized harvester of large, carbon rich diatoms. The average clearance rate on diatoms is consistently higher compared to ciliates and hdinos. Furthermore, clearance on diatoms rises rapidly with increasing size of the cell and then is maintained stable for a whole range of biomass important species (see Figure 6a). Feeding on the biomass dominant particle type is referred to as "peak tracking" (Atkinson 1995) and is not necessarily tied to large sized cells, as demonstrated in studies by Schnack (1983, 1985) and Perissinotto (1992). The latter reports preferential feeding of *C. simillimus* on the particle fraction < 20 µm that represented the biomass peak during his investigation. Chain formation adds further complexity to the "size" discussion. The single *Chaetoceros* cell, excluding the spines, is only up to 20 µm in diameter but the spines and colony formation seem to enhance grazing mortality considerably in comparison to cells of similar size. In comparison, *P. turgidula* and *P. lineola* are grazed at rates similar to the average rate of their size class despite considerable elongation through chain formation. Finally, the average carbon content per cell is 246 pg for *Chaetocero*s but only 53 pg for *P. lineola*, which might be another reason to feed on one more intensely than average but not on the other. No sharp line can be drawn on whether size or biomass determines the efficiency with which a diatom is preyed upon. This seems reasonable considering that a grazer has to survive in an environment with highly variable food supply in terms of quality and quantity as has been impressively demonstrated by the floristic shift following the fertilization. This variability is also observed *in situ*. In November 1992, *Polarstern* expedition ANT X/6 encountered three distinct blooms in the PFZ, each dominated by a different diatom species (Smetacek et al. 1997).

R. gigas showed a very different feeding strategy to *C. simillimus* that seems to be associated with a change in foraging behavior in response to the diatom bloom. In the initial and out-patch experiment, motile cells are positively selected compared to diatoms. Exceptions make again large diatoms, especially *Chaetoceros* and *Corethron*. Both diatoms have something in common, they possess spines. An important feature of ambush feeding is detection, which is either accomplished through mechano- or chemoreception (Price 1988). Detection of motile prey via mechanoreception, by using the receptors on the first antennae to sense hydrodynamic disturbances generated by prey movement, has been demonstrated (e.g. Landry 1980, De Mott & Watson 1991). Also algal cells can be detected over several hundred

microns distance (Price 1988). A hypothesis by Legier-Visser et al. (1986) speculates on the mechanism to detect non-motile cells via mechanoreception which according to them is based on deformations that the cell creates in the flow streamlines of the feeding current. Similar to the generation of a pressure wave these deformations extend in front of the particle (Price 1988). Spines will generate a stronger deformation in the streamlines than a more homogeneous particle of spherical shape for example and a *Chaetoceros* colony could therefore be more easily detected than other cells. Alternatively, the spines of both species simply increase the detectable surface. All results strongly suggest that *R. gigas* resorts predominantly to ambush feeding in times of scarce supply of phytoplankton. Inside the bloom, ciliates are still positively selected and diatoms are cleared according to their contribution to abundance. Probably, *R. gigas* switches between the feeding modes at this stage, still taking advantage of the highly nutritious ciliates via ambush feeding but maximizing ingestion of diatoms through rather passive accumulation. Similar behavior is described for *Acartia tonsa* feeding in a mixture of diatoms and ciliates at varying concentrations (Kiørboe et al. 1996). Atkinson & Shreeve (1995) also termed *R. gigas* an "indiscriminate feeder" at the bloom stations of their study in the Bellingshausen Sea. The flexible feeding behavior of *R. gigas* is possibly one reason why *R. gigas* is so widespread throughout the Southern Ocean, a fact that is known since the Discovery Cruises (Ommaney 1936), whereas *C. simillimus* is restricted to the comparably productive waters of the PFZ (Froneman et al. 2000).

With progression of the diatom bloom, hdinos are apparently "avoided" by *C. simillimus* and *R. gigas*. The size spectrum of the dinoflagellate community remained unchanged and absolute biomass even increased. However, the relative contribution to total biomass and abundance decreased. Reduction of feeding on one type of prey with increased availability of an alternative food source is called "prey switching" and appears to be a common strategy among copepods to maximize energy gain (e.g. Kiørboe et al. 1996, Meyer-Harms et al. 1999). One mechanism that leads to prey switching is a change in feeding behavior from ambush feeding to suspension feeding. Furthermore, suspension feeding copepods increase the rate of flapping of the cephalothoraic appendages with increasing cell concentrations (Price & Paffenhöfer 1986). Especially dinoflagellates are able to escape the feeding current that is created during suspension feeding. Some ciliate species, however, can be entrained in the feeding current (Kiørboe et al. 1996). This is a possible explanation for the continued ingestion of ciliates by all copepods. It is proposed that prey switching in *C. simillimus* and *R. gigas* occurred due to modifications in their feeding mode, for *R. gigas*

from ambush feeding to suspension feeding and for *C. simillimus* to increased activity in the suspension feeding mode with development of the bloom.

Dinoflagellates and ciliates can intensely graze on diatoms of both the nanophytoplankton and the size range of diatoms available for copepods (e.g. Klaas 1997) and hence are in competition for food with their mesozooplankton predators. This blend of competition and predation is called intraguild predation (Polis & Holt 1992) and has several stabilizing effects on the co-existence of the predator and the prey as demonstrated in a modeling study of Gismervik & Anderson (1997). An important side effect of intraguild predation and prey switching by copepods is a higher and more stable algal biomass in the modeled system. Thereby, copepods not only increase their own nutritional intake by preying on micrograzers but they indirectly promote growth of their "preferred" food. Furthermore, releasing the grazing pressure on hdinos and ciliates makes nutrients available for the larger microphytoplankton that is usually out-competed for them by the nanophytoplankton (Gismervik & Anderson 1997).

Atkinson conducted several studies similar to this one near South Georgia (Atkinson 1994, Atkinson 1996) and in the Bellingshausen Sea (Atkinson 1995) and interpreted grazing selectivity solely on the basis of clearance rates. All of them concur in that clearance of diatoms and non-diatom taxa increases with size. Selective grazing on motile prey in comparison to similar sized diatoms is attributed to predatory behavior, i.e. ambush feeding on microzooplankton, and is associated with specific grazers or low phytoplankton concentrations in pre-bloom waters. Results obtained for the grazing patterns of *C. simillimus* and *R. gigas* during EisenEx confirm this view.

Differential diatom mortality and grazing impact on prey populations

All copepods clearly showed an influence of size on their efficiency to clear diatoms from the incubation water. Large diatoms are cleared with highest rates. Assuming that changes in handling effort are minor, this bears the advantage of supplying a maximum carbon ration with few feeding "operations", i.e. detection, pursuit, capture, handling and ingestion of the prey (*sensu* Frost 1972, Price 1988). The fact, that few diatom species, *Corethron pennatum* and *Rhizosolenia chunii* for example (see Figure 3b), contribute a major fraction of ingested carbon to the copepods reflects this principle very well. Consequently, the mortality that these species suffer from copepod grazing is proportionally higher than for species cleared with lower efficiency. Overall mortality of diatoms due to the grazing activity of *C. simillimus*, the dominant grazer, decreases with development of the bloom so grazing

activity is not sufficient to prevent accumulation of diatoms (Table 6). Mortality calculated for day 2 can be seen as a maximum estimate. Zooplankton abundance was exceptionally high with phytoplankton standing stock still being low. Such local zooplankton peaks are frequently observed throughout the study, indicated by results from acoustic measurements (S. Krägefsky pers. comm.). Although not of great significance when interpolated on temporal and spatial scales they can potentially alter the composition of the phytoplankton standing stock locally. The situation on day 8 probably reflects the average mortality of certain diatoms. Assmy (2004) determined four response types for the accumulation of diatom species following the iron fertilization. Two of them shall be presented here as we think that diatom mortality due to copepod grazing has the potential to shape the accumulation pattern. Type I is characterized by fast growing, weakly silicified diatoms with sustained exponential growth rates. Diatoms representative of this type are *Pseudonitzschia lineola* and *Chaetoceros curvisetus*. The initially high mortality for *P. lineola* and *Chaetoceros* spp. is substantially reduced in the fully developed bloom which is well in accordance with the highest accumulation rates determined for these diatoms by Assmy (2004). Copepod grazing could not cope with the high intrinsic growth rates of these species. The second accumulation pattern, Assmy's "type III", is linear growth without initial lag phase. Representatives of this group are *C. pennatum* and several other large diatoms like *Guinardia cylindrus*, *Haslea* sp., *Leptocylindrus mediterraneus*, *Membraneis imposter* and *R. chunii*. Daily loss of the population due to grazing is still high for *Corethron* spp, *R. chunii* and *Guinardia* spp. on day 21. Linear accumulation indicates growth limitation via bottom-up or top-down factors. Assuming that all macronutrients were non-limiting and that the three consecutive infusions of iron also completely relieved the cells from iron limitation, the heavy grazing pressure is a possible explanation for the linear accumulation pattern observed for the large diatoms. The initial phytoplankton community was numerically dominated by *Fragilariopsis kerguelensis* and *Thalassionema nitzschioides* that both suffer lowest grazing mortality from large grazers. This reduced mortality is most likely due to their small size in the present study. Calculation of selectivity indices for various diatoms shows unselective feeding of *C. simillimus* on *F. kerguelensis* and avoidance of *T. nitzschioides* (data not shown) which we believe reflects merely the influence of their size on grazing efficiency. Alternatively, the high degree of silicification of *F. kerguelensis* is argued to protect it from grazing (Verity and Smetacek 1996, Hamm et al. 2003). *Calanus propinquus*, however, fed intensely on a culture of *F. kerguelensis* and was able to crush the solid frustules (Schultes unpublished data). As long as larger, carbon rich diatoms are present, these will be grazed preferentially by copepods.

These findings and the continued presence of a part of the copepod community in the surface layer during the austral winter may partly explain the dominance of *F. kerguelensis* and other small species in the initial population and in spring populations of the Southern Ocean in general (Hart 1934).

Overall diatom mortality due to the grazing activity of *C. simillimus* alone reaches 15.2 % of the standing stock per day and decreases to 5.6 % with the development of the bloom. Studying the grazing impact of this copepod in a bloom and non-bloom situation near the Prince Edward Archipelago, Perissinotto (1992) found *C. simillimus* ingesting 1.2 to 2.1 % of the standing stock per day during the bloom and 13.4 to 15.7 % in the non-bloom situation. As previously mentioned, abundance of *C. simillimus* during EisenEx was comparable to the study of Perissinotto (1992) and the same holds true for the grazing impact estimates. *C. simillimus* frequently dominates the copepod communities of the PFZ and within these often shows highest daily ingestion rates among grazers (Perissinotto 1992, Atkinson et al. 1996, Pakhomov et al. 1997, Froneman et al. 2000). Overall grazing impact of copepods in these studies reaches 25 % of phytoplankton standing stock removed per day with the major fraction being attributable to *C. simillimus*. Due to low diatom ingestion rates or low grazer standing stock, the combined grazing impact of *R. gigas* and the copepods < 2 mm estimated from the incubations did not exceed 1.5 %. However, gut fluorescence measurements indicate that this number might be an underestimation (Schultes & Bathmann in prep.). A complete grazing and export budget, including also the feeding impact of *Oithona* sp., will be presented elsewhere (Krägefsky et al. in prep.).

Conclusion

Already Froneman et al. (2000) stated that *C. simillimus* should be considered a key organism of the PFZ. The results of this study support this conclusion. The grazer dominated the mesozooplankton community, showed highest ingestion rates, consistently preyed on diatoms most effectively and can possibly structure the phytoplankton composition of blooms developing in this area of enhanced primary production. Furthermore, importance of heterotrophic prey for copepod grazers in the Southern Ocean has been confirmed and future investigations on feeding should include methods to estimate both autotrophic and heterotrophic carbon sources. Differences in foraging behavior in response to the variable supply of microplankton standing stock as observed for *R. gigas* possibly determines the ability of a grazer species to adapt to a wider range of productivity regimes.

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Table 1:
Sampling details for casts with a multiple opening closing net (MN) and copepod standing stock estimated from these casts integrated over the upper 150 m of the water column.

MN Station -Cast	Days since 1st Fe fertilization	Time at depth	Position	Depth intervals (m)	Copepod standing stock (ind m^{-2}) > 2 mm	1-2 mm
09-02	-2	0:19	initial	0-50, 50-110, 110-190, 190-300, 300-1000	21,380	40,030
11-04	0	22:23	in patch	0-100, 100-200, 200-300, 300-400, 400-750	26,100	73,950
12-03	1	10:35	out patch	0-100, 100-250, 250-400, 400-500, 500-700	11,350	79,150
14-03	2	2:33	in patch	0-50, 50-100, 100-200, 200-300, 300-500	62,350	206,650
38-09	3	16:30	in patch	0-150, 150-300, 300-500, 500-1000, 1000-1500	41,100	44,100
45-06	7	12:50	in patch	0-65, 65-130, 130-400, 400-500, 500-750	57,755	180,730
48-06	9	16:07	out patch	0-50, 50-100, 100-250, 250-500, 500-750	13,730	31,350
49-06	10	16:05	in patch	0-80, 80-200, 200-300, 300-400, 400-500	51,490	91,630

Table 2:
Details on experimental set-up of the incubation series including time and position of sampling for grazers and prey populations, initial chl *a* concentrations of the incubation water, duration of the incubation, number of grazers per 1 liter bottle and developmental stage where applicable.

Expt.	Date	Days since 1st Fe fertilization	Station	Position	Initial chl *a* ($\mu g\ l^{-1}$)	Grazers (no/stages)	Duration (h)
1	7 nov	-2	9	initial	0.56	Copepods < 2mm (10/n.d.) *R. gigas* (5/C6♀)	24
2	10 nov	2	14	initial	0.52	*C. simillimus* (5/C6♀)	27
3	13 nov	5	42	out patch	0.55	*R. gigas* (4/ C6♀)	43
4	15 nov	7	45	in patch	1.0	Copepods < 2mm (20/n.d.)	41
5	16 nov	8	46	in patch	1.15	*C. simillimus* (5/C6♀)	36
6	24 nov	16	88	in patch	1.4	*R. gigas* (4/ C6♀)	30
7	25 nov	17	89/90	out patch (ctd) edge patch (bongo)	0.47	Copepods < 2mm (15/n.d.) *C. simillimus* (5/C6♀)	34

Table 3:
Prey categories for the calculation of clearance and ingestion rates and list of species regrouped in theses categories when determined. Numbers in brackets in the category section indicates the min-max values of pg carbon per cell. In the species section the average elongation of the cell in the largest dimension is indicated.

Category (pg C cell^{-1})	**Species (mean length µm)**
Chaetoceros spp. (21-582; mean 246)	*Chaetoceros aequatorialis* (25) *Chaetoceros atlanticus* (18) *Chaetoceros bulbosus* (15) *Chaetoceros convolutes* (22) *Chaetoceros curvisetus* (12) *Chaetoceros dichaeta* (16) *Chaetoceros neglectus* (5) *Chaetoceros peruvianus* (20) *Chaetoceros* indet. (n.d.)
Corethron spp. (231-2001)	*Corethron inerme* (90) *Corethron pennatum* (90)
Fragilariopsis kerguelensis (35-182)	*Fragilariopsis kerguelensis* (40)
Guinardia spp. (202-2318)	*Guinardia cylindrus* (80) *Guinardia delicatula* (40)
Pseudonitzschia lineola (31-77)	*Pseudonitzschia lineola* (95)
Pseudonitzschia turgidula (20-61)	*Pseudonitzschia turgidula* (45)
Rhizosolenia chunii (202-1072)	*Rhizosolenia chunii* (80)
Thalassionema nitzschioides (8-49)	*Thalassionema nitzschioides* (25)
other diatoms (variable)	*Cylindrotheca closterium* (80) *Dactyliosolen antarcticus* (250) *Fragilariopsis rhombica* (25) *Fragilariopsis obliquecostata* (70) *Haslea* sp. (90) *Leptocylindrus mediterraneus* (80) *Membraneis imposter* (90) *Navicula* spp. (35) *Nitzschia* spp. (10) *Pleurosigma atlantica* (120) *Pseudonitzschia heimii* (90) *Pseudonitzschia prologatoides* (60) *Pseudonitzschia turgiduloides* (90) *Proboscia* spp. (300) *Rhizosolenia hebetata* (500) *Thalassionema nitzschioides var. lanceolatum* (90) *Thalassiothrix antarctica* (1500) undet. centric diatoms (< 20 µm, 20-50 µm, > 50 µm)
silicoflagellates (626)	*Dictyocha speculum* (25)
phototrophic nanoflagellates (8)	n.d.
heterotrophic nanoflagellates (10)	n.d.
heterotrophic dinoflagellates (160)	n.d.
ciliates (254)	n.d.

Table 4:
Clearance rates for various prey species estimated before the bloom, inside and outside the fertilized patch

clearance rate (ml ind^{-1} h^{-1})	*C. simillimus*			*R. gigas*			copepods < 2 mm		
	initial day 2	**in day 8**	**out/edge day 17**	**initial day -2**	**out day 5**	**in day 16**	**initial day -2**	**in day 7**	**out/egde day 17**
***Chaetoceros* spp.**	20.9 (5.2)	21.5 (0.3)	22.0 (1.8)	13.4 (5.5)	n.d.	9.3 (2.0)	1.0 (0.8)	0.5 (0.9)	0.5 (0.8)
***Corethron* spp.**	22.1 (3.1)	25.3 (6.0)	17.4 (6.3)	4.4 (4.2)	3.1 (1.5)	9.4 (3.9)	0.7 (0.2)	0.3 (0.3)	0.8 (0.5)
F. kerguelensis	2.2 (4.3)	7.6 (2.0)	8.7 (4.9)	-6.0 (1.1)	-1.3 (0.6)	1.9 (4.8)	-4.0 (1.4)	0.5 (0.3)	0.5 (1.3)
***Guinardia* spp.**	18.3 (6.6)	9.5 (1.7)	19.9 (3.9)	n.d.	n.d.	-1.0 (4.5)	n.d.	n.d.	1.0 (1.0)
P. lineola	23.5 (4.4)	19.3 (0.3)	14.8 (4.5)	0.7 (4.8)	3.1 (1.6)	12.1 (3.7)	-2.5 (0.4)	-0.5 (0.1)	0.6 (0.4)
P. turgidula	9.4 (2.7)	13.2 (1.8)	10.3 (3.5)	-3.6 (3.7)	-0.7 (1.6)	14.3 (6.9)	-0.7 (2.9)	0.3 (0.4)	0.9 (1.0)
R. chunii	n.d.	18.9 (1.3)	19.9 (10.6)	n.d.	n.d.	19.3 (9.3)	n.d.	n.d.	1.3 (0.6)
T. nitzschioides	0.5 (1.0)	-2.5 (1.0)	1.1 (0.6)	-4.7 (1.3)	0.2 (0.4)	2.2 (3.4)	-2.5 (1.6)	0.0 (0.1)	0.0 (0.3)
diatoms < 20 µm	4.8 (4.6)	-1.4 (0.7)	-0.91 (1.5)	-1.3 (2.7)	0.2 (1.6)	3.2 (3.8)	-1.0 (1.6)	-0.2 (0.4)	0.1 (0.8)
diatoms 20-50 µm	3.4 (1.4)	4.1 (1.9)	3.8 (1.9)	-4.6 (1.3)	0.0 (0.5)	6.1 (4.2)	-2.8 (1.1)	0.2 (0.2)	0.4 (0.7)
diatoms 50-100µm	16.3 (4.2)	15.2 (3.1)	14.3 (5.0)	1.1 (0.9)	1.5 (1.5)	9.4 (3.4)	-1.1 (0.4)	0.0 (0.1)	1.0 (0.2)
diatoms > 100 µm	37.0 (24.3)	17.9 (8.6)	25.4 (1.5)	10.1 (14.7)	3.7 (1.9)	16.6 (5.1)	1.8 (2.7)	-0.5 (0.6)	2.7 (0.4)
diatoms (average)	12.7 (3.5)	12.4 (2.2)	14.9 (3.5)	2.5 (1.0)	1.5 (0.3)	7.7 (1.4)	0.4 (0.1)	0.2 (0.2)	0.8 (0.4)
D. speculum	4.5 (2.9)	8.4 (3.0)	1.8 (1.6)	-3.3 (6.1)	-1.5 (2.1)	1.7 (4.0)	1.1 (3.7)	0.0 (0.3)	0.2 (0.7)
pnanos	-1.8 (0.2)	-0.9 (0.2)	-0.2 (0.1)	-2.3 (0.2)	-2.1 (0.6)	-1.2 (0.1)	0.1 (0.1)	0.1 (0.0)	0.1 (0.0)
hnanos	-0.7 (0.6)	-0.7 (0.2)	-1.2 (0.1)	-2.8 (0.3)	-0.6 (0.3)	-1.8 (0.2)	0.3 (0.1)	0.1 (0.0)	0.1 (0.1)
hdinos	3.9 (0.2)	1.8 (0.2)	0.3 (0.2)	6.7 (0.4)	4.7 (0.5)	3.1 (0.5)	0.3 (0.1)	0.2 (0.0)	0.3 (0.1)
ciliates	7.0 (0.3)	4.0 (0.2)	2.9 (0.4)	12.0 (1.2)	9.8 (2.1)	7.6 (0.5)	0.7 (0.1)	0.7 (0.0)	0.6 (0.1)

Table 5:
Results of the chi-square ($\chi 2$) goodness-of-fit test (Sokal & Rohlf 1969) between the relative contribution of prey taxa in the environment and to the diet.

Experiment	Grazer	χ^2	Significance
1 – initial	copepods < 2 mm	69.4	$p < 0.001$
	R. gigas	80.9	$p < 0.001$
2 – initial	*C. simillimus*	1.9	n.s.
3 – out	*R. gigas*	52.7	$p < 0.001$
4 – in	copepods < 2 mm	59.1	$p < 0.001$
5 – in	*C. simillimus*	9.1	$p < 0.05$
6 – in	*R. gigas*	11.7	$p < 0.01$
7 – out/edge	copepods < 2 mm	8.6	$p < 0.05$
	C. simillimus	27.3	$p < 0.001$

Table 6:
Mortality calculation (% of standing stock d^{-1}) of various diatoms due to the grazing activity of *Calanus simillimus*. See text for further explanation.

	Day 2 - Station 14			**Day 8 - Station 45/46**			**Day 21 - Station 107**		
	community I mg C m^{-2} d^{-1}	prey standing stock (80 m) mg C m^{-2}	mortality % s.s. d^{-1}	community I mg C m^{-2} d^{-1}	prey standing stock (80 m) mg C m^{-2}	mortality % s.s. d^{-1}	community I mg C m^{-2} d^{-1}	prey standing stock (80 m) mg C m^{-2}	mortality % s.s. d^{-1}
Chaetoceros spp.	13.5	36	37.8	12.6	68	18.6	17.1	218	9.4
Corethron spp.	45.2	103	44.0	63.4	211	30.0	86.2	519	19.9
F. kerguelensis	0.5	30	1.6	3.9	68	5.7	5.2	137	4.6
Guinardia spp.	18.5	54	34.1	7.7	126	6.1	18.6	80	27.9
P. lineola	3.6	19	19.4	11.9	52	22.8	16.1	883	2.2
P. turgidula	0.9	8	11.0	2.7	22	12.3	3.7	82	5.4
R. chunii	n.d.	60	n.d.	7.7	31	24.9	24.4	117	25.0
T. nitzschioides	0.2	21	0.7	n.d.	28	n.d.	0.2	83	0.3
total diatoms	135.7	895	**15.2**	121.7	1304	**9.3**	165.1	3508	**5.6**
C. simillimus ind m^{-2} (150 m)	51 400			32 100			52 400		

Figure Captions

Figure 1:
Composition of copepod standing stock > 2 mm estimated from casts with a multiple opening closing net for the first 10 days of the fertilization experiment. "*" indicates casts taken in unfertilized control waters

Figure 2:
Frequency distribution of the major prey taxa in the control bottles over the course of the experimental series. Open symbols: out-patch; full symbols: in-patch.

Figure 3:
Daily ingestion rates and prey composition of *Calanus simillimus* estimated from the incubation experiments. A) Total ingestion. B) Diatom ingestion.

Figure 4:
Daily ingestion rates and prey composition of *Rhincalanus gigas* estimated from the incubation experiments. A) Total ingestion. B) Diatom ingestion.

Figure 5:
Daily ingestion rates and prey composition of copepods < 2 mm estimated from the incubation experiments. A) Total ingestion. B) Diatom ingestion. Note the different y-axis scaling compared to Fig. 3 and 4.

Figure 6:
Development of clearance rate with diatom cell size for A) *Calanus simillimus*, B) *Rhincalanus gigas* and C) copepods < 2 mm. For *C. simillimus* results from all three incubations are plotted. For *R. gigas* and the copepods < 2 mm the results from the experiment with highest diatom ingestion are plotted. Every data point represents the result from a single replicate bottle. Bars indicate the average clearance rate for the respective diatom size fraction. See text for further explanations.

Figure 7:

Change of clearance rate on the major prey taxa with absolute abundance of the taxa in the incubation water of the control bottle as estimated in the three incubations with *Rhincalanus gigas*. Error bars indicate the standard deviation calculated for the mean of three replicate bottles.

Figure 8:

Selectivity indices α calculated for the major prey taxa in the three incubations with A) *Calanus simillimus*, B) *Rhincalanus gigas* and C) copepods < 2 mm. Error bars indicate the standard deviation calculated for the mean of three replicate bottles.

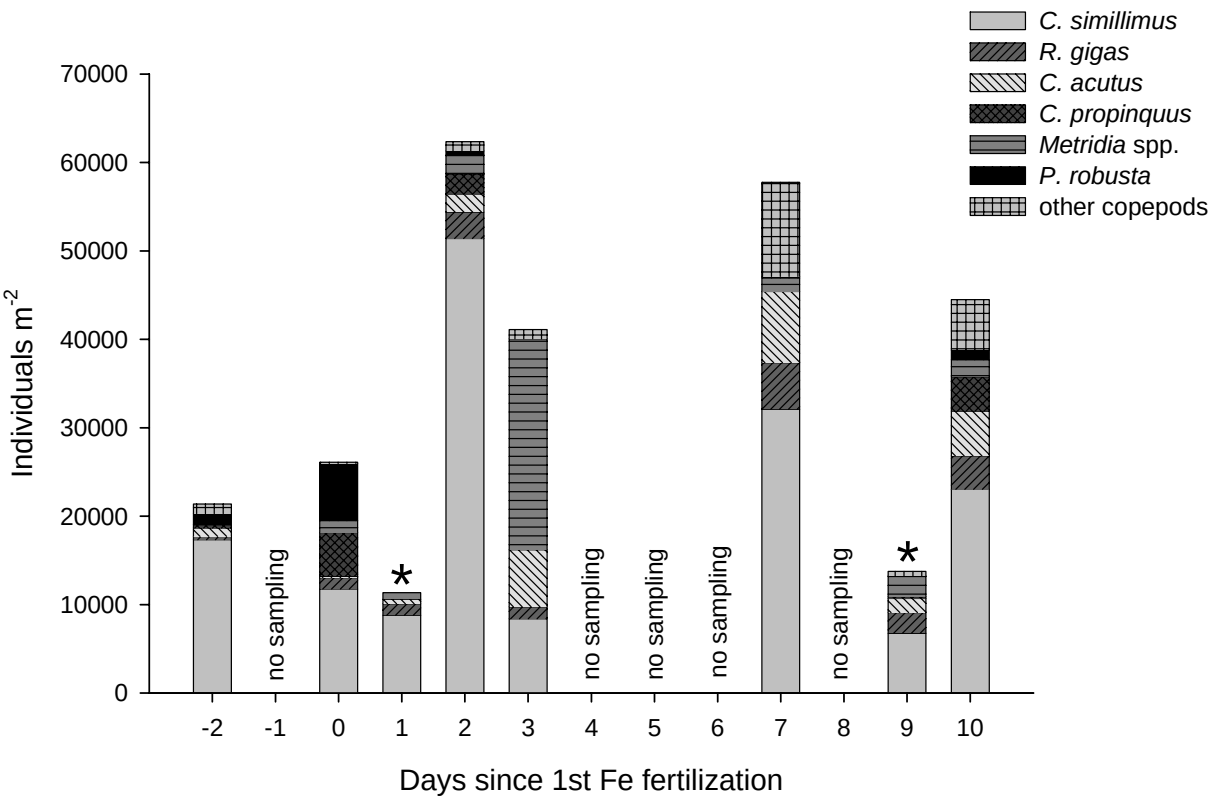
C. simillimus
R. gigas
C. acutus
C. propinquus
Metridia spp.
P. robusta
other copepods
Individuals m-2
70000
60000
50000
40000
30000
20000
10000
0
no sampling
no sampling
no sampling
no sampling
no sampling
*
*
-2
-1
0
1
2
3
4
5
6
7
8
9
10
Days since 1st Fe fertilization

Figure 1

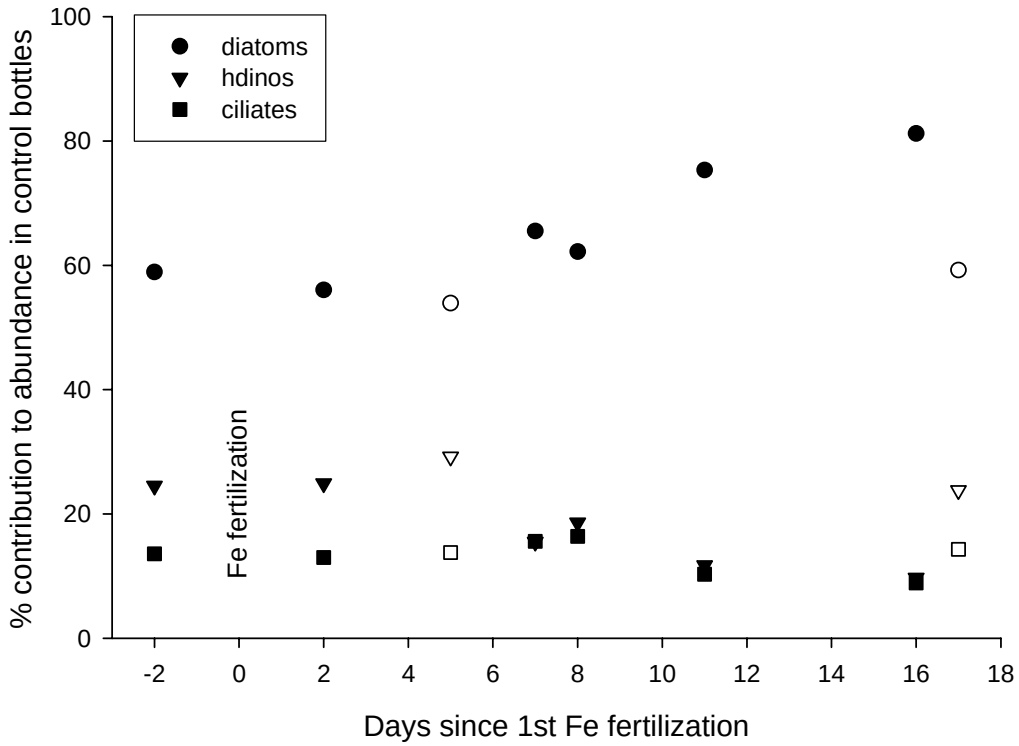
diatoms
hdinos
ciliates
% contribution to abundance in control bottles
Fe fertilization
Days since 1st Fe fertilization
0
20
40
60
80
100
-2
0
2
4
6
8
10
12
14
16
18

Figure 2

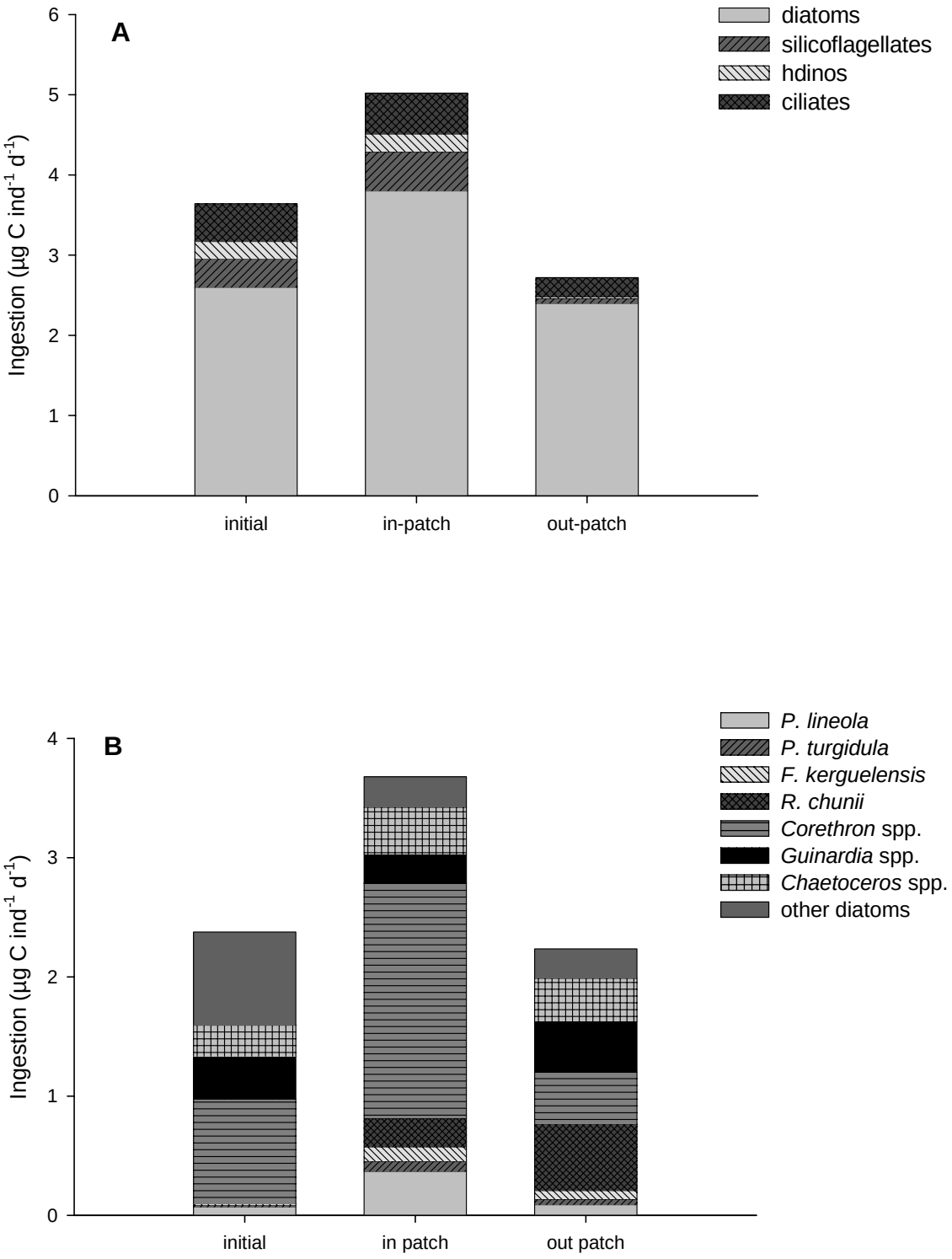
A
diatoms
silicoflagellates
hdinos
ciliates
Ingestion (µg C ind-1 d-1)
0
1
2
3
4
5
6
initial
in-patch
out-patch
B
P. lineola
P. turgidula
F. kerguelensis
R. chunii
Corethron spp.
Guinardia spp.
Chaetoceros spp.
other diatoms
Ingestion (µg C ind-1 d-1)
0
1
2
3
4
initial
in patch
out patch

Figure 3

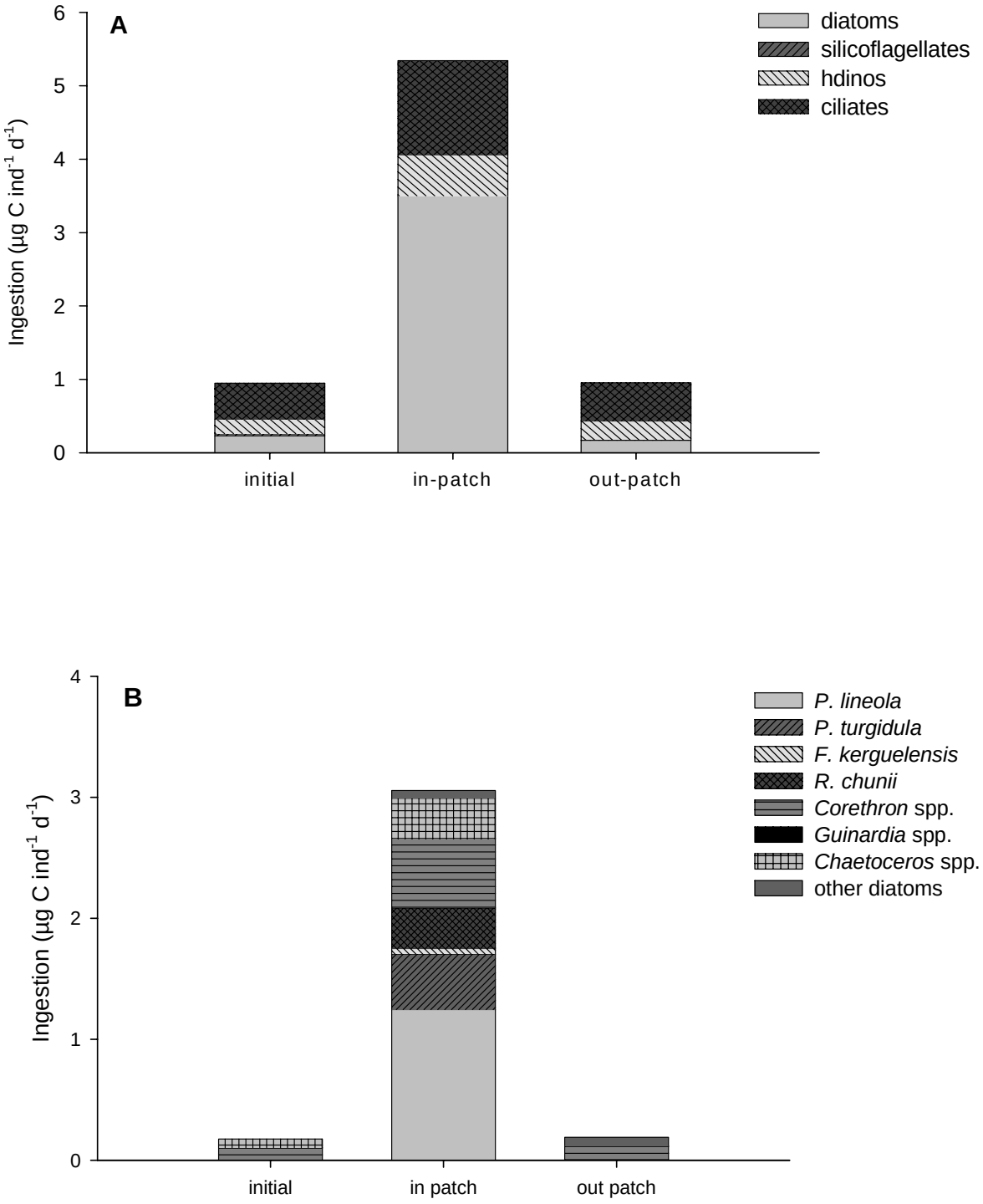
A
Ingestion (µg C ind-1 d-1)
0
1
2
3
4
5
6
initial
in-patch
out-patch
diatoms
silicoflagellates
hdinos
ciliates
B
Ingestion (µg C ind-1 d-1)
0
1
2
3
4
initial
in patch
out patch
P. lineola
P. turgidula
F. kerguelensis
R. chunii
Corethron spp.
Guinardia spp.
Chaetoceros spp.
other diatoms

Figure 4

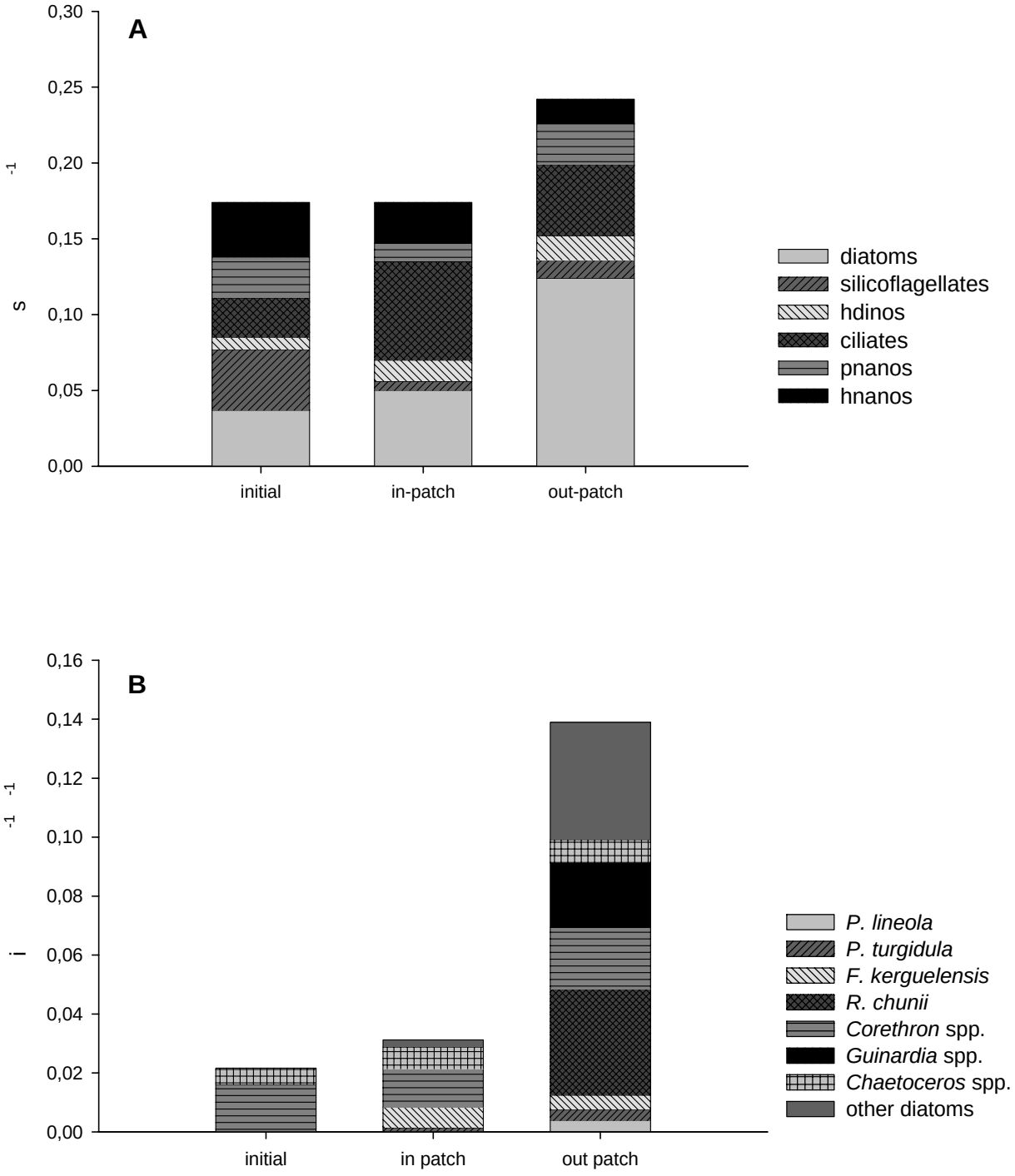
A
0,30
0,25
0,20
0,15
0,10
0,05
0,00
s -1
initial
in-patch
out-patch
diatoms
silicoflagellates
hdinos
ciliates
pnanos
hnanos
B
0,16
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
i -1 -1
initial
in patch
out patch
P. lineola
P. turgidula
F. kerguelensis
R. chunii
Corethron spp.
Guinardia spp.
Chaetoceros spp.
other diatoms

Figure 5

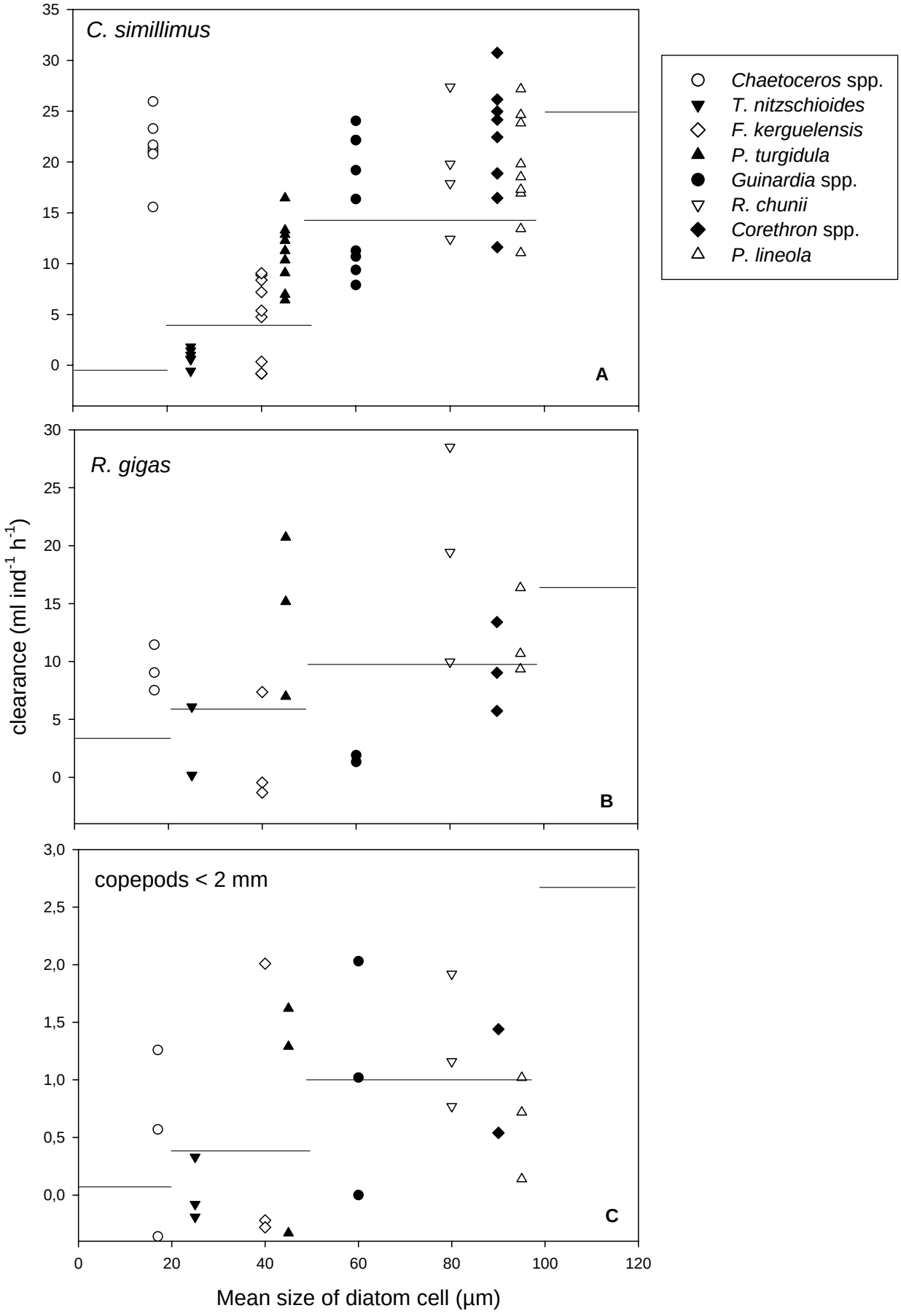
C. simillimus
R. gigas
copepods < 2 mm
A
B
C
clearance (ml ind-1 h-1)
Mean size of diatom cell (µm)
Chaetoceros spp.
T. nitzschioides
F. kerguelensis
P. turgidula
Guinardia spp.
R. chunii
Corethron spp.
P. lineola

Figure 6

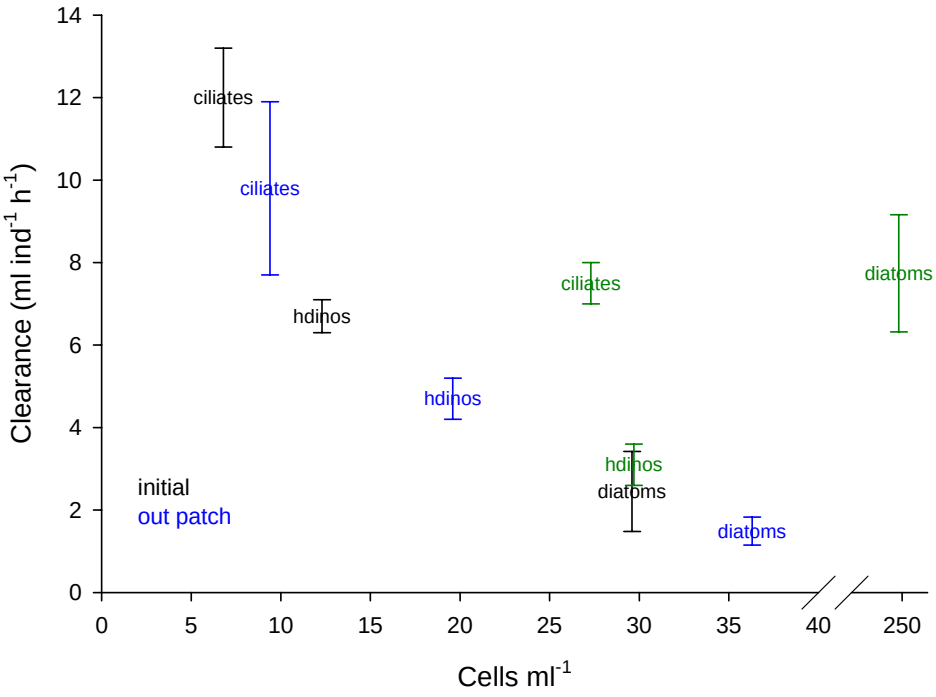
14
12
10
8
6
4
2
0
Clearance (ml ind-1 h-1)
ciliates
ciliates
hdinos
hdinos
ciliates
diatoms
hdinos
diatoms
diatoms
initial
out patch
0
5
10
15
20
25
30
35
40
250
Cells ml-1

Figure 7

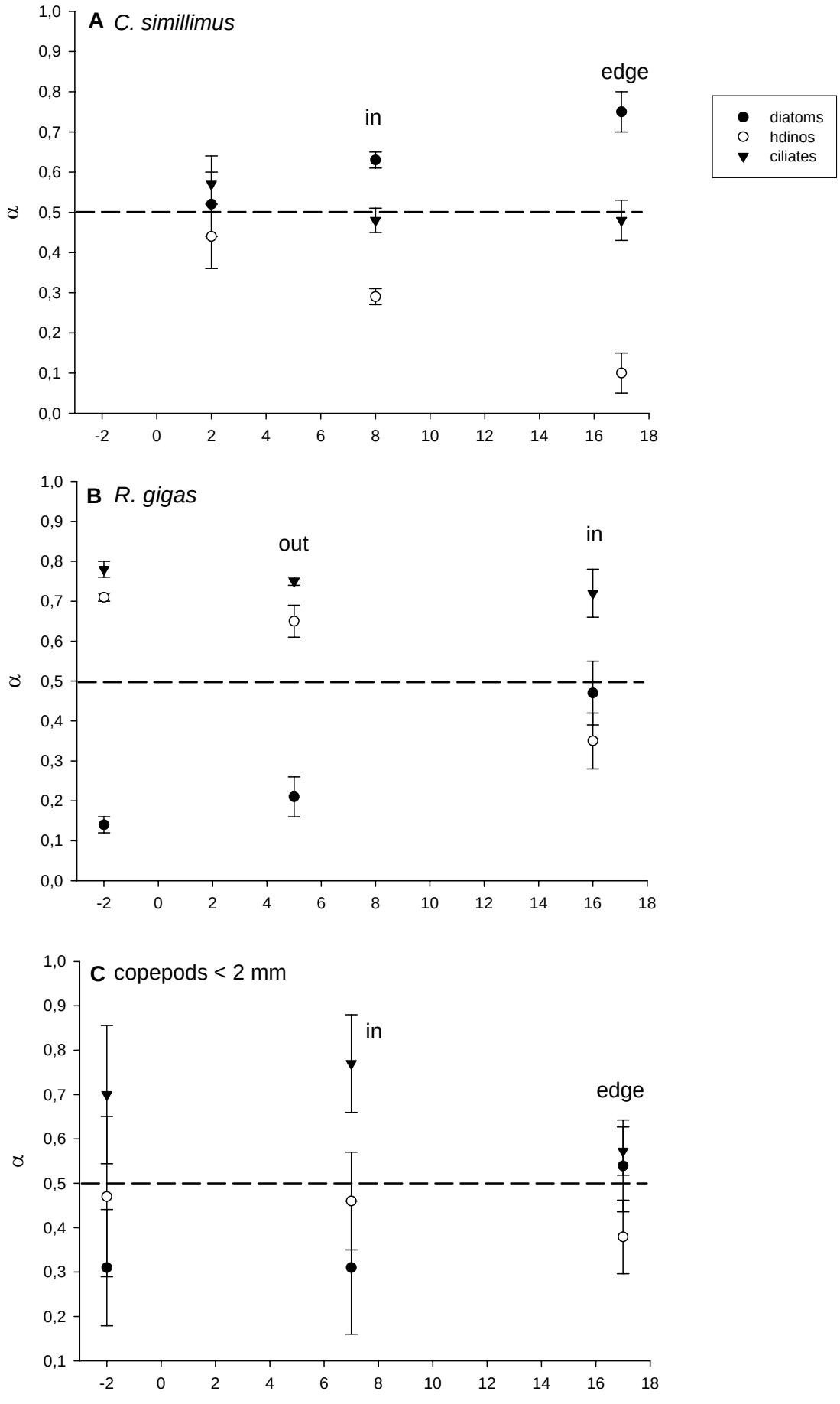
A C. simillimus
edge
in
diatoms
hdinos
ciliates
α
B R. gigas
out
in
C copepods < 2 mm
in
edge
Days since 1st Fe fertilization

Figure 8

MANUSCRIPT 2

Grazing and metabolic activity of copepods at the Antarctic Polar Front – How well agree ingestion estimates from gut fluorescence and *in vitro* incubations with respiratory carbon demand?

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Abstract

In the framework of an iron-fertilization study near the Antarctic Polar Front, grazing and metabolic activity of copepods was investigated. O_2 respiration and N excretion rates of *Calanus simillimus*, *Rhincalanus gigas* and copepods < 2 mm agreed with previously published results and showed allometric relationships with dry weight. Ingestion rates based on the gut fluorescence technique indicated that *C. simillimus*, *R. gigas* and copepods < 2 mm on average ingested sufficient pigmented food to cover their basic respiratory carbon demand. Ingestion rates from incubation experiments with subsequent microscopic count of un-eaten food items agreed with rates from gut fluorescence only for *C. simillimus*. Ingestion rates for *R. gigas* and the small copepods were underestimated by up to a factor of 20 in incubation experiments. This discrepancy is apparently related to differences in feeding behavior. *C. simillimus* grazed on live diatoms before and during the bloom, which can be determined with both, the gut fluorescence measurement and the *in vitro* incubations. *R. gigas* and the small copepods additionally preyed on microzooplankton and detritus in varying intensity, both leading to underestimation of ingestion rates in incubations.

Introduction

Grazing on phytoplankton influences the biological pump of carbon, modifies the quality and quantity of the particle flux (Kiørboe 1997, Le Fèvre et al. 1998, Wassmann 1998) and potentially structures plankton communities (Verity & Smetacek 1996). A correct determination of *in situ* grazing rates is hence important for evaluating the role and impact of grazers in the pelagic ecosystem and on biogeochemical cycles.

Zooplankton communities in the ice-free waters of the Southern Ocean are dominated by copepods (Pakhomov et al. 2000). Estimates of their grazing impact range from less than 10 % (e.g. Atkinson 1996, Zeldis 2001) to more than 100 % of daily primary production (Mayzaud et al. 2002a) or up to 36 % of the phytoplankton standing stock per day (Bernard & Froneman 2003). Frequently, studies report ingestion rates below the minimum respiratory carbon demand of grazers for both, rates determined with gut fluorescence (Atkinson 1996) and in incubation experiments (Atkinson 1994, Zeldis 2001). This problem is not inherent to the Southern Ocean, but also known from temperate marine systems (e.g. Peterson et al. 1990). It is also not inherent to the grazing methods in general, since the derived ingestion estimates have been shown to agree with metabolic carbon needs based on egg production and/or oxygen respiration (Kiørboe et al. 1985). Gut fluorescence measurements have been criticized to underestimate feeding rates due to pigment destruction in the gut for example (Conover et al. 1986), bottle incubations due to trophic cascading (Roman & Rublee 1980, Nejstgaard et al. 2001). Recent investigations from the Southern Ocean thus propose that studies on copepod feeding should rely on several approaches, e.g. carbon ingestion and fecal pellet production rates, and additionally consider the balance with respiratory carbon demand (Alcaraz et al. 1998). Furthermore, attention should be given to interactions with the microbial food web and the fine scales at which copepods aggregate, migrate, and interact with potential food (Zeldis 2001).

In this study, we monitored mesozooplankton grazing activity in response to a diatom bloom induced by iron-fertilization. Several methods were used: gut fluorescence measurements for a rapid and simple assessment of the grazing impact of various mesozooplankton organisms on the developing phytoplankton bloom; *in vitro* incubations in order to determine grazing selectivity and changes in feeding behavior of dominant copepods; oxygen respiration and ammonia excretion measurements to monitor changes in overall metabolic activity of grazers from Antarctic Polar Front (APF) in spring and in response to the bloom. Additional information on the spatial distribution of the mesozooplankton

community was obtained with multi-frequency acoustics (Krägefsky et al. in prep.). Results of respiratory carbon demand and estimated carbon ingestion are compared for two dominant copepods, notably *C. simillimus* and *R. gigas*, and the copepod size class < 2 mm. The objectives were 1) to inter-calibrate methods in a given environmental setting and thereby overcome shortcomings of each approach alone, 2) to diversify the information that is drawn from the investigation in order to gain a more profound understanding of feeding behavior of copepods before and in response to the diatom bloom and 3) to investigate reasons for the experimentally observed mismatch between carbon demand and carbon ingestion. Finally, we provide a brief review on published daily carbon rations for *C. simillimus* and *R. gigas* derived with gut fluorescence and *in vitro* incubations.

Material and Methods

All measurements on copepod feeding and metabolism were performed during the iron fertilization experiment "EisenEx" on FS *Polarstern* leg ANT XVIII/2 at ~21°E and 48°S, north of the Antarctic Polar Front (APF). In response to the iron fertilization a diatom bloom developed with chl *a* concentrations and phytoplankton carbon (PPC) increasing from 0.51 to 2.3 mg m^{-3} and from 9.9 to 43.9 mg m^{-3} respectively (Assmy 2004, Gervais et al. 2002). The mesozooplankton community was sampled in regular intervals inside and outside the fertilized patch, subsequently referred to as "in patch" and "out patch".

Grazer collection and sorting

Copepods were collected with vertical Bongo net hauls through the top 100 to 350 m of the water column. Nets (200 µm mesh gauze) had a closed cod-end and were towed at 0.3 m sec^{-1} to minimize damage to the individuals during the haul. Once on deck, the content of the cod-end was immediately diluted in 10 liters of seawater. Samples for gut fluorescence measurements were diluted in a 20 liter carboy cooler filled with 0.7 µm filtered seawater. Individuals used in incubation or respiration experiments were rapidly sorted from the diluted catch under a stereomicroscope in a cooled (4-7°C) laboratory container. Only actively swimming and apparently healthy individuals were chosen and maintained in filtered seawater until the start of the experiment, no longer than 5 hours after collection of the grazers.

Dry weight and elemental analysis

Sub-samples of the Bongo net hauls were collected on a fine mesh, immediately frozen at -80°C and stored at -20°C for later determination of mesozooplankton dry weight, carbon and nitrogen content. On land, samples were thawed, re-suspended in 0.2 µm filtered seawater and dominant copepods in the catch sorted in a Petri dish under a stereomicroscope. Animals were rapidly washed twice in de-ionized water and 1 to 15 individuals of the same size or stage were transferred to a cleaned (acetone, chloroform) and pre-weighed tin recipient. The recipients with the animals were weighed, dried for 24 h at 50-60°C and weighed again to determine wet and dry weight. Samples were then analyzed for carbon and nitrogen content with a Carlo-Erba elemental analyzer.

Respiration and NH_4 excretion

For the measurements of oxygen uptake, grazers were incubated for 24 h, in the dark, at *in situ* temperature (4 °C) in sealed glass bottles filled with 100 ml 0.2 µm filtered seawater. For larger copepods 3-5 individuals and for copepods < 2 mm 10-20 individuals were incubated per bottle. Respiration measurements were done on triplicate incubations with grazers and on two control incubations without added grazers. For the determination of the initial concentration of O_2 in the incubation water, two bottles were filled and fixed immediately. Dissolved oxygen was determined by Winkler titration (Strickland and Parson 1972) using a 716 DMS Titrino (METROHM). Oxygen saturation of seawater used in the experiments was on average 84 % compared to 104 % *in situ* (Y. Bozec pers. comm.). The decrease in dissolved oxygen during the incubation was usually less than 10 % with exception of the experiments with *R. gigas* in which up to 20 % of the oxygen was respired leading to a minimum saturation of 68 %.

To estimate ammonia excretion rates, incubations were run in parallel to the respiration measurements but the grazers were incubated in 50 ml PE bottles filled with 0.2 µm filtered seawater. NH_4 concentrations at t0, in the control and the bottles with grazers were measured from 10 ml sub-samples within 6 hours from the start or the termination of the experiments following routine methods (Hartmann et al. 2001) with a Technicon Autoanalyser II system.

Daily respiratory carbon requirement (DRR) was derived from O_2 consumption rates. Based on average elemental composition of phytoplankton (Laws 1991, Anderson 1995) and measured ammonia excretion, the fraction of O_2 respired to metabolize proteins was calculated and converted to carbon with a metabolic quotient of 0.81. The left-over O_2 was

converted to carbon with a quotient of 0.71 or 1.0, assuming either lipids or carbohydrates as metabolic substrate. Summation of the protein and lipid or carbohydrate fraction yields a minimum (protein + lipid) and maximum (protein + carbohydrate) estimate of DRR in µg C ind^{-1} d^{-1}.

Gut content and gut passage time

Zooplankton samples for determination of initial gut pigment content were collected as rapidly as possible. Still on deck, a time zero sub-sample of the haul was retrieved from the diluted catch on a piece of fine mesh, the mesh was packed in aluminum foil and shock-frozen in liquid nitrogen (- 80 °C) to avoid pigment loss. A time series for estimation of gut evacuation rate was established from subsequent samples taken after 2, 5, 10, 15, 25, 40, 70 and 110 min respectively. Frozen samples were thawed and copepods sorted in a cooled Petri dish under a stereomicroscope and dim light. 1 to 20 individuals were placed in a 20 ml PE centrifuge vial, covered with 5 ml 90% acetone/water and left for extraction of pigments for a period of 2 hours in a fridge. Pigment concentration was measured on a Tuner fluorometer before and after acidification. Values were not corrected for pigment destruction to non-fluorescent components. Calculation of the gut clearance coefficient k was based on the exponential model following the method of Dam & Petersen (1988):

$$k = (\ln G_0 - \ln G_t)/t$$

with G_0 representing the initial gut content (ng pigment ind^{-1}) and G_t the gut content at a given time t. The quotient of 1/k represents the gut passage time (GPT) in minutes. Daily ingestion (ng pigment ind^{-1} d^{-1}) is derived from G_0 and k according to the following equation:

$$I = G_0 * k * 60 * 24.$$

Ingestion rates were obtained by multiplying the initial gut content with the according gut passage time that was experimentally determined at the same station. Pigment was converted to phytoplankton carbon (PPC) using a C:chl *a* ratio of 40 (Riebesell unpubl.).

Incubation experiments

Mesozooplankton grazing activity was also investigated in incubation experiments following the method of Frost (1972). Copepods were incubated in 1 liter bottles filled with natural seawater as prey assemblage. Grazer densities ranged from 4 to 20 individuals per liter. For details on experimental treatments see Schultes et al. (in prep.). Sub-samples from every treatment, i.e. three replicate bottles with grazers and two control bottles, were fixed with acidic Lugol's solution, a 20-50 ml aliquot was allowed to settle for 24 h in an Utermöhl

chamber and diatoms counted if possible to the species level under an inverted microscope (Utermöhl 1958). Diatom carbon was calculated using the geometric formulas according to Edler (1979) and the volume to carbon conversion factors proposed by Menden-Deuer et al. (2000). Ciliates, heterotrophic dinoflagellates (hdinos), phototrophic nanoflagellates (pnanos) and heterotrophic nanoflagellates (hnanos) were enumerated from sub-samples preserved in glutaraldehyde (final concentration = 0.3 %). Cell abundance, dimensions, and biovolumes were determined via quasi-automated color image analysis (Verity & Sieracki 1993). Cell biovolume measurements were converted to carbon biomass using conversion factors based on literature values of carbon density of microplankton (Verity et al. 1992 and references therein). When more than 30 cells of a species or genus could be counted in the control (Atkinson 1995) clearance rates for each prey organism (i) were calculated following the equation of Frost (1972) modified to:

$$F_i = \ln(C_{c,i}/C_{g,i})*V/(n*t)$$

(Atkinson 1996) where F_i is the clearance rate (ml ind^{-1} h^{-1}), $C_{c,i}$ the final concentration of the prey organism in the control, $C_{g,i}$ the final concentration of the prey organism in the grazing treatment, V the experimental volume (ml), n the number of grazers (l^{-1}) and t the duration (h) of the experiment. Ingestion rates (I_i; ng C ind^{-1} h^{-1}) were calculated by multiplying positive single clearance rates F_i of a given prey organism with its final abundance in the control bottle $C_{c,i}$. Total ingestion (ΣI_i; µg C ind^{-1} d^{-1}) was achieved by summation of all I_i.

Results

Elemental composition and metabolic activity of copepod grazers

The species composition of the mesozooplankton population did not differ in patch compared to out patch and was a typical sub-Antarctic, copepod dominated assemblage (Schultes et al. in prep.). In the size class > 2 mm, *Calanus simillimus* dominated community biomass and abundance. The *C. simillimus* population was dominated by copepodites of stage V and adult copepods. Dry weight and elemental composition remained constant throughout the study and the average dry weight of *C. simillimus* CVI ♀ was to 0.118 mg ind^{-1} (n = 15). Carbon accounted for 38 % of DW, nitrogen for 9 %, resulting in a C/N ratio (by weight) of 4.4 (Table 1). Respiration and excretion rates showed no significant differences for grazers from fertilized and control waters, nor a temporal development (Table 2). Daily average respiration and excretion rates for *C. simillimus* were 11.8 µl O_2 ind^{-1} d^{-1} and 0.45 µg N ind^{-1} d^{-1} respectively.

Rhincalanus gigas contributed only a small fraction (approximately 4 %) to total community abundance in the upper 150 m of the water column. The dry weight of CVI ♀ showed more variability with values higher by a factor of three towards the end of the study both in patch and out patch. This is mirrored in both the carbon and nitrogen content. Average dry weight is was 0.827 mg ind^{-1} with a carbon and nitrogen content of 30 % and 7 % of DW respectively. The C/N ratio was 4.4 (Table 1). Adult females of *R. gigas* respired on average 31.4 µl O_2 ind^{-1} d^{-1} and excreted 0.86 µg N ind^{-1} d^{-1} and no significant spatial nor temporal difference was observed (Table 2).

Taxonomic information on the copepod size class < 2 mm is not available. The standing stock of this grazer fraction in the upper 150 m of the water column was on average a factor 2.7 higher than the grazers > 2 mm (Schultes et al. in prep.) and represents an important feeding potential. Average dry weight of the copepods < 2 mm was 0.006 mg ind^{-1}, with an average carbon and nitrogen content of 47 % and 9 % of DW, respectively, and a C/N ratio of 5.5, somewhat higher than the ratio of *C. simillimus* and *R.gigas* (Table 1). Oxygen uptake of the small copepods was on average 1.7µl O_2 ind^{-1} d^{-1}, excretion rates 0.07 µg N ind^{-1} d^{-1} (Table 2). Atomic O/N ratios did not differ between copepods and were 19.5 (±6.2) on average (Table 2).

Daily respiratory carbon requirement (DRR) ranged from 11-14 % of body carbon for *C. simillimus*, 5.0-6.5 % for *R. gigas* and 28-36 % for the copepods < 2 mm depending on whether lipids or carbohydrates were metabolized in addition to proteins respectively (Table 3). Assuming that N excretion rates reflect the amount of protein metabolized by the organism the contribution of proteins to fulfill DRR ranged from 23-29 % for *C. simillimus*, 17-22 % for *R. gigas*, and 24-30 % for the copepods < 2 mm.

Allometric relationships between metabolic rates and dry weight were found. These are:

$R_O = 0.027*DW^{0.597}$

$r = 0.998$

$R_N = 0.002*DW^{0.518}$

$r = 0.988$

where R_O is the rate of oxygen respiration (µmol O_2 ind^{-1} d^{-1}), R_N the rate of ammonia excretion (µmol N ind^{-1} d^{-1}). DW is given as µg ind^{-1}. Based on the calculation of DRR (see

Material & Methods) rates of carbon catabolism and allometric relationships with dry weight were derived. The resulting power functions are:

$$R_{C,\ prot+lipid} = 0.024*DW^{0.572}$$
$$r = 0.997$$

$$R_{C,\ prot+carbohydrate} = 0.030*DW^{0.577}$$
$$r = 0.998$$

where $R_{C,\ prot+lipid}$ and $R_{C,\ prot+carbohydrate}$ are rates of carbon catabolism (µmol C ind^{-1} d^{-1}) assuming protein and lipid or carbohydrate as substrate respectively.

Gut fluorescence measurements

Initial gut content of *C. simillimus* ranged from 0.9 to 1.9 ng pigm ind^{-1}, of *R. gigas* from 0.5 to 3.0 ng pigm ind^{-1}, and of copepods < 2 mm from 0.15 to 0.25 ng pigm ind^{-1}. Gut contents were normalized to dry weight and are presented in Figure 1. Highest weight specific gut contents were determined for the fraction of copepods < 2 mm, followed by *C. simillimus* and *R. gigas*. For all grazers highest gut content was recorded in night samples. An increase of pigment concentration in the gut with an increase in the phytoplankton, as determined by chl *a* (µg l^{-1}) concentration over the top 80 m of the water column, was only observed for copepods < 2 mm.

Gut passage time (GPT) did not differ significantly between copepods and ranged from 2 to 43 min with an average value of 12 min (Figure 2). For *C. simillimus* GPT decreased from 21 min to 6 min with increasing phytoplankton concentration in the field. Disregarding the daytime measurement which gives a lower value than dusk or nighttime samples, a highly significant exponential relationship ($r^2 = 0.98$; $p = 0.001$) between dusk or nighttime GPT and chl *a* concentrations was found (Figure 3). No relationship was found for *R. gigas* and the small fraction of copepods (Figure 2).

Ingestion rates derived from gut fluorescence ranged from 0.7 – 17.1 µg C ind^{-1} d^{-1} for *C. simillimus*, 0.7 - 32.4 µg C ind^{-1} d^{-1} for *R. gigas*, and 0.7 – 1.8 µg C ind^{-1} d^{-1} for the small copepods respectively. No difference between rates from inside and outside the patch was found. Rates were expressed as daily ration (% body C ind^{-1} d^{-1}) and the average, as well as minimum and maximum values are presented in Table 3.

Incubation experiments

In addition to gut fluorescence measurements, ingestion rates (µg PPC ind^{-1} d^{-1}) for *C. simillimus*, *R. gigas* and copepods < 2 mm were derived from bottle incubations. A separate manuscript on feeding behavior with the developing diatom bloom is in preparation (Schultes et al. in prep.) but in the following we briefly summarize results. Throughout the fertilization study, *C. simillimus* was predominantly feeding on diatoms and increased its ingestion rates during the developing bloom. Nevertheless, 10 to 19 % of its daily carbon intake originated from preying on microzooplankton. *R. gigas* modified its feeding behavior from predominantly ambush feeding on ciliates and dinoflagellates before the bloom (> 70 % of daily carbon intake according to results from the bottle experiments only) to a diatom dominated diet inside the fertilized patch, with microzooplankton carbon accounting for merely 35 % of its carbon ration. The small copepods also increased their clearance on diatoms in response to the bloom but continuously preyed on microzooplankton and nanoflagellates that taken together supplied 46 to 72 % of the daily ingested carbon. Frequently, growth of prey organisms in excess of the control incubations was determined. For *C. simillimus* and *R. gigas* these negative clearance rates were determined mostly for small food items, i.e. the diatoms < 20 µm in general (Figure 4 a+b) or *Thalassionema nitzschioides* in particular, as well as pnanos and hnanos (data not shown). In the case of the copepods < 2 mm, a positive clearance rate was resolved for pnanos and hnanos in all experiments but negative clearance rates were found for all counted diatom size classes with the exception of the larger fraction (Figure 4 c). Clearance rates on diatoms increased with the size class of the diatom in all incubations (Figure 4). These results suggest that copepods, by grazing on other zooplankton (protozoa or metazoan larvae), promoted growth of small diatoms and nanoflagellates.

Diatom ingestion estimated from bottle incubations ranged from 2.35 (±0.54) to 3.80 (±0.68) µg PPC ind^{-1} d^{-1} for *C. simillimus*, from 0.17 (±0.04) to 3.52 (±0.64) µg PPC ind^{-1} d^{-1} for *R. gigas*, and from 0.038 (±0.013) to 0.124 (±0.067) µg PPC ind^{-1} d^{-1} for the small copepods. Diatom ingestion rates were recalculated using the highest clearance rates determined in each experiment. This correction increases ingestion rates of *C. simillimus*, *R. gigas* and the small copepods by a factor of 2, 2.5 and 3.4 respectively (Table 4).

Comparison of ingestion rates with respiratory requirements

All ingestion rates were expressed as daily ration (DR, % body C ind^{-1} d^{-1}) and are presented together with daily respiratory C requirement (DRR, % body C ind^{-1} d^{-1}) in Table 3.

Daily ration obtained from microzooplankton and nanoflagellates ("other C sources") is also indicated. Ingestion estimated from gut fluorescence is for all grazers comparable to DRR (Table 3). Average daily rations derived from incubation experiments generally remain below the respiratory C demand, even when autotrophic and heterotrophic food sources are combined.

Ingestion rates derived with the gut fluorescence method are presented together with average (open triangles) and corrected (closed triangles) estimates from incubation experiments in Figure 5. For *C. simillimus* estimates from incubation experiments fall in the same range as ingestion derived from gut fluorescence Average rations calculated from the uncorrected incubations are a factor of two lower than from gut fluorescence but maximum values from either the corrected or the uncorrected *in vitro* method fall into the range of values from the gut fluorescence estimate (Table 3, Figure 5a). For *R. gigas,* the two incubation experiments carried out at low chl *a* concentrations yield ingestion rates a factor of three to four lower than the lowest estimate from gut fluorescence. However, estimates of both methods come into closer agreement inside the diatom bloom, especially when ingestion is corrected to maximum clearance efficiency (Figure 5b). In the case of the small copepods, un-corrected and corrected ingestion rates from the incubation experiments remain at all times below values based on gut fluorescence, methods differ up to a factor of 20 (Figure 5c, Table 3).

Discussion

Physiological characteristics of the copepod community

The copepod population studied during EisenEx displayed the typical physiological characteristics of an actively feeding grazer community in ice-free waters of the Southern Ocean. Ammonia excretion rates of *R. gigas* and *C. simillimus* are comparable to previously published values (Atkinson & Whitehouse 2001). The body-mass scaling coefficient of the allometric relationship between N excretion and dry weight is 0.518 and situated at the lower end of the range presented by Atkinson & Whitehouse (2001). In comparison, the respiration rates determined for *R. gigas* and *C. simillimus* are within the upper range of published values (Kawall et al. 2001, Schnack-Schiel 2001, Mayzaud et al. 2002b). Razouls et al. (1998) determined O_2 uptake for size fractionated zooplankton in the area of the Kerguelen Islands (approx. 50 °S) and grazers with a cephalothorax length of 1.2 to 2.4 mm respired on average 2.9 µl O_2 ind^{-1} d^{-1} which is well in accordance with our results for the copepods < 2 mm.

Dagg et al. (1982) present an allometric relationship between respiratory carbon demand and grazer size for zooplankton from the Bering Sea. Established at the same temperature as in this study, the regression explains 96 % of the variation and should provide a good estimate of respiratory needs. A comparison of the allometric relationship of Dagg et al. (1982) with results for copepods during EisenEx is shown in Figure 6. Estimates according to Dagg et al. (1982) are up to a factor of three lower than determined in this study with exception of the minimum estimate (protein + lipid) for *R. gigas*. Ikeda (1989) explored the question whether Antarctic zooplankton were metabolically more cold-adapted than arctic zooplankton with an intra-generic comparison of oxygen consumption rates. Although results were not consistent enough to draw the general conclusion that Antarctic zooplankton had higher weight specific rates at the same temperature than their Arctic counterparts, the comparison did show significantly increased respiration of pteropods and calanoid copepods and results from this study further support this notion. A more thorough investigation on the metabolic cold adaptation of Antarctic zooplankton is required as respiration rates are a robust method (Ikeda 1977) to determine the minimum carbon requirement of grazers.

The relative amount of carbon, but not that of nitrogen, shows variability in this study that appears to be related to the size of the grazer. Carbon accounted for 30 % of dry weight in *R. gigas*, in comparison to 38 % in *C. simillimus* and 47 % in copepods < 2 mm. This difference in relative carbon content, increasing from the largest to the smallest copepod has to our knowledge not been determined so far. Schnack et al. (1985) published remarkably similar relative carbon contents, 45 to 51 % for copepods varying over almost two orders of magnitude in dry weight and sampled from three different sites. Numerous studies hence assume an average carbon content of 45 % for Southern Ocean copepods (e.g. Atkinson et al. 1996, Pakhomov et al. 1997). However, carbon content of total dry weight in an investigation by Froneman et al. (1996) varies from 33 % for *Calanoides acutus* to 67 % for *Metridia gerlachei*, lending some credibility to the variability observed during EisenEx. Additionally, weight specific metabolic activity in general is inversely related to body size in Antarctic zooplankton (Ikeda & Mitchell 1982), which is also evident in results from this study, and it seems not counterintuitive that relative carbon content should follow a similar trend.

Typical C/N ratios of Antarctic copepods range from 3.5 to 5 which is comparable to the range of 4.4 to 5.5 found in this study. The higher C/N ratio of the small copepods can be due to a difference in N excretion. Comparison of the allometric relationships of C catabolism and N excretion indeed reveals a lower slope for N excretion which could account for the observed difference in elemental composition. Due the analytical uncertainty associated with

the N excretion measurement, however, the difference between slopes is not significant. Alternatively, the difference in C/N can be the result of a difference in the relative amount of protein ingestion and nitrogen assimilation in the feeding history of the grazers.

Frequently, values of 15 and lower are reported for O/N ratios of Antarctic copepods. This led to the assumption that Antarctic copepods have a protein based metabolism contrary to their Arctic counterparts (Ikeda & Mitchell 1982, Conover & Huntley 1991, Schnack-Schiel 2001). O/N ratios in this study range from 14 to 31, similar to values from 18 to 35 found by Hernández-Léon et al. (1999). Contribution of protein to basic metabolism did not exceed 30 % (Table 3) indicating that also lipids and carbohydrates are important metabolic substrates.

Ingestion rates based on the gut fluorescence method

Over the last decade, a substantial amount of copepod grazing estimates based on gut fluorescence has been collected in the Southern Ocean, especially from more productive areas near the APF (Dubischar & Bathmann 1997, Urban-Rich et al. 2001, Bernard and Froneman 2003) and in the vicinity of islands such as South Georgia (Atkinson et al. 1992 b, Atkinson et al. 1996, Pakhomov et al. 1997), the Prince Edward Archipelago (Perissinotto 1992) or the Kerguelen Islands (Razouls et al. 1998, Mayzaud et al. 2002a). Variability between studies but also for estimates for a single grazer species within the framework of the same investigation is large. Published gut contents for *C. simillimus* stretch from a minimum of 0.1 ng pigm ind^{-1} (Perissinotto 1992, Mayzaud et al. 2002a) to a maximum of 44.5 ng pigm ind^{-1} (Pakhomov et al. 1997). Similarly, gut fluorescence of *R. gigas* can range from minimum values of 0.1 ng pigm ind^{-1} (Mayzaud et al. 2002a) to a maximum of 34 ng pigm ind^{-1} (Atkinson et al. 1992 b). Gut contents determined during EisenEx are situated at the lower range of the published values.

A major problem that leads to underestimation of gut content is pigment destruction that can vary from 1-100 % (Head & Harris 1992; see also Table 1 of Dam & Peterson 1988). No pigment budgets were constructed for gut fluorescence measurements during this study and considering the large variability observed for pigment destruction, gut content was not corrected. This could lead to underestimation of ingestion. On the other hand, most experiments were run during dusk or night time and the extrapolation of night time gut content to the entire day might lead to overestimation of ingestion. Further sources of variation for gut fullness are diel changes in gut content and ambient chl *a* concentrations. In an investigation of Perissinotto (1992), gut content of *C. simillimus* varied between 4 an 12 ng

pigm ind^{-1} over a diel cycle at chl *a* concentrations of 1-2 µg l^{-1}, and from less than 0.5 to 2 ng pigm ind^{-1} in waters with 0.2 µg chl *a* l^{-1}. Although gut content during this study was also highest at night, it generally was not related to the ambient phytoplankton concentrations.

Gut content also varies with the size of the grazer according to the relationship established by Morales et al. (1990). The equation (log G = 1.61 + 0.72 log W) predicts a maximum gut content of 8.5 ng pigm ind^{-1} for *C. simillimus*, 35.5 ng pigm ind^{-1} for *R. gigas*, and 1.0 ng pigm ind^{-1} for the small copepods, roughly a factor of 4 to 12 higher than the measured maximum pigment concentrations from this study. Whereas the theoretical value for *R. gigas* is in good agreement with the published maximum, the theoretical estimate for *C. simillimus* is a lot lower than 44.5 ng pigm ind^{-1}, determined by Pakhomov et al. (1997) for grazers of similar dry weight as in this study. Already Morales et al. (1990) noted that the relationship between dry weight and maximum gut content has mainly "descriptive purposes". Southern Ocean copepods were not included in the regression analysis and the relationship should be revised taking into account the data that has been accumulated from this marine system. Furthermore, high ingestion rates do not appear to be necessarily associated with high gut contents as shall be seen further below.

As for gut content, variability of GPT estimates in the literature is substantial. Based on data from Atkinson et al. (1992 a, b), Atkinson (1996), Bernard & Froneman (2003), Dubischar & Bathmann (1997), Froneman et al. (2000), Perissinotto (1992) and Tirelli & Mayzaud (1999) average values of 67 min (std ± 51; n = 9) and 107 min (std ± 70; n = 12) were calculated for *C. simillimus* and *R. gigas* respectively. The shortest gut passage times for both copepods were determined by Froneman et al. (2000), specifically 14 min and 13 min. Therefore, the GPT measured in this study are among the lowest determined in the Southern Ocean.

The rate at which copepods evacuate their gut contents depends upon a range of variables, including food concentration (Dagg & Walser 1987), food quality (Mayzaud et al. 1998), temperature (Dam & Peterson 1988) and initial gut content (Irigoien 1998). GPT was not related to gut content (Pearson correlation coefficient α = - 0.14; n = 26, n.s.) in our study. An influence of food concentration on gut passage time can be resolved for *C. simillimus* but not for *R. gigas*. Mayzaud et al. (1998) demonstrate that gut passage time of *Acartia clausi* decreases with increasing food concentration when feeding on a diet of pure diatoms. This trend was not resolved with a pure dinoflagellate, mixed diatom-detritus or pure detritus diet. Bottle incubations indicate that *C. simillimus* grazed predominantly diatoms whereas *R. gigas* preyed intensively on microzooplankton (Schultes et al. in prep.) and possibly detritus. Thus,

not only the feeding environment, but also the feeding preferences of a grazer may influence the GPT.

Dam & Peterson (1988) proposed the possibility to predict gut clearance rate coefficient from temperature for conditions in which food is not limiting and the linear relationship was confirmed by Irigoien (1998) for a temperature range from -1 to 25° C. Based on the equation given by Dam & Peterson (1988; k = 0.0117+0.001794*T) a gut clearance rate coefficient of 0.018876 min^{-1} (i.e. a gut passage time of 53 min) is predicted for a temperature of 4° C. The GPT determined in this study is more than four times faster, and applying the theoretical value to the measured gut content would lead to a dramatic underestimation of ingestion.

Dagg and Walser (1987) stipulate that the conversion of gut content measurements to an accurate estimate of ingestion rate must be made with a gut passage time representative of the *in situ* food conditions. Results from EisenEx and especially the large variability of both, gut content and gut passage time reported from the Southern Ocean only reconfirm the importance of this issue. Rapid GPTs together with low gut contents measured in this study nevertheless result in ingestion rates sufficiently high to cover respiratory carbon demand of all grazers (Figure 6). In the case of *C. simillimus* up to 17.1 µg PPC ind^{-1} d^{-1} or 38 % body C d^{-1}. This feeding rhythm also indicates that copepods quickly digest the ingested food and will not transport a large amount of fecal material out of the mixed layer with their diel vertical migration. A contrary situation is depicted by Bernard & Froneman (2003) who worked in environmental settings very similar to EisenEx, in the Polar Frontal Zone of the Indian Sector of the Southern Ocean with average integrated chl *a* concentrations from 1.1 to 2.8 µg l^{-1}, albeit in autumn. They report gut contents of 20-30 ng pigm ind^{-1} for *C. simillimus* of 0.093 mg ind^{-1} dry weight and a gut passage time of 187 min. Although gut content and gut passage time differ by more than an order of magnitude from this study the resulting ingestion rate for *C. simillimus* is only a factor of two higher, on average 31 µg PPC ind^{-1} d^{-1}. Clearly, two very different feeding scenarios lead to similar carbon ingestion. If assimilation efficiency is constant then digestive processes must be equally contrasting.

Ingestion rates determined from bottle incubations

The estimation of ingestion rates in bottle experiments suffers from the interactions of trophic levels when predation of copepods on microzooplankton is strong (Nejstgaard et al. 2001). Mortality of microzooplankton due to mesozooplankton grazing alleviates the grazing pressure of ciliates and dinoflagellates on other components of the food web. Unmistakable

proof for such trophic cascades is the determination of apparently negative clearance rates, which were frequently measured during the incubation study, especially for small food items such as diatoms < 20 µm and nanoflagellates. Whether diatom ingestion is limited by small cell size is controversial in the literature. Atkinson (1994, 1995 and 1996) repeatedly reports most efficient grazing on large cells from bottle incubations and this result was confirmed in the incubation study from EisenEx (Schultes et al. in prep.). Perissinotto (1992) however demonstrates selective grazing on pico- (1-5 µm) and nanophytoplankton (5-20 µm) in incubations, and Bernard & Froneman (2003) present evidence from gut fluorescence measurements that copepods can efficiently harvest phytoplankton < 20 µm in the Southern Ocean. Although even culture experiments without interference of other trophic levels demonstrate that clearance rate of copepods increases with the size of the cell (Frost 1972) and that optimum cell size is related to the size of the grazer (Berggreen et al. 1988) it cannot be ruled out that reduced grazing activity of microzooplankton on small cells amplifies the influence of cell size on copepod grazing efficiency in the incubation approach. A correction for the effect of the trophic interaction in the sense of Nejstgaard et al. (2001) can only be accomplished when mortality rates of each prey item due to microzooplankton grazing are determined via dilution experiments (Landry & Hassett 1982) run in parallel with mesozooplankton grazing experiments and counted to the same taxonomic detail. As this could not be accomplished in the framework of EisenEx only a rough correction for diatoms was attempted for comparison of ingestion rates derived from gut fluorescence and from incubations. This correction increased the ingestion estimate for all grazers and represents a maximum value assuming that the grazers harvest the available diatom standing stock with equal efficiency regardless of cell size.

Comparison of ingestion rates obtained from the gut fluorescence and incubation methods

I. *Calanus simillimus*

Ingestion rates from both methods are in reasonable agreement, with exception of two peak values derived from gut fluorescence. In addition, both methods succeed in resolving the tendency of ingestion to increase with enhanced phytoplankton availability in the environment. Bottle incubations yield the average feeding activity of a grazer integrated over a certain time interval, e.g. 24 h, but no information on peak activity that is either associated with diel feeding behavior (Atkinson et al. 1992 a, Perissinotto 1992, Pahkomov et al. 1997, Bernard & Froneman 2003) or with food patchiness in the field. Gut fluorescence bears the advantage to demonstrate this peak activity given that sampling is carried out accordingly.

The average ration based on gut fluorescence of 13.2 % matches the DRR of 11 to 14 % but only peak values of maximum 38.2 % will allow the grazer to channel carbon into growth or reproduction.

Table 5 presents daily rations available for *C. simillimus* in the literature. When rations were not explicitly given in the manuscript, daily ingestion rates were converted to daily rations based on published carbon content or assuming the body carbon measured in this study. Daily rations available in the literature range from 0.26 to 84 % body C d^{-1} with most rations being 10 % and higher. The results of Atkinson (1996) demonstrate that both methods can yield daily rations below the respiratory requirement. In this particular study, carbon rations of all tested grazers were below the threshold for respiratory maintenance. Also the incubation study of Atkinson in 1994 yielded a daily ration < 1 % which is in contradiction with the overall accordance of methods for *C. simillimus* during EisenEx. Our findings confirm previous results of method comparisons. A study of Kiørboe et al. (1985) during a spring bloom in the Kattegat and Skagerrak area concludes that gut fluorescence and incubation methods yield comparable results of *in situ* feeding rates for seven different copepod species. *C. simillimus* shows clear herbivorous grazing before and during the EisenEx bloom (Schultes et al. in prep.). As shall be demonstrated below, differences in feeding behavior are probably at the origin of the discrepancy observed between the experimental approaches for *R. gigas* and the copepods < 2 mm.

II. *Rhincalanus gigas*

Published values of daily carbon rations for *R. gigas* (Table 5) do not exceed 8 % and both methods agree on this finding. Low daily rations therefore appear to be the rule for *R. gigas* and concur well with the low weight specific metabolic rates and low relative body carbon presented above. Average carbon rations of 4.5 % derived from gut fluorescence are close to the estimated DRR of 5.0 to 6.5 % but again, only peak values of grazing activity will provide the grazer with sufficient carbon to enable growth or reproduction. Gut fluorescence measurements show strong feeding on pigmented material in the early stages of the bloom, a fact that is in disagreement with the low grazing rates on diatoms emanating from incubation experiments. The discrepancy observed between gut fluorescence and incubation seems not to be inherent to the methodology in general, as both experimental approaches are in good agreement for *C. simillimus*. Atkinson et al. (1996) report similar rations determined with gut fluorescence and incubations in the framework of the same study.

Dagg (1993a, 1993b) observed a disagreement between methods for one particular copepod during an investigation in the sub-arctic Pacific Ocean. Pigment-derived rates and bottle incubations agreed for *Neocalanus plumchrus* and *Neocalanus flemingeri* but incubations resulted in ingestion rates a factor of four lower than gut fluorescence measurements for *Neocalanus cristatus*. *N. cristatus* fed on microzooplankton with clearance rates a factor of six higher than on phytoplankton, which is similar to results for *R. gigas* (Schultes et al. in prep.). However, neither in Dagg's study nor during EisenEx, abundance of microzooplankton was sufficient to cover the respiratory C demand of the grazer (Table 4), which points to the existence of an alternative food source. Dagg (1993b) demonstrated with a microscopic analysis of the gut content that the additional pigmented material detected via gut fluorescence originated from feeding on aggregates. This signal cannot be reproduced in bottle experiments which only give information about feeding on homogenously distributed plankton cells.

R. gigas and *N. cristatus* have similar adult body size of 8-9 mm but their morphology differs in that *R. gigas* does not posses the long plumes on the distal ends of the antennae and the caudal furcae which, according to Dagg (1993b) enable *N. cristatus* to detect and capture aggregates at greater distance. It is especially the similarity of behavioral traits of both copepods that support the idea that *R. gigas* obtains carbon from the same detrital sources as *N. cristatus*. The latter copepod is described to remain hanging motionless in the water column (Dagg 1993b and references therein) and a similar behavior can be observed for *R. gigas* when maintained in the laboratory (pers. observation). Furthermore, compared to the two other *Neocalanus* species that maintain themselves in the upper mixed layer the majority of the population of *N. cristatus* is situated at the base or below the mixed layer (Dagg 1993b). Although *R. gigas* does not display such a clear maximum, its distribution over the top 300 m is more homogenous than for *C. simillimus* that concentrates the bulk of its population in the upper 150 m (S. Schultes, unpublished data). Atkinson et al. (1992b) also encountered *R. gigas* grazing below the chl *a* maximum in comparison to other copepods. Additionally, Dubischar & Bathmann (1997) speculate, that *R. gigas* feeds on fecal pellets as they fail to retrieve pellets from incubation containers of actively feeding specimens whereas pellets were numerous for *Calanus propinquus* in the same experimental approach. These observations strongly suggest, that *R. gigas* at times obtains a large percentage of pigmented food from aggregates and detritus. With progression of the bloom, *R. gigas* increased its clearance on diatoms (Schultes et al. in prep.). Indeed, at high chl *a* concentrations, gut fluorescence and *in vitro* incubations regain comparability.

III. Copepods < 2 mm

For the small copepods, ingestion rates estimated with the two methods clearly cannot be brought into agreement. Average daily ration based on gut fluorescence is estimated to 49.4 %, slightly higher than the measured respiratory requirement of 28 to 36 %. Respiration rates agree with previously published results (see above) and daily rations for small grazers in the Southern Ocean can reach several hundred percent of body carbon (e.g. Atkinson 1994, Atkinson 1996, Atkinson et al. 1996, Bernard & Froneman 2003).

Atkinson (1996) reports significantly lower daily rations from incubations in comparison to gut fluorescence measurements, in the case of *Oithona* spp. 9.5 % and 34 % for incubation and gut fluorescence, respectively, and interprets the results as a possible artifact of different handling procedures of the small grazers. Determination of grazing activity of small copepod grazers is more difficult due to the delicacy of the organisms, and due to low individual grazing rates which requires a larger number to be sorted and incubated. Mortality of the grazers could not be verified at the end of our incubation experiments as retrieving them from the bottles with a sieve or a pipette would have possibly changed the composition of the uneaten food items. If mortality was large, then ingestion rates per added grazer are underestimated. Concerning the gut fluorescence technique the necessity to collect enough grazers for a single pigment extraction increases the sorting time which could lead to pigment destruction (Morales et al. 1990) and to underestimation of gut content. On the other hand, small grazers have a higher surface to volume ratio and contamination with phytoplankton on an individual basis may be more important for small than for large grazers. In the end, both factors may eventually compensate each other. Ingestion based on gut fluorescence measurements concurs well the estimated respiratory requirement and severe overestimation of rates cannot be explained with sound reasoning.

Again, difference in feeding behavior might be at the origin of the large underestimation associated with bottle experiments. Ingestion of microzooplankton and nanoflagellates increased the daily ration small copepods by another 4.5 % (Table 3) but the additional carbon intake is not sufficient to cover respiratory C demand. Also the correction of phytoplankton carbon ingestion with a maximum clearance rate still underestimates ingestion and daily ration by more than a factor of five compared to results from gut fluorescence. Proof for the possible utilization of detritus as alternative food source is indicated by field data. The copepods < 2 mm were initially associated with deeper water layers below 150 m but accumulated in the phytoplankton rich surface layer over the course of the bloom. They were significantly correlated with diatom standing stock in the second half of the fertilization

experiment (S. Krägesfky pers. comm.). An increase of the clearance rate for diatoms with the development of the bloom (Schultes et al. in prep.) further supports the idea of a diet shift in the small grazers from detritus to newly available phytoplankton.

The difference between phytoplankton ingestion determined with bottle incubations and the ingestion of pigmented material, derived with gut fluorescence measurements, possibly yields information on the magnitude of grazing on detritus. To convert this unaccounted pigmented material to carbon requires first of all knowledge on the type of detritus, i.e. whether fecal pellets or algal aggregates dominate the detrital pool in the upper water column. Additionally, an appropriate conversion factor from pigment to carbon must be available. Detritus during EisenEx was present in the form of metazoan and protozoan fecal pellets, integrated standing stock (150 m) ranging from 77 to 369 mg C m^{-2} (J. Henjes pers. comm.). Assuming a minimum factor of C:chl *a* similar to live phytoplankton, i.e. 40, the grazer population < 2 mm in the upper 150 m of the water column would have consumed between 14 and 65 % of the fecal pellet standing stock per day.

Summary and conclusions

In this study, grazing activity determined in bottle incubations and with the gut fluorescence technique were compared with respiratory C demand derived from oxygen uptake experiments for two copepod species and the size class of grazers < 2 mm cephalothorax length. Comparison of gut content measurements and gut clearance rates with published values for Southern Ocean copepods shows large variability in the literature and no agreement with dry weight (Morales et al. 1990) or temperature (Dagg & Peterson 1988) relationships established for temperate and boreal zooplankton. It is therefore suggested that both parameters be always determined together to achieve a precise estimate of ingestion. Possibly similar caution has to be given to respiration estimates for Southern Ocean copepods via allometric equations established with boreal zooplankton (Dagg et al. 1982) but data of this paper are too scant to draw a general conclusion on this issue.

All three grazer types show good agreement between basic respiratory carbon needs and ingested carbon when ingestion is derived with the gut fluorescence method. Bottle incubations agree on gut fluorescence measurements and minimum carbon requirements for *C. simillimus* that shows clear herbivorous grazing activity. Methods differ for *R. gigas* and the small copepod fraction where bottle incubations seriously underestimate daily carbon ration below the minimum carbon requirement. From the results of this study the

underestimation appears to be related to differences in feeding behavior between the grazers, particularly predation on microzooplankton and ingestion of pigmented detritus, i.e. algal aggregates or fecal pellets. Bottle incubations detect predation on microzooplankton that cannot be quantified with pigment based methods but is important in copepod feeding (Froneman et al. 1996). Feeding on detritus is retained in the signal of gut fluorescence measurements. The complete spectrum of carbon ingestion can thus only be inferred with a combination of methods. Until molecular methods (Nejstgaard et al. 2003) to determine prey types qualitatively and quantitatively in copepod guts and feces will be routinely applicable in the field, we suggest that incubations should only be used in combination with alternative methods for inter-calibration of results, or if herbivorous feeding on suspended algae of the grazer is known.

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Table 1:
Dry weight, relative carbon and nitrogen content, and C/N ratios (by weight) for *Calanus simillimus*, *Rhincalanus gigas* and small copepod grazers. For dry weight the average weight as well as minimum and maximum values are indicated, for the C/N ratios the average and standard deviation.

Grazer	n	DW (mg ind^{-1}) average min - max	% C	% N	C/N
C. simillimus CVI ♀	15	0.118 0.072 – 0.143	38	9	4.4 ± 0.4
R. gigas CVI ♀	14	0.827 0.477 – 1.960	30	7	4.4 ± 0.5
copepods < 2 mm	20	0.006 0.003 – 0.008	47	9	5.5 ± 0.8

Table 2:
Respiration rates, ammonia excretion rates and atomic O:N ratios for *Calanus simillimus*, *Rhincalanus gigas* and small copepod grazers near the Antarctic Polar Front in spring. Values indicate the mean of three replicate measurements with the standard deviation given in brackets.

Grazer	Date	Position	µl O2 ind^{-1} d^{-1}	µg N ind^{-1} d^{-1}	O:N
C. simillimus CVI ♀	1 Nov	pre-bloom	7.0 (±0.4)	0.32 (±0.15)	14
	16 Nov	in patch	17.6 (±4.2)	0.58 (±0.10)	19
	28 Nov	in patch	10.9 (±3.3)	-	-
	29 Nov	out patch	11.7 (±1.1)	-	-
R. gigas CVI ♀	13 Nov	pre-bloom	32.9 (±4.5)	1.11 (±0.86)	19
	24 Nov	in patch	29.8 (±1.3)	0.60 (±0.61)	31
copepods < 2 mm	8 Nov	pre-bloom	2.1 (±1.9)	0.09 (±0.03)	15
	16 Nov	in patch	1.7 (±0.1)	0.05 (±0.01)	19
	29 Nov	out patch	1.3 (±0.5)	-	-

Table 3:
Daily carbon rations (DR) for *Calanus simillimus*, *Rhincalanus gigas* and small copepod grazers estimated from gut fluorescence and incubations. For incubations, daily ration derived from phytoplankton carbon, from corrected phytoplankton carbon (see text for explanation) and carbon obtained from sources other than phytoplankton are presented. Values indicate the average of all available measurements with the minimum and maximum estimates in brackets. For comparison, the daily respiratory carbon requirement (DRR) is also presented. CarboH = carbohydrate.

Grazer	**DR** **% body C d^{-1}** average (min – max)				**DRR** **% body C d^{-1}** (% protein of DRR)	
	Gut fluorescence	Incubation PPC	Incubation PPC corrected	Incubation other C sources	Protein + Lipid	Protein + CarboH
C. simillimus	13.2 (1.5 – 38.2)	6.6 (3.9 – 10.2)	12.4 (8.9 – 17.4)	1.9 (0.7 – 2.7)	11 (29)	14 (23)
R. gigas	4.5 (0.3 – 13.0)	0.5 (0.1 – 1.7)	1.4 (0.2 – 3.5)	0.4 (0.3 – 0.7)	5.0 (22)	6.5 (17)
copepods < 2 mm	49.4 (25.6 – 69.1)	2.6 (0.8 – 7.6)	8.8 (4.4 – 15.9)	4.7 (4.4 – 5.1)	28 (30)	36 (24)

Table 4:
Ingestion rates, maximum diatom clearance rates, diatom carbon concentration in control bottles, and corrected diatom ingestion rates for *Calanus simillimus*, *Rhincalanus gigas* and small copepod grazers determined in bottle incubations. Numbers in brackets indicate the standard deviation.

Grazer	Experiment	IR ($\mu g\ C\ ind^{-1}\ d^{-1}$)	max F ($ml\ ind^{-1}\ h^{-1}$)	Diatom PPC ($ng\ C\ ml^{-1}$)	corr IR ($\mu g\ C\ ind^{-1}\ d^{-1}$)
C. simillimus	pre-bloom	2.6 (±0.7)	23.5	8.6	4.86
CVI ♀	in patch	3.8 (±0.7)	25.3	12.8	7.74
	out patch	2.4 (±0.5)	25.4	6.5	3.96
R. gigas	pre-bloom	0.2 (±0.1)	13.4	3.8	1.23
CVI ♀	out patch	0.2 (±0.0)	5.0	4.7	0.57
	in patch	3.5 (±0.6)	19.3	19.0	8.78
copepods	pre-bloom	0.04 (±0.01)	1.8	3.8	0.17
< 2 mm	in patch	0.05 (±0.05)	0.5	9.2	0.11
	out patch	0.12 (±0.08)	2.7	6.5	0.42

Table 5:
Daily carbon rations for *Calanus simillimus* and *Rhincalanus gigas*. The experimental method from which rations were estimated and the source are indicated additionally. Text in bold indicates when rations were determined with both methods in the same study.

Grazer	Daily ration %	Method	Source
C. simillimus	< 1	incubation	Atkinson 1994
	0.26 – 0.63	**incubation**	**Atkinson 1996**
	6.6 – 12.4	**incubation**	**this study**
	12	gut fluorescence	Atkinson et al. 1992 b
	12 – 70	gut fluorescence	Perissinotto 1992
	1.5	**gut fluorescence**	**Atkinson 1996**
	10	gut fluorescence	Atkinson et al. 1996
	14	gut fluorescence	Pakhomov et al. 1997
	12	gut fluorescence	Mayzaud et al. 2002
	83.5 (±42.2)	gut fluorescence	Bernard & Froneman 2003
	13.2	**gut fluorescence**	**this study**
R. gigas	1.8 – 7.6	incubation	Schnack 1985
	< 1	incubation	Atkinson 1994
	0 – 6.9	**incubation**	**Atkinson et al. 1996**
	0.5 – 1.4	**incubation**	**this study**
	1.5 – 2.0	gut fluorescence	Atkinson et al. 1992 b
	4.8 – 7.9	**gut fluorescence**	**Atkinson et al. 1996**
	1.8 – 7.6	gut fluorescence	Dubischar & Bathmann 1997
	0.1 -0.4	gut fluorescence	Pakhomov et al. 1997
	8	gut fluorescence	Mayzaud et al. 2002
	4.5	**gut fluorescence**	**this study**

Figure Captions

Figure 1:
Variation of initial gut content with environmental chl *a* concentrations for *Calanus simillimus*, *Rhincalanus gigas* and copepods < 2 mm.

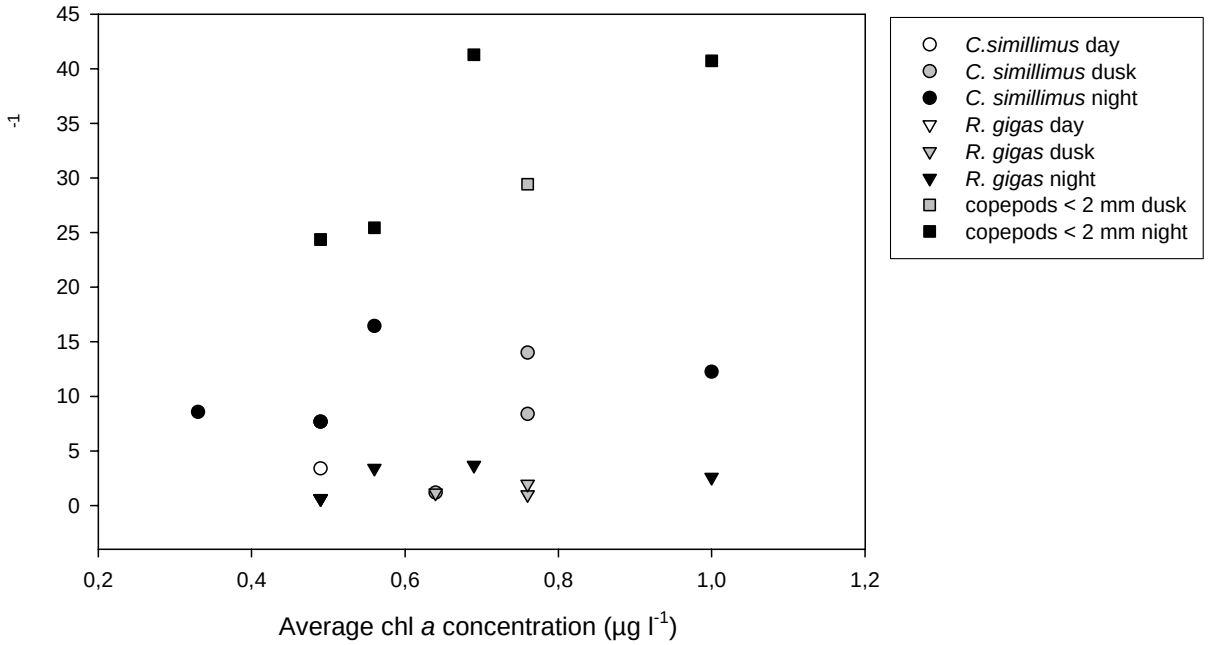
Figure 2:
Gut passage times for *Calanus simillimus*, *Rhincalanus gigas* and copepods < 2 mm estimated at different environmental chl *a* concentrations.

Figure 3:
Decrease of gut passage time of *Calanus simillimus* with increasing environmental chl *a* concentrations. The line represents the results of a negative exponential fit to the data with exception of the daytime measurement (open symbol; see text for further explication). The equation of the fit and its statistical significance are indicated.

Figure 4:
Clearance rates of a) *Calanus simillimus* b) *Rhincalanus gigas* and c) copepods < 2 mm for four diatom size classes. Results are presented for all incubation experiments and each data point represents a single replicate bottle.

Figure 5:
Ingestion rates determined with gut fluorescence and in bottle incubations at different environmental chl *a* concentrations. a) *Calanus simillimus*, b) *Rhincalanus gigas*, c) copepods < 2 mm.

Figure 6:
Respiratory carbon demand based on protein and lipid or carbohydrate catabolism in relation to grazer mass. The broken lines represent the fit to the experimental data (Prot+CarboH: $\ln(y) = -0.661+0.634*\ln(x)$, $r = 0.999$; Prot+Lipid: $\ln(y) = -0.898+0.629*\ln(x)$, $r = 0.998$). The solid line indicates the allometric relationship between respiratory demand and grazer mass published in Dagg et al. (1982). Additionally, carbon ingestion based on gut fluorescence measurements is indicated.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1,2
Average chl a concentration (µg l-1)
C.simillimus day
C. simillimus dusk
C. simillimus night
R. gigas day
R. gigas dusk
R. gigas night
copepods < 2 mm dusk
copepods < 2 mm night

Figure 1

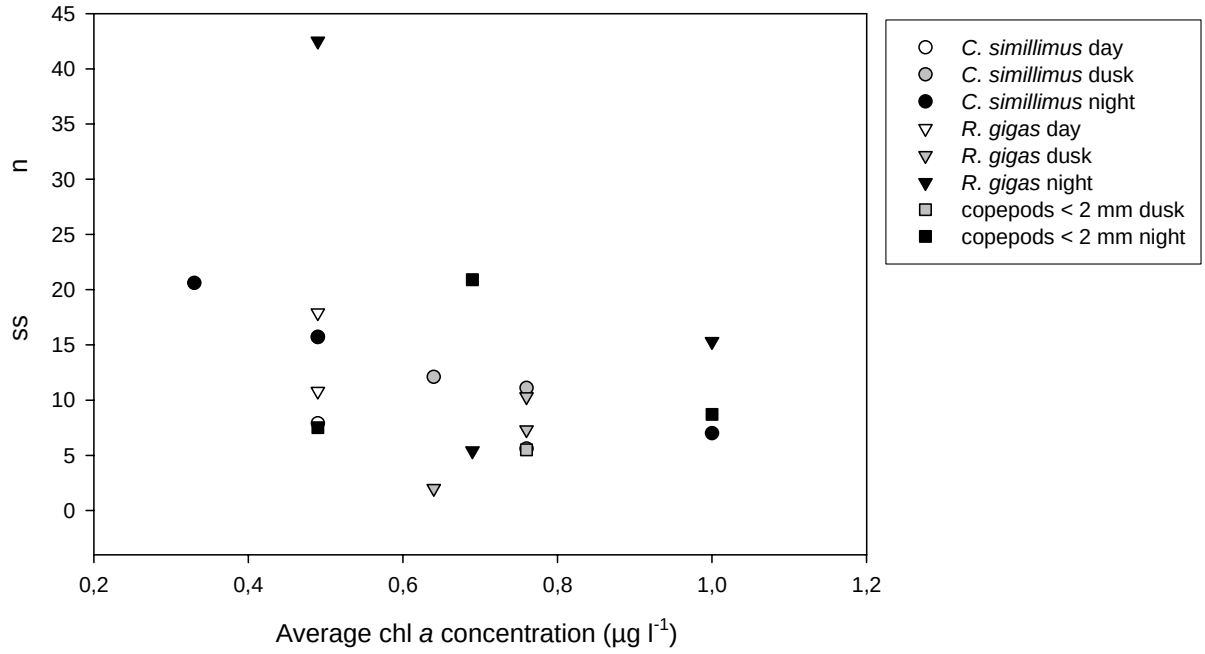
45
40
35
30
25
20
15
10
5
0
n
ss
0,2
0,4
0,6
0,8
1,0
1,2
Average chl a concentration (µg l-1)
C. simillimus day
C. simillimus dusk
C. simillimus night
R. gigas day
R. gigas dusk
R. gigas night
copepods < 2 mm dusk
copepods < 2 mm night

Figure 2

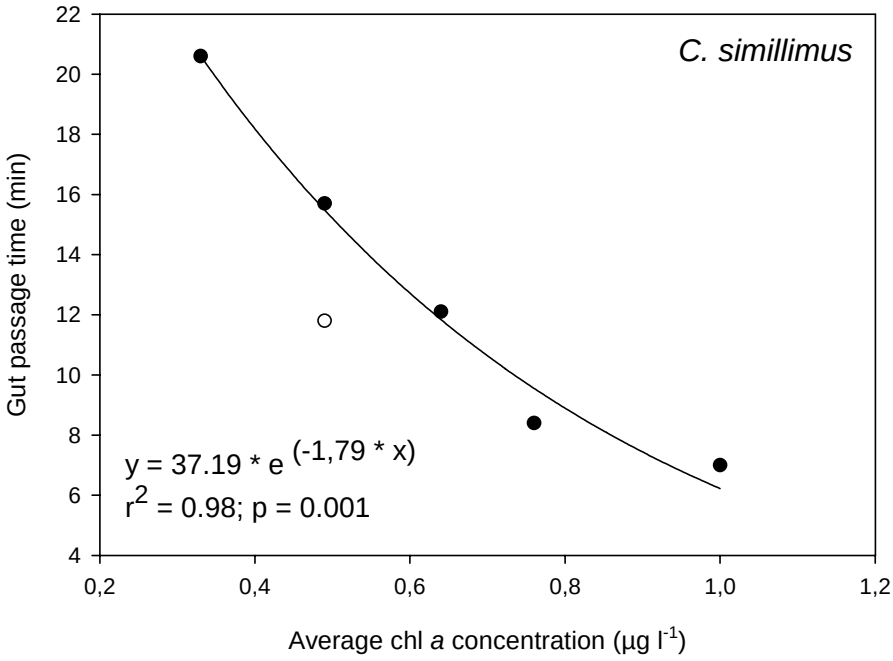
C. simillimus
Gut passage time (min)
22
20
18
16
14
12
10
8
6
4
0,2
0,4
0,6
0,8
1,0
1,2
y = 37.19 * e (-1,79 * x)
r2 = 0.98; p = 0.001
Average chl a concentration (µg l-1)

Figure 3

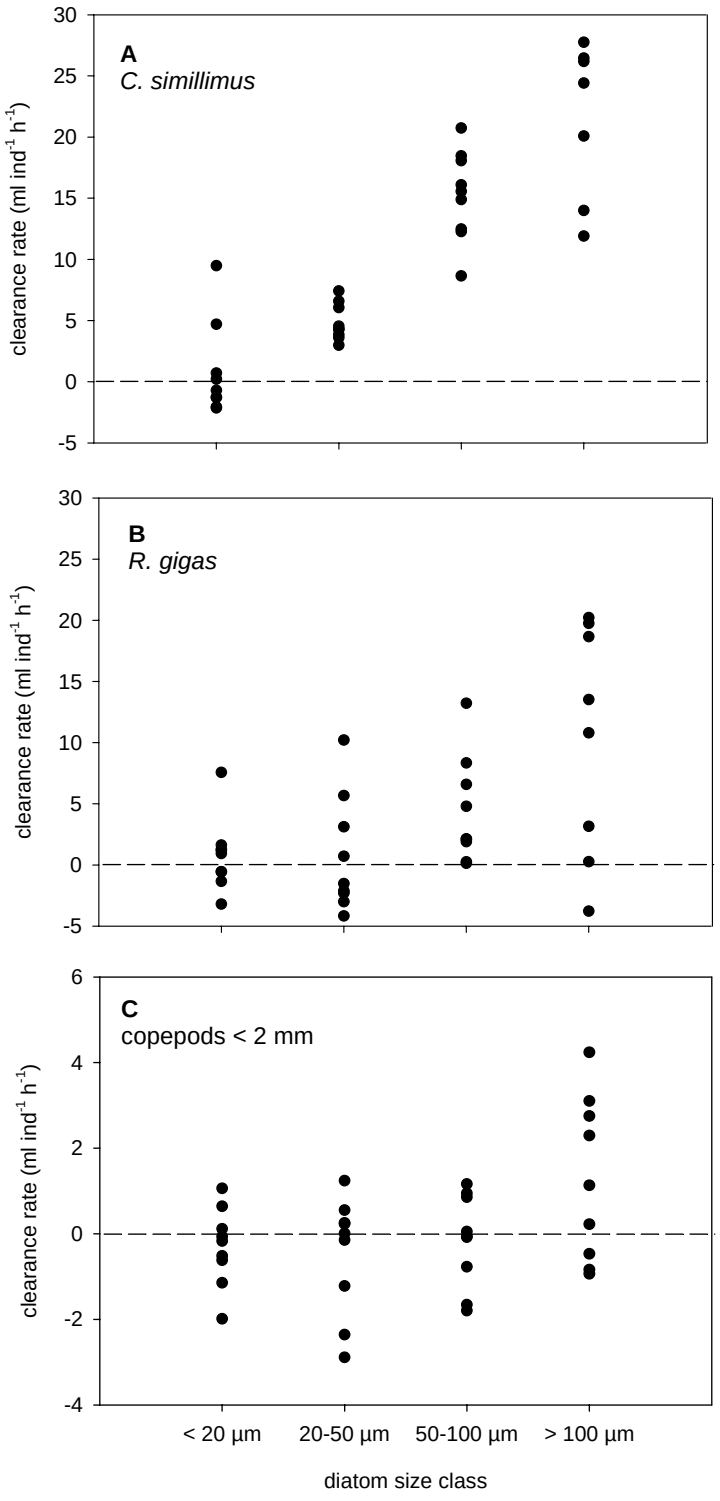
A
C. simillimus
B
R. gigas
C
copepods < 2 mm
clearance rate (ml ind-1 h-1)
< 20 µm
20-50 µm
50-100 µm
> 100 µm
diatom size class

Figure 4

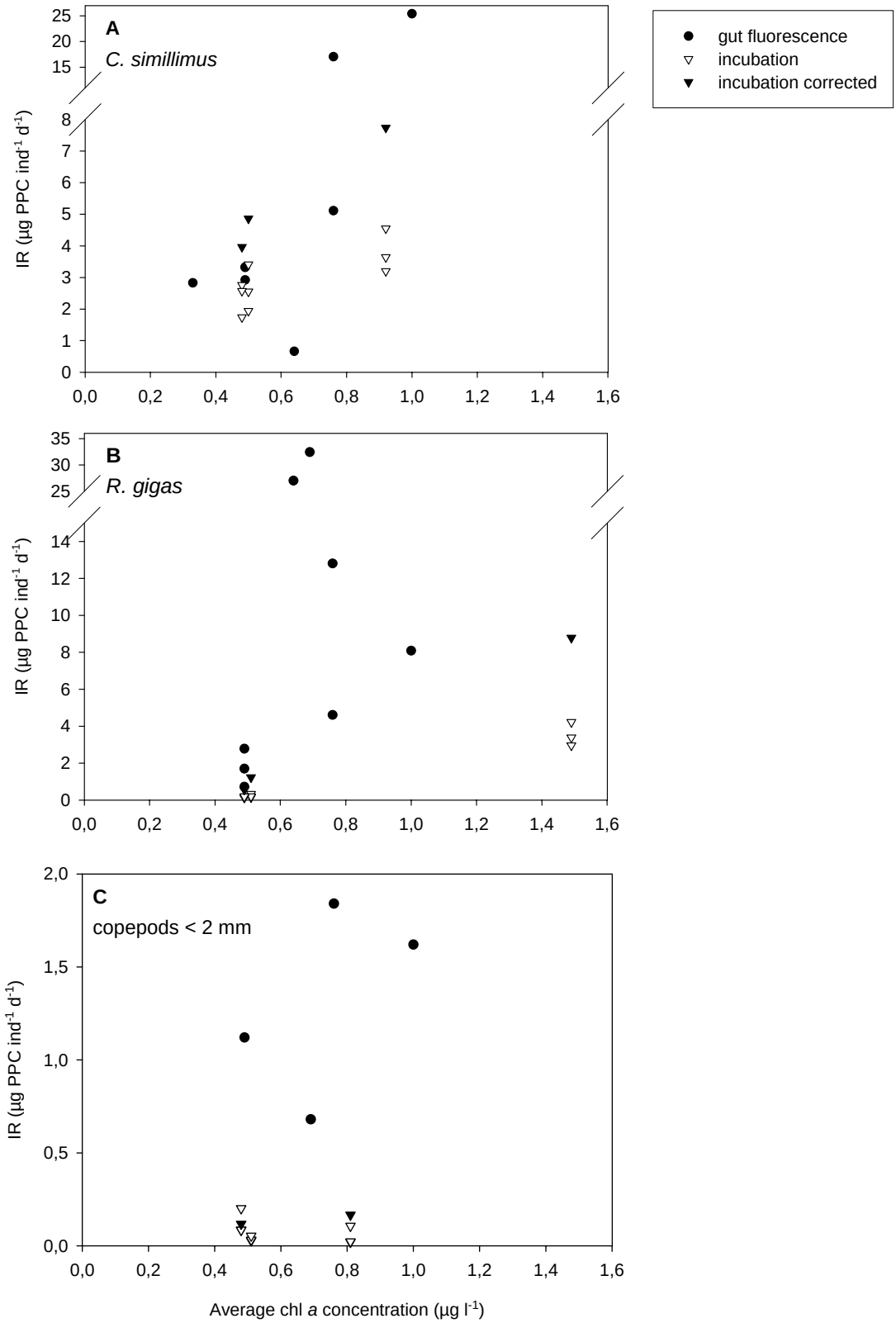
A
C. simillimus
B
R. gigas
C
copepods < 2 mm
gut fluorescence
incubation
incubation corrected
IR (µg PPC ind-1 d-1)
Average chl a concentration (µg l-1)

Figure 5

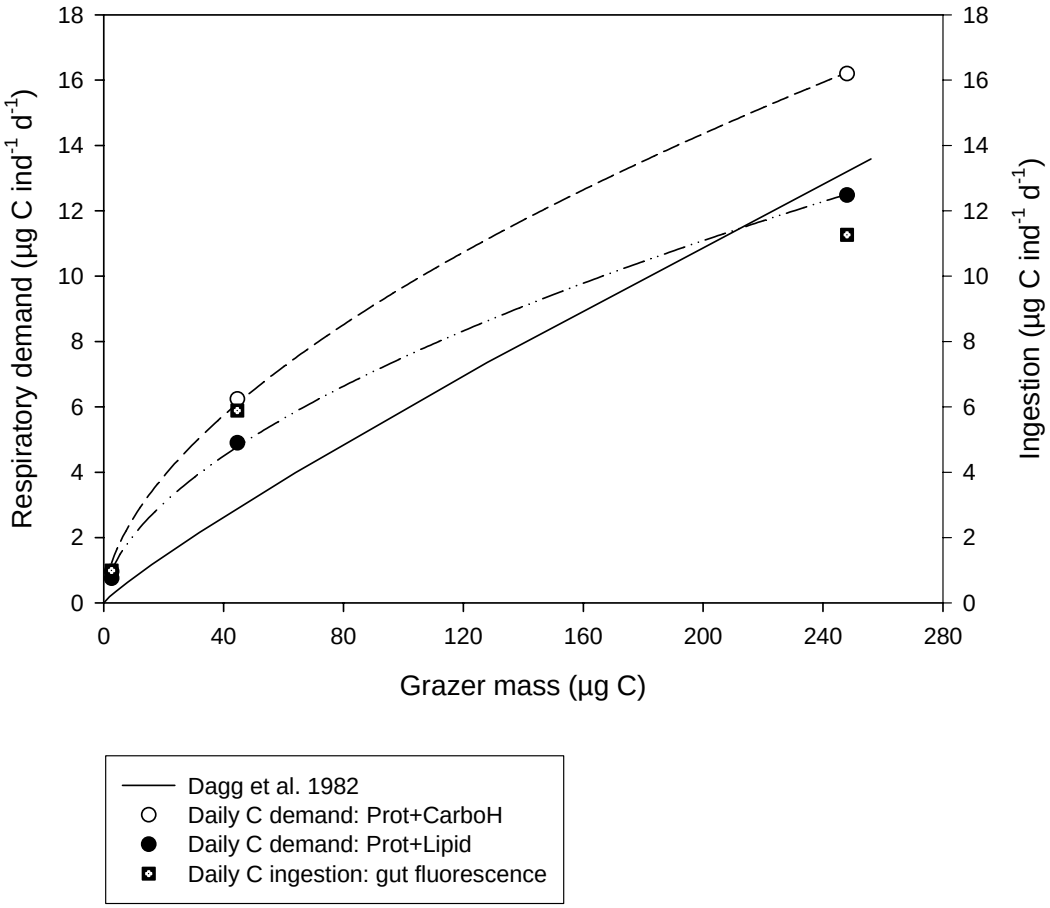
Respiratory demand (µg C ind-1 d-1)
Ingestion (µg C ind-1 d-1)
Grazer mass (µg C)
0
2
4
6
8
10
12
14
16
18
0
40
80
120
160
200
240
280
Dagg et al. 1982
Daily C demand: Prot+CarboH
Daily C demand: Prot+Lipid
Daily C ingestion: gut fluorescence

Figure 6

MANUSCRIPT 3

Influence of mesozooplankton grazing on the dissolution rate of Antarctic diatom silica

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Abstract

The impact of mesozooplankton grazing on the dissolution rate of Antarctic diatom frustules was investigated in a series of experiments during a SO GLOBEC cruise on FS Polarstern to the Bellingshausen Sea in austral autumn 2001. *Calanus propinquus*, *Metridia gerlachei* and furcilia stages of *Euphausia superba* grazed on natural diatom communities from the surface water and from first year sea ice as well as on cultures of *Fragilariopsis kerguelensis* and *Thalassiosira* sp. for 3 to 4 days in the dark. Following the removal of grazers, batch incubation of grazed diatom assemblages and non-grazed controls continued at 4°C for a period of up to 3 months. Results indicate a reduction in relative dissolution of biogenic silica (BSi) in the grazed communities by 32 to 95 % compared to the control. This effect appears to be attributable to the packaging of BSi in fecal pellets. Rate coefficients calculated from the experimental data are in the range previously determined for natural diatom assemblages dissolving at low temperatures. The variability of rate coefficients is related to the species composition of the diatom community and to the amount of BSi freely available for dissolution, calculated from the percentage of diatoms that has been ingested by mesozooplankton grazers and thus enclosed in feces. Scanning electron microscope analysis confirms that BSi escapes rapid dissolution when it is aggregated or packed into fecal pellets. Furthermore, the solidity of fecal pellets seems to be a crucial factor regulating pelagic dissolution of diatoms.

Introduction

Diatoms dominate new production in the oceans and drive the biological pump of CO_2 that leads to export of organic carbon to the deep sea (Goldmann 1993). Diatom frustules are built of biogenic silica (opal; BSi) and key to the taxonomy of > 1000 marine species (Hasle & Syvertsen 1996; Sournia et al. 1991). Sedimentary deposits of these frustules are omnipresent in today's ocean independent of depth, latitude or climate zone. Substantial accumulation of BSi originating from diatoms occurs in HNLC areas, especially the Southern Ocean that is thought to play an important role in regulating the Earth's climate on geological timescales by modulating the glacial/interglacial pCO_2 concentrations of the atmosphere (Sigman & Boyle 2000). Based on the assumption that spatial variations of biogenic silica production in the surface waters are mirrored by its spatial distribution in the sediment, silica depositions hold a strong potential as proxy, both paleo and present, for the functioning of the biological pump. Reliable interpretation of the sedimentary record requires calibration of the proxy for the mechanisms controlling its production, export and preservation in a process orientated approach (Ragueneau et al. 2000).

Annually, diatom growth leads to a build-up of 2.8×10^{14} moles of BSi and a mean of 60 % dissolves in the upper 50 to 100 m of the water column (Nelson et al. 1995). Export of opal from the upper ocean is controlled by its production and dissolution. Factors that govern the recycling intensity are poorly understood (Ragueneau et al. 2000) and on a global average only 3% of the surface production of BSi are preserved in the sediments (Tréguer et al. 1995). According to Hurd & Birdwhistell (1983) the specific dissolution rate of BSi (h^{-1}) is a function of the solubility of opal (mol cm^{-3}) corrected for the ambient concentration of silicic acid ($Si(OH)_4$; mol cm^{-3}), the specific surface area of the opal present (cm^2 mol^{-1}) and a first order rate constant k (cm h^{-1}). The solubility and the rate constant of BSi dissolution both increase with temperature (Hurd 1972; Kamatani 1982) and therefore, the temperature of the surface layer and factors that retain diatoms in the upper water column are of major importance in controlling opal dissolution. In that respect, aggregation of diatoms and mass sinking of cells associated with diatom life cycles or seeding strategies (Crawford 1995; Smetacek 1985; Smetacek 2000) attain biogeochemical significance concerning the recycling of biogenic silica in the water column. Ambient $Si(OH)_4$ concentrations are far from saturation concentrations and are assumed to have a minor effect on the dissolution rate (Hurd 1973; Ragueneau et al. 2000). Variability of BSi dissolution observed for different diatom species and diatom communities is attributed to changes in specific surface area of the opal

(Kamatani & Riley 1979; Lawson et al. 1978). The morphology and silicification of the frustules, influenced by growth conditions such as light and nutrient availability (Brzezinski 1985; Harrison et al. 1977; Hutchins and Bruland 1998) may additionally modulate the rate at which diatoms dissolve. Furthermore, opal solubility decreases due to incorporation of trace elements, for example Al, into the matrix (van Bennekom et al. 1991). Living diatom cells are protected from dissolution through the presence of an organic coating composed of polysaccharides (Coombs & Volcani 1968; Hecky et al. 1973; Lewin 1961). Bacterial degradation of this protective cover accelerates the rate of dissolution (Bidle & Azam 1999) and microzooplankton grazing or diatom mortality introduced by viral infection (Fuhrman 1999; Jacobsen & Anderson 1986) can be expected to have a similar rate enhancing effect. The impact that mesozooplankton grazing has on the remineralization rate of BSi in the water column has only received limited attention so far. While feeding on diatoms, grazers break up the diatom frustules and selectively digest the organic carbon with no measurable effect on the silica content of the prey (Cowie & Hedges 1996; Tande & Slagstad 1985). Recent scanning electron microscope investigations in the laboratory on diatom frustules from freshly produced feces, however, show an increase in pore size of the frustules relative to diatoms that have not been grazed, indicating that gut passage already initiates dissolution (Jansen 2002). Generally, it is assumed that the undigested diatom material in fecal pellets rapidly transfers biogenic silica to the seafloor and that dissolution of BSi is prevented by the presence of a peritrophic membrane that encases the pellets (Kamatani 1982; Schrader 1971; Tréguer et al. 1989). If the pellet membrane is disrupted or microbially degraded, an increase in the surface area exposed to seawater of diatom debris compared to intact cells could indirectly hasten dissolution of BSi. Ingestion (coprophagy) and destruction (coprorhexy, coprochaly) of fecal pellets through metazoan grazers is well documented (Lampitt et al. 1990; Smetacek et al. 1980). For example, coprophagy seems to be important aspect in the feeding ecology of the cyclopoid copepod *Oithona* (González & Smetacek 1994).

The present study focuses on the net effect of mesozooplankton grazing on the dissolution rate of diatom silica. Natural communities of Antarctic diatoms and diatom cultures were submitted to grazing activity of copepods and krill. Silica dissolution was followed during subsequent batch incubation with the objective to study the remineralization of BSi from a previously grazed and control diatom assemblage while settling through the water column.

Material and Methods

Sampling

Influence of mesozooplankton grazing on the dissolution of BSi was investigated during the expedition ANT XVIII/5b of RV Polarstern in the Bellingshausen Sea. Incubation experiments were carried out with diatom communities from the surface water and sea ice as well as cultures of *Fragilariopsis kerguelensis* and *Thalassiosira* sp. The surface diatom community was collected in a bloom encountered over the shelf off Adelaide Island (station 58-303; 66° 51'S – 70° 29'W; Bathmann (2002)) via the ships seawater pump system. The algal material was retained in a 55µm Apstein net and the concentrate collected from the cod-end was cleared of large metazoan grazers with a pipette. For the sea ice community pieces of the bottom layer of a 79 cm thick first year sea ice flow were recovered at station 58-314 (71° 05'S – 85° 23'W), melted according to Garrison & Buck (1986) and the algae were re-suspended in 0.2µm filtered seawater. The cultures of *F. kerguelensis* and *Thalassiosira* sp. were isolated from the Atlantic sector of the Antarctic Polar Front during ANT XVIII/2. Isolates were grown at the Alfred Wegener Institute (AWI) in Schreiber medium (Schreiber 1927; von Stosch & Drebes 1964), at 0 °C under 24 h light, and the cultures were taken back to the ship continuously cooled. On board, the cultured diatoms were harvested from the growth media on a fine mesh and re-suspended in ambient, 0.2 µm filtered seawater. Mesozooplankton was caught with vertical tows of Bongo nets (mesh size 100 to 335µm) over the upper 100 to 500 m of the water column. Late copepodite and adult stages of *Metridia gerlachei*, *Calanus propinquus* as well as furcilia stages of *Euphausia superba* were sorted in a cooled Petri dish under a stereomicroscope and either kept in filtered seawater (several hours) or maintained in bottles with ambient seawater on a plankton wheel (several days) until transferred to experimental containers. Individuals of *Oithona* sp. for use in experiment 4 were caught and sorted shortly before the start of the incubation.

Experimental design

The re-suspended diatoms, ice algae and cultures were diluted with 0.2 µm filtered seawater to approximately 2.5 µg chl *a* l^{-1} and sampled for initial determination of $Si(OH)_4$, BSi, chl *a* and microscopy. For practical purpose, the diatom incubation medium for controls and grazing treatments (6 liters each) was then split in 3x2 or 6x1 liter flasks. In a first step, mesozooplankton grazers that had previously been acclimatized to feeding conditions for 24h were added to one half of the bottles. Control and grazing bottles were incubated on a

plankton wheel (~ 1 rpm), in the dark, at ambient temperature. In total, four experimental series were carried out (Table 1). Experiment 1 to 3 used combinations of a single grazer and natural or cultured diatom material. This allowed investigating differences in dissolution rate for BSi of different origin, i.e. the control treatments, and BSi subjected to the grazing activity of copepods and furcilia stages of Antarctic krill. In experiment 4, we tried to evaluate the effect of re-ingestion and destruction of *C. propinquus* fecal pellets by *Oithona* sp. on BSi dissolution through a combination of both grazers in one of the experimental treatments. In general, the grazing phase lasted for approximately four days (see Table 1) to ensure that a substantial fraction of the diatom material was ingested in the grazing bottles compared to the control. Following the incubation, grazers were carefully removed with a wide-mouth pipette and checked for mortality. The volume of diatom incubation medium of grazing and control bottles, respectively, was pooled again to achieve the total of 6 liters each, the volume needed for the time series experiment. The latter was done to homogenize variability in the algal material between parallel grazing incubations before the start of the dissolution experiment. The grazed and un-grazed diatom material was again sampled for t0 determination of $Si(OH)_4$, BSi, chl *a* and microscopy. The remaining volume was redistributed into 50 ml centrifuge vials, filled without airspace (oxygen measurements after 3 months still indicated ~ 95% saturation). Vials were incubated on a plankton wheel at 4°C in the dark. In weekly, later monthly, intervals six vials were sacrificed, three for measuring $Si(OH)_4$ and three for scanning electron microscope (SEM) analysis. The dissolution experiment was started on board the ship within 6-12 hours after the end of the grazing experiment. At the end of the cruise, approximately two weeks later, all vials were transferred steadily cooled to the AWI and incubation continued for up to 96 days.

Analytical methods

Measurements of BSi were performed from 500 ml sample water collected on a 0.8 µm cellulose acetate filter and the filter kept frozen at -20°C until analysis according to Müller & Schneider (1993). Chl *a* measurements on 250 ml sub-samples followed standard JGOFS procedures. For determination of $Si(OH)_4$ 50 ml sub-samples were filtered over 0.7µm polycarbonate membranes and the filtrate fixed with $HgCl_2$ to a final concentration of 3.5 % (Kattner 1999). Samples were stored at 4°C until measuring with an auto analyzer following the method of Grasshoff et al. (1999). Analytical precision of the method is 1 % of the employed silicate standard, in this study ± 0.35 µmol $Si(OH)_4$ l^{-1} (H. Johannsen pers. comm.).

Microscopy

Preparation of diatom material for SEM involved filtration of several ml of sample onto a 0.2 µm polycarbonate membrane with help of a syringe and a Swinnex Millipore filtration unit. To remove salts, the sample was washed with the same volume of de-ionized water before drying simply exposed to air (Jansen 2002). The dry filter was mounted on a stub and sputtered with Gold/Palladium under Argon gas using an EMSCOPE SC 500 Sputter Coater (25 mA, 3 min). Additionally, a sub-sample was fixed with buffered formaldehyde (4 % final concentration) and counted, if possible to species level, under an inverted microscope (Utermöhl 1958).

Calculation of the rate coefficient

To describe the dissolution of BSi the simplified first order reaction equation

$$dC/dt = - k (C_0-C) \qquad (1)$$

proposed by Kamatani & Riley (1979) was applied to the data. Integration of equation (1) gives:

$$kt = - \ln ((C_0-C)/C_0) \qquad (2)$$

k, expressed in h^{-1}, is not a true kinetic rate constant and will be termed "rate coefficient" subsequently (Kamatani 1982; Tréguer et al. 1989). It can be calculated from the initial concentration of BSi ($c_{initial}$ [BSi]; C_0) added per unit volume to the solution and the concentration of $Si(OH)_4$ (c[$Si(OH)_4$]; C) in the solution at a certain point in time (t). We estimated k from the slope of the linear regression (least squares fit, $p < 0.05$) of $\ln ((C_0-C)/C_0)$ plotted against time. Errors represent estimations of the standard error of the slope estimate. The terms "rate coefficient" and "specific dissolution rate" will be used as synonyms subsequently.

Results

Grazing on diatoms by mesozooplankton

In all experiments, chl *a* concentration of the grazed diatom material decreased relative to the control (Fig.1). Delta chl *a* ranged from -0.8 to -3.6 µg l^{-1}. Microscopic counts also revealed a decrease in cell abundance by 29 and 53 % in experiment 1 for *M. gerlachei* and *E. superba*, respectively, by 48 % in experiment 3, and by 96 and 92 % for *C. propinquus* and *C. propinquus* and *Oithona* sp. combined in experiment 4. The percentage of diatoms ingested, i.e. BSi that has been processed and passed through the gut of the grazer, was

calculated from changes in chl *a* concentration, and also from changes in cell concentration where a count is available, and ranged from 25 to 96 % (Table 1). Aggregation of sea ice diatoms, dominated by several spiny diatom species such as *Chaetoceros* sp. and *Corethron pennatum*, precluded a reliable count in experiment 2. The shelf bloom diatom community was numerically dominated by *Chaetoceros* spp. and *Pseudonitzschia* spp., accounting for 46 % and 24 % of the total diatom abundance respectively. Analytical precision of the chl *a* measurements was always better than 10 %. The error associated with the microscopic count is also estimated to a maximum of 10 % (Edler 1979).

Dissolution of diatoms

Processes and rates of diatom dissolution are inferred from $Si(OH)_4$ accumulation dynamics as c[BSi] measurements are only available for time zero of the dissolution experiment. Any increase in $c[Si(OH)_4]$ during the dissolution phase is assumed to be the result of BSi dissolution. To correct for differences in initial $Si(OH)_4$ concentration ($c_{initial}\,[Si(OH)_4]$) all results are presented as delta $c[Si(OH)_4]$ relative to time zero of the dissolution phase. The influence of varying $c_{initial}\,[Si(OH)_4]$ on the rate of dissolution will be discussed in a later section.

Dynamics of $Si(OH)_4$ accumulation in the control

All diatom control treatments showed a non-linear increase in $c[Si(OH)_4]$ throughout the incubation (Fig. 2 a, b). Initial rates of $Si(OH)_4$ accumulation, estimated from the change in $c[Si(OH)_4]$ between t0 and the first sampling after 7 to 9 days, are 0.15 (std 0.03) µmol $Si(OH)_4\ l^{-1}\ d^{-1}$ for shelf bloom diatoms, 0.28 (std 0.04) for sea ice diatoms, 0.22 (std 0.02) for *F. kerguelensis* and 0.03 (std 0.08) for *Thalassiosira* sp. Derived from the total increase in $c[Si(OH)_4]$ and initial concentrations of BSi, 39.7 to 104.7 % of the suspended biogenic silica dissolved during the incubation of the control treatments (Table 2). *F. kerguelensis* and the shelf bloom diatoms show a marked decrease in dissolution rate in the 3rd and 5th week of the incubation after 79.7 % (std 17.8) and 32.1 % (std 8.5) of $c_{initial}$ [BSi] had dissolved respectively.

Dissolution of diatoms in the grazing treatments

For shelf bloom diatoms and sea ice diatoms, i.e. natural communities, the initial change of $c[Si(OH)_4]$ in the grazing treatments with copepods or krill larvae closely followed the one of the control (Figs 3&4). Initial rates of $Si(OH)_4$ accumulation (Table 2) were not

significantly different (Student t-Test, $p < 0.05$) from their respective control with exception of *C. propinquus* grazed sea ice diatoms where accumulation was slowed to rate of 0.08 (std 0.08) µmol $Si(OH)_4$ l^{-1} d^{-1} compared to 0.28 (std 0.04) µmol $Si(OH)_4$ l^{-1} d^{-1} in the control. After 3 to 9 weeks, in 3 of the 4 grazing treatments, the accumulation of $c[Si(OH)_4]$ was significantly diminished and remained lower than the control value until the end of the experiment. The grazing activity of *M. gerlachei* seemed to have no influence on the dynamics of diatom dissolution in the present study.

F. kerguelensis and *Thalassiosira sp.* cultures, when grazed by *C. propinquus*, displayed a decrease in the initial rate compared to the control, both to negative values. Moreover, $Si(OH)_4$ accumulation in the grazed cultures was slower than in the control over the whole period of sampling (Figs 5&6), in contrary to the experiments with natural diatom communities. The *Thalassiosira* sp. culture that had been subjected to the combined grazing pressure of *C. propinquus* and *Oithona* sp. revealed very different dynamics of $Si(OH)_4$ accumulation. It yields a strongly increased initial rate of 0.17 (std 0.04) µmol $Si(OH)_4$ l^{-1} d^{-1} with a final delta $c[Si(OH)_4]$ of 2.1 µmol l^{-1} compared to 0.2 µmol l^{-1} and -0.5 µmol l^{-1} in the control and in the exclusively by *C. propinquus* grazed culture respectively (Fig.6).

The percentage of initially added biogenic silica that dissolved in the grazing treatments is reduced with respect to the control (Table 2). For example, in the experiment with *F. kerguelensis* only 4.9 (std 12.4) % of the initially present biogenic silica dissolved when the diatom had been grazed by *C. propinquus* compared to 88.3 (std 4.8) % in the control.

Variation of specific dissolution rates

Rate coefficients k estimated for the dissolution of shelf diatoms, sea ice diatoms and *F. kerguelensis* and the respective grazing treatments are also presented in Table 2 and vary over two orders of magnitude. For the shelf bloom diatom control, k is derived in two ways ($p < 0.05$; Fig.7). Once to a maximum value of 13.3 x 10^{-3} d^{-1} from the first five measurements, for use in correlation analyses, and secondly to a mean value of 6.3 x 10^{-3} d^{-1} from the overall timecourse for comparison with results from parallel experiments. Scatter in the data precluded the calculation of a significant initial value of k for the other control and grazing treatments and only overall values are presented. Mean rate coefficients of grazed diatoms were, with exception of the *M. gerlachei* grazed shelf bloom diatoms, by a factor 4 to 26 lower than the ones calculated for the respective control treatments.

Discussion

Pelagic dissolution of Antarctic diatoms

Several approaches to model the dissolution rate of BSi have been considered (Greenwood et al. 2001) and the surface area available for dissolution plays a central role as rate limiting factor in most models. According to the surface reaction approach (Greenwood et al. 2001) the net rate of dissolution is determined by the balance between forward reaction, proportional to the available surface area, and the backward reaction, proportional to both the surface area and the concentration of the reaction product ($Si(OH)_4$) in the bulk solution. In the majority of the models the surface area is assumed to remain constant, a condition that is only met when BSi is added in excess of the amount required for reaching saturation and in which case the change in surface area is negligible for the duration of the batch incubation. However, when studying the water column dissolution of diatoms the system is far from equilibrium concentrations. For example, the saturation concentration of *Thalassiosira decipiens* dissolving at 4.5°C is 74.4 mg BSi l^{-1} (Kamatani & Riley 1979). In the present study, we tried to mimic *in situ* concentrations with $c_{initial}$ [BSi] ranging from 0.26 to 0.41 mg l^{-1}, i.e. three orders of magnitude lower. Therefore, the available surface area is likely to be a major aspect when it comes to the interpretation of variability observed for initial dissolution rates and rate coefficients. Although application of the "decreasing surface area model" (Greenwood et al. 2001; Kamatani et al. 1980) seems to be most appropriate the simple description developed by Kamatani & Riley (1979) was used to calculate rate coefficients and thus enable their comparison with those of previous studies.

Initial dissolution rates determined in this study vary by a factor of two, the highest rate being associated with the sea ice diatoms, followed by *F. kerguelensis*, the shelf bloom diatoms and *Thalassiosira* sp. Differences in dissolution rate between diatom species are well established (Kamatani 1982; Lawson et al. 1978; Tréguer et al. 1989) and related to the material properties of the biogenic silica, more specifically the available surface area and porosity, but determinations for neither are available in the framework of this study. *F. kerguelensis* is a key species of Southern Ocean phytoplankton and frequently contributes to blooms in the area (e.g. Smetacek et al. 1997). It is well preserved in the sediment, and a major constituent of the silica belt beneath the Antarctic Circumpolar Current (ACC) (Zielinski & Gersonde 1997). This strong contribution to silica deposits is attributed to comparably low grazing mortality and slow dissolution both due to the high degree of silicification of this diatom (Verity & Smetacek 1996). Therefore, it is surprising that

F. kerguelensis displays the second highest initial dissolution rate and rate coefficient determined in this study. Speculation on whether the comparably fast dissolution is due to the fact that *F. kerguelensis* originated from a culture, causing weaker silicified frustules (U. Freier pers. comm.) and possibly higher bacterial activity associated with the cells is opposed by the extremely slow dissolution of *Thalassiosira* sp., also grown in culture. Diatoms used in the dissolution experiments of this study were not pre-treated by acid cleaning (e.g. Kamatani 1982), heat killing (Patrick & Holding 1985) or freeze-thaw lysing (Bidle and Azam 1999). In addition, Peters & Thomas (1996) have shown that the viability of Antarctic diatoms under prolonged darkness is species dependent and in the order of several months. Consequently, it has to be assumed that the presence of the protective coating of living diatom cells (Lewin 1961) also modulated the rate of dissolution.

The diatoms sampled from a dense autumn bloom over the shelf off Adelaide Island and *F. kerguelensis*, grown in culture, dissolved with a marked decrease in dissolution rate over the course of the incubation. This decrease has previously been observed in experiments where BSi was present in concentrations less than necessary for saturation (Kamatani 1982; Kamatani & Riley 1979; Kamatani et al. 1980). The different stages of dissolution are interpreted as a result of selective dissolution of more or less soluble parts of the frustules, for example spines or girdle bands, tantamount to decreases in specific surface area. In comparison, over the same period of time, diatoms melted out of first year sea ice dissolved much more progressively and until virtually no biogenic silica was left.

To explain the differences in dissolution rate between shelf bloom and sea ice diatoms, the concept of selective dissolution may be extrapolated from the different components of the frustule to the composition of a diatom community. The taxonomic composition of shelf bloom and sea ice diatom communities was not strikingly different, both being numerically dominated by diatoms of the genera *Chaetoceros* and *Pseudonitzschia*. Surface phytoplankton communities in polar seas are trapped in newly forming sea ice and ice algae that are released in the water column during ice melt in spring serve as seeding population for developing ice edge blooms (Légendre et al. 1992), a mechanism that explains the similarity in phytoplankton community composition. However, *Thalassiothrix antarctica* and *Trichotoxon reinboldii*, both very large pennate diatoms (mean apical length 1400µm and 800µm, respectively), were present in the shelf bloom but not in the sea ice community. Although of little importance in terms of abundance with 1.0 % and 0.6 % of the total, taken together they represent 10 % of diatom carbon (M. Brichta pers. comm.) or, assuming a constant Si:C ratio, 10 % of the BSi in the sample. Furthermore, *T. antarctica* is highly silicified and has been

shown to account for a lot of export (Kemp et al. 2000). If we infer that these giants of siliceous phytoplankton are also more refractory to dissolution their presence in the shelf bloom diatom community may partly serve as explanation for the lower initial dissolution rate and rate coefficient of shelf bloom diatoms compared to the sea ice diatoms.

Rate coefficients determined for the dissolution of common Antarctic diatoms showed considerable variation but are comparable to rate coefficients and specific dissolution rates previously published for diatoms dissolving at low temperatures. In the laboratory, Kamatani & Riley (1979) estimated rate coefficients for acid cleaned diatoms dissolving at 4.2 °C ranging from 0.04 to 0.36 d^{-1} for cultured *Thalassiosira decipiens* and 0.02 to 0.06 d^{-1} for a natural community dominated by *Rhizosolenia hebetata*. Pre-treating diatom frustules with acid increases their dissolution coefficient by one to two orders of magnitude (Kamatani 1982; Kamatani & Riley 1979). *In situ*, diatom communities of the Southern Ocean dissolve with specific dissolution rates of 0.01 to 0.04 d^{-1} in the Pacific sector of the Antarctic Circumpolar Current (Nelson & Gordon 1982) and 0.01 to 0.02 d^{-1} in the Ross Sea (Nelson et al. 1991). All these estimates from the laboratory and the field compare well to the values of k varying from 0.006 to 0.066 estimated from the control incubations in this study (Table 2). Additionally, our initial dissolution rates of shelf bloom diatoms and sea ice diatoms, 0.15 and 0.28 µmol $l^{-1}d^{-1}$, respectively, resemble the rates of 0.2 to 0.5 µmol Si l^{-1} d^{-1} determined for biogenic silica in the surface water of the Ross Sea (Nelson et al. 1991).

Effect of grazing on biogenic silica dissolution

Feeding activity of a single type of mesozooplankton grazer (experiments 1 to 3) on diatoms reduced the rate coefficient of dissolving biogenic silica by a factor of 4 to 26 (Table 2). For a similar period of incubation, 32 to 95 % less BSi dissolved in the grazing treatments compared to the control. Only exception makes the feeding activity of *M. gerlachei*, which had no significant effect on the dissolution of shelf bloom diatoms. From all experiments, *M. gerlachei* ingested the least amount of diatoms (Fig. 1, Table 1). The estimated changes in chl *a* and cell concentrations are both larger than the analytical error and the presence of fecal pellets in the microscopic samples confirm that *M. gerlachei* has actively been feeding on the diatoms. Possibly, the amount of BSi that was ingested by *M. gerlachei* was not sufficient to reach a threshold level that is necessary to lead to a measurable effect on BSi dissolution.

Initial dissolution rates of grazed natural diatom communities do not change compared to the control in three out of four cases and the decrease in initial dissolution rate for *C. propinquus* grazed sea ice diatoms is not maintained (Fig. 4a). Clear differences in BSi

dissolution between control and grazing treatments only become apparent after several weeks of incubation. Also regarding cultured diatoms, grazing reduced the amount of BSi that dissolved relative to the control. Contrasting to the natural communities, the reduction in dissolution is already detectable at the first sampling after 8 to 9 days. Both grazing treatments of cultured diatoms initially display a negative initial rate, i.e. a decrease in $c[Si(OH)_4]$. For the *C. propinquus* grazed *F. kerguelensis*, this trend is reversed to a slow but overall net dissolution of BSi during further incubation. However, for the *C. propinquus* grazed *Thalassiosira* sp. delta $c[Si(OH)_4]$ appears to be negative throughout the rest of the experiment. The changes in $c[Si(OH)_4]$ are 0.79 and 0.29 µmol l^{-1} at most for the *Calanus* grazed *Thalassiosira* and the control, respectively. Considering a background signal of approximately 40 µmol $Si(OH)_4$ l^{-1} and an analytical error of ± 0.35 µmol $Si(OH)_4$ l^{-1} this variation in $c[Si(OH)_4]$ is barely above or within the limits of analytical resolution and the results should therefore not be over interpreted. Despite the slightly different results obtained from natural communities compared to cultured diatoms, the overall effect to be observed is a reduction in BSi dissolution when diatoms were submitted to grazing pressure.

Changes in dissolution rate are hypothesized to be associated with variations in surface area available for the dissolution to take place. A previously grazed diatom community or culture is composed of remaining, not ingested cells, some cell debris and fecal pellets. Considering that, in natural communities, initial dissolution of the grazing treatments continued at a rate similar to the control and that the net decrease in biogenic silica dissolution became apparent only after several weeks, we attribute the different dissolution dynamics of a grazed diatom community to the enclosure of a part of the BSi in fecal pellets. The initial rate of dissolution is maintained as long as unchanged opal surface area, i.e. not ingested diatoms, is available for dissolution. The reduction in dissolution rate becomes only effective once the supply of the surface area as it was present in the original diatom community runs out. Therefore, a relationship should exist between the specific dissolution rate and the amount of BSi that has been packed in fecal pellets.

Variation observed for all rate coefficients, control and grazing treatments combined, is not significantly correlated with differences in $c_{initial}$ [BSi] ($r = 0.58$, $n = 8$). Assuming that all biogenic silica that has been ingested by the grazers is enclosed in fecal pellets and therefore not available for dissolution anymore, a corrected value for $c_{initial}$ [BSi] has been calculated, thereafter referred to as free biogenic silica (c_{free} [BSi]; Table 1). The correlation between k and c_{free} [BSi] is significant ($r = 0.75$, $n = 8$, $p<0.05$) and a linear regression between the two variables ($y = 0.0085x - 0.016$; $r^2 = 0.56$) reveals that 56 % of the observed

variability in k can be explained by the free amount of biogenic silica in suspension when the modification introduced by the grazers is taken into account.

Further, no correlation can be established between k and $c_{initial}$ [$Si(OH)_4$] (r = 0.22, n = 8). This result seems to confirm the general assumption that ambient seawater concentrations of $Si(OH)_4$ have no influence on the rate of BSi dissolution. Seawater is largely under-saturated in $Si(OH)_4$ with respect to biogenic silica dissolution, saturation concentrations being in the order of 1 mmol $Si(OH)_4$ l^{-1} (Hurd 1973). The change in specific dissolution rate is inferred to be less than 10 % for an increase in c[$Si(OH)_4$] from 0 to 100µmol l^{-1} (Ragueneau et al. 2000). We closer examined the relationship between c_{free} [BSi], $c_{initial}$ [$Si(OH)_4$] and k, as the use of different batches of filtered seawater to re-suspend the sea ice diatoms in experiment 2 had introduced large differences (~ 20 µmol l^{-1}) in $c_{initial}$ [$Si(OH)_4$]. Plotting k for sea ice diatoms and the respective *C. propinquus* and *E. superba* grazing treatments against the ratio of c_{free} [BSi]/$c_{initial}$ [$Si(OH)_4$] yields an almost perfect regression (Fig. 8), indicating that a combination of both, the particulate and dissolved silica species, influences the velocity of dissolution. Both grazers had ingested 70 % of the BSi but the mean rate coefficient for the *E. superba* grazed material is 58 % higher than the one estimated for *C. propinquus* (Table 2). The slower dissolution of BSi in the *C. propinquus* treatment is apparently caused by a 1.5 fold higher initial $Si(OH)_4$ concentration, 62 µmol l^{-1} compared to 41 µmol l^{-1} in the *E. superba* treatment. As predicted by the surface reaction model, the net rate of dissolution is slowed by the back reaction when bulk concentrations of $Si(OH)_4$ increase. Already at *in situ* values of BSi and $Si(OH)_4$ concentrations typical for the surface waters in the coastal current of the Antarctic Peninsula as well as oceanic intermediate and deep waters this effect seems be of considerable importance. The correlation between k and the ratio c_{free} [BSi]/$c_{initial}$ [$Si(OH)_4$] still remains significant when results from all experiments are pooled (r = 0.64, n = 8) but is weaker than the correlation with c_{free} [BSi] only that has been presented above. The primary rate limiting factor therefore seems to be the amount of free biogenic silica available for dissolution.

How important is the solidity of fecal pellets?

Copepods break down the diatom frustules to debris with their strong, silica edged mandibles, krill in its gastric mill. The debris is then packed into more or less compact, fast sinking fecal pellets and strings. Dense zooplankton fecal material is possibly an important conveyor of particulate material out of the surface layer (Angel 1984; Turner & Ferrante 1979) with sinking speeds of several hundred meters per day. However, cyclopoid copepods

of the genus *Oithona* have been shown to feed on feces (González & Smetacek 1994), counteracting the vertical flux and leading to retention of material in the upper water column (Lampitt et al. 1990). In the light of this evidence a second grazing treatment was added to experiment 4, a combination of *C. propinquus* and *Oithona* sp. speculating that *Oithona* might feed on the fecal pellets of *C. propinquus* causing disruption of the pellets and liberation of the diatom debris into the medium. The time courses of $Si(OH)_4$ accumulation for this experiment are shown in Figure 7. Whereas virtually no dissolution of BSi took place in the control and *Calanus* treatment, c[$Si(OH)_4$] in the *Calanus*-*Oithona* treatment increases by approximately 2 µmol l^{-1}, almost one order of magnitude more than in the control. Unfortunately, we have no reliable determination of $c_{initial}$ [BSi] for this experiment. However, the initial concentration of chl *a* in this experiment was 1.9 µg l^{-1}. Assuming a chl *a* to carbon ratio of 40 and a Si:C of 0.3 (determinations of molar Si:C ratios for the diatoms used in experiment 1 to 3 ranged from 0.3 to 0.45) the chl *a* corresponds to about 2 µmol Si l^{-1}. *C. propinquus* was feeding heavily on *Thalassiosira* in both experiments and produced fecal pellets, as has been confirmed by light microscopy. We have no proof, that *Oithona* has actually been feeding on the *Calanus* feces but the signal in the dissolved silicate appears reliable to us and lends support to the speculation that *Oithona* did destroy the fecal pellets causing the free diatom fragments to dissolve rapidly.

SEM analysis of samples from the sea ice diatom experiment corroborates the thought that breakup of feces accelerates dissolution of biogenic silica. During the course of the incubation a calculated 105% of the initially added BSi dissolved in the control, 47 % in the *C. propinquus* and 72 % in the *E. superba* grazed medium despite the fact that a similar amount of BSi had been ingested by both grazers. One explanation for the higher percentage of dissolution in the krill treatment is possibly the initial concentration of $Si(OH)_4$, as has been elaborated in the previous section. A second reason might be found in the integrity of the feces of both grazers. Copepods produce solid fecal pellets that are completely enclosed by a peritrophic membrane. Krill discards so called fecal strings into the water column, where the digestive leftovers are mantled by a skin that remains open at the ends. Pictures representative of the remnant diatom silica at the end of experiment 2, i.e. after 3 months of dissolution, are shown in Figure 9. Stubs prepared with the control medium were virtually empty and the only remaining material were aggregated diatoms dominated by *Chaetoceros* cells (Plate a). This aggregation had also been observed in samples for the light microscope and had made a count impossible. Fecal pellets, overall intact and compacted as the display detail presented in plate b, dominated the overall view of stubs with material from the *Calanus* treatment. Diatoms

packed in these pellets show only minor signs of dissolution with respect to the beginning of the experiment. For the *E. superba* grazed sea ice diatoms, fecal material that was still discernible as such (plate c) was the exception and disintegration of the feces obvious. The stubs prepared from this treatment were scattered with what can be described as feces crumbs (plate d). Moreover, fine debris of diatom frustules that was observed on the stubs of both grazing treatments at the beginning of the dissolution experiment was not noticed in the preparation from the final sampling. Lose debris and free cells apparently dissolved rapidly. BSi in aggregates or packed in fecal pellets escaped dissolution provided that the fecal pellets stayed intact. Preservation of diatom frustules in fecal pellets is reported from marine and limnic environments, and the peritrophic membrane has been shown to persist for up to 20 days at 5°C (Ferrante & Parker 1977, Honjo & Roman 1978). The results of the present study suggest that the membrane may stay intact for up to 3 months. The state of the membrane as it can be observed in Figure 9b should be viewed and compared cautiously with the images from Honjo & Roman (1978, their figure 1g for example), as the preparation of the material for SEM in the present study did not involve critical point drying which possibly altered the state of the membrane. A more recent laboratory study on the degradation of copepod fecal pellets (Hansen et al. 1996) demonstrated that pellets from a diatom diet were denser, more solid, less colonized by bacteria and degraded an order of magnitude slower than the ones produced on nanoflagellate and dinoflagellate diets. All these results confirm that mesozooplankton grazing on diatoms is an effective means of transport for Si and C to significant depth. Moreover, considering dissolution rates for diatoms of 10 yr^{-1} and settling velocities of 1 m d^{-1} (O. Ragueneau pers. comm.) a free diatom cell has little chance to leave the surface layer unless it is protected from dissolution or subjected to accelerated sinking, both effects being provided by fecal pellets. In that respect, mesozooplankton grazing gains significant importance in driving the surface to sea bed flux of silica.

Conclusion

The influence of the diatom species on the dissolution rate of BSi remains an important but still very little understood factor, lacking a quantitatively palpable material property. The viability of diatoms under physiological stress, prolonged darkness in polar regions for example, might additionally modulate the water column dissolution of opal. Sea ice diatoms dissolved up to one order of magnitude faster than a diatom community that was numerically dominated by the same genera, sampled from an autumn bloom over the shelf on

the western rim of the Antarctic Peninsula. The presence of low numbers of highly silicified species such as *Thalassiothrix antarctica* might be sufficient to alter the rate of solubilization of the shelf bloom community. The comparably high specific dissolution rate for *F. kerguelensis*, a key planktonic organism of the Southern Ocean and important contributor to silica deposits beneath the ACC, is an unexpected result.

Mesozooplankton grazers modify biogenic silica in the pelagic realm by breaking down the diatom frustules and enclosing the fragments in fecal pellets. In this study, grazing by copepods and krill reduced the rate coefficient of dissolving diatoms by a factor of 4 to 26. The initial rate of dissolution is generally unchanged. Rate coefficients are correlated with the amount of freely available BSi when the initially added concentration is corrected for the percentage that has been ingested by the grazers. Disintegration of feces by coprophagy or due to less solid pellets seems to re-enhance the rate of dissolution. Concluding, the rate limiting effect of mesozooplankton grazing activity on the pelagic dissolution of diatoms appears to be the packaging of BSi in fecal pellets leading to a decrease in specific surface area. Numerous authors have speculated on this effect (Kamatani 1982; Nelson et al. 1995; Ragueneau et al. 2000; Tréguer et al. 1989) but to our knowledge we present the first comprehensive study on this issue.

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Table 1:
Details on experimental treatments including origin and quality of diatom material, chl *a* concentrations at the start of the grazing phase, density of grazers, duration of grazing phase, amount of BSi ingested by the grazers and concentrations of BSi, $Si(OH)_4$ and free BSi at the beginning of the dissolution phase. See text for further explanations.

Expt.	Origin of diatom material	Dominant diatom species/genera	Chl *a* µg l^{-1}	Grazers ind l^{-1}	Duration of grazing phase h	BSi ingested %	$c_{initial}$ [BSi] µmol l^{-1}	c_{free} [BSi] µmol l^{-1}	$c_{initial}$ [$Si(OH)_4$] µmol l^{-1}
1	shelf bloom	*Pseudonitzschia* *Chaetoceros*	2.6	control	107	-	5.9	5.9	47.6
				M. gerlachei (2.9)	107	25-29*	5.0	3.6	45.4
				E. superba (1.7)	107	37-53*	5.4	3.0	47.0
2	sea ice	*Chaetoceros* *Pseudonitzschia*	4.1	control	90	-	6.9	6.9	56.7
				C. propinquus (n.d.)	90	70	6.5	1.9	61.7
				E. superba (1.3)	96	70	5.7	1.7	41.0
3	culture	*F. kerguelensis*	2.9	control	90	-	4.4	4.4	56.7
				C. propinquus (n.d.)	90	33-48*	4.5	2.7	59.6
4	culture	*Thalassiosira* sp.	1.9	control	87	-	-	-	42.3
				C. propinquus (3.4)	87	90-96*	-	-	43.0
				C. propinquus (3.4) + *Oithona* sp. (42)	87	86-92*	-	-	40.1

*) higher percentage always calculated from cell counts

Table 2:
Initial dissolution rates, rate coefficients and final percentage of initially added BSi that dissolved over the course of the dissolution experiment.

diatom material	initial rate µmol $Si(OH)_4$ l^{-1} d^{-1} (std)	rate coefficient k x 10^{-3} d^{-1} (SE)	BSi dissolved % (std)
shelf bloom diatoms	0.15 (0.03)	6.3 (1.3) - 13.3 (0.8)	39.7 (7.6)
M. gerlachei grazed	0.15 (0.07)	8.0 (2.6)	50.1 (6.0)
E. superba grazed	0.06 (0.06)	1.6 (0.6)	14.9 (6.4)
sea ice diatoms	0.28 (0.04)	65.8 (6.7)	104.7 (3.4)
C. propinquus grazed	0.08 (0.08)	7.1 (2.9)	47.4 (8.5)
E. superba grazed	0.37 (0.07)	12.3 (2.4)	71.5 (19.7)
F. kerguelensis	0.22 (0.02)	18.4 (7.4)	88.3 (4.8)
C. propinquus grazed	-0.04 (0.05)	0.7 (0.2)	4.9 (12.4)
Thalassiosira sp.	0.03 (0.08)	-	-
C. propinquus grazed	-0.06 (0.01)	-	-
C. propinquus & *Oithona* sp. grazed	0.17 (0.04)	-	-

Figure captions

Figure 1:
Difference in chl *a* concentration of grazed diatom suspensions relative to the control at the end of the grazing phase.

Figure 2:
Change in $Si(OH)_4$ concentration relative to time zero during dissolution of (a) natural and (b) cultured Antarctic diatoms.

Figure 3:
Change in $Si(OH)_4$ concentration relative to time zero during dissolution of shelf bloom diatoms grazed by (a) *Metridia gerlachei* and (b) *Euphausia superba* .

Figure 4:
Change in $Si(OH)_4$ concentration relative to time zero during dissolution of sea ice diatoms grazed by (a) *Calanus propinquus* and (b) *Euphausia superba*. Note the different y-axis scaling compared to Fig. 3

Figure 5:
Change in $Si(OH)_4$ concentration relative to time zero during dissolution of *Fragilariopsis kerguelensis* grazed by *Calanus propinquus* .

Figure 6:
Change in $Si(OH)_4$ concentration relative to time zero during dissolution of *Thalassiosira* sp. control (white triangles), grazed by *Calanus propinquus* (black circles) or a combination of *Calanus propinquus* and *Oithona* sp. (black squares).

Figure 7:
Dissolution of shelf bloom diatoms as a function of time. The linear regression between $\ln((C_0-C)/C_0)$ and incubation time is indicated as estimated for the first five measurements (solid bar) and the complete time course (dashed bar).

Figure 8:

Linear regression (least squares fit; p = 0.05) between the rate coefficient determined in experiment 2 and the ratio of free biogenic silica to initial dissolved silicic acid.

Figure 9:

Scanning electron microscope pictures of diatoms from experiment 2 after 3 months of dissolution. Material typical for the control (plate a), the *Calanus propinquus* treatment (plate b) and the *Euphausia superba* grazed diatoms (plate c, d) are shown.

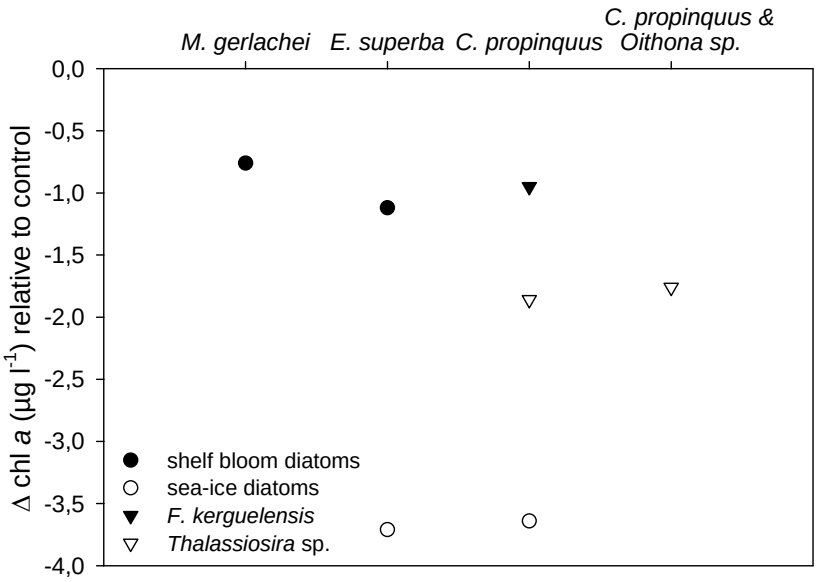

M. gerlachei
E. superba
C. propinquus
C. propinquus & Oithona sp.
0,0
-0,5
-1,0
-1,5
-2,0
-2,5
-3,0
-3,5
-4,0
Δ chl a (µg l-1) relative to control
shelf bloom diatoms
sea-ice diatoms
F. kerguelensis
Thalassiosira sp.

Figure 1

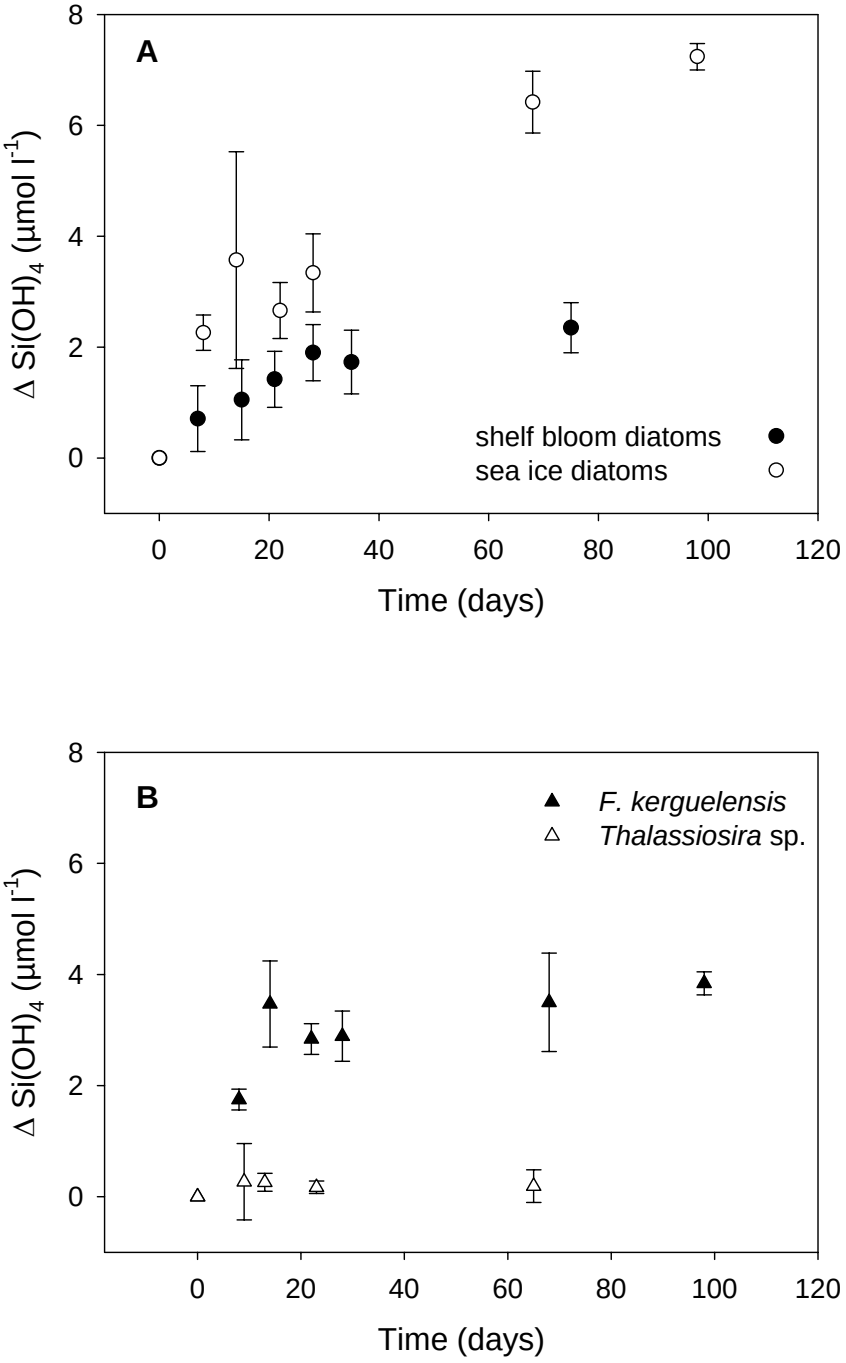

A
B
shelf bloom diatoms
sea ice diatoms
F. kerguelensis
Thalassiosira sp.
Δ Si(OH)4 (µmol l-1)
Time (days)
0
2
4
6
8
0
20
40
60
80
100
120

Figure 2

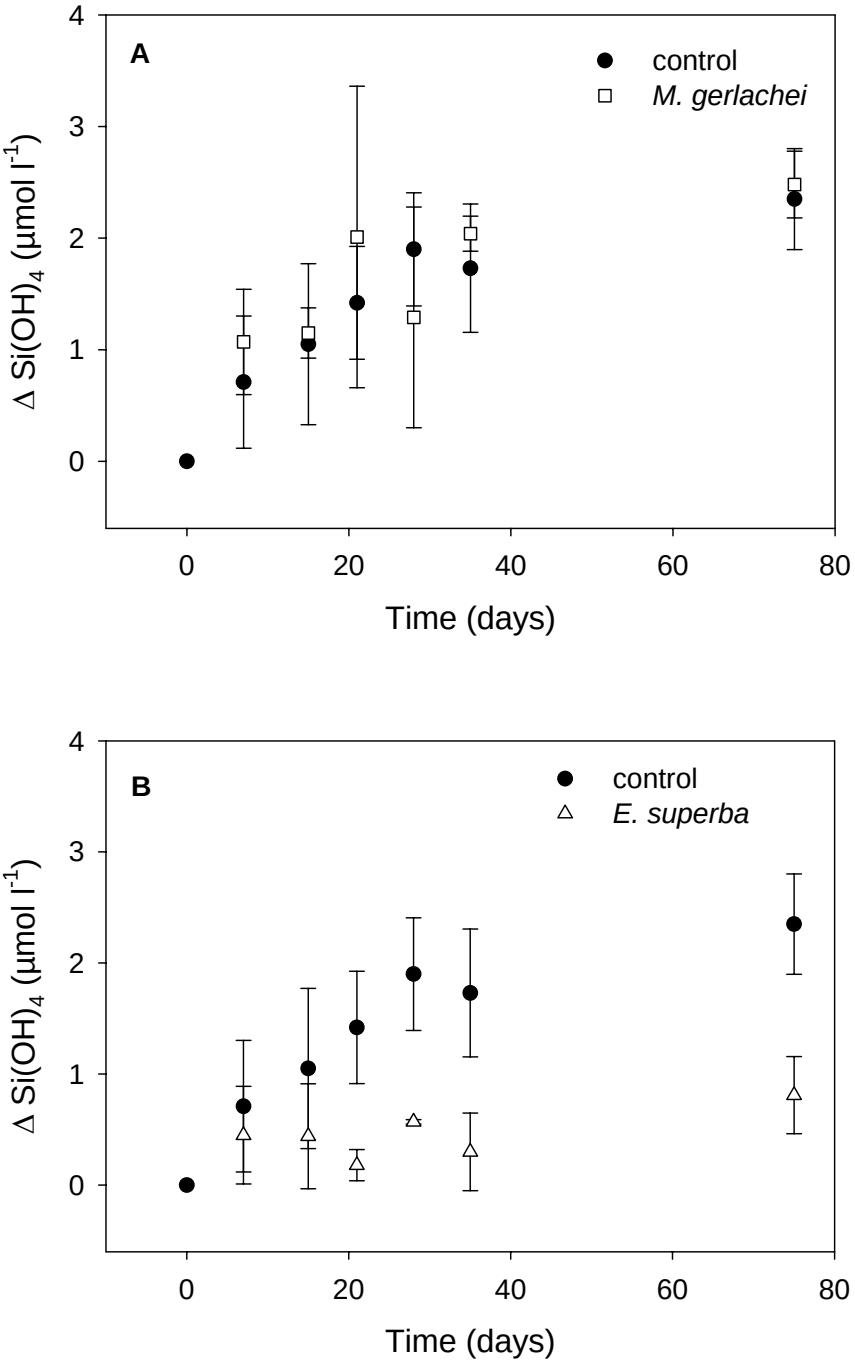

A
control
M. gerlachei
Δ Si(OH)4 (µmol l-1)
Time (days)
B
control
E. superba
Δ Si(OH)4 (µmol l-1)
Time (days)

Figure 3

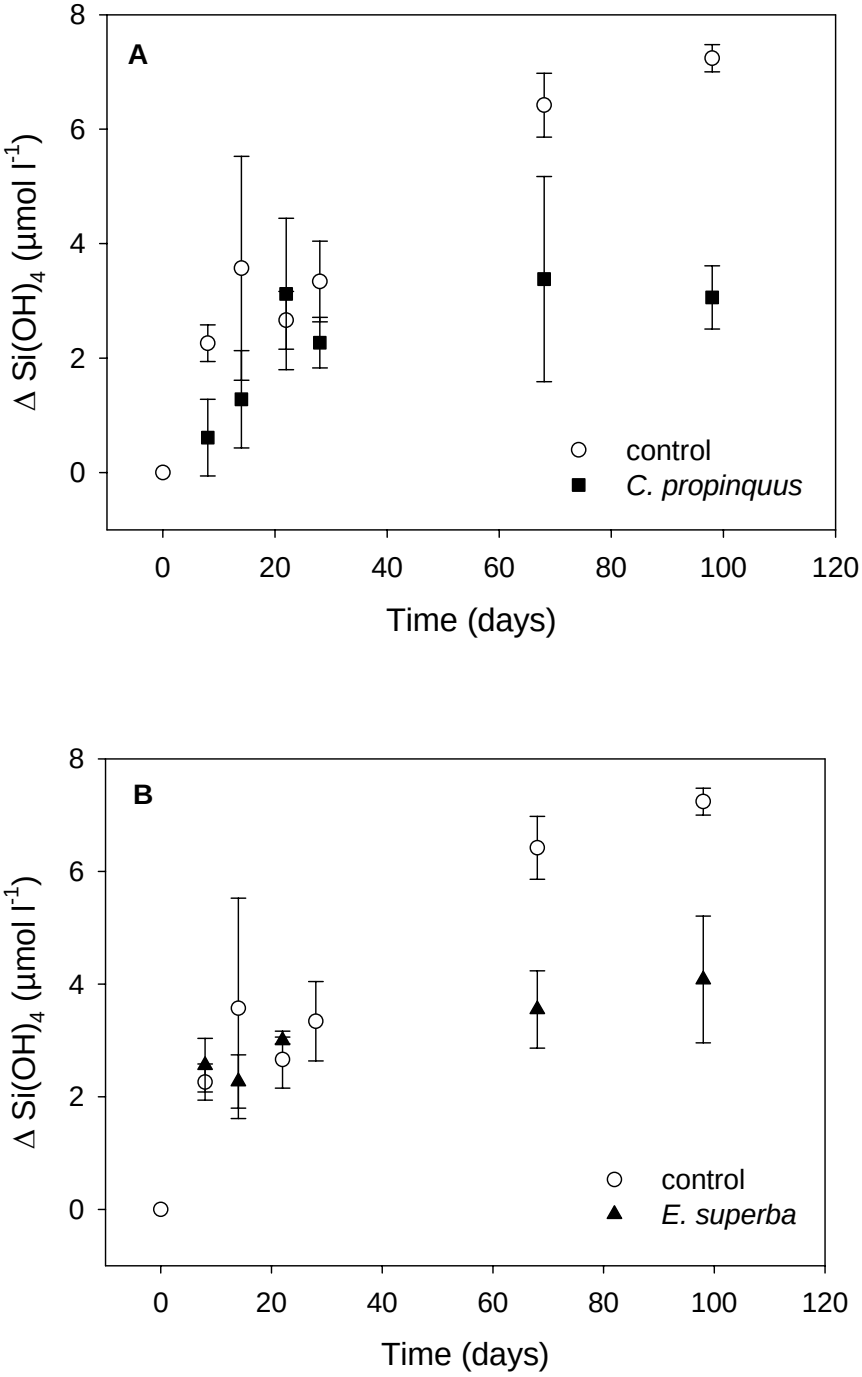

A
B
Δ Si(OH)4 (µmol l-1)
Time (days)
control
C. propinquus
E. superba
0
2
4
6
8
0
20
40
60
80
100
120

Figure 4

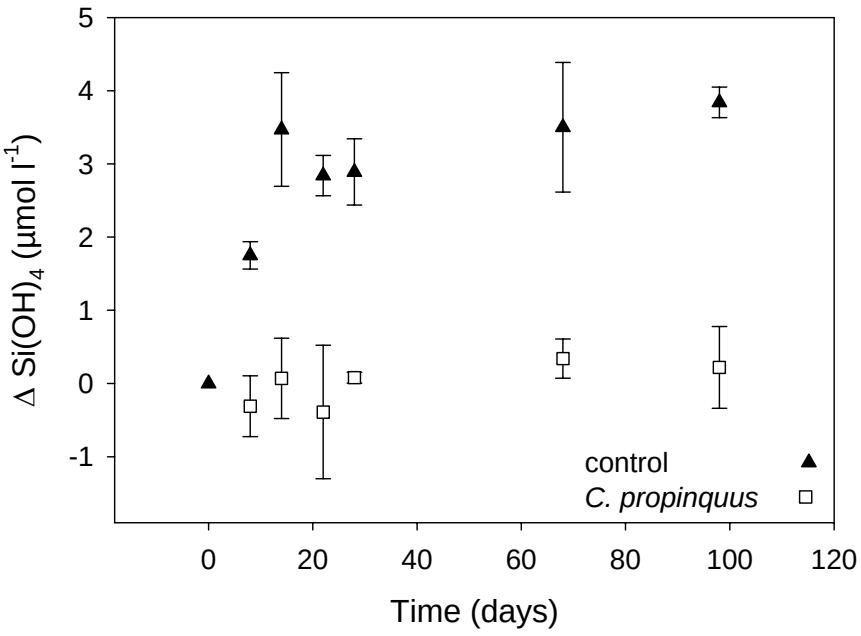

5
4
3
2
1
0
-1
Δ Si(OH)4 (µmol l-1)
0
20
40
60
80
100
120
Time (days)
control
C. propinquus

Figure 5

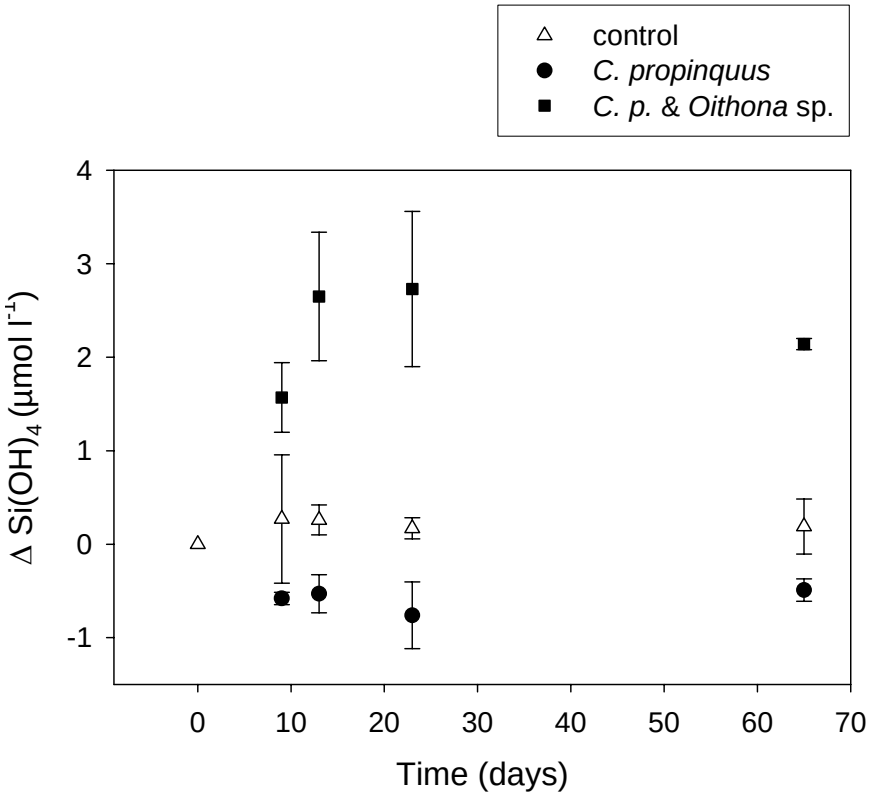

control
C. propinquus
C. p. & Oithona sp.
Δ Si(OH)4 (µmol l-1)
4
3
2
1
0
-1
0
10
20
30
40
50
60
70
Time (days)

Figure 6

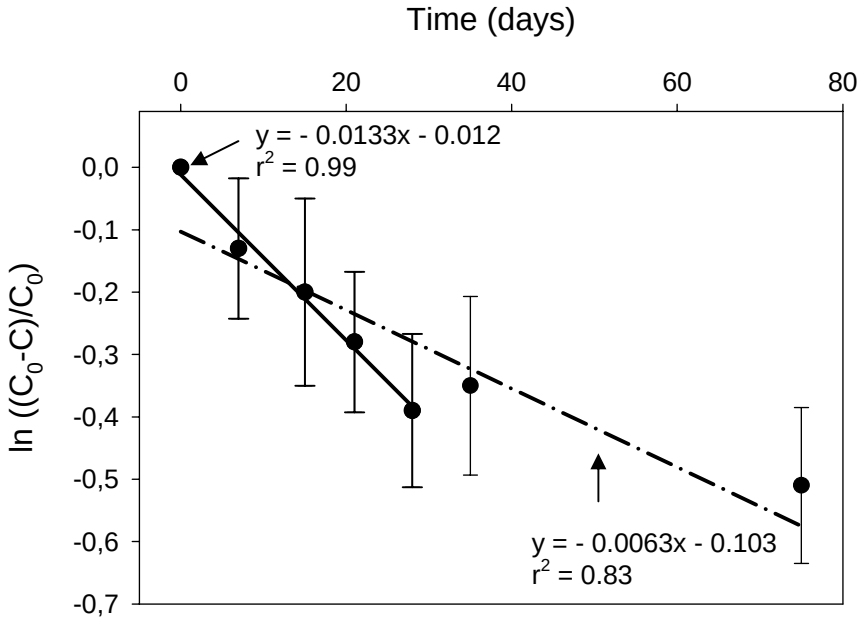

Time (days)
0
20
40
60
80
0,0
-0,1
-0,2
-0,3
-0,4
-0,5
-0,6
-0,7
ln ((C0-C)/C0)
y = - 0.0133x - 0.012
r2 = 0.99
y = - 0.0063x - 0.103
r2 = 0.83

Figure 7

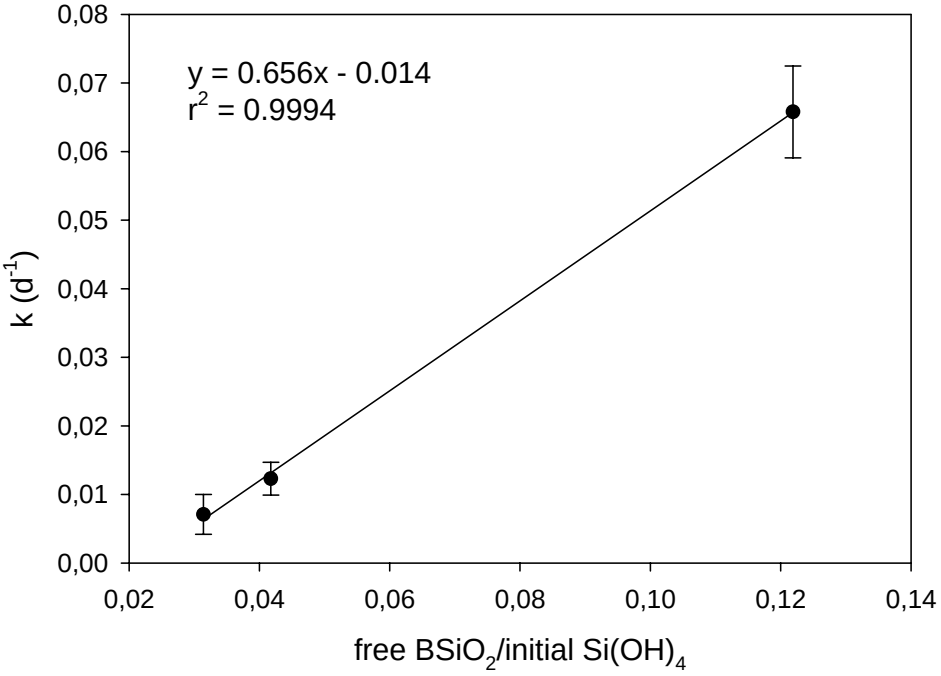
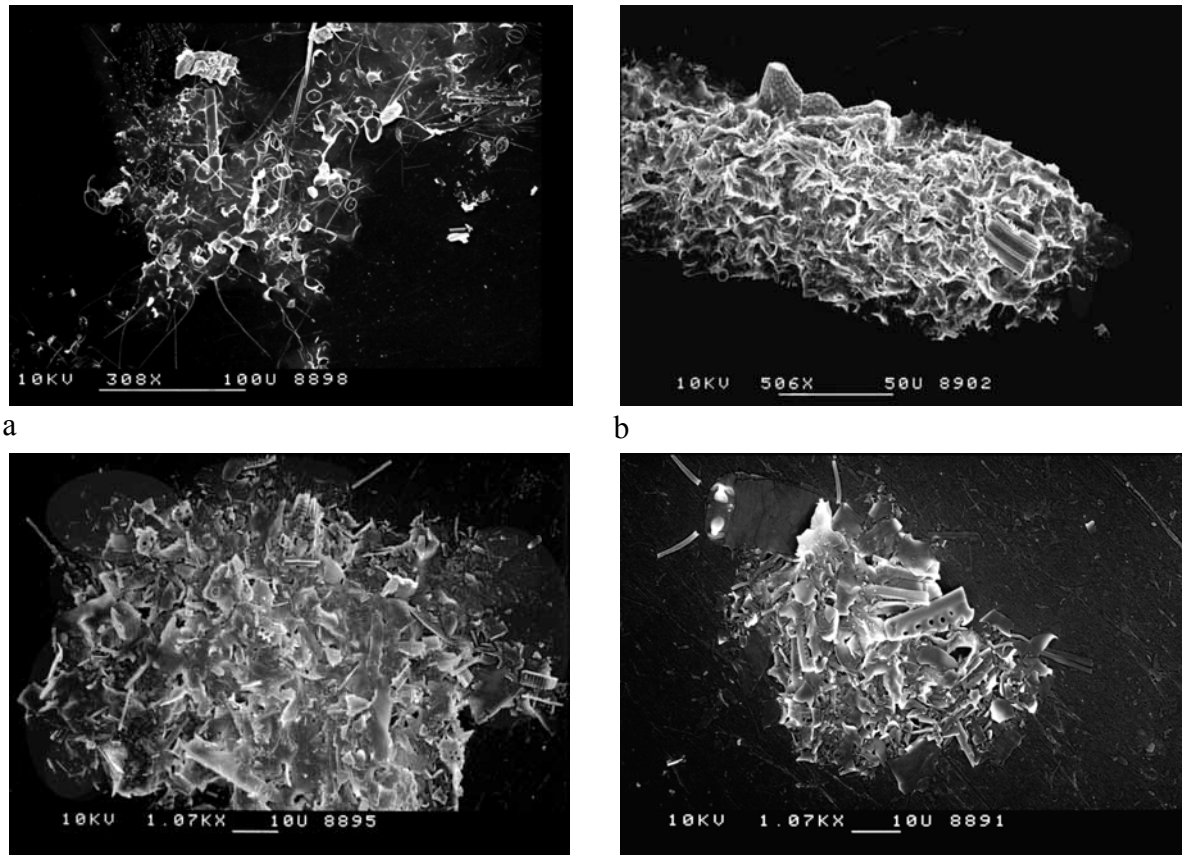

y = 0.656x - 0.014
r2 = 0.9994
k (d-1)
free BSiO2/initial Si(OH)4
0,00
0,01
0,02
0,03
0,04
0,05
0,06
0,07
0,08
0,02
0,04
0,06
0,08
0,10
0,12
0,14

Figure 8



a b

c d

Figure 9

SYNTHESIS

The overall objective of this dissertation was to shed light on the role of metazoan grazers, particularly copepods, in the pelagic Si and C cycles. Subjects wide open to investigation were the identification of mechanisms by which grazing could alter the build-up and flux of BSi, and a general appreciation of whether grazing was responsible for increased recycling or strong export of BSi. Furthermore, an approach on the species level should give insight into the existence of key grazers in a given zooplankton assemblage and whether all members of this assemblage had similar effects on the cycling of Si and C. These broad objectives were investigated in a twofold manner. In a purely experimental approach, the net effect of grazing on the BSi dissolution rate of Antarctic diatom assemblages was studied **(manuscript 3)**. Secondly, during the *in situ* iron-fertilization experiment EisenEx the grazing activity and selectivity of dominant copepods were investigated in response to the iron-induced diatom bloom **(manuscript 1)**. This Lagrangian type experiment offered the possibility to study presumably the same zooplankton assemblage for 21 consecutive days, which by itself has so far not been achieved in the Southern Ocean. Acoustic methods provided information on the fine scales at which copepods aggregated, migrated, and interacted with potential food (Krägefsky unpublished data). Additionally, experimental results on grazing activity could be related to an extensive and detailed description of temporal developments in the microplankton community (Assmy 2004, Henjes 2004). Finally, the parallel use of three classic methods in mesozooplankton research – i.e. incubations, gut fluorescence measurements and O_2-uptake experiments – in the framework of EisenEx incited an inter-calibration of results for the studied copepods **(manuscript 2)** to increase our understanding of the factors that are at the origin of variability and discrepancy observed in the current literature (e.g. Zeldis 2001).

Estimating feeding rates of grazers in the Southern Ocean – how reliably can carbon ingestion be measured with current methods?

Carbon ingestion is classically inferred from bottle incubations with subsequent count of eaten food items and measurement of chl *a* disappearance respectively (Frost 1972), or based on gut fluorescence measurements (Mackas & Bohrer 1976). In order to construct a C budget and to follow C flow from primary to secondary production, ingestion rates are

commonly compared to basic respiratory needs, determined in O_2-uptake experiments, and/or growth estimates, inferred from egg production rates (EPR; e.g. Schnack et al. 1985, Alcaraz et al. 1998, Mayzaud et al. 2002a/b). Frequent reports on daily rations substantially below minimum respiratory requirements emanated from these comparisons (e.g. Atkinson 1994, Atkinson 1996, Mayzaud et al. 2002a) and have led Zeldis (2001) to assert an "enigma of copepod nutrition". This problem is not inherent to the ecosystem of the Southern Ocean. Also in temperate waters, *in situ* estimates of carbon ingestion have been shown to fall short of estimated carbon requirements (e.g. Irigoien et al. 1998). The discrepancy cannot be due to one of the methods. Overall comparison of daily carbon rations derived from incubations and gut fluorescence yields similar estimates and both methods can fall short of required carbon uptake (**manuscript 2**, but see also Kiørboe et al. 1985, Peterson et al. 1990).

Results from this dissertation allow the conclusion that DIFFERENCES IN FEEDING BEHAVIOR OF SOUTHERN OCEAN COPEPODS CAN BE ONE REASON WHY THE EXPERIMENTAL DETERMINATION OF DAILY RATIONS SOMETIMES POINTS TO STRONG FOOD LIMITATION OF COPEPODS. Carbon ingestion of *R. gigas* and the copepods < 2 mm inferred from bottle incubations was far below minimum respiratory needs whereas for *C. simillimus* the same method yielded satisfying values **(manuscript 2)**. The diet of *C. simillimus* was clearly dominated by diatoms, but for *R. gigas* and the small copepods apparently by heterotrophic prey **(manuscript 1)**. Microzooplankton can be of significant importance in the diet of Southern Ocean copepods (Froneman et al. 1996) and hence is justifiably assumed to make up for the missing carbon in some studies that had only used pigment-based methods (e.g. Urban-Rich et al. 2001, Mayzaud et al. 2002a), However, additional carbon rations obtained from predation on ciliates and dinoflagellates were insufficient to cover respiratory losses for the grazers during EisenEx. Correcting for the underestimation of phytoplankton ingestion due to trophic cascading (*sensu* Nejstgaard et al. 2001) still left a large mismatch between respiratory requirements and ingestion for incubations carried out at low phytoplankton concentrations. Gut fluorescence measurements, however, indicated carbon ingestion equivalent to respired amounts via pigmented food throughout the entire study. It was hence concluded that *R. GIGAS* AND THE COPEPODS < 2 MM DREW A LARGE PERCENTAGE OF DIETARY CARBON FROM FEEDING ON DETRITUS BEFORE THE EISENEX BLOOM.

FOR A PRECISE PICTURE OF GRAZING ACTIVITY IT IS NECESSARY TO COMBINE THE PRESENTLY AVAILABLE METHODS. Bottle incubations with a subsequent count of uneaten prey allow quantification of grazing on homogeneously distributed microplankton, both pigmented and un-pigmented. Gut fluorescence measurements give a total estimate of pigmented food

sources including detritus, which in turn cannot be inferred from incubation experiments. Converting gut pigment to detrital carbon is challenging. It requires knowledge on the type of detritus and its pigment to carbon ratio. Both, carbon and chl *a* content of feces for example are highly variable (Bathmann & Liebezeit 1986, Riebesell et al. 1995, Gowing et al. 2001) and would require concurrent measurement of both parameters for a reliable estimate of carbon ingestion. The attempt to calculate grazing pressure on fecal pellet standing stock via the comparison of incubation method and gut fluorescence (**manuscript 2**) has therefore only theoretical value. The simple deduction, however, that a grazer has been feeding on detritus is important considering the role of grazing in modulating particle flux.

Average carbon ingestion from autotrophic and heterotrophic sources was in agreement with respiratory requirements based on O_2-uptake **(manuscript 2)**. This, however, does not necessarily indicate that ingestion rates were sufficient for secondary production, i.e. growth or egg production, to take place. Mayzaud et al. (2002a) demonstrated that ingestion concurred with respiration but could not explain the observed EPR. Based on maximum ingestion rates from EisenEx, assuming an assimilation efficiency of 70 % and deducting the respiratory needs, 6.2 and 7.7 µg C ind^{-1} d^{-1} would have been available for egg production of *C. simillimus* and *R. gigas* respectively (calculation based on estimates presented in manuscript 2, Table 3). Average EPRs for these species are 15.5 and 8.9 eggs $female^{-1}$ d^{-1} (Ward & Shreeve 1995) equivalent to 7.8 and 8 µg C ind^{-1} d^{-1} (µg C egg^{-1}: *C. simillimus* ~ 0.5, *R. gigas* ~ 0.9; Mayzaud et al. 2002a). Ingestion rates estimated during EisenEx would therefore be sufficient to fuel observed rates of reproduction which are linked to food availability for *C. simillimus* and *R. gigas* (Ward & Shreeve 1995). The fact that only maximum estimates of carbon ingestion provide sufficient energy for reproduction appears less dramatic in the light of recent findings that reproductive processes in copepods rely also on the feeding history (Rey-Rassat et al. 2002), i.e. previously stored energy for example in the form of lipids. Antarctic zooplankton indeed establishes extensive lipid stores (Hagen 1988). Shreeve et al. (2002) actually report that population growth of *R. gigas* is related to past productivity levels of the system. Mass specific growth rates of this species decrease from 0.06 to 0.04 d^{-1} for copepodite stages CI to CIII (Shreeve et al. 2002) and the authors conclude that measured growth rates concur with developmental times and life cycle duration derived from cohort analyses. Assuming excess carbon ingestion of 7.7 µg C ind-1 d^{-1} (see above), mass specific "growth" of 0.03 d^{-1} can be estimated for *R. gigas* CVI♀ during EisenEx. Considering also previously published daily carbon rations and the compliance with *in situ* measurements of growth and egg production, IT IS CONCLUDED THAT PRESENTLY

AVAILABLE METHODS IN MESOZOOPLANKTON RESEARCH YIELD REALISTIC ESTIMATES OF CARBON INGESTION.

On the biogeochemical significance of copepod grazing

Two dominant copepod species of the ACC and the size class of copepods < 2 mm were in the focus of my investigation. In every respect, interpretation and discussion of the results for the size class of small copepods was hampered by the fact that it represented a mixture of organisms. The approach to regroup the mesozooplankton community into experimentally amenable size classes is a relict from the purely budget oriented studies of the JGOFS decade but has not proven fruitful in addressing the objectives of this dissertation. The most valuable piece of information that emanated from the experiments with the copepods < 2 mm is the fact that they can tap the nanoflagellate carbon pool **(manuscript 1)**. This efficiently partitions resources among copepod grazers and places the small metazoans in a connective position between the microbial loop and the phytoplankton-herbivore food chain. In the context of the current research effort in biological oceanography, for example the "Integrated Marine Biogeochemistry and Ecosystem Research (IMBER)", the identification of key species and their feeding behavior has regained importance. One of the major findings of this dissertation is a difference in feeding behavior between *C. simillimus* and *R. gigas* which determines, as shall be seen below, their role and importance in pelagic biogeochemistry. This reconfirms the validity of the IMBER approach and the necessity to work on the species level.

Introductory it had been elaborated that grazing effects on the export of particulate material could be twofold and antagonistic. Feeding on the phytoplankton standing stock enhances particle flux via fecal pellet production. Coprophagy or detritivory reduces the downward transport of biogenic material. THE FEEDING ACTIVITY OF *C. SIMILLIMUS* IS CLEARLY CONDUCIVE TO ENHANCE EXPORT OF PRIMARY PRODUCED CARBON AND SILICON. The organism dominated the copepod community in the top 150 m of the water column and fulfilled its metabolic demand entirely from grazing on diatoms before and during the bloom **(manuscript 1)**. In general, this copepod has an important impact on diatom communities in the PFZ with grazing impact by this single species reaching 25 % of the phytoplankton standing stock per day (Perissinotto 1992, Froneman et al. 2000). The high abundance and strong grazing pressure on diatoms even at low chl *a* concentration noted during EisenEx

reconfirm the ecological and biogeochemical significance of this copepod in the ecosystem of the ACC.

IN THE HNLC-STATE OF THE PELAGIC ECOSYSTEM, *R. GIGAS* IS THE COMPLEMENTARY TO *C. SIMILLIMUS*. Opportunistic feeding on fresh phytoplankton, microzooplankton and detritus is reported for *R. gigas* (Atkinson 1998) and is reflected in its lipid composition (Graeve et al. 1994) and in the morphology of its mandibles (J. Michels pers. comm.). Assuming a respiratory requirement of 6.5 % body C d^{-1} and a maximum ingestion of 1.2 % body C d^{-1} via diatoms and microzooplankton in the pre-bloom an out-patch experiments **(manuscript 1+2)**, *R. gigas* needs to cover approximately 82 % of its respiratory carbon demand by feeding on detritus, equivalent to 13 µg C ind^{-1} d^{-1}. Based on average ingestion rates and 70 % assimilation efficiency, *C. simillimus* egests 2 µg C ind^{-1} d^{-1} so the grazing activity of one *R. gigas* would be sufficient to balance the fecal carbon flux of six *C. simillimus*. Peak abundances of both grazers are generally found in the PFZ (Pakhomov et al. 2000). Based on the available abundance estimates, the average ratio of *R. gigas/C. simillimus* during EisenEx varied from 1:2 (Bongo; upper 100 m to 350 m) to 1:11 (MN; 150 m integrated). The range arises from the apparently more homogeneous distribution of *R. gigas* over the upper 500 m water column compared to *C. simillimus* which concentrates its population in the mixed layer. Also Atkinson et al. (1992b) encountered *R. gigas* grazing below the chl *a* maximum and the major part of the grazer community. Although very speculative, IT IS PROPOSED THAT *C. SIMILLIMUS* AND *R. GIGAS* REPRESENT THE TWO ECOLOGICAL COUNTERPARTS OF A "COPEPOD-RETENTION-SYSTEM" that minimizes loss of biogenic carbon and associated elements from the surface-layer (*sensu* Smetacek et al. 1990). Further research is necessary to holster this perception and to identify other "simillimus-type" and "gigas-type" grazers within the prevailing copepod assemblage. The small cyclopoid copepod *Oithona* sp. for example is thought to intercept much of the fecal pellet flux out of the surface layer (González & Smetacek 1994) and would hence be considered a "gigas-type".

The sub-arctic Pacific Ocean resembles in many ways the ecosystem of the ice-free Southern Ocean. Likewise an HNLC system, phytoplankton standing stock in the sub-arctic Pacific is continuously low and the community is dominated by small cells. For a long time, the absence of substantial blooms was thought to be due to grazing control on phytoplankton communities (see Miller 1993 and references therein) but iron limitation has been shown to be of greatest importance (Dagg 1993a, Boyd et al. 1999, Tsuda et al. 2003, Boyd et al. 2004). Within the stable system of the sub-arctic gyre, copepod biomass is dominated by three species of the genus *Neocalanus,* which feed efficiently on the low phytoplankton standing

stock and microzooplankton (Frost et al. 1983, Gifford 1993b). The habitat is vertically partitioned with *N. plumchrus* and *N. flemingeri* occupying the surface layer and *N. cristatus* together with *Eucalanus bungii* dwelling at the bottom of the mixed layer (Mackas et al. 1993). *N. plumchrus* is categorized as an omnivorous suspension feeder with high efficiency to clear diatoms even at low phytoplankton concentrations (Frost et al. 1983). *N. cristatus* is described as an omnivorous grazer with tendency to predatory feeding on microzooplankton (Frost et al. 1983). Both descriptions align well with the appraisal on the feeding behavior of *C. simillimus* and *R. gigas* in this study **(manuscript 1)**. Dagg (1993b) reports that the deep living *N. cristatus* feeds on sinking aggregates. This feeding behavior resulted in a similar discrepancy of ingestion estimates from gut fluorescence and incubation methods (Dagg 1993a), and first stimulated the comparison of *R. gigas* and *N. cristatus* (**manuscript 2;** see also above). As it appears, *R. gigas* occupies a similar niche in the ACC as *N. cristatus* in sub-arctic Pacific gyre. Both are encountered at greater depth compared to other species and feed on detritus sinking out of the surface layer. Therefore, both species can be placed in the group of flux-feeders that play an important role in reducing particle flux out of the mixed layer (Jackson 1993). Near the surface the grazing activity of *N. plumchrus* has a similar potential to impact microplankton communities (Landry & Lehner-Fournier 1988) as is hypothesized for *C. simillimus* during EisenEx **(manuscript 1,** see also below). IT IS THEREFORE PROPOSED THAT THE *C. SIMILLIMUS – R. GIGAS* CONSTELLATION SHOULD BE SEEN AS THE ACC ANALOGUE TO THE *N.PLUMCHRUS/FLEMINGERI – N. CRISTATUS* SYSTEM IN THE SUB-ARCTIC PACIFIC GYRE.

In the ACC, phytoplankton blooms are frequently observed despite high grazer abundances (e.g. Smetacek et al. 1997). This difference to the ecosystem of the sub-arctic Pacific is probably rooted in the physical particularities of the ACC (Strass et al. 2002). The high grazing impact of *C. simillimus* is not capable of preventing the development of phytoplankton blooms once cells are relieved of iron limitation. In relative numbers, diatom mortality due to copepod grazing decreases with progression of the bloom. The grazing selectivity on large diatoms, however, is speculated to play a role in shaping the composition of the pre-bloom microplankton community, and in the differential accumulation patterns (Assmy 2004) observed for various diatom species following iron-fertilization **(manuscript 1)**. A conclusive link between population dynamics of ciliates and heterotrophic dinoflagellates (hdinos) with copepod grazing could not be established. This can be partly due to the fact that grazing mortality, i.e. clearance rates, for these prey organisms was determined globally, without taking into consideration size classes or morphotypes such as loricate or aloricate tintinnids. Also in this respect, it appears therefore more fruitful to work on the

species level. Grazing pressure on ciliates is generally higher than on hdinos which could reflect an influence of size or motility. With increased availability of diatoms all copepods additionally release grazing pressure on hdinos concomitant with an increase in diatom clearance. Such prey switching behavior is not only nutritionally advantageous for grazers (Kiørboe et al. 1996) but favors as well the stability of the ecosystem (Gismervik & Andersen 1997). For *R. gigas* and the copepods < 2 mm the increase in diatom clearance is presumably accompanied with a decrease in ingestion of detritus. Hence, WITH RESPECT TO THE BIOGEOCHEMICAL CYCLING OF C AND SI PREY SWITCHING "SWITCHES" THE "COPEPOD-RETENTION-SYSTEM" TO A "COPEPOD-EXPORT-SYSTEM".

IN THE BLOOM SITUATION, ALL COPEPODS INCEASED INGESTION OF DIATOMS AND THUS CONTRIBUTED TO THE EXPORT OF PARTICULATE C AND SI. Especially *R. gigas* showed a disproportionate increase of its feeding rate on diatoms with development of the bloom **(manuscript 1)**, which has also been shown for *N. cristatus* (Frost et al. 1983). Grazing activity of *R. gigas* in the HNLC-state was sufficient to balance detrital flux produced by *C. simillimus*, making it a key organism to be taken into account in ecosystem modeling. In contrast, the low abundance of *R. gigas* results in an only minor grazing impact on the phytoplankton standing stock compared to *C. simillimus*. Also the copepods < 2 mm, that are an order of magnitude more abundant than the large grazers, cleared the newly growing diatoms with greater efficiency **(manuscript 1)**. Eventually, this generalized change in feeding preference leads to a complete disintegration of the coprophagous filter (González & Smetacek 1994) that is put in place by the "gigas-type" grazers in the HNLC-state. Potentially, the sudden increase in export of carbon and silicon observed during a spring bloom near the APF (Rutgers van der Loeff et al. 1997, see also Rutgers von der Loeff et al. 2002) is a reflection of the changes in the "gearing" of the copepod community.

Concluding, it can be said that THE BIOGEOCHEMICAL ROLE OF COPEPODS DEPENDS ON THE STATE OF THE PELAGIC ECOSYSTEM. In the HNLC-state, habitat and resource partitioning of dominant copepod grazers leads to efficient retention of organic material in the surface layer. In the bloom-situation, the copepod community will enhance particle flux out of the surface layer altogether. Considering the Si cycle in particular, the COPEPOD-EXPORT-SYSTEM and the disappearance of the coprophagous filter most certainly accelerate BSi-flux out of the surface layer via fecal pellet sedimentation. The COPEPOD-RETENTION-SYSTEM will potentially have a stronger decoupling effect on the C and Si cycle due to the selective digestion of carbon over silicon (Cowie & Hedges 1996). The Si:C ratio of particulate material will increase with the successive ingestion of first, the diatoms and subsequently the detritus.

Although organic material is retained that way, the quantity of opal leaving the surface layer in theory remains unchanged and might proceed even faster as particle settling rates are correlated with the loss of organic carbon (Berelson 2002). However, a decisive factor that needs to be taken into account in that respect is whether and how the rate at which BSi dissolves is also altered by grazing.

The influence of grazing on the preservation of opal in the water column

As for the export of particulate material, the effect of grazing on the dissolution of opal can be twofold. Destruction of the diatom frustule during feeding can enhance the dissolution rate of BSi, enclosure of intact cells and debris into compact feces can reduce it. Results of the experimental study presented in **manuscript 3** lead to the conclusion that both effects do occur and that again feeding behavior of copepods decides the net effect.

ZOOPLANKTON GRAZING CAN EFFECTIVELY REDUCE THE SPECIFIC DISSOLUTION RATE OF A DIATOM ASSEMBLAGE. Rate coefficients for grazed assemblages are a factor of 4 to 26 lower and are correlated with the amount of BSi that has been ingested by the grazers and thus enclosed in fecal pellets. *Per se*, the peritrophic membrane of fecal pellets therefore prevents diatoms from dissolution. This has generally been assumed (Schrader 1971, Ferrante & Parker 1977; see also Kamatani 1982, Tréguer et al. 1989, Nelson et al. 1995, Ragueneau et al. 2000) but previously not been demonstrated in a comprehensive manner. The initial rate of dissolution of natural diatom communities is generally not affected through grazing. Additionally, the differences in sinking velocity, that have not been able to act in the batch incubations used in this study, will vertically separate the BSi enclosed in feces from the free, still intact cells. Hence, no immediate effect of grazing on the recycling of Si in the surface layer can be expected, unless feces are mechanically disrupted in the upper water column.

COPROPHAGOUS FEEDING POTENTIALLY INCREASES THE DISSOLUTION RATE OF BSI. This conclusion can unfortunately only be drawn with caution as the experimental set-up did not provide conclusive evidence. The combined grazing treatment with *Calanus propinquus* and *Oithona* sp. showed remarkably higher dissolution of BSi than the control and *C. propinquus* treatment **(manuscript 3).** Proof that *Oithona* sp. actually did feed on the fecal pellets produced by *C. propinquus* is not available. Nevertheless, the results indicate that in the HNLC-state not only carbon is retained in the upper water column but also silicon, which would counteract the potentially strong decoupling of the C and Si cycles proposed above. Depending on the depth at which fecal pellets are fragmented (coprorhexy), disrupted

(coprochaly) and ingested (coprophagy) by other mesozooplankton, the recycled $Si(OH)_4$ is available again for diatom production. In the current nutrient state of the ice-free Southern Ocean, this would be of greatest importance for the waters north of the Polar Front that are the only ones prone to Si limitation (Laubscher et al. 1993). Eventually, BSi dissolution in the upper 400 m of the water column slightly alleviates the strong Si deficit of the Sub-Antarctic Mode Water that originates in the ACC and supplies nutrients to lower latitudes (SAMW; Sarmiento et al. 2004).

Dubischar & Bathmann (2002) concluded that "sedimentation of biogenic material in the Polar Frontal region as well as in the ACC probably does not occur via copepod fecal pellets but during special events such as the occurrence of larger swarms of krill or salps". Substantial export of feces is indeed associated with areas near the Antarctic Peninsula and the marginal ice zone, where krill is abundant (Dubischar & Bathmann 2002). González (1992), however, reports that krill feces dominated fecal material in the top 50 m of the water column but was retained and probably recycled in the upper 150 m. The author attributes these observations either to coprophagy on krill feces or to mechanical disintegration of the frail fecal strings. FECAL STRINGS OF *EUPHAUSIA SUPERBA* ARE LESS EFFICIENT IN PREVENTING BSI DISSOLUTION THAN FECAL PELLETS OF *C. PROPINQUUS* **(manuscript 3)**. Observations made with the scanning electron microscope revealed that krill fecal strings broke into small pieces leaving behind "feces crumbs" of diatom debris which apparently facilitated dissolution. Copepod fecal pellets, however, remained completely intact for up to three months. The faster dissolution rate of BSi from krill feces is probably compensated by its higher sinking speed compared to copepod pellets. THE RELATIVE IMPORTANCE OF KRILL OR COPEPOD FECES FOR EXPORT IS THEREFORE NOT SO MUCH A QUESTION OF FECES QUALITY BUT ON THE INTERACTIONS WITHIN THE PELAGIC FOOD WEB.

The loss of the coprophagous filter in bloom situations (see above) will also allow the smaller and slower copepod fecal pellets to sediment out of the surface layer. The combined grazing impact of the entire copepod community during EisenEx was approximately 20 % of the diatom or BSi standing stock per day (S. Krägefsky pers. comm.). Contrary to the view of Dubischar & Bathmann (2002), it is proposed that also COPEPOD GRAZING CAN BE RESPONSIBLE FOR THE EXPORT OF AN IMPORTANT FRACTION OF BSI IN THE ACC. Published sinking speeds (e.g. Honjo & Roman 1978) of copepod fecal pellets are certainly high enough to transport the biogenic material below the depth of the winter mixed layer, which is approximately at 200 m in the ACC (Park et al. 1998). Depending on the final export depth, the exported Si will be unavailable for diatom production at the surface for increasingly

longer periods of time. Assuming a sinking rate of 100 m d^{-1}, the observed durability of copepod pellets of three months is in theory sufficient to reach the ocean floor beneath the ACC. So far, no study has addressed the solubility of fecal pellets with geochemical methods in order to be integrated in the investigations on processes taking place at the sediment-water-interface and in the sediment itself. On geological time-scales however, fecal pellets can be entirely preserved in the sediment, especially under anoxic conditions (see Turner 2002 and references therein).

Does copepod grazing really matter? - Future perspectives for zooplankton research

If copepod grazing really plays a noteworthy role in the biogeochemical cycling of C and Si it remains elusive. This appreciation is certainly not a satisfying one and should preferably not precede the final considerations of a dissertation on this subject. An all-embracing review on the role of zooplankton fecal pellets, marine snow and sinking phytoplankton blooms (Turner 2002) in vertical flux and water column recycling ends on the very same note. Synthesizing the results of over 500 publications, Turner leaves the reader with an excellent impression of the high variability of processes that govern the relative contribution of the three components that appear to be "situation specific and dependent on multiple interacting factors".

Although marine snow and phytodetritus are increasingly recognized to dominate vertical flux in the ocean (Wassmann 1998), zooplankton fecal pellet contribution to total particulate organic carbon flux varies from "insignificant" to "almost all" (see Turner 2002, Table 3). The share of copepod fecal pellets in this zooplankton mediated flux depends on the importance of other grazers in the same system: protozooplankton, euphausids, tunicates and fish. Therefore, a first step in answering the above question is to assess the composition of the zooplankton community on temporal and spatial scales, whereas the latter includes important vertical components. Especially information on the meso- and bathypelagic zone is scarce. A reoccurring reason for the variability of flux and recycling in Turner's review is the structure of the pelagic community and the trophic position of consumers. This includes changes in feeding behavior. Feeding on detritus in general and on zooplankton fecal material in particular seems to be of crucial importance. Investigation of its temporal and spatial occurrence should be routinely included in zooplankton grazing studies. Coprophagy is an important ecological process that is difficult to quantify in the pelagic ecosystem. The only

way to identify feeding on fecal pellets or detritus is through a combination of classical grazing methods and microscopic observations.

At that point the answer to whether copepods really matter must be “It depends...“. It depends in part on the just mentioned factors as results of this dissertation have demonstrated. So how can we make the role of grazers in biogeochemical cycling of elements less elusive and more tangible? With another 500 studies on flux, derived from sediment traps? With more bottle incubations and gut fluorescence measurements to study copepod grazing? Of course answering “no” to these questions would be heresy. I would like to give an account of my personal learning experience as one possible answer:

The turning point in perceiving and finally making sense out of my data came with the discovery of the apparently different feeding behavior of *Rhincalanus gigas*. Until then this grazer had only marginally caught my interest. Experimental results for it were blurred. Ingestion rates were too low - (too low for what?). All seemed to concur with the deception in the face of zooplanktologists when it dominates the catch, followed by the comment “it doesn’t feed”. This of course is a snap judgment, and a final one considering mitigation of elemental fluxes. *Calanus simillimus* was the diatom glutton, the relevant organism. The end is known: *R. gigas* does feed and is important. Not more or less important than *C. simillimus* but important. Elucidating hints came from the comparison of several methods, from reports on similar discoveries of grazers in other HNLC-systems, from personal observations of peculiar behavior of the copepod when sorting it or maintaining it in the ship’s laboratory. The point I wish to make is a) most of the evidence was of indirect nature and b) the apparently “strange” behavior of a species had provided the key. Almost 20 years ago, William Hamner held a keynote address at the International Symposium on Marine Plankton on “The Importance of Ethology for Investigations of Marine Zooplankton” (Hamner 1985). I very much recommend reading the written transcript of his talk. He criticizes the complete lack of behavioral studies in biological oceanography. A situation that has barely changed and that is certainly due to the transient nature of pelagic ecosystems. Immense technological difficulties have to be overcome to observe a copepod in the wild. Therefore, “ethological information for pelagic animals will (…) accumulate slowly”, or indirectly, as seen in the results of this dissertation. High speed cinematography for example has profoundly changed our perception of copepod feeding (e.g. Price & Paffenhöfer 1986). The development and calibration of new methods, for example acoustics to study zooplankton distribution in the water column, is only one part of the effort that needs to be made. The autecology of the single organism must be investigated. Once it is known how fast it grows and how long it

lives, we will know how much it needs to ingest. If we know where in the pelagic ecosystem it lives in relation to potential prey but also other zooplankton we can tell what it feeds on. In a world with nowhere to hide, survival is more important than feeding and the way a copepod feeds most certainly is related to its strategy to avoid predators. It can be easily envisaged that *R. gigas* floats so still and motionless in the water column not only to pounce on prey, i.e. to ambush feed, but also to perceive when it will be pounced on itself. Its glassy look is possibly a camouflage.

The final statement of Hamner is harsh: "Biological Oceanography cannot continue to dismiss the central importance of behavior and evolution in its attempts to describe oceanic communities or it will continue to fall even further behind as a contemporary discipline in the field of modern biology". Since the talk of Hamner, a large amount of investigations on zooplankton in all systems has accumulated. Although still "primarily concerned with quantitative aspects of feeding ecology" – we still lack the methods for more – continued integration and cross-comparison of results among systems has the potential to improve our understanding also with respect to behavior and life cycle strategies of the grazers. The Southern Ocean is difficult to sample whereas "Ocean Station Papa" in the sub-arctic Pacific is more accessible, hence the greater knowledge on the genus *Neocalanus* for example. The effort to condense the existing knowledge from all systems in terms of analogies and with the principles of behavioral ecology in mind goes beyond the scope of this dissertation but the approach has proven fruitful in assessing the role of *R. gigas* and *C. simillimus* in the biogeochemical cycling of elements.

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