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Degradation of copepod faecal pellets in the upper layer: role of microbial community and *Calanus finmarchicus*

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ABSTRACT: Copepod faecal pellets (FP) are considered important contributors to vertical carbon flux, but investigations comparing FP production with FP export using sediment traps conclude that vertical export is not their only fate. FP are degraded to a large extent in the upper 60 m, and even among large, fast-sinking FP, only a fraction reaches sediment traps deeper than 200 m. Retention mechanisms for copepod FP are still not well understood. In order to investigate the relative importance of the small (<180 µm) compartment of the plankton community versus larger filter-feeding copepods for degradation of large, fast-sinking FP, we incubated FP produced by *Calanus finmarchicus* (Gunnerus) in 180 µm-filtered water from the chlorophyll *a* maximum. From a series of experiments, we found that the degradation of large FP is time-dependent, as no degradation was apparent after 20 or 48 h of incubation, but after 72 h FP volume was reduced by 32%. We also found that large filter-feeding copepods may facilitate the degradation process, since FP degradation increased from 0 to 75% after 48 h of incubation in the presence of 5 *C. finmarchicus*. We conclude that ciliates and dinoflagellates are able to degrade large copepod FP, but that this process is too slow to explain observed retention of large FP in the upper 200 m of the water column due to fast sinking of large particles. Rather than looking for single-factor explanations for flux-regulating processes, we stress the importance of investigating combined effects in relevant time frames to understand the complexity of carbon flux regulation in natural systems.

KEY WORDS: Faecal pellet · Faecal pellet retention · Microbial degradation · Faecal pellet fragmentation · *Calanus finmarchicus* · Carbon cycling

INTRODUCTION

Copepod faecal pellets (FP) are fast-sinking packages of carbon and are assumed to be a main contributor to the vertical flux of carbon in the ocean (Turner & Ferrante 1979, and references therein). Over the last 20 yr, however, comparison of FP production and the actual downward export measured with sediment traps has revealed that highly variable, but generally strong, retention mechanisms limit FP export and facilitate recycling in the upper layers (Viitasalo et al. 1999, Wassmann et al. 1999, Wexels Riser et al. 2001, 2007, Turner 2002). The mechanisms and organisms involved in FP retention are still not fully understood, although some previous studies exist. For instance, as early as 1979, Paffenhöfer & Knowles described the ingestion of FP material by copepods (Paffenhöfer & Knowles 1979). Later, Lampitt et al. (1990) demonstrated that copepods are able to break up FP mechanically or remove the peritrophic membrane. Both mechanisms facilitate increased microbial degradation, i.e. degradation by heterotrophic bacteria as well as unicellular eucaryotes, such as heterotrophic ciliates and flagellates. Iversen & Poulsen (2007) also suggest that FP fragmentation is caused by filter feeding and FP rejection

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by copepods. González & Smetacek (1994) hypothesised that the abundant cyclopoid copepods *Oithona* spp. play a key role in the retention of copepod FP, but evidence is conflicting, indicating that *Oithona* spp. may be indicators for a retention community rather than the retaining organisms (Reigstad et al. 2005, Poulsen & Kiørboe 2006). This shows that organisms other than *Oithona* spp. could play a major role in FP retention. In an experiment investigating the effect of various size-fractions of grazers, Poulsen & Iversen (2008) concluded that heterotrophic ciliates and dinoflagellates are the main degraders of small FP from the calanoid copepod *Acartia tonsa* and that the effect of copepods on degradation rates is comparatively minor. At high latitudes, the copepod community is dominated by larger copepod species producing larger FP. It is therefore relevant to ask if the same groups of organisms and mechanisms are important for degradation of larger FP at high latitudes.

Sediment trap investigations revealed considerable attenuation of particulate organic carbon (POC) over a limited depth range (30–60 m) in areas where *Calanus* spp. dominate the larger copepod community biomass, such as the relatively productive areas on- and off-shelf along the northwest Atlantic (Andreassen et al. 1999, Olli et al. 2001), and in the Barents Sea (Reigstad et al. 2008). This depth range is often associated with a deep chlorophyll *a* maximum (chl a_{max}), and/or the pycnocline. These layers have recently received increased attention as bioactive layers and sites for particle transformations (Checkley et al. 2008). Comparing vertical FP export to *in situ* FP production rates, there is a high but variable retention of larger and fast-sinking FP, ranging from 30 to 98% in the upper 100 m (Urban-Rich et al. 1999, Wexels Riser et al. 2002, 2007). High food concentrations in the chl a_{max} provide suitable conditions for high concentrations of grazers, including unicellular grazers such as heterotrophic ciliates and dinoflagellates (hereafter referred to as 'protozooplankton'). We therefore believe that this layer of high grazing activity can be important for FP degradation, and that the degradation of larger and faster-sinking FP requires efficient processes that might differ from those of smaller and slower-sinking FP.

To investigate the role of different organisms or size groups in the removal of large FP, we investigated degradation rates of *Calanus finmarchicus* FP (156 µm equivalent spherical diameter, ESD) by organisms <180 µm in a northern Norwegian fjord. More specifically, we performed 3 experiments to assess (1) whether FP are degraded at different rates at and below the chl a_{max} (due to the presence or absence of bioactive layers), (2) the effect of time on FP degradation, by investigating differences in degradation rate of FP after 20, 48 and 72 h of incubation in water from chl a_{max}, and (3) the effect of *C. finmarchicus* on FP degradation.

MATERIALS AND METHODS

Sampling and FP production

Water was collected on 12 and 19 May 2009 at station Svartnes in Balsfjord, a 45 km long and 2–3 km wide fjord with SSE orientation, (69° 22′ N, 19° 07′ E) (Fig. 1). Balsfjord has a maximum depth of 190 m, and sills situated outside the mouth (~30 m deep) that separate the fjord from the coastal water (Svendsen 1995, Reigstad 2000). Vertical mixing in Norwegian fjords is generally through winter convection, and fjord circulation is generated by wind forcing (Svendsen 1995). Tidal amplitudes are 2.5 m, but the turbulence regime is not yet known. The spring bloom peaks in mid-April before any major stratification is established in the fjord (Eilertsen et al. 1981, Wexels Riser et al. 2010). The annual primary production of Balsfjord is between 105 and 130 g C m^{-2} yr^{-1} (Eilertsen & Taasen 1984) and new production is approx. 30 to 40% of this (Wassmann et al. 2000). Incubation water for Expt 1 (see detailed description

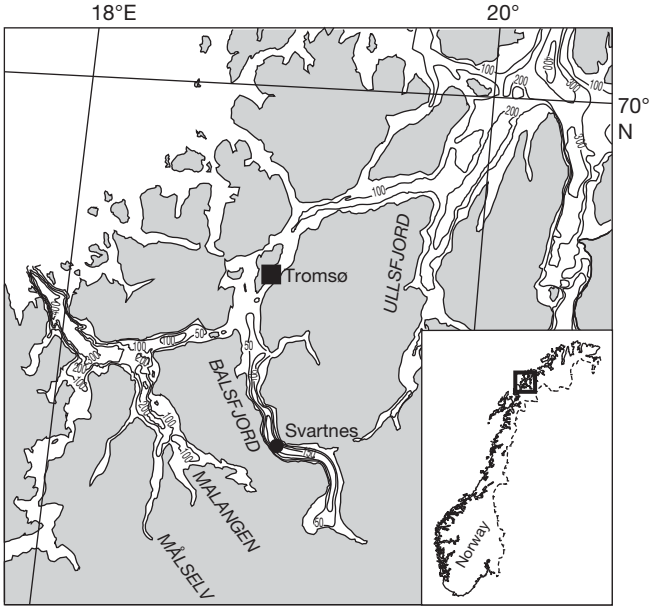

Fig. 1. Sampling station Svartnes (●), in Balsfjord, northern Norway, with bathymetry (m)

below) was collected at depths of 60 and 90 m on 12 May, in order to sample both the chl a_{max} and below the pycnocline, respectively. For Expts 2 and 3, water was only collected at the depth of the chl a_{max} (20 m) on 19 May, in order to investigate time-dependent FP degradation.

At the time of sampling, a CTD profile was taken and samples for suspended chl *a*, POC and particulate organic nitrogen (PON) were collected with Niskin water bottles at 10, 20, 30, 40, 50, 90, 120 and 200 m. Depth of chl a_{max}, and hence sampling depth of incubation water for the experiments, was determined from the fluorescence profile obtained with the CTD. Samples for enumeration and identification of protozooplankton were collected from the incubation water depths and preserved in 2% acid Lugol's solution.

Copepods for the experiments were collected near the station at least 1 d prior to the start of the experiments to allow production of FP. Large individuals (Stage CV and females between 2.2 and 2.7 mm prosome length) of *Calanus finmarchicus* were sorted under a dissecting microscope equipped with a cold light in a temperature controlled room at 6°C. FP were produced in two 10 l beakers by allowing approx. 65 *C. finmarchicus* to graze on a culture of *Rhodomonas* sp. (3×10^6 cells l^{-1}) in darkness at *in situ* temperature (6°C). After approx. 10 h, the bottom content of the beakers was siphoned off into smaller beakers and the FP were sorted under a dissecting microscope. FP were always kept on ice during the sorting process and were rinsed 3 times in filtered seawater before being added to the experimental bottles. Only intact FP were selected for the experiments, and total handling time for the FP was a maximum of 6 h. The mean ± SD size of individual FP was 590 ± 97 µm length and 66 ± 10 µm width (n = 100) (Table 1), corresponding to an average ESD of 156 µm. The approximate sinking rate for similar-sized FP was estimated in settling experiments to be between 100 and 200 m d^{-1} (E. Arashkevich and C. Wexels Riser, unpubl. data). The *Rhodomonas* sp. culture originated from the Canadian Arctic and was grown at 6°C in f/2 medium.

Table 1. Properties of faecal pellets (FP) used in the degradation experiments. Length, width, volume, carbon content, nitrogen content and carbon:volume (C:V) ratio of pellets produced by *Calanus finmarchicus* feeding on *Rhodomonas* sp. (mean ± SD, n = 100)

Length (µm)	590 ± 97
Width (µm)	66 ± 10
Volume (μm^3)	$2.12 \times 10^6 \pm 0.82 \times 10^6$
Carbon (µg C FP^{-1})	0.26 ± 0.016
Nitrogen (µg N FP^{-1})	0.041 ± 0.0038
C:V (µg C mm^{-3})	123.2

Experiments

Three experiments were performed to investigate FP degradation caused by the microbial community (Expts 1 and 2) and FP removal caused by *Calanus finmarchicus* (Expt 3) (Table 2). All experiments were run in a temperature-controlled room (6°C) in the dark. Freshly produced FP were incubated in 600 ml bottles on a slowly rotating (1 rpm) plankton wheel to keep FP in suspension during the experiments. All incubation water was screened through 180 µm Nitex mesh to exclude patchy occurrence of large organisms, but keep the protozooplankton community at natural concentrations.

Expt 1

To investigate the differences in microbial degradation of FP at 2 depths, we incubated 80 freshly produced FP for 20 h in (1) water from below the pycnocline (90 m depth), (2) water from the chl a_{max} (60 m depth) and (3) 0.22 µm-filtered seawater (FSW) as a control. All treatments had 4 replicates. At the end of the experiment, before collecting the remaining FP, bottles were left for 30 min on the bench to allow the FP to sink. Thereafter, incubation water was gently reduced to 200 ml using a silicon tube covered with a 10 µm filter to prevent accidental removal of FP. The remaining water was fixed with formalin (2% final concentration). FP were counted and their length and width measured after 24 h sedimentation in Utermöhl chambers using an inverted microscope (Leica DM IL) at 100× magnification. Note that counting and measuring all FP fragments at 100× magnification is necessary for obtaining the high precision biovolume measurements needed for investigating FP degradation, as counting but not measuring FP at lower magnification would result in higher and erroneous degradation rates.

Expt 2

The design of Expt 2 was similar to Expt 1, but the incubation water was only collected at the chl a_{max} (20 m depth). To investigate the effect of time on FP

Table 2. Experimental set-up, incubation time (inc. time) and properties of incubation water (Inc. water) for Expts 1–3. FP: faecal pellets; chl a_{max}: depth of chlorophyll *a* maximum; FSW: filtered seawater

Expt	Date (d.mo.yr)	Inc. time (h)	Inc. water (replicates)	Purpose
1	12.05.09	20	Chl a_{max}: 60 m (4) *In situ* 90 m (4) Control: FSW (4)	FP degradation from microbial community <180 µm after 20 h
2	19.05.09	48	Chl a_{max}: 20 m (4) Control: FSW (4)	FP degradation from microbial community <180 µm after 48 and 72 h
		72	Chl a_{max}: 20 m (4) Control: FSW (4)	
3	20.05.09	48	Chl a_{max}: 20 m (4) Control: chl a_{max} 20 m (4)	Mechanical fragmentation of FP by *Calanus finmarchicus*

degradation by the <180 µm community, the incubation time was prolonged to 48 and 72 h. In total, 16 bottles containing 80 FP each were incubated. Eight bottles were incubated for 48 h (4 replicates, 4 controls), and 8 bottles were incubated for 72 h (4 replicates, 4 controls). Treatment bottles contained FP and 180 µm-screened water from 20 m depth, and the control bottles contained FP and FSW. The procedure for ending the experiment was the same as for Expt 1.

Expt 3

The last experiment investigated FP degradation in the presence of both *Calanus finmarchicus* and the microbial community <180 µm. We used incubation water from the chl a_{max} (20 m), as for Expt 2. Four bottles, each containing 80 FP, were incubated with filtered (180 µm) water from the chl a_{max}, while 5 *C. finmarchicus* were added to another 4 incubation bottles containing filtered water from the chl a_{max} and 80 FP. An additional 4 bottles filled with FSW and 80 FP served as a control. Prior to the experiment, the copepods were left overnight in FSW to empty their guts. The experiment was terminated after 48 h as described for Expt 1 and 2.

Chl *a*, POC, and FP analyses

Chl *a* concentrations in the incubation water and *in situ* profiles were measured on a Turner Designs model 10-AU fluorometer calibrated using pure chl *a* (Sigma C6144). Samples were filtered onto GF/F (total chl *a*) and membrane filters (chl *a* >10 µm) and extracted in 100% methanol overnight (Holm-Hansen et al. 1965). Samples for POC and PON were filtered in triplicate onto pre-combusted GF/F filters, frozen (–20°C) and analysed on a Leeman Lab 440 elemental analyser. Prior to analyses, inorganic carbon was removed by fuming filters with concentrated HCl. We also determined carbon content of the FP used in the degradation experiments (3 replicates containing 100 FP each). The average volume of 100 measured FP was used to estimate a carbon:volume conversion for *Calanus finmarchicus* FP. The carbon content of the FP used in the experiments was 123.2 µg C mm^{-3}, equivalent to 0.26 µg C FP^{-1}. This implies that 20.5 µg of faecal pellet carbon (FPC) was added to each experimental bottle. Incubation water was checked for 'background' FP.

In situ plankton community determinations

Ciliates and heterotrophic dinoflagellates were enumerated under an inverted microscope (Leica DM IL) at 200× magnification in accordance with Utermöhl (1958). Cell sizes were measured and geometric proxies were used to calculate biovolume of the cells. The biovolume was converted to carbon using a volume to carbon conversion factor of 0.19 pg C $µm^{-3}$ (Putt & Stoecker 1989) for aloricate ciliates. The carbon content of dinoflagellates was calculated in accordance with Menden-Deuer & Lessard (2000) as pg C $cell^{-1} = 0.76 \times Vol^{0.819}$, where Vol = cell volume ($µm^3$). The samples were screened for small metazoan zooplankton (i.e. copepod nauplii and meroplankton), which could have passed through the 180 µm screen. No such grazers were found; therefore, the grazer community <180 µm used in the experiments consisted entirely of protozooplankton.

Statistical analyses

Data were checked for normality, and differences in FPC in control (FSW) and treatment (chl a_{max}) bottles were tested for statistical significance using a 2-way ANOVA with time and treatment as factors. Differences between the treatments in the 48 h incubations (chl a_{max}, *Calanus*) and the control (FSW) were tested for statistical differences using paired Student's *t*-tests (SPSS16.0).

Table 3. Depth of chlorophyll *a* maximum (chl a_{max}), temperature and salinity at the sampling station Svartnes on the 2 dates sampled. Water for Expt 1 was sampled 12.05.09, and water for Expts 2 and 3 was sampled 19.05.09

Date (d.mo.yr)	Depth of chl a_{max} (m)	Temperature at chl a_{max} (°C)	Salinity at chl a_{max}	Temperature range, 0–60 m (°C)	Salinity range, 0–60 m	Average temperature, 60–100 m (°C)	Average salinity, 60–100 m
12.05.09	60	2.7	33.56	2.7–5.5	32.0–33.6	2.76	33.6
19.05.09	20	4.5	33.28	2.8–7.5	31.1–33.6	2.81	33.6

RESULTS

Physical and biological background for experiments

Physical and biological parameters were measured at the sampling station Svartnes in Balsfjord on 2 occasions in order to obtain information about the incubation water for the experiments (Table 3). The main difference between the 2 sampling occasions was the depth of chl a_{max}, which was 60 m on May 12 due to a wind event prior to sampling, and 30 m on May 19, when the wind had eased (Fig. 2). The deep chl a_{max} at 60 m on May 12 had concentrations of chl *a* and POC of 3 and 250 mg m^{-3}, respectively. On May 19, the concentrations were within the same range as the first sampling date (Fig. 2). The chl *a* concentrations were dominated by phytoplankton cells <10 µm (91 and 84% on May 12 and May 19, respectively) (data not shown). The deep mixing also influenced water-column properties such as average temperature and salinity in the upper 60 m (Table 3).

Protozooplankton in the incubation water

Water for Expt 1, sampled at the chl a_{max} at 60 m depth on May 12, had a protozoan biomass of 12 µg C l^{-1} (Table 4). Athecate dinoflagellates dominated in both numbers and biomass. Small athecate dinoflagellates (ESD <30 µm) were numerically most abundant, but dominated in terms of biomass only in the water from 90 m (Table 4). At 60 m, larger athecates (ESD >30 µm) constituted the largest fraction of dinoflagellate biomass, dominated by dinoflagellates of the genus *Gyrodinium* (average ESD 41 µm). The dinoflagellate assemblage at 60 m was on average 20 µm larger than the assemblage at 90 m in terms of ESD. The same trend was observed for the ciliate assemblage from these 2 depths, with ciliates being on average 12 µm larger at 60 m. At 60 m, oligotrich ciliates of the

Table 4. Concentrations of chlorophyll *a* (chl *a*), particulate organic carbon (POC) and protozooplankton in the incubation water collected at 60, 90 and 20 m depth at the start (t_0) of Expts 1–3. ESD: equivalent sperical diameter

Concentration	Experiment:	1	1	2 + 3
	Sampling depth:	60 m	90 m	20 m
Chl *a* (µg l^{-1})		3	0.3	2
POC (µg l^{-1})		254	98	209
Total ciliates (µg C l^{-1})		3	0	50
Strombidium spp.		3	0	22
Laboea strobila		0	0	18
Other ciliates		1	0	10
Total dinoflagellates (µg C l^{-1})		9	2	26
Athecate ESD 10–30 µm		1	2	16
Athecate ESD 30–50 µm		5	0	4
Other dinoflagellates		3	0	7
Total protozooplankton (µgC l^{-1})		12	2	76

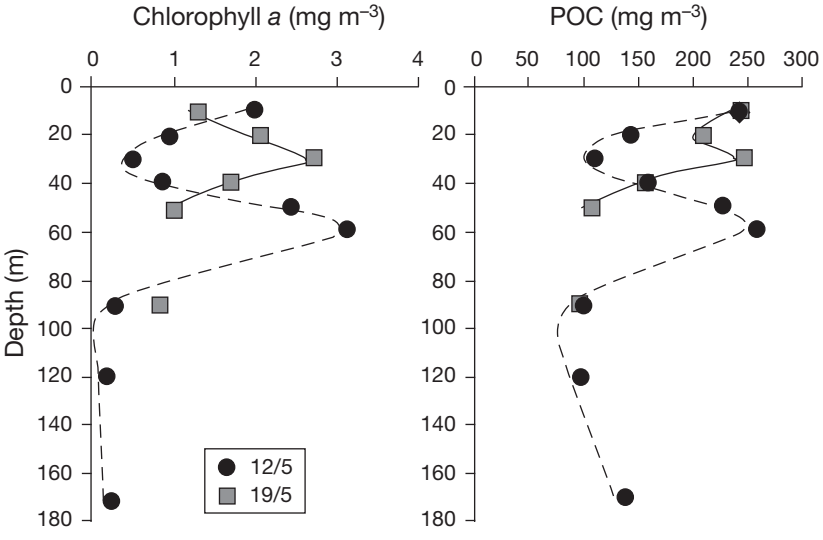

Fig. 2. Vertical profiles of chlorophyll *a* (chl *a*) and particulate organic carbon (POC) on 12 and 19 May 2009

genus *Strombidium* dominated the ciliate assemblage, constituting ≥60% of the numbers and biomass. At 90 m, small (average ESD 15 µm) aloricate choreotrichs (genera *Lohmanniella* and *Leegaardiella*) were numerically most abundant, but strombidiids (average ESD 20 µm) dominated the biomass.

The incubation water for Expts 2 and 3 was collected on May 19 from 20 m depth, at the chl a_{max} (according to the CTD profile). Total biomass of protozoans was 76 µg C l^{-1} (Table 4). Numerically, small (ESD <30 µm) heterotrophic dinoflagellates dominated the protozoan assemblage. In terms of biomass, however, ciliates were more important due to the occurrence of the large oligotrichs, *Strombidium* cf. *constrictum* and *Laboea strobila*, with average ESDs of 63 and 103 µm, respectively. Large strobilidiids (ESD ≥70 µm) were also present. The larger dinoflagellates were dominated by *Gyrodinium* spp., which ranged in ESD from 49 to 97 µm.

Degradation of faecal pellets

Copepod FP were incubated in FSW and water from the chl a_{max} for 20, 48 and 72 h. In addition, FP were incubated for 20 h in water collected from below the pycnocline at 90 m depth, as part of Expt 1. No statistically significant difference in FP recovery was found after 20, 48 and 72 h in FSW ($p > 0.05$) (Fig. 3A). This was confirmed by microscopic observations of FP after incubation (Fig. 4).

FP incubated with water containing the microbial community <180 µm, i.e. containing protozooplankton, were not significantly degraded after 20 or 48 h incubation, and FPC was not statistically significantly different from the control bottles containing FP in FSW (Fig. 3A,B). FP incubations (20 h) in water from 90 m depth were not included in Fig. 3, since there was no significant difference between incubations from 60 and 90 m ($p > 0.05$). The only significant effect of the microbial community on FP degradation occurred after 72 h incubation time ($p < 0.05$), where 32% of the initial FPC was removed compared to the control bottles containing FP in FSW. The extent of FP fragmentation also increased from ~0% after 48 h to ~40% after 72 h (data not shown). Compared to the shorter incubations, the FP appeared more fragmented after 72 h (Fig. 4).

In the bottles where *Calanus finmarchicus* were added to the incubation water containing the microbial community (Expt 3), FPC was significantly reduced compared to FPC in the FSW and chl a_{max} treatments after 48 h ($p < 0.01$) (Fig. 3C). Visual inspection demonstrated that the FP from the *C. finmarchicus* treatment were broken into many pieces, in a different way than the FP incubated for 72 h in water from the chl a_{max} (Fig. 4). The total FPC removal was 75%, corresponding to a daily removal rate of 37%. However, as we must assume that the degradation took place between 48 and 72 h, it is misleading to operate with a daily removal rate calculated as the average over 72 h.

DISCUSSION

We investigated the degradation of large *Calanus finmarchicus* FP after different time intervals by exposure to FSW and the microbial community, with or without the presence of *C. finmarchicus*. Incubations in FSW did not demonstrate a time-dependent

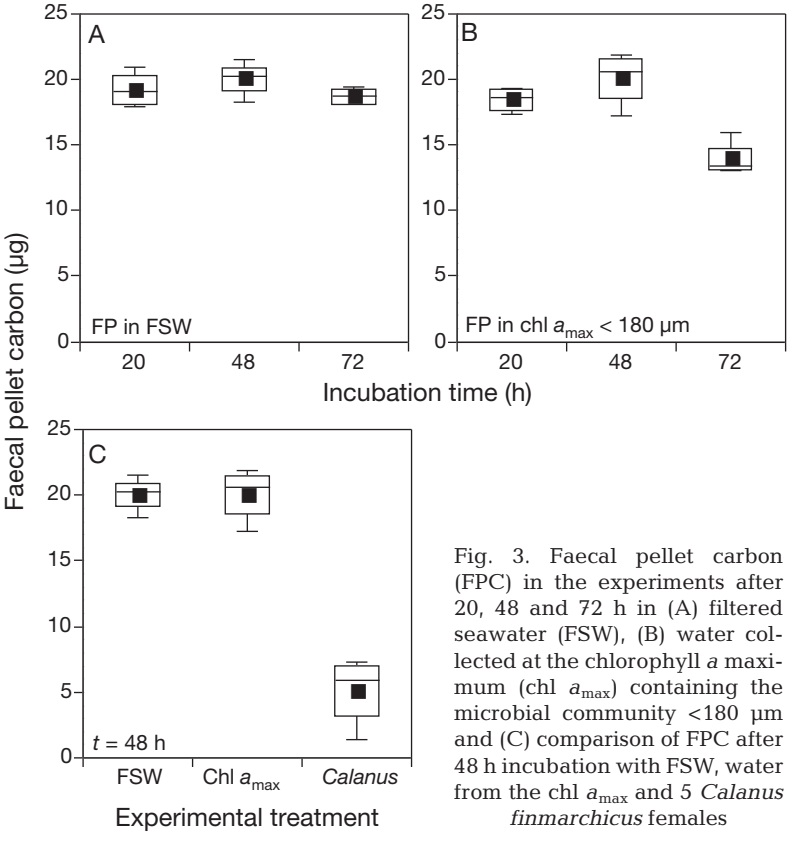

Fig. 3. Faecal pellet carbon (FPC) in the experiments after 20, 48 and 72 h in (A) filtered seawater (FSW), (B) water collected at the chlorophyll *a* maximum (chl a_{max}) containing the microbial community <180 µm and (C) comparison of FPC after 48 h incubation with FSW, water from the chl a_{max} and 5 *Calanus finmarchicus* females

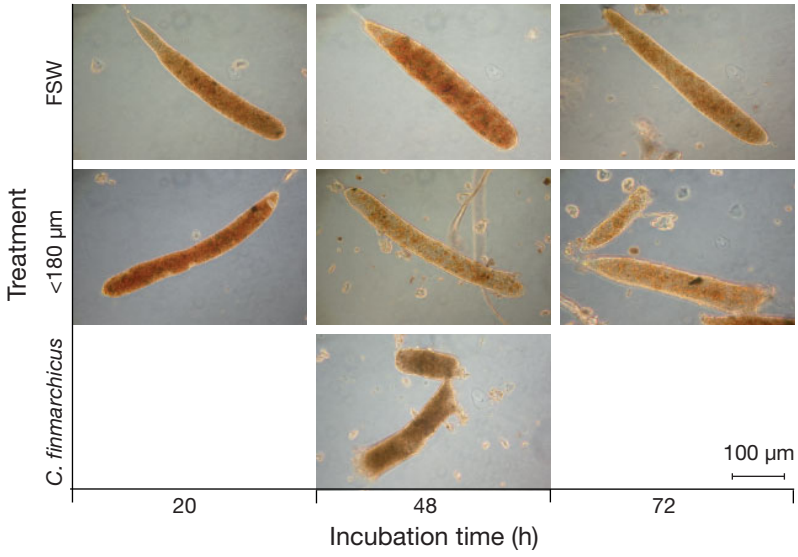

Fig. 4. Faecal pellets incubated with filtered seawater (FSW), the microbial community <180 µm and *Calanus finmarchicus*, with time

difference in FP degradation and fragmentation within 72 h, and we therefore conclude that FP degradation due to ageing, or fragmentation due to handling and incubation on the plankton wheel, were not of importance in our experiments.

Time-dependent FP degradation by the microbial community

Our results indicated that (1) the <180 µm microbial community was capable of significantly degrading the large FP produced by *Calanus finmarchicus*, and (2) this was a relatively slow process, as loss of faecal material was only detected after 72 h of incubation, in contrast to previous investigations (Poulsen & Iversen 2008).

Degradation rates of small FP produced by *Acartia tonsa* range from 22 to 87% d^{-1}, and biota measuring <200 µm, particularly dinoflagellates, are reported to be the main degraders (Poulsen & Iversen 2008). The daily pellet degradation rates found by Poulsen & Iversen (2008) after 24 and 48 h of incubation are much higher than in our study, raising some important points. (1) Faecal pellet degradation rates are likely size-dependent. Pellets produced by *A. tonsa* are considerably smaller (volume 3–12 × 10^4 μm^3, ESD ~50 µm) than those produced by *Calanus finmarchicus* (volume 2 × 10^6 μm^3, Table 1, ESD = 156 µm) and thus closer to the preferred prey size of protozooplankton. (2) Degradation rates may be a function of pellet concentration, due to increased encounter rates between the pellets and the degrader organisms. The pellet concentration in the experiments conducted by Poulsen & Iversen (2008) was 0.5 FP ml^{-1}; about 10-fold greater than our experiments (0.08 FP ml^{-1}). A FP volume equal to 0.26 mm^3 l^{-1} was added in the present experiments. This is within the natural range of suspended FP concentrations previously observed in Balsfjord (up to 0.11 mm^3 l^{-1}, Wexels Riser et al. 2010) and on the northern Norwegian shelf (up to 0.36 mm^3 l^{-1}, Wassmann et al. 1999). (3) Differences in degradation rates may be explained by differences in the abundance and composition of the degrader organisms. In Expt 2, where the highest microbial degradation rates were found, the protozooplankton community was dominated by ciliates (50 µg C l^{-1}), although heterotrophic dinoflagellates were also abundant (26 µg C l^{-1}). However, most dinoflagellates were <30 µm ESD, and dinoflagellates >30 µm only contributed 4 µg C l^{-1}. In comparison, large dinoflagellates (>20 µm) contributed a maximum of 15 µg C l^{-1} in the study by Poulsen & Iversen (2008).

While ciliates have a prey preference of 1/10 of their own body size and select prey <20 µm, dinoflagellates may feed on particles several times larger than themselves, with an optimal prey size of 1/1 (Hansen 1992, Jakobsen & Hansen 1997). Although the FP in our experiments were large (590 × 97 µm on average, average ESD 156 µm), they could potentially, but not optimally, have been fed upon by *Gyrodinium* spp. (average ESD 41 µm), which dominated the large dinoflagellate assemblage. Poulsen et al. (2011) suggested that FP >400 µm might be too large for most phagotrophic dinoflagellates to graze upon. Difficulties with handling large FP might explain why degradation of *Calanus finmarchicus* FP was only apparent after 72 h in our study. However, the FP were certainly too large for ciliates to feed upon, though they may have caused physical damage to the periotrophic membrane, as reported by Lampitt et al. (1990). Total protozooplankton biomass was low in the water collected at 60 (chl a_{max}) and 90 m depths, and this, in addition to the short incubation time, may explain why *C. finmarchicus* FP were not degraded in Expt 1. We thus conclude that both incubation time and FP size are important variables for FP degradation rates.

FP degradation by copepods

The role of copepods and large zooplankton in FP degradation is still under debate. Copepods may impact FP through ingestion (coprophagy), mechanical break-up (coprorhexy) or by destroying the membrane (coprochaly) (Lampitt et al. 1990, Noji 1991). Paffenhöfer & Van Sant (1985) showed that the copepod *Eucalanus pileatus* feeds on, but does not positively select for, FP from *Paracalanus* spp. Harpacticoid copepods such as *Oncea* spp. feed on the surfaces of FP, and have the potential to retain the FP material in the upper layers of the southern Indian Ocean (Møller et al. 2011). The benthic harpacticoid copepod *Paramphiascella fulvofasciata* also ingests FP (De Troch et al. 2009). Iversen & Poulsen (2007) showed that *Calanus helgolandicus* actively reject FP from *Acartia tonsa* during filter feeding, resulting in FP fragmentation. Recent studies on the relative impact of smaller versus larger zooplankton on FP degradation concluded that copepods and zooplankton >200 µm do not play an important role in the degradation of pellets from the natural zooplankton community in the Baltic Sea, where *Oithona* spp. and smaller calanoid copepods, such as *A. tonsa*, are most abundant (Poulsen & Kiørboe 2006, Poulsen & Iversen 2008, respectively). In these studies, heterotrophic dinoflagellates and late stage nauplii were the most important degraders.

Most of the aforementioned experiments are concerned with the degradation of FP of smaller copepods, with an ESD in the feeding size range of protozooplankton. From field studies, we know that the retention of small FP is high, as their contribution to vertical export at depth is minor despite high abundance of small zooplankton (Wexels Riser et al. 2008, 2010). However, the retention mechanism of larger FP, which contribute to vertical export, but are also partly retained (Wexels Riser et al. 2002) is still uncertain.

Our experiments showed that *Calanus finmarchicus* had a significant effect on removal rates of larger *C. finmarchicus* FP. The average FP removal from bottles containing both microbes <180 µm and *C. finmarchicus* was 37 % d^{-1} (Expt 3). This corresponds to a pellet clearance rate, calculated in accordance with Frost (1972), of 83 ± 14 ml copepod^{-1} d^{-1} (mean ± SD at 6°C). In comparison, *C. helgolandicus* has a clearance rate of up to 172 ± 125 ml female d^{-1} (at 14°C) when feeding on pellets from *Acartia tonsa* (Iversen & Poulsen 2007). Correcting for the difference in temperature using a Q_{10} of 2.8 (Hansen et al. 1997), our rates for *C. finmarchicus* feeding on FP are higher (234 ml female^{-1} d^{-1}), but in the same order of magnitude, as clearance rates reported by Iversen & Poulsen (2007). Our results therefore support the conclusions of Iversen & Poulsen (2007) that when a filter-feeding copepod encounters a FP, the pellet is often rejected, and ingestion occurs only when fragments are so small that they are ingested unintentionally with other food particles. Based on the photos of FP incubated with *C. finmarchicus* (Expt 3), it seems that FP were cut open, and when counting the FP material we noted many small FP fragments (Fig. 4). We suggest that the missing FP material in Expt 3 was ingested by *C. finmarchicus*, although FP were perhaps not the target food. The need to look at additive effects also gives rise to the hypothesis that larger copepods facilitate the degradation of FP material by making it more available for complex microbial processes (Ploug et al. 2008). In our case, it is probable that successive processes are present, where the 'FP breakers' are the first step in the degradation of larger FP.

In situ downward flux and degradation of copepod FP

The size and sinking speed of FP determines its residence time in the upper layer of the ocean, as increased residence time implies greater exposure to degrader organisms in the 'retention filter' (sensu Wexels Riser et al. 2001). Several functional or compositional models defining such retention filters have been suggested, such as the coprophagous filter (Gonzales & Smetacek 1994) and the protozooplankton filter (Poulsen & Iversen 2008). We suggest that the retention filter that most adequately describes the observed retention processes is the layer of increased grazing activity observed at the base of the surface mixed layer, related to the chl a_{max}, as described by Checkley et al. (2008). The high concentration of grazers attracted to this area by increased food availability increases the retention of FP through fragmentation as well as feeding on FP or FP fragments.

The sinking rate of FP varies greatly and is a function of size, shape and density. Also, measured sinking velocities on ellipsoid pellets can be higher than those calculated from Stoke's law, which assumes a spherical shape (Ploug et al. 2008). For ellipsoid FP produced by *Eucalanus pileatus* (volume 2.5 ± 0.2 × 10^6 µm^3, mean ± SD) fed with *Thalassiosira weissflogii,* the sinking velocity is 36.4 ± 3.8 m d^{-1} (Patonai et al. 2011). Small et al. (1979) reported sinking

velocities for similar-sized FP ranging from 30 to 100 m d^{-1}, and attributed the variability to different diets of the FP producers. A clear effect of copepod diet on FP sinking velocities was also found for *Temora longicornis*; FP produced on a *Rhodomonas* sp. diet sink more slowly (35 ± 29 m d^{-1}) than those produced on a *Emiliania huxleyi* (200 ± 93 m d^{-1}) and *T. weissflogii* diet (322 ± 169 m d^{-1}) (Ploug et al. 2008). In a seasonal study in Balsfjord, the mean sinking rate of copepod FP was100 to 200 m d^{-1} (A. Arashkevich & C. Wexels Riser unpubl. data). As pellets produced on a diatom diet have lower carbon content and higher sinking rates than pellets produced on a flagellate diet, FP produced on a flagellate diet are more likely to be recycled within the euphotic zone than those produced on a diatom diet (Urban-Rich 2001). This implies that the content of the FP also affects degradation and hence contribution to the vertical flux of organic carbon from the euphotic zone.

Consequently, the efficiency of the retention filter is not only dependent on the composition of the filter, but also on the properties of the particles that pass through it. We must therefore distinguish between retention mechanisms relevant for small and large FP. In our experiments, the microbial degradation of the large FP produced by *Calanus finmarchicus* was only detectable after 72 h, and given the residence time of natural FP in the upper 100 m in Balsfjord, the FP would have reached the seafloor within that time frame. Hence, large FP sink quickly through the euphotic zone where the most efficient degradation takes place. The low sinking rate of small FP is one likely explanation why few FP produced by small copepods, such as *Acartia* spp. and similar, are found at depth and why the size of FP increases with depth of capture (Hofmann et al. 1981, Ayukai & Hattori 1992, Cadée et al. 1992, Wexels Riser et al. 2008).

For plankton communities, export of organic material (including copepod FP) represents a loss of nutrients that could potentially fuel continued production. Regenerated production, which is typical in most systems after the spring bloom, is dependent on recycling of the nutrient pool. Recycling of nutrients in the euphotic zone is linked to processes within the retention filter. To fully comprehend export and retention processes, we must address the relevant time scales. Not only must processes and key players be investigated experimentally, but results must be interpreted in light of field data and existing knowledge. Vertical export of biogenic particles and retention processes have been examined from a deep ocean perspective, but there is a need to understand the mechanisms involved in the retention of biogenic particles at the surface (Wassmann 1997, Boyd & Trull 2007). Iversen et al. (2010) argue that residence time of particles in the upper water column is important for addressing the relative importance of bacteria versus flux-feeding zooplankton. Our results suggest that in highly productive regions, where mesozooplankton producing fast-sinking FP are important, rapid degrading processes (<48 h), such as fragmentation by filter-feeding copepods, are more import than processes involving microbial degraders.

CONCLUSIONS

Large FP produced by *Calanus finmarchicus* can contribute significantly to the vertical flux of biogenic matter, but generally, FP captured in sediment traps fall short of pelagic FP production. Our experiments demonstrate that large *C. finmarchicus* FP are relatively difficult to degrade, as a detectable effect of the plankton community <180 µm was only visible after 72 h. However, combined effects of both the microbial community and large copepod grazers resulted in more efficient degradation rates.

Our first hypothesis predicting a more efficient retention community close to the chl a_{max} compared to deeper water was not tested appropriately, as the time frame of the experiment (20 h) was too short to detect a response from the microbial community. Our second hypothesis, that large FP requires a more efficient retention community than smaller FP, was supported. Also, our results imply that mesozooplankton such as *Calanus finmarchicus* may be more important than protozooplankton for the degradation of large FP. This contrasts with previous studies on smaller FP pellets.

We suggest that the degradation of FP can prolong the pelagic productive season by retaining organic material and nutrients in the euphotic zone. A strong pelagic food web could thus provide positive feedback through retention processes.

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