Limnol. Oceanogr., 53(2), 2008, 469–476

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

Helle Ploug[1]
Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D - 27570 Bremerhaven, Germany; Max Planck Institute for Marine Microbiology, Celsiusstr. 1, D-28359 Bremen, Germany

Morten Hvitfeldt Iversen
Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D - 27570 Bremerhaven, Germany

Marja Koski
Danish Institute for Fisheries Research, Kavalergården 6, DK-2920 Charlottenlund, Denmark

Erik Theodoor Buitenhuis
University of East Anglia, NR4 7TJ Norwich, United Kingdom

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

[1] To whom correspondence should be addressed. Present address: Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D - 27570 Bremerhaven, Germany.

Acknowledgments

We are grateful for advice from Dirk deBeer, Armin Giseke, and Lubos Polerecky. We thank Gaby Eickert and Karin Hohmann for constructing microsensors and Klaus-Uwe Richter for measuring particulate organic carbon. The data acquisitioning program "μ-profiler" has been developed by Lubos Polerecky. Thomas Kiørboe is thanked for providing laboratory facilities to measure fecal pellet sinking velocity.

This study was supported by the Alexander von Humboldt Foundation (DAN 1072992 STP to H.P.), the Carlsberg Foundation (to M.K.), the Max Planck Society, the Helmholtz Association ("Wiedereinstiegsstelle" to H.P.), the Alfred Wegener Institute for Polar and Marine Research, and the Research Center Ocean Margins ([RCOM] to H.P. and M.I.).

This is AWI publication No. 16636 and RCOM publication No. 0530.

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*

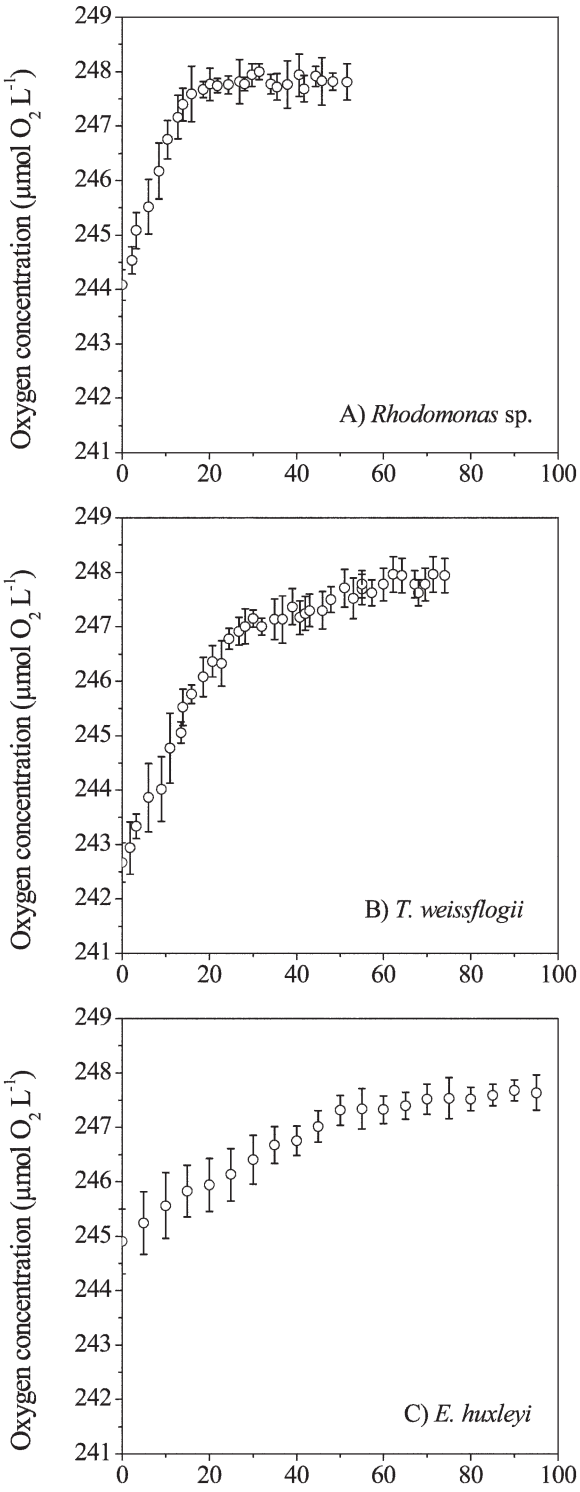

Fig. 1. Oxygen concentration gradients measured at the pellet–water interface of pellets produced on (A) *Rhodomonas* sp., (B) *T. weissflogii*, and (C) *E. huxleyi* as food sources. The symbols represent the mean value with the standard deviation of the mean value shown as bars ($n = 5$).

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).

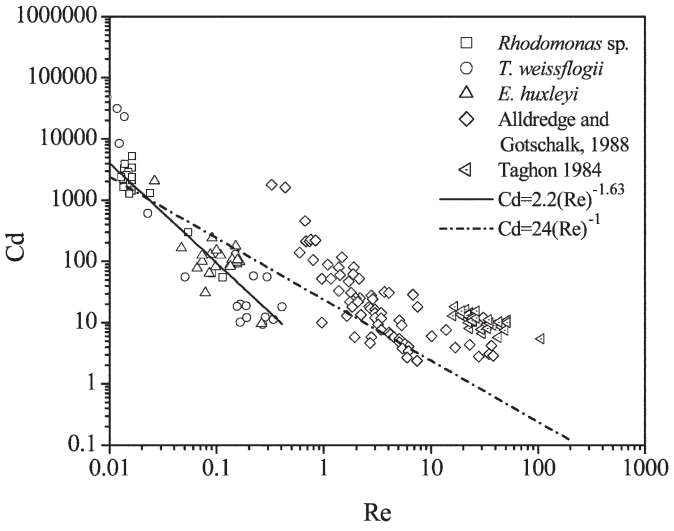

Fig. 2. Drag coefficient (C_D) as a function of Reynolds (Re) number, including previously published data by Alldredge and Gotschalk (1988) and Taghon et al. (1984) and Stokes' relationship for solid spheres at $Re < 0.5$.

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.

References

Alldredge, A. L., and Y. Cohen. 1987. Can microscale chemical patches persist in the sea? Microelectrode study of marine snow and fecal pellets. Science **235:** 689–691.

———, and C. C. Gotschalk. 1988. In situ settling behavior of marine snow. Limnol. Oceanogr. **33:** 339–351.

Armstrong, R. A., C. Lee, J. I. Hedges, S. Honjo, and S. G. Wakeham. 2002. A new, mechanistic model for organic carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals. Deep-Sea Res. I **49:** 219–236.

Besiktepe, S., and H. G. Dam. 2002. Coupling of ingestion and defecation as a function of diet in the calanoid copepod *Acartia tonsa*. Mar. Ecol. Prog. Ser. **229:** 151–164.

Broecker, W. S., and T. H. Peng. 1974. Gas exchange rates between air and sea. Tellus **26:** 21–35.

Bruland, K. W., and M. Silver. 1981. Sinking rates of fecal pellets from gelatinous zooplankton, slaps, pteropods doliolids. Mar. Biol. **63:** 295–300.

Buitenhuis, E. T., P. van der Wal, and H. J. W. Baar. 2001. Blooms of *Emiliania huxleyi* are sinks of atmospheric carbon dioxide: A field and mesocosm study derived simulation. Global Biogeochem. Cycles **15:** 577–587.

Båmstedt, U., J. C. Nejstgaard, and P. Solberg. 1999. Utilisation of small-sized food algae by *Calanus finmarchicus* (Copepoda: Calanoida) and the significance of feeding history. Sarsia **84:** 19–38.

Dutz, J., M. Koski, and S. H. Jónasdóttir. 2008. Copepod reproduction is unaffected by diatom aldehydes or lipid composition. Limnol. Oceanogr. **53:** 225–235.

Feinberg, L. R., and H. G. Dam. 1998. Effects of diet on dimensions, density and sinking rates of fecal pellets of the copepod *Acartia tonsa*. Mar. Ecol. Prog. Ser. **175:** 87–96.

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. **6:** 805–815.

Guillard, J. K., and J. H. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* (Hustedt) and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. **8:** 229–239.

Harris, R. P. 1994. Zooplankton grazing on the coccolithophore *Emiliania huxleyi* and its role in inorganic carbon flux. Mar. Biol. **119:** 431–439.

Huskin, I., R. Anadón, F. Álvarez-Marqués, and R. P. Harris. 2000. Ingestion, faecal pellet and egg production rates of *Calanus helgolandicus* feeding coccolithophorid versus non-coccolithophorid diets. J. Exp. Mar. Biol. Ecol. **248:** 239–254.

Hansen, B., F. L. Fotel, N. J. Jensen, and S. D. Madsen. 1996. Bacteria associated with a marine planktonic copepod culture. II. Degradation of fecal pellets produced on a diatom, a nanoflagellate or a dinoflagellate diet. J. Plankton Res. **18:** 275–288.

Jansen, H., and D. A. Wolf-Gladrow. 2001. Carbonate dissolution in copepod guts: A numerical model. Mar. Ecol. Prog. Ser. **221:** 199–207.

———, R. E. Zeebe, and D. A. Wolf-Gladrow. 2002. Modeling the dissolution of settling CaCO3 in the ocean. Global Biogeochem. Cycles **16:** 1027, doi: 10.1029/2000GB1001279.

Kiørboe, T., H.-P. Grossart, H. Ploug, and K. Tang. 2002. Mechanisms and rates of bacterial colonization of sinking aggregates. Appl. Environ. Microbiol. **68:** 3996–4006.

Klaas, C., and D. E. Archer. 2002. Association of sinking organic matter with various types of mineral ballast in the deep sea: Implications for the rain ratio. Global Biogeochem. Cycles **16:** 1116, doi: 10.1029/2001GB1001765.

Knappertsbusch, M., and G.-J. A. Brummer. 1995. A sediment trap investigation of sinking coccolithophorids in the North Atlantic. Deep-Sea Res. I **42:** 1083–1109.

Komar, P. D., A. P. Morse, L. F. Small, and S. W. Fowler. 1981. An analysis of sinking rates of natural copepod and euphausiid fecal pellets. Limnol. Oceanogr. **26:** 172–180.

Lee, B-G., and N. S. Fisher. 1992. Decomposition and release of elements from zooplankton debris. Mar. Ecol. Prog. Ser. **88:** 117–128.

Milliman, J. D., P. J. Troy, W. M. Balch, A. K. Adams, Y.-H. Li, and F. T. Mackenzie. 1999. Biologically mediated dissolution of calcium carbonate above the chemical lysocline? Deep-Sea Res. I **46:** 1653–1669.

Nejstgaard, J. C., U. Båmstedt, E. Bagøien, and P. T. Solberg. 1995. Algae constraints on copepod grazing. Growth state, toxicity, cell size, and season as regulating factors. ICES J. Mar. Sci. **52:** 347–357.

———, I. Gismervik, and P. T. Solberg. 1997. Feeding and reproduction by *Calanus finmarchicus*, and microzooplankton grazing during mesocosm blooms of diatoms and the coccolithophore *Emiliania huxleyi*. Mar. Ecol. Prog. Ser. **147:** 197–217.

Olsen, S. N., P. Westh, and B. Hansen. 2005. Real time quantification of microbial degradation of copepod fecal pellets monitored by isothermal microcalorimetry. Aquat. Microb. Ecol. **40:** 259–267.

Ploug, H. 2001. Small-scale oxygen fluxes and remineralization in sinking aggregates. Limnol. Oceanogr. **46:** 1624–1631.

———, and H.-P. Grossart. 1999. Bacterial production and respiration in suspended aggregates—A matter of the incubation method. Aquat. Microb. Ecol. **20:** 21–29.

———, and ———. 2000. Bacterial growth and grazing on diatom aggregates: Respiratory carbon turnover as a function of size and sinking velocity. Limnol. Oceanogr. **45:** 1467–1475.

———, ———, F. Azam, and B. B. Jørgensen. 1999. Photosynthesis, respiration, and carbon turnover in sinking marine snow from surface waters of Southern California Bight: Implications for the carbon cycle in the ocean. Mar. Ecol. Prog. Ser. **179:** 1–11.

———, M. Kühl, B. Bucholz, and B. B. Jørgensen. 1997. Anoxic aggregates—An ephemeral phenomenon in the pelagic environment. Aquat. Microb. Ecol. **13:** 285–294.

Pond, D. W., R. P. Harris, and C. Brownlee. 1995. A microinjection technique using a pH-sensitive dye to determine the gut pH of *Calanus helgolandicus*. Mar. Biol. **123:** 75–79.

Poulsen, L. K., and T. Kiørboe. 2005. Coprophagy and coprorhexy in the copepods *Arcartia tonsa* and *Temora longicornis*: clearance rates and feeding behaviour. Mar. Ecol. Prog. Ser. **299:** 217–227.

———, and ———. 2006. Vertical flux and degradation rates of copepod fecal pellets in a zooplankton community dominated by small copepods. Mar. Ecol. Prog. Ser. **323:** 195–204.

Revsbech, N. P. 1989. An oxygen microelectrode with a guard cathode. Limnol. Oceanogr. **34:** 474–478.

Schwinghamer, P., D. M. Anderson, and D. M. Kulis. 1991. Separation and concentration of living dinoflagellate resting cysts from marine sedimentation via density-gradient centrifugation. Limnol. Oceanogr. **36:** 588–592.

Simon, M., H.-P. Grossart, B. Schweitzer, and H. Ploug. 2002. Microbial ecology of organic aggregates in aquatic ecosystems. Aquat. Microbial. Ecol. **28:** 175–211.

Taghon, G. L., A. R. M. Nowell, and P. A. Jumars. 1984. Transport and breakdown of fecal pellets: Biological and sedimentological consequences. Limnol. Oceanogr. **29:** 64–72.

Thor, P., H. G. Dam, and D. R. Rogers. 2003. Fate of organic carbon release from decomposing copepod fecal pellets in relation to bacterial production and ectoenzymatic activity. Mar. Ecol. Prog. Ser. **33:** 279–288.

Turner, J. T. 2002. Zooplankton fecal pellets, marine snow and sinking phytoplankton blooms. Aquat. Microb. Ecol. **27:** 57–102.

Urban-Rich, J. 1999. Release of dissolved organic carbon from copepod fecal pellets in the Greenland Sea. J. of Exp. Mar. Biol. Ecol. **232:** 107–124.

van der Wal, P., R. S. Kempers, and M. J. W. Veldhuis. 1995. Production and downward flux of organic matter and calcite in a North Sea bloom of the coccolithophore *Emiliania huxleyi*. Mar. Ecol. Progr. Ser. **126:** 247–265.

Weast, R. C. [ed.] 1968. Handbook of chemistry and physics. 49th ed. The Chemical Rubber Co., Cleveland.

White, F. M. 1974. Viscous fluid flow. McGrawHill.

Received: 6 June 2007
Accepted: 10 October 2007
Amended: 19 October 2007