CARBON TURNOVER IN SINKING PARTICLES IN THE MARINE ENVIRONMENT

Dissertation

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Morten Hvitfeldt Iversen

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LIST OF PAPERS

This thesis is based on the following papers. In the text they will be referred to by roman numerals.

Paper I: Iversen M. H., Poulsen L. K. (2007) Coprorhexy, coprophagy, and coprochaly in the copepods *Calanus helgolandicus*, *Pseudocalanus elongatus*, and *Oithona similis*. Mar. Ecol. Prog. Ser. **350**:79-89

Content: This paper investigated fecal pellet feeding behavior by *Calanus helgolandicus*, *Pseudocalanus elongatus*, and *Oithona similis* through grazing experiments and by visual observation of adult females. The importance of an alternative food source for pellet clearance rate was also investigated.

Conclusions: *O. similis* did not seem to view fecal pellets as suitable food items. Coprorhexy was the main feeding behavior on fecal pellets by the calanoid copepods. No support for intensive feeding on fecal pellets by copepods was found, and, thus, other organisms seem important for the high fecal pellet degradation in the upper ocean.

These experiments were performed during my master period. During the Ph.D. periods the samples were re-counted, data analyzed, and scientific paper written and submitted.

Paper II: Poulsen L. K., Iversen M. H. (2008) Degradation of copepod fecal pellets: key role of protozooplankton. Mar. Ecol. Prog. Ser. **367**:1-13

Content: This paper investigated the pellet degradation from different size fractions of a plankton community from Øresund (Denmark) throughout a year. The size fractions consisted of a non-fractionated (total community), and five additionally size fractions (<0.2 µm, <2 µm, <20 µm, <100 µm, and <200µm). Each size fraction was incubated in triplicates with a know amount of added fecal pellets, to identify which size fraction contained the major pellet degraders.

Conclusions: Large heterotrophic dinoflagellates seem to have a very important role in the degradation of fecal pellets, and may form a ´protozoan filter´ which can retain the fecal pellets in the upper ocean. Copepods mainly played an indirect role in the pellet degradation, either via grazing on the protozooplankton organisms or via fragmentation of the fecal pellets.

These experiments were performed during my master period. However, zooplankton samples were re-counted during the Ph.D. period. No phytoplankton and protozooplankton samples

were counted during the master work, and those data were not counted until the Ph.D. period. Since the main pellet degraders were found within the protozooplankton organisms a significant change in conclusions was made before the paper was written and submitted during the Ph.D. period.

Paper III: Ploug H., Iversen M. H., Koski M., Buitenhuis E. T. (2008) Production, oxygen respiration rates, and sinking velocity of copepod fecal pellets: Direct measurements of ballasting by opal and calcite. Limnol. Oceanogr. **53**(2):469-476

Content: This paper presents information on the sinking rates and loss of carbon from copepod fecal pellets as a function of food type. Investigations were performed on pellets without biominerals (produced on flagellates) and on pellets containing biominerals (produced on diatoms or coccolithophorids). The feeding rates and pellet production rates of the copepod *Temora longicornis* were investigated for the different diet types. The sinking speeds were measured in a settling column and respiration rates were calculated from small scale oxygen fluxes to the pellets measured with O_2 microelectrodes.

Conclusions: Freshly produced fecal pellets containing ballast minerals had increased sinking speeds compared to non-ballasted pellets. Biominerals did not seem able to protect the freshly produced pellets from decomposition. Carbon preservation was estimated to be 10-fold higher in fecal pellets ballasted by biominerals compared to pellet without biominerals.

Paper IV: Ploug H., Iversen M. H., Fischer G. (2008) Ballast, sinking velocity, and apparent diffusivity within marine snow and zooplankton fecal pellets: Implications for substrate turnover by attached bacteria. Limnol. Oceanogr. **53**(5):1878-1886

Content: This paper investigated the hypothesis that ballast minerals in aggregates promote organic matter export. Coccolithophorid and diatom aggregates were produced in roller tanks and fecal pellets were collected from sediment traps or produced by *Temora longicornis* feeding on flagellates, diatoms, or coccolithophorids. Apparent diffusivity was measured by injecting hydrogen into aggregates and pellets and observing the diffusion out. The oxygen diffusivity was calculated from measurements of oxygen gradients to the aggregates and apparent diffusivity inside the aggregates. Volume, dry weight, and composition were measured and used to calculate the porosity and sinking speed of the aggregates.

Conclusions: The presence of ballast minerals did not affect the apparent diffusivity in the aggregates, and no support for protection from decomposition by biominerals or lithogenic material was found. The ballasted aggregates had increased sinking speeds which may lead to

increased oxygen supply to the aggregates, benefitting the carbon-specific respiration from the microbes associated with the aggregates.

Paper V: Iversen M. H., Nowald N., Ploug H., Jackson G. A., Fischer G. (submitted) High resolution profiles of vertical particulate organic matter export off Cape Blanc, Mauritania: degradation processes and ballasting effects. Manuscript submitted to Deep Sea Research I.

Content: In this paper vertical fluxes were calculated from *in situ* camera profiles of aggregate size-specific abundances using estimated sinking speeds and aggregate masses. The calculated fluxes were compared to deep ocean sediment trap data. Carbon consumption was estimated from the fluxes and used to identify degradation processes at different depths. The different carbon consumptions were compared with on board measurement of aggregate associated microbial degradation rates and calculated copepod abundances. Both aggregate sinking speeds and aggregate degradation were considered in relation to carbon export.

Conclusions: The majority of carbon removal occurred in the upper 220 m of the water column. Mesozooplankton activity seemed dominant for aggregated carbon removal in the depth layer between 20 and 80 m. Microbes dominated the aggregate carbon removal at depths below 80 m. The microbial carbon removal seemed limited between 220 and 2500 m depth. The presence of ballast minerals in aggregates increased their sinking speeds and, thus, potentially reduced the retention time in the upper 220 meters. Hence, ballasting of aggregates seemed an important parameter for vertical carbon export.

ZUSAMMENFASSUNG

Kotballen (´fecal pellets´) und Aggregate (´Marine Snow´) sind Bestandteile der Biologischen Pumpe, die organischen Kohlenstoff vom Oberflächenozean in den tiefen Ozean transportieren und damit CO_2 über viele Jahre bis Jahrhunderte speichern können. Allerdings sind die vielfältigen Mechanismen von Kohlenstoffexport und Transfer in die Tiefsee noch weitgehend ungeklärt. Die Produktionsraten von Kotballen sind z.B. wesentlich höher als der Kohlenstoffffluss von Kotballen in der Tiefsee (sehen Turner, 2002). Dies wird durch hohen Fraßdruck von Copepoden (Ruderfußkrebsen), besonders von cyklopoiden Arten der Gattung *Oithona* sp. erklärt (Gonzalez und Smetacek 1994), direkte Beobachtungen dafür fehlen jedoch.

Neuere Ergebnisse von Sinkstofffallen aus der Tiefsee haben gezeigt, dass Karbonat- und die organischen Kohlenstoffflüsse gut korrelieren (z. B. Francois et al., 2002; Armstrong et al. 2002). Dies führte zur so genannten Ballasthypothese, wobei das Vorhandensein verschiedener Biominerale (z.B. Karbonat) im Oberflächenozean zu höheren Kohlenstoffflüssen in der Tiefsee führen soll. Erhöhte Partikelsinkgeschwindigkeiten aufgrund der höheren Dichte von Aggregaten, und/oder der Mineralschutz gegen den Abbau der organischen Substanz in den Zellen kommen als mögliche Ursachen in Frage (Armstrong et al. 2002; Francois et al. 2002; Klaas und Archer 2002). Der Einfluss verschiedener Ballastminerale auf die Partikelsinkgeschwindigkeiten und den Kohlenstoffabbau in der Wassersäule sind jedoch weitgehend ungeklärt.

Diese Dissertation hat den Kohlenstoffumsatz in Kotballen und Phytoplanktonaggregaten zum Thema, um wichtige Prozessen zu identifizieren, die den Abbau und Export von Kohlenstoff in der Wassersäule bestimmen. Fraßexperimente und visuelle Beobachtungen der Copepoden *Calanus helgolandicus*, *Pseudocalanus elongatus* und *Oithona similis* haben gezeigt, dass die meisten Kotballen nicht aufgenommen wurden, z. B. bei *C. helgolandicus* und *P. elongatus* (**Paper I**). *O. similis* wurde als effektiver Kotballen-Grazer in der Literatur angesehen (sehen Turner 2002), dies konnte hier jedoch nicht bestätigt werden (**Paper I**). Das Vorkommen von Copepoden führte häufig zur Kotballenfragmentierung (´coprorhexy´), jedoch sind sie nicht die wichtigsten Organismen für die Kohlenstoffremineralisation in Kotballen (**Paper I**). Aus Fraßuntersuchungen mit Kotballen verschiedener Größenfraktionen einer natürlichen Planktongemeinschaft aus dem Øresund (Dänemark) wurde festgestellt, dass große Protozooplanktonorganismen (20 bis 100 µm) die wichtigsten Organismen für den Abbau von Kotballen waren (**Paper II**).

Die größte Bedeutung des Mesozooplanktons, neben der Produktion von Kotballen, erscheint indirekter Art über das Grazing von Protozooplankton (potentielle Zunahme des Exportes) und mittels Kotballenfragmentierung (´coprorhexy´; potentielle Abnahme des Exportes) (**Paper I**; **Paper II**). Die Protozooplanktonorganismen können als wirksamer "Protozoenfilter" funktionieren, die die Kotballen aus dem vertikalen Stoffffluss weitgehend entfernen (**Paper II**). Es wurde weiterhin festgestellt, dass Kotballen von Phytoplankton durch den hohen Anteil an Biomineralen eine hohe Dichte und daher erhöhte Sinkgeschwindigkeiten haben (**Paper III**; **Paper IV**). Es wurden keine Hinweise auf den Schutz gegen mikrobiellen Abbau als Funktion der Biominerale in frisch produzierten Kotballen gefunden (**Paper III**). Von Phytoplankton produzierte Kotballen mit Biomineralen wurden 10-fach mehre Kohlenstoff exportiert als solche ohne Mineralanteile (**Paper III**). Durch erhöhte Sinkgeschwindigkeiten in ballastreichen Kotballen war jedoch die Sauerstoffzufuhr erhöht, da die Biominerale keinen Einfluss auf die scheinbare Sauerstoff-Diffusivität in den Kotballen haben (**Paper IV**). Hiermit war viel Sauerstoff für den Abbau labiler organischer Kohlenstoffverbindungen während der Sedimentation zur Verfügung.

Das Vorkommen von Biomineralen und lithogener Minerale (z.B. Tonminerale, Quarz) konnte als wichtiger Faktor für die Partikelsinkgeschwindigkeiten in Aggregaten bestimmt werden (**Paper IV**; **Paper V**). Die Sinkgeschwindigkeiten von Aggregaten sind abhängig von der Aggregatquelle, der Dichte und vom Alter und weniger abhängig von der Größe der Aggregate (**Paper IV**). Allerdings müssen die bestimmten Sinkgeschwindigkeiten aus Laborexperimenten als Maximalwerte betrachtet werden, die *in situ* Werte sind sicherlich geringer (Alldredge und Gotschalk 1988). Die Gründe für längere Verweilzeiten im Oberflächenwasser sind physikalischer Natur (Strömungen, Turbulenz) sowie biologisch bedingt z.B. durch Abbau, Lösung und Disaggregation.

Die biologisch bedingten größeren Verweilzeiten von Partikeln in der oberen Wassersäule wurden durch die Veränderungen der Kohlenstoffflüsse mit zunehmender Tiefe abgeschätzt (**Paper V**). Die größten Kohlenstoffabbauraten waren in den oberen 220 m zu finden und konnten in zwei wichtige Prozesse mit Tiefenstufen aufgeteilt werden, zwischen 20 und 80 m und zwischen 80 und 220 m. In den oberen 20 bis 80 m war der Abbau von Mesozooplanktonorganismen dominiert, unterhalb von 80 m waren die bakterielle Atmung und die Hydrolyse die entscheidenden Faktoren. In Tiefen zwischen 220 und 2500 m wurden sehr niedrige Kohlenstoffabbauraten durch bakteriellen Aktivität gefunden, begrenzt durch niedrige Temperaturen, geringe Bakterienmengen bzw. geringe Bakterienmengen an den Aggregaten (**Paper V**).

Daher wird der organische Kohlenstoff, der die Zone unterhalb 220 m erreicht, nur noch wenig abgebaut und kann in die Sedimente gelangen. Eine kurze Verweilzeit von organisch-reichen Partikeln in der oberen Wassersäule ist offenbar entscheidend für einen erhöhten Kohlenstofftransfer und die Kohlenstoffspeicherung. Ballastminerale sind ein wichtiger Faktor für die Partikelsinkgeschwindigkeiten und kann dadurch die biologische Pumpe auch kontrollieren (**Paper III**; **Paper IV**; **Paper V**).

SUMMARY

Fecal pellets and marine snow aggregates drive the biological carbon pump via sedimentation of organic matter from the surface ocean to the deep ocean where carbon can be sequestered for hundreds to thousands of years. However, the controlling mechanisms for carbon export from the surface ocean are still unclear. It is often observed that fecal pellet fluxes comprise less of the POC flux than would be expected from their production rates (see Turner 2002). This has been explained by high grazing on the fecal pellets by copepods, especially the cyclopoid copepods *Oithona* sp. (Gonzalez and Smetacek 1994), but direct evidence from the field is still missing. Recent observations have shown that carbonate and organic carbon fluxes have close correlations in the bathypelagic zones of the ocean (e.g., Armstrong et al. 2002). This has lead to the hypothesis that biominerals in phytoplankton, e.g., carbonate and opal, promote carbon preservation in the sinking flux either via increasing the sinking velocity of aggregates due to increased aggregate densities and/or via protection of the organic matter in the cells from degradation (Armstrong et al. 2002; Francois et al. 2002; Klaas and Archer 2002). However, the effect of ballast minerals on sinking velocity and degradation rates in sinking aggregates is still unclear.

This dissertation investigated carbon turnover in fecal pellets, marine snow, and phytoplankton-derived aggregates to identify important processes promoting either retention or export of aggregated organic carbon. The terms used for the different aggregate types are defined as; ***fecal pellets***: aggregates surrounded by a peritrophic membrane and produced via excretion of ingested material by zooplankton organisms; ***marine snow***: large aggregates (>0.5 mm) formed *in situ* or from *in situ* collected material in roller tanks; ***phytoplankton-derived aggregates***: aggregates formed in roller tanks from phytoplankton cultures.

From grazing experiments and visual observations of the feeding behavior of the copepods *Calanus helgolandicus*, *Pseudocalanus elongates*, and *Oithona similis* it was observed that most encountered pellets were rejected by the calanoid copepods (*C. helgolandicus* and *P. elongatus*) (**Paper I**). No pellet encounters were observed for the cyclopoid copepods *O. similis* (**Paper I**), though *O. similis* have been suggested to be an effective pellet grazer (see Turner 2002). Pellet rejections often caused damage to the pellets and occasionally cut them in halfs. It was therefore concluded that the main impact of copepods on pellet degradation is via coprorhexy (pellet fragmentation) and that copepods are not the main pellet degraders (**Paper I**). From experiments with different size fractions of a natural plankton community from Øresund (Denmark) incubated with a known amount of pellets, large protozooplankton (20 to 100 µm) was found to be the main degraders of fecal

pellets (**Paper II**). Thus, the main impact from mesozooplankton organisms, apart from pellet production, seemed indirect via grazing on the protozooplankton (potentially increasing export) and via coprorhexy (potentially decreasing export) (**Paper I**; **Paper II**). The protozooplankton organisms formed an effective ´protozoan filter´ which could remove fecal pellets from the vertical flux (**Paper II**). It was further found that pellets produced from phytoplankton containing biominerals became ballasted and experienced elevated sinking speeds (**Paper III**; **Paper IV**). However, no indications of protection against microbial degradation as a function of biominerals were observed in freshly produced pellets. Thus, it was demonstrated that fresh fecal pellets produced from phytoplankton containing biominerals potentially had 10-fold higher carbon preservation than pellets produced without biominerals (**Paper III**). Further, the oxygen supply to the pellets was potentially increased via the elevated sinking speeds of ballasted pellet, since ballasting had no influence on the apparent diffusivities in the pellets (**Paper IV**). Hereby, high amounts of oxygen were available for respiration of labile organic carbon during sedimentation.

The presence of biominerals and lithogenic material was indicated as an important factor for the sinking speeds in marine snow and phytoplankton-derived aggregates (**Paper IV**; **Paper V**). Sinking speeds of aggregates were found dependent on aggregate source, density, and age, rather than on the size of the aggregates (**Paper IV**). However, sinking speeds measured in laboratories indicate the potential maximum sinking speeds of aggregates and the residence times of aggregates *in situ* will likely be much longer in the upper ocean than estimated from laboratory experiments (Alldredge and Gotschalk 1988). This is due to the increased retention times caused by physical processes, e.g., turbulence, and biological processes, e.g., consumption, dissolution, and disaggregation. The biological retention processes in the upper ocean was estimated from changes in carbon fluxes with increasing depths (**Paper V**). These estimates revealed the main carbon removal occurred in the upper 220 m off Cape Blanc, and could be divided into two important processes, one between 20 and 80 m and one between 80 and 220 m. Mesozooplankton organisms dominated the carbon removal in the depth layer between 20 and 80 m, and below 80 m the carbon removal was dominated by bacterial respiration and hydrolysis. At depths between 220 and 2500 m very low carbon removal rates were observed and assumed via bacterial activity which seemed limited potentially due to temperature decrease, decrease in bacterial abundance and/or activity, or detachment of bacteria (**Paper V**). This indicated that carbon escaping the upper 220 m was likely to settle to the deep ocean where it can be sequestered. Therefore, a short residence time in the upper ocean seems important for carbon export and deep-ocean carbon

sequestration. Thus, ballast minerals was concluded an important factor controlling aggregate sinking speed and carbon export in the ocean (**Paper III**; **Paper IV**; **Paper V**).

1 INTRODUCTION

1.1 Organic matter in the ocean

When carbon dioxide (CO_2) is fixated and turned into organic material during photosynthesis by phytoplankton, the carbon is converted from dissolved inorganic to particulate organic carbon. This transformation enables downward transport of carbon, since particles can sink in the ocean, whereas solutes cannot (Kiørboe 2001). On short time scales, the amount of vertically transported material is controlled by the sinking speeds and degradation rates of settling particles. On long time scales the vertical flux is balanced by the input of limiting nutrients to the sunlit areas of the ocean (euphotic zone) since export cannot exceed input. In this perspective the pelagic degradation mainly influences the magnitude of recycling and, thus, the size and structure of the pelagic biomass (Kiørboe 2001) (Fig. 1).

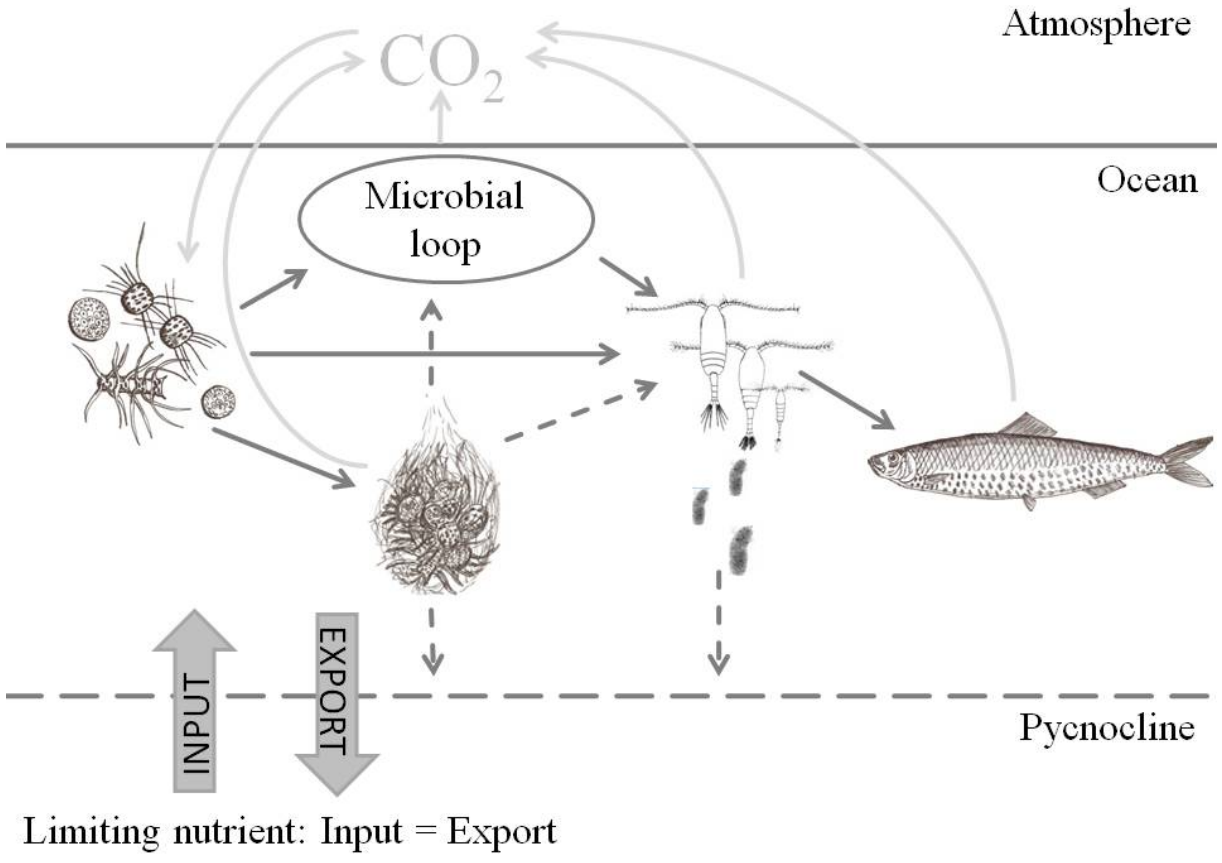

Fig. 1. Simplified pelagic ecosystem. Limiting nutrient are entering the euphotic zone from the aphotic zone and taken up together with CO_2 by growing phytoplankton. Vertical flux occurs via sedimentation of aggregation of phytoplankton and zooplankton fecal pellets. At steady state the sinking aggregates balance the nutrient input rate. Thus, the sum of both marine snow and fecal pellets must balance the nutrient input, meaning the more marine snow contribute to settling the less is the ´need´ for zooplankton to the flux, resulting in lower zooplankton biomass. However, remineralization

of both aggregates and phytoplankton also occurs via the microbial loop and grazing. Remineralization retain the material in the upper ocean, governing increased aggregate formation or zooplankton biomass, if steady state between input and export of limiting nutrients are maintained. This increased biomass can benefit higher trophic levels as well. If remineralization of the sinking aggregates occurs below the euphotic zone, the net burial rate of carbon in the deep ocean is reduced. (The figure is modified from Kiørboe (2001)).

Recycling of organic material in the euphotic zone creates a temporary mismatch between nutrient input and output (export) which eventually will be at steady state. However, degradation of settling particles occurs throughout the whole water column and within the sediment until the particles are finally buried and stored. These settling and degradation processes maintain higher concentrations of biologically active elements and compounds at depths compared to the ocean surface. This process is called ´the biological pump´, and when focusing on carbon transformations it is called ´the biological carbon pump´ (Volk and Hoffert 1985). When carbon is transported to the deep ocean via the biological carbon pump, CO_2 is removed from the surface ocean enable more CO_2 absorbtion from the atmosphere (Sarmiento and Bender 1994). Once the organic carbon reaches the deep ocean sediment it can be sequestered for up to millions of years (Raven and Falkowski 1999). Thus, the understanding of the biological carbon pump is very important for the global carbon cycle. The efficiency of the biological carbon pump is determined by the processes taking place in the time span between the fixation of carbon in the surface ocean and the burial of carbon in the deep ocean. Simplified, the biological carbon pump can be divided into the production of particulate organic carbon (POC), the settling of POC, and the decomposition of POC. Photosynthesis is light-dependent and therefore spatially restricted to the euphotic zones in the upper <200 meters of the water column. Below the euphotic zone, most ecosystems are feed by export of organic matter produced via photosynthesis. This has been recognized since Agassiz (1888):

> "…deep-sea organisms are nourished by a ´rain´ of organic detritus from overlying surface waters"

The amount of ´organic rain´ to the deep-sea is determined by settling and degradation of organic matter (Alldredge and Gotschalk 1989; Fowler and Knauer 1986; Suess 1980). Therefore a short transit time from the surface ocean to the sea floor, shortens the time available for decomposition of the organic particles and, thus, enhances the organic carbon export. If organic matter were to settle as individual phytoplankton cells they would need ~10

years to reach the sea floor in the open ocean (Smayda 1969). Within that time the organic matter would be grazed or remineralized by herbivores and microbes. Therefore, the sinking flux mainly consists of fast-sinking, large aggregates such as marine snow and zooplankton fecal pellets (Fowler and Knauer 1986), with sinking speeds greater than 100 m d^{-1} (e.g., Alldredge and Gotschalk 1988; Shanks and Trend 1980; Turner 2002).

1.2 Large marine aggregates

Marine snow is defined as large aggregates with diameters greater than 0.5 mm (Alldredge and Silver 1988). Marine snow can form from physical coagulation of smaller particles (Jackson and Burd 1998) (Fig. 2A), discarded houses from appendicularians (Fig. 2B), and mucus feeding nets from foraminiferas and pteropods (e.g., Alldredge and Silver 1988; Hansen et al. 1996b) (Fig. 2C). As these aggregates sink, they may scavenge additional particles helped by the sticky nature of transparent exopolymer particles (TEP) which form a matrix of the aggregates (see Passow 2002). The fecal pellets investigated in this thesis are produced by mesozooplakton organisms, mainly copepods. The fecal pellets produced by copepods are typically cylindrical and surrounded by a peritrophic membrane (Fig. 2D). Just as marine snow, fecal pellets might have high nutritional value depending on the food source, and on the assimilation efficiency of the ingested particles before they are excreted (Hansen et al. 1996a). The assimilation efficiency is inversely proportional to food concentration and pellet production time (Hansen et al. 1996a). Intact and viable phytoplankton cells have been found in pellets produced during bloom conditions (Dubischar and Bathmann 2002; Jansen and Bathmann 2007; Wotton 1994). The high nutritional value of both marine snow and fecal pellet makes them a desired food source for many marine organisms. Since the retention time in the water column potentially shortens with increased sinking velocity, rapid sinking marine snow and fecal pellets are likely candidates in the carbon export.

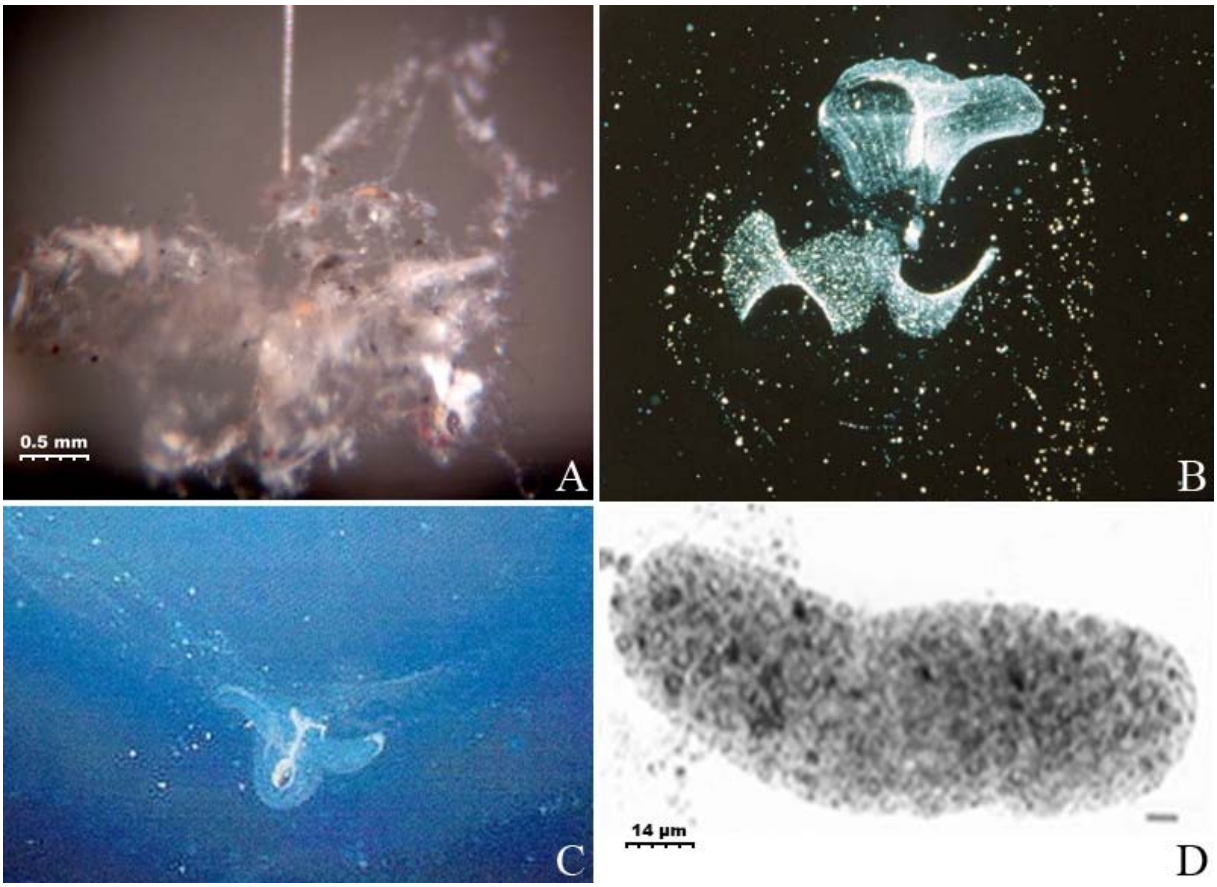

Fig. 2. Photographs of (A) a marine snow aggregate formed in a roller tank from *in situ* material collected at the depth of fluorescence maximum, (B) a larvacean in its house (Photo: Alice Alldredge), (C) a pteropod (*Gleba chordata*) and its mucus feeding web (photo: Alice Alldredge), and (D) a copepod fecal pellet produced by *Acartia tonsa* feeding on *Rhodomonas* sp.

1.3 Sinking of marine aggregates

1.3.1 Fecal pellet sinking speed

A large range of sinking speeds for fecal pellets has been reported in the literature, ranging from 5 to 220 m d^{-1} for copepods, from 16 to 862 m d^{-1} for euphausiids, and from 25 to 166 m d^{-1} for small appendicularians (see Table 2 in Turner 2002). Komar et al. (1981) suggested a modified version of the Stokes equation to estimate sinking speed (w) of cylindrical fecal pellets:

$$w = 0.0790 \frac{1}{\mu} (\rho_s - \rho) g L^2 \left(\frac{L}{D}\right)^{-1.664} \qquad (1)$$

where μ is the water dynamic viscosity (g cm^{-1} s^{-1}), ρ_s is the pellet density (g cm^{-3}), ρ is the water density (g cm^{-3}), g is the acceleration due to gravity (981 cm s^{-2}), L is the pellet length (cm), and D is the diameter (width, cm) of the pellet. Eq. 1 only apply for particles with a cylindrical shape and within Stokes region ($\rho wL/\mu < 2$). According to Eq. 1 the sinking speed of a fecal pellet increases with increasing length, width, and density. However, since food type, quality, and quantity affect the pellet size and density (e.g., Bienfang 1980; Dagg and Walser 1986; Turner 1977) the sinking speed is very difficult to predict at *in situ* conditions. The pellet density depends on the density of the compounds contained within the pellet and on the level of compaction of these compounds (Bienfang 1980; Urban et al. 1993). The pellet size depends on food type and concentration (Dagg and Walser 1986; Feinberg and Dam 1998; Tsuda and Nemoto 1990). Sinking speeds of copepod fecal pellets show variations with food conditions (Butler and Dam 1994) and size of the producing copepod (Harris 1994). Therefore, a range of pellets with different sizes and densities, and, thus, different sinking speeds, may be produced from a pelagic mesozooplankton community in a given ecosystem. This implies that the characteristics of pellets may vary temporally and spatially according to the species composition and the biomass of the plankton community.

Feinberg and Dam (1998) investigated the effects of diet type and concentration on pellet length, width, and density. They identified pellet width as the most influential parameter on sinking speed and pellet length as the least influential parameter. I compiled settling speeds from three different studies and plotted them against length, width, and equivalent spherical diameter (ESD) (Fig. 3).

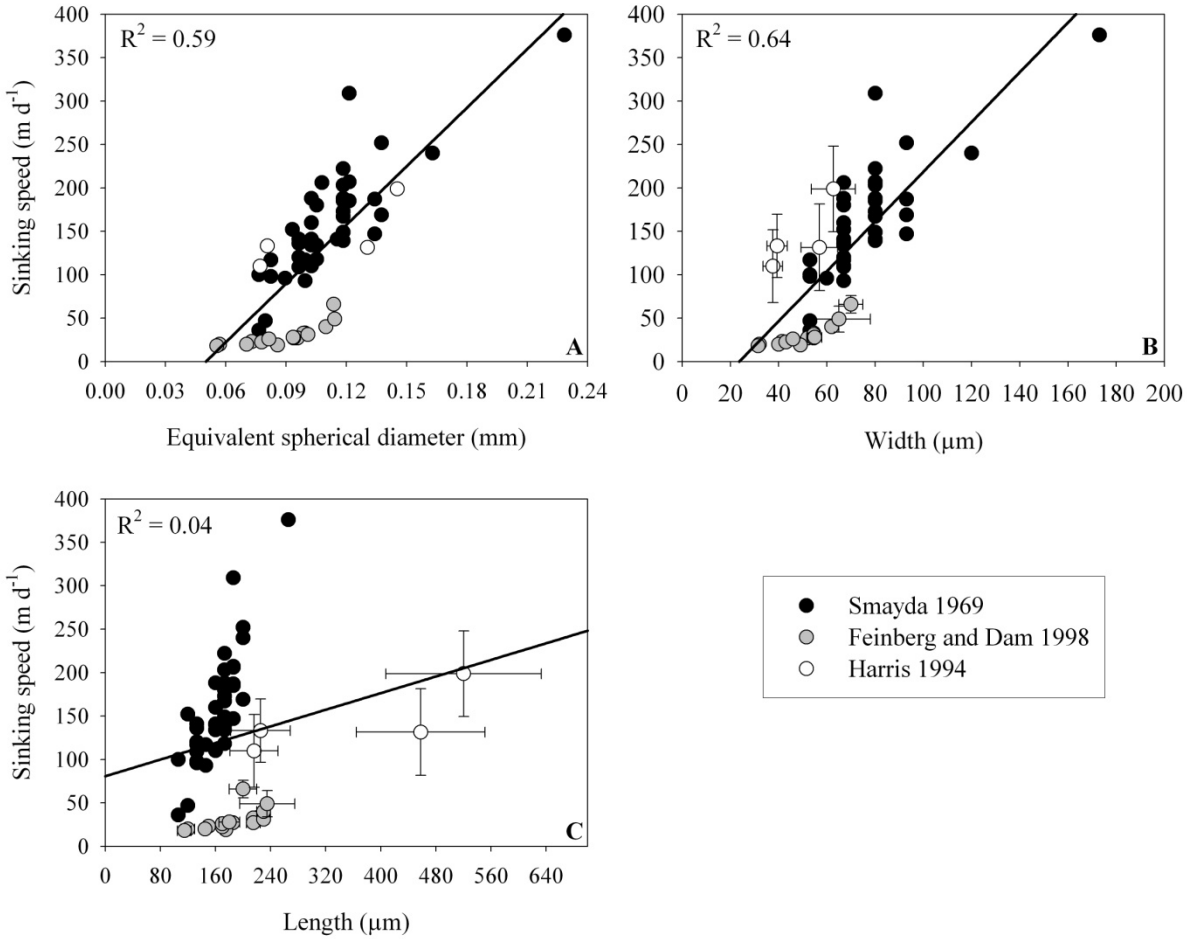

Fig. 3. Fecal pellet sinking speed plotted as a function of (A) equivalent spherical diameter (ESD), (B) width, and (C) length. Three data sets from the literature are plotted. Black circles indicate the data set from Smayda (1969), grey circles indicate the data set from Feinberg and Dam (1998), and the white circles indicate the data set from Harris (1994). The solid lines are linear regression to all three data sets in each plot.

I found the ESD (Fig. 3A) and width (Fig. 3B) of the pellets as the best general size parameters in describing the sinking speeds of fecal pellets (R^2 = 0.59 and R^2 = 0.64, respectively), while the pellet length (Fig. 3C) had very poor general correlation with sinking speeds between the different studies (R^2 = 0.04). When observing the pellets within the three studies (Feinberg and Dam 1998; Harris 1994; Smayda 1969), a better correlation between pellet length and sinking speed seemed to exist (Fig. 3C), indicating pellet length as a possible describing parameter of sinking speeds for similar pellets, e.g., similar composition and /or same producer. Pellet density may also be an important controlling parameter for sinking speed since the pellets containing dense particles such as calcium carbonate producing coccolithophorids (Harris 1994) and dense pellets possible containing minerals (Smayda 1969) had higher size-specific sinking speeds than the pellets produced from less dense monocultures of either flagellates or diatoms (Feinberg and Dam 1998) (Fig. 3A). This

indicates that both the shape and density of fecal pellets might be important parameters controlling their sinking speeds.

1.3.2 Marine snow sinking speed

Marine snow aggregates are fractal (Logan and Wilkinson 1990) and therefore have different scaling properties than assumed in settling speed calculations using Stokes' law (Johnson et al. 1996). The fractal nature of marine snow complicates the predictions of their sinking speeds. However, direct studies of sinking speeds and sizes of marine snow can be described using power relationships (Alldredge and Gotschalk 1988). Still, size-specific sinking speeds found in different studies can vary largely (Alldredge and Gotschalk 1988; Hamm 2002). This is likely due to the different types of particles within the aggregates resulting in different densities and porosities of the aggregates. Therefore, the sinking speeds of marine snow seems controlled by aggregate size, density, and porosity, making the *in situ* conditions just as influential on the sinking speeds of marine snow as they are on the sinking speeds of fecal pellets.

The sinking speeds of marine snow and fecal pellets are often measured under controlled conditions without any changes in aggregate structure or water parameters. Such measurements are maximum speeds and a similar sized and composed aggregate likely settle at lower rate *in situ* due to changes in both aggregate characteristics, water turbulence, and ocean currents (Alldredge and Gotschalk 1988). Thus, retention times of aggregates in the water column may be longer than predicted from laboratory measurements. Since increased retention times in the upper ocean potentially leads to increased degradation, it seems equally important to identify key degradation processes and controlling mechanisms for the sinking speed when attempting to determine the efficiency of the biological carbon pump.

1.4 Degradation of large aggregates

Vertical profiles of particulate organic carbon (POC) often show exponential decline with increasing depth (Martin et al. 1987; Schlitzer 2000; Suess 1980), indicating that most of the POC is decomposed in the surface ocean. Therefore, the surface ocean is likely to have the most intensive degradation of marine aggregates. In fact less than half of the primary production are exported out of the euphotic zone and only a few percent reach the deep ocean and sediment (e.g., Martin et al. 1987) (Fig. 4).

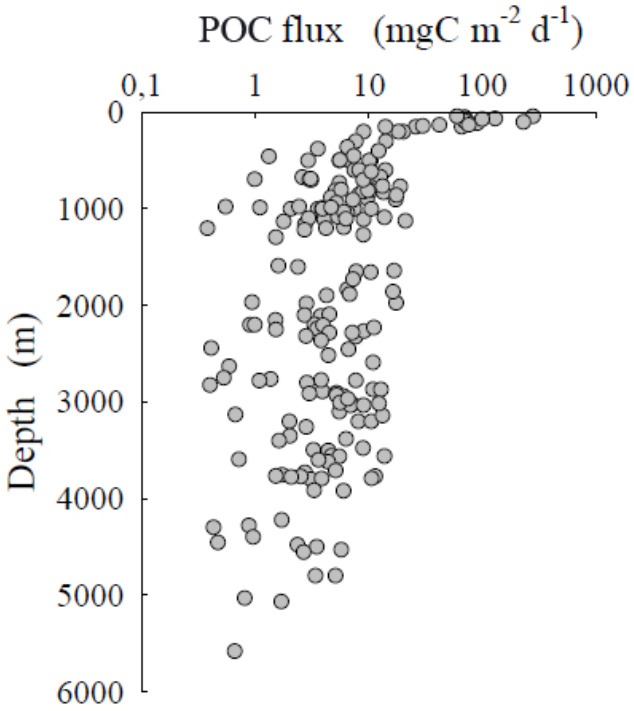

Fig. 4. Mean annual global POC flux at different depths in the ocean. The data is compiled from Martin et al. (1987), Suess et al. (1980), Lutz et al. (2002), Andersson et al. (2004) (Figure is modified from Lutz et al. (2002) by C. De La Rocha).

The actual shape of vertical POC profiles is determined by the settling, retention, and removal processes of the organic carbon formed in the upper ocean (Buesseler et al. 2007). Physical disaggregation from shear tearing the aggregates apart has been suggested important for carbon degradation (Milligan and Hill 1998), but this process seems of little importance at typical turbulent shear rates in the upper ocean (Alldredge et al. 1990). The high degradation of organic carbon seems more likely via respiration and hydrolysis by bacteria attached on the aggregates (e.g., Smith et al. 1992) and from fragmentation and consumption of the aggregates by mesozooplankton (Dilling and Alldredge 2000; Kiørboe 2000; Steinberg 1995). These processes convert the POC into CO_2, dissolved organic carbon (DOC), and convert the large, sinking aggregates into smaller aggregates with reduced sinking speeds. It has been shown that attached bacteria have higher cell-specific hydrolytic enzyme activity, but not elevated carbon demands, relative to the free-living bacteria (Cho and Azam 1988; Smith et al. 1992). Therefore, it seems that a large part of the DOC produced from aggregated carbon is leaking out of the aggregates and oxidized by free-living microbes (Kiørboe and Jackson 2001; Thor et al. 2003). The part of DOC leakage which is not oxidized by free-living microbes may potentially remain within the water body until the overturning circulation returns it to the upper ocean. The depth of DOC leakage determines the residence time of the

DOC, with time scales of decades for the mesopelagic, and centuries for bathypelagic and abyssal zones.

While microbes attached on marine snow mainly seem to colonize the aggregates after they have formed and started to settle (Kiørboe et al. 2002), microbes on fecal pellets can both have arrived from the surrounding water (Honjo and Roman 1978) and from the gut of the zooplankton and packed within the pellet (Gowing and Silver 1983). This has raised the question whether fecal pellets are degraded from the ´inside out´ or from the ´outside in´ (see Turner 2002). Hansen et al. (1996a) have suggested that bacterial degradation of fecal pellets depends on the diet on which the pellets are produced. Though both marine snow aggregates and fecal pellets seem to be hotspots for bacterial abundances and growth (Alldredge and Silver 1988; Azam and Long 2001; Jacobsen and Azam 1984) this high bacterial colonization may not be the major remineralization pathway of the aggregates (Jacobsen and Azam 1984). On average the bacteria have a net assimilation equivalent to 15% of the carbon fixed through photosynthesis (Ducklow 2000). However, 97-99% of the net primary production is oxidized back to CO_2 (De La Rocha and Passow 2007), supporting that other degradation mechanisms, in addition to bacterial remineralization, must be important. Therefore mesozooplankton organisms have been suggested important in the degradation of both marine snow aggregates (Kiørboe 2000; Lampitt et al. 1993; Steinberg et al. 1994) and fecal pellets (Gowing and Silver 1983; Green et al. 1992; Paffenhöfer and Knowles 1979).

1.4.1 Why Visit aggregates

There may be many reasons why organisms settle on or visit marine aggregates. Bacteria gain rich food supplies when attached to aggregates, but experience elevated predation pressure and removal from the surface ocean as the aggregates sink. Protozoans are abundant on aggregates where they are likely to feed on both bacteria and other particulate constituents of the aggregate (Caron 1987; Tiselius and Kiørboe 1998). Bacteria that have colonized an aggregate may detach again after ~3 hours, perhaps to avoid both predation and removal from the surface ocean (Kiørboe et al. 2002). The high ecto-enzymatic activity observed for attached bacteria (Smith et al. 1992) might also be a strategy to avoid both the risk of predators and removal from the surface ocean. High rates of DOC leak from the settling aggregates and create a plume in their wake (Kiørboe and Jackson 2001). The colonizing bacteria may detach and feed in this plume where both the risk of removal and predation is lowered. A side-effect of this high DOC production is the formation of important growth habitats for free-living bacteria, whereby the once aggregated organic carbon may

feed a significant proportion of the water column bacterial production (Kiørboe and Jackson 2001). The plume of DOC in the wake of an aggregate might also enable horizontal cruising mesozooplankters to detect and encounter the aggregates via chemical perception (Kiørboe and Thygesen 2001), bacteria have also been suggested to encounter aggregates using the DOC plumes (Kiørboe and Jackson 2001). The numerous mesozooplankton species visiting the aggregates may feed on the constituents and the microbes associated with the aggregate. Some copepods explore settling aggregates so successfully that they have adapted to feeding on solid surfaces, as aggregates, despite their pelagic lifestyle (Kiørboe 2001). Aggregates also provide ´public transportation´ as observed for several invertebrate larvae, which appear to use aggregates as sinking vehicles when they are ready to metamorphose and settle (Shanks and Del Carmen 1997).

1.4.2 Degradation in rates

1.4.2.1 Marine snow degradation rates

The diverse group of organisms recycling the aggregates makes it difficult to determine which fraction of the carbon removal is carried out by zooplankton and which fraction is removed by bacteria. Average carbon specific respiration rates due to microorganisms have been found at ~0.1 d^{-1} and seem size-independent in both natural and laboratory made marine snow aggregates (Ploug and Grossart 2000; Ploug et al. 1999). It has been shown that specific leakage of DOC are similar to the respiratory losses (Grossart and Simon 1998; Kiørboe and Thygesen 2001; Smith et al. 1992), indicating specific carbon removal rates by attached microbes in the order of ~0.2 d^{-1}. The disaggregation of marine snow by swimming zooplankton organisms (Dilling and Alldredge 2000; Graham et al. 2000; Stemmann et al. 2004) may be important since it leads to longer residence times, and thereby to further degradation of the marine snow in the upper ocean. Dilling and Alldredge (2000) calculated that the euphasiid *Euphausia pacifica* at natural abundances could disrupt aggregates contained in 3 – 33 % of the upper 100 m of the water column. Kiørboe (2000) calculated that invertebrate zooplankters could degrade between 20 and 70 % of the aggregates before they leave a 50 m deep euphotic zone. Thus, it seems that large parts of the marine snow aggregates may be degraded within the euphotic zone due to activity from microbes and zooplankters attaching to or visiting the aggregates.

1.4.2.2 Fecal pellet degradation rates

Microbial carbon specific degradation of fecal pellets has suggested rates between 0.06 and 0.19 d^{-1} (Lee and Fisher 1992; Thor et al. 2003; Urban-Rich 1999). Even degradation rates of 90% within the first 24 hours have been suggested for pellets produced at excess food concentrations (Hansen et al. 1996a). Diffusion of DOC from pellets has been shown at rates more than 50% of the total organic carbon in the pellet (Thor et al. 2003), reaching 28% within the first 15 min (Møller et al. 2003). In addition to these high microbial degradation and hydrolyzation rates, the pellets also seem an attractive food source for zooplankton. Several mechanisms have been suggested as an outcome of the encounter between copepods and fecal pellets. They can break up the pellets (coprorhexy) (Lampitt et al. 1991), ingest parts of, or the whole pellet (coprophagy), and they can disrupt the peritrophic membrane around the pellet (coprochaly) (Noji et al. 1991). Coprophagy removes the pellets and they will not be part of the export flux. Coprorhexy converts large pellets into smaller pellet fragments and coprochaly results in partial dispersal of fecal pellet contents into the water. Both coprorhexy and coprochaly potentially increase the residence time of fecal material and enhance the time for degradation in the upper water column. All encounters between copepods and fecal pellets, thus, increase the recycling of fecal pellets in the upper ocean, either directly via coprophagy or indirectly via coprorhexy or coprochaly. The cyclopoid copepods *Oithona* spp. have been suggested as the main pellet grazer (Gonzalez and Smetacek 1994; Svensen and Nejstgaard 2003). However, most of the conclusions for copepod grazing behavior on pellets have been made without direct observations, and the rates and triggering conditions for the different mechanisms are still unclear. It is, however, know that the contribution from fecal pellets to the vertical flux of organic matter varies both temporally and spatially (Poulsen and Kiørboe 2006; Wexel-Riser et al. 2001). Some studies have reported increased pellet flux during bloom conditions (Wexel-Riser et al. 2002) while other have observed maximum pellet degradation during bloom conditions (Dubischar and Bathmann 2002; Sampei et al. 2004; Urban-Rich 2001). It therefore seems that an effective pellet retention filter may prevent pellet export from the surface ocean (e.g., Wexel-Riser et al. 2001), but the relative contributions to a retention filter by microbes and zooplankters are still unclear.

1.5 Marine snow vs. fecal pellets in the vertical flux

Though numerous studies of the POC flux exist, there is still no clear understanding on the relative roles of marine snow aggregates and fecal pellets in the export of POC. At times, fecal pellets seem to dominate in the POC flux and contribute with more than 70% to the total carbon flux (e.g., Bishop et al. 1977; Gonzalez et al. 1994), while other studies indicate that marine snow aggregates are dominant and fecal pellets contribute with less than 10% to the total carbon flux (e.g., Andreassen et al. 1996; Knauer et al. 1979; Lundsgaard et al. 1999; Viitasalo et al. 1999).

Smetacek (1980) found low carbon contribution from pellet to the total POC flux throughout a year in Kiel Bight, and observed the highest pellet removal rate during summer. Sampei et al. (2004) also observed seasonal variation in the contribution of pellet to the total POC flux, and found that 99% of the produced fecal pellets did not reach depths of 200 m during the period with highest primary production. Landry et al. (1994) calculated that grazing from mesozooplankton could remove between 16 to 44% of the primary production, while only 23 to 32% of the pellets produced reached the deep ocean. Interestingly, the highest percentage of the produced pellets often seems removed during periods with plenty of food for the mesozooplankters, indicating that other degradation processes than coprophagy may be important. This may even indicate other organisms than copepods as the major pellet degraders.

The use of long term sediment traps has revealed that the deep ocean sedimentation is highly variable. However, a linear relationship between surface primary production and POC flux at 2000 m has been suggested (Lampitt and Antia 1997). Even so, it is unclear how much of the primary production is exported, which aggregate types are responsible, and how this changes with area, over time, and composition of pelagic communities (see Turner 2002). The association with minerals have been suggested to ballast the aggregates and thereby increase the POC flux by either protecting the organic matter from oxidation or by increasing the sinking speed of the aggregates (Armstrong et al. 2002; Francois et al. 2002; Klaas and Archer 2002). Good correlations between calcium carbonate and POC have suggested calcium carbonate as the controlling ballast mineral for the POC flux (Francois et al. 2002; Klaas and Archer 2002). This hypothesis has been termed the ballast hypothesis. However, the actual mechanisms triggering the close correlation between fluxes of POC and minerals are still unclear. Alternative suggestions are that the settling POC scavenge suspended minerals which otherwise would not sink (Passow 2004; Passow and De La Rocha 2006).

There is still no conclusive evidence for either protection of organic aggregates by minerals or increased sinking velocity due to loading with mineral. Incorporation of minerals have been suggested to decrease the porosity and increase the density, and thereby increase the sinking speed of marine aggregates (De La Rocha and Passow 2007), despite the fact that the presence of minerals seemed to fragment marine snow into smaller aggregate (Passow and De La Rocha 2006).

1.6 Questions to be answered

Intense investigations of the processes controlling the vertical flux of organic carbon through the water column have been made since Martin et al. (1987) observed rapid carbon removal in the upper 1000 m. Despite numerous investigations, essential pieces of the puzzle still need to be brought together before we have the whole picture. In this thesis I will attempt to answer some of the open questions:

Is Oithona sp. the main pellet degrader?

What is the degradation mechanism and impact from copepods on fecal pellets?

Are other organisms than copepods important for degradation of fecal pellets?

What is the influence from ballast minerals on aggregate sinking speed?

Can ballast minerals protect aggregates from microbial degradation?

What is the relative contribution from zooplankters and microbes to carbon removal, and how is this relationship at different depths?

2 RESULTS AND DISCUSSION

There exist only few investigations of the carbon flux above 200 to 500 m depth since long-term sediment traps have limitations at these depths. Therefore we lack basic understanding on how vertical carbon flux is regulated between the depth of primary production and the depths where most carbon fluxes are measured. This has been described as a hundreds of meter thick black box (Wassmann et al. 2003). However, it has been shown that there is a rapid decrease in vertical POC flux in the twilight zone (Olli et al. 2001; Wassmann et al. 2003). To get a better insight to the processes taking place in the twilight zone, a series of investigations of processes important for carbon turnover and export of fecal pellets, marine snow, and phytoplankton-derived aggregates were performed. The terms used for the different aggregate types in the following discussion are defined as; ***fecal pellets***: aggregates surrounded by a peritrophic membrane and produced via excretion of ingested material by zooplankton organisms; ***marine snow***: large aggregates (>0.5 mm) formed *in situ* or from *in situ* collected material in roller tanks; ***phytoplankton-derived aggregates***: aggregates formed in roller tanks from phytoplankton cultures. Table 1 provides an overview of the publications based on these investigations.

Table 1: Overview of the conducted experiments. Objectives, applied methods, main results, and conclusions.

	Experiment parameters	Objectives and questions	Method applied	Main results	Conclusions
Paper I	• Copepods species: *C. finmarchicus*, *P. elongatus*, and *O. similis*. • Fecal pellets offered as sole food or together with an alternative food source. • Experiments were performed at *in situ* temperature and salinity.	• Investigations of copepod feeding behavior while grazing on fecal pellets. • Are copepods the main fecal pellet degraders?	• Grazing incubations and visual observations of copepod females offered pellets as sole food or together with an alternative food source. • Methods: plankton wheel incubations and video recordings.	• The calanoid copepods had high fragmentation rate of encountered pellets. • The calanoid copepods mainly ingested small fecal pellet and pellet fragments, large pellets were rejected. • The presence of an alternative food source increased the pellet encounter rate by calanoid copepods and, thus, the ingestion of fecal pellets by the calanoid copepods. • *O. similis* did not seem to have an efficient grazing on fecal pellets.	• Coprorhexy was the main feeding behavior by calanoid copepods. • Ingestion of fecal pellets by the calanoid copepods mainly seemed unintentional and increased when the pellets were offered together with an alternative food source. • *O. similis* did not seem important for fecal pellets retention in the upper ocean.
Paper II	• Fecal pellets were offered to size a fractionated plankton community from Øresund (DK). • The incubations were performed app. every second month. • Incubated in laboratory at *in situ* conditions.	• Degradation of pellets by different size fractions of a natural plankton community from Øresund (DK). • Identify the most important organisms in pellet degradation and their seasonal effect.	• Grazing incubations on plankton wheel. • The natural plankton community was fractionated in <0.2 µm, <2 µm, <20 µm, <100 µm, <200 µm, and non-fractionated.	• Seasonality of pellet degradation followed the phytoplankton (high degradation during bloom etc.). • The highest fecal pellet degradation impact was observed in the 20 to 100 µm size fraction. • The abundance of the heterotrophic dinoflagellate *G. spirale* could be used as a predictor for the pellet degradation rate in the 20 to 100 µm size fraction.	• Large heterotrophic dinoflagellates were indicated as the main pellet degraders throughout the year. • Late stages of copepod nauplii may have an impact on pellet degradation in the >200 µm size-fraction. • Copepods may have high indirect influence on pellet degradation via grazing on the main pellet degraders (protozooplankters). • A ´protozoan filter´ seemed to retain pellets in the upper ocean.

Table 1 (continued): Overview of the conducted experiments. Objectives, applied methods, main results, and conclusions.

	Experiment parameters	Objectives and questions	Method applied	Main results	Conclusions
Paper III	• Grazing incubations with *T. longicornis* feeding on either, *Rhodomonas* sp. (only organic matter), *T. weissflogii* (opal), or *E. huxleyi* (calcite).	• Microbial degradation and sinking speed measurements of fecal pellets produced on different food types. • Does ballasting of fecal pellets affect degradation and sinking speed?	• Direct measurements of high resolution small O_2 fluxes to fecal pellets, sinking speed, density, and chemical composition. • Determination of grazing and pellet production rate.	• Fecal pellet production was significantly higher with *T. weissflogii* than with other food sources. • Pellets containing ballast minerals had significantly higher sinking speeds and densities as compared to pellets without ballast minerals. • No differences were observed in carbon-specific respiration rates between fecal pellet with and without ballast minerals.	• It was demonstrated that ballasting of fecal pellets increased the sinking speed of freshly produced pellets but could not protect the pellets from decomposition. • The presence of ballast minerals in freshly produced fecal pellets appeared to be an important factor controlling vertical carbon fluxes in the ocean.
Paper IV	• Measurements on field-sampled marine snow, laboratory-made aggregates from diatoms, coccolithophorids, or *in situ* collected material, small and large fecal pellets containing biogenic and/or lithogenic ballast minerals were investigated.	• Does ballasting increase carbon export via increased sinking speeds and/or protect from decomposition due to lowered apparent diffusivities in marine sinking aggregates?	• Direct measurements of high resolution small O_2 fluxes to aggregates. • Diffusivity measurements within aggregates. • Direct measurements of aggregate sinking speed and density.	• Increased sinking speeds were observed for aggregates containing ballast minerals. • No significant change in apparent diffusivity was observed between aggregates with or without ballast minerals.	• Aggregates ballasted by biominerals and lithogenic material did not show any change in apparent diffusivity of solutes and oxygen. Therefore, no support for protection from decomposition by ballast minerals was found. • Increased sinking speeds of ballasted aggregates may even increase the oxygen supply to the aggregates as they settle and, thus, maintain carbon specific respiration in sinking aggregates as they settle.

Table 1 (continued): Overview of the conducted experiments. Objectives, applied methods, main results, and conclusions.

	Experiment parameters	Objectives and questions	Method applied	Main results	Conclusions
Paper V	• Aggregates formed from water and material collected at the depth of fluorescence maximum off Cape Blanc, Mauretania (NW Africa). • *In situ* vertical determination of particle size distributions and abundances from vertical camera profiles between the surface and 2500 m depth.	• Which processes are important for the vertical carbon flux off Cape Blanc? • How does the relative contribution from zooplankton organisms and microbes influence the carbon removal at different depths?	• Direct measurements of high resolution small scale O_2 fluxes to aggregates. • Direct aggregate sinking speed measurements. • *In situ* camera profiles. • Deep ocean sediment trap collections. • Vertical flux calculations.	• High carbon removal from mesozooplanton organisms in the upper 80 m of the water column. Microbial carbon removal dominated from 80 to 2500 m depths, but was low between 220 and 2500 m depth. • The presence of lithogenic material and carbonate in aggregates resulted in high sinking velocities. • The carbon specific respiration rates of the aggregates were size-independent.	• The upper 220 m of the water column showed the highest carbon retention, potentially via high carbon removal by mesozooplankton organisms and microbes. • Ballasting of aggregates seemed an important contolling factor aggregate sinking speed and, thus, important for vertical carbon fluxes.

2.1 Carbon turnover in fecal pellets

The majority of the produced copepod fecal pellets seem recycled within the water column (Smetacek 1980; Turner 2002) and often at shallow depths. Therefore, a large part of the POC removal in the twilight zone may occur as fecal pellet recycling. Copepods have been suggested as the main degraders of fecal pellets, and coprophagy are believed to be the most important degradation mechanism (see Turner 2002) (Fig. 5). However, degradation mechanisms as coprorhexy and coprochaly have also been suggested important (Lampitt et al. 1991; Noji et al. 1991). The most important copepod species in pellet degradation are believed to be the cyclopoid copepods *Oithona* spp. (Gonzalez and Smetacek 1994; Suzuki et al. 2003; Svensen and Nejstgaard 2003). Gonzalez and Smetacek (1994) suggested that populations of *Oithona* spp. ´sit´ below the depth of pellet production and act as a ´coprophagous filter´ removing most of the produced pellets and, thereby, reduce the vertical export of fecal pellets (Fig. 5).

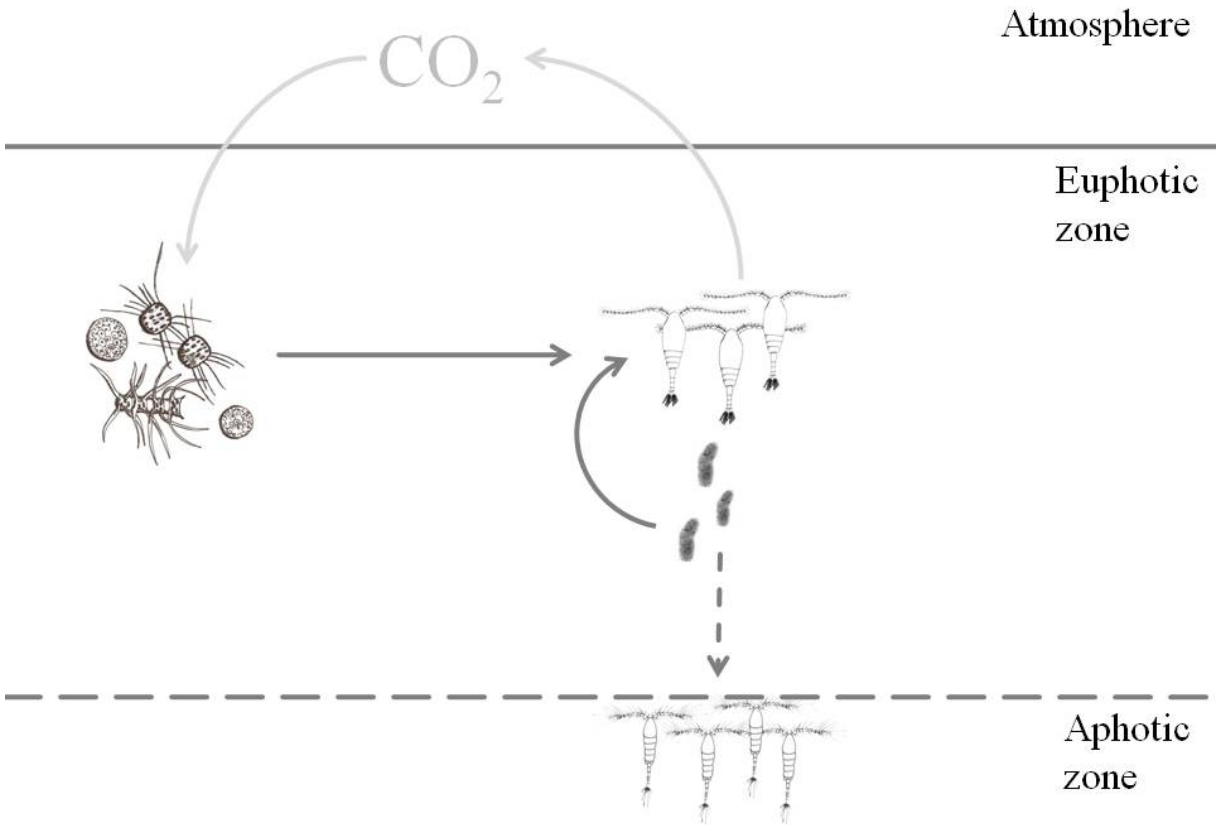

Fig. 5. Present view of the role of copepods in the fecal pellet degradation. In this simplified illustration large, calanoid, filter feeding copepods graze on phytoplankton in the euphotic zone. Part of the organic matter grazed by the calanoid copepods are excreted as fecal pellets, which sink out of the euphotic zone, if not removed via coprophagy by the calanoid copepods. *Oithona* sp. are believed

to form a ´coprophagous filter´ at the base of the euphotic zone, whereby the fecal pellets are removed from the export flux at high rates (Gonzalez and Smetacek 1994).

Despite the general acceptance of copepods being the most important pellet degraders, there exists no direct evidence supporting their role in pellet degradation. The coprophagous behavior of *Oithona* sp. is only based on the study by Gonzalez and Smetacek (1994), who conducted their experiments in upright bottles and reported *Oithona similis* to graze fecal pellets from the bottom of these bottles. *O. similis* require a hydrodynamic signal from its prey, either created from the swimming motion of the prey or from the sinking of fecal pellets (Svensen and Kiørboe 2000). To detect pellets on the bottom of a bottle, chemosensory detection is needed. Chemosensory detection requires a feeding current (Andrews 1983), and since *O. similis* lacks the ability to filter feed (Svensen and Kiørboe 2000) it cannot detect pellet from the bottom of a bottle.

To investigate the role of copepods in fecal pellet degradation, a series of experiments were conducted to determine the fecal pellet feeding behavior of three common copepod species in temperate areas (*Calanus helgolandicus*, *Pseudocalanus elongatus*, and *O. similis*) (**Paper I**). By combining visual observations of feeding behavior with grazing rate measurements, the mechanism and impact on pellet degradation from the copepods could be measured. The copepods were offered fecal pellets as sole food and fecal pellets in combination with an alternative food source. The copepod species were chosen due to their different sizes and different feeding strategies. The calanoid copepods *C. helgolandicus* and *P. elongatus* are suspension feeders and detect their food via chemosensory perception using a feeding current while cruising slowly through the water (Andrews 1983). The cyclopoid *O. similis* is an ambush feeder ´hanging´ in the water and only launching an attack when a prey particle is detected via hydromechanical disturbances generated by the prey item (Kiørboe and Visser 1999; Svensen and Kiørboe 2000).

2.1.1 Is Oithona sp. the main pellet degrader?

Despite the fact that *O. similis* seem unlikely to have grazed pellets from the bottom of a bottle in the experiment by Gonzalez and Smetacek (1994), *O. similis* may still be the main component of a ´coprophagous filter´. Supporting this, ingestion of fecal pellets was observed from the grazing incubations (**Paper I**). However, the visual observations of the feeding behavior of *O. similis* contradict that it forms a coprophagous filter, since no observations of launched attacks on pellets were observed during 7 h of analyzed video recordings (**Paper I**).

The pellet ingestion increased when pellet were offered together with *Rhodomonas salina*, indicating *O. similis* were triggered into a feeding mode by the presence of alternative food. In this feeding mode *O. similis* seemed more likely to encounter pellets with pellet fragmentation or ingestion as an outcome (**Paper I**). However, the apparent lack of launced attacks on pellets during the visual observations, despite pellets being within detection distance (Kiørboe and Visser 1999) and observed feeding on *R. salina*, indicates that *O. similis* does not view pellets as suitable food items (**Paper I**). Thus, it does not seem likely that *O. similis* function as a coprophagous filter in the upper ocean. This is supported by **Paper II** and other studies (Poulsen and Kiørboe 2006; Reigstad et al. 2005; Sampei et al. 2004) in which *Oithona* sp. did not seem to impact the pellet degradation. However, *O. similis* may be an indicator species for high pellet degradation regimes, but does not necessarily degrade the pellets themself (Reigstad et al. 2005; **Paper I**).

2.1.2 What is the degradation mechanism and impact from copepods on fecal pellets?

From the visual observations, it was observed that the calanoid copepods did not view fecal pellets as a suitable food item. This was concluded since pellets caught in the feeding current of the copepods were either avoided or rejected (**Paper I**). Pellets were avoided either by pausing the filtration activity whereby the copepod settled away from the pellet, by jumping away from the pellets, or by kicking with their swimming legs and, thus, creating a small water thrust pushing the pellets away. For all avoidance occasions the copepods detected the pellets via chemosensory perception before an actual encounter between copepod and fecal pellet occurred. When the avoidance was performed by pausing the filtration activity and by jumping away, the pellets were left ´unharmed´. However, when avoidance occurred via generation of a water thrust, the pellets were occasionally observed to break apart due to the physical stress from the water current. Rejections of pellets occurred when the pellets reached the feeding appendages of the copepods. The rejections were performed via continues jumps, kicks with the swimming legs, or rapid circular swimming. Often the pellets were fragmented after such rejection episodes (**Paper I**). Actual pellet ingestions were mainly observed when small pellet fragments were caught in the feeding current and ingested, seemingly unintentionally, along with suitable food particles, indicating pellet size as a controlling factor for the pellet ingestion. This lead to the conclusion that the calanoid copepods did not view fecal pellets as suitable food items (**Paper I**). When pellets were offered together with an alternative food source, a feeding response in the copepods was

induces, whereby suspension feeding activity was increased, leading to increased pellet encounters. This indicates that pellets are mainly encountered as a side-effect of feeding activity on other particles (**Paper I**).

The main fecal pellet degradation mechanism was rejection, which occasionally lead to membrane rupture, opening and/or fragmentation of the pellets (**Paper I**). Hence, the main degradation mechanism for calanoid copepods seems via coprorhexy. This has also been observed in other studies (Lampitt et al. 1991; Noji et al. 1991; Paffenhöfer and Van Sant 1985; Poulsen and Kiørboe 2005). **Paper I** and Poulsen and Kiørboe (2005) observed that copepods preying via ambush feeding (*O.similis* and *Acartia tonsa*) have little impact on pellet degradation. It therefore seems that copepods in general have little direct impact on fecal pellet degradation. However, the calanoid filter feeding copepods may have a large indirect impact due to coprorhexy, whereby the pellets may be fragmented. Once fecal pellets are fragmented, their sinking speeds may be reduced and the peritrophic membrane opened. This potentially increases the retention time of pellets in the upper water column, and, thus, increases the time for degradation processes to take place. Further, the smaller pellet size enable grazing from smaller organisms (Fig. 6). Therefore copepods do not seem the main pellet degraders and other organisms must play a crucial role in the pellet degradation in the upper ocean.

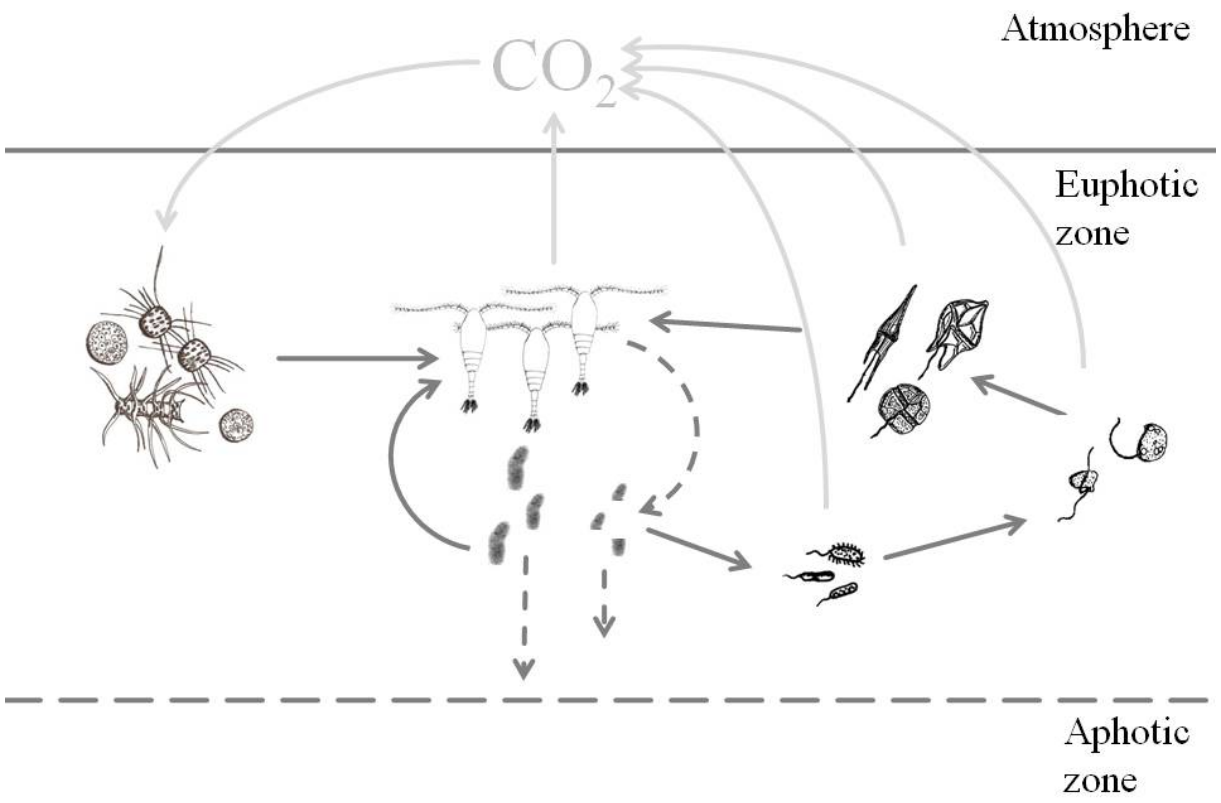

Fig. 6. Modified simple illustration of the role of copepods for the fecal pellet degradation. It is observed that the main role in pellet degradation from copepods is via coprorhexy, fragmenting the pellets into smaller parts. This prolongs their residence time in the euphotic zone, and, thus, increases the impact via the microbial loop (pellet fragments → bacteria → nano-flagellates → heterotrophic dinoflagellates). The heterotrophic dinoflagellates may then be grazed by copepods, adding to the pelagic biomass in the euphotic zone. Note that there no longer exists a coprophagous filter, and that *Oithona similis* do not seem to have a significant role in the degradation of fecal pellets (**Paper I**).

Careful estimations of the ecological impact from copepods on the fecal pellet export can be made by assuming a simple system consisting only of fecal pellets and the two copepods *C. helgolandicus* and *P. elongatus*. In the following estimations the two copepod species are assumed to ´sit´ below fecal pellets which sink towards them. The concentration of sinking fecal pellets is 300 L^{-1}, equivalent to 0.3 pellet per ml (Emerson and Roff 1987). *C. helgolandicus* are assumed to have an abundances of 1 L^{-1}, which is higher than expected *in situ* (Miralto et al. 2003) and the abundance of *P. elongatus* are assumed to be 3 L^{-1}, which is in the high end of *in situ* abundances (**Paper II**). We assume a pellet production rate of 20 pellet d^{-1} from both *C. helgolandicus* and *P. elongatus* (Huskin et al. 2000; Vargas et al. 2002). The encounter rate and feeding behavior is estimated according to the values found for the low concentrations of pellets offered together with an alternative food source (Table 2 and

3, **Paper I**). The suspension feeding activity from Fig. 5 and 6 (**Paper I**) is used as a measure for the filtration rate during an eight hour feeding period. The eight hours illustrate a feeding period from ten o´clock in the evening until dawn (Fig. 7).

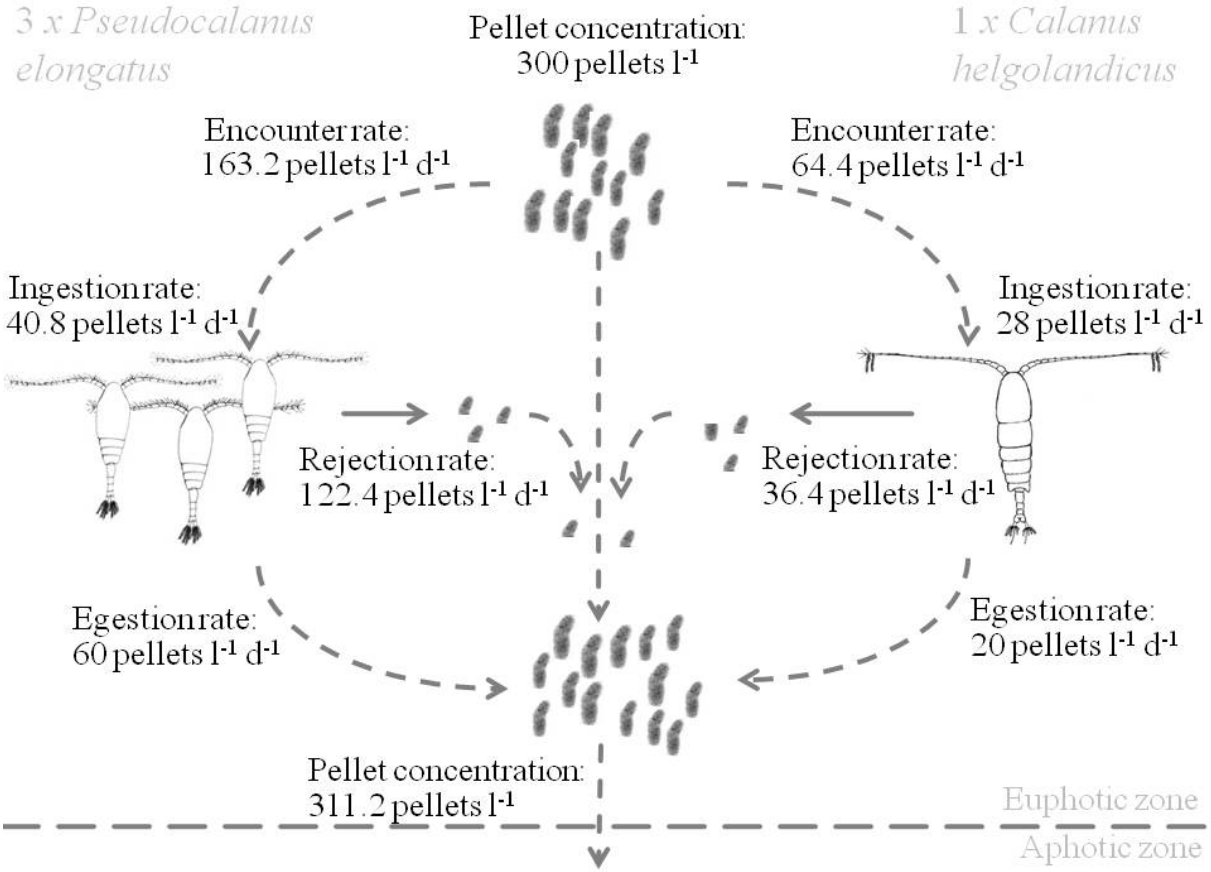

Fig. 7. Simple estimation of impact from copepods on fecal pellet export. Only the two copepod species *Calanus helgolandicus* and *Pseudocalanus elongatus* are considered in this estimation. Pellet encounter rate, ingestion rate, and rejection rate are from **Paper I**. See text above for explanaition. A concentration 300 fecal pellet L^{-1} are sinking towards the copepods. *P. elongatus* encounter 163 pellets and ingest 41 of those pellets while the rest are rejected. *C. helgolandicus* encounter 64 pellets and ingest 28 of those, rejecting 36 pellets. In addition, a total of 80 pellets are produced, assuming that the whole daily pellet production takes place during the 8 h feeding period. As an outcome it is observed the copepods only have little influence on the pellet removal, and may even cause an increase in pellet fluxes.

Despite the simplicity of this estimation (Fig. 7), it illustrates that copepods are unlikely to have a large role in the high recycling of pellets in the upper ocean. It is even seen that copepods potentially may increase the pellet concentration via their pellet production (Fig. 7). Therefore, copepods do not seem to have a direct role in pellet degradation and, thus, other

organisms than copepods may be key players in the pellet degradation, but have been overlooked so far.

2.1.3 Are other organisms than copepods important for degradation of fecal pellets?

To identify the key players responsible for the high retention rate of fecal pellets in the upper ocean, experiments were conducted with incubations of different size fractions of a natural plankton community (**Paper II**). Approximately every second month, throughout one year, the whole plankton community from Øresund (Denmark) was sampled and divided into 5 size fractions (<0.2 µm, <2 µm, <20 µm, <100 µm, and <200 µm) and an unfractionated fraction containing the whole plankton community. The different size fractions were incubated with a known amount of fecal pellets. After incubation the pellet degradation from each size fraction was determined, and the species composition of the plankton for each size fraction identified. This investigation confirmed the finding in **Paper I**, and indicated that copepods, and other large mesozooplankters, in general do not have a significant impact on the pellet degradation (**Paper II**). This has also been found by Poulsen and Kiørboe (2006) in the North Sea, where they indicated plankton organisms <200 µm to have an important role in pellet recycling. **Paper II** was able to narrow the size range containing the key degraders further, and found the major impact on pellet degradation from the microplankton organisms between 20 and 100 µm. Therefore, protozooplankters seemed the likely candidates in pellet recycling, and especially the presence of the heterotrophic dinoflagellate *Gyrodinium spirale* seemed to explain the pellet removal (**Paper II**). Supporting this, *G. spirale* and *Protoperidinium* spp. have been observed to feed on fecal pellets during microscopic observations (pers. com. P. J. Hansen and L. K. Poulsen). Earlier studies have observed protozooplankton associated with fecal pellets, but these observations were interpreted as grazing on the microbes attached to the peritrophic membrane surrounding the pellet (Gowing and Silver 1983).

It may seem surprising that protozooplankton within the size range of 20 to 100 µm can have a huge impact on the fecal pellet removal. *G. spirale* normally have a length ranging between 40 and 200 µm and a width between 20 to 45 µm, which is the same dimensions as most fecal pellets produced by calanoid copepods in the Baltic (e.g., *Temora longicornis*, *Pseudocalanus elongatus*, and *Acartia tonsa*). However, large heterotrophic dinoflagellates have an optimum prey size similar to their own size, i.e., the predator to prey size ratio of 1:1 (Hansen 1992; Jacobsen and Hansen 1997), indicating that they may feed on both pellet

fragments and most intact pellets produced in the Baltic. *Euplotes* sp. which is a benthic ciliate has been observed to degrade fecal pellets at high rates in **Paper II** and in a study by Hansen et al. (1996). However, this does not seem a general behavior from ciliates since most ciliates are smaller than fecal pellets and have an optimum prey size of 1:10, resulting in prey sizes <20 µm (Jacobsen and Hansen 1997).

G. spirale have a pellet clearance rate of 1 ml cell^{-1} d^{-1} (**Paper II**). This may seem rather low, but when considering that abundances of heterotrophic diniflagellates can reach >1000 cells L^{-1} (Nakamura et al. 1995) it becomes likely that protozooplankton may be important pellet degraders and could function as an effective 'protozoan filter' for fecal pellets (**Paper II**). Kiørboe (2003) found a linear relationship between the abundances of the heterotrophic dinoflagellate *Noctiluca scintillans* and specific fecal pellet remineralization rates off the coast of Brazil, and suggested *N. scintillans* to clear the water for pellet at ~0.61 pellets cell^{-1} d^{-1}. During a ship cruise in the North Sea in 2007, *N. scintillans* were measured to clear fecal pellet at rates of 2.84 ± 0.35 pellets cell^{-1} d^{-1} at concentrations of 0.08 fecal pellets ml^{-1} (unpublished data, M. H. Iversen). Microscopic observations often revealed pellets within *N. scintillans* (Fig. 8). Thus, it seems that not only *G. spirale* are able to feed on fecal pellets, but potentially more protozooplankters have adapted to exploit these sinking food packages.

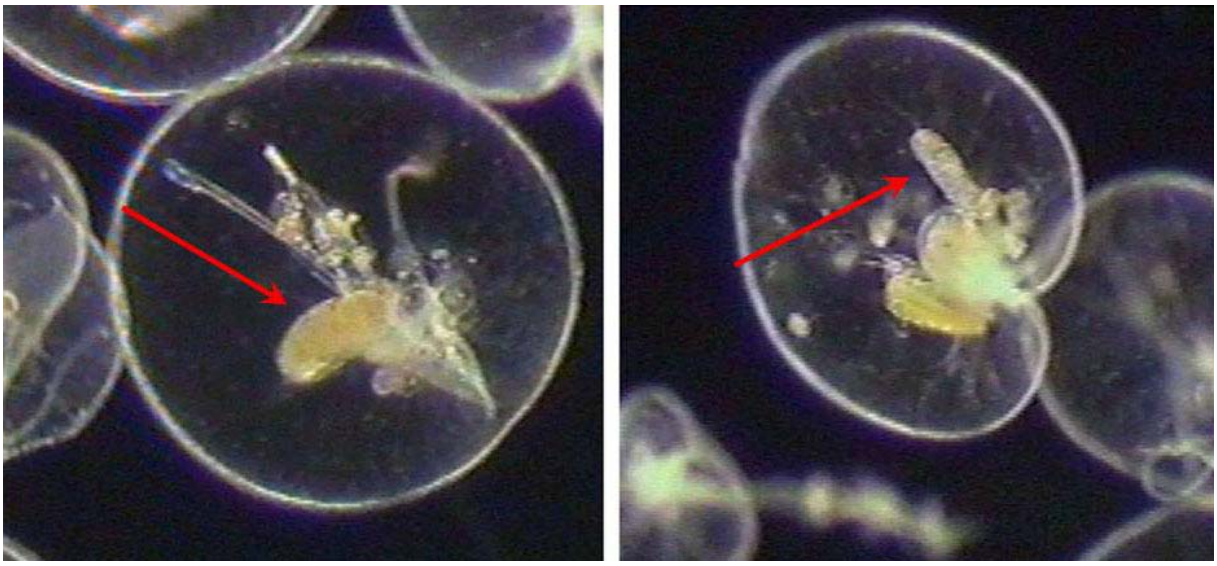

Fig. 8. Microscopic pictures of *Noctiluca scintillans* with a fecal pellet within them. The red arrows indicate the fecal pellets (Photo: M. H. Iversen).

It may be argued that the pellets used in **Paper II** provided more desired food items than *in situ* produced pellets, since the experiments were performed with pellets produced on

excess food concentrations of *Rhodomonas salina*, and therefore likely to contain high amounts of organic carbon. To investigate if the use of *in situ* pellets changes the degradation patterns found between the size fractions of a plankton community offered laboratory produced pellets in **Paper II**, a similar experiment was performed using *in situ* produced pellets and a plankton community from the North Sea (2007) (unpublished data, M. H. Iversen). These investigations supported the importance of plankton organisms <100 µm in the recycling of fecal pellets (Fig. 9), and it seems confirmed that large (>20 µm to <100 µm) protozooplankton organisms are the main pellet degraders. Thus, an effective protozoan filter may prevent pellet export in both the Baltic, off the coast of Brazil, and in the North Sea.

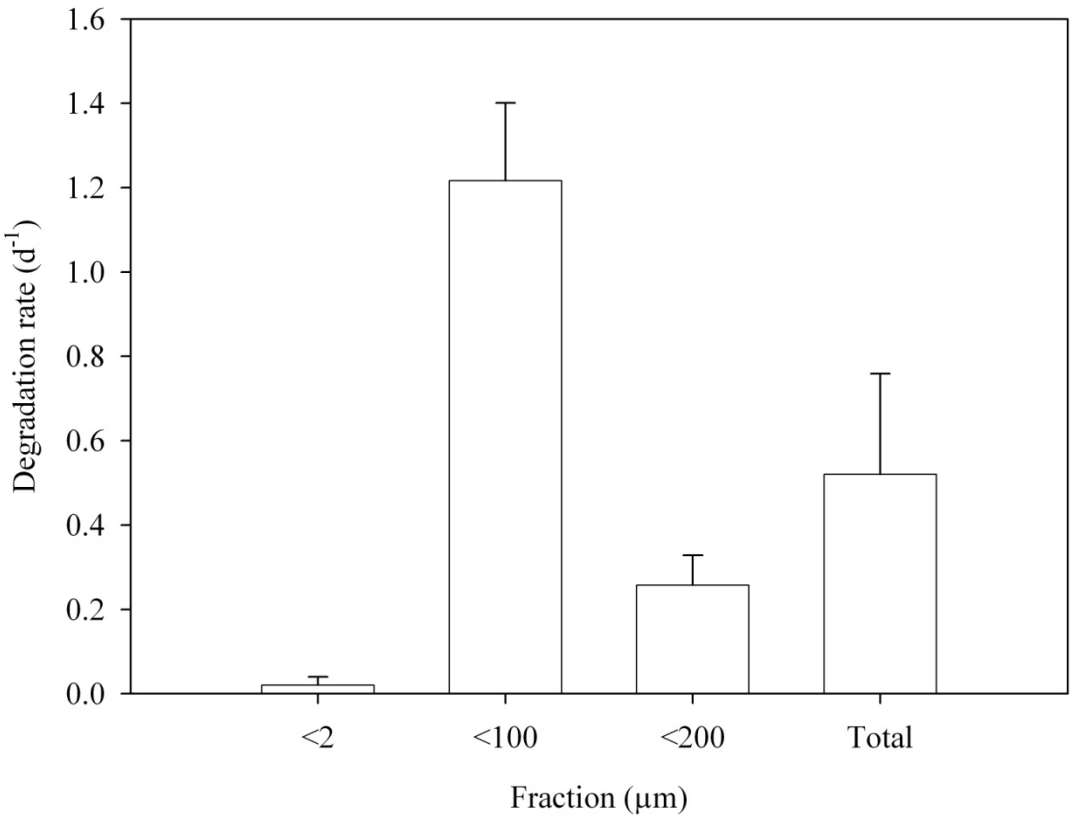

Fig. 9. Fecal pellet degradation rate for each size fraction from a natural plankton community collected in the North Sea during a ship cruise in July 2007. The offered fecal pellets were produced by copepods feeding on the plankton community *in situ*. The pellets were collected by letting the copepods defecate one hour after they were collected with plankton net hauls. These pellets were incubated together with different size fractions of the natural plankton community (Unpublished data, M. H. Iversen).

Often maximum pellet degradation have been observed at bloom conditions, where high concentrations of alternative food sources for copepods are available (Dubischar and Bathmann 2002; Sampei et al. 2004; Urban-Rich 2001). With the introduction of a ´protozoan filter´ instead of a ´coprophagous filter´ (**Paper II**) an explanation to the high pellet

degradation at bloom conditions may be provided, since the biomass of protozooplankton seems closely related to the patterns of phytoplankton biomass (Fig. 3 in **Paper II**). Therefore, the pellet degradation by protozoans is likely to reach its maximum during bloom situations due to the concomitant peak in protozooplankton biomass. The suggestion of a ´protozoan filter´ complicates the role of mesozooplankton in the pellet degradation, since mesozooplankton organisms can increase the pellet degradation via coprorhexy or decrease the pellet degradation via grazing on the protozoan community. The impact of high grazing pressure from copepods on protozooplankton was observed in July 2005 (**Paper II**), and lead to a decrease in pellet degradation rate for the size fraction containing copepods (Fig. 5H in **Paper II**). Finally, the ability of protozooplankton to graze directly on fecal pellets may influence the pelagic biomass since the microbial loop may be short-circuited and the respiration losses from bacteria and nanoflagellates may be avoided in situations with an effective ´protozoan filter´. This provides a more efficient carbon transfer to the copepods grazing on protozoans (Fig. 10).

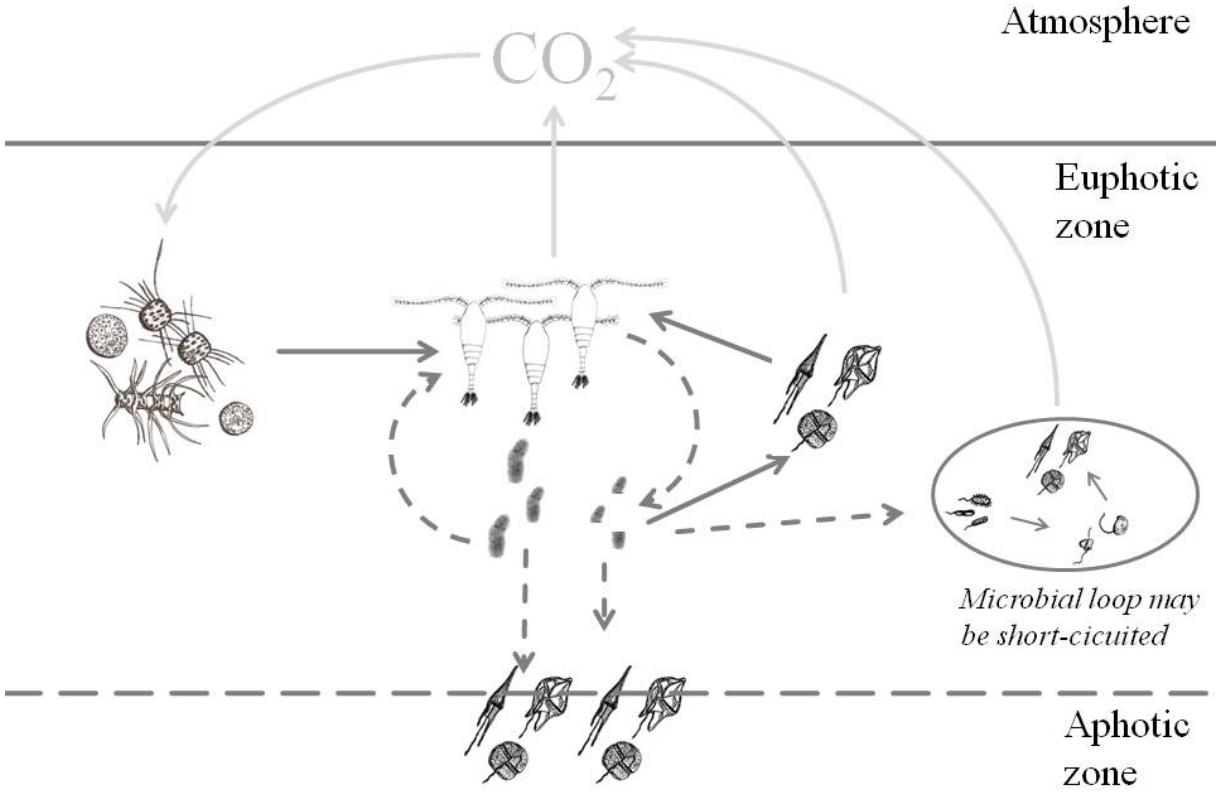

Fig. 9. In this simple illustration, protozooplankton organisms are the main pellet degraders, and form a ´protozoan filter´ preventing high flux rates of fecal pellets. The copepods have a positive influence on the pellet degradation via coprorhexy. Coprorhexy transforms intact pellets into smaller pellet fragments, whereby pellet sinking speeds are reduced and their residence time in the upper ocean increased. The smaller pellet sizes enable small protozoans to graze on the pellets. The copepods also

graze on protozoans and, thus, reduce the impact of the ´protozoan filter´. When directly ingesting fecal pellets, the protozooplankton organisms short-circuit the microbial loop, and provide a food source for mesozooplankters without respiratory losses from the bacteria or the nanoflagellates.

The observations of protozooplankton organisms being the main pellet degraders (**Paper II**) explain why the high turnover rates of organic matter within pellets in the upper ocean are not matched by bacterial activity alone (Jacobsen and Azam 1984; Lampitt et al. 1991; **Paper II; Paper III)**. Organic carbon degradation rates in copepod fecal pellets have been measured to vary between 0.06 and 0.17 d^{-1} (Thor et al. 2003), which agree well with the estimations of microbial degradation rates and respiratory turnover of carbon in pellets found in **Paper II** and **Paper III**. The microbial carbon turnover time is therefore between 6 and 17 days. This would result in larger pellet fluxes from the upper ocean than observed, due to the high sinking speeds of pellets. However, the activity from the bacteria associated with the fecal pellets may still play a significant role in the pellet degradation, since attached bacteria have high ecto-enzymatic hydrolysis activities (Smith et al. 1992; Thor et al. 2003; Ziervogel and Arnosti 2008), which may be closely coupled to their respiration (**Paper III**). The hydrolytic activity forms microenvironments within the pellets, with elevated concentrations of dissolved organic carbon (DOC) relative to the surrounding water (Smith et al. 1992). As the pellets sink, the DOC leaks away from the pellets and forms a plume in its wake extending 10 to 100 fold larger than the volume of the pellet (Kiørboe et al. 2001). It is due to the formation of such plumes that bacteria may play an important role in the high fecal pellet retention in the upper ocean, since zooplankters, protozooplankters, and detached bacteria may use chemosensory behavior to detect the plumes and thereby encounter the pellets at high rates (Kiørboe 2001; Kiørboe et al. 2002). Hereby, higher pellet ingestions by protozooplankters (**Paper II**) and higher pellet fragmentation via coprorhexy (**Paper I**) are promoted. Pellet fragmentation decreases the pellet size, and, thus, their sinking speeds (**Paper III**) and increases the residence time in the upper ocean.

2.1.4 What is the influence from ballast minerals on aggregate sinking speed?

The residence time of fecal pellets in the upper ocean is influenced by the sinking speed of the fecal pellets, affected by physical processes, e.g. turbulence, and biological processes, e.g. coprohexy, in the upper ocean. Such processes often cause mesozooplanktonic fecal pellets to remain in the upper ocean much longer than expected from their sinking speeds (Alldredge et al. 1987). However, a fast-sinking fecal pellet is still more likely to reach the deep ocean than a slow-sinking pellet. To investigate the influence of diet type on sinking

velocity, fecal pellets were produced from nanoflagellates (only organic matter), diatoms (producing opal), and coccolithophorids (producing calcite) (**Paper III**).

When fecal pellets were produced on a diet containing biogenic ballast minerals (e.g. diatoms and coccolithophorids) they had significant higher sinking speeds compared to pellets produced on a diet without these ballast minerals (e.g. nanoflagellates) (**Paper III**). Very dense appendicualrian pellets containing high amount of lithogenic material and carbonate even had sinking speeds <1000 m d^{-1} (**Paper IV**). A significant increase in pellet density was observed when ballast minerals were present as compared to pellets without ballast minerals, indicating pellet density important for sinking speed (**Paper III**). It is therefore indicated that biominerals within fecal pellets can be an important factor controlling pellet sinking speed, and potentially induce 10-fold higher vertical pellet fluxes (**Paper III**).

2.1.5 Can ballast minerals protect organic matter from microbial degradation?

Bacterial degradation rates have been observed to differ with different food sources, leading to higher microbial degradation rates for pellets produced on flagellates as compared to diatoms (Hansen et al. 1996; Lee and Fisher 1992; Thor et al. 2003). **Paper III** showed similar, or even higher, carbon specific respiration rates in fecal pellets produced on coccolithophorids and diatoms compared to carbon specific respiration rates in fecal pellets produced on flagellates. Hence, no evidence was found for higher microbial degradation rates for pellets produced on a flagellate diet compared to a diatom or coccolithophorid diet (**Paper III**). However, the pellets produced on a flagellate diet were two to four times smaller by volume than those produced on diatoms and coccolithophorids, and had higher average oxygen fluxes to the pellets. This resulted in two to six times higher volumetric respiration rates in the flagellate pellets compared to the two other pellet types. Despite the differences in volumetric respiration rates between the pellet types, we did not observe differences in the carbon specific respiration rates. Hence the higher volume-specific O_2 uptake reflected high volume-specific carbon content in the flagellate pellets compared to the two other pellet types, and there was a tight coupling between respiration rate and carbon content of the pellets. Therefore, ballasting did not seem to protect freshly produced pellets against microbial degradation (**Paper III**).

The findings in **Paper III** demonstrate that sinking speeds of fecal pellets were more affected by the presence of biominerals than the specific carbon remineralization rates were. It therefore appeared that ballast minerals (e.g., carbonate and opal) are important in controlling the vertical carbon fluxes in the ocean. The promotion of carbon preservation in the presence

of biominerals was due to increasing sinking speeds of the pellet, caused from their higher densities when produced on phytoplankton containing biominerals (**Paper III**) and/or on lithogenic material (**Paper IV**). Within freshly produced pellets, no evidence for protection of the organic matter by the presence of biominerals was found. Further, it was shown that biominerals have little influence on apparent diffusivity within the fecal pellets (**Paper IV**). In contrast, the potential oxygen flux to the pellets may increase as they settle due to the increased sinking speeds. Thus, the presence of biominerals in pellets may even cause a more efficient turnover of labile carbon at depths, since they maintain an oxic environment inside the pellets at great depths (**Paper IV**).

In summary, the main role of copepods in the pellet degradation seems to be fragmentation of the pellets via coprorhexy. This decreases the sinking speeds of the pellets and, thus, increases their residence times in the upper ocean. Further, the small pellet size makes them available for smaller grazing organisms. The main pellet degraders are protozooplankters which form an effective protozoan filter removing the pellets from the vertical POC flux. This protozoan filter decreases the respiratory losses by short-circuiting the microbial food web. The main role of the bacterial remineralization seems to lie in the formation of a DOC plume in the wake of the pellets, providing a signature trail behind them and, thus, increasing the encounter rates from grazers detecting their prey via chemosensory behavior. Incorporation of biominerals produced by diatoms and coccolithophorids and/or lithogenic materials can increase the sinking speeds of fecal pellets, but does not protect freshly produced pellets from decomposition. Hereby, either fast-sinking or slow-sinking fecal pellets may be produced, depending on the type of phytoplankton the pellets are produced from. Fast sinking fecal pellets may be transformed into slow sinking pellets via coprorhexy from the copepods. The ballasting of the fecal pellets may potentially increase the vertical flux of fecal pellets (Fig. 10).

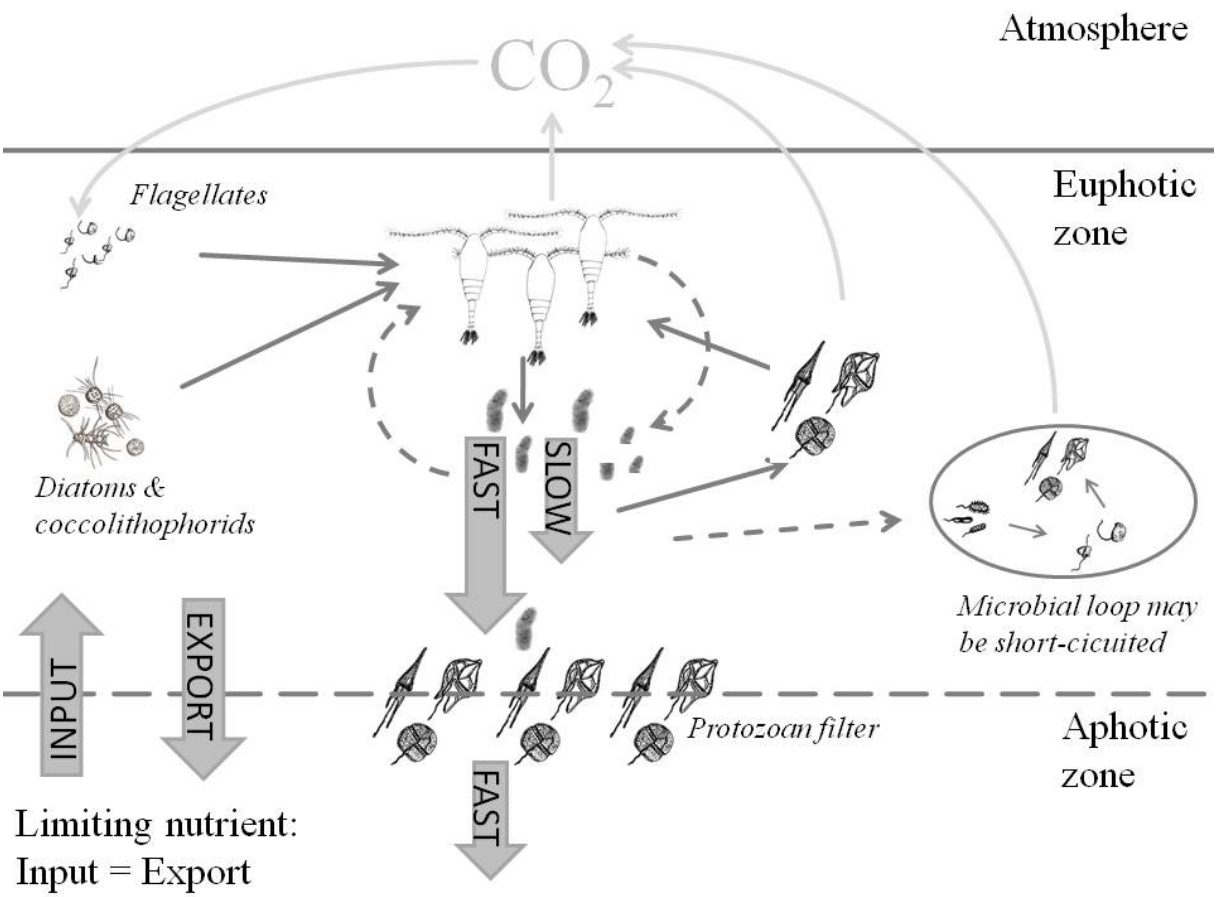

Fig. 10. Simplified system only considering fecal pellet processes. It is assumed that the downward export of limiting elements equals their input. The degradation and remineralization of fecal pellets lead to recycling of the limiting elements in the euphotic zone. This may increase the phytoplankton biomass and thereby increase the copepod biomass and fecal pellet production. The copepods fragment pellets via coprorhexy, and, thus, increase their residence time in the upper ocean and enable small grazers to feed on the pellets. Protozooplankters form an effective protozoan filter which remove pellets from the vertical POC flux and decrease the respiratory losses by short-circuiting the microbial food web. Bacterial remineralization can form a DOC plume in the wake of the pellets. This DOC plume acts as a signature trail behind the pellets. Hereby encounter rates from grazers may be increased via chemosensory behavior. If the fecal pellets are produced on phytoplankton containing biominerals (e.g., diatoms and coccolithophorids) their sinking speeds are likely high compared to pellets produced on phytoplankton without biominerals (flagellates). Thus, biominerals from diatoms and coccolithophorids may increase the vertical flux of fecal pellets due to the ballasting of the pellets. However, if the fast-sinking pellets are fragmented via coprorhexy they may add to the pool of slow-sinking pellets.

2.2 Carbon turnover in aggregates

As observed in figure 4 there is a rapid decrease in carbon flux in the upper few hundreds of meters of the surface ocean (see introduction, 1.4). This strongly indicates that investigations of aggregate associated processes in the upper ocean are very important in

understanding the controls on the efficiency of particle transport between the surface and the deep ocean. The discussion of fecal pellet fluxes already illustrated the complexity of the processes controlling export and/or retention of pellets (see discussion, 2.1). Despite the fact that pellets are easier to handle in experiments and collect *in situ* due to their more robust nature compared to marine snow and phytoplankton-derived aggregates, the process leading to the current understanding of the role of pellets in the POC flux has a long history and was already reviewed three decades ago (Turner and Ferrante 1979). The role of particulate organic matter in microbial decomposition and recycling of nutrient has been studied the last four decades, but the intense study of marine snow and phytoplankton-derived aggregates only started in the 1990s (see Simon et al. 2002). Still, more questions are often raised than answered, and even for the role of fecal pellets in the vertical export recent studies has called for a major revision of our understanding (e.g., **Paper II**).

To identify regulating processes for retention and export of marine snow and phytoplankton-derived aggregates in the surface ocean a series of experiments were conducted. These experiments included analysis of the influence from biominerals on sinking speed, apparent diffusivity, and small scale oxygen fluxes to sinking aggregates. Further, estimated vertical carbon fluxes between the surface ocean and 2500 m depth were used to identify important degradation processes at different depths intervals. In the following discussion I will focus on controlling processes for marine snow and phytoplankton-derived aggregates in the vertical carbon flux. However, when appropriate I will include the role of fecal pellets in the vertical flux.

2.2.1 Can ballast minerals protect aggregates from microbial degradation?

Concentrations of solutes within marine snow are significantly different from those of the surrounding water (Alldredge 2000; Alldredge and Cohen 1987; Brzezinski et al. 1997; Ploug et al. 1999). These concentration differences form steep gradients of gases, nutrients, and other solutes in marine snow and have led to the speculations that diffusion may be slow within these aggregates (Alldredge 2000; Brzezinski et al. 1997; Shanks and Reeder 1993). However, a recent study by Ploug and Passow (2007) showed that apparent diffusivity of gasses within diatom aggregates was close to that of (stagnant) seawater. This was explained by high water content of the aggregates. Other studies have shown that mass transfer in aggregates appears to be dominated by diffusion (Ploug 2001; Ploug et al. 1999; Ploug et al. 2002), indicating the advective flow within aggregates very low or absent (Ploug and Passow

2007). The high volume fraction of TEP may limit advective flow rather than diffusion rates within marine snow (Ploug and Passow 2007). The apparent diffusivity in a wide range and types of porous marine snow and phytoplankton-derived aggregates showed only slightly lower values than that in (stagnant) sea water (**Paper IV**). Further, apparent diffusivity within large fecal pellets with a high volume-specific content of carbonate and lithogenic material was only approximately 25% lower than that in sea water, indicating high content of biominerals in aggregates may limit advection rather than diffusion within aggregates (**Paper IV**). Thus, the presence of biominerals in aggregates did not show evidence for protection mechanisms against degradation of labile organic matter that might result from a lower diffusivity (**Paper IV**). In fact, ballasting of aggregates may increase the total mass transfer of oxygen to the sinking aggregates (**Paper IV**), via increased sinking speed of ballasted aggregates (**Paper III**; **Paper IV; Paper V**).

Large variations in oxygen consumption across the aggregate size spectrum are observed both for fecal pellets, marine snow, and phytoplankton-derived aggregates (**Paper III**; **Paper IV**; **Paper V**). However, the respiration rate from the microbial community associated with the aggregates seems closely connected to the content of aggregated POC. This leads to relatively similar carbon specific respiration rates for different types and sizes of aggregates (**Paper III**; **Paper IV**), and compares well with previous studies of *in situ* diatom aggregates (Ploug et al. 1999) and aggregates formed on diatom detritus (Ploug and Grossart 2000). The influence of biominerals on the carbon specific respiration rate was investigated in pure diatom aggregates, in pure coccolithophorid aggregates, and in aggregates formed from a mixture of both diatoms and coccolithophorids. These measurements confirmed a tight coupling between respiration rate and POC content of the aggregates, and the average carbon specific respiration rate was similar to those measured for the aggregates in other studies (Table 2) (unpublished data, M. H. Iversen and H. Ploug). Thus, it seems that carbon specific respiration rates are relatively similar within fecal pellets, marine snow, and photyplankton-derived aggregates irrespective of biogenic or lithogenic ballast material content, origin, and size (**Paper III**; **Paper IV**; **Paper V**; unpublished data, M. H. Iversen and H. Ploug). The carbon specific respiration rate ranged between 0.08 and 0.21 d^{-1} and averaged ~0.13 d^{-1} (Table 2). These rates were measured in relatively fresh aggregates with high hydrolysis rates and high apparent diffusivities of solutes and oxygen supply for respiration (**Paper IV**). Thus, the microbial community within the aggregates was not limited by oxygen availability and an efficient carbon turnover was supported (**Paper IV**). Since all aggregate types and sizes seem to be degraded at similar specific rates, the extent of carbon remineralization in the upper

ocean may be controlled by the residence times of the aggregates at these depths. Therefore, sinking speeds of marine snow and phytoplankton-derived aggregates may be a controlling factor for organic carbon export, as observed for fecal pellets.

Table 2. Carbon specific respiration rates of aggregates (agg) and fecal pellets (pellets) of different type, composition, and origin from five different studies. F-max indicates aggregates formed from water collected at the depth of fluorescence maximum off Cape Blanc. Unpub. indicates unpublished data.

Aggregate composition (type of aggregate)	C-specific resp. rate (d^{-1})	Reference
F-max water Cape Blanc (agg)	0.13 ± 0.07	**Paper V**
Skeletonema costatum (agg)	0.13 ± 0.09	Unpub. Iversen and Ploug
E. huxleyi (agg)	0.13 ± 0.13	Unpub. Iversen and Ploug
S. costatum & *E. huxleyi* (agg)	0.12 ± 0.07	Unpub. Iversen and Ploug
Rhodomonas sp. (pellets)	0.16	**Paper III**
T. weissflogii (pellets)	0.20	**Paper III**
T. weissflogii (pellets)	0.12	**Paper III**
E. huxleyi (pellets)	0.21	**Paper III**
E. huxleyi (pellets)	0.08	**Paper III**
Diatoms & <80 µm Øresund water (agg)	0.08 ± 0.03	Ploug and Grossart 2000
In situ collected (California) (agg)	0.10 to 0.12	Ploug et al. 1999

2.2.2 What is the influence from ballast minerals on sinking speed?

To investigate if phytoplankton-derived aggregates experience the same elevated sinking speeds when containing biominerals as observed for fecal pellets in **Paper III**, sinking speeds were measured for aggregates formed from diatoms, from coccolithophorids, and from a mixture of both diatoms and coccolithophorids (Fig. 11) (unpublished data, M. H. Iversen and H. Ploug).

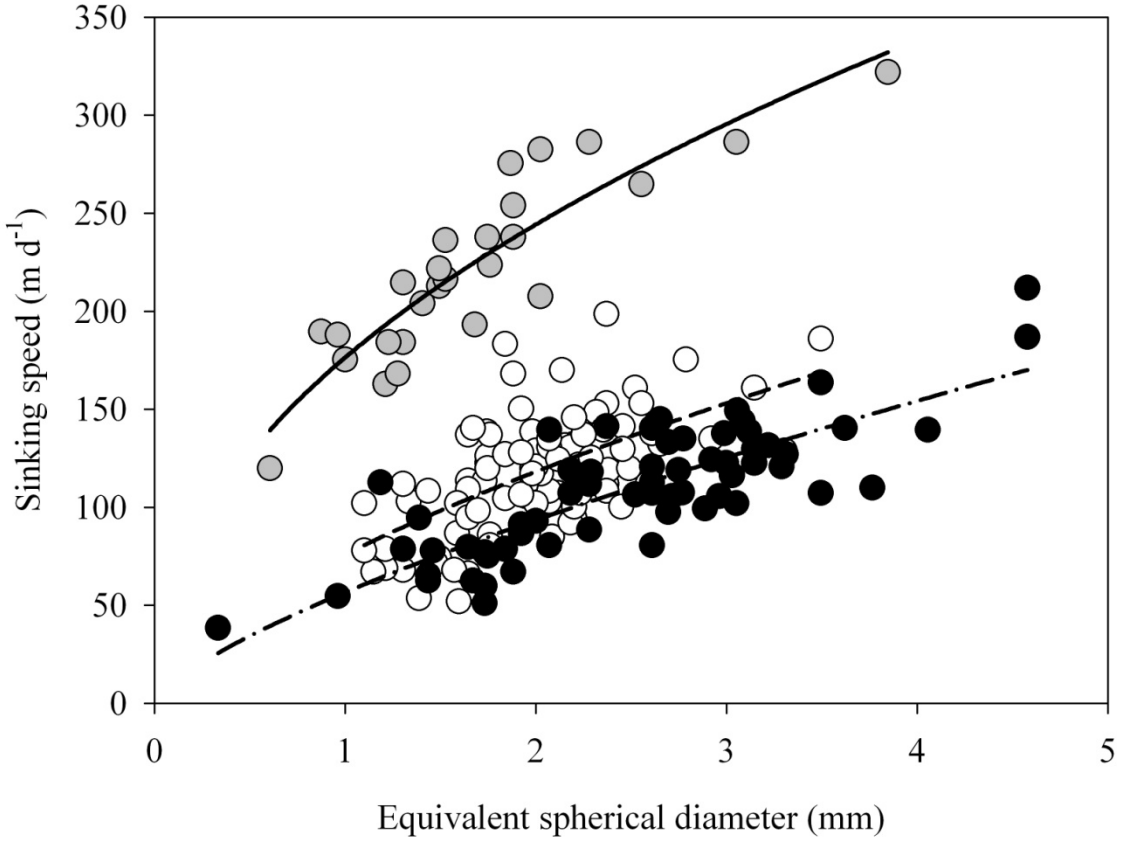

Fig. 11. Sinking speeds as a function of aggregate size for aggregates formed from *Skeletonema costatum* (black circles), *Emiliania huxleyi* (grey circles), and a mix of *S. costatum* and *E. huxleyi* (white circles). The lines represent a power fit to the measurements. Solid line (*E. huxleyi*): $y = 176.3x^{0.47}$, $R^2 = 0.8$; dashed line (mix of *S. costatum* and *E. huxleyi*): $y = 75.8x^{0.64}$, $R^2 = 0.28$; and dashed and dotted line (*S. costatum*): $y = 56.6x^{0.72}$, $R^2 = 0.65$.

The measurements in Fig. 11 show higher sinking speeds for aggregates formed from coccolithophorids as compared to aggregates formed from either diatoms or a mix of diatoms and coccolithophorods. The elevated sinking speeds were explained by higher densities of the coccolithophorid aggregates. Two factors lead to higher densities in the coccolithophorid aggregates; 1) a high content of calcium carbonate produced by the coccolithophorids, and 2) the formation of more compact aggregates compared to aggregates containing diatoms since the coccolithophorid aggregates mainly contained the small sized coccoliths (~2 µm). When diatoms were present in the aggregates, spines from the diatoms maintained a more porous aggregate structure which resulted in less compact, and, thus, less dense aggregates (unpublished data, M. H. Iversen and H. Ploug).

Sinking speeds of aggregates depend on aggregate composition and density. Thus, similar sized aggregates with different compositions may have very different sinking speeds. In **Paper IV** the density of similar composed aggregates increased as they aged, leading to elevated sinking speeds over time for individual aggregates. This may be explained by a decrease in TEP to dry mass ratio in aging aggregates (Ploug and Passow 2007), likely due to degradation of TEP. This may also explain observations of increasing particle sinking speeds with depth in the ocean (Berelson 2002), but direct observations are still needed.

Measurements of high sinking speeds for aggregates formed off Cape Blanc (**Paper V**) illustrates the difficulties of predicting sinking speeds from aggregate sizes alone. The sinking speeds of aggregates off Cape Blanc (**Paper V**) were 5-10 times higher than previous measurements for similar sized aggregates (Alldredge and Gotschalk 1988; Ploug et al. 1999). This seemed due to high a content of carbonate and lithogenic material within the aggregates off Cape Blanc, resulting in higher size specific densities as compared to previous measurements (Alldredge and Gotschalk 1988). Therefore, ballasting of aggregates appears to have a large influence on the sinking speeds of fecal pellets, marine snow, and phytoplankton-derived aggregates, but hardly any effect on their carbon specific respiration rates and apparent diffusivities. Thus, biominerals and lithogenic material seem an important factor controlling aggregate sinking speed and, therefore, vertical carbon fluxes in the ocean (**Paper III**; **Paper IV**; **Paper V**).

Though, the presence of biominerals and lithogenic material is indicated as an important factor for the sinking speeds of aggregates, it does not simplify the estimations of aggregate sinking speeds *in situ*. Illustrating this, the small sized fecal pellets investigated in **Paper III** showed comparable sinking speeds to the much larger marine snow and phytoplankton-derived aggregates (Fig. 12). From Fig. 12 it is also observed that sinking speeds of similar-sized aggregates can vary greatly. Thus, sinking speeds of aggregates depend on source, density, and age rather than size (**Paper IV**). Therefore, we have to deal with similar-sized aggregates sinking at different speeds and/or different aggregate sizes sinking at same speed, when attempting to predict carbon fluxes. Further, when measuring sinking speeds in laboratories the potential maximum sinking speeds are observed, since aggregates *in situ* potentially will have much longer residence times in the upper ocean (Alldredge and Gotschalk 1988).

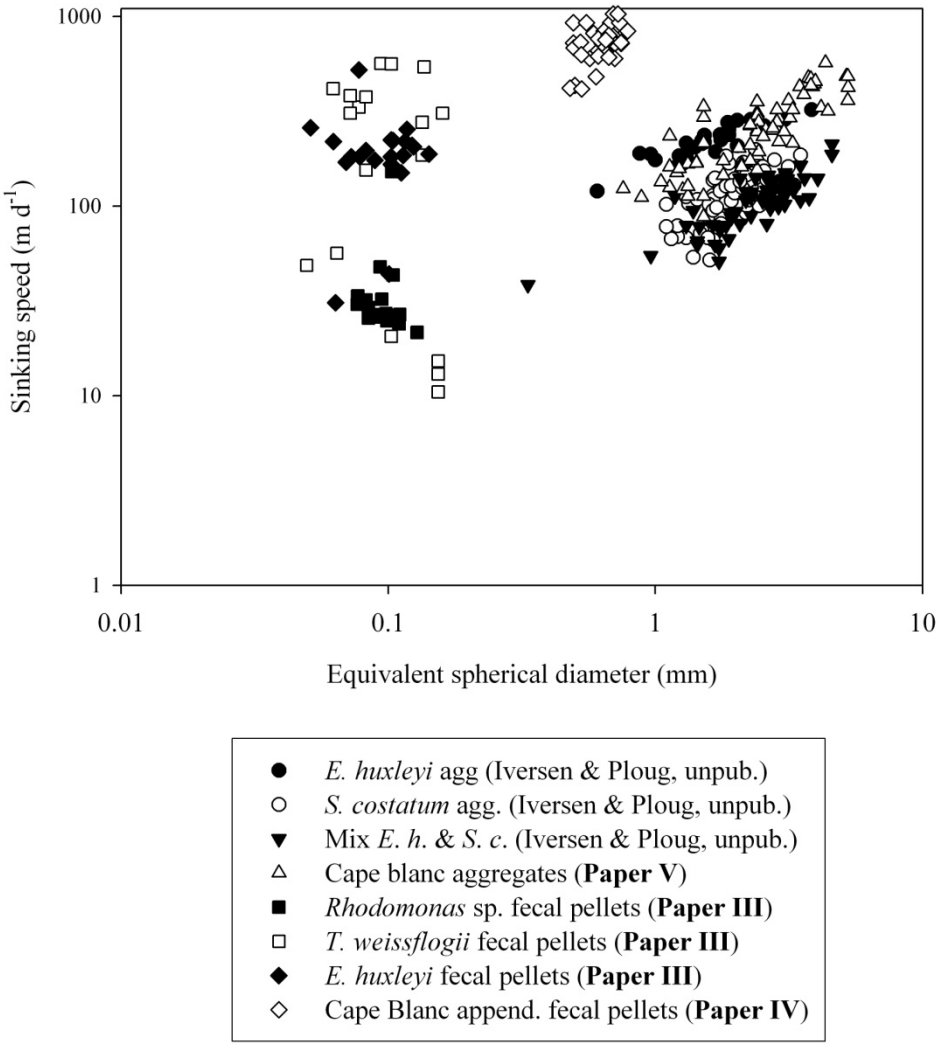

Fig. 12. Sinking speed as a function of aggregate size for a wide range of fecal pellets, marine snow, and phytoplankton-derived aggregates. See legend box for further information about the different aggregates.

2.2.3 What is the relative contribution from zooplankton organisms and microbes to carbon removal, and how is this relationship at different depths?

To investigate the relative importance of zooplankton organisms and microbes for the vertical flux down through the water column, carbon flux profiles were calculated and used to identify important depths for carbon removal. The main carbon removal occurred in the upper 220 m and seemed divided into two important processes; one between 20 and 80 m and one between 80 and 220 m. At greater depths only little carbon removal was observed (**Paper V**). The upper 20 to 80 m was dominated by carbon removal from mesozooplankton organisms (135 ± 69 µgC m^{-3} d^{-1}) in combination with bacterial respiration and hydrolysis (60 ± 48 µgC m^{-3} d^{-1}). Below 80 m and down to 220 m the impact from zooplankton organisms seemed

absent and the carbon removal could be explained by the bacterial activity. The estimated production of DOC from attached bacterial could not equal the measured respiration rates from the free-living microbes and, thus, alternate DOC production may also have fed the free-living bacteria; e.g., leakage and/or exudation from algae (Lignell 1990), release from zooplankton feeding (Jumars et al. 1989; Møller et al. 2003), and diffusion out of fecal pellets (Jumars et al. 1989). At depths between 220 m and 2500 m very low carbon removal rates (2.5 ± 3.2 µgC m^{-3} d^{-1}) were observed and presumably resulted from bacterial activity. However, the low rates indicated that the bacterial activity is limited, i.e., due to temperature decrease, decrease in bacterial abundance or activity, detachment of bacteria, etc. (**Paper V**). Fig. 13 presents a simple overview of estimated aggregated carbon fluxes and estimated carbon removal rates found during a ship cruise off Cape Blanc (**Paper V)**.

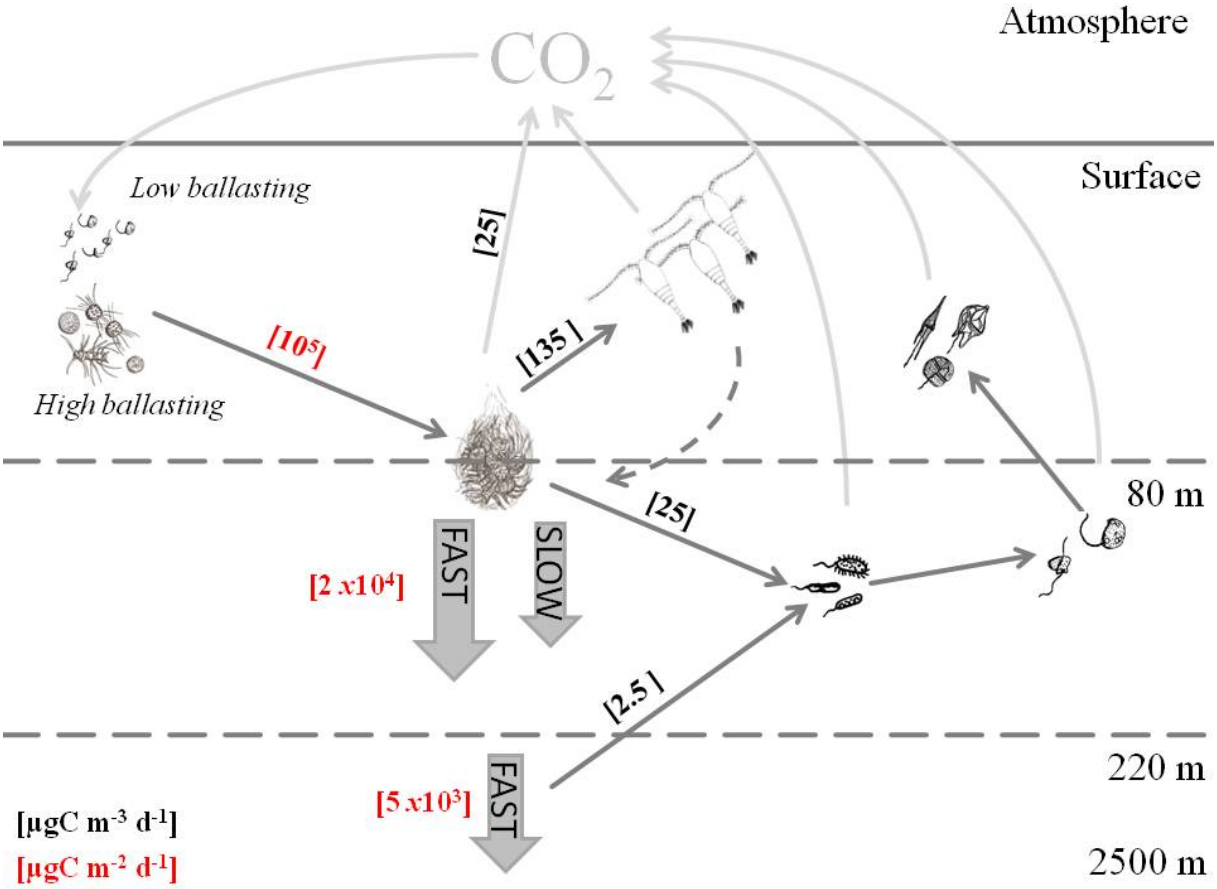

Fig. 13. Simple overview based on the estimated carbon fluxes and carbon removal rates found for aggregated carbon during the cruise off Cape Blanc (**Paper V**). It is observed that two types of marine snow aggregates can be formed; fast-sinking ballasted aggregates and slow-sinking non-ballasted aggregates. In the upper 80 m of the water column mesozooplanktonic activity will dominate the carbon removal rates either via direct consumption of the aggregated carbon or by disaggregation whereby fast-sinking aggregates can be converted to slow-sinking aggregates. Microbial respiration and hydrolysis is rather high in the upper 220 m of the water column, and thus transform particulate organic carbon (POC) into dissolved organic carbon (DOC) and CO_2. DOC can feed the free-living

bacteria and, thus, the microbial food web. However, it can also increase the encounter rates between chemosensory microbes and zooplankters via formation of a DOC plume in the wake of sinking aggregates. At greater depths than 220 m the microbial carbon removal decreases to very low levels. The black numbers indicate carbon removal rates (µgC m^{-3} d^{-1}). The red numbers indicate carbon fluxes (µgC m^{-2} d^{-1}). Both fluxes and removal rates are only given as rough estimates; see **Paper V** for more detailed information.

In summary, mesozooplankton organisms seem to dominate carbon removal in the upper water column. In the deeper midwater zones microbial biota become more important due to decreasing abundances of zooplankton organisms. However, the microbial degradation seems limited at greater depths (**Paper V**). Similar tendencies for the relative importance of zooplankton and microbes at different depths have been suggested by Stemmann et al. (2004). Despite mesozooplankters have two times higher carbon turnover rates compared to the microbial activity in the upper 80 m, the bacteria still account for twice as much carbon removal per day compared to mesozooplankton, when considering the full depth range from 20 to 2500 m. The low carbon removal rates estimated below the upper 220 m indicate that once the sinking carbon has traversed the upper few hundred meters it is likely to settle to the deep ocean where it can be sequestered. Therefore, a short residence time in the upper ocean seems important for carbon export and, thus, ballast minerals may be an important factor controlling aggregate sinking speed and carbon export in the ocean.

3 CONCLUSIONS

Fecal pellets and marine snow remain much longer in the surface water than expected from their sinking speeds alone. This is due to a series of complex retention processes taking place in the surface ocean. The export of fecal pellets is retained by rapid degradation from protozooplankton, fragmentation from copepods, and microbial decomposition. Marine snow can disaggregate due to fragmentation by zooplankton, whereby their sinking speeds are reduced. Bacteria associated with marine snow and fecal pellets are characterized by high hydrolytic enzyme activities (Smith et al. 1992; Thor et al. 2003) forming microenvironments with elevated concentrations of DOC relative to those of the surrounding water. These microenvironments extent 10 to 100 fold larger than the volume of sinking aggregates (Kiørboe et al. 2001). This enables organisms with chemosensory abilities to find and encounter the aggregates (Kiørboe 2001; Kiørboe et al. 2002) whereby aggregates are colonized at high rates leading to a significant carbon turnover of the aggregates in the surface ocean. All these processes result in long residence times in the upper ocean and work against vertical carbon export, indicating ballasting of aggregates as a main factor controlling carbon export, due to its potential to increase the sinking speeds of aggregates and, thus, reduce the residence times of aggregates in the surface ocean.

4 OUTLOOK

Though, this thesis provides some estimates of surface ocean degradation and export processes there are still many unanswered questions. We know little about which large plankton organisms feed on marine snow aggregates. We are still missing direct estimates of the metazoan aggregate degradation and how this may change seasonally and spatially. The relative importance of zooplankton organisms and microbes for aggregate degradation found in this thesis also call for more direct measurements and investigations of how such relationships change with composition of pelagic food webs. Understanding such processes and knowing how they change with changing environments are essential for the understanding of carbon export from the surface ocean. Additionally we need to obtain a good understanding of the deep water aggregate processes before we can make realistic estimates of the efficiency of the biological carbon pump and the sequestration of carbon in the deep ocean. For instance, how does aging of aggregates influence the sinking speed and degradation rates? Is it possible that ballast minerals can protect ´old´ aggregates from degradation and which part of the organic matter would then be protected?

I have listed some of the questions that have puzzled me during the progress of this Ph.D. work:

- *How important is the degradation impact in the euphotic zone for the aggregate fluxes in the deep ocean?*

- *To what extent does aggregate transformation occur down through the water column, and which processes are important; e.g., repackaging, disaggregation, re-aggregation, consumption, degradation, and solubilization?*

- *How does the changing community structure in different environments affect aggregate fluxes?*

- *Does sedimentation proceed mainly by short episodic events or as continuous settling during the productive period?*

- *How does season and region affect aggregate fluxes and the organisms important for aggregate degradation?*

- Are attached bacteria mainly degrading aggregated carbon via respiration or solubilization?

- How does bacterial activity affect aggregate size, density, settling rate, and stickiness?

- Does bacterial activity and community change with depth?

- Does aggregate sinking speed change with depth, and if, why?

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Coprorhexy, coprophagy, and coprochaly in the copepods *Calanus helgolandicus*, *Pseudocalanus elongatus*, and *Oithona similis*

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ABSTRACT: Studies of fecal pellet flux show that a large percentage of pellets produced in the upper ocean is degraded within the surface waters. It is therefore important to investigate these degradation mechanisms to understand the role of fecal pellets in the oceanic carbon cycle. Degradation of pellets is mainly thought to be caused by coprophagy (ingestion of fecal pellets) by copepods, and especially by the ubiquitous copepods *Oithona* spp. We examined fecal pellet ingestion rate and feeding behavior of *O. similis* and 2 other dominant copepod species from the North Sea (*Calanus helgolandicus* and *Pseudocalanus elongatus*). All investigations were done with fecal pellets as the sole food source and with fecal pellets offered together with an alternative suitable food source. The ingestion of fecal pellets by all 3 copepod species was highest when offered together with an alternative food source. No feeding behavior was determined for *O. similis* due to the lack of pellet capture in those experiments. Fecal pellets offered together with an alternative food source increased the filtration activity by *C. helgolandicus* and *P. elongatus* and thereby the number of pellets caught in their feeding current. However, most pellets were rejected immediately after capture and were often fragmented during rejection. Actual ingestion of captured pellets was rare (<37% for *C. helgolandicus* and <24% for *P. elongatus*), and only small pellet fragments were ingested unintentionally along with alternative food. We therefore suggest coprorhexy (fragmentation of pellets) to be the main effect of copepods on the vertical flux of fecal pellets. Coprorhexy turns the pellets into smaller, slower-sinking particles that can then be degraded by other organisms such as bacteria and protozooplankton.

KEY WORDS: Fecal pellet · Grazing · Functional response · Visual observation · Video recording

INTRODUCTION

Fecal pellets in the sea are degraded by bacteria (Cho & Azam 1988) and possibly by zooplankton such as copepods (Paffenhöfer & Strickland 1970). Copepods degrade pellets through coprophagy (ingestion of pellets), coprorhexy (fragmentation of pellets), and coprochaly (loosening of pellets) (Paffenhöfer & Strickland 1970, Lampitt et al. 1990, Noji et al. 1991). Coprophagy is the best documented of the degradation processes. Paffenhöfer & Strickland (1970) observed *Calanus helgolandicus* ingesting fecal pellets even in the presence of algal food. This ability was also shown for *C. helgolandicus* nauplii (Green et al. 1992). Further, grazing rates of fecal pellets have been obtained for many other species of copepods; for example, *Acartia omorii* ingests fecal pellets at high rates (Viitasalo et al. 1999), and *Eucalanus pileatus* consumes nauplii fecal pellets at the same rate as algae of similar size (Paffenhöfer & Knowles 1979). Coprorhexy was suggested by Lampitt et al. (1990), who observed copepods to be highly adept at breaking up their own fecal

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pellets while only ingesting small fragments. Noji et al. (1991) observed coprochaly in *Acartia clausi, C. helgolandicus,* and *Pseudocalanus elongatus*.

Although these studies have indicated the existence of fecal pellet grazing behavior by copepods, only 2 studies have visually observed feeding behavior, in *Acartia tonsa* (Poulsen & Kiørboe 2005) and *Eucalanus pileatus* (Paffenhöfer & Van Sant 1985). Visual observations of the feeding behavior of *A. tonsa* grazing on fecal pellets suggested that *A. tonsa* mainly degrades fecal pellets via coprorhexy (95%; Poulsen & Kiørboe 2005). Paffenhöfer & Van Sant (1985) observed *E. pileatus* ingesting fewer fecal pellets than live and dead phytoplankton cells, indicating a preference for food particles other than pellets. Furthermore, pellets arriving at the mouth were opened or slightly macerated before ingestion when rejection did not occur (Paffenhöfer & Van Sant 1985), indicating coprorhexious feeding behavior for *E. pileatus*.

We investigated the feeding behavior of *Calanus helgolandicus, Oithona similis*, and *Pseudocalanus elongatus* on copepod fecal pellets produced by *Acartia tonsa* feeding on *Rhodomonas salina*. This was done through simultaneous incubation experiments and video recordings of feeding behavior in the presence and absence of alternative food. The species investigated in this study were chosen due to their differences in size and feeding behavior, and due to their common presence in temperate areas. *C. helgolandicus* and *P. elongatus* are suspension feeders that detect their food via chemosensory perception using a feeding current while cruising slowly through the water (Andrews 1983). *O. similis* is an ambush feeder that 'hangs' in the water and only launches an attack when a prey particle is detected via hydromechanical disturbances generated by the prey (Kiørboe & Visser 1999, Svensen & Kiørboe 2000).

MATERIALS AND METHODS

Phytoplankton. *Thalassiosira weissflogii* (a diatom), *Rhodomonas salina* (a cryptophyte), and *Heterocapsa triquetra* (a dinoflagellate) were maintained in exponential growth in f/2 medium (Guillard 1975). The cultures were kept at a constant light:dark cycle (12:12 h) in filtered (0.2 µm) seawater (~33‰) at 18°C and used as food for zooplankton cultures. Only *R. salina* (9 × 12 µm in size) was used as a food item during the production of fecal pellets.

Zooplankton. *Calanus helgolandicus* females were collected in October 2004 and August 2005 in the north-eastern North Sea and kept in culture in filtered (0.2 µm) seawater (~33‰) at 14°C, reared on a mixed diet of *Rhodomonas salina*, *Thalassiosira weissflogii*, and *Heterocapsa triquetra*. *Oithona similis* was collected in November 2004 in The Sound (Øresund, Denmark) and kept in natural seawater from The Sound (~22‰) at 12°C. *Pseudocalanus elongatus* was collected in August 2003 in the North Sea, kept in culture in filtered seawater (~33‰) at 14°C, and reared on a mixed diet of *R. salina*, *T. weissflogii*, and *H. triquetra*. *Acartia tonsa* was kept in a laboratory culture in filtered (0.2 µm) seawater (~33‰) at The Danish Institute for Fisheries Research at 18°C and reared on *R. salina* (Støttrup et al. 1986). All copepods were acclimated to the experimental conditions for >24 h prior to each experiment (Table 1). Fecal pellets offered as food for the copepods in both grazing and behavior experiments were produced by adult *A. tonsa* grazing on *R. salina* at excess concentration (>713 µg C l^{-1}; Kiørboe et al. 1985) for <18 h. This simulated pellets produced under bloom conditions or when the copepod locates a food patch such as a subsurface bloom. Such pellets may be attractive food particles due to high pellet production rates, low gut passage times, and relatively low assimilation efficiencies (Besiktepe & Dam 2002) resulting in fecal pellets of high nutritional value. Thus, fecal pellets were between 0.5 and 18 h old at the start of the incubation. The average fecal pellet length and width for pellets offered in the experiments was (mean ± SD) 96 ± 26 and 28 ± 5 µm, respectively.

Grazing experiments. Fecal pellet clearance rates and ingestion rates of adult females were determined in both the presence and absence of alternative food (Table 1). Incubations were run for 24 h, in a 12:12 h light:dark cycle, and on a plankton wheel rotating at ~1 rpm. This rotation speed was chosen to keep the pellets suspended and to have minimum effect on the copepods. Ploug et al. (in press) found sinking velocities of 28 ± 3 m d^{-1} for similar sized pellets produced by *Temora longicornis* feeding on *Rhodomonas salina*. At a rotation speed of 1 rpm, the experimental bottles (8 cm diameter) were in each position (upright, sideways, and upside down) for ~15 s. The pellets sank 0.5 ± 0.005 cm during 15 s; therefore, only pellets near the side of the vessel at the beginning of the experiment had their motion affected during incubation. When an alternative food source was present, it was always at a concentration of 3×10^6 cells l^{-1} to ensure the presence of alternative food during the whole incubation. A wide range of pellet concentrations was used to determine the functional response in pellet clearance to pellet concentration (Table 1). Only pellet concentrations <2 pellets ml^{-1} represent pellet concentrations found *in situ*. The pellets offered as food were distinguished from the pellets produced during the experiments due to a visually recognizable size difference. However, when offering *Pseudocalanus elongatus* fecal pellets together with an alternative food

Table 1. Experimental design. Copepod species (*Calanus helgolandicus*, *Oithona similis*, and *Pseudocalanus elongatus*), alternative food source (*Thalassiosira weissflogii*, natural plankton <20 µm, and *Rhodomonas salina*), female concentration, equivalent spherical diameter (ESD), and concentrations of fecal pellets offered during the incubations. rep.: number of replicates at each pellet concentration; con.: number of replicates of control bottles run in parallel with the experimental bottles at 3 different concentrations (low, intermediate, and high); P: fecal pellets as sole food source; P + alga: fecal pellets in the presence of an alternative food source; –: not investigated

Copepod species	Alternative food source	Female conc. (ind. l^{-1})	Temp. (°C)	Salinity (‰)	Bottle volume (ml)	ESD (µm)	Fecal pellet conc. (pellets ml^{-1})	
							P	P + alga
Grazing experiment								
C. helgolandicus	*T. weissflogii*	1.6 (5 rep., 5 con.)	14	32	615	50	0.2–4.8[a]	
O. similis	Natural plankton <20 µm *R. salina*	25 (3 rep., 3 con.)	12	22	320	47	0.3–8[a]	
P. elongatus	–	13 (3 rep., 3 con.)	15	36	615	52	0.2–4.8	–
Behavior experiment								
C. helgolandicus	*T. weissflogii*	7.8	14	33	637	45	0–11	0.3–9
O. similis	*R. salina*	29.4	14	21	408	50	0.1–10	0–9
P. elongatus	*T. weissflogii*	19.6	12	33	408	50	0–23	0.1–15

[a]Same pellet concentration both with and without algae

source, it was not possible to distinguish between 'food pellets' and pellets produced by *P. elongatus* itself, and thus fecal pellet clearance and ingestion rates could not be estimated. It was possible to distinguish between pellets produced by *P. elongatus* and food pellets in the absence of an alternative food source due to color and differences in structure. Ingestion and clearance rates were calculated using the equations of Frost (1972).

Behavior experiments. Grazing behavior was investigated by filming adult females feeding on a range of fecal pellet concentrations in the presence and absence of alternative food (Table 1). Filming was conducted in a cylindrical aquarium (*Calanus helgolandicus*: 10 cm diameter, 7.9 cm depth; *Oithona similis* and *Pseudocalanus elongatus*: 7.9 cm diameter, 7.9 cm depth). The aquarium was placed on a rolling table that was rotated at ~1 rpm to keep fecal pellets suspended. The rotation of the aquarium opposed the sinking velocity of the pellets, and they were not near the side of the rotating aquarium. The filming equipment consisted of a CCD video camera (Mintron MTV-1802CB) equipped with a 105 mm lens (Nikon Micro Nikkor 1:2.8) and connected to a video cassette recorder, a time-code generator, and a monitor. Infrared illumination was provided from behind by a light-emitting diode (LED), which was collimated through a condenser. Females were acclimated to the fecal pellet concentration in the aquarium for 1 h prior to filming. Alternative food was added to the aquarium after 40 min of filming, and the females were again acclimated for 1 h prior to filming (Table 1). Fecal pellet concentrations were measured before and after each period of filming (40 min). The average fecal pellet concentration during filming was used as the experimental concentration. One female at a time was kept in focus and followed for at least 1 min if possible. The video tapes were analyzed frame by frame, and the fraction of time a female spent filtering, sinking, or jumping was recorded for approximately 1 min per female. The behavior of 4 females was analyzed at each fecal pellet concentration, in the absence and presence of alternative food. Periods when females were close to the aquarium wall were excluded from the analysis. The numbers of rejected and ingested fecal pellets observed during a total of 30 min of filming with a female in focus were recorded for each pellet concentration in the presence and absence of alternative food (half-hour analysis, see Tables 2 & 3).

Counting procedure. After incubation, the fecal pellets were collected on a 15 µm screen, preserved in acid Lugol's solution, and counted under an inverted microscope. The filtrate from the 15 µm screen did not contain pellets or recognizable fragments. Pellet fragments (end parts only) were counted as a half-pellet. The average volume and equivalent spherical diameter (ESD) of the pellets were calculated from measurements of length and width of at least 30 intact fecal pellets in each experiment.

Statistical analysis. Student's *t*-test (SigmaStat version 3.1) was used to test for differences between treatments in fecal pellet clearance rates. The ingestion

rate, suspension feeding activity (%), feeding bout duration, number of jumps, jump duration, and sink duration were tested for differences between treatments with an analysis of covariance (ANCOVA; SPSS version 13.0), using the interactions between the treatments (absence/presence of alternative food) and average fecal pellet concentration (covariate). Suspension feeding activity (%) was the percentage of time a copepod spent filtrating the water: (time spent filtrating/sum of all activity) × 100.

RESULTS

Clearance and ingestion

Calanus helgolandicus

The clearance of fecal pellets was variable with no clear trend and was independent of pellet concentration both with and without the alternative food (*Thalassiosira weissflogii*; Fig. 1A). Maximum pellet clearance (mean ± SD) was 172 ± 125 ml female^{-1} d^{-1} in the presence of *T. weissflogii* and 205 ± 198 ml female^{-1} d^{-1} when pellets were offered alone (Fig. 1A). The ingestion rate increased with increasing pellet concentration in the presence of alternative food to a level of 671 ± 389 pellets female^{-1} d^{-1} (Fig. 1B), equivalent to 43 ± 32 × 10^6 µm^3 pellet material female^{-1} d^{-1}. When offered only pellets, however, the pellet ingestion reached a maximum (443 ± 242 pellets female^{-1} d^{-1}) at food concentrations of ~2 pellets ml^{-1}, whereupon it decreased again (Fig. 1B); the maximum ingestion was 23 ± 16 × 10^6 µm^3 female^{-1} d^{-1}. Clearance and ingestion of fecal pellets by *C. helgolandicus* were statistically independent of the presence or absence of *T. weissflogii* (clearance maximum: p = 0.757, Student's *t*-test; ingestion rate p = 0.287, ANCOVA; Fig. 1).

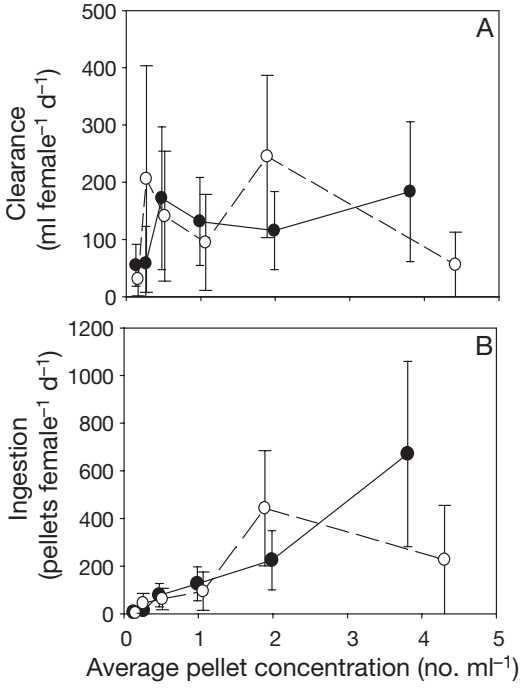

Fig. 1. *Calanus helgolandicus*. Average (A) clearance and (B) ingestion rates of fecal pellets with (●) or without (○) *Thalassiosira weissflogii* as an alternative food source. Fecal pellets were produced by *Acartia tonsa* adults feeding on *Rhodomonas salina*. Fecal pellet clearance and ingestion rates were estimated according to Frost (1972). Error bars indicate ±SD

Pseudocalanus elongatus

Clearance rate with pellets as the sole food for *Pseudocalanus elongatus* was constant at pellet concentrations from 0 to 1.5 pellets ml^{-1}, whereupon it decreased. Maximum clearance was 72 ± 14 ml female^{-1} d^{-1} (Fig. 2A).

The ingestion rate of fecal pellets increased asymptotically with pellet concentration toward a maximum ingestion rate of 121 ± 55 pellets female^{-1} d^{-1} at pellet concentrations >1.5 pellets ml^{-1}, resulting in a volume ingestion of 9 ± 5 × 10^6 µm^3 female^{-1} d^{-1} (Fig. 2B).

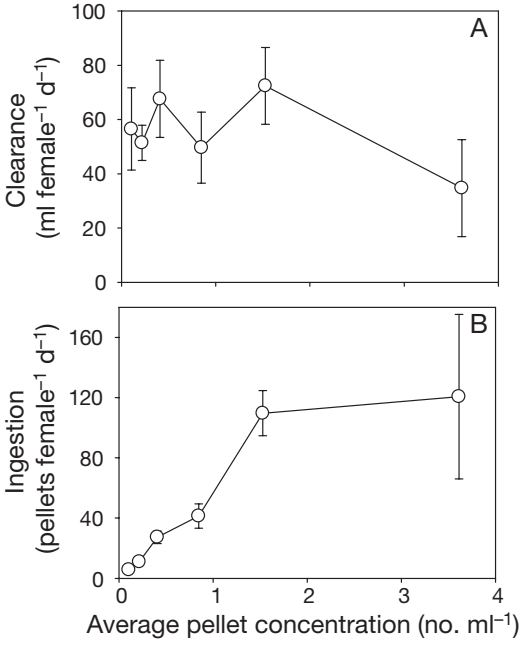

Fig. 2. *Pseudocalanus elongatus*. (A) Clearance and (B) ingestion rates of fecal pellets offered as sole food source. Fecal pellets were produced by *Acartia tonsa* adults feeding on *Rhodomonas salina*. Fecal pellet clearance and ingestion rates were estimated according to Frost (1972). Error bars indicate ±SD

Oithona similis

The clearance rate of fecal pellets was dependent on pellet concentration in both the presence and absence of an alternative food source (Fig. 3A). The presence of a monoculture of *Rhodomonas salina* induced significantly higher maximum clearance rates than when the pellets were offered as the sole food ($p = 0.007$, Student's *t*-test). No significant difference was found between maximum clearance of pellets offered as the sole food and fecal pellets offered with a natural plankton assemblage (<20 µm), or between the 2 treatments with alternative food ($p > 0.1$, Student's *t*-test; Fig. 3A,B). Maximum clearance rates (24 ± 1, 29 ± 17, and 9 ± 5 ml female^{-1} d^{-1} for pellets offered with *R. salina*, natural plankton <20 µm, and as the sole food, respectively) were reached at fecal pellet concentrations of ~0.5 pellets ml^{-1} in both the presence and absence of alternative food.

The ingestion rates of fecal pellets by *Oithona similis* increased with increasing pellet concentration in the presence of alternative food (Fig. 3C,D). The maximum ingestion in the presence of an alternative food source was 0.9 ± 1.2 × 10^6 µm^3 female^{-1} d^{-1} for the monoculture and 1.1 ± 1.3 × 10^6 µm^3 female^{-1} d^{-1} for the natural phytoplankton <20 µm at concentrations of 7 and 2.8 pellets ml^{-1}, respectively (Fig. 3C,D). When pellets were offered as the sole food, high variation in ingestion rates was observed. The maximum pellet ingestion was found at 6 pellets ml^{-1}, at which 0.6 ± 1 × 10^6 µm^3 female^{-1} d^{-1} was ingested (Fig. 3C). No significant difference in the ingestion rates was found between the different treatments ($p > 0.1$, ANCOVA), although higher ingestions were observed at concentrations <4 pellets ml^{-1} when an alternative food source was present (Fig. 3C,D).

Thus, the copepods cleared fecal pellets in both the absence and presence of alternative food. Further, the presence of an alternative food source seemed to increase the clearance rates and ingestion rates of fecal pellets.

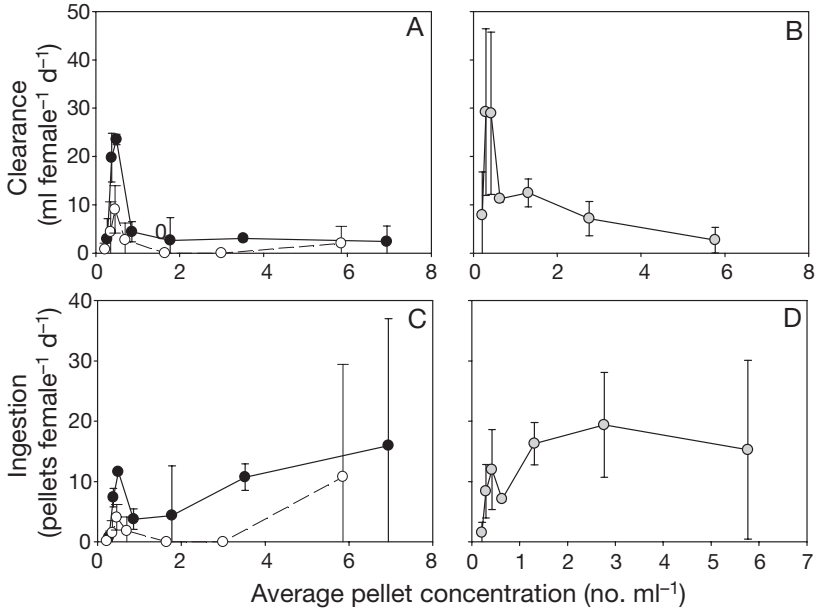

Fig. 3. *Oithona similis*. (A,B) Clearance rate and (C,D) ingestion rate of fecal pellets by *O. similis* are measured with fecal pellets as the sole food (○), offered together with *Rhodomonas salina* (●), and as pellets offered with a natural plankton community (<20 µm) from The Sound (Øresund, Denmark; ◍). Fecal pellets were produced by *Acartia tonsa* adults feeding on *Rhodomonas salina*. Fecal pellet clearance and ingestion rates were estimated according to Frost (1972). Error bars indicate ±SD

Feeding behavior

For all experiments, the suspended fecal pellets sank slowly, but as the aquarium turned, the pellets were kept suspended as did the animals when not moving.

Oithona similis is an ambush feeder and perceives only moving particles. It attacks a particle by jumping toward it and making a short burst of filtration upon encounter. Ambush feeding and ingestion of *Rhodomonas salina* cells were observed during filming, but no pellets were ingested. Since *O. similis* is an ambush feeder, its feeding activity was recorded as jump activity (jumps min^{-1}; Fig. 4A). No observations of encounters of fecal pellets were recorded. Further, the presence or absence of *R. salina* did not influence the feeding behavior of *O. similis*, as no significant difference was observed in the number of jumps, jump duration, and sink duration ($p > 0.05$, ANCOVA; Fig. 4). Clearly, *O. similis* viewed *R. salina* to be more attractive food particles than fecal pellets, which elicited no feeding response during the ~7 h of filming.

The feeding behavior of *Calanus helgolandicus* consisted of short continuous feeding bouts interrupted by brief periods of sinking. During the feeding bouts, a feeding current was generated, which pulled the copepod forward while it collected the particles caught in the current ('suspension feeding'). Pellet encounters occurred only during suspension feeding and often seemed unintentional with effort made to reject the pellets (Table 2, Fig. 5). The rejection was made with a

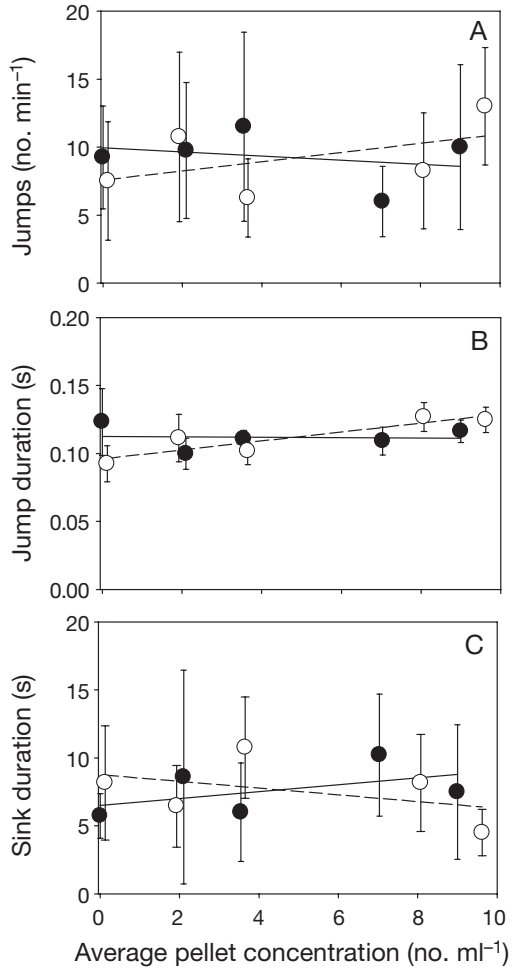

Fig. 4. *Oithona similis.* Female feeding behavior as a function of fecal pellet concentration in the absence (○, dashed lines) and presence (●, solid lines) of an alternative food source (*Rhodomonas salina*). Average (A) number of jumps per minute, (B) jump duration in seconds, and (C) sink duration. Each point is the mean ± SD of the behavior of 3 females analyzed for approximately 1 min each

powerful water thrust created by kicking the swimming legs. Coprorhexious behavior was observed from the rupture of some pellets during rejection. *C. helgolandicus* was also observed to feed coprochalously but only on large fecal pellets. In case of coprochaly, the pellet was handled by the feeding appendages for several seconds (5 to ≤20 s), whereupon it was rejected. After rejection, the shape of the pellet was clearly more fuzzy and ruptured due to apparent removal of parts of the periotrophic membrane. Only small or fragmented pellets were ingested, mainly along with the alternative food. Selective capture of fecal pellets was observed on a few occasions. This was seen as purposeful circling around a pellet while continuously filtering in short bursts until the pellet was captured. However, this effort rarely resulted in actual pellet ingestion. More often the pellet was rejected immediately after capture.

The presence of *Thalassiosira weissflogii* as an alternative food source induced a significant increase in suspension feeding activity of *Calanus helgolandicus* (>35%; p = 0.0001, ANCOVA) whereas the average suspension feeding activity was low (<21%) when it grazed fecal pellets as the sole food source (Fig. 5A). No significant difference was found between the duration of feeding bouts with or without *T. weissflogii* as an alternative food source (p > 0.1, ANCOVA; Fig. 5B). The sink duration was significantly higher when pellets were offered as the sole food (p = 0.006, ANCOVA; Fig. 5C). The increase in suspension feeding activity when *T. weissflogii* was present led to increased encounters of fecal pellets. The percentage of rejections of total encounters remained constant between 75 and 90%, independent of the total number of encounters, and the presence or absence of an alternative food source.

Pseudocalanus elongatus is a suspension feeder, and encounters of fecal pellets were only observed

Table 2. *Calanus helgolandicus*. Fecal pellet feeding behavior in the presence (+alga) or absence (–alga) of alternative food (*Thalassiosira weissflogii* at concentrations of 3×10^6 cells l^{-1}), during the half-hour analysis. Rejection—Thrust: when the pellet caught in the feeding current was forcibly thrust away from the copepod; Filtration: when the pellet caught in the filtration current smoothly moved through the feeding appendages and out again. Avoidance: when the presence of the pellet induced an escape jump in the female. Ingestion: when the pellet was actually ingested. Encounter: number of observed encounters of fecal pellets (rejection, avoidance, or ingestion) during the half-hour analysis. –: not investigated

Pellet conc. (pellets ml^{-1})	Rejection				Avoidance		Ingestion		Encounter	
	Thrust		Filtration							
–alga/+alga	–alga	+alga	–alga	+alga	–alga	+alga	–alga	+alga	–alga	+alga
0.25/0	0	0	0	0	0	0	0	0	0	0
2.25/2.38	2	4	9	9	1	2	9	10	24	40
–/4	–	1	–	–	–	5	–	2	–	22
5.63/5.38	1	2	0	0	1	3	0	12	13	97
8.75/8.75	0	2	17	17	0	18	17	9	86	79
11.25/–	2	–	5	5	4	–	5	–	38	–

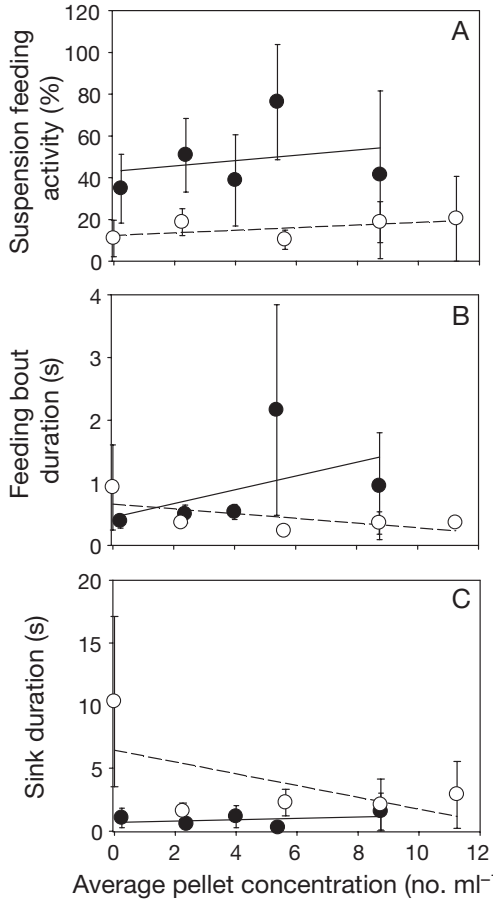

Fig. 5. *Calanus helgolandicus*. Female feeding behavior as a function of fecal pellet concentration in the absence (○, dashed lines) and presence (●, solid lines) of an alternative food source (*Thalassiosira weissflogii*). Average (A) suspension feeding activity as a percentage of total activity, (B) feeding bout duration in seconds, and (C) sink duration in seconds. Each point is the mean ± SD of the behavior of 3 females analyzed for approximately 1 min each

during filtration bouts. Often the encounters with fecal pellets seemed unintentional, with efforts made to reject the pellets. The rejection often involved large bursts of water creating a strong current that pushed the pellet away. At other times, the rejection was accomplished by swimming in circles while kicking with both feeding and swimming appendages in an attempt to reject the pellet, as if it caused handling problems. Rejection caused physical stress, which at times was observed to rupture and fragment the fecal pellets (coprorhexy). Only small pellets or pellet fragments were ingested, mainly along with alternative food.

The presence of *Thalassiosira weissflogii* induced a significantly higher suspension feeding activity by *Pseudocalanus elongatus* ($p = 0.002$, ANCOVA; Fig. 6A), although this difference was most pronounced at low pellet concentrations of <2 pellets ml^{-1}. No significant difference was observed in feeding bout duration in the presence of alternative food ($p = 0.9$, ANCOVA; Fig. 6B). Sink duration increased significantly when pellets were offered as the sole food ($p < 0.001$, ANCOVA; Fig. 6C). A difference in the number of pellet encounters was observed depending on the presence or absence of phytoplankton (Table 3).

Fecal pellet encounter and ingestion rates

The total number of encounters between fecal pellets and calanoid copepods (*Calanus helgolandicus* and *Pseudocalanus elongatus*) was estimated from the video observations (Tables 2 & 3). Fecal pellets were

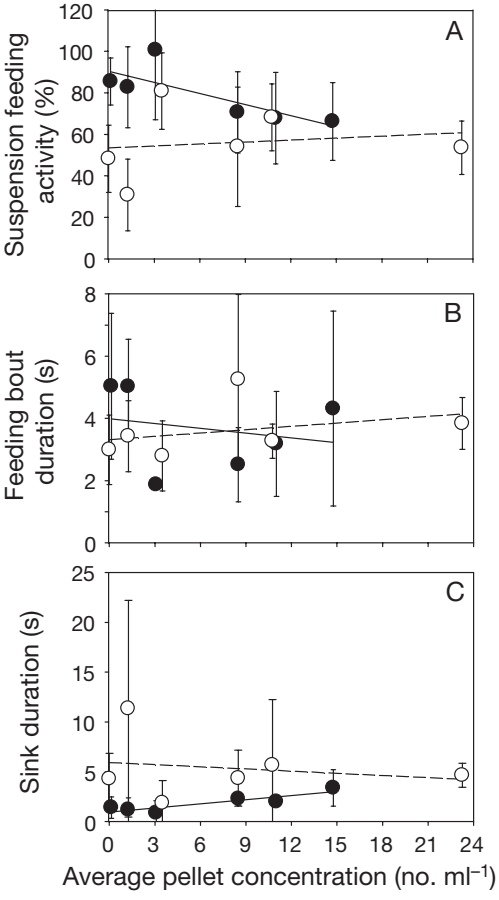

Fig. 6. *Pseudocalanus elongatus*. Female feeding behavior as a function of fecal pellet concentration in the absence (○, dashed lines) and presence (●, solid lines) of an alternative food source (*Thalassiosira weissflogii*). Average (A) suspension feeding activity as a percentage of total activity, (B) feeding bout duration in seconds, and (C) sink duration in seconds. Each point is the mean ± SD of the behavior of 3 females analyzed for approximately 1 min each

Table 3. *Pseudocalanus elongatus*. Fecal pellet feeding behavior in the presence (+alga) or absence (–alga) of alternative food (*Thalassiosira weissflogii* at concentrations of 3×10^6 cells l^{-1}), during the half-hour analysis. Further details as in Table 2

Pellet conc. (pellets ml^{-1}) –alga/+alga	Rejection				Avoidance		Ingestion		Encounter	
	Thrust		Filtration							
	–alga	+alga	–alga	+alga	–alga	+alga	–alga	+alga	–alga	+alga
0/0.1	0	0	0	3	1	1	0	1	1	5
1.25/1.25	0	0	0	1	1	3	0	0	1	4
3.5/3.1	9	5	8	11	2	0	6	0	25	16
8.5/8.5	5	2	3	8	0	2	1	1	9	13
10.75/11	11	5	16	49	1	2	1	7	29	63
23.25/–	10	–	62	–	0	–	3	–	75	–

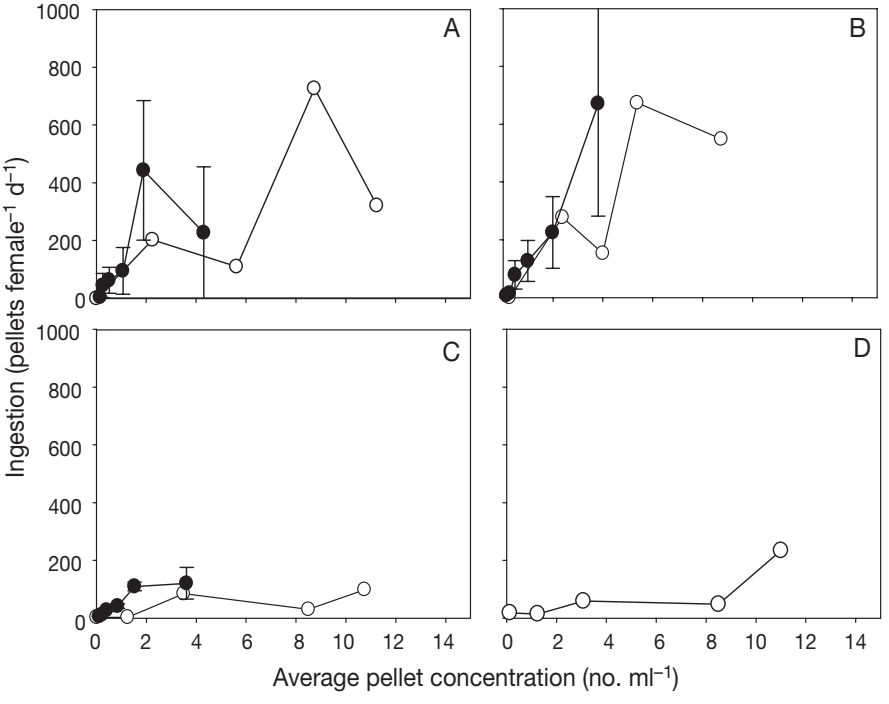

Fig. 7. (A,B) *Calanus helgolandicus* and (C,D) *Pseudocalanus elongatus*. Comparison between the ingestion rates of fecal pellets obtained from grazing experiments (●) and visual observations (○). Ingestion rates by *C. helgolandicus* and *P. elongatus* (B,D) with or (A,C) without an alternative food source (*Thalassiosira weissflogii*) are shown; ingestion rates from the visual observations were calculated from the percentage of observed ingestions and the total encounters of fecal pellets. Each point was calculated from the analysis of 30 min of video recording with a female in focus, assuming the behavior to be constant (half-hour analysis). Ingestion rates from the grazing experiments were estimated according to Frost (1972). Error bars indicate ±SD

encountered more frequently when an alternative food source was present. Less than 20% of the encountered fecal pellets were ingested, independent of pellet concentrations (calculated from Tables 2 & 3).

Ingestion rates were estimated from the observed ingestions during the video recordings, assuming a constant ingestion rate during 24 h. Daily ingestion was calculated from the percentage of ingestions of the total encounters. The ingestion rates of *Calanus helgolandicus* and *Pseudocalanus elongatus* estimated from both video observations and from the grazing experiments (Figs. 1 & 2) were compared by plotting them together (Fig. 7). No significant difference was found between the ingestion rates estimated from the video recordings and the grazing experiments for either *C. helgolandicus* or *P. elongatus* ($p > 0.5$, ANCOVA). The ingestion rates obtained from the video observations showed that pellet ingestion rates of both *C. helgolandicus* and *P. elongatus* increased by a factor of 1.4 and 2.8, respectively, in the presence of an alternative food source. Further, the ingestion of *P. elongatus* was 3.4 times lower than the ingestion rate of *C. helgolandicus* in the presence of alternative food and 7 times lower when pellets were offered as the sole food (Fig. 7).

Thus, visual observation revealed that the increased clearance of fecal pellets in the presence of alternative food was caused by an increased suspension feeding activity by the calanoid copepods. The presence of alternative food induced unintentional ingestion of small pellet fragments along with the algae. Further, intact fecal pellets were avoided by both calanoid species. No feeding response was observed for *Oithona similis* during 7 h of filming, indicating that fecal pellets were generally not viewed as attractive food particles by any of the copepods.

DISCUSSION

Fecal pellet degradation by calanoid copepods

The similarity of the ingestion rates obtained by visual observations and incubation experiments demonstrates that these 2 methods are consistent for the measurement of grazing rates.

Visual observations revealed that the presence of an alternative food source increased the ingestion rates of fecal pellets for both *Calanus helgolandicus* and *Pseudocalanus elongatus*. The same tendency was observed in the grazing experiments, but not at significant levels. The increased ingestion was due to increased suspension feeding activity initiated by the presence of the alternative food source. Increased feeding activity resulted in higher encounter rates of fecal pellets, and thus more modulation of the pellets. Other calanoid copepods (*Acartia tonsa, Centropages hamatus*, *Eucalanus pileatus*, and *Temora longicornis*) show similar increases in their functional response to fecal pellets in the presence of alternative algal food (Paffenhöfer & Van Sant 1985, Lampitt et al. 1990, Poulsen & Kiørboe 2005). Therefore, the degradation of fecal pellets by these suspension feeding calanoid copepod species is dependent on the feeding activity induced by other food particles.

Calanus helgolandicus and *Pseudocalanus elongatus* were observed to reject large fecal pellets at high rates, seemingly due to handling difficulty of the large particle sizes. Poulsen & Kiørboe (2005) found that the clearance of pellets by calanoid copepods decreased with the relative increase in pellet size. Our pellet clearances obtained for the calanoid copepods fit well with the data from Poulsen & Kiørboe (2005) (Fig. 8). We therefore support the view that pellet size is a controlling factor for the ability of a copepod to recognize the pellet as an unsuitable food particle. Further, pellet ingestion occurred only when the fragments were so small that they were ingested unintentionally along with the alternative food particles.

The high rejection rate was independent of pellet concentration or the presence or absence of an alternative food source. The total number of rejections increased when an alternative food source was present, due to increased pellet encounter rates. Often rejections led to membrane rupture, opening, and/or fragmentation of the pellet. The most common degradation process by *Calanus helgolandicus* and *Pseudocalanus elongatus* was therefore coprorhexy. Other investigators have also observed that calanoid copepods damage fecal pellets during rejection (Paffenhöfer & Van Sant 1985, Noji et al. 1991, Poulsen & Kiørboe 2005), supporting the view that the direct response to intact, large pellets from several calanoid copepods is rejection and coprorhexy.

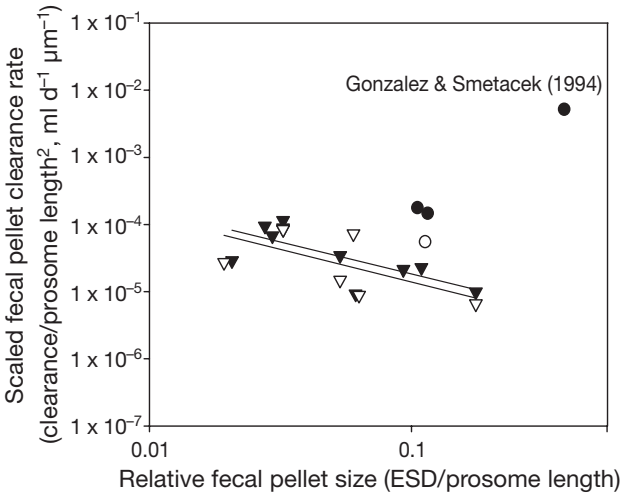

Fig. 8. Copepod fecal pellet clearance rate (normalized by copepod prosome length squared) as a function of relative pellet size (ESD/copepod prosome length) for calanoid copepods (▽, ▼) and *Oithona similis* (○, ●), in the absence (open symbols) or presence (closed symbols) of alternative food. Clearance rates and pellet sizes for the calanoid copepods were obtained from Table 5 in Poulsen & Kiørboe (2005) and from the present study. Clearance rates and pellet sizes for *O. similis* were obtained from González & Smetacek (1994) and the present study. The data point obtained from González & Smetacek (1994) for *O. similis* is labeled with the reference. The regression for pellets as the sole food for the calanoid copepods is: $\log(y) = -0.97\log(x) - 5.83$, $R^2 = 0.39$. The regression for pellets offered together with an alternative food source for the calanoid copepods is: $\log(y) = -0.95\log(x) - 5.69$, $R^2 = 0.53$

Coprochaly may also be an important degradation mechanism for *Calanus helgolandicus*. This was evident as females of this species were observed to remove and possibly ingest parts of the peritrophic membrane. This behavior was also observed for *C. helgolandicus* and *Acartia clausi* by Noji et al. (1991), who named the behavior coprochaly. It is therefore likely that *C. helgolandicus* feeds on bacteria and other organisms, which rapidly colonize the peritrophic membrane of fecal pellets after egestion (Hargrave 1975, Tezuka 1990), increasing the nutritional value of the pellet (Turner & Ferrante 1979, Simon et al. 2002). The occurrence of coprochaly can be mistaken for coprorhexy without the inclusion of direct observations, since both coprochaly and coprorhexy impose physical stress on the pellet, causing pellet loosening or fragmentation. *In situ* loosening or fragmentation of pellets increases the residence time and thereby the microbial degradation of fecal pellets within the water column because the reduced particle size and/or density decreases the sinking velocity of the pellets. Therefore, coprorhexy and coprochaly are important degradation processes, which seem to be more com-

mon feeding behaviors for calanoid copepods than coprophagy.

In conclusion, the importance of calanoid suspension feeding copepods for pellet degradation mainly seems to be indirect via the modulation of fecal pellets. The rate of the modulation is controlled by the presence of the alternative food, which induces suspension feeding. For copepods to have a significant effect on pellet degradation, situations with very high abundances of copepods in combination with small pellets would be needed (M. H. Iverson & Poulsen unpubl. data). Further, plankton organisms <200 µm (Poulsen & Kiørboe 2006) and more specifically large protozooplankton (>20 µm) play a dominant role in the degradation of fecal pellets (M. H. Iverson & Poulsen unpubl. data).

Fecal pellet degradation by *Oithona similis*

Visual observation of numerous situations in which *Oithona similis* was within detection distance of a fecal pellet did not result in the launch of an attack (calculation of critical detection distance was done according to Kiørboe & Visser 1999). Thus, *O. similis* did not seem to view fecal pellets as suitable food items. However, the grazing experiments indicated ingestion of fecal pellets by *O. similis*. Further, ingestion and clearance rates increased when an alternative food source was present, although only at significant levels when pellets were offered together with *Rhodomonas salina*. These findings are contrary to our expectations. We expected the grazing rates to be independent of the presence or absence of an alternative food source, since *O. similis* uses hydromechanical signals to perceive its prey particles. These signals do not change in the presence or absence of small food particles, and the feeding behavior should stay constant in both situations. Therefore, since a suitable alternative food source increased pellet removal, we suggest that the presence of alternative food triggers *O. similis* into a feeding mode in which it fragments or ingests fecal pellets at increased rates.

The obtained average fecal pellet removal rates for *Oithona similis* are low compared to removal rates reported by González & Smetacek (1994). At similar pellet concentrations, we found that the removal of pellets by *O. similis* was lower than the rates found by González & Smetacek (1994) by a factor of 5000 when offering calanoid pellets with an algal monoculture, and lower by a factor of 500 when offered with a natural plankton assemblage. In contrast, Reigstad et al. (2005) observed a complete lack of fecal pellet removal by *O. similis* when offering the same pellet type as used by González & Smetacek (1994). In the study by González & Smetacek (1994), *O. similis* presumably grazed large pellets lying on the bottom of standing experimental bottles. This contradicts the results of several studies of grazing behavior, which show that *O. similis* is an ambush feeder that perceives its prey by detecting the hydromechanical disturbance generated when the prey is sinking or swimming (Paffenhöfer 1993, Kiørboe & Visser 1999, Svensen & Kiørboe 2000). Accordingly, the pellets must be sinking in order to be detected. To detect pellets on the bottom of a bottle, chemosensory perception is needed. Chemosensory perception of small prey such as fecal pellets requires a feeding current (Andrews 1983), and since *O. similis* lacks the ability to suspension feed (Svensen & Kiørboe 2000), the detection of pellets lying on the bottom is unlikely. We therefore question the fact that the high removal rate of pellets lying on the bottom of the bottles was due to ingestion by *O. similis*.

In our study and in the study by Reigstad et al. (2005), constant generation of hydromechanical signals from the pellets was generated by rotation of the incubation bottles on a plankton wheel. This approaches the situation *in situ* where *Oithona similis* only encounters sinking fecal pellets. Thus, evidence from our study and 3 field studies shows that *O. similis* does not have a significant effect on fecal pellet degradation (Sampei et al. 2004, Reigstad et al. 2005, Poulsen & Kiørboe 2006, M. H. Iverson & Poulsen unpubl. data).

It may be argued that the low degradation rates of pellets obtained by Reigstad et al. (2005) and in our experiments were caused by the inability of *Oithona similis* to perceive the pellets hydromechanically due to water mixing in the rotating incubation bottles (~1 rpm). However, after a short spin up and down, there will be no turbulence in the bottles. Further, in our study, the rotation speed was slow enough not to disturb *O. similis* and fast enough to prevent the pellets from encountering the sides of the bottle when sinking. Thus, hydromechanical perception was not disturbed, and water mixing cannot explain the lower degradation rates. Another study of visual observation of ambush feeding *Acartia tonsa* females in a rotating aquarium (similar setup as in this study) supports this, as *A. tonsa* was able to detect, attack, and ingest fecal pellets using hydromechanical signals (Poulsen & Kiørboe 2005). We therefore agree with Reigstad et al. (2005), who hypothesized that *O. similis* is an indicator species for high degradation regimes but does not necessarily degrade the pellets itself. This is possible since *Oithona* spp. are often abundant in plankton communities that mainly consist of small copepods. Thus, the produced fecal pellets are small, with low sinking rates that increase the time available for degradation.

Poulsen & Kiørboe (2006) found high degradation of pellets (61 to 97%) in the upper 50 m of the water column during a field study of vertical flux and pellet degradation. However, they observed low pellet degradation when incubating only large zooplankton organisms (>200 µm), indicating that copepods and other mesozooplankton did not affect pellet degradation significantly. We previously investigated the degradation of different size fractions of the plankton community in The Sound (Denmark) in more detail and likewise found that copepods and other mesozooplankton did not affect pellet degradation significantly (M. H. Iverson & Poulsen unpubl. data). Further, we (unpubl. data) found protozooplankton and bacteria to be the main degraders of fecal pellets in the sea. We therefore suggest that copepods play a minor role in direct pellet degradation *in situ* and mainly influence the degradation through fragmentation of the pellets. However, via the grazing pressure imposed by the copepods on the effective pellet degraders such as large protozooplankton (>20 µm) and possibly nauplii, they are indirectly very important for the downward export of fecal pellets (M. H. Iverson & Poulsen unpubl. data).

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FEATURE ARTICLE

Degradation of copepod fecal pellets: key role of protozooplankton

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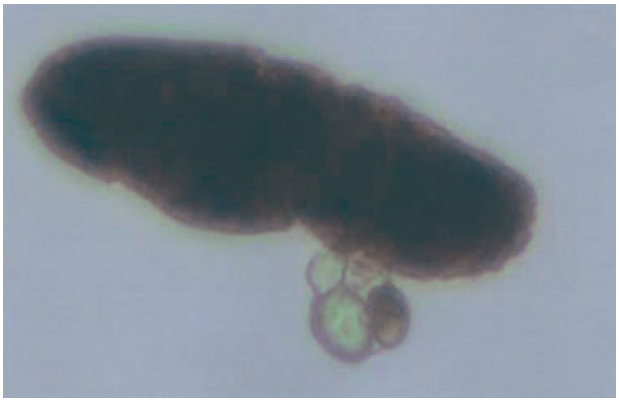
ABSTRACT: Copepod fecal pellets are often degraded at high rates within the upper part of the water column. However, the identity of the degraders and the processes governing the degradation remain unresolved. To identify the pellet degraders we collected water from Øresund (Denmark) approximately every second month from July 2004 to July 2005. These water samples were divided into 5 fractions (<0.2, <2, <20, <100, <200 µm) and total (unfractionated). We determined fecal pellet degradation rate and species composition of the plankton from triplicate incubations of each fraction and a known, added amount of fecal pellets. The total degradation rate of pellets by the natural plankton community of Øresund followed the phytoplankton biomass, with maximum degradation rate during the spring bloom (2.5 ± 0.49 d^{-1}) and minimum (0.52 ± 0.14 d^{-1}) during late winter. Total pellet removal rate ranged from 22% d^{-1} (July 2005) to 87% d^{-1} (May). Protozooplankton (dinoflagellates and ciliates) in the size range of 20 to 100 µm were the key degraders of the fecal pellets, contributing from 15 to 53% of the total degradation rate. Free-living *in situ* bacteria did not affect pellet degradation rate significantly; however, culture-originating bacteria introduced in association with the pellets contributed up to 59% of the total degradation rate. An effect of late-stage copepod nauplii (>200 µm) was indicated, but this was not a dominating degradation process. Mesozooplankton did not contribute significantly to the degradation. However, grazing of mesozooplankton on the pellet degraders impacts pellet degradation rate indirectly. In conclusion, protozooplankton seems to include the key organisms for the recycling of copepod fecal pellets in the water column, both through the microbial loop and, especially, by functioning as an effective 'protozoan filter' for fecal pellets.



Protozooplankton organisms (dinoflagellates and ciliates) are key degraders of copepod fecal pellets: *Gyrodinium dominans* draws pellet material into a food vacuole through the open transversal groove.

Photo: L. K. Poulsen

KEY WORDS: Bacteria · Copepod · Degradation rate · Dinoflagellates · Fractionated plankton community · Protozoan filter · Nauplii

INTRODUCTION

The processes governing the degradation of copepod fecal pellets in the sea are poorly known. Fecal pellets produced in the upper ocean are often degraded in the water column at high rates (Turner 2002, Poulsen & Kiørboe 2005). Bacteria degrade pellets, and copepods can degrade pellets through coprorhexy

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(fragmentation of pellets), coprophagy (ingestion of pellets) and coprochaly (loosening of pellets) (Lampitt et al. 1990, Noji et al. 1991). Especially copepods have been proposed as the main degraders of copepod fecal pellets, since several copepods have been shown to degrade fecal pellets in the laboratory (Paffenhöfer & Strickland 1970, Paffenhöfer & Knowles 1979, Paffenhöfer & Van Sant 1985, Ayukai & Nishizawa 1986, Lampitt et al. 1990, Noji et al. 1991, González & Smetacek 1994, González et al. 1994). However, evidence for the major role of copepods in the field is lacking. To our knowledge, copepod fecal pellet degradation has only been investigated in one field study by Poulsen & Kiørboe (2006), who found that copepods were not important degraders of fecal pellets and suggested that plankton organisms <200 µm were the main degraders. Furthermore, recent studies combining grazing experiments and visual observation of feeding behavior have shown that some copepods do not view fecal pellets as attractive food particles, and actual ingestion by suspension-feeding calanoids appears to consist of small fragments. These fragments are mainly ingested unintentionally along with food particles contained within the feeding current (Poulsen & Kiørboe 2005, Iversen & Poulsen 2007).

Evidence for fecal pellet degradation by planktonic organisms other than adult copepods does exist but is very scarce. Nauplii of the hapacticoid copepod *Amonardia normanni* (Koski et al. 2005) and *Calanus helgolandicus* (Green et al. 1992) consume fecal pellets. Kiørboe (2003) found high clearance rates of the heterotrophic dinoflagellate *Noctiluca scintillans* on fecal pellets in an upwelling plume off the coast of Brazil (~600 ml cell^{-1} d^{-1}). Ciliates and heterotrophic flagellates may colonize fecal pellets and have been observed to increase pellet degradation (Hansen et al. 1996).

This study investigates the degradation of copepod fecal pellets by the plankton community of Øresund (Denmark). The degradation rate of fecal pellets by the unfractionated plankton community, as well as of the 5 size classes (ultra-, pico-, nano-, micro- and mesoplankton), was investigated through incubations of fecal pellets with the total plankton community and the 5 size fractions. The species composition of the plankton community was quantified to evaluate the degradation of specific plankton groups and species in detail. Our main goal was to pinpoint the plankton organisms responsible for pellet degradation and to quantify the impact of these organisms throughout the year. Thus, only 1 type of fecal pellets was used in the degradation experiments, eliminating the influence of other possible governing factors for pellet degradation rate, such as the food source of the pellets, pellet size, pellet age, etc.

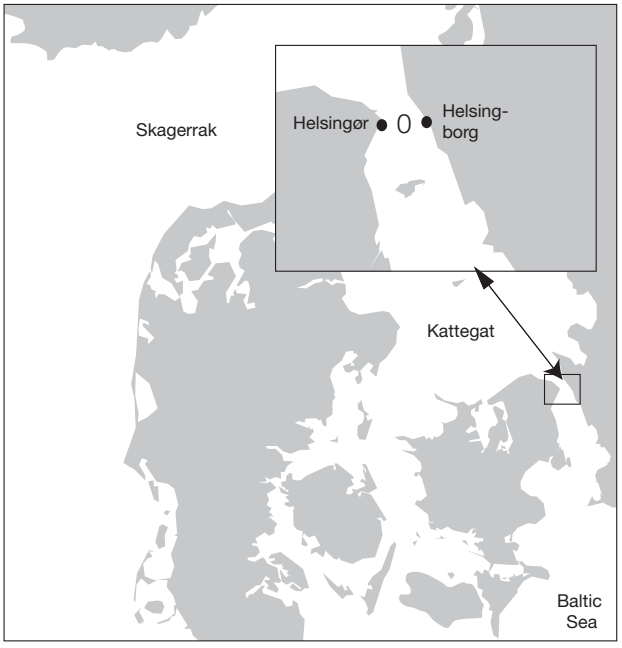

Fig. 1. Sampling area. Sampling was conducted approximately every second month during a year (2004/2005) in the strait of Øresund between Denmark and Sweden. Sampling of the plankton community was conducted at the depth of the fluorescence maximum

MATERIALS AND METHODS

Sampling and location. Sampling for plankton was conducted approximately every second month throughout a year in the narrow strait of Øresund, located between Denmark and Sweden (Fig. 1, Table 1). Øresund is characterized by a strong halocline throughout the year with low-saline (~10‰) surface water from the Baltic Sea and saline (~30‰) bottom water from the Kattegat (Lintrup & Jakobsen 1999). Strong wind events may break up the halocline periodically. Water samples for experimentation were collected at the depth of the fluorescence maximum in the lower part

Table 1. Sampling data. Sampling depth was the depth of the fluorescence maximum in the lower part of the halocline

Sampling date	Sampling depth (m)	Total depth (m)	Temperature (°C)	Salinity (‰)
6 Jul 2004	9	20	16	28
18 Aug 2004	12	25	16	28
8 Sep 2004	12	25	14	28
2 Nov 2004	9	20	12	28
24 Feb 2005	19	35	5	22
16 Mar 2005	8	20	4	26
17 May 2005	15	25	7	18
28 Jul 2005	16	39	14	33

of the halocline (~8 to 19 m) (Table 1) by use of a water pump. The fluorescence maximum was determined through vertical profiles of temperature, salinity and fluorescence using a SeaBird SBE11 CTD equipped with an *in situ* fluorometer. The water was immediately transported to the laboratory in 30 l plastic containers. In the laboratory the containers were kept at *in situ* temperature and gently aerated.

Cultures and pellet production. *Rhodomonas salina* (Wislouch) Hill & Wetherbee (a cryptophyte) was maintained in exponential growth in *f*/2 medium (Guillard 1975). The cultures were kept in a constant light:dark cycle (12:12 h) in filtered seawater (~33‰) at 18°C. *Acartia tonsa* was kept in culture at 18°C and reared on *R. salina* (Støttrup et al. 1986). The fecal pellets added in the degradation experiment of each sampling month were produced by 1 cohort of *A. tonsa* females feeding on *R. salina* at saturating food concentrations (>713 µg C l^{-1}; Kiørboe et al. 1985). Screen insets (180 µm) in the bottom of the pellet production containers minimized coprophagous and coprorhexious grazing of the pellets by *A. tonsa*. The copepods were allowed to feed for <18 h before the pellets were separated from phytoplankton and copepods by filtration through a double filter with an upper 180 µm screen (removes copepods) and a lower 30 µm screen (collects pellets but not *R. salina*). The pellets were then gently washed and suspended in a single bottle with filtered seawater (<0.2 µm micropore filter), which was used as the stock solution of pellets. The pellet concentration in the stock solution was calculated from pellet counts (pellet fragments [end parts only] were counted as a one-half pellet). The average volume (cylindrical shape) of the pellets was calculated from measurements of length and width of at least 30 fecal pellets (Table 2). Subsamples were immediately transferred to the incubation bottles containing the different plankton fractions (within 1 h of production) corresponding to a final fecal pellet concentration of ~0.5 pellets ml^{-1}.

Table 2. Experimental data and fecal pellet characteristics at incubation start. Pellet volume was calculated from measurements of pellet length and width. Pellet length and volume (± SD) given as means

Sampling date	Incubation time (h)	Length (µm)	Volume (10^4 µm^3)	Fragmentation (%)
6 Jul 2004(a)	48	66 (±42)	3.4 (±5.4)	44
6 Jul 2004(b)	48	80 (±30)	6.0 (±4.8)	34
18 Aug 2004	48	–	–	–
8 Sep 2004	48	93 (±23)	5.2 (±2.0)	26
2 Nov 2004	24	91(±24)	7.4 (±3.9)	34
24 Feb 2005	24	79 (±17)	5.5 (±2.5)	45
16 Mar 2005	24	95 (±37)	9.1 (±5.6)	39
17 May 2005	24	111 (±34)	12.4 (±6.2)	19
28 Jul 2005	24	85 (±16)	8.2 (±4.2)	30

Degradation experiments. Water from the fluorescence maximum of Øresund was fractionated into 5 fractions (<0.2, <2, <20, <100, <200 µm) and total (unfractionated) to evaluate the pellet degradation rate attributable to the different plankton size groups at *in situ* concentrations (0.2 to 2 µm = bacteria, 2 to 20 µm = nanoplankton, 20 to 200 µm = microplankton and >200 µm = mesozooplankton). The water was fractionated into the size classes <20, <100 and <200 µm by inverse filtration through 20, 100 and 200 µm filters and into the <0.2 and <2 µm size fractions by use of, respectively, a 0.2 µm and 2 µm micropore filter mounted on a suction system. Water from each fraction and the unfractionated (total) sample was incubated in triplicate for 48 h (July and September 2004) or 24 h (other months) (Table 2) after addition of 300 fecal pellets from the pellet solution per 615 ml blue cap incubation bottle (= 0.5 pellets ml^{-1}). Control bottles (3 replicates), without addition of pellets, were made for the size fractions containing *in situ* copepod fecal pellets and copepods: <20, <100, <200 µm and total. These controls were used to correct for *in situ* pellets and pellets produced by copepods present in the water samples during incubation. Bottles were incubated on a plankton wheel (1 rpm), kept at *in situ* temperatures (i.e. temperature at sampling depth; Table 1), and on a 12 h light:12 h dark cycle. After incubation, fecal pellets and zooplankton were collected on a 15 µm filter, stored in 50 ml plastic containers and preserved by acid Lugol's solution (1% final concentration) for later counting.

The degradation experiment carried out in August 2004 was contaminated by the mainly benthic ciliate *Euplotes* sp. (from the *Rhodomonas salina* culture), and therefore discarded; in this experiment, all fecal pellets were degraded within 48 h. Two degradation experiments (Expts a and b) were run in parallel in July 2004, with water from the same sample and same size fractions. The parallel experiments were conducted to investigate how the initial degree of pellet fragmentation influenced the pellet degradation rate. The pellets were produced by the same cohort of *Acartia tonsa* females as described above. However, different pellet fragmentation percents were obtained by filtration, such that fragmentation was 44% in Expt a and 34% in Expt b (Table 2). Pellets for Expt a were filtered through a double filter with an upper 180 µm screen (removes copepods) and a lower 15 µm screen (collects pellets). Pellets for Expt b were filtered through a double filter with an upper 180 µm screen and a lower 30 µm screen. The fragmentation percent (F) of the fecal pellets was calculated as:

$$F = \frac{(\text{fragments}/2)}{\text{Intact} + (\text{fragments}/2)} \times 100\% \quad (1)$$

where fragments are a one-half pellet (end parts only) and intact is whole pellets.

The fecal pellet degradation rate was calculated from the loss of fecal pellet material described by:

$$N_t = N_0 e^{-rt} \quad (2)$$

where N is the total number of fecal pellets in the incubation bottle at incubation start (N_0) and at the end of incubation (N_t), t is incubation time (days) and r is the degradation rate (d^{-1}). The degradation rate estimated in this study is a cumulative degradation rate in the sense that organisms present in the fraction <0.2 µm are also present in all other fractions, the organisms present in the fraction <2 µm are present in all other fractions except <0.2 µm and so forth. The degradation rate of fecal pellets in the unfractionated samples is called *the total degradation rate*.

Control experiments. A control experiment was conducted to ensure that the handling procedure did not affect the pellet degradation rates. Two subsamples of 5 ml were taken from a pellet stock solution. One of the samples was placed directly in a Petri dish. The other sample was added to a Bluecap bottle filled with filtered seawater (<0.2 µm), collected on a 15 µm filter and flushed into a Petri dish, following the same procedure as in the degradation experiments of the present study. The 2 subsamples were fixed with acid Lugol's solution simultaneously and counted. The procedure was repeated 6 times with new subsamples from the pellet solution, and a *t*-test (SigmaStat 3.1) was conducted to test for significant differences between the 2 treatments. No differences between the recovered pellets in the 2 treatments were found ($p > 0.05$), i.e. the handling procedure did not affect the pellet degradation rate.

Counting. The total number of pellets in all size fractions and in the controls of the size fractions of <20, <100, <200 µm and total were counted after incubation (3 replicates in all size fractions). The concentration of *Acartia tonsa* pellets after incubation was calculated by subtracting the average number of pellets in the control bottles (3 replicates) from the total number of pellets left in each incubation bottle (3 replicates) of the corresponding size fraction. After incubation all 6 size fractions (3 replicates in each fraction) were fixated with acid Lugol's solution, and the zooplankton species composition was assessed by use of a stereo microscope. Thus, the zooplankton in each fraction represents the *in situ* concentration of the zooplankton. All animals were identified to species or genus level. Samples for enumeration and identification of phytoplankton and protozooplankton were taken in each of the 6 size fractions *prior* to incubation and fixed in acid Lugol's solution (1% final concentration). Counting was conducted in Utermöhl settling chambers (settling time >24 h) (Utermöhl 1958). Only cells >10 µm were counted, since smaller cells are not satisfactorily determined with the Utermöhl method. At least 400 cells were counted per sample. Biovolumes of protists were estimated from linear dimensions using appropriate geometric shapes, and were converted to biomass using 2 linear regression equations of carbon:volume relationships, 1 for diatoms (Mullin et al. 1966):

$$\log C = 0.76 \log V - 0.29 \quad (3)$$

and 1 for flagellates (autotrophic and heterotrophic) (Menden-Deuer & Lessard 2000):

$$\log C = 0.94 \log V - 0.6 \quad (4)$$

where C is cell carbon mass in pg and V is cell volume in μm^3.

Identification of ciliates to species, group, or morphotype was based on Montagnes & Lynn (1991) and Hansen & Nielsen (1999). Dinoflagellates were identified according to Tomas (1997) and Thomsen (1992). No correction for cell shrinkage was applied. Protozooplankton was divided into the size classes <20 and >20 µm according to the shortest dimension. Separation of autotrophic and heterotrophic dinoflagellates was not complete, as this is only possible with 100% precision using epifluorescence microscopy.

Chlorophyll *a*. Samples (1 to 3 l) for chl *a* measurements of the unfractionated water were filtered onto GF/F filters, extracted in 96% ethanol and measured on a Perkin Elmer spectrophotometer. No chl *a* measurements were conducted in July 2004 and September 2004.

Statistical analysis. The difference between the degradation rates of fecal pellets (dependent variable) in the 5 size fractions and the unfractionated water sample of each month (factor) was tested with 1-way ANOVA. The Tukey *post hoc* test was used for pairwise comparisons (SigmaStat 3.1).

The importance of the initial degree of pellet fragmentation for the pellet degradation rate of the parallel experiments in July 2004 (Expts a and b; Table 2) was tested with a *t*-test comparing the average degradation rates (see Fig. 5A, B) of each of the 5 size fractions and total between Expts a and b (SigmaStat 3.1).

The correlation between temperature and average bacterial degradation rate (<0.2 and <2 µm fractions) was determined through a Pearson product moment correlation (SigmaStat 3.1).

Stepwise linear regression was used to find the best model describing the relationship between pellet degradation rate (dependent variable) and the abundance of different protozooplankton and zooplankton organisms (independent variables) in the size ranges of 20 to 100 µm (microzooplankton) and ≥200 µm (mesozooplankton). The degradation rate in the 2 size ranges

was obtained by subtraction ($r_{20–100\ \mu m} = r_{<100\ \mu m} - r_{<20\ \mu m}$ and $r_{\geq 200\ \mu m} = r_{total} - r_{<200\ \mu m}$); the same was done for animal abundances. September was excluded from the analysis due to contamination of the <0.2 and <2 µm fractions with protozooplankton, making it impossible to separate the bacterial contribution from that of the other fractions. Linear regression was done for each independent variable, and significant variables ($p < 0.05$) were investigated in all possible combinations through stepwise linear regression (SigmaStat 3.1) to search for the best model, describing the dependence of the degradation rate on the relevant independent variables in the 2 size ranges. Residuals were analyzed to confirm that model assumptions were satisfied, and the models were tested for autocorrelation and multicollinearity (correlation between independent variables).

RESULTS

Hydrography and plankton

A strong halocline was present at 10 to 20 m depth, except in February, when strong winds broke it up. The fluorescence maximum was generally located in the lower part of the halocline (8 to 19 m; Table 1). Phytoplankton blooms were observed, 1 in March, May and July 2005 (267 to 512 µg C l^{-1}, 3 to 7 µg chl *a* l^{-1}) and 1 in the autumn (September, 167 µg C l^{-1}) (Figs. 2 & 3A). During the rest of the sampling months concentrations of 2 to 39 µg C l^{-1} and 0.3 to 1.7 µg chl *a* l^{-1} prevailed in the subsurface maximum.

The heterotrophic biomass was dominated by protozooplankton (dinoflagellates and ciliates) in all months and followed the seasonal variation in phytoplankton biomass (Figs. 2 & 3B). Dinoflagellates dominated the protozooplankton in all months (Fig. 3B). The large protozooplankton groups (>20 µm) present in the <100 µm fraction included the autotrophic/mixotrophic dinoflagellates *Ceratium* spp. and *Dinophysis* spp., heterotrophic dinoflagellates >20 µm, *Gyrodinium spirale* (Bergh) Kofoid & Swezy, *Protoperidinium* spp. and ciliates >20 µm.

The zooplankton community of Øresund consisted of relatively few species, which were dominated by small copepods (Fig. 4). Copepod nauplii were found in the plankton throughout the year, with a maximum abundance in July 2005 (65 ± 16 ind. l^{-1}) and minimum in February (4 ± 3 ind. l^{-1}). Maximum copepod abundances of 22 to 60 ind. l^{-1} were found in July and September (Fig. 4). *Oithona similis* was present in all months except March and dominated the zooplankton community except in spring, when *Acartia* spp. dominated. Evidence of high grazing pressure exerted by the mesozooplankton during their maximum abundance was observed in the degradation experiment of July 2005. This was due to the general disruption and senescence of the phytoplankton, large amounts of dead organic matter originating from disrupted fecal pellets, and to a low abundance of large (>20 µm) protozooplankton (Fig. 3B).

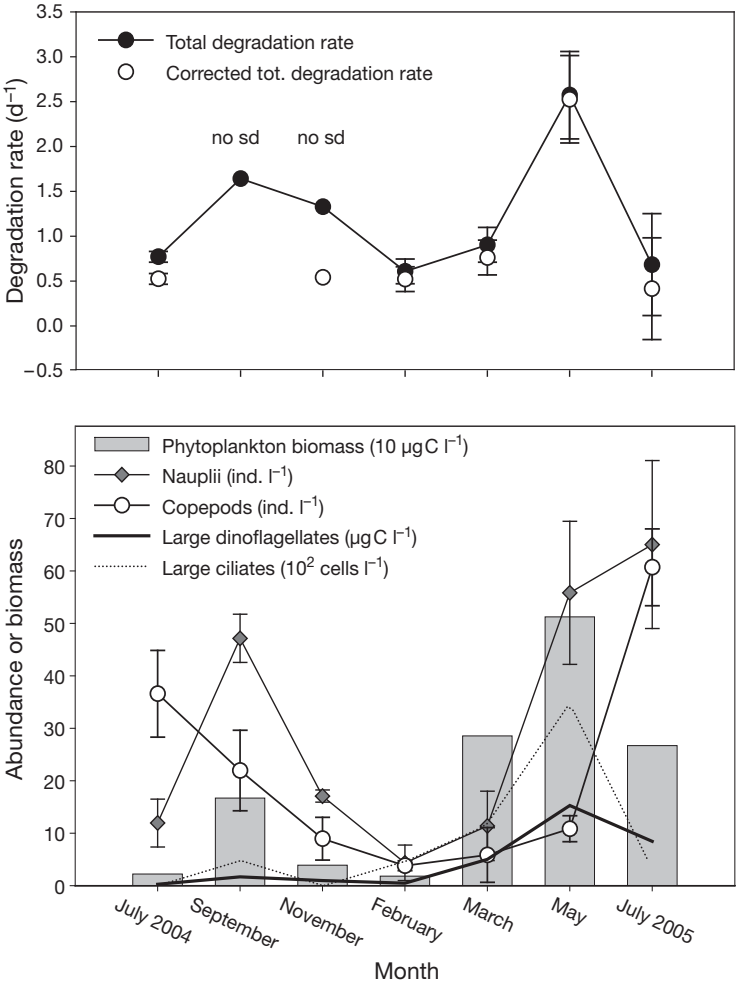

Fig. 2. Total degradation rate and plankton abundances in the unfractionated water samples (total). The total degradation rate (d^{-1}) and the corrected total degradation rate (d^{-1}) are presented in the upper panel. The corrected total degradation rate represents the total degradation rate of the natural plankton community, from which the contribution of the culture-originating bacteria (<0.2 µm fraction) has been subtracted (excluding September). Phytoplankton biomass (×10 µg C l^{-1}); biomass of large (>20 µm), heterotrophic dinoflagellates (µg C l^{-1}); abundance of large (>20 µm) ciliates (×10^2 cells l^{-1}); nauplii abundance (ind. l^{-1}) and copepod abundance (ind. l^{-1}) are presented in the lower panel. Total degradation rate, nauplii and copepod abundances are represented as the average (±SD) for July 2004 Expts a and b

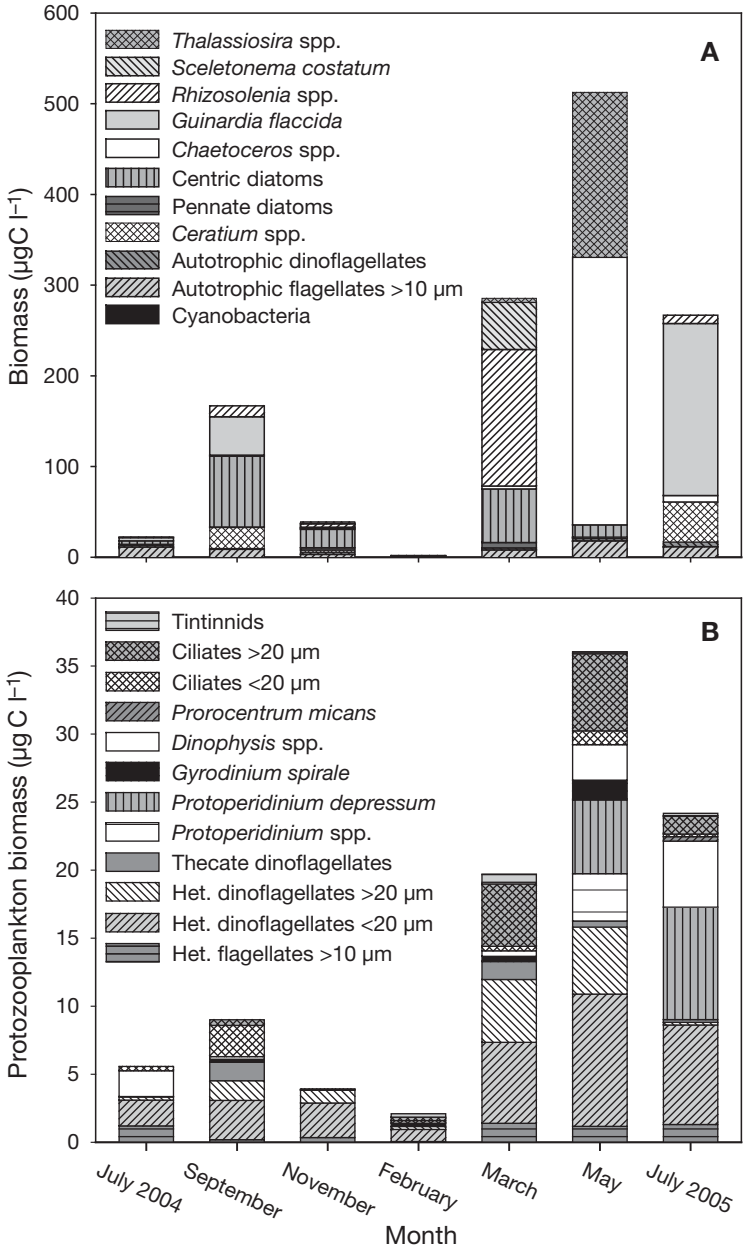

Fig. 3. Plankton biomass and species composition. (A) phytoplankton and (B) protozooplankton in the unfractionated water samples of each month. The phytoplankton community was dominated by *Chaetoceros* spp. and autotrophic flagellates > 10 µm in February. Only autotrophic flagellates >10 µm could be satisfactorily determined with the Utermöhl method. Cyanobacteria here are colonial forms. Dinoflagellates and ciliates were determined from shortest dimension

Degradation of fecal pellets

The results of the pellet degradation experiments in the present study revealed that bacteria associated with the pellets and protozooplankton in the size range of 20 to 100 µm were the major plankton groups responsible for pellet degradation.

The degradation rate was not significantly different between the <0.2, <2 and the <20 µm size fractions, except in May (Fig. 5). Furthermore, the degradation rate was significantly different from zero in the 0.2 µm fraction (except in May). All *in situ* bacteria were removed from the seawater via filtration in this fraction; we therefore suggest that bacteria associated with the pellets (culture-originating) were responsible for the degradation rate in these size ranges. The degradation rate provided by the naturally occurring bacteria was small, as seen from the insignificant contribution of the <2 µm size fraction compared to that of the <0.2 µm fraction. The average degradation contributed by the naturally occurring bacteria (the size range of 0.2 to 2 µm) ranged from 15 to 4% of the total degradation rate in all months (Fig. 6), except in July 2005, when the contribution was 29%, corresponding to a degradation rate of 0.2 d^{-1} (Fig. 5H). The degradation rate of the culture-originating bacteria ranged from 0.05 to 0.79 d^{-1} (<0.2 µm fraction) contributing from 2 to 59% of the total degradation rate. September was not included, since the <0.2 and <2 µm fractions were contaminated by protozooplankton. There was no correlation ($p > 0.05$) between temperature and average degradation rate of pellets by *in situ* bacteria (<2 µm fraction).

In May, the significantly higher degradation rate in the <20 µm size fraction corresponded to the unique presence of the large, heterotrophic, dinoflagellate *Gyrodinium spirale*. The absence of nauplii and other zooplankton organisms (Fig. 5G) indicated that *G. spirale* had an important impact on pellet degradation rate.

Fecal pellet degradation rate was significantly higher in the <100 µm size fraction as compared to the <20 µm fraction in all months (except September). Additionally, the pellet degradation rates in the fractions <100 and <200 µm were not significantly different in any month (Fig. 5). Thus, microplankton organisms in the size range of 20 to 100 µm had a major impact on the degradation rate of fecal pellets in all months (except September) (Fig. 6). In September, the microplankton presumably had no impact ($r_{20–100\ \mu m} = 0$), but since we do not know the size of the bacterial contribution to the degradation rate we cannot say whether this was caused by a high bacterial degradation rate or by a high contribution from organisms in the <20 µm fraction (size range 2 to 20 µm). The possible microplankton candidates for causing the significant degradation rate in the specific size range of 20 to 100 µm were large (>20 µm) protozooplankton (dinoflagellates and ciliates) and nauplii, since copepods

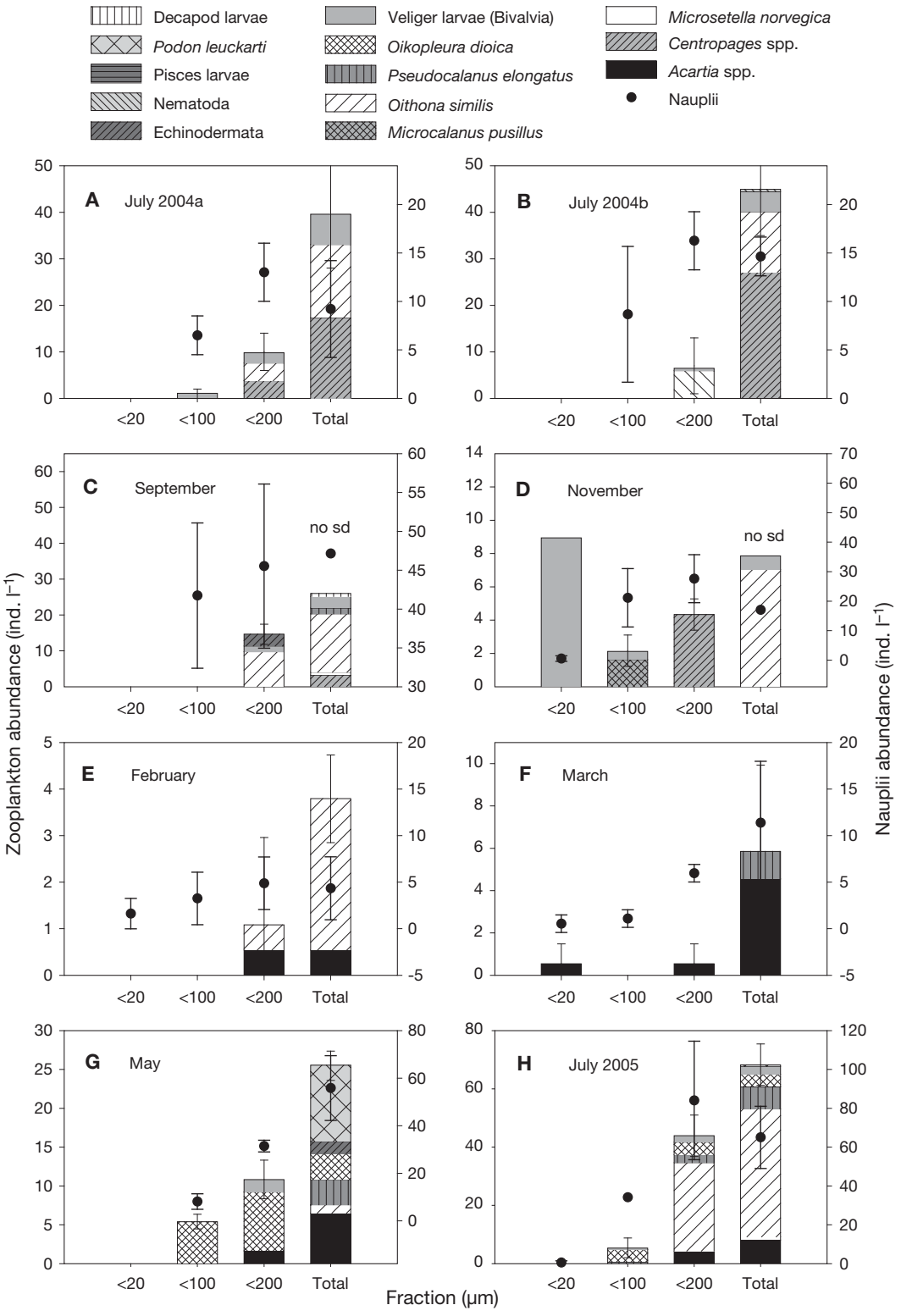

Fig. 4. Zooplankton. Abundance and species distribution of the dominating zooplankton groups and species in the 4 relevant fractions of each degradation experiment (mean of triplicate incubations) during different sampling months (A to H). No mesozooplankton organisms were found in the fractions <0.2 or <2 µm. Naupliar abundance is represented separately by dots. The 2 degradation experiments in July 2004 were conducted with the same plankton community and fecal pellets, but the fragmentation percent F (see Eq. 1) was 44 and 34 % in July 2004 Expt a and b, respectively. The 'total' fraction represents the complete plankton community present in the unfractionated water samples. Note different scaling and different y-axes of nauplii and zooplankton

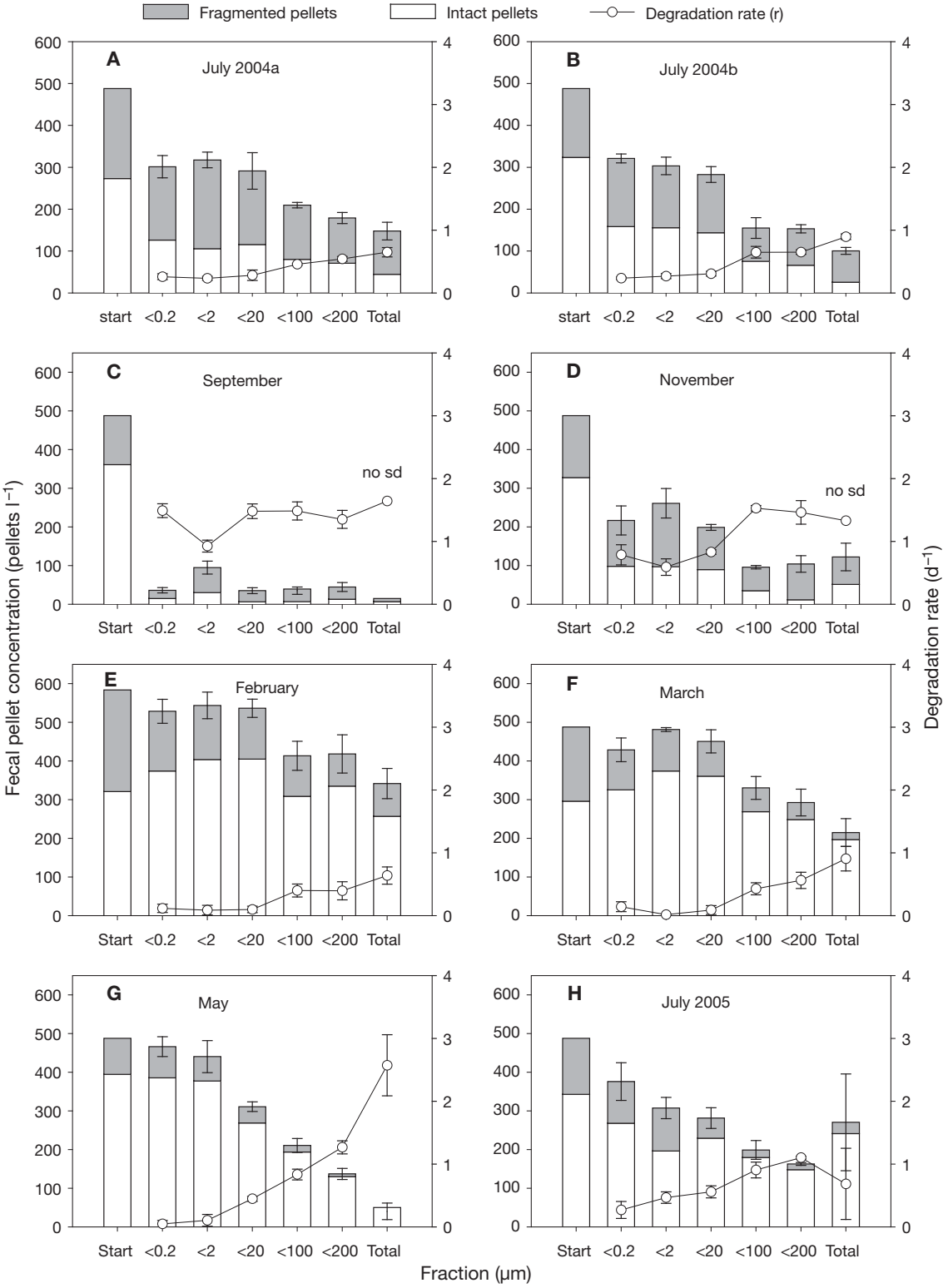

Fig. 5. Fecal pellets. Initial fecal pellet concentration, pellet concentration in each fraction and in the unfractionated plankton samples (total) after incubation during different sampling months (A to H; triplicate incubations). The fragmentation percent *F* (see Eq. 1) is the grey area of the columns and the white area represents the percentage of intact pellets. The degradation rate estimated in each fraction is represented separately by dots. The 2 degradation experiments in July 2004 were conducted with the same plankton community and fecal pellets, but the fragmentation percentage *F* (see Eq. 1) was 44 and 34 % in July 2004 Expt a and b, respectively. Note different *y*-axes

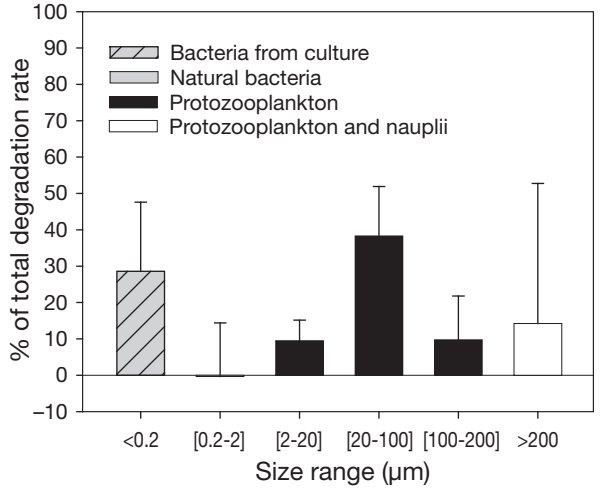

Fig. 6. Total degradation rate contributed by the different size ranges. The total degradation rate is split into the different size ranges and each size range is represented as the average (±SD) of each month. September was not included in the calculation of the percent degradation due to the contamination of the <0.2 and <2 µm fractions. The degradation rate in the size ranges was calculated by subtracting the fractions from each other (e.g. $r_{20-100\ \mu m} = r_{<100\ \mu m} - r_{<20\ \mu m}$). The plankton organisms dominating the degradation in each size fraction are indicated by different patterns. Natural bacteria dominated the degradation in the <2 µm size fraction (this is difficult to see due to the minuscule size of the bar)

and other zooplankton occurred in the <200 µm to total fractions (Fig. 4). The microplankton in the size range of 20 to 100 µm contributed from 15 to 53% of the total degradation rate ($r_{20-100\ \mu m}$ = 0.18 to 0.70 d^{-1}), excluding September. Linear regression showed that only the abundance of *Gyrodinium spirale* could be used as a predictor for the degradation rate in the 20 to 100 µm size range ($r^2 = 0.88$, $p < 0.01$). However, *G. spirale* was not present in all months (present in November, March and May) and was not abundant (40 to 600 cells l^{-1}). Other protozooplankton species >20 µm were, therefore, also important for the fecal pellet degradation rate, but could not be identified by the linear regression.

Copepods and other mesozooplankton were not important for the fecal pellet degradation rate in the present study, since the <200 µm and total fractions, containing the main part of the copepods and other zooplankton species, were not significantly different from the <100 µm fraction in most months (July 2004a, September, November, February, July 2005), including July 2004a and July 2005, when copepod abundance peaked (Figs. 4 & 5). The total degradation rate of the unfractionated plankton (total) was significantly different ($p < 0.05$) from the other fractions only in July 2004b, March and May. In these months mesozooplankton (≥200 µm) contributed from 27 to 51% of the total degradation rate ($r_{(\geq 200\ \mu m)}$ = 0.25 to 1.3 d^{-1}). Possible mesozooplankton candidates for causing the significant degradation rate in this size range were large protozooplankton, nauplii, copepods and other zooplankton organisms ≥200 µm. Linear regression showed that only the abundance of copepod nauplii could be used as a predictor for the degradation rate in the specific size range of ≥200 µm, including all months ($r^2 = 0.98$, $p < 0.001$).

The total degradation rate followed the phytoplankton biomass with a maximum in May (2.57 ± 0.49 d^{-1}) and a minimum in February (0.64 ± 0.14 d^{-1}), corresponding to a pellet removal rate of, on average, 42 to 95% d^{-1} (Fig. 2). However, this rate included the degradation rate provided by the <0.2 µm size fraction, which was caused by factors other than the natural plankton community of Øresund. Correcting for this contribution ($r_{(total*)} = r_{(total)} - r_{(<0.2\ \mu m)}$, excluding September), the corrected total degradation rate of the natural plankton community of Øresund ranged from 0.52 to 2.53 d^{-1} (Fig. 2), corresponding to a pellet removal rate of 22 to 87% d^{-1}.

The degree of pellet fragmentation did not influence the degradation rates of the parallel degradation experiments in July 2004 (Table 2, Fig. 5A,B), since no significant difference was found between the degradation rates of the different fractions in Expts a and b (*t*-test, $p > 0.05$).

DISCUSSION

Contrary to the general belief that copepods are the major degraders of fecal pellets in the sea (see review by Turner 2002) recent studies have shown that their role is minor (Reigstad et al. 2005, Poulsen & Kiørboe 2006, Iversen & Poulsen 2007). The present study investigates the degradation rate of fecal pellets in a plankton community and reveals which plankton organisms actually degrade fecal pellets.

The total degradation rate (corrected) of fecal pellets by the natural plankton community of Øresund ranged from 0.42 to 2.53 d^{-1}, corresponding to a pellet removal rate of 22 to 87% d^{-1}, which is within the range found in other field studies (Viitasalo et al. 1999, Wexels Riser et al. 2002, Sampei et al. 2004, Poulsen & Kiørboe 2006), but lower than the maximum pellet degradation rates (12 to 13 d^{-1}) found in Kiørboe (2003). The total degradation rate in the present study generally followed the seasonal variation of the phytoplankton. Maximum pellet degradation rates during maximum phytoplankton concentrations have been found in other studies in temperate and polar regions (Urban-Rich 2001, Dubischar & Bathmann 2002, Sampei et al. 2004).

Bacteria

Bacterial degradation rates could be separated into the degradation rate contributed by culture-originating bacteria (<0.2 µm size fraction) and by free-living bacteria from Øresund (<2 µm size fraction). Bacteria do not pass through a 0.2 µm filter, and the degradation rates observed in this size fraction, therefore, originate from possible mechanical stress and bacteria supplied to the incubation bottles in association with the *Acartia tonsa* fecal pellets. The pellet degradation rate of the <0.2 µm size fraction in May was nearly zero (0.05 ± 0.06 d^{-1}; Fig. 6), the method used for handling and incubation was the same for all months; hence, the impact of mechanical stress in general was insignificant. Supporting this handling of the pellets did not cause a significant increase in pellet degradation rate in a control experiment (see 'Materials and methods'). We therefore conclude that the fecal pellet degradation rate in the size fraction <0.2 µm was caused by culture-originating bacteria associated with the added pellets.

The degradation rates were not significantly different in the <0.2, <2 and <20 µm fractions (except May), indicating that the culture-originating bacteria associated with the fecal pellets were the main bacterial degraders, whereas the contribution of the free-living naturally occurring bacteria was small. The bacterial degradation rates supplied by the culture-originating bacteria in the present study was an important part of the total degradation rate in some months, but highly variable. It ranged from approximately 0 to 0.031 h^{-1}, which is within the range found in laboratory studies for flagellate-based pellets (Hansen et al. 1996, Thor et al. 2003, Olsen et al. 2005, Ploug et al. 2008).

Some impact of free-living bacteria in late-bloom situations was indicated in July 2005, where the degradation rate of the free-living bacteria was within the range of bacterial degradation rates obtained in laboratory studies for flagellate-based pellets (Hansen et al. 1996, Thor et al. 2003, Olsen et al. 2005, Ploug et al. 2008).

The bacteria were not enumerated in this study, and we are therefore unable to say whether or not the culture-originating bacteria were mainly attached to the surface of the pellets (Honjo & Roman 1978), or within the pellets (Gowing & Silver 1983). Dense populations of bacteria seeded within copepod fecal pellets have been observed in several studies (Gowing & Silver 1983, Jacobsen & Azam 1984, Tang 2005). Bacteria seeded within *in situ* fecal pellets may also be important for fecal pellet degradation *in situ*, and we can therefore only conclude that free-living natural bacteria did not have a significant impact in the present study. Further investigation into the bacterial degradation of *in situ* fecal pellets is obviously needed.

Protozooplankton and nauplii

The major impact of microplankton organisms in the size range of 20 to 100 µm corresponded to the presence of large protozooplankton and early-stage nauplii (<100 µm). However, only the abundance of *Gyrodinium spirale* could be used as a predictor for the degradation rate in this size range (linear regression). The importance of the protozooplankton as opposed to that of the mesozooplankton was supported by the fact that both the total degradation rate and corrected total degradation rate followed the abundance of the protozooplankton, and not that of nauplii or copepods. Thus, large protozooplankton (>20 µm) were the main degraders of fecal pellets in this study. Plankton organisms <200 µm were also the main degraders of fecal pellets in a field study in the North Sea in August 2002 (Poulsen & Kiørboe 2006) and in June 2007 (Iversen unpubl. data), and Kiørboe (2003) found even higher degradation rates (0.3 to 13 d^{-1}) of the heterotrophic dinoflagellates *Noctiluca scintillans* on fecal pellets. Microscopic observations of *Gyrodinium dominans*, *G. spirale* and *Protoperidinium* spp. feeding on fecal pellets additionally confirm the ability of dinoflagellates to feed on fecal pellets (L. K. Poulsen unpubl. data, P. J. Hansen pers. comm.). Pellet degradation by the mainly benthic ciliate *Euplotes* sp. was directly observed in the present study. Faster degradation of pellets due to contamination with *Euplotes* sp. was also found by Hansen et al. (1996); and Lampitt et al. (1990) mentioned microscopic observations on ciliates with fecal pellets that suggested that they could cause substantial physical damage to the pellet after the peritrophic membrane was removed.

Taking the prey size spectrums into account, it becomes clear that dinoflagellates, and not ciliates, were most likely the main degraders in this study. Ciliates generally feed on prey <20 µm, corresponding to an optimum prey size of 1/10 of their own size (Hansen 1992, Jakobsen & Hansen 1997). Large ciliates were present in all months except July 2004 and November and were generally between 21 and 52 µm in length, making them likely degraders only of small pellet fragments. The ciliates present in this study would therefore only be capable of degrading already fragmented pellets. However, large heterotrophic dinoflagellates are able to feed on particles many times larger than themselves with an optimum prey size of 1/1 (Hansen 1992, Jakobsen & Hansen 1997). Large dinoflagellates are therefore able to consume intact fecal pellets, and their feeding strategy of attaching themselves to the food particle by a filament prior to consumption through direct engulfment, pallium feeding, or peduncle feeding (Hansen 1992) enables them to tow the fecal pellet, thereby counteracting the sinking rate of

the pellet (L. K. Poulsen unpubl. data). Chemosensory capabilities and positive chemotaxis to food particles has been shown for heterotrophic dinoflagellates (reviewed by Verity 1991, Martel 2006). Likewise heterotrophic dinoflagellates detect fecal pellets by registering the chemical trail released by the pellet (L. K. Poulsen unpubl. data). Heterotrophic dinoflagellates are therefore easily able to detect, handle and consume copepod fecal pellets.

The optimum prey particle size for *Gyrodinium spirale* corresponds to its own size, and it is able to ingest prey that is 5.3 times its own volume with an efficiency as high as 75% (Hansen 1992). Average pellet volume was 1.2 to 4.1 times the average volume of *G. spirale* in the relevant months, and, thus, within the optimum size for *G. spirale*. The average fecal pellet clearance rate of *G. spirale* was approximately 1 ml ind.$^{-1}$ d^{-1}, as estimated from 2 separate sources. Linear regression in the microplankton size range showed that the degradation rate increased at a rate of 0.0012 d^{-1} for every increase by 1 ind. l^{-1}, corresponding to a clearance rate ($F_{G.spirale}$) of 1.2 ml ind.$^{-1}$ d^{-1}. A similar clearance rate was obtained from the <20 µm fraction in May by assuming that *G. spirale* was the main degrader ($r_{2-20\ \mu m}$ = 0.34 d^{-1}, *G. spirale* = 0.36 cells ml^{-1}), yielding a clearance of 0.95 ml cell^{-1} d^{-1} equal to a pellet volume removal of 4 times the volume of an average *G. spirale* cell per day. These estimates are realistic since *G. spirale* clear similar-sized algae (~50 µm equivalent spherical diameter) at a rate of approximately 3 ml ind.$^{-1}$ d^{-1} (Hansen 1992). Using the average clearance rate of 1 ml ind.$^{-1}$ d^{-1}, *G. spirale* contributed from 7 to 52% of the corrected, cumulative degradation rate in the <100, <200 µm and total fractions. Hence, *G. spirale* had a large impact on the degradation rate, but could not alone explain the degradation rate in these fractions. Other large dinoflagellates therefore also contributed.

The pellet degradation rate was dependent on copepod nauplii abundance in the total fraction ($r_{\geq 200\ \mu m}$), but not in the <100 or <200 µm fractions. This suggests that late stages of nauplii >200 µm contributed significantly to pellet degradation. However, the organisms of the >200 µm size range only contributed significantly to the total degradation rate in 3 months, whereas late-stage nauplii were present in all months, indicating a species-specific impact of nauplii. Naupliar consumption of fecal pellets is supported by 2 other studies (Green et al. 1992, Koski et al. 2005).

Mesozooplankton

Copepods (adults and copepodite stages) and other mesozooplankton did not influence the degradation rate significantly in the present study, since none of the copepod species, individually or together, could be used as predictors of the degradation rate of fecal pellets. This contradicts the former paradigm stating that copepods are the main degraders of fecal pellets (see review by Turner 2002). This paradigm was understandably based on the fact that adults of several copepod species have been found to clear fecal pellets in laboratory studies (Turner 2002). Likewise, *Oithona similis* females had clearance rates of 29 ml ind.$^{-1}$ d^{-1} in a grazing experiment conducted in parallel with the degradation experiment in November (see Iversen & Poulsen 2007). The cause of this seeming discrepancy between the high impact in laboratory grazing experiments and the low impact of copepods in natural plankton communities is that the laboratory studies have been conducted only with C5 or adult copepods, whereas natural copepod communities consist of a mix of stages. The copepod clearance rate of fecal pellets is size dependent, and the clearance rate of a given pellet size increases with increasing size of the copepod grazer (Poulsen & Kiørboe 2005). Thus, the clearance rates of fecal pellets by *in situ* copepod communities consisting of a mix of copepodite stages and adults will always be much lower than those obtained for adult copepods only in the laboratory, as supported by the insignificant impact of copepods on pellet degradation rate found in recent studies (Reigstad et al. 2005, Poulsen & Kiørboe 2006, Iversen & Poulsen 2007). Behavioral studies of *Acartia tonsa*, *Calanus helgolandicus* and *Pseudocalanus elongatus* have shown that even the adult female copepods avoid or reject fecal pellets when possible, and that ingestion by suspension feeders appears to consist mainly of small fragments, which are ingested unintentionally along with food particles contained within the feeding current (Poulsen & Kiørboe 2005, Iversen & Poulsen 2007).

The direct impact of copepods on pellet degradation under natural conditions is therefore insignificant. However, copepods may be important for pellet degradation rate indirectly, since they exert a high grazing pressure on the main pellet degraders, such as the protozooplankton. This was illustrated in July 2005 when peak copepod abundances coincided with low abundances of large protozooplankton, and a corresponding decrease in the fecal pellet degradation rate in incubations with the total (unfractionated) plankton community.

CONCLUSION

Large protozooplankton (ciliates and dinoflagellates), and likely mainly heterotrophic dinoflagellates, were the main degraders of fecal pellets in this study.

Free-living bacteria present in the natural seawater from Øresund did not have a significant impact on pellet degradation rate; however, culture-originating bacteria had a variable, but in some months important impact on the degradation rate of fecal pellets. Mesozooplankton in general did not have a significant impact on pellet degradation; however, an indirect impact was apparent through their grazing on the pellet degraders.

To our knowledge, this is the first study to demonstrate a general importance of protozooplankton for fecal pellet degradation. Hence, the effective 'coprophagous filter' responsible for the removal of fecal pellets within the water column (González & Smetacek 1994) is more likely a 'protozoan filter'. The present study demonstrates that further investigation is required to identify the protozooplankton and nauplii species that degrade fecal pellets. Also, further investigation into the processes governing their impact is needed to fully understand and predict fecal pellet degradation and vertical material flux in the oceans.

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Paper III

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Production, oxygen respiration rates, and sinking velocity of copepod fecal pellets: Direct measurements of ballasting by opal and calcite

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Abstract

Production, oxygen uptake, and sinking velocity of copepod fecal pellets egested by *Temora longicornis* were measured using a nanoflagellate (*Rhodomonas* sp.), a diatom (*Thalassiosira weissflogii*), or a coccolithophorid (*Emiliania huxleyi*) as food sources. Fecal pellet production varied between 0.8 pellets ind^{-1} h^{-1} and 3.8 pellets ind^{-1} h^{-1} and was significantly higher with *T. weissflogii* than with the other food sources. Average pellet size varied between 2.2 $\times 10^5$ μm^3 and 10.0 $\times 10^5$ μm^3. Using an oxygen microsensor, small-scale oxygen fluxes and microbial respiration rates were measured directly with a spatial resolution of 2 μm at the interface of copepod fecal pellets and the surrounding water. Averaged volume-specific respiration rates were 4.12 fmol O_2 μm^{-3} d^{-1}, 2.86 fmol O_2 μm^{-3} d^{-1}, and 0.73 fmol O_2 μm^{-3} d^{-1} in pellets produced on *Rhodomonas* sp., *T. weissflogii*, and *E. huxleyi*, respectively. The average carbon-specific respiration rate was 0.15 d^{-1} independent on diet (range: 0.08–0.21 d^{-1}). Because of ballasting of opal and calcite, sinking velocities were significantly higher for pellets produced on *T. weissflogii* (322 ± 169 m d^{-1}) and *E. huxleyi* (200 ± 93 m d^{-1}) than on *Rhodomonas* sp. (35 ± 29 m d^{-1}). Preservation of carbon was estimated to be approximately 10-fold higher in fecal pellets produced when *T. longicornis* was fed *E. huxleyi* or *T. weissflogii* rather than *Rhodomonas* sp. Our study directly demonstrates that ballast increases the sinking rate of freshly-produced copepod fecal pellets but does not protect them from decomposition.

The biological carbon pump of the ocean is driven by sedimentation of phytoplankton aggregated in marine snow and fecal pellets. Through this mechanism, a substantial fraction of CO_2 assimilated by phytoplankton is exported from the upper mixed surface waters into the deep ocean and sediments (Turner 2002). Sinking velocities of these particles range between 5 m d^{-1} and 400 m d^{-1} (Alldredge and Gotschalk 1988; Turner 2002). Fecal pellet sinking velocity is largely determined by its composition and size (Komar 1981). Fecal pellet size and density depend on copepod species, food concentrations, and food source (Feinberg and Dam 1998). The contribution of zooplankton fecal pellets to the overall vertical flux varies from a few percent up to 99% in the ocean (Turner 2002). A key feature determining potential vertical fluxes in the water column is the carbon-specific degradation rate of organic matter relative to its sinking velocity. Zooplankton fecal pellets produced on a dinoflagellate diet show high degradation rates and low sinking velocities leading to fast turnover in the water column (Hansen et al. 1996; Thor et al. 2003). In contrast, pellets produced on coccolithophorids show high sinking velocities, and a large fraction of these pellets reach depths below 1,000 m in the ocean (Harris 1994; Knappertsbusch and Brummer 1995). Fecal pellet degradation rate and sinking velocity is thus largely determined by the phytoplankton available as food source.

Copepods are ubiquitous in the ocean and in neritic seas. Copepods play an important and dual role in mediating vertical carbon flux as well as nutrient retention within the

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euphotic zone depending on the available food source (Thor et al. 2003). We here present the first direct and high-resolution measurements of small-scale oxygen fluxes to copepod fecal pellets. We measured fecal pellet egestion rate by the copepod *Temora longicornis*, small-scale oxygen fluxes to the pellets, and sinking velocity as a function of diet to analyze the potential impact on carbon recycling and export. As key groups of food sources, we used a nanoflagellate (only organic carbon), a diatom (producing opal, SiO_2), and a coccolithophorid (producing calcite, $CaCO_3$). This enabled us to test whether the ballasting effect of opal and calcite is caused by increased density and sinking speed or by adsorptive protection and reduced degradation in fresh fecal pellets (van der Wal et al. 1995; Buitenhuis et al. 2001; Klaas and Archer 2002). Their potential contribution to vertical flux and carbon turnover relative to that of marine snow across the particle size spectrum in the ocean is discussed.

Materials and methods

Cultures—Phytoplankton cultures were grown in ca 1-liter batch cultures using a F/2-medium (+silicate for diatoms) at 15°C, and a 12 : 12 light : dark cycle (Guillard and Ryther 1962). The calanoid copepod *T. longicornis* originated from the central North Sea, but was cultured in the laboratory for >10 generations. The copepod cultures were kept in dim light at 15°C and fed an excess mixture of *Rhodomonas* sp., *Thalassiosira weissflogii*, and the dinoflagellate *Heterocapsa* sp. The copepods used in experiments originated from a recently (<1 week) matured generation.

Grazing, ingestion, and fecal pellet production by copepods—Grazing and fecal pellet production by *T. longicornis* were measured using single species diets of the diatom *T. weissflogii* (218 pg C $cell^{-1}$), the coccolithophorid *E. huxley* (12 pg C $cell^{-1}$) or the cryptophyte *Rhodomonas* sp. (42 pg C $cell^{-1}$) at a concentration of 430 (±50) μg C L^{-1}. Four to five *T. longicornis* females were sorted out from a stock culture and placed into rotating 0.6-liter bottles containing the above food suspensions. After ~24 h of adaptation, actively swimming individuals were transferred into a new food suspension for 2-h pellet production incubations and subsequent 24-h grazing and pellet production experiments. After the first 2 h, the contents of bottles were carefully filtered on a net of 11 μm and flushed into Petri-dishes. Pellets were gently collected using a mouth pipette and counted and measured under a dissection microscope with an ocular micrometer. Actively swimming individuals were transferred back to the bottles into a new food suspension for the start of the 24-h incubations. A 10-mL sample was preserved in 2% acid Lugol at the start and end of the 24-h incubations for later microscope counts. The rest of the bottles were filtered on an 11-μm sieve, and the number of pellets and surviving : dead females were counted. Twenty to sixty pellets were sized at the end of the 2-h and/or the 24-h incubations. For each food species, 5–6 replicates were performed. Triplicate grazing controls without animals were incubated in parallel for each food species. Grazing samples (at least two replicates per sample) were counted using an inverted microscope by counting fields of view until >150 cells were counted. The counting errors varied between 5% and 12% of the mean value. Clearance and ingestion rates were calculated according to Frost (1972). Clearance, ingestion, and pellet production rates as well as pellet volume were tested for differences between the diets by using a one-way analysis of variance (ANOVA). Tukey HSD (honestly significantly different) post-hoc test was used for pair-wise comparisons.

Particulate organic carbon—Pellets for measurements of particulate organic carbon (POC) were counted and sized under a dissection microscope. Afterward, they were gently collected using a mouth pipette and placed on silver filters (Milipore, 25 mm, 0.45 μm). Fecal pellet carbon content was measured on an elemental analyzer mass spectrometer (ANCA-SL 20-20), Sercon Ltd.) with a precision of ±0.7 μg C. Inorganic carbon was assumed to be 30% of total carbon in pellets produced on *E. huxleyi* (Harris 1994).

Small-scale measurements of oxygen—Pellets (1–3 h old) were transferred to a thermostated container (15°C) coated by a 5-mm-thick agar layer (1% w:w) at the bottom and covered by filtered (0.2 μm) seawater. The diffusion in 1% agar is very close to that in seawater (Revsbech 1989). Using a container coated with agar we avoided artifacts from a solid boundary, which limits solute exchange between pellets and the surrounding water (Ploug and Grossart 1999). Oxygen concentrations were measured during darkness using a Clark type oxygen microsensor (Revsbech 1989) attached to a motor-controlled micromanipulator. The current was measured by a picoamperemeter (Unisense, PA2000) connected to a computer for direct data acquisitioning using the program "μ-profiler" (MPI, Bremen). The electrode was calibrated at anoxic conditions and at air-saturation. Its 90% response time was <1 s and the stirring sensitivity <0.3%. The oxygen microelectrode tip was 2 μm wide, and its position was observed under a dissection microscope with an ocular micrometer. The electrode was carefully positioned at the pellet surface as observed through the dissection microscope. Oxygen distributions were measured backwards toward the free water phase at 2–5-μm step increments. Five concentration gradients were measured in the pellet-water boundary layer of each pellet. The fluxes of oxygen (nmol O_2 cm^{-2} s^{-1}) were calculated as the product of the measured concentration gradients and the diffusion coefficient of oxygen in seawater at 15°C (1.71×10^{-5} cm^2 s^{-1}; Broecker and Peng 1974). Area-integrated fluxes were calculated assuming cylindrical geometry of the pellets. Carbon respiration rates were calculated assuming a respiratory quotient of 1 mol O_2 : 1 mol CO_2.

Fecal pellet density and sinking velocity—Fecal pellet density was measured in a density gradient using a modified version of the centrifugation method (Schwinghamer et al. 1991; Feinberg and Dam 1998). Seven dilutions were made using Ludox TM colloidal silica, sucrose, and distilled water. The dilutions ranged in density from 1.05 g cm^{-3} to

Table 1. Average food concentration (μg C L^{-1}), clearance (mL ind^{-1} h^{-1}) and ingestion (μg C ind^{-1} d^{-1}) rates, pellet production (pellets ind^{-1} h^{-1}) and volume (μm^3) in 2- and 24-h incubations and average pellet production in carbon (μg C ind^{-1} d^{-1}) (mean ± SD). If there was more than one parallel experiment (*T. weissflogii* and *E. huxleyi*), the results are pooled. Number of replicates is indicated in parenthesis. (–) Missing data.

Food source and concentration (μg C L^{-1})	Grazing		Pellet production (pellets ind^{-1} h^{-1})		Pellet volume ($\times 10^5$ μm^3)		Pellet production (μg C ind^{-1} d^{-1})
	C (mL ind^{-1} h^{-1})	I (μg C ind^{-1} d^{-1})	2 h	24 h	2 h	24 h	24 h
Rhodomonas sp. (420±40)	0.5±0.3 (5)	4.5±2.9 (5)	2.6±0.2 (5)	0.8±0.2 (5)	2.2±0.6 (16)	–	1.4±0.3* (5)
T. weissflogii (420±40)	1.1±0.2 (10)	9.6±1.7 (10)	3.8±1.0 (15)	1.7±0.5 (10)	10±7.7 (20)	4.1±3.3 (26)	2.9±0.9 (10)
E. huxleyi (390±20)	0.6±0.2 (9)	6.2±1.5 (9)	1.9±0.7 (19)	1.2±0.3 (10)	4.8±4.6 (23)	5.9±0.43 (59)	2.1±0.5 (10)

* Calculated on the basis of 2-h incubations.

1.43 g cm^{-3}. The dilutions were buffered to pH 8.1 with 0.0125 mol L^{-1} Tris plus 0.0125 mol L^{-1} Tris-HCl (final concentration). Hence, the gradient produced was isosmotic with seawater of salinity ~32 (Handbook Chem. Phys. 1968). From each dilution 2 mL was gently transferred to a 20-mL centrifuge tube, beginning with the densest and finishing with the lightest dilution. The density gradients were stored at 5°C overnight but were at room temperature before use. Fecal pellets were rinsed in filtered seawater (~32), placed in 1 mL of seawater, and pipetted on top of the density gradient. The samples were centrifuged at 3,000 rpm for 30 minutes. After centrifugation, 1 mL from the different density layers was removed from the tube using a peristaltic pump and weighed using a Mettler Toledo balance to 0.1 mg. The size and number of pellets layer^{-1} were recorded using a dissection microscope.

Fecal pellets sinking velocities were measured in a settling cylinder. The cylinder (40 cm high and 3 cm in diameter) was filled with filtered seawater (~32), and kept in a 15°C thermostated room, surrounded by a water jacket for thermal stabilization. The settling cylinder was closed at both ends, only allowing insertion of a Pasteur pipette at the top. Pellets were rinsed in filtered seawater and collected in a Pasteur pipette with filtered seawater (~32). Pellets sank out of the Pasteur pipette, which was centered in the cylinder. The descent of the pellets was recorded by two charge-coupled device (CCD) video cameras (Mintron MTV-1802CB) equipped with 105-mm lenses (Nikon Micro Nikkor 1:2.8). The cameras were placed along the x- and z-axis of the pellets, giving a three-dimensional view of the settling. A time-code generator was connected to the cameras, making timing of the pellets possible. Infrared illumination was provided from behind by light-emitting diodes (LED) and collimated through condensers. The setup was calibrated by recording a length scale before sinking velocity measurements.

Sinking velocity was, furthermore, calculated from density and fecal pellet dimensions according to (Komar et al. 1981)

$$\omega_s = 0.0790 \times \frac{1}{\mu} \times (\rho_s - \rho) \times \left(\frac{L}{D}\right)^{-1.664} \quad (1)$$

where ω_s is the sinking velocity, μ is the dynamic viscosity of sea water, ρ_s is the density of the pellets, ρ is the density of sea water, and L and D are fecal pellet length and diameter, respectively.

We also compared measured sinking velocities with those predicted from Stokes' law

$$\omega_s = \frac{g(\rho_s - \rho)Vd}{12\mu A} \quad (2)$$

where g is the gravitational acceleration (981 cm s^{-2}), V is the volume of the particle, and A is the cross-sectional area of the particle perpendicular to the direction of sinking.

Stokes' law is a special case of the drag equation for which the drag coefficient is described by (White 1974)

$$C_D = \frac{24\mu}{\omega_s d\rho} \quad (3)$$

where d is the equivalent spherical diameter $(4A/\pi)^{0.5}$. We also calculated the drag coefficient for the general case

$$C_D = \frac{2g(\rho_s - \rho)V}{\rho A \omega_s^2} \quad (4)$$

and the Reynolds number as

$$\mathrm{Re} = \frac{d\omega_s\rho}{\mu} \quad (5)$$

Results

Grazing and fecal pellet production—Average clearance, ingestion, and fecal pellet production rates using different food species are summarized in Table 1. There were significant differences in clearance, ingestion, and pellet production rates between the different diets (one-way ANOVA; $F_2 = 19$, 13, and 11, respectively; $p < 0.001$), with highest rates observed on the *T. weissflogii* diet (Tukey HSD; $p < 0.001$). Ingestion rates ranged from 5 μg C ind^{-1} d^{-1} to 10 μg C ind^{-1} d^{-1} (Table 1), which corresponded to a weight-specific ingestion of ca 0.7–1.4 μg C (μg C)$^{-1}$ d^{-1} (assuming an average female carbon content of 7.4 μg C ind^{-1}; Dutz et al. 2008). In 2-h incubations, grazing and pellet production rates were significantly higher, and pellets were significantly larger when produced on *T. weissflogii*

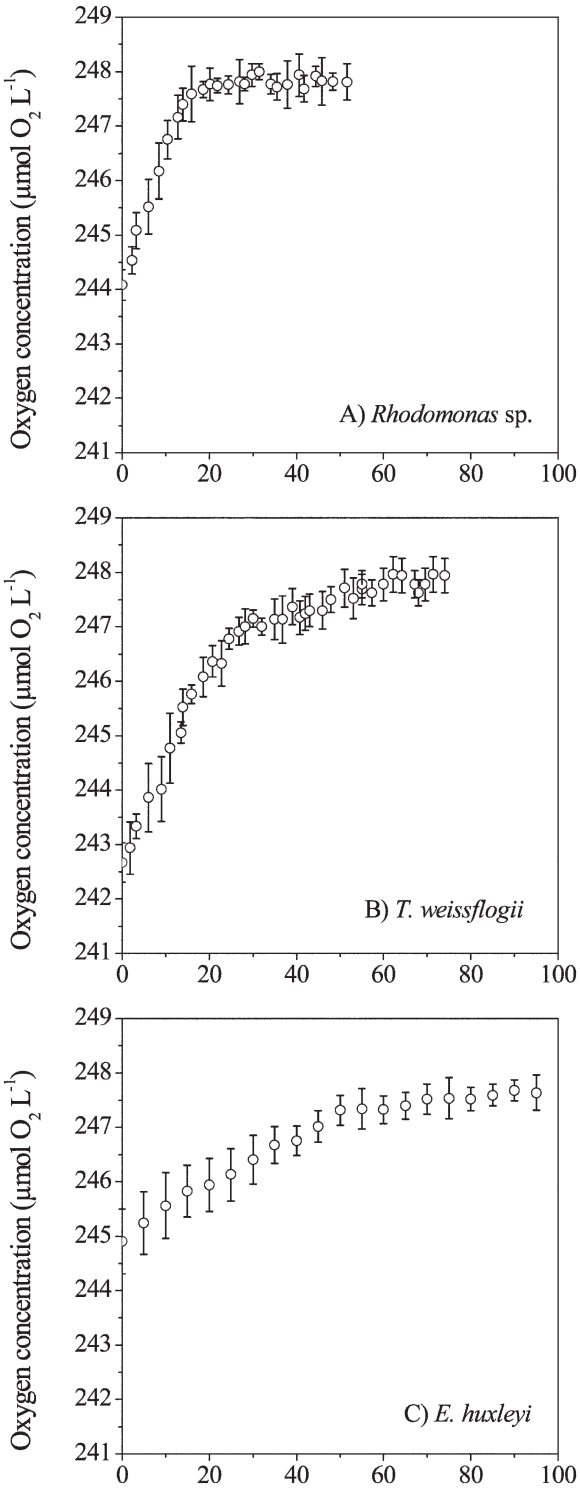

Fig. 1. Oxygen concentration gradients measured at the pellet–water interface of pellets produced on (A) *Rhodomonas* sp., (B) *T. weissflogii*, and (C) *E. huxleyi* as food sources. The symbols represent the mean value with the standard deviation of the mean value shown as bars ($n = 5$).

compared to those measured using *E. huxleyi* or *Rhodomonas* sp. as food sources (ANOVA; $F_2 = 25$, $p < 0.001$; Tukey HSD; $p < 0.01$). No significant differences of these variables were found in 24-h incubations. The fraction of organic carbon egested relative to organic carbon ingested after 24 hours was similar with *Rhodomonas* sp. and *T. weissflogii* as a diet (respectively, 0.48 ± 0.3 and 0.49 ± 0.2), but substantially lower with *E. huxleyi* diet (0.19 ± 0.06).

Microbial respiration on fecal pellets—Fecal pellets were surrounded by a thin diffusive boundary layer through which all exchange of gases and nutrients occur. These boundary layers were only 10–30 µm wide, depending on the pellet size. Pellets produced on *Rhodomonas* sp. were ca. two to four times smaller by volume compared to those produced on *T. weissflogii* and *E. huxleyi* (Table 1). Examples of oxygen gradients measured in the boundary layer of the three different pellet types are shown (Fig. 1). The average oxygen fluxes to the fecal pellets were 0.043 nmol O_2 cm^{-2} s^{-1}, 0.032 nmol O_2 cm^{-2} s^{-1}, and 0.011 nmol O_2 cm^{-2} s^{-1} for pellets produced on *Rhodomonas* sp., *T. weissflogii*, and *E. huxleyi*, respectively. Thus, the oxygen flux was four times higher to a pellet produced on *Rhodomonas* sp. compared to that to a pellet produced on *E. huxleyi*. Average values of O_2-fluxes to pellets and respiration rates are shown (Table 2). The volumetric respiration rate was up to 5.6 times higher in pellets produced on *Rhodomonas* sp. compared to that in pellets produced on *E. huxleyi*. The volumetric respiration rates were 4.12 fmol O_2 μm^{-3} d^{-1}, 1.64–2.86 fmol O_2 μm^{-3} d^{-1}, and 0.77–2.12 fmol O_2 μm^{-3} d^{-1} in pellets produced on *Rhodomonas* sp., *T. weissflogii*, and *E. huxleyi*, respectively. These rates corresponded to average carbon respiration rates ranging between 183 pg C $pellet^{-1}$ h^{-1} and 840 pg C $pellet^{-1}$ h^{-1}. Carbon-specific respiration rates, however, appeared to be relatively similar in pellets produced on different food sources. Pellets produced on *E. huxleyi* showed the highest variability in respiration rates.

Density and sinking velocity varied considerably in pellets produced on different food sources (Table 3). Density and sinking velocity of pellets produced on *Rhodomonas* sp. were significantly lower than those measured with *T. weissflogii* and *E. huxleyi* as food sources (Student's *t*-test; $p < 0.01$). Measured sinking velocity of all types of pellets were higher than predicted from Eq. 1 (Komar et al. 1981). Measured sinking velocity of pellets produced on *Rhodomonas* sp. was similar to that predicted by Stokes' law, but Stokes' law underestimated sinking velocities of pellets produced with *T. weissflogii* and *E. huxleyi* as food sources. We estimated the influence of particle shape and Reynolds number on the drag coefficient for the fecal pellets measured in this study and for a compilation of marine particles from previous studies (Taghon et al. 1984, Alldredge and Gotschalk 1988; Fig. 2). The drag coefficient varied three orders of magnitude for particles sinking with Reynolds numbers varying between 0.01 and 100. The drag coefficient which encompassed fecal pellets was best described by $Cd = 2.2 \times Re^{-1.63}$; $R^2 = 0.87$ ($n = 54$). Whereas the drag coefficient appeared to be higher for marine snow than predicted by Stokes' law for spheres with similar Re, that of fecal pellets was often lower than that predicted by Stokes' law (Fig. 2).

Table 2. Size and respiration rate of pellets produced on different food sources. Number of replicates is indicated in parentheses.

	Volume ($\times 10^5$ μm^3)	O_2-flux (nmol O_2 cm^{-2} s^{-1})	Total O_2-uptake (nmol O_2 $pellet^{-1}$ d^{-1})	Volumetric O_2-uptake (fmol O_2 μm^{-3} d^{-1})	Carbon resp. rate (pg C $pellet^{-1}$ h^{-1})	C-specific resp. rate (d^{-1})
Rhodomonas sp.*	1.7±0.4	0.048±0.016 (45)	0.69±0.24 (9)	4.11±1.42 (9)	345±119	0.16
*T. weissflogii**	3.0±1.7	0.038±0.026 (60)	0.86±0.55 (12)	2.86±1.84 (12)	428±275	0.20
T. weissflogii†	10±7.7	0.032±0.014 (45)	1.68±0.74 (9)	1.68±0.74 (9)	840±370	0.12
*E. huxleyi**	2.6±1.7	0.030±0.009 (55)	0.55±0.16 (11)	2.12±0.63 (11)	273±81	0.21
E. huxleyi†	4.8±4.6	0.014±0.004 (40)	0.37±0.09 (8)	0.77±0.19 (8)	183±46	0.08

* Belong to the same experiment.
† Belong to the same experiment.

The L-ratio (m^{-1}) is calculated as the ratio of the carbon-specific degradation rate (d^{-1}) relative to measured sinking velocity (m d^{-1}) (Feinberg and Dam 1998). It thus describes the carbon-specific degradation m^{-1}, and it can be used as an index of the carbon degradation or preservation in sinking organic matter. The L-ratio was approximately 10-fold lower for pellets produced on *T. weissflogii* and *E. huxleyi* as food sources compared to that in pellets produced on *Rhodomonas* sp. (Table 3).

Discussion

Copepod feeding and pellet production rates on *T. weissflogii* and *Rhodomonas* sp. were similar to those measured previously for *T. longicornis* females, confirming the generally highest proportional egestion on diatom diets (Besiktepe and Dam 2002, Dutz et al. 2008). Although data on copepod feeding on coccolithophorids is relatively scarce and restricted to a few copepod species of the genus *Calanus* and *Pseudocalanus*, substantial ingestion rates seem to be generally observed only at high concentrations of cells (Nejstgaard et al. 1995,1997). The clearance and ingestion rates measured in this study were in the range of these previous observations of ca 5 μg C ind^{-1} d^{-1} for *Calanus helgolandicus* (Huskin et al. 2000), up to 20 μg C ind^{-1} d^{-1} for *Calanus finmarchicus* (Båmstedt et al. 1999) and 6.9 μg C ind^{-1} d^{-1} for *Pseudocalanus elongatus* (Harris 1994) in corresponding food concentrations of ≥300 μg C L^{-1}. Similar ingestion rates of 6.3 ± 2.3 μg C ind^{-1} d^{-1} (n = 5) were also measured for *T. longicornis* in previous incubations with *E. huxleyi* (J. Dutz, M. Koski, S. Jónasdóttir, unpubl. data). Therefore, it seems that *T. longicornis* can use *E. huxleyi* as a food source, irrespective of the small size or coccolith cover of the cells.

The pellet production rates on *E. huxleyi* diet seem to be more controversial: whereas some studies show moderate to high pellet production on *E. huxleyi* (Harris 1994; Båmstedt et al. 1999), nearly no pellets were produced in other studies, irrespective of ingestion (Huskin et al. 2000; Dutz, Koski, Jónasdóttir, unpubl. data). In the present study the lowest proportion of ingested carbon was egested with *E. huxleyi* diet, although there was no difference in the number of pellets produced between *Rhodomonas* sp. and *E. huxleyi*. This is similar to observations of Båmstedt et al. (1999), who, irrespective of a relatively high number of pellets produced, observed a substantially lower proportional egestion by *C. finmarchicus* on *E. huxleyi* than on *Rhodomonas* sp. diet. Harris (1994) suggested that the low proportional egestion of coccolithophorids would be a result of acid digestion in copepod gut because of gut pH, which may approach 6 depending on feeding conditions (Pond et al. 1995). In our study, however, pellet production of *T. longicornis* on decalcified and half-decalcified *E. huxleyi* (1.6 ± 0.5 and 1.4 ± 0.4, respectively) was not different from the untreated *E. huxley* (1.9 ± 0.7), thus there was no direct reason to suspect substantial dissolution of coccolith calcite in copepod gut during the present experiments (data not shown).

This is the first study reporting direct measurements of microbial respiration in copepod fecal pellet. Degradation of these small fecal pellets has been estimated from recordings of pellet volume over time (Hansen et al. 1996), ^{14}C-labeling, bacterial production, and dissolved organic carbon (DOC) production (Lee and Fischer 1992; Urban-Rich 1999; Thor et al. 2003), and by isothermal microcalorimetry (Olsen et al. 2005). Using ^{14}C-labeling, total organic degradation rates in 0–3-day-old copepod fecal pellets vary between 0.06 d^{-1} and 0.17 d^{-1}, which

Table 3. Density, sinking velocity, and L-ratio (carbon-specific respiration rate: measured sinking velocity) of pellets. Number of pellets indicated in parenthesis.

	Density (g cm^{-3})	POC (g cm^{-3})	Komars' settling* (m d^{-1})	Stokes' settling† (m d^{-1})	Measured sinking velocity (m d^{-1})	L-ratio ($\times 10^{-3}$ m^{-1})
Rhodomonas sp.	1.08±0.01 (122)	0.34 (600)	18±5 (19)	39±18 (19)	35±29 (19)	4.0
T. weissflogii	1.14±0.02 (56)	0.17 (600)	41±24 (14)	86±68 (14)	322±169 (14)	0.37–0.63
E. huxleyi	1.17±0.04 (46)	0.12 (600)	42±20 (21)	94±49 (21)	200±93 (21)	0.38–1.0

* Calculated according to Komar et al. 1981 (Eq. 1).
† Calculated according to Stokes law (Eq. 2).

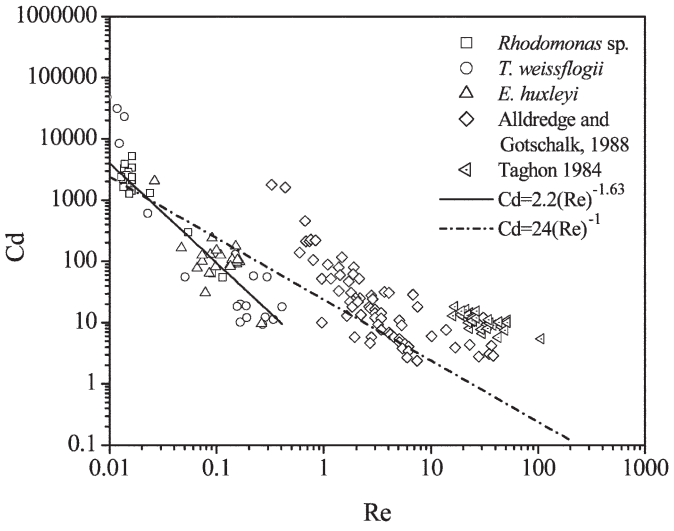

Fig. 2. Drag coefficient (C_D) as a function of Reynolds (Re) number, including previously published data by Alldredge and Gotschalk (1988) and Taghon et al. (1984) and Stokes' relationship for solid spheres at $Re < 0.5$.

encompasses most of our estimates of respiratory turnover of carbon in pellets. Ectoenzymatic hydrolysis rates measured on fecal pellets produced by *Acartia tonsa* fed on *Rhodomonas* sp. and *T. weissflogii* are high, but bacterial production rates were approximately 160-fold lower than respiration rates measured in our study (Thor et al. 2003). Thus, it appears that hydrolysis and respiration are more closely coupled than bacterial production and respiration are, and that net growth efficiency of attached bacteria is low. Another possibility is that bacterial production is underestimated in pooled samples because of diffusion limitation as demonstrated for pooled samples of small aggregates incubated under stagnant conditions (Ploug and Grossart 1999).

Using microelectrodes, it was previously demonstrated that millimeter-long crustacean fecal pellets attached to marine snow can be anoxic in their interior (Alldredge and Cohen 1987). Millimeter-long aggregates formed by detritus including fecal pellets from a copepod culture (*A. tonsa*) grown on *Rhodomonas* sp. are anoxic in their center during the initial degradation stage. The average volumetric O_2-respiration rate in pellets produced on *Rhodomonas* sp. in the present study was 7.8 times higher than the volumetric O_2-respiration rates measured in anoxic aggregates (Ploug et al. 1997), and up to 400 times higher than those of 1.5-mm-long, porous marine snow sampled in the Southern California Bight (Ploug et al. 1999). Copepod fecal pellets have a large surface to volume ratio, and mass transfer by diffusion in seawater occurs on the order of milliseconds at a micrometer scale. Anoxia was thus argued to be unlikely to occur in copepod fecal pellets because of their small size (Alldredge and Cohen 1987). The oxygen flux (i.e., the oxygen concentration gradient) to the millimeter-sized fecal pellets of that study was four times higher than the average flux measured to 130-μm-long pellets produced on *Rhodomonas* sp. of our study. Oxygen concentrations were high at the surface of the small copepod pellets because the diffusive boundary layer surrounding a copepod fecal pellet is very thin and approximately extends two radii into the surrounding water from the center of the pellet. Thus, there was no indication of anoxic conditions within the copepod fecal pellets. Pellets disintegrated upon penetration of the peritrophic membrane surrounding them, and it was therefore not possible to measure the exact oxygen concentrations within intact pellets.

Studies of alkalinity and vertical fluxes have suggested significant (60–80%) calcite dissolution to occur in the upper 500–1,000 m of the water column despite the fact that surface waters of the sea are supersaturated with calcite (Milliman et al. 1999). Model calculations have suggested that respiration in marine snow and fecal pellets is too low and sinking velocities are too high to create microenvironments with pH $\sim$ 6 at which calcite dissolution could occur (Jansen and Wolf-Gladrow 2001). Our study showed high respiration rates in copepod pellets produced on *E. huxleyi*. The pH was above 8.18 in the vicinity of the pellets at which carbonate dissolution at supersaturation of calcite cannot occur (data not shown). The pH in anoxic aggregates produced on zooplankton detritus was previously shown to be 7.2 (Ploug et al. 1997). Other experimental and modeling studies, however, have pointed at other pelagic microenvironments like copepod guts as loci for carbonate dissolution (Harris 1994; Pond et al. 1995; Jansen et al. 2002).

It has been hypothesized that biominerals in phytoplankton, e.g., carbonate and opal, promote carbon preservation of the sinking flux because these biominerals protect the organic matter in the cells from being degraded and/or increase its sinking velocity due to their high densities (Armstrong et al. 2002). Whereas carbon-specific respiration rates were estimated to be similar to or even higher in fecal pellets produced on *E. huxleyi* or *T. weissflogii* compared to those in fecal pellets produced on *Rhodomonas* sp., measured densities and sinking velocities of pellets produced on *E. huxleyi* or *T. weissflogii* were significantly higher than those of fecal pellets produced on *Rhodomonas* sp. Our study directly demonstrates that oxygen consumption varies less than sinking velocity does in pellets produced on a nanoflagellate, diatom, or coccolithphorid diet, showing that at least for freshly-produced particles there is evidence for ballasting, but not for protection. As the protection mechanism includes a free POC fraction, our results do not exclude the possibility that protection could occur for more degraded particles.

Fecal pellet composition, density, and size are important factors determining their sinking velocity. Our measurements of sinking velocities of fecal pellets produced by *T. longicornis* on *E. huxleyi* as diet were close to those of similar-sized fecal pellets produced by *Pseudocalanus elongatus* on the same diet (Harris 1994), but higher than those of similar-sized copepod fecal pellets reported by Bruland and Silver (1981). Sinking velocities of fecal pellets produced on *Rhodomonas* sp. were similar to those of similar-sized copepod fecal pellets reported by Bruland and Silver (1981). Fecal pellet density was close to those previously measured for *T. weissflogii* and *Rhodomonas*

sp. (Feinberg and Dam 1998). In that study, the equation by Komar et al. (1981) was used to estimate sinking velocity of different types of pellets. However, our observed sinking velocities of fecal pellets were two to eight times higher than estimated by the relationship between sinking velocity and pellet dimensions and density reported by Komar et al. (1981), and 0.9–4 times higher than estimated by Stokes' law. This is in contrast to marine snow aggregates, which sink slower than predicted by Stokes' law (Alldredge and Gotschalk 1988). Fecal pellets are not spheres. Their orientation during sinking thus influences the drag considerably. Moreover, the equation by Komar et al. (1981) and Stokes' law are formally only valid for Reynolds numbers (Re) <0.5. When either or both of these conditions are not met, an empirical relationship between C_D and Re must be derived. For the fecal pellets in this study we derived the relationship $C_D = 2.2 \times Re^{-1.63}$ ($R^2 = 0.87$, $n = 54$; Fig. 2). Pellets produced on *T. weissflogii* and *E. huxleyi* had the highest Re and showed the highest deviation from theoretical sinking velocities. Despite their small size, sinking velocities of copepod fecal pellets can be as high as those of larger marine snow (200–400 m d^{-1}). The drag coefficients of sinking marine snow appear to be higher compared to those predicted for spheres by Stokes' law as well as to those of copepod fecal pellets with similar Re. This observation may be explained by high porosities (>0.999) of marine snow (Alldredge and Gotschalk 1988). Drag coefficients of sinking fecal pellets were often lower than those predicted by Stokes' law, which may be explained by the cylindrical shape of the fecal pellets and their orientation during sedimentation. Sinking velocities of particles measured in the laboratory, however, should be considered as maximum sinking velocities. The residence time of sinking particles in the upper mixed layer in the sea is longer than that predicted by the sinking velocity measured in still water (Alldredge and Gotschalk 1988).

Fecal pellets and marine snow are important sites for nutrient recycling within the mixed layer in the ocean, as well as comprising an important fraction of vertical carbon flux. A large fraction of fecal pellets does not leave the upper mixed layer of the ocean by sedimentation (Turner 2002). Coprophagy and coprorhexy (fragmentation) have been considered as mechanisms responsible for this observation (Poulsen and Kiørboe 2005). However, it has been shown that zooplankton >200 μm only play an insignificant role in degradation of fecal pellets (Poulsen and Kiørboe 2006). Microbial food web interactions are complex, and coprorhexy by zooplankton results in fragmentation of pellets, which may lead to increased degradation by bacteria and protozoa because of increased surface : volume ratio and increased leakage of DOC and nutrients. Many particle-attached bacteria are motile and can actively search for particles. Substantial colonization of particles by pelagic bacteria thus occurs within minutes. Bacteria continuously attach to and detach from particles with an average residence time of 3 h (Kiørboe et al. 2002). Hence, the interaction between bacteria and particles is more complex than previously thought, leading to an efficient utilization of nutrients within the upper mixed layer of the ocean.

Oxygen uptake on 0.5–10 mm large aggregates in aquatic systems varies two to three orders of magnitude (Ploug 2001). Measured carbon-specific respiration rates, however, appear to be relatively similar and high across the particle spectrum in the sea. Carbon-specific respiration rates in 1–6-mm-sized diatom aggregates and other types of marine snow are 0.08–0.12 d^{-1} (Ploug et al. 1999; Ploug and Grossart 2000), similar to those in fecal pellets with an equivalent spherical diameter of <100 μm of the present study. Total oxygen consumption in 5–25 fecal pellets equaled that of one 1.5-mm-large marine snow aggregate with a similar carbon-specific respiration rate (Ploug et al. 1999). Marine snow abundance often ranges between 0.1 aggregates L^{-1} and 10 aggregates L^{-1} (Simon et al. 2002). Copepods are the most common metazoans in the pelagic ocean, with a typical abundance of $\sim$1 ind L^{-1}. With a production rate of one pellet h^{-1}, the daily respiration on pellets produced by one copepod equals that on 1–5 single (1.5 mm large) marine snow aggregate. Thus, carbon remineralization can be quantitatively similar in copepod fecal pellets and marine snow in the ocean. The present study demonstrates that sinking velocity varies much more than the specific carbon remineralization rates in copepod fecal pellets and marine snow does. Ballast material (e.g., opal and carbonate) indeed appears to be an important factor controlling particle sinking velocity and vertical carbon fluxes in the ocean.

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Paper IV

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Ballast, sinking velocity, and apparent diffusivity within marine snow and zooplankton fecal pellets: Implications for substrate turnover by attached bacteria

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Abstract

We analyzed size-specific dry mass, sinking velocity, and apparent diffusivity in field-sampled marine snow, laboratory-made aggregates formed by diatoms or coccolithophorids, and small and large zooplankton fecal pellets with naturally varying content of ballast materials. Apparent diffusivity was measured directly inside aggregates and large (millimeter-long) fecal pellets using microsensors. Large fecal pellets, collected in the coastal upwelling off Cape Blanc, Mauritania, showed the highest volume-specific dry mass and sinking velocities because of a high content of opal, carbonate, and lithogenic material (mostly Saharan dust), which together comprised ~80% of the dry mass. The average solid matter density within these large fecal pellets was 1.7 g cm^{-3}, whereas their excess density was 0.25 ± 0.07 g cm^{-3}. Volume-specific dry mass of all sources of aggregates and fecal pellets ranged from 3.8 to 960 μg mm^{-3}, and average sinking velocities varied between 51 and 732 m d^{-1}. Porosity was >0.43 and >0.96 within fecal pellets and phytoplankton-derived aggregates, respectively. Averaged values of apparent diffusivity of gases within large fecal pellets and aggregates were 0.74 and 0.95 times that of the free diffusion coefficient in sea water, respectively. Ballast increases sinking velocity and, thus, also potential O_2 fluxes to sedimenting aggregates and fecal pellets. Hence, ballast minerals limit the residence time of aggregates in the water column by increasing sinking velocity, but apparent diffusivity and potential oxygen supply within aggregates are high, whereby a large fraction of labile organic carbon can be respired during sedimentation.

Marine snow and fecal pellets comprise a significant fraction of the sinking carbon flux in the ocean (Alldredge and Silver 1988; Simon et al. 2002; Turner 2002). Hence, sedimentation of these particles into the bathypelagic zone is important for the ocean's capacity to sequester CO_2 from the atmosphere, i.e., the ocean's biological carbon pump (De La Rocha and Passow 2007). The recent observation that carbonate and organic carbon fluxes show close correlations in the bathypelagic zone of the ocean has led to the hypothesis that biominerals in phytoplankton, e.g., carbonate and opal, promote carbon preservation of the sinking flux because these biominerals increase sinking velocity because of their high densities and/or protect a fraction of the organic matter in the cells from being degraded in the deep ocean (Armstrong et al. 2002; Francois et al. 2002; Klaas and Archer 2002). The effect of ballast minerals on sinking velocity relative to that on small-scale oxygen fluxes and degradation rates in sinking particles, however, is largely unknown.

The physical and chemical microenvironment of sinking particles is significantly different from that of the surrounding water. High concentrations of inorganic and organic matter, ecto-enzymatic activities, and remineralization rates by attached bacteria lead to oxygen and pH gradients within sinking marine snow and fecal pellets (Alldredge and Cohen 1987; Smith et al. 1992; Ploug et al. 1999). The observations that dissolved organic carbon (DOC), silicic acid, ammonium, and phosphate concentrations are higher inside marine snow compared to the surrounding water have led to the hypothesis that diffusion within marine snow is significantly slower than in sea water (Shanks and Trent 1979; Brzezinski et al. 1997; Alldredge 2000).

The flux of a solute within an aggregate equals the product of the apparent diffusivity and the radial concentration gradient of the solute, i.e., Fick's first law of diffusion:

$$J = \phi D_s \frac{dC}{dr} \quad (1)$$

where J is the flux of the solute, ϕ is the porosity, D_s is the effective diffusion coefficient, and dC/dr is the radial concentration gradient of the solute. The combined parameter (ϕD_s) is the apparent diffusivity of the solute. The effective diffusion coefficient is a function of porosity,

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tortuosity, and the free diffusion coefficient in water (D_0). The tortuosity relates the actual distance a molecule or an ion travels within an aggregate to the distance it travels in water per unit length of the aggregate. Direct measurements of apparent diffusivity in marine snow are scarce. Recently, apparent diffusivity of gases was measured directly within diatom aggregates containing transparent exopolymer particles (TEP) using a microsensor (Ploug and Passow 2007). Apparent diffusivity of gases within these aggregates was close to the free molecular diffusion coefficient of these in the surrounding water, independently of size and age of the aggregates. Steep concentration gradients of oxygen within diatom aggregates thus reflected high biological activity (fluxes) within these aggregates rather than low diffusivity.

In the present study, we analyze the effect that ballast has on sinking velocity, apparent diffusivity, and small-scale oxygen fluxes to sinking particles of different sources with naturally varying content of ballast material, e.g., field-sampled marine snow; laboratory-made aggregates formed by diatoms or coccolithophorids; and small and large zooplankton fecal pellets, containing biogenic and/or lithogenic ballast minerals.

Methods

Aggregates and fecal pellets—Cultures of diatoms (*Skeletonema costatum*) and coccolithophorids (*Emiliania huxleyi*) were grown at 15°C in F/2 medium in a 12 : 12 light : dark cycle during 14–28 d (Guillard and Ryther 1962). Aggregates of the respective cultures were formed in roller tanks rotating at 3 rounds min^{-1} in darkness (Shanks and Edmonson 1989). Diatom aggregates formed during the first day after the culture had been transferred to roller tanks. Aggregates from the coccolithophorid culture formed only after 5 d of rotation. Large zooplankton fecal pellets, most probably produced by giant mesopelagic larvaceans (appendicularians), had been collected in sediment traps from 1,296-m water depth from the eutrophic site CBi-2 off Cape Blanc, Mauritania, NW Africa (20°45′N, 18°42′W). The sample cups were filled with filtered seawater and poisoned with $HgCl_2$ before deployment. The coastal upwelling area is characterized by high production of coccolithophorids and diatoms, and a high input of dust from the Sahara (Fischer et al. in press). The fecal pellets (up to 1.5 mm in length) were pipetted individually from three sediment trap samples collected from 10 November 2004 to 18 January 2005 (sampling intervals 23 d each). Copepod fecal pellets were produced in culture experiments using *Temora longicornis* grown on *E. huxleyi* as previously described (Ploug et al. 2008).

Size measurements—Aggregates and large fecal pellets were placed on a net in a vertical flow system, and their size was determined under the dissection microscope using a calibrated ocular meter (Ploug and Jørgensen 1999). The aggregate or fecal pellet was turned during measurement, which allowed for size determination of all three axes. The dimensions of small fecal pellets were determined directly under a dissection microscope. The volume of aggregates was calculated assuming an ellipsoid, $V = (1/6)\ \pi \times \text{length} \times \text{width} \times \text{height}$, and that of fecal pellets was calculated assuming cylindrical geometry.

Dry mass and fractal dimension—Single aggregates or single appendicularian fecal pellets with known volumes were filtered onto preweighed 0.4-μm polycarbonate filters, washed and dried at 60°C for 48 h, and reweighed. Copepod fecal pellets were pooled in triplicate samples with 600 pellets $filter^{-1}$. The volume of 30 copepod fecal pellets was calculated from their dimensions. The sensitivity of the scale was 0.1 μg (Mettler Toledo, UMX 2). The fractal dimension (D_3) of aggregates was estimated from the relative distribution of mass in aggregates of different sizes measured as equivalent spherical diameter (Logan and Wilkinson 1990).

Sinking velocity—Aggregate sinking velocity was measured in a vertical flow system wherein an upward-directed flow velocity was adjusted to balance the sinking velocity of aggregates (Ploug and Jørgensen 1999). The sinking velocity was calculated from the volume of water passing through the flow chamber per unit time divided by the cross-sectional area of the flow chamber. An advantage of this method is that the aggregate is not destroyed and can easily be collected for further analysis afterwards. However, a comparison of aggregate sinking velocities measured within this flow system with those measured in a sedimentation column have shown that sinking velocities tend to be underestimated by 18% in the flow system relative to the values obtained in a sedimentation column (H. Ploug unpubl.). Sinking velocities were corrected by this value in the present study to normalize sinking velocity data to those measured in a sedimentation column (*see* below).

Fecal pellets do not disaggregate as easily as do marine snow and other porous aggregates. Sinking velocities of appendicularian fecal pellets were too high to be measured in the flow system because of air-bubble formation within the system at the high inflow rates needed to keep particles in suspension. Sinking velocities of fecal pellets were therefore measured in a sedimentation column. The column (40 cm high and 3 cm in diameter) was filled with filtered seawater (~32) and kept in a 15°C thermostated room, surrounded by a water jacket for thermal stabilization. The settling column was closed at both ends, allowing only insertion of a Pasteur pipette at the top. Pellets were rinsed in filtered seawater and collected in a Pasteur pipette with filtered seawater (~32). Pellets sank out of the Pasteur pipette, which was centered in the column. The descent of the pellets was recorded by two charge-coupled device video cameras (MTV-1802CB, Mintron) equipped with 105-mm lenses (Micro Nikkor 1 : 2.8, Nikon). The cameras were placed along the *x*- and *z*-axes of the pellets, giving a three-dimensional view of the settling. A time-code generator was connected to the cameras, making timing of the pellets possible. Infrared illumination was provided from behind by light-emitting diodes and collimated through condensers. The setup was

calibrated by recording a length scale before sinking velocity measurements.

Theoretical sinking velocities of aggregates and fecal pellets were calculated from

$$U = \left(2g\Delta\rho V / \rho_f C_D A\right)^{0.5} \quad (2)$$

where U is the sinking velocity, g is the acceleration caused by gravity, $\Delta\rho$ is the excess density of the aggregates, and ρ_f is the density of sea water (1.02488 g at 15°C). C_D is the drag coefficient and A is the cross-sectional area of the particle.

The Reynolds number was calculated as (White 1974)

$$Re = \frac{dU}{v} \quad (3)$$

where d is the diameter and v is the kinematic viscosity of sea water (1.19×10^{-2} cm^2 s^{-1} at 15°C).

The drag coefficient for $Re > 0.5$ was calculated as (White 1974)

$$C_D = \frac{24}{Re} + \frac{6}{1 + Re^{0.5}} + 0.4 \quad (4)$$

Excess density and solid matter density—The excess density of aggregates was calculated from measured sinking velocities (Eq. 2) using the drag coefficient (Eq. 4). The solid matter density was calculated from Alldredge and Gotschalk (1988):

$$\Delta\rho = \frac{W}{V} \times \left(1 - \frac{\rho_f}{\rho_s}\right) \quad (5)$$

where W is the dry mass, ρ_s the solid matter density, and V the volume of the aggregate.

The densities of fecal pellets were determined experimentally in a density gradient, using a modified version of the centrifugation method (Schwinghamer et al. 1991; Feinberg and Dam 1998). Seven dilutions were made using Ludox TM colloidal silica, sucrose, and distilled water. The dilutions ranged in density from 1.05 to 1.43 g cm^{-3}. The dilutions were buffered to pH 8.1 with 0.0125 mol L^{-1} Tris plus 0.0125 mol L^{-1} Tris-HCl (final concentration). Hence, the gradient produced was isosmotic with seawater of salinity ~32 (Weast 1968). Two milliliters of each dilution was gently transferred to a 20-mL centrifuge tube, beginning with the densest and finishing with the lightest dilution. The density gradients were stored at 5°C overnight but were at room temperature before use. Whole fecal pellets were rinsed in filtered seawater (~32), placed in 1 mL of seawater, and pipetted on top of the density gradient. The samples were centrifuged at 3,000 rpm for 30 min. After centrifugation, 1 mL from each of the different density layers was removed from the tube using a peristaltic pump and weighed using a Mettler Toledo balance to 0.1 mg. The size and number of pellets layer^{-1} were recorded using a dissection microscope. No pellets were observed to be disrupted by the centrifugation process.

Porosity—The porosity of aggregates and fecal pellets was calculated as (Alldredge and Gotschalk 1988):

$$p = 1 - \frac{W/\rho_s}{V} \quad (6)$$

The calculated porosity in aggregates is relatively insensitive to the density of dry mass because aggregate porosity is very high, as also previously noted by Alldredge and Gotschalk (1988).

Scanning electron microscopy—Aggregate or fecal pellet composition was visually analyzed using scanning electron microscopy (SEM). Aggregates and fecal pellets were filtered onto 0.4-μm polycarbonate filters, rinsed with deionized water, and dried >24 h at 40°C. The filters were covered with a 5-nm gold–palladium layer (SC500, Emscope). Random areas of the filters were chosen for visual determination of the content. SEM analysis was performed using an FEI Quanta 200F scanning electron microscope together with xTmicroscope software.

Elemental analysis of fecal pellets—About 50 appendicularian pellets were collected with a pipette from the wet splits (1/5) from three samples from the CBi-2 trap positioned at 1,296-m water depth. Pellets were freeze-dried and homogenized with a mortar. We performed organic carbon, total nitrogen, and carbonate analysis using a HERAEUS-CHN analyzer. Organic carbon was measured using the freeze-dried material, from which calcium carbonate was removed with 6 mol L^{-1} HCl in silver boats and dried on a hot plate prior to the analysis. Carbonate was determined from the difference between nonacidified samples and acidified samples. Biogenic opal was determined with a sequential leaching technique with 1 mol L^{-1} NaOH (Müller and Schneider 1993). Lithogenic material was calculated according to: lithogenic = total mass – opal – calcium carbonate – 2 × organic carbon. Organic carbon was multiplied by a factor of 2 to estimate total organic matter (Wakeham et al. 1980).

Diffusivity—Apparent diffusivity was measured directly within aggregates or fecal pellets using a diffusivity microsensor (Unisense) connected to a picoammeter (PA2000, Unisense) and a strip chart recorder (Revsbech et al. 1998; Ploug and Passow 2007). The detection principle is based on a tracer gas (hydrogen) diffusing away from the microsensor tip. These sensors are very stable. Hydrogen, however, is not a completely inert gas in biological systems. Production or consumption of hydrogen can be recognized by non–steady-state signals within biological samples. Such signals were never observed during our measurements. The microsensor was attached to a micromanipulator. The tip diameter was 50 μm and its position was observed under a dissection microscope. The microsensor was calibrated in glass beads with a diameter of 5–20 μm, and in stagnant water in the boundary layer of a piece of 1% agar (Revsbech et al. 1998; Ploug and Passow 2007). Single aggregates or fecal pellets were placed on a net to ensure free diffusion in all dimensions during measurements, i.e., to avoid wall effects (Ploug and

Table 1. Source and culture age, sample size, equivalent spherical diameter (*ESD*), volume-specific dry mass (Vol.-spec. dry mass), fractal dimension (D_3), porosity, apparent diffusivity ($\phi D_s : D_0$) within aggregates, sinking velocity (U), and Sherwood number (Sh) for marine snow, phytoplankton-derived aggregates, and fecal pellets (fp). nd: no data.

Source	No. in sample	*ESD* (mm)	Vol.-spec. dry mass (μg mm^{-3})	D_3	Porosity	$\phi D_s : D_0$	U (m d^{-1})	*Sh*
Marine snow*	12	2.9±1.3	3.8±1.0	nd	0.996	nd	51±56	5.9
S. costatum (2 weeks)	35	2.8±0.5	8.3±2.8	1.60±0.22	0.992±0.003	0.95±0.01	140±41	8.3
S. costatum (5 weeks)	22	2.0±0.7	9.3±5.3	1.47±0.22	0.989±0.009	nd	250±97	9.0
S. costatum (2 weeks)	12	1.7±0.3	16.2±4.5	1.63±0.18	0.984±0.004	0.95±0.01	80±26	5.9
E. huxleyi (3 weeks)	12	1.5±0.3	44±19	2.16±0.82	0.959±0.018	0.95±0.02	216±31	7.7
Appendicularian fp	38	0.63±0.09	960±171†	—	0.434±0.100†	0.74±0.06†	732±153	8.8
Copepod fp	20	0.10±0.02	550‡	—	0.65	nd	199±92	3.1

* Collected in the Southern California Bight (Ploug et al. 1999).
† n=7.
‡ 3×600 pooled fp.

Jørgensen 1999; Ploug and Passow 2007). The apparent diffusivity was measured at multiple positions within each aggregate or fecal pellet at stagnant conditions. After measurements, aggregates and fecal pellets were further processed for dry mass measurements.

Oxygen measurements—Oxygen distributions were measured within and around marine snow and phytoplankton-derived aggregates using a microelectrode with a tip diameter of 10 μm attached to a micromanipulator (Revsbech 1989). The microsensor was calibrated at air saturation and under anoxic conditions. Its 90% response time was <1 s, and its stirring sensitivity was <0.3%. Its current was read by a picoammeter connected to a strip chart recorder. The aggregate was placed on a net in the vertical flow system and the flow was adjusted to suspend the aggregate one diameter above the net, whereby its sinking velocity was balanced by the upward-directed flow velocity (Ploug and Jørgensen 1999; Kiørboe et al. 2001). The microelectrode was slowly brought to the aggregate surface as observed under the dissection microscope. The gradients across the aggregate–water interface were measured at 50–100-μm depth increments, and afterwards analyzed using a diffusion-reaction model to calculate the oxygen uptake rates and to estimate the apparent diffusivity inside aggregates (Ploug et al. 1997). This model is based on mass balance of fluxes at the aggregate–water interface assuming aggregates to be impermeable to flow. The flux of oxygen into the aggregate, which is the product of the apparent diffusivity and the oxygen concentration gradient below the aggregate surface, must equal the diffusive supply from the surrounding water at the aggregate–water interface. This model has previously been shown to give very accurate estimates of oxygen uptake in model systems like agar beads (Ploug et al. 2002).

The Sherwood number for oxygen transport to sinking aggregates and fecal pellets was calculated as (Kiørboe et al. 2001)

$$Sh = 1 + 0.619 \times \left(\frac{Ur_0}{\nu}\right)^{0.421} \left(\frac{\nu}{D_0}\right)^{1/3} \quad (7)$$

where r_0 is the aggregate radius (cm) and D_0 is the molecular diffusion coefficient of oxygen in sea water (1.74×10^{-5} cm^2 s^{-1} at 15°C; Broecker and Peng 1974).

Results

Equivalent spherical diameter, volume-specific dry mass, fractal dimension (D_3), porosity, apparent diffusivity ($\phi D_s : D_0$), sinking velocity (U), and Sherwood number (Sh) in marine snow, phytoplankton-derived aggregates, and fecal pellets are summarized in Table 1. The diameters varied approximately 30-fold across the different sources of aggregates and fecal pellets. The porosity of field-sampled marine snow and of aggregates derived from phytoplankton cultures was high (>0.95). Volume-specific dry mass, porosity, sinking velocity, and Sherwood number of diatom aggregates varied greatly depending on average aggregate size and age as observed in our three diatom cultures (*S. costatum*). The densest and most compact phytoplankton aggregates were those formed from the *E. huxleyi* culture. These aggregates had on average a higher fractal dimension and lower porosity compared to those formed by the diatom. Scanning electron microscopy showed that these aggregates had a very high content of liths. The volume-specific dry mass in appendicularian fecal pellets was approximately 250-fold higher than that in field-sampled marine snow. Forty-four percent of their dry mass was lithogenic material (mostly Sahara dust), 27% was calcium carbonate, and organic carbon comprised 12% of the dry mass content (Table 2). The mean C_{org} : N ratio was 11. The estimated porosity was significantly lower in fecal pellets compared to that measured in phytoplankton-derived aggregates and marine snow. Apparent diffusivity within phytoplankton aggregates was close to the free diffusion coefficient in sea water in contrast to that within appendicularian fecal pellets, which was significantly lower than the free diffusion coefficient in sea water (Table 1). Sinking velocity varied up to 14-fold for different sizes and sources of aggregates. Estimated excess densities varied more than 1,000-fold. The estimated solid matter density varied between 1.08 and 1.7 g cm^{-3} (Table 3).

The Sherwood number measures the enhancement of mass transfer (diffusion and advection) to a sinking particle relative to that to a stagnant particle (diffusion only) in the water column. Hence, the potential mass transfer to sinking aggregates and fecal pellets was enhanced 3- to 9-fold compared to that to stagnant aggregates and pellets. The

Table 2. Average composition (in % of dry mass with the standard deviation of the mean value; $n=3$) of appendicularian fecal pellets collected in sediment traps (1,296-m water depths) off Cape Blanc, Mauritania, from 10 November 2004 to 18 January 2005.

	Composition
C_{org}	12±2.7
Nitrogen	1.31±0.37
Calcium carbonate	27±8
Biogenic opal	8.8±0.4
Lithogenic material	44±6

potential mass transfer of oxygen to denser and faster-sinking aggregates was enhanced by 31–41% relative to that to similar-sized aggregates with lower density and sinking velocity (Table 1). Diatom aggregates formed by an older culture showed higher sinking velocities compared to those formed by the younger one, although the average dry mass (wet volume)$^{-1}$ was similar in aggregates of the two cultures.

The fluxes of oxygen to sinking aggregates are determined by the potential mass transfer of oxygen by diffusion and/or advection and by the oxygen consumption rates within aggregates. The oxygen gradients within aggregates, which are not permeable to fluid flow, depend on aggregate size, oxygen consumption rates, the apparent diffusivity within aggregates, and the concentration boundary layer thickness at the aggregate–water interface during sinking, i.e., the Sherwood number (Ploug et al. 1997; Ploug 2001). Here, we estimated the apparent diffusivity of oxygen within aggregates and marine snow from measured oxygen gradients using a diffusion-reaction model assuming our aggregates to be impermeable to flow. Three examples of oxygen gradients measured in field-sampled marine snow (Ploug et al. 1999), diatom aggregates, and aggregates formed by the *E. huxleyi* culture are shown in Fig. 1. The oxygen consumption rates in the aggregates were calculated from the oxygen gradient in the water phase immediately adjacent to the aggregate surface, i.e., they can be determined independently of the apparent diffusivity inside the aggregates, because all oxygen being consumed inside the aggregate must cross the aggregate–water interface. The measured oxygen gradients were consistent with those modeled for similar-sized aggregates with an apparent diffusivity of oxygen within the aggregate of 0.95 times the free diffusion coefficient of oxygen in sea water in all three cases. The variation in oxygen gradients between aggregates was explained by variations in oxygen consumption rates. The oxygen consumption rate in field-sampled marine snow was 2.3 nmol O_2 h^{-1}, whereas that of the diatom aggregate was 4.6 nmol O_2 h^{-1}, and that of the aggregate formed by the *E. huxleyi* culture was 14.3 nmol O_2 h^{-1}. Hence, aggregates formed by the *E. huxleyi* culture were rich in liths as well as in labile organic matter. Assuming an apparent diffusivity of oxygen within aggregates to be 0.1 or 0.01 times that of the free diffusion coefficient in sea water resulted in significantly different oxygen gradients compared to those measured within the aggregates (Fig. 2). The aggregates would have been anoxic if the apparent diffusivity had been only 1–10% of the free diffusion coefficient. Diffusion of oxygen, therefore, did not limit the bacterial respiration of organic matter within these aggregates.

Discussion

Sinking velocity and remineralization rates of particles are two major factors determining vertical carbon fluxes and the efficiency of the biological carbon pump in the ocean (De La Rocha and Passow 2007). Phytoplankton-derived aggregates and zooplankton fecal pellets are important components in the vertical carbon flux to the deep sea and sediments (Turner 2002). The present study is the first to directly measure aggregate or fecal pellet composition, density, sinking velocity, apparent diffusivity, and O_2 fluxes across a variety of particle sources occurring in the ocean. Hereby we could experimentally assess the effect of ballast on sinking velocities and mineralization rates of phytoplankton-derived aggregates and fecal pellets in the upper ocean. We used volume-specific dry mass, excess density, and solid matter density as measures of ballast, where chemical composition was not measured directly. The volume-specific dry mass varied 250-fold across the different particle and aggregate sources. It was shown that ballast minerals have little influence on

Table 3. Source and culture age, excess density, solid matter density (ρ_s), Reynolds number (Re), drag coefficient (C_D), theoretical sinking velocity (U_{theory}), and the ratio between measured and theoretical sinking velocity. fp: fecal pellet.

Source	Excess density (mg cm^{-3})	ρ_s (g cm^{-3})	Re	C_D	U_{theory} (m d^{-1})	$U: U_{theory}$
Marine snow*	0.19†	1.08	1.43	20	52	0.98
S. costatum (2 weeks)	0.63†	1.11	3.81	3.8	139	1.00
S. costatum (5 weeks)	2.23†	1.35	4.86	4.9	243	1.03
S. costatum (2 weeks)	0.83†	1.08	1.32	1.3	79	1.01
E. huxleyi (3 weeks)	3.36†	1.11	3.15	3.2	217	0.99
Appendicularian fp	250±70	1.7	4.48	4.5	1,233	0.59
Copepod fp	150±40	1.6	0.19	0.20	105	1.88

† Calculated from measured sinking velocity (Eq. 2).
* Collected in the Southern California Bight (Ploug et al. 1999).

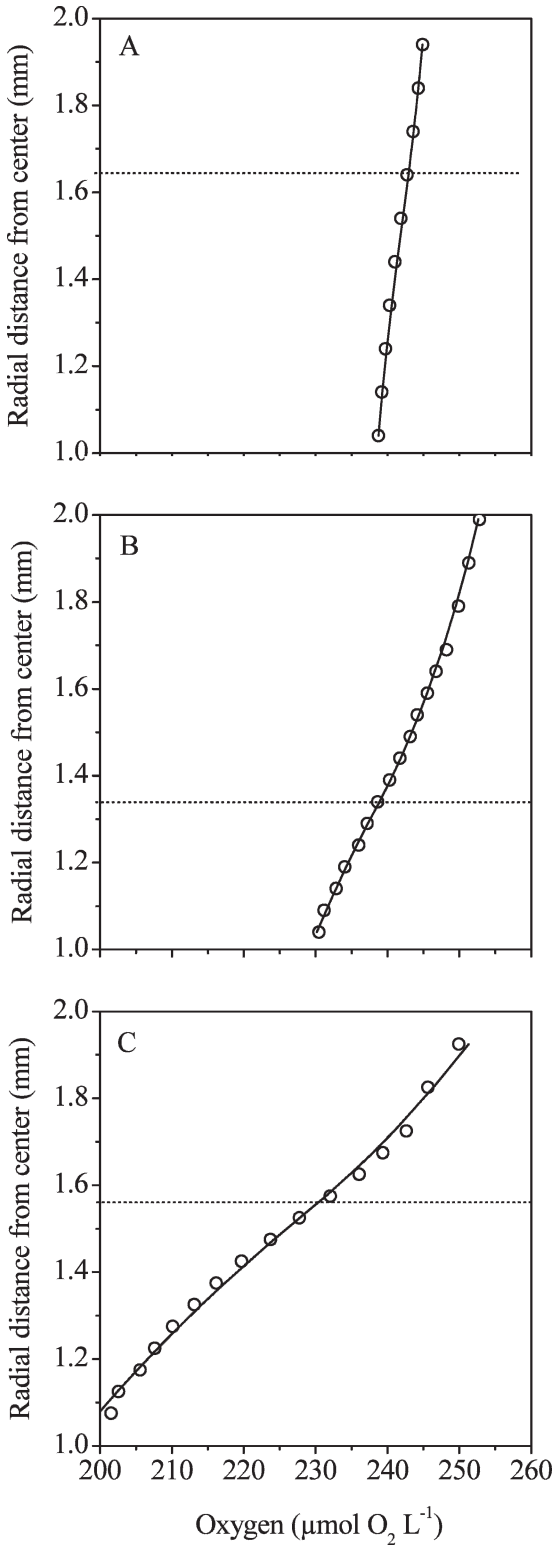

Fig. 1. (A) Oxygen gradients measured (circles) and modeled (line) across the aggregate–water interface assuming an apparent diffusivity of oxygen to be 0.95 times the free diffusion coefficient inside sea water in marine snow, (B) an aggregate formed by *S. costatum*, and (C) an aggregate formed by *E. huxleyi*. The horizontal dotted lines indicate the aggregate surface. *See* text for details.

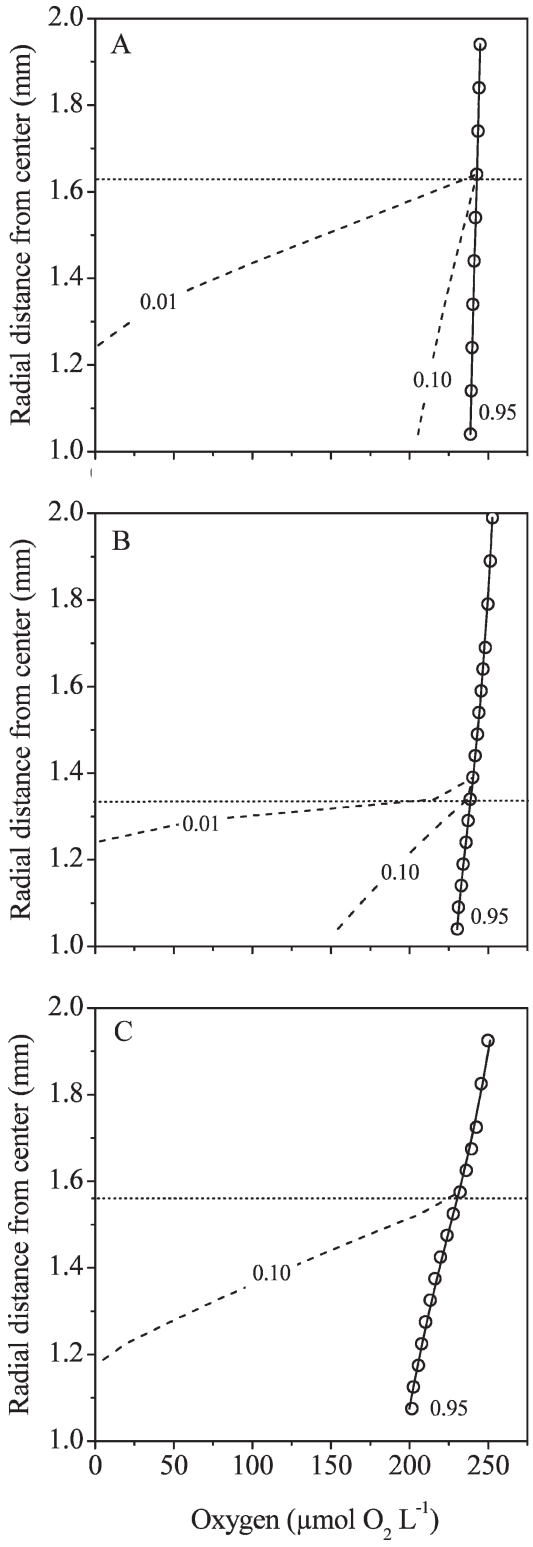

Fig. 2. (A) Oxygen gradients measured (circles) and modeled (line) across the aggregate–water interface assuming an apparent diffusivity of oxygen to be 0.95, 0.10, or 0.01 times the free diffusion coefficient in sea water inside marine snow, (B) an aggregate formed by *S. costatum*, and (C) an aggregate formed by *E. huxleyi*. The horizontal dotted lines indicate the aggregate surface. *See* text for details.

apparent diffusivity within aggregates, but these minerals increase sinking velocity and thereby also the potential O_2 fluxes to sedimenting aggregates and fecal pellets.

Sinking velocities of fecal pellets and marine snow cannot be predicted by Stokes' law, because their Reynolds numbers are well above 0.5, their geometry is nonspherical, and marine snow is porous. Furthermore, sinking velocities of similar-sized aggregates vary greatly (Alldredge and Gotschalk 1988; Khelifa and Hill 2007; Ploug et al. 2008). Our study demonstrates that sinking velocities of different particle types depend on source, density, and age rather than on size. Sinking velocity of similar-sized aggregates and fecal pellets is largely controlled by excess density, which depends on solid matter density, i.e., composition of primary particles, porosity, and fractal dimension (Khelifa and Hill 2007). A high content of lithogenic material (mostly fine-grained dust) and calcium carbonate in zooplankton fecal pellets coincides with high densities and sinking velocities of these pellets. By comparison, the excess density of large, porous phytoplankton-derived aggregates is much lower than that of fecal pellets. TEP are significant components in phytoplankton aggregates, where they occupy a high fraction of the aggregate volume but contribute little to dry mass (Ploug and Passow 2007). TEP can diminish sinking velocity of aggregates because of low specific density of these particles (Engel and Schartau 1999; Azetsu-Scott and Passow 2004). The TEP : dry mass ratio tends to decrease in aging aggregates, which may partly explain the increased sinking velocities and apparently higher solid matter densities in older aggregates of the present study (Ploug and Passow 2007).

It has been argued that diffusivity of gases and solutes within marine snow may be significantly lower than the respective diffusion coefficients in water because of their fractal geometry (Alldredge 2000). The fractal dimensions of our aggregates were similar to those reported from the field (Alldredge and Gotschalk 1988; Logan and Wilkinson 1990), but the apparent diffusivity of gases within porous marine snow and phytoplankton-derived aggregates was only slightly lower than that in (stagnant) sea water. High concentrations of cells, minerals, and TEP may limit advection rather than diffusion within aggregates. The apparent diffusivity in large fecal pellets with a very high volume-specific content of carbonate and lithogenic material was only approximately 25% lower than that in sea water. Although ballast material had little influence on apparent diffusivity within aggregates and fecal pellets, it had a large effect on sinking velocity and thus total mass transfer (diffusion and advection) of oxygen to sinking particles. Hence, our study provides no evidence for protection mechanisms against degradation of labile organic matter that might result from a lower diffusivity because of packaging of aggregates and fecal pellets during sedimentation.

It has previously been shown that millimeter-long euphausidian fecal pellets attached to marine snow can be anoxic (Alldredge and Cohen 1987). The aggregates analyzed in the present study were far from being anoxic. The half-saturation constant for oxygen uptake in bacteria is approximately 1 μmol L^{-1} (Fenchel and Finlay 1993), and bacterial respiration was thus not limited by oxygen availability within aggregates. The oxygen gradients occurring within the aggregates of our study were explained by the oxygen consumption rates in combination with a high apparent diffusivity of oxygen. This was also the case in anoxic aggregates formed by zooplankton detritus produced on a nanoflagellate diet (Ploug et al. 1997). Anoxic aggregates and particles, however, may be common within oxygen minimum zones with <25 μmol O_2 L^{-1}, rather than in fully oxygenated sea water (Ploug 2001, 2008).

Production, degradation, and residence time of organic carbon in sinking particles are complex processes that depend on physical processes, e.g. turbulence, as well as on chemical and biological processes in the upper mixed layer of the ocean. Macrocrustacean fecal pellets can remain in surface water much longer than could be expected from their sinking velocity alone (Alldredge et al. 1987). Marine snow and fecal pellets can disaggregate because of fragmentation by zooplankton, leading to decreased sinking velocities and increased substrate turnover within the upper mixed layer of the ocean (Dilling and Alldredge 2000; Iversen and Poulsen 2007). Bacteria associated with marine snow and fecal pellets are characterized by high enzymatic activities (Smith et al. 1992; Thor et al. 2003; Ziervogel and Arnosti 2008), leading to microenvironments with elevated concentrations of DOC, inorganic nutrients, and amino acids relative to those of the surrounding water (Smith et al. 1992; Brzezinski et al. 1997; Alldredge 2000). These microenvironments are 10- to 100-fold larger than the volume of sinking aggregates themselves because of advection and diffusion acting at the aggregate–water interface (Kiørboe et al. 2001; Ploug and Passow 2007). Chemosensory behavior has been demonstrated in marine bacteria as well as in zooplankton that feed on marine snow (Kiørboe 2001; Kiørboe et al. 2002). Hence, bacterial colonization of sinking marine particles is a very fast process leading to a significant turnover of the aggregate carbon and nitrogen pools in the surface ocean (Smith et al. 1992; Ploug et al. 1999; Kiørboe et al. 2002). Carbon-specific respiration rates are relatively similar within copepod fecal pellets, irrespective of biogenic ballast material content, and within marine snow (0.08 to 0.21 d^{-1}) (Ploug et al. 1999; Ploug and Grossart 2000; Ploug et al. 2008). This observation may largely be explained by high hydrolysis rates combined with high apparent diffusivities of solutes and oxygen supply for respiration, which support an efficient turnover of labile carbon across different particle sources and sizes in the ocean.

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Paper V

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High resolution profiles of vertical particulate organic matter export off Cape Blanc, Mauritania: Degradation processes and ballasting effects

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Running head: Particle remineralization and sedimentation

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Abstract

We estimated vertical carbon fluxes from *in situ* profiles of aggregate size-specific abundances measured off Cape Blanc (Mauritania), using size-specific relationships of aggregate sinking speeds and carbon content. Estimated carbon fluxes were compared with fluxes measured by deep-ocean sediment traps for the same area and period. We estimated carbon removal rates from vertical POC fluxes and identified the important degradation processes at different depth intervals. Copepod grazing and bacterial activity were the dominant degradation processes in the depth layer between 20 and 80 m. In water depths between 80 and 220 m, the carbon removal rate was dominated by microbial activity. Below 220 m, the carbon removal rate was constantly low and likely limited by low bacterial activity due to either abundance or physical parameters, e.g., temperature and pressure. Hence, most carbon degradation occurred in the upper 220 meters and was mainly limited by aggregate residence time at these depths. The aggregates off Cape Blanc contained high amounts of ballast material due to the presence of coccolithophorid liths and presumably fine-grained dust from the Sahara desert. Aggregate sinking velocities were high and increased with increasing aggregate size. Hence, ballast minerals and aggregate sinking velocity appeared to be important parameters controlling export of carbon to greater depths in the deep ocean and the sediment.

Keywords: aggregate, flux, camera profile, respiration, sinking velocity, particle size spectrum

1. Introduction

A significant fraction of organic carbon produced by photosynthesis in the surface ocean is vertical transported to depth as large sinking aggregates and fecal pellets (Fowler and Knauer 1986). This transfer of carbon from the surface ocean to the deep ocean is termed ´the biological carbon pump´.

In order to predict the efficiency of the biological carbon pump in time and space it is important to understand the controlling mechanisms for the export flux of organic carbon from the euphotic zone to the mesopelagic and deep ocean (e.g., Boyd and Trull 2007; Buesseler et al. 2007). Quantifying these processes is fundamental to understanding the global cycling of carbon. The efficiency of the biological pump depends on the balance between remineralization and sinking of aggregated particulate organic carbon (POC) (Sarmiento et al. 2004). Low sinking rates of aggregates increase their residence times and hence degradation time in the upper ocean. Significant correlations between vertical fluxes of POC and inorganic material in the deep ocean has lead to the suggestion that inorganic minerals (calcite, opal, clay, and other lithogenic material) in aggregates may increase the organic carbon export by production of dense aggregates with high sinking velocities (Klaas and Archer 2002) or by providing protection from bacterial remineralization (Armstrong et al. 2002). These mechanisms are known as the ballast effect or ballast hypothesis. Though rapid settling of aggregates prevents significant dissolution of opal and calcite and significant remineralization of organic carbon (Passow et al. 2003; Ragueneau et al. 2000), the underlying mechanisms supporting the ballast effect are still unclear (see De La Rocha and Passow 2007), and the relationship between organic carbon export and ballast minerals needs further investigation with respect to the mechanisms involved.

One reason for our lack of knowledge about the underlying mechanisms for carbon export is the fragile nature of large marine aggregates (marine snow), which makes them very difficult to collect and study, particularly in deeper waters. Sediment traps are frequently used to collect and estimate the vertical sinking particle flux. However, marine aggregates are often transported laterally by currents as they fall, which makes the determination of their origins difficult. Further, standard sediment traps can only be used to estimate the total flux because they average over all sinking aggregates and particles. The problems of estimating size distribution and abundance of the aggregates has partly been overcome by use of polyacrylamide gel in the sediment trap collectors (Jannasch et al. 1980; Kiørboe et al. 1994) and by the use of *in situ* camera systems (Asper 1987; Nowald et al. 2006). The use of *in situ* camera systems has enabled measurements of high resolution depth profiles of aggregate size distribution and abundances. However, neither the use of gel in sediment trap collectors nor the use of *in situ* camera systems can provide detailed information on the chemical composition or remineralization rates of the aggregates, which are needed to understand the processes controlling the biological pump.

Several studies have combined the use of sediment traps and high resolution vertical profiles of aggregate size distribution and abundances to estimate relationships between physical properties and sizes of aggregates (e.g., Guidi et al. 2008b; Stemmann et al. 2002). Models have been based on the combination of trap fluxes and *in situ* camera profiles to investigate the role of physical coagulation, microbial activity, zooplankton grazing (Stemmann et al. 2004a; Stemmann et al. 2004b), and to identify special flux patterns and the importance of different abiotic and biotic factors on the export flux (Guidi et al. 2008a). These studies as well as early studies of POC fluxes (e.g., Martin et al. 1987; Suess 1980) have all shown that most carbon is remineralized in the upper ocean (<500 m). No studies, however,

101 have yet combined small scale direct measurements of settling velocities and carbon
102 degradation on aggregates with high resolution vertical fluxes from sediment traps and *in situ*
103 camera systems.
104
105 The aim of this study is to identify regulating processes for the export fluxes off Cape
106 Blanc and to identify important degradation processes at different depth intervals. We
107 estimated vertical carbon fluxes through the water column between the ocean surface and
108 2500 m depth by using particle size distributions determined by an *in situ* camera system in
109 combination with previous determined size-specific relationships of aggregate mass and
110 sinking velocity (Guidi et al. 2008b). We compared our estimated fluxes against fluxes
111 measured using deep-ocean sediment traps. Rates of carbon loss for different depth layers
112 were calculated and compared with on board measurements of size-specific O_2-uptake,
113 sinking velocity, and composition of roller tank made surface aggregates. This enabled us to
114 identified depth-specific key degradation processes.
115

2. Materials and Methods

We investigated the carbon remineralization and sedimentation of particles during a cruise (RV Maria S. Merian) in the NW African upwelling area off Cape Blanc (Mauritania) from 23 to 29 March 2007 (Fig. 1).

2.1. Vertical flux

For particle sampling in deeper waters (1204 to 1866 m), large-aperture time-series sediment traps of the Kiel-type were used. They were equipped with 20 cups and had openings of 0.5 m². The cups were poisoned with $HgCl_2$ and NaCl was added to increase the density (40‰) of the filtered seawater. The pH was checked after recovery of the traps and was generally ~8. Large swimmers were removed by hand and small swimmers were removed by carefully wet-sieving the collected material through 1 mm nylon mesh. The > 1mm size fraction only contained a few crustaceans and no large particles were observed. Particle flux data therefore refer to the < 1mm size fraction, which also includes material settling as large aggregates, which disrupt in the trap cups or during the handling of the material due to their fragile nature (Fischer and Wefer 1991). The homogenized samples were split into sub-samples for further analysis. Total carbon, organic carbon and nitrogen were obtained by combustion with a HEREAUS-CHN-analyzer. Organic carbon was measured after removal of carbonate with 2N HCl. Carbonate was determined by subtracting organic carbon from total carbon, the latter being measured by combustion without a pre-treatment with 2N HCl. Organic carbon was multiplied by a factor of 2 to estimate total organic matter (Wakeham et al. 1980).

2.2. Particle size and depth distribution

We measured particle size distributions between the surface and 2500 meter depth using *in situ* photographs taken at ten meter intervals with a particle camera (ParCa). The ParCa system used was an improved version of the system used in Nowald et al. (2006). It consisted of a NIKON Coolpix 995 still image camera (3.34 megapixel) with microcontroller and adapted software for use in depth down to 4000m. A collimated light source (strobe) mounted perpendicular to the camera illuminated a volume of 12.4 L (12 cm width). A SeaBird 19 CTD (equipped with an oxygen sensor and a CHELSEA flourometer) transmitted the *in situ* pressure values to the camera and triggered it at depth intervals of ten meters, while it was lowered at 0.3 m s^{-1}. To prevent interference with ambient light, the ParCa was only deployed during night. The images were analyzed using the image analyze software ´Optimas´ (Media Cybernetics). The software recognizes foreground objects (particles) below a given threshold value, returning the area within an outer perimeter and abundance of each aggregates in an image. We calculated the equivalent spherical diameter (ESD) from the returned area of the each particle. Zooplankton was manually removed from the returned data for each image. No stringers or comet shaped aggregates were observed on the images. We calculated the particle size spectrum n as a function of particle diameter d by dividing the concentration of particles (ΔC) in a given small size range (Δd): $n=\Delta C/\Delta d$. We found the usable size range to be between 140 and 6500 µm, with the lower bound set by optical limitation and the upper bound by the low concentrations of the large aggregates.

The particulate organic carbon flux (F_{POC}) can be calculated from the profiles of aggregate size-specific abundance and distribution if the size dependent aggregate carbon content and sinking velocity is known. Aggregate carbon mass (POC) and sinking velocity (w) are often expressed using power relationships ($y=Ax^b$) as a function of aggregate diameter (e.g., Alldredge and Gotschalk 1988). We used the size-dependent sinking velocity and

carbon content to calculate the fluxes from our aggregate size distributions. The F_{POC} was calculated by integrating the flux at a given size over all particle sizes (Guidi et al. 2008b):

$$F_{POC} = \int_0^\infty n(d)POC(d)w(d)dd \quad (1)$$

where *POC(d)* is the carbon content of an individual aggregate of diameter *d*, and *w(d)* is its sinking velocity.

We used two different relationships for sinking velocity and carbon content to test the sensitivity of the results to the choice. One relationship for *wPOC* was developed by Guidi et al. (2008b) from a large number of profiles made between the surface and 1000 m depth using camera imaging at different seasons and locations. They used a minimization procedure to find parameters for the power law relationships for $POC(d)$ $w(d)=Ad^b$ that provided the best fits between the calculated fluxes and fluxes measured with sediment traps. Guidi et al. (2008b) calculated A=12.5 x 10^{-3} gC m^{-3} mm^{-b} d^{-1} and b=3.81.

The second relationship between w_{POC} and d was determined from measurements of properties of aggregates formed in shipboard roller tanks (see section 2.6.). Carbon fluxes were calculated using the two different relationships and the observed particle size distributions between 140 to 6500 µm ESD and compared to concurrent sediment trap collections.

2.3. Roller tank aggregates

We incubated water collected from the fluorescence maximum in shipboard roller tanks. Fluorescence maximum was identified from a Seabird SBE CTD-O_2 and chl-a-fluorescence sensors, and particles at this depth were the expected source of sinking aggregates. Water samples were collected at four stations (1, 2, 4, and 6) using a rosette water sampler equipped with a SeaBird CTD (Table 1). Within one hour of collection the water was

190 incubated in ten 1.15 L Plexiglas cylinders (roller tanks) with a diameter of 14 cm and depth
191 of 7.47 cm. The tanks were rotated on a rolling table at 3 rotations per minute (rpm) at near *in*
192 *situ* temperature (20 °C) in dim light (Shanks and Edmondson 1989).

193

194 *2.4. Sinking velocity*

195 Size-specific sinking velocity of aggregates was measured in a vertical flow system
196 (Ploug and Jørgensen 1999). Individual aggregates were gently transferred from the roller
197 tanks to the open flow-through chamber using a wide bore pipette. The flow chamber was a
198 10 cm high Plexiglas tube (5 cm diameter) with a net extended in the middle. The net created
199 a relative uniform flow field across the upper chamber when a flow was supplied from below
200 (Ploug and Jørgensen 1999). The flow was adjusted with a needle valve until the aggregate
201 was suspended one diameter above the net, whereby its sinking velocity was balanced by the
202 upward-directed flow velocity. The sinking velocity of an aggregate was calculated by
203 dividing the flow rate by the cross-sectional area of the flow chamber. Triplicate
204 measurements of sinking velocity were made for each aggregate. The length of all three
205 aggregate axes (x, y, and z direction) was measured in the flow system using a horizontal
206 dissection microscope with a calibrated ocular. The aggregate volume was calculated by
207 assuming an ellipsoid shape. For comparison with other aggregate shapes we calculated the
208 diameter of a sphere with equivalent volume (ESD) compared to that of the ellipsoid.

209

210 *2.5. Respiration rates*

211 Oxygen concentration gradients at the aggregate-water interface were measured at 50
212 µm resolution in darkness at steady state using a Clark-type oxygen micro-electrode with a
213 guard cathode (Revsbech 1989). The microelectrode was calibrated in air-saturated and in

anoxic seawater. The electrode current was measured by a pico-ammeter (Unisense, PA2000) connected to a strip chart recorder (Kipp and Zonen). The tip of the micro-electrode was 2 µm wide and was moved gently towards the surface of the aggregate. The aggregates appeared highly porous, and we could not detect any oxygen gradients when they were suspended in the flow field. Oxygen distributions were therefore measured at stagnant conditions at *in situ* temperature in darkness. To avoid any limitation in oxygen diffusion due to wall effects, we placed single aggregates on the net in the flow chamber while measuring oxygen distributions, and no aggregate was anoxic in its interior (Ploug and Jørgensen 1999). Oxygen flux was calculated from the measured oxygen gradient at the aggregate-water interface (Ploug et al. 1997). We used a temperature and salinity corrected oxygen diffusion coefficient of 1.95 x 10^{-5} cm^2 s^{-1} in the calculations. The surface area of ellipsoids (Maas 1994) was used to calculated total oxygen consumption. Oxygen consumption rate was converted to carbon respiration assuming a respiratory quotient of 1.2 mol O_2 to 1 mol CO_2.

2.6. Aggregate dry mass and POC content

Two to five aggregates with known and similar volumes were filtered onto pre-weighted 0.45 µm, 25 mm silver filters (Millipore, Bedford). The filters were gently rinsed with de-ionized water, and dried at 40 °C (>48 hours) before being re-weighed on a Mettler Toledo UMX2 balance (sensitivity: 0.1 µg). The total particulate organic carbon (POC) content of the aggregates was then measured with an EA mass spectrometer (ANCA-SL 20-20, Sercon Ltd. Crewe, UK) having a precision of ±0.7 µg C or 0.3 %. Half of the filters (total of 15 filters) were fumed with hydrochloric acid (HCl) before the POC measurements. The difference in POC content between the fumed and non-fumed samples was used to determine the inorganic carbon content. The POC content of each aggregate was assumed to equal its volume fraction times the total POC determined for the filter.

239

240 *2.7. Density of aggregates*

241 We used the Navier-Stokes drag equation to calculate the excess density ($\Delta\rho$) of our
242 aggregates (Stokes 1851):

243 $$\Delta\rho = \frac{C_D\, \rho_\omega\, w^2}{\frac{4}{3}\, g\, ESD} \quad (2)$$

244 where C_D is the dimensionless drag force defined in equation (4), ρ_w is the density of the
245 ambient fluid in g cm^{-3}, w is the measured sinking velocity in cm s^{-1}, g is the gravitational
246 acceleration of 981 cm s^{-2}, and *ESD* is the equivalent spherical diameter in cm. We calculated
247 C_D using the drag equation for Re>1 given by White (1974):

248 $$C_D = \left(\frac{24}{Re}\right) + \left(\frac{6}{1+Re^{0.5}}\right) + 0.4 \quad (3)$$

249 where Reynolds number were defined as:

250 $$Re = w\, ESD\, \frac{\rho_\omega}{\eta} \quad (4)$$

251 where η is the dynamic viscosity.

252

253 *2.8. POC content of sea water*

254 To determine the content of particulate organic carbon at the depth of fluorescence
255 maximum, we made five replicates of 2.5 L water filtered through pre-combusted (450°C, 12
256 h) and pre-weighed Whatman GF/F filters (diameter: 25 mm) at each investigated station
257 (Table 1). Additionally we treated three filters with milliQ water and used those as blanks.
258 Both the filters treated with sea water and milliQ water were dried at 40°C (>48 hours).
259 Before determination of the carbon content, the filters were re-weighed and the dry weight of
260 the suspended material in the sea water determined. The filters were fumed in hydrochloric
261 acid (HCl) before carbon analyses were performed on a EuroEA 3000 CN Element Analyzer.

262

263 *2.9. Sea water respiration*

264 Respiration at the depth of fluorescence maximum in the bulk water at station 2, 4, 5,
265 and 6 was measured by Winkler titration (Carignan et al. 1998; Carritt and Carpenter 1966).
266 Five replicates were used to measure the concentration of oxygen at time zero (t_0) and five
267 replicates of both non-filtered sea water (test) and 0.2 µm filtered sea water (blank) were
268 incubated in 11 ml gas-tight glass vials with glass stoppers. The vials were kept dark at
269 constant temperature during the 24 hours incubation time. The accuracy of O_2 determinations
270 was 0.1 µmol L^{-1}, and the average total decrease in O_2 concentration during the incubations
271 was 43.1 ± 16.2 µmol L^{-1} (range: 28 to 63.5 µmol L^{-1}).
272

3. Results

3.1. Characteristics of roller tank aggregates

After 2.5 hours of incubation in the roller tanks the first macro aggregates (>0.5 mm in diameter) appeared. The aggregate abundances increased during incubation period, reaching 2.2 ± 1.4 aggregates per rolling tank after 19 hours. The average ESD was 2.60 ± 1.18 mm (SD). The ESD of collected aggregates ranged between 0.76 and 5.26 mm, limited by difficulties of handling very small aggregates in our experimental setup and limitations on maximum aggregate size from the incubation time and roller tank volume. The ratio of the longest to the shortest axis was 5.7 ± 4.9. Because of the asymmetry, the aggregates were treated as ellipsoids when calculating their volumes and surface areas.

The dry weight increased with increasing aggregate diameter, from 2.55 to 450.98 µg per aggregate. One aggregate with dry weight (855.73 µg) more than twice that of other aggregates in its size range (~5.2 mm ESD) was excluded in further data analyses (Fig. 2A). Settling velocity increased with increasing aggregate sizes, from 87.6 to 568.8 m d^{-1} (Fig. 2B). We observed no significant differences in aggregate sizes (p = 0.87, one-way ANOVA), dry weights (p = 0.74, one-way ANOVA), or settling velocities (p = 0.09, one-way ANOVA) between the four sampling stations and have therefore pooled all aggregates from the different stations in subsequent analyses.

The excess densities ($\Delta\rho$) of roller tank aggregates ranged from 0.68 to 5.6 mg cm^{-3} and decreased with increasing aggregate diameter (Fig. 2C). The largest variation occurred among the small aggregates (d <1.6 mm ESD) (Fig. 2C). The average $\Delta\rho$ was 1.89 ± 1.09 mg cm^{-3}.

297 The material forming aggregates was dominated by diatoms, with *Chaetoceros* sp. as
298 the dominant species but *Thallassiosira* sp., *Nitzschia* sp., and pennate diatoms were also
299 present. Coccolithophores were abundant in the aggregates, but at lower abundances than
300 diatoms, with *Emiliania huxley* as the dominant species. The abundance of *Chaetoceros* sp.
301 was 2 $x10^4$ to 30 $x10^4$ fold higher than that of *E. huxley*. Liths of *E. huxley* were 10 to 10^6
302 times more abundant in the aggregates than whole coccolithophorids. The liths inside the
303 aggregates appeared partly dissolved (Fig. 3C), unlike liths collected from water samples (Fig.
304 3D), but the dissolution was not quantified. The aggregates also contained empty frustules,
305 setae, phytoplankton detritus, copepod fecal pellets, clay minerals, small grains of quartz, and
306 unidentified lithogenic material. The aggregates appeared highly porous (Fig. 3A & B). The
307 aggregation potential of the suspended material in the water was found by dividing amount of
308 aggregated material with its start concentration in the roller tank. The aggregation potential
309 after 19 hours of incubation was 0.33 ± 0.58 for whole *E. huxley* cells, 0.44 ± 0.33 for *E.*
310 *huxley* liths, 0.07 ± 0.1 for *Thallassiosira* sp., 0.18 ± 0.32 for *Chaetoceros* sp. spines, and 0.65
311 ± 0.42 for POC.

312

313 POC in aggregates ranged between 1.8 and 591.1 µgC agg^{-1}. It increased with
314 increasing diameter of the aggregate (Fig. 4A) and was ~40 % of dry weight. No significant
315 differences were found for the POC content of the aggregates formed from water at the four
316 stations sampled (p = 0.4, Student´s t-test). The inorganic carbon to dry weight ratio was
317 0.075 ± 0.014; the $CaCO_3$:POC ratio was 0.09. The respiration rate per aggregate ranged from
318 18.96 to 728.76 ngC h^{-1} (Fig. 4B) and increased with increasing aggregate size. One ~5 mm
319 aggregate had a very high respiration rate (1.66 µgC h^{-1}) and was excluded from the analysis.
320 The POC-specific respiration rate (R) was 0.13 ± 0.07 d^{-1} and was independent on size (Fig.

4C). The carbon turnover times ranged between 2.8 and 45.9 days, with an average of 11.4 ± 8.9 days.

Because the aggregates are settling, the L-ratio (L-ration= *R* divided by *w*) expresses the fractional degradation per meter settled, providing a different water column perspective of the degradation (Fig. 4D). L-ratio ranged between 0.05 and 3.23 km^{-1} and decreased with increasing aggregate size (Fig. 4D & Fig. 2B).

3.2. Suspended POC content and respiration in the bulk water

POC content and respiration in the bulk water at the depth of fluorescence maximum was investigated at station 2, 4, 5, and 6 (Table 1). The POC content at the fluorescence maximum was 253.78 ± 163.45 μgC L^{-1}; C:N was 5.3 ± 0.36. The dry weight of particulate material was 2.09 ± 0.62 mg L^{-1}. The community respiration rate ranged between 163 and 467 μgC L^{-1} d^{-1}, averaging 286 ± 130 μgC L^{-1} d^{-1}.

3.3. ParCa profiles

ParCa profiles were collected at station 1, 2, 3, 4, and 6 (Table 1). There was a rapid decrease in total aggregate volume in the upper 220 m at all stations (Fig. 5A). Between 220 and 1500 m there was relatively little variation in the integrated volume, but its value varied among the stations, being largest at station 3 and smallest at station 2. For station 1, 3, and 6 there was a gradual increase in particles below 1500 m depth. This was most pronounced at station 3. The profile at station 4 extended only to ~720 m depth because of its position close to the continental slope.

344 The particle number spectrum as a function of *d* for station 1 (Fig. 5B) was similar to
345 that at all the stations, with high values for small *d* and small values for large *d*. Particle
346 concentrations were higher at shallower depth. The values of *n* changed at about 75 m depth
347 to lower abundances characteristic of the deeper water column. Although the small particles
348 dominated by number, the large particles dominated by volume (Fig. 5C).

349

350 *3.4. Estimated F_{POC} from aggregate distribution*

351 Downward vertical fluxes were calculated from *in situ* camera profiles of aggregate
352 size-specific abundance and size-specific sinking velocities and aggregate carbon content
353 either measured in this study or previously developed (Guidi et al. 2008b). The trends for
354 calculated F_{POC} were the same using either the relationship from our aggregates or the
355 relationships determined by Guidi et al. (2008b), with rapid declines in the upper 220 m and
356 relatively constant fluxes deeper. The inferred carbon fluxes were compared with the carbon
357 fluxes collected with two sediment trap sites (CBi-4 and CB-17) from the same area and time
358 period (Table 2, Fig. 1). The F_{POC} estimated with our size-specific POC and sinking velocity
359 measured in aggregates overestimated the trap recovered F_{POC} by factors of 26 to 137 (R^2 =
360 0.70) while F_{POC} using those of Guidi et al. underestimated F_{POC} by factors of 4 to 15 (R^2 =
361 0.87). We therefore used estimated F_{POC} from the Guidi et al. relationship in the following
362 analysis (Fig. 6A). When comparing total mass fluxes from the sediment traps with the
363 estimated mass fluxes, we found no difference between the correlation coefficients for our
364 size-specific mass and sinking velocity measured in aggregates (R^2= 0.62) and for those of
365 Guidi et al. (R^2= 0.67) (data not shown). The mass fluxes estimated using our ship-board data
366 of size-specific mass and sinking velocity overestimated the trap recovered mass fluxes by
367 factors of 5 to 10 while mass fluxes using Guidi et al. relationship underestimated by factors
368 of 7 to 11. Similar size-specific sinking velocities were used in both mass fluxes and F_{POC},

therefore, the better fit between sediment trap F_{POC} and the Guidi et al. relationship might be due to high organic carbon content in our roller tank aggregates formed from particles in the fluorescence maximum rather than high sinking velocities of these aggregates.

The highest flux was calculated at station 3 and lowest at station 1 (Fig. 6A). This was observed from the sediment traps (Table 2). The depth profile of average carbon flux for all stations could be divided into three parts (Fig. 6B); a fast carbon removal at 20 – 80 m, an intermediate removal at 80 – 220 m, and a low carbon removal at 220 – 2500 m. We fitted straight lines to the carbon concentrations in each depth interval for all the stations to estimate the carbon removal rate within the three depth intervals (Fig. 6C). At station 3, the carbon flux showed a large increase at depths deeper than 1500 m, likely due to advection (Karakas et al. 2006; Nowald et al. 2006). Therefore the linear fit for the deep layer at station 3 was only performed between 220 and 1500 m. The layer between 20 and 80 m had a carbon removal rate of 195.06 ± 77.73 μgC m^{-3} d^{-1}. The carbon removal rate decreased with increasing depth and was found at 59.85 ± 48.44 μgC m^{-3} d^{-1} for the middle layer between 80 and 220 m, and 2.11 ± 3.17 μgC m^{-3} d^{-1} for the deep layer between 220 and 2500 m. Hence, the upper 220 m of the water column was most important for carbon removal, with the most intense removal in the upper 80 meters. Once the settling organic carbon had reached depths below 220 meters, it was only exposed to low removal rates and seemed likely to be exported to greater depths and to the sea floor.

3.5. Impact of aggregate associated O_2 consumption on the environment

The impact from aggregate associated O_2 consumption on the O_2 concentration in the bulk water was estimated for the depth of fluorescence maximum (F-max) and for the oxygen

393 minimum zone (OMZ). The total respiration from the microbial community attached to
394 aggregates at F-max what calculated from the size-specific respiration rate (Fig. 4B) and the
395 size distribution and abundance of aggregates found from the ParCa images (Table 3). The
396 total aggregate associated respiration at F-max (microbial and mesozooplanktonic) and at the
397 OMZ (microbial) was found from the fitted carbon consumption rates (Fig. 6C), assuming a
398 respiratory quotient of 1.2 mol O_2 to 1 mol CO_2 (Table 3). The bulk respiration at F-max was
399 estimated from the Winkler titrations (see section 3.2.). By relating these O_2 consumption
400 rates with the observed O_2 concentration in the bulk water (from ParCa CTD profiles) at F-
401 max and at OMZ, we can express the percentage of O_2 removed due to aggregate associated
402 processes (at F-max and OMZ) and due to bulk respiration (OMZ). Respiration on aggregates
403 was ~5 % of the total respiration (aggregate and bulk) at F-max, and it potentially had a trivial
404 impact, only, on O2 concentrations in the OMZ (Table 3).

405

406 **4. Discussion**

407 The average respiration rate in the roller tank-formed aggregates was proportional to
408 the aggregated particulate organic carbon. As a consequence, the carbon-specific respiration
409 rates of the aggregates were size-independent. This has also been found in previous studies of
410 diatom aggregates collected off the coastal California (Ploug et al. 1999) and aggregates
411 formed on diatom detritus (Ploug and Grossart 2000). The specific respiration rates in this
412 study and from Ploug et al. (1999) were both ~0.12 d^{-1}. Since all aggregate sizes are degraded
413 at the same specific rates, the extent of their remineralization in the upper water column is
414 determined by their residence times, and sinking velocity may be a controlling factor for
415 organic carbon export.

416

417 To illustrate the importance of aggregate sinking velocities for carbon export, we
418 calculated the fate of aggregated POC in fast and slow settling aggregates. For simplicity, we
419 only considered degradation from aggregate community respiration. Slowly sinking
420 aggregates have long residence times in the upper water column. From the L-ratio (Fig. 4D),
421 we found a carbon specific degradation of 0.001 m^{-1} for aggregates with sinking velocities of
422 90 m d^{-1}. Assuming a perfect vertical settling at a constant speed of 90 m d^{-1}, half of the
423 aggregated POC was degraded at 480 m depth ((ln 0.5)/(-0.001 m) = 480 m). Large
424 aggregates that settled with 570 m d^{-1} were exposed to a carbon specific degradation of
425 0.0002 m^{-1} (Fig. 4D). This implied that half of the aggregated POC was degraded at 3039 m
426 depth. However, when observing the modeled carbon flux (Fig. 6A), half of the sinking
427 carbon is degraded at much shallower depths than ~500 m. One reason for this rapid carbon
428 removal in the upper ~200 m may be that aggregates do not settle in a perfect vertical
429 direction at constant speed, but are retained by currents, turbulence, density gradients, and
430 carbon flux may be dominated by very small, slowly sinking aggregates in the upper water

column. Other degradation processes like ecto-enzymatic hydrolysis with a net release to the surrounding water (Smith et al. 1992), and fragmentation and degradation by mesozooplankton might also be important for the rapid carbon removal in the upper 500 m (Banse 1990; Dilling and Alldredge 2000; Kiørboe 2000).

Calculated carbon flux profiles suggested that the majority of the carbon removal occurred in the upper 220 m (Fig. 6C). Therefore, the carbon removal rate in the upper 220 m was used to identify the importance of key processes (e.g., sinking velocity, respiration, and zooplankton grazing and fragmentation) for the biological carbon pump in the coastal upwelling area off Cape Blanc. The carbon removal rates varied largely between three major depth intervals, with highest removal rate in the upper layer and lowest removal rate in the deep layer; 20 to 80 m, 80 to 220 m, and 220 to 2500 m (Fig. 6B). Half of the carbon removal rate in the layer between 80 and 220 m was accounted by respiration on aggregates. Attached bacteria may have markedly higher cell-specific hydrolytic enzyme activity relative to the free-living bacteria (Cho and Azam 1988; Grossart et al. 2007; Smith et al. 1992). Hence, the other half of the carbon removal between 80 and 220 m may occur via rapid solubilization of the aggregated organic matter by attached bacteria and net release to the bulk (Cho and Azam 1988). The dissolved organic carbon (DOC) released from the aggregates are then partly assimilated and partly respired by free-living bacteria (Kiørboe and Jackson 2001; Thor et al. 2003). The measured respiration in bulk water (146 ± 284 μgC m^{-3} d^{-1}) at the depth of fluorescence maximum was much higher than the potential production of DOC by attached bacteria (~25 μgC m^{-3} d^{-1}). Therefore, alternate DOC production was also feeding the free-living bacteria; e.g., leakage and/or exudation from algae (Lignell 1990), release from zooplankton feeding (Jumars et al. 1989; Møller et al. 2003), and diffusion out of fecal pellets (Jumars et al. 1989). At the depth of fluorescence maximum the microbial community in the

456 bulk water respired ~13% of the total O_2 per day. This suggested the bulk microbial
457 community to be more important for the remineralization of organic matter than attached
458 biota on aggregates, since the latter only respired ~0.7% of the bulk O_2 concentration per day.
459 Still, 65 ± 42% of the total POC was found as aggregated carbon, assuming 40% of the total
460 dry weight was POC in *in situ* aggregates.

461

462 Assuming the microbial community on the aggregates respired carbon at similar rate
463 in the upper layer as in the middle layer, there was an additional carbon removal rate of 135 ±
464 69 μgC m^{-3} d^{-1} between 20 and 80 m compared to the depth between 80 and 220 m. This
465 additional carbon removal may be explained by zooplankton activity (e.g., Banse 1990).
466 Several investigations have suggested copepods as degraders of marine aggregates (Green and
467 Dagg 1997; Koski et al. 2005; Steinberg et al. 1994). We calculated the copepod abundances
468 needed to remove the additional removed carbon in the upper layer. The equation for oxygen
469 consumption rate was used to estimate respiration of one epipelagic copepod (Ikeda et al.
470 2001):

471 $$Respiration\ (\mu l\ O_2 h^{-1}) = 1.132 B^{0.78} e^{0.073T} \qquad (5)$$

472 where B is the copepod mass (mg carbon) and T is the surface temperature (°C). We assumed
473 B = 1.5 μg carbon for a copepod colonizing an aggregate (Kiørboe 2000). The estimated
474 respiration rate of a single copepod was 0.394 μgC d^{-1}. Assuming the grazing rate is about
475 three times the metabolic rate (Kiørboe 1989), a copepod abundance of 114 ± 41 copepods m^{-3}
476 was needed to remove carbon at similar rates as the estimated carbon removal. The reported
477 copepod abundance in the upper 100 meter depth off Cape Blanc in 1998 was 100 – 250
478 copepods m^{-3} (Somoue et al. 2005). Our estimations only indicate that the carbon removal
479 may be accounted for by realistic copepod abundances. Therefore, both attached microbes and

480 mesozooplankters appeared to play important roles in the degradation of aggregates in the
481 upper 80 meters of the water column. However, other zooplankton organisms and
482 mechanisms are possible (Dilling and Alldredge 2000; Kiørboe 2000; Shanks and Walters
483 1997). The aggregate associated respiration, including both attached microbial community
484 and potential mesozooplankton respiration, accounted for ~1% of the bulk O_2 concentration
485 per day. Even when including mesozooplankton respiration it seems that the aggregate
486 associated respiration is small as compared to the total remineralization of organic matter in
487 the upper ocean. Hence, the free-living bacteria appear fueled via processes such as leakage
488 and exudation from algae (Lignell 1990) and sloppy feeding by zooplankton (Jumars et al.
489 1989; Møller et al. 2003).

490

491 We observed low carbon removal rates (2.5 ± 3.2 μgC m^{-3} d^{-1}) below 220 m.
492 Consistent with bacterial activity, but the low rates indicate that such bacterial activity would
493 be limited, i.e., due to temperature decrease, bacterial abundance decrease, detachment of
494 bacteria, etc. Assuming all carbon was removed via respiration, the microbial community on
495 aggregates only consumed 0.03 ± 0.05% of the total O_2 concentration per day in the oxygen
496 minimum zone.

497 The POC flux is determined by degradation processes, aggregation and
498 disaggregation, particle sinking velocity, and physical mixing processes in the water column.
499 The majority of the POC is remineralized in the upper ocean twilight zone > 1000 m (Martin
500 et al. 1987; Suess 1980). Our study and other studies by Stemmann et al. (2004a) in the
501 Mediterranean suggest that mesozooplankton dominated carbon removal in the upper water
502 column whereas microbial biota were more important in the deeper midwater zones where
503 zooplankton are rarer. Since high aggregate sinking velocities potentially shorten the
504 residence time in the upper ocean, sinking velocities may be an important controlling factor of

505 the carbon pump. The factors controlling the sinking velocity of aggregates *in situ* are
506 complex, as indicated by the large range of sinking rates observed for a given aggregate size
507 (Ploug et al. 2008a; Stemmann et al. 2004b; Trull et al. 2008).

508

509 Our study shows that, whereas our data derived from measurements of POC and
510 sinking velocity of single aggregates produced in surface waters generally overestimated
511 carbon flux, the relationship for sinking velocities of POC developed by Guidi et al. (2008b),
512 based on data from different seasons and locations, generally underestimated carbon flux at
513 the Cape Blanc region. Size-specific sinking velocities of our aggregates were 5-10 times
514 higher than previous measurements of sinking velocities for similar sized aggregates in other
515 regions, e.g., Southern California Bight (Alldredge and Gotschalk 1988; Ploug et al. 1999).
516 The area off Cape Blanc is characterized by high coccolithophorid production (Fischer and
517 Karakas 2009) and it receives large input of dust containing fine grained lithogenic
518 components from the Sahara desert (Chiapello et al. 1995). Previous studies have found that
519 incorporation of dust and coccolithophorids into fecal pellets and algae aggregates increase
520 their excess densities and thereby sinking rates (Fischer and Karakas 2009; Ploug et al.
521 2008b). We observed <10 times higher size-specific excess densities compared to those of
522 aggregates in the Southern California Bight (Alldredge and Gotschalk 1988). The study area
523 has low carbonate production and dust input. Therefore, the increased size-specific sinking
524 velocities in our study may partly be explained by the high amount of both carbonate and
525 lithogenic material found in our aggregates. However, a bottle effect originating from
526 interactions with the sides of the roller tank might also increase the aggregate density. The
527 incubation time in the roller tanks allowed the aggregates to collect high amounts of material
528 from the depth of fluorescence maximum which demonstrates the high aggregation potential
529 of particles in surface waters. However, aggregates may be less compact in the field because

these sink out to deeper waters where less material presumably is scavenged during descent. Field studies have demonstrated high sinking velocities of large particles formed in carbonate-dominated areas (Fischer and Karakas 2009). Sediment trap studies have shown correlations between organic matter and mineral content (Armstrong et al. 2002; Francois et al. 2002; Klaas and Archer 2002). Whether this correlation occurs via enhanced sinking velocities (ballast hypothesis), as protection of organic matter against remineralization (ballast ratio hypothesis) (Armstrong et al. 2002), from carrying capacity of minerals by organic matter (see Passow and De La Rocha 2006) or vice versa (see Armstrong et al. 2002), is unclear. The presence of ballast minerals potentially enhances carbon export via the higher sinking velocities. Francois et al. (2002) suggested that the higher organic carbon transfer efficiency in carbonate production systems compared to biogenic opal production systems reflects a difference in the particle transport mode, with slow sinking diatom aggregates in opal production systems and fast sinking fecal pellets in carbonate productive systems. Our study shows that particle flux not only consist of fast sinking fecal pellets in the Cape Blanc region, but potentially also of fast sinking diatom aggregates loaded with Saharan dust and carbonate from liths.

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Table 1: Physical parameters and experiments for each station sampled. Depth of fluorescence maximum (F-max depth [m]), in situ temperature, in situ salinity, and experiments performed for the different stations. The parenthesis after roller tank shows the total number (*N*) of aggregates formed during the roller tank incubation. POC & Winkler indicate that the particulate organic carbon (POC) content and respiration in the sea water collected from F-max was measured. ParCa indicates that an image profile of particle size distribution was made at the station. The squared brackets below the station name indicate the position of the station given as longitude and longitude.

Station Number	Station Name [longitude, latitude]	F-max depth (m)	Temperature (°C)	Salinity (psu)	Experiments
1	**GeoB-11833 Trap site CB-17** **[21°17.170N, 20°48.223W]**	48	20.4	36.6	Roller tank (*N*=17) ParCa
2	**GeoB-11834** **[20°59.989N, 19°50.014W]**	35	20	36.5	Roller tank (*N*=18) POC & Winkler ParCa
3	**GeoB-11835 Trap site CBi-4** **[20°44.796N, 18°42.021W]**	20	18.3	36.1	ParCa
4	**GeoB-11836** **[20°34.969N, 17°58.761W]**	25	18	36.1	Roller tank (*N*=18) POC & Winkler ParCa
5	**GeoB-11838** **[20°44.897N, 18°41.998W]**	23	18.7	36.2	POC & Winkler
6	**GeoB-11839** **[21°19.997N, 18°50.475W]**	25	19.1	36.5	Roller tank (*N*=16) POC & Winkler ParCa

Table 2: Station number, trap site, trap depth, collection period, and flux for the two deep ocean sediment traps. The organic carbon flux (F_{POC}) is showed for the recovered trap flux (F_{POC} – trap) and the calculated flux (Eq. 1) with our data (F_{POC} – this study) or data from Guidi et al. (2008b) (F_{POC} – Guidi et al. 2008).

Station Number (Trap site)	**Trap depth (m)**	**Collection Start/ End (date)**	**F_{POC} – trap (mg C m^{-2} d^{-1})**	**F_{POC} - this study (mg C m^{-2} d^{-1})**	**F_{POC} – Guidi et al. (2008) (mg C m^{-2} d^{-1})**
1 (CB-17)	1204	15.03.07 /23.03.07	8.02	1100	2
3 (CBi-4)	1256	15.03.07 /23.03.07	76.81	2000	5
	1866	15.03.07 /23.03.07	111.66	6000	10

Table 3: Depth of fluorescence maximum (F-max) and oxygen minimum zone (OMZ), oxygen (O_2) concentration, O_2 respiration from microbes in the bulk water (Bulk), microbial community on the aggregates (Microbial), and respiration from mesozooplankton grazing on the aggregates (Zoo.). The impact from the bulk or aggregate associated respiration on the bulk O_2 concentration is showed for the bulk microbial community (Bulk), microbial community attached to the aggregate (Microbial), and for the mesozooplankton and the attached microbial community (Zoo. & microbial).

		O_2 respiration ($\mu molO_2\ l^{-1}\ d^{-1}$)			**Impact on bulk O_2 concentration (% d^{-1})**		
Depth (m)	O_2 concentration ($\mu molO_2\ l^{-1}$)	Bulk (water)	Microbial (agg)	Zoo. & microbial (agg)	Bulk (water)	Microbial (agg)	Zoo. & microbial (agg)
F-max (30.6 ± 11.1)	213.52 ± 8.03[a]	28.55 ± 12.95[b]	1.55 ± 0.98[c]	2.34 ± 0.93[d]	13.2 ± 6.06	0.72 ± 0.44	1.09 ± 0.4
OMZ (342.5 ± 106.6)	71.7 ± 16.83[a]	-	0.03 ± 0.04[d]	-	-	0.04 ± 0.05	-

[a] measured with a SeaBird 19 CTD equipped with an oxygen sensor.

[b] estimated fromWinkler titration

[c] estimated from size-specific respiration rate (Fig. 4B) and in situ size distribution and abundance of aggregates

[d] estimated from fitted carbon removal rates

Figure legends

Fig. 1. *Location of the sampling sites*. Six stations were sampled during a cruise (MSM 04b) off Cape Blanc, NW Africa (Mauritania). Each closed circle indicates the station number and position (see Table 1 for GeoB station number). Deep ocean sediment trap sites are indicated with rectangulars enclosing the station numbers. Sediment trap site CB-17 and CBi-4 were positioned at station 1 and 3, respectively. The line beneath the station number indicates roller tank incubation. Station 3 and 5 are at same position, but sampled at different times.

Fig. 2. *Size-specific measurements of marine snow aggregates*. (A) Aggregate dry weight as a function of diameter. The solid line represents a power fit to the measurements. The * indicates that the aggregate is excluded from the regression. (B) Aggregate settling velocity as a function of size. The solid line represents a power fit to the measurements. (C) Excess density of the aggregates as a function of diameter.

Fig. 3. *Pictures of aggregate and constitutes*. (A) Three mm large aggregate seen from above during suspension. (B) Three mm large aggregate seen from the side during suspension. (C) Scanning electron microscopic picture of coccoliths from *Emiliania huxleyi* found within an aggregate. (D) Scanning electron microscopic picture of coccoliths from *Emaliana huxley* collected from a sea water sample.

Fig. 4. *Size-specific measurements of marine snow aggregates*. (A) Particulate organic carbon (POC) content as a function of aggregate size. The solid line represents a power fit to the measurements. The * indicates that the aggregate is excluded from the regression. (B) Measured respiration rate as a function of aggregate size. The solid line represents a power fit to the measurements. The * indicates that the aggregate is excluded from

the regression. (C) POC-specific respiration rate as a function of aggregate diameter. (D) L-ratio as a function of aggregate size.

Fig. 5. *Comparison of mass fluxes*. (A) Mean vertical distribution of integrated particle volume (cm^3 per m^3, ppm) from the particle camera profiles (ParCa) made at the different stations. The integrated particle volume is calculated as average for 50 m bins below the upper 50 m depth. (B) Observed mean particle number size spectra (n) against aggregate equivalent spherical diameter (ESD, cm) for station 1 at different depths, $n = -(\Delta N/\Delta ESD)$. N is the number of particle greater than a given size. The particle number size spectrum is calculated as average for 50 m bins below the upper 50 m depth. (C) Average particle volume spectra ($cm^3\ L^{-1}\ ESD^{-1}$) against equivalent spherical diameter (ESD, cm) for different depths at station 1.

Fig. 6. *Carbon fluxes*. (A) The aggregated carbon fluxes ($gC\ m^{-2}\ d^{-1}$) estimated from the particle camera (ParCa) measurements of aggregates size distribution and abundance and the size-specific relationship for aggregate carbon content and sinking velocity provided by Guidi et al. (2008b). Measurements for the coastal station were only made to a depth of 700 m due to shallower water depth. The dotted lines indicate the position of the ParCa profile (in eastward direction: station 1, 2, 3, and 4). (B) Average POC flux profile ($gC\ m^{-2}\ d^{-1}$) of all calculated carbon fluxes against depths. The carbon fluxes are calculated as 50 bins below the upper 50 m depth. (C) Average carbon removal rate ($gC\ m^{-3}\ d^{-1}$) for the three identified important depth intervals of carbon loss. Error bars indicate standard deviation.

Fig. 1. Iversen et al.

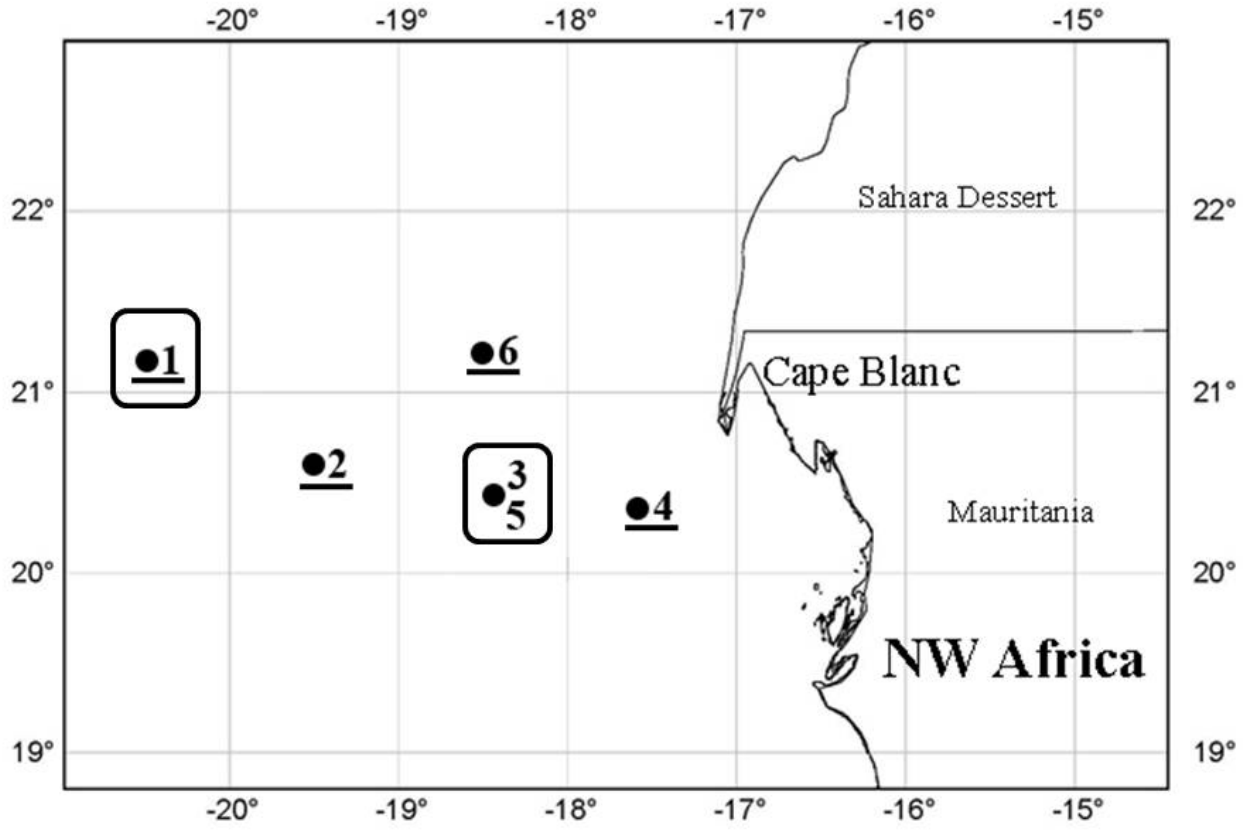
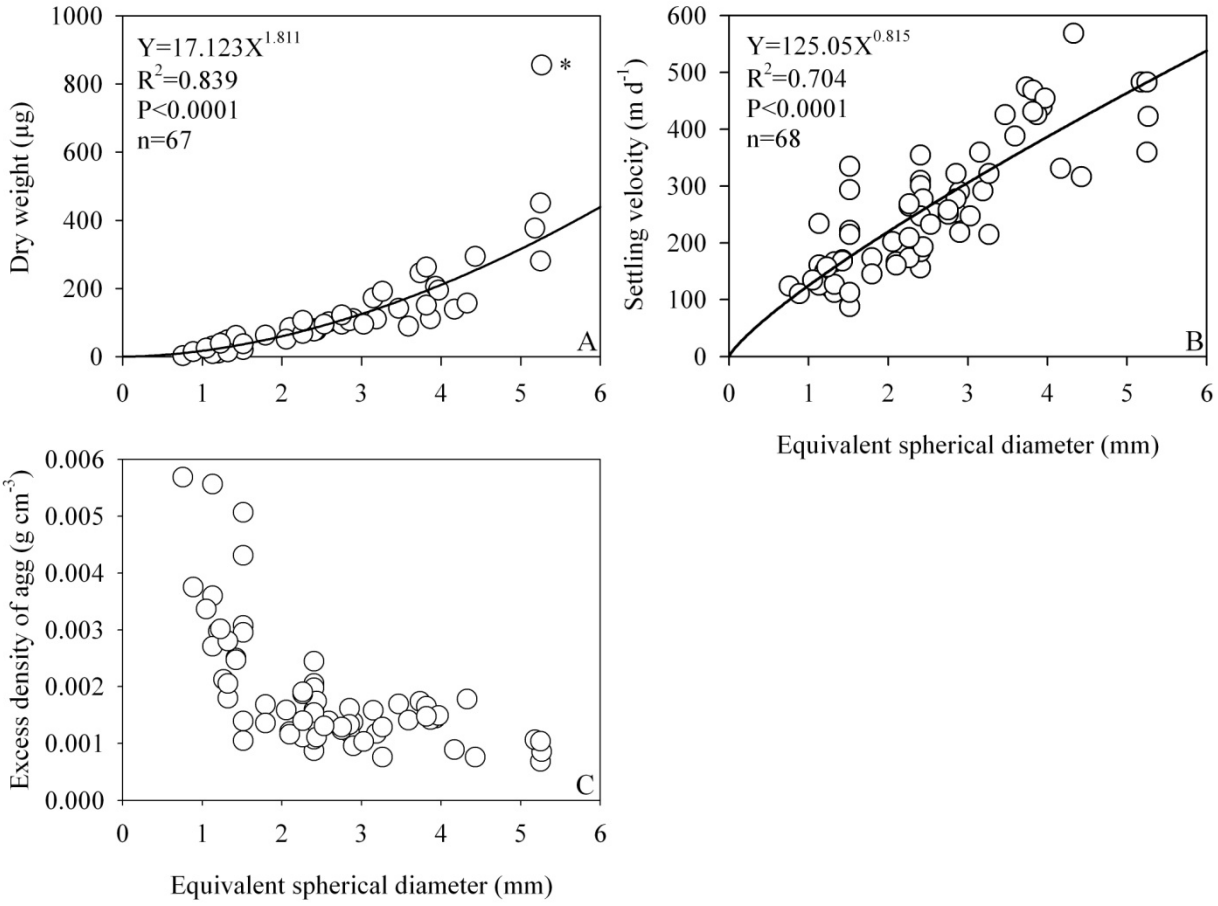


Fig. 2. Iversen et al.



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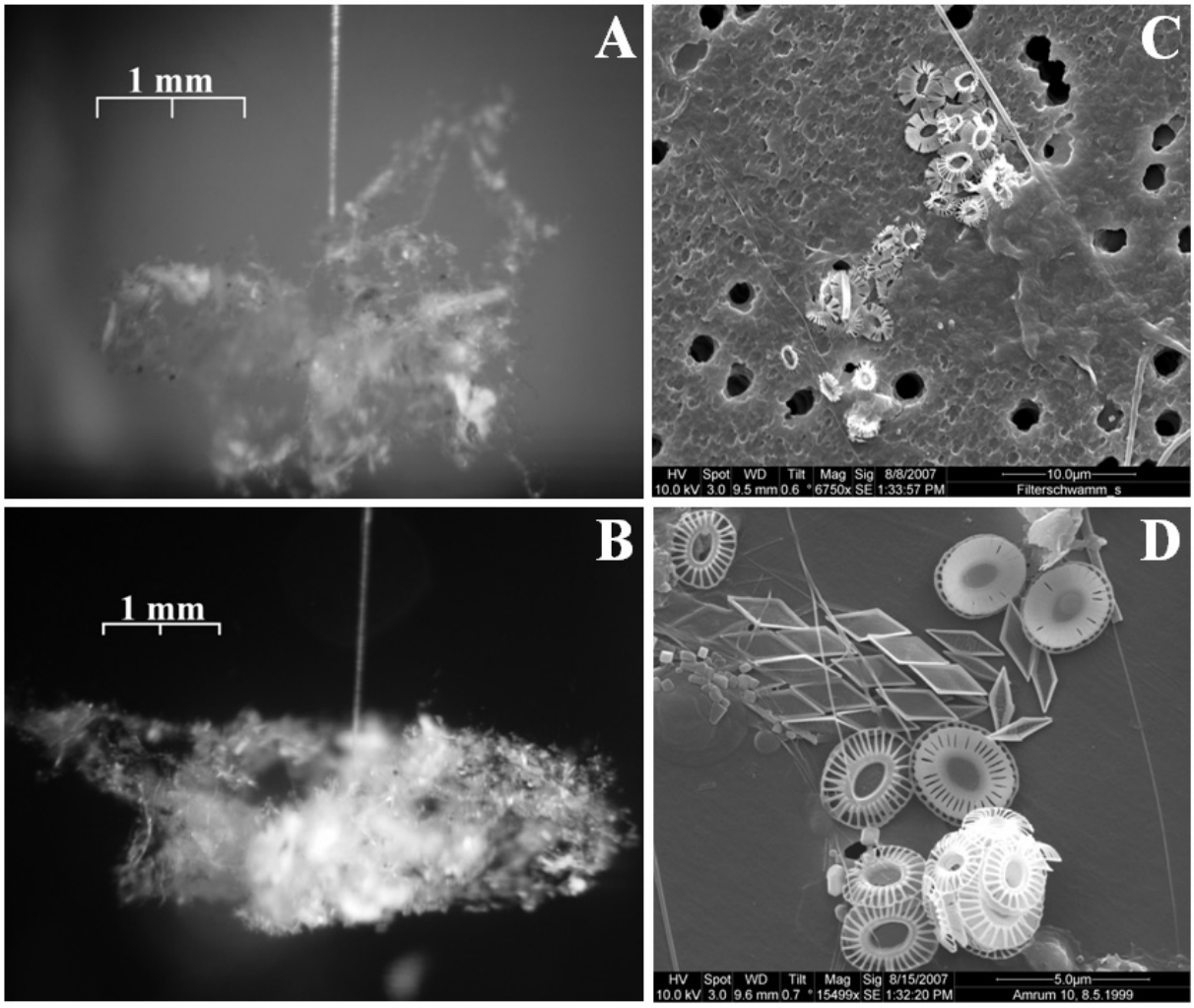

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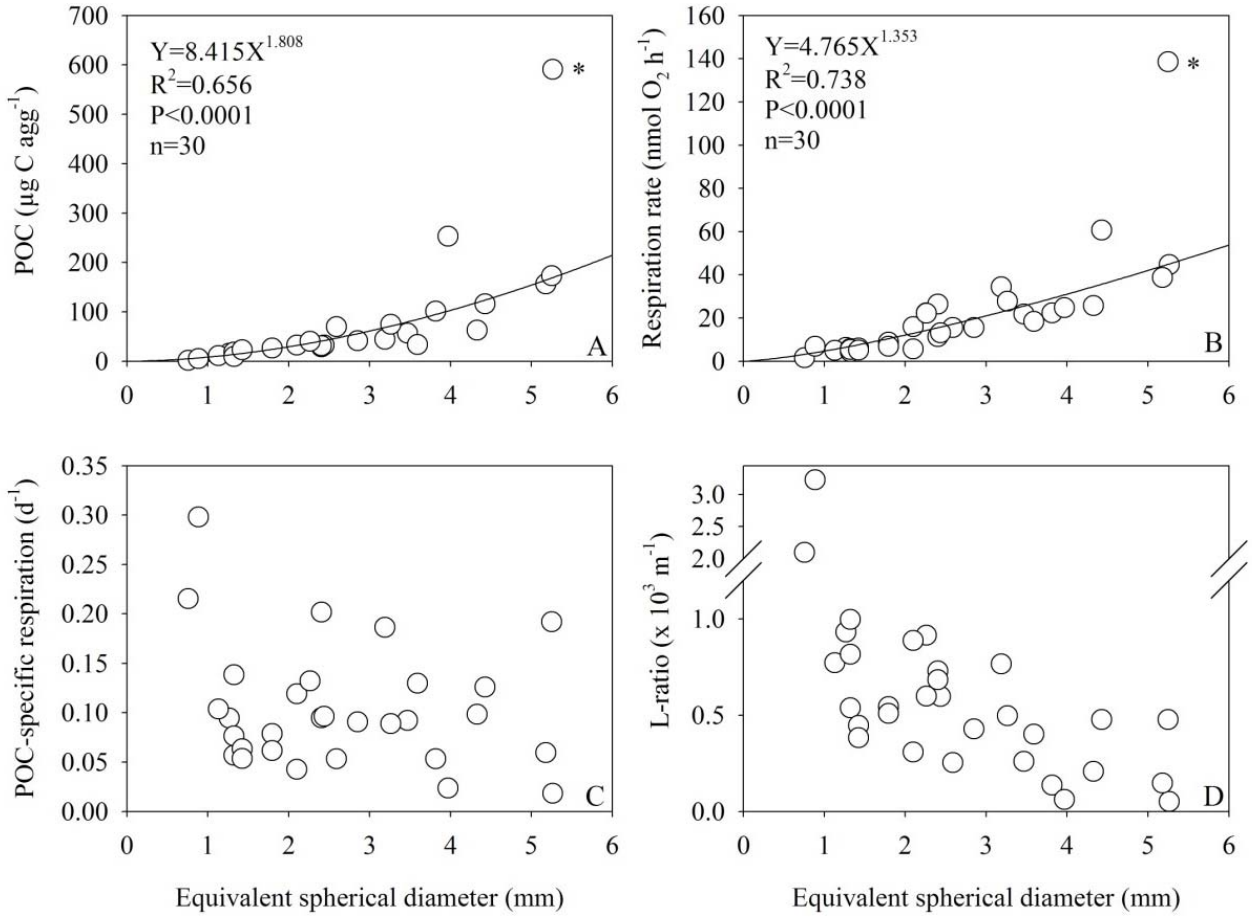

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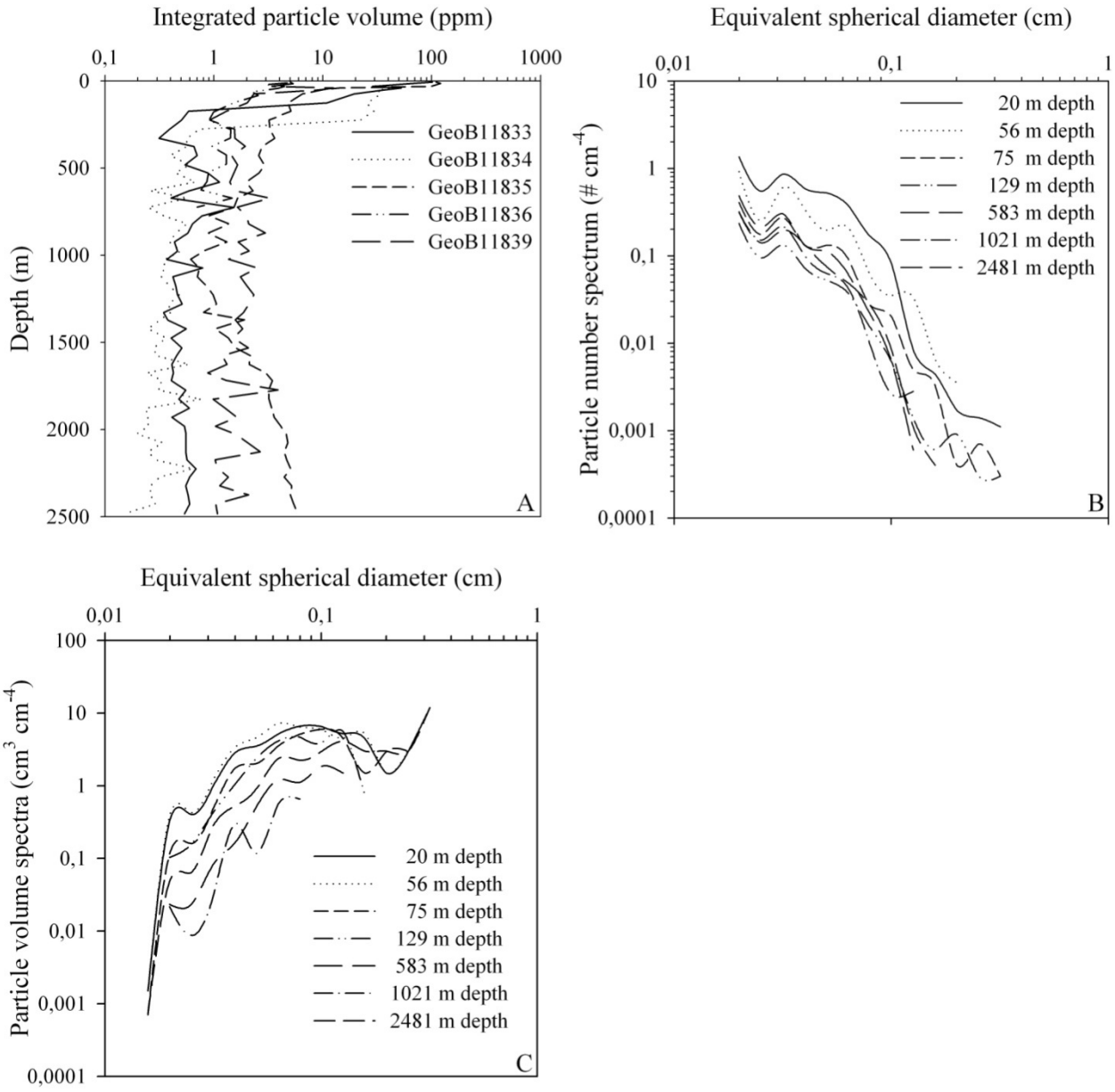

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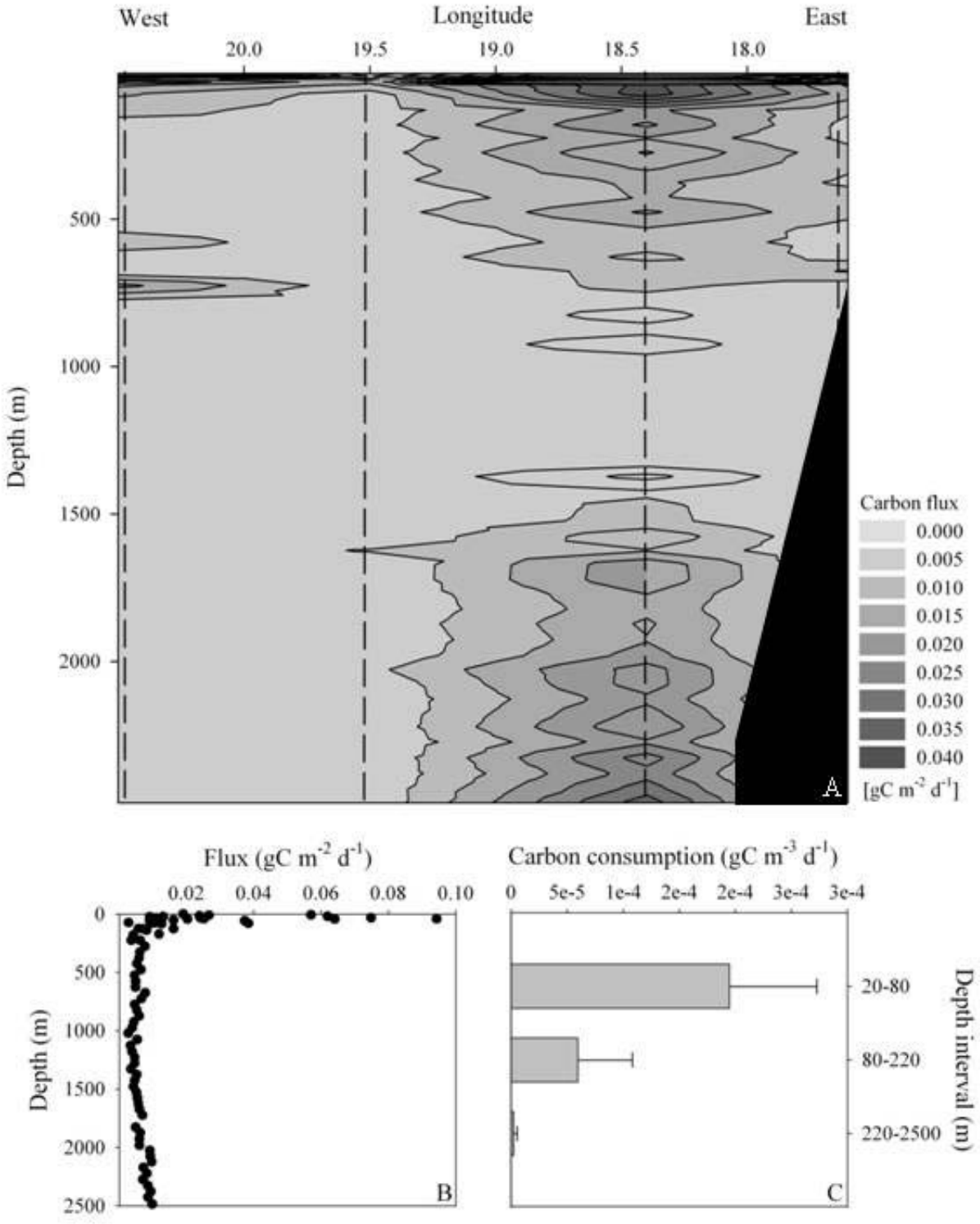

SPECIFIC CONTRIBUTION TO EACH PAPER

Paper I: Iversen M. H., Poulsen L. K. (2007) Coprorhexy, coprophagy, and coprochaly in the copepods *Calanus helgolandicus*, *Pseudocalanus elongatus*, and *Oithona similis*. Mar. Ecol. Prog. Ser. **350**:79-89

Specific contribution: M. H. Iversen contributed to the planning, execution and publication of the above named work. More specific; M. H. Iversen did most of the laboratory work, data analysis, and data treatment. M. H. Iversen was the primary author and did most of the writing and revision of the paper.

Paper II: Poulsen L. K., Iversen M. H. (2008) Degradation of copepod fecal pellets: key role of protozooplankton. Mar. Ecol. Prog. Ser. **367**:1-13

Specific contribution: M. H. Iversen contributed to the planning, execution and publication of the above named work. More specific; M. H. Iversen and L. K. Poulsen contributed equal to the experimental work in the period from July 2004 to November 2004. From February 2005 to July 2005 the experimental work was performed by M. H. Iversen. M. H. Iversen contributed to the data analysis, data treatment, writing, and revision of the paper.

Paper III: Ploug H., Iversen M. H., Koski M., Buitenhuis E. T. (2008) Production, oxygen respiration rates, and sinking velocity of copepod fecal pellets: Direct measurements of ballasting by opal and calcite. Limnol. Oceanogr. **53**(2):469-476

Specific contribution: M. H. Iversen contributed to the planning, execution and publication of the above named work. More specific; M. H. Iversen did not participate in the quantitative measurements of grazing and fecal pellet production rates. M. H. Iversen maintained plankton cultures and performed pellet production incubations for the pellets used in small-scale measurements of oxygen, particulate organic carbon content, pellet density, and pellet sinking velocity. M. H. Iversen performed the measurement of fecal pellets sinking velocities, densities, sizes, and volumes and made the preparations for the organic carbon content measurements. M. H. Iversen contributed in the measurements of small-scale oxygen fluxes to

the pellets. M. H. Iversen contributed to the data analysis, data treatment, writing, and revision of the paper.

Paper IV: Ploug H., Iversen M. H., Fischer G. (2008) Ballast, sinking velocity, and apparent diffusivity within marine snow and zooplankton fecal pellets: Implications for substrate turnover by attached bacteria. Limnol. Oceanogr. **53**(5):1878-1886

Specific contribution: M. H. Iversen contributed to the planning, execution and publication of the above named work. More specific; M. H. Iversen maintained plankton cultures and performed pellet production and aggregate formation incubations. M. H. Iversen contributed to the measurements of aggregate sizes, dry masses, sinking velocities, diffusivity, and contributed to the oxygen measurements. M. H. Iversen contributed to the data analysis, data treatment, writing, and revision of the paper.

Paper V: Iversen M. H., Nowald N., Ploug H., Jackson G. A., Fischer G. (submitted to Deep-Sea Res. I) High resolution profiles of vertical particulate organic matter export off Cape Blanc, Mauritania: degradation processes and ballasting effects. Manuscript submitted to Deep-Sea Res. I.

Specific contribution: M. H. Iversen contributed to the planning, execution and publication of the above named work. More specific; M. H. Iversen performed aggregate formation incubations, measurements of dry mass and preparations for measurements of particulate organic carbon (POC) content in the aggregates, measurements of respiration rates of POC content in the bulk sea water, measured POC content in the bulk water, and contributed to measurements of sinking velocities and small-scale oxygen fluxes to the aggregates. M. H. Iversen contributed to the determination of particle size spectra, calculations of vertical fluxes, and comparison of those to sediment trap data. M. H. Iversen did a large part of the data analysis, most of the data treatment, writing, and revision of the submitted paper.

EIDESSTATTLICHE ERKLÄRUNG

Gem. §6(5) Nr. 1-3 PromoO

Hiermit erkläre ich, dass ich die vorliegende Arbeit ohne unerlaubte, fremde Hilfe angefertigt habe und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe. Die in den benutzen Werken wörtlich oder inhaltlich entnommenen Stellen sind als solche kenntlich gemacht.

Bremen, 21.02.2009

Morten Hvitfeldt Iversen