# Egestion of non-pellet-bound fecal material from the copepod *Acartia tonsa*: implication for vertical flux and degradation

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ABSTRACT: Only a minor fraction of copepod defecation appears to leave the upper water column as fast-sinking fecal pellets in coastal waters. This study suggests that most egested matter from copepods is retained in the water column because (1) >50 % of fecal matter is released as small, slow-sinking particles that are not surrounded by a peritrophic membrane and (2) small fecal pellets sink slowly and are degraded rapidly. The production, appearance and fate of fecal material from the calanoid copepod *Acartia tonsa* (fed on 2 different phytoplankton species, the cryptophyte *Rhodomonas salina* and the diatom *Skeletonema costatum*) was followed in association with the grazing activity of the copepod in a laboratory experiment. For both diets, >50 % of the defecation was released as dispersed small (<10 µm) non-pellet-bound particles. The diatom was less suitable as a food item than the flagellate and led to a 3 times higher rate of grazing and egestion. Nevertheless, specific assimilation and egg production per female were 2 times higher for the *Rhodomonas* diet versus the *Skeletonema* diet. As a result, the total egestion comprised 18 % of the ingestion of *Rhodomonas* and 27 % of the *Skeletonema* ingestion. In terms of vertical loss, sinking rates for both types of fecal pellets were ca. 5 m d$^{-1}$ and, in terms of degradation, ca. 0.5 d$^{-1}$ (18°C) in the absence of copepods. Transferring these findings to similar neritic conditions suggests that 60 % of the fecal pellets from copepods will be recycled within a 15 m deep mixed layer and that >80 % of the total fecal matter can be expected to be retained when the unbound fecal material is also included.

KEY WORDS: Zooplankton · Grazing · Chlorophyll · Fecal pellets · Egestion · Assimilation · Degradation · Vertical flux

## INTRODUCTION

Whether copepods stimulate *in situ* mineralization or increase sinking rates of C, N, P has been questioned over the past few years (Wassmann 1998, Banse 2002). Fecal pellets have long been considered to be the main component of autochthonous matter in the vertical flux of stratified neritic waters (cf. Steele 1974). Copepods, like many other pelagic crustaceans, produce distinct fecal pellets surrounded by a peritrophic membrane. Fecal pellets are generally known to have much higher rates of sinking than phytoplankton cells (Smayda 1969, Bienfang 1980, Fowler & Knauer 1986). However, the relatively low contribution of recognizable fecal pellets in sediment traps deployed around world

coastal waters has challenged the role of fecal pellets in the downward transport of organic matter within the sea (Smetacek 1980, Bathmann et al. 1987, Peinert et al. 1989, Lampitt et al. 1990, Ayukai & Hattori 1992, Landry et al. 1994, Lane et al. 1994, Olesen & Lundsgaard 1995, Viitasalo et al. 1999, Wassmann et al. 1999, González et al. 2000).

The high sinking velocity and low proportion of fecal pellets in sediment traps have led to the assumption that fecal pellets degrade rapidly within the mixed layer. There have been many speculations for the reason, including bacterial degradation and leakage. In a theoretical paper, Jumars et al. (1989) suggested that most of the dissolved organic carbon (DOC) in fecal pellets is lost to the surrounding water by diffusion on

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a minute time scale. There is, however, disagreement in the literature as to the relative importance of the different mechanisms. Leakage from fecal pellets has been reported as the major flux of dissolved organic matter from zooplankton (Riemann et al. 1986, Hygum et al. 1997), while other studies report no such loss (Tande & Slagstad 1985, Strom et al. 1997, Xu & Wang 2003). By measuring the DOC production relative to newly voided pellets from calanoid copepods, Møller et al. (2003) found that pellets lose 28% of their carbon within the first hour and apparently without alteration of shape. While following the decay of fecal pellets from copepods fed with different common phytoplankton species at natural food concentrations, Hansen et al. (1996) found degradation rates between only 0.03 and 0.3 d$^{-1}$ at 18°C. However, even if a degradation rate of 0.3 d$^{-1}$ applies to natural conditions, this would not prevent the majority of fecal pellet matter from sinking out of the mixed layer. Alternative mechanisms must, therefore, be considered. Feeding on fecal pellets (coprophagy) may contribute to retaining material in the mixed layer because fecal pellets can either be reingested or destroyed by zooplankton (Johannes & Satomi 1967, Gonzáles & Smetacek 1994), but so far no thorough evidence that this mechanism is decisive for the retention of matter has been given (Turner 2002).

All the proposed mechanisms, involving the degradation of fecal pellets, do not satisfactorily explain why only a small fraction of copepod fecal material appears to leave the mixed layer as sinking pellets. We have, therefore, performed a laboratory experiment that is designed to follow the relationship between copepods feeding on different unialgal diets and their association with the egestion, degradation and sinking of fecal material.

## MATERIALS AND METHODS

**Food and copepod cultures.** We used the common and widely distributed calanoid copepod *Acartia tonsa*. The copepods were offered 2 kinds of different diets: the cryptomonad *Rhodomonas salina*, an autotrophic nanoflagellate, and *Skeletonema costatum*, a chain-forming diatom. Cohorts of adult *A. tonsa* were obtained by hatching several thousand eggs from a laboratory culture 4 wk before the start of any experimentation. For the first 2 wk, all nauplii were fed on *Rhodomonas*. Thereafter, half of the copepods were fed *Skeletonema*, while the other half remained on the *Rhodomonas* diet. Female copepods constituted approximately one third of the cohort population. Phytoplankton was added once per day until a slight coloration of the water (just visible to the naked eye) was apparent; this corresponds to a concentration

between 10 and 20 µg chlorophyll *a* l$^{-1}$. The temperature was 16 to 18°C and the salinity was 28 PSU during the experiments. The investigations were divided into 2 parts: an enclosure and a tracer study. Statistical analyses were performed using simple regression analyses. Linear and curved relationships were described with Excel 2002 for Windows.

**Enclosure experiment.** The first part of the study was performed as a week-long enclosure experiment consisting of four 100 l (1 m height) containers in which homogeneous mixing and deployment of sediment traps was made possible. The purpose was to follow egg production and egestion of fecal pellets from copepods relative to the sinking of the different constituents and on differences in diet. The 2 phytoplankton species were added to a final concentration of 10 to 20 µg chlorophyll *a* l$^{-1}$ in each of 2 containers to ensure food-saturating conditions for the copepods. The light was dimmed to reduce the growth rate of phytoplankton during the experiment. Ca. 50 copepods l$^{-1}$ were added to one of the containers containing *Rhodomonas* (R+) and *Skeletonema* (S+). Two controls (i.e. without copepods) were called R– and S– respectively. Moderate air bubbling was applied in all 4 containers. After 72 h, a new batch of algae, similar to the initial amount, was added to all containers. Concentrations of chlorophyll *a* (chl *a*), pheopigment (Pheo), particulate organic carbon (POC) and nitrogen (PON) were measured twice per day. Copepods (male and female), eggs and fecal pellets were enumerated simultaneously. Sedimentation of pellets, eggs, POC, PON, chl *a* and Pheo were measured every second day. Bacterial production was measured once per day.

A homogenous distribution of particles in the containers with copepods was obtained with a vertically oscillating disc. The disc, 18 cm in diameter with holes corresponding to a solid stroke area of 188 cm$^2$, was attached to an adjustable electromotor via a stainless steel frame. The grid moved up and down in the lower half of the container with a stroke length of 32 cm. We employed an oscillation frequency of 10 rpm creating enough turbulent energy to keep the produced eggs and fecal pellets homogenously suspended in the water column. The level of turbulent kinetic energy in the containers, expressed as the average dissipation rate, could be calculated following Peters & Gross (1994) to be ca. 0.3 cm$^2$ s$^{-3}$. The arrangement yielded a turbulent dissipation rate that corresponded to turbulence generated in the upper mixed layer by moderate winds (Kiørboe & Saiz 1995)

Chl *a* and Pheo were measured on subsamples from the water and sediment traps by filtration on Whatmann GF/C filters and extraction in 96% ethanol. Trapping of copepods in the filters was avoided by prescreening the subsamples through a 180 µm screen.

Samples were frozen (–18°C) and stored for a maximum of 2 wk. The extract was centrifuged (4000 × *g*) and measured on a Pharmacia LKB Ultrospec + spectrophotometer at 665 nm before and after acidification with 100 ml of 1 M HCl per 3 ml of extract. Concentrations of chl *a* and Pheo were calculated according to Lorenzen (1967) using an absorption coefficient of 83.4 l cm$^{-1}$ g$^{-1}$ for chl *a* in 96 % (v/v) ethanol (Wintermans & DeMots 1965). Ingestion rates were calculated from the change in chl *a* concentration between the control and grazer enclosures. The degradation of chlorophyll was estimated by comparing the disappearance of chl *a* with the appearance of pheopigments according to Shuman & Lorenzen (1975).

For measuring carbon (POC) and nitrogen (PON) content of the particles in water and sediment traps, a pre-screened subsample (180 μm) was filtered onto combusted (450°C) 13 mm Whatmann GF/F filters and stored at –18°C. Filters were dehydrated at 60°C for 24 h before analysis using a Carlo Erba CHN-elemental analyzer (model 240 C).

Samples of fecal pellets, eggs and copepods, from both sediment traps (200 ml) and ambient water (500 ml), were preserved with acid Lugol's solution and counted and measured using a stereo microscope (40× magnification, Olympus SZ40). Subsamples were analyzed until 100 pellets from each diet type were measured. Intact and broken pellets were enumerated separately. The volume of pellets was calculated based on the length and width of intact pellets assuming a cylindrical shape. Estimates of the carbon content of the fecal pellets were obtained by assuming a carbon:volume ratio of 0.085 pg C μm$^{-3}$ (Besiktepe & Dam 2002).

Sinking rates were measured with sediment traps deployed 3 times for ca. 4 h during the enclosure experiment. The arrangement of the trap consisted of 2 acrylic cylinders fixed in a vertical position mid-way between the bottom and surface of the containers on thin stainless shafts. The cylinders were 5.2 cm in diameter and 33 cm high (aspect ratio = 6.3). The mouth of the cylinders was above the upper position of the oscillating disk. Neither salt to increase the density of the trap water content nor preservatives were added. Pilot experiments using the same design, but with dye in the 2 trap cylinders, showed that the bottom water layer of the cylinders remained stable and unmixed for more than 6 h of deployment, while the surrounding water was efficiently mixed (the dye was transferred to the bottom of the cylinders after being placed in the containers through a thin silicone tube mounted to a syringe). Sinking flux (*S* [cm$^{-2}$ h$^{-1}$]) was calculated as $S = (c_s - c_w)V/(At)$, where $c_s$ is the biomass concentration (cm$^{-3}$) in the sediment trap, $c_w$ the average concentration (cm$^{-3}$) in the ambient water of

the container during deployment, *V* the resuspended volume (cm$^3$) of the sediment trap sample, *A* the cross section area of the sediment trap (cm$^2$) and *t* the deployment time. The sinking velocity (*W* [m d$^{-1}$]) was calculated as $W = S/c_w$.

**Tracer experiment.** The second part of the study was conducted as a tracer experiment and followed the fate of labeled phytoplankton that were offered as food. Inocula of *Skeletonema costatum* and *Rhodomonas salina* were grown for 6 to 8 doublings in 10 l B1-medium with silicate (Hansen 1989) to which $3.7 \times 10^6$ Bq of NaH$^{14}$CO$_3$ were added. The algae were incubated at an irradiance of about 200 μmol m$^{-2}$ s$^{-1}$ for 3 to 4 d and to a final concentration of 15 to 30 μg chl l$^{-1}$. Four liters of each of the labeled algae were twice transferred to 5 l beakers and placed in dim light with gentle air bubbling for 2 h before the start of the experiment. At the same time, adults of *Acartia tonsa* were transferred to 4 semi-closed acrylic cylinders with a false bottom (made of 240 μm plankton netting) and submerged into 5 l beakers filled with 0.22 μm-filtered seawater without food. The number of copepods used for the experiment was 840 l$^{-1}$ for the *Rhodomonas* diet and 141 l$^{-1}$ for the *Skeletonema* diet. Temperature was held at 17°C during the whole experiment.

***Step 1 — Phytoplankton growth:*** The development of labeled algae was first followed for 1 h with no grazers by frequent sampling of approximately 50 ml aliquots. An open acrylic cylinder (without net) was placed in the beaker with labeled algae to create the same light conditions as the subsequent grazing experiment. The radioactivity of the algae was measured on 8 ml subsamples, which were either filtered through a 5 μm nylon filter for *Skeletonema* and a GF/C filter for *Rhodomonas* or transferred directly to scintillation vials for the measurement of total labeled matter ('Total' in Fig. 2). The filters were rinsed several times with 0.22 μm-filtered seawater. Inorganic $^{14}$C was removed from the filters in a fume of concentrated HCl for 1 h before the filters were placed into scintillation vials and 10 ml of scintillation cocktail (Packard Ultima Gold) added. Inorganic carbon in the 8 ml total fraction was removed by adding 1 ml of 1N HCl and the vials were left for 24 h without lids before the addition of 10 ml Packard Insta-Gel. All vials were counted in a liquid scintillation analyzer (Packard 1500) approximately 12 h after the scintillator was added. Chlorophyll was measured at the start and at the end as described above.

***Step 2 — Zooplankton grazing:*** The open cylinders of Step 1 were replaced with cylinders containing copepods enclosed by 240 μm netting. The copepods were allowed to feed on the labeled phytoplankton for 1 to 2 h. To follow the change in concentration as the animals grazed, aliquots of 50 ml were taken every

10 min with a syringe mounted with a 240 µm net to exclude zooplankton. The subsamples were treated in the same way as in Step 1.

**Step 3—Defecation:** The cylinders with copepods were removed and carefully rinsed in 2 beakers with clean sea water to remove any labeled material not incorporated by the copepods. The cylinders were then placed in new beakers containing 4 l of unlabeled phytoplankton to allow the animals to empty their guts of any labeled material. After 1 h, cylinders with copepods were removed, leaving the labeled egested matter <240 µm in the beaker.

**Step 4A—Fecal matter production and degradation:** The labeled fecal matter was gently resuspended and evenly distributed in twelve 300 ml bottles. Fresh copepods (10 to 12 individuals) were added to half of the bottles. The remaining amount (ca. 400 ml) was preserved with Lugol's for microscopical counting. Two of the bottles were used immediately for measuring labeled carbon in the fractions >10 µm and <10 µm ($t = 0$). The fraction >10 µm was sieved gently by gravity filtration through a 15 µm circular Nitex mesh net, mounted in a filter holder. The <10 µm particulate fraction consisted of the filtrate retained on a GF/C filter. Glass fiber filters may have absorbed some dissolved organic matter too but probably only a minor fraction of the total volume filtrated. Søndergaard & Middelboe (1993) measured that a maximum of 3% of the filtrated DOC are retained by 13 mm Whatman

GF/F filters. The 2 × 5 other bottles were placed on a rotating plankton wheel (1 rpm) for later measurements of the same 2 fractions ($t = 1, 3, 8, 20$ and 50 h). Temperature was held at 16 to 17 °C during this step.

**Step 4B—Assimilation:** The copepods used in the tracer experiment were allowed another 30 min in clean sea water and counted. The dead copepods were removed and the living animals were collected on a 240 µm circular screen for determination of incorporated $^{14}C$.

## RESULTS

### Enclosure experiment

*Effect of grazing on particulate organic matter*

Chl *a* development differed significantly in the containers with and without copepods (Fig. 1). The slopes of the regression lines between the first (0 to 72 h) and second (76 to 172 h) time period were not statistically different (p > 0.1, 2-tailed *t*-test) and the results were, therefore, pooled (Table 1). Differences between the regression line slopes for the enclosure with copepods and the respective control were highly significant (p < 0.01, 2-tailed *t*-test), and revealed a gross removal rate of chl *a* in the presence of copepods of 0.154 µg chl l⁻¹ h⁻¹ (SE ± 0.048) for the *Rhodomonas* diet and of 0.104 µg chl l⁻¹ h⁻¹ (SE ± 0.050) for the *Skeletonema* diet. Using the C/chl conversion factors found (Table 2), this corresponds to a specific grazing of 76.7 ng C cop⁻¹ h⁻¹ (SE ± 23.8) for *Rhodomonas* and 76.5 ng C cop⁻¹ h⁻¹ (SE ± 25.0) for *Skeletonema*.

The Pheo concentration remained close to zero in containers without copepods (Table 1). The acidification factors were 1.70 (SE = ±0.01) and 1.68 (SE = ±0.02) for R– and S– respectively. In both containers with copepods, Pheo concentration gradually increased as the acidification factor decreased from an initial value of 1.7 to 1.3 at the end of the experiment. Since the regression coefficient for the controls did not significantly differ from zero (p > 0.1, 2-tailed *t*-test), the regression-line slope for Pheo development in the presence of copepods is taken as the Pheo production rate (Table 1). Pheo concentration was, therefore, found to increase by 0.132 µg l⁻¹ h⁻¹ in the container with *Rhodomonas* and by 0.066 µg l⁻¹ h⁻¹ in the *Skeletonema* container. Values of Pheo are given in terms of the equivalent weight of chl *a*.
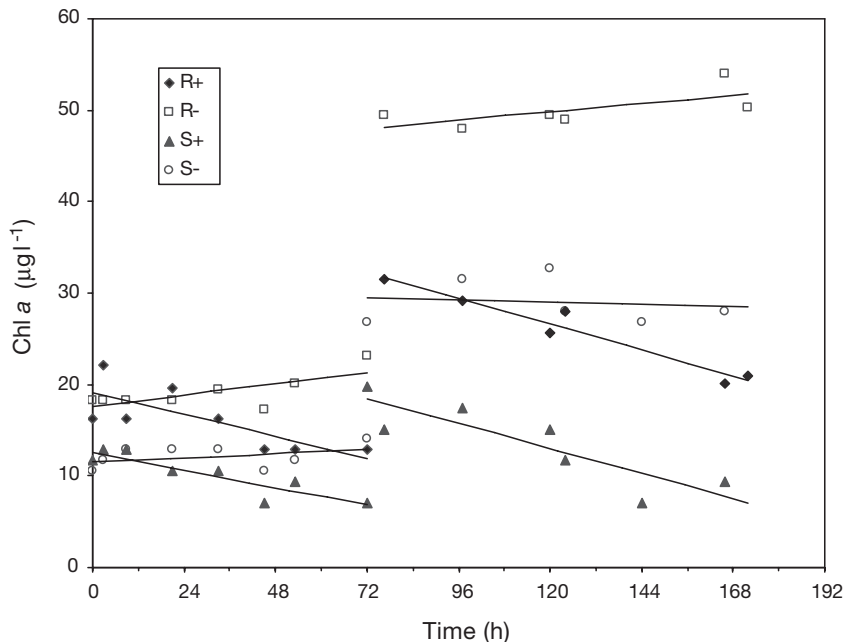


Fig. 1. Chl *a* development in enclosures with *Rhodomonas salina* (R) and *Skeletonema costatum* (S). R+ and S+ are enclosures with copepods, while R– and S– are controls without copepods. A new portion of cells was added after 72 h

Table 1. Slopes of the regression lines for the chlorophyll *a* (chl) and pheopigment (Pheo) development in the enclosures (Fig. 1). R+ and R–, *Rhodomonas salina* enclosures with (R+) or without (R–) copepods. S+ and S–, *Skeletonema costatum* enclosures with (S+) or without (S–) copepods. Standard error (SE) of the estimated slope coefficients and total numbers of observations (n) are shown. Avg: average regression line slopes for chl development

|  | R+ ($\mu$g l$^{-1}$ h$^{-1}$) | R– ($\mu$g l$^{-1}$ h$^{-1}$) | S+ ($\mu$g l$^{-1}$ h$^{-1}$) | S– ($\mu$g l$^{-1}$ h$^{-1}$) |
|---|---|---|---|---|
| n | 14 | 14 | 15 | 14 |
| Chl 0 to 72 h | −0.100 ± 0.035 | 0.052 ± 0.020 | −0.080 ± 0.016 | 0.018 ± 0.018 |
| Chl 76 to 172 h | −0.118 ± 0.014 | 0.037 ± 0.021 | −0.119 ± 0.026 | −0.010 ± 0.035 |
| Avg | −0.109 ± 0.038 | 0.045 ± 0.029 | −0.100 ± 0.031 | 0.004 ± 0.039 |
| Pheo 0 to 172 h | 0.132 ± 0.020 | 0.017 ± 0.010 | 0.066 ± 0.010 | 0.006 ± 0.008 |

Table 2. Slope coefficients for the regression lines of particulate carbon (C) versus particulate nitrogen (N) and particulate carbon (C) versus chlorophyll *a* (chl) in the control enclosures with *Rhodomonas salina* (R–) and *Skeletonema costatum* (S–). Values in weight/weight. Standard error (SE) of the coefficients and number of observations (n) are shown

|  | R– | S– |
|---|---|---|
| C/N | 4.7 ± 0.2 (15) | 5.0 ± 0.3 (15) |
| C/chl | 26.9 ± 1.2 (14) | 39.0 ± 0.8 (14) |

POC and PON developed similarly to chl *a*. Linear regression coefficients between POC and chl *a* in the controls revealed a C/chl weight-based ratio of 26.9 for *Rhodomonas* and 39.0 for *Skeletonema* (Table 2). In the R+ and S+ containers, this relationship was less pronounced and was likely due to the presence of fecal matter and other debris from the copepod activity. Furthermore, the C:N ratio showed a noticeable difference in nitrogen content between the 2 algal species (Table 2).

### Production and sinking of fecal pellets and eggs

The number of fecal pellets and eggs in the enclosures increased asymptotically with time, which was probably due to increased significance in density-dependent changes (hatching and decomposition). The fecal and egg production rates were, therefore, calculated from the initial slope of the increment of fecal pellets and eggs (Table 3). Egestion and egg production showed remarkable differences that were dependent on diet. Copepods that were fed diatoms produced, on average, 3.5 fecal pellets h$^{-1}$ but only 1.0 h$^{-1}$ when fed with flagellates. The volume of the *Skeletonema*-based fecal pellets was almost 50% larger than pellets produced on the *Rhodomonas* diet (Table 4).

Egg production showed an opposite trend to pellet production: 0.25 eggs female$^{-1}$ h$^{-1}$ on the *Skeletonema*

Table 3. Specific production of fecal pellets, eggs and nauplii (mean ± SE) in enclosures with diet of *Rhodomonas salina* (R+) and *Skeletonema costatum* (S+). cop: coppepods; n: number of observations

|  | R+ | n | S+ | n |
|---|---|---|---|---|
| Fecal pellet production |  |  |  |  |
| (pellets cop$^{-1}$ h$^{-1}$) | 0.96 ± 0.02 |  | 3.45 ± 0.42 |  |
| ($\mu$g C cop$^{-1}$ h$^{-1}$) | 0.0043 ± 0.0001 | 8 | 0.0227 ± 0.0027 | 6 |
| Egg production |  |  |  |  |
| (eggs female$^{-1}$ h$^{-1}$) | 0.56 ± 0.04 | 9 | 0.25 ± 0.05 | 9 |
| Hatching |  |  |  |  |
| (nauplii female$^{-1}$ h$^{-1}$) | 0.19 ± 0.02 | 9 | 0.06 ± 0.01 | 6 |

Table 4. Size and volume (mean ± SE) of newly egested fecal pellets from *Acartia tonsa* fed on *Rhodomonas salina* and *Skeletonema costatum*

| Fecal pellets | *R. salina* | *S. costatum* |
|---|---|---|
| Measured | 93 | 83 |
| Length ($\mu$m) | 102 ± 2.9 | 117 ± 3.8 |
| Width ($\mu$m) | 24 ± 0.7 | 28 ± 0.5 |
| Volume ($\times 10^5$ $\mu$m$^3$) | 0.52 ± 0.04 | 0.77 ± 0.05 |

diet and 0.56 eggs h$^{-1}$ when fed on *Rhodomonas* (Table 3). The ratio between nauplii recruitment and egg production rates, an expression of hatching success, was found to be 34% for the *Rhodomonas*-fed copepods and 25% with the *Skeletonema* diet.

Despite differences in size, the sinking rates of the 2 types of fecal pellets were similar with average sinking velocities of 4.5 and 5.1 m d$^{-1}$ for pellets based on the *Skeletonema* and *Rhodomonas* diets, respectively (Table 5). Sinking rates for eggs, chl *a*, and Pheo did not show any difference between the 2 types of food (Table 5). For POC and PON, there was an insignificant tendency towards a relatively higher rate of sinking in the R+ container when compared to the S+ container (Table 5).

Table 5. Sinking rates (mean ± SE) of different entities from enclosures with *Rhodomonas salina* (R+) and *Skeletonema costatum* (S+). TPC: total particulate carbon; PON: particulate organic nitrogen

|  | R+ | S+ |
|---|---|---|
| Fecal pellet (m d$^{-1}$) | 5.1 ± 0.3 | 4.5 ± 0.5 |
| Egg (m d$^{-1}$) | 11.2 ± 2.3 | 9.1 ± 1.7 |
| Chl *a* (m d$^{-1}$) | 0.56 ± 0.31 | 0.57 ± 0.12 |
| Pheopigment (m d$^{-1}$) | 1.85 ± 0.41 | 2.25 ± 0.37 |
| TPC (m d$^{-1}$) | 0.59 ± 0.14 | 0.22 ± 0.05 |
| PON (m d$^{-1}$) | 0.65 ± 0.10 | 0.15 ± 0.03 |

## Tracer experiment

### Grazing and ingestion

The specific activity achieved for the phytoplankton food in the tracer experiment was 1081 dpm µg C$^{-1}$ for the radio-labeled *Rhodomonas* cells and 2165 dpm µg C$^{-1}$ for the *Skeletonema* cells. The carbon content of phytoplankton was estimated from the initial chl *a* concentration by applying the C/chl conversion factor from the enclosure experiment (Table 2). The difference between the rates of change in concentration of labeled material before (Step 1) and after addition of copepods (Step 2) represents the removal rate of phytoplankton by zooplankton (Fig. 2). Thus, by subtracting the slope of the linear regression of labeled phytoplankton versus time before and after addition of copepods, grazing could be estimated. The differ-

ence between the 2 regression lines was significant for the *Rhodomonas* diet (p < 0.02, 2-tailed *t*-test) but, due to few control measurements being taken with *Skeletonema*, this difference was not significant for the *Skeletonema* diet (p > 0.1). Instead, the regression line for the data of both *Skeletonema* fractions without copepods was used as the rate of change in the control. A significance of p < 0.1 (2-tailed *t*-test) was then obtained between the slopes before and after addition of copepods. The removal of total labeled material by copepods (Step 2) corresponds to a grazing activity of 66 ng C cop$^{-1}$ h$^{-1}$ (SE ± 16) for the *Rhodomonas* cells and 237 ng C cop$^{-1}$ h$^{-1}$ (SE ± 131) for the *Skeletonema* chains. On the other hand, the removal rate of labeled *Skeletonema* cells (>5 µm fraction) was 396 ng C cop$^{-1}$ h$^{-1}$ (SE ± 150), which is almost twice the removal rate of total labeled material. The decline of *Rhodomonas* cells (GF/C fraction) was 83 ng C cop$^{-1}$ h$^{-1}$ (SE ± 9.1) and therefore, only slightly higher than the net reduction of total labeled material. The difference between the slopes of the regression lines representing the 2 fractions was not significant (p > 0.1, 2-tailed *t*-test). The greater removal of *Skeletonema* cells than total labeled material is probably the result of intact cells being transformed into small debris by copepod grazing. Whilst estimated grazing rates on *Rhodomonas* in the 2 types of experiments were similar, the grazing rate on *Skeletonema* (based on the removal rate of total labeled material in the tracer experiment) was found to be more than 5 times that of the enclosure experiment.
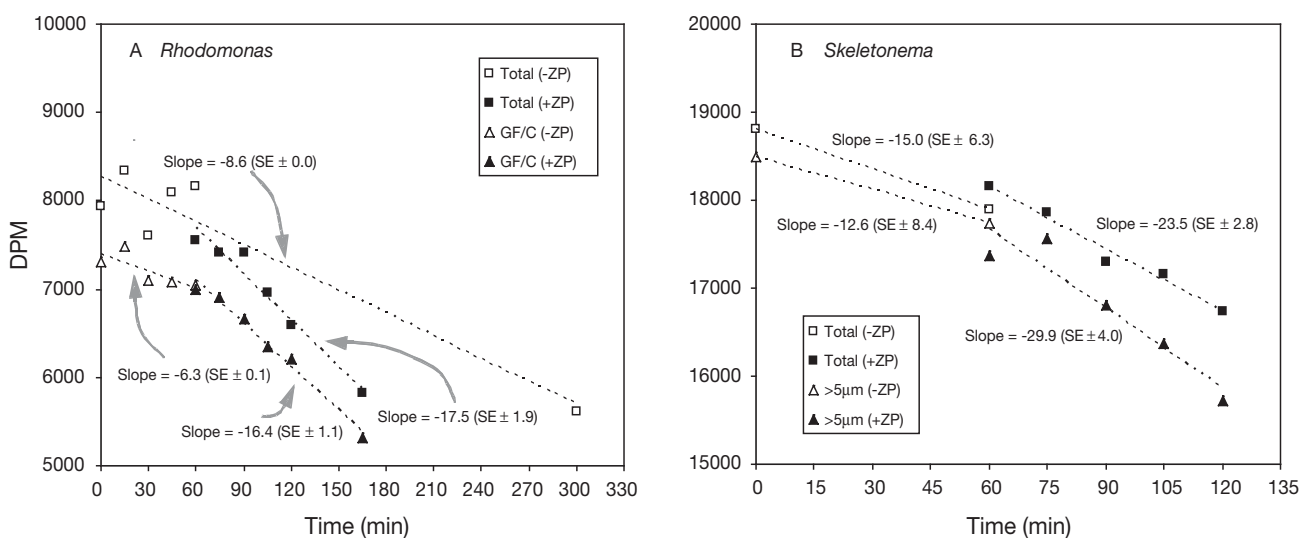


Fig. 2. Concentration of labeled cells of (A) *Rhodomonas salina* and (B) *Skeletonema costatum*, in terms of radioactive tracer (DPM), before (−ZP) and after addition of copepods (+ZP). Two subsamples of 8 ml prescreened for copepods were taken regularly: 1 containing all labeled material (Total) and 1 retained on a filter for mainly intact algal cells (>GF/C for *R. salina* and >5 µm for *S. costatum*)

### Retention and egestion

The incorporation of labeled carbon into copepods (Step 4B) fed on *Rhodomonas* corresponds to a retention of 19.3 ng C $cop^{-1}$ $h^{-1}$ (SE ± 1.0). The retention of carbon for the copepods fed on labeled *Skeletonema* cells was only 9.1 ng C $cop^{-1}$ $h^{-1}$ (SE ± 0.8). The labeled particulate matter collected within 1 h after defecation (Step 3) corresponded to a total particulate matter egestion rate of 11.8 ng C $cop^{-1}$ $h^{-1}$ (SE ± 2.0) for *Rhodomonas*-fed copepods and of 64 ng C $cop^{-1}$ $h^{-1}$ (SE ± 12.4) for *Skeletonema*-fed copepods (Fig. 3). The egestion of non-pellet matter corresponded to 8.0 ng C $cop^{-1}$ $h^{-1}$ (SE ± 1.5) and to 36 ng C $cop^{-1}$ $h^{-1}$ (SE ± 14.3) for copepods fed on *Rhodomonas* and *Skeletonema*, respectively. Thus, the fraction below 10 µm accounted for more than half of the total egested matter (i.e. 67 % for the *Rhodomonas* diet and 57 % for the *Skeletonema* diet). It was examined whether the fecal pellets are broken up when collected on 10 µm filters. Fecal pellets were produced over a 30 min period by *Rhodomonas*-fed *Acartia* in clean GF/C-filtered water. After the copepods were removed and the pellets allowed to settle, the supernatant was siphoned out and the bottom layer gently transferred to Petri dishes for counting under the microscope. This was repeated after filtration through a 10 µm nylon gauze. No change in the number of intact pellets was observed.

The degradation of fecal material was followed for 2 d with and without copepods present (Fig. 3). Assuming that the exponential curve fitted to the measured values expresses degradation, an instantaneous degradation rate of 0.036 $h^{-1}$ (SE ± 0.013) was found for fecal pellets in the presence of copepods for the *Rhodomonas* pellets and 0.023 $h^{-1}$ (SE ± 0.004) for the *Skeletonema* pellets. Without copepods, the rate of degradation was 0.027 (SE ± 0.009) and 0.016 $h^{-1}$ (SE ± 0.022) respectively. Although the degradation rate of fecal pellets seems faster in the presence of copepods, the difference was not statistically significant (p > 0.1, 2-tailed *t*-test). *Rhodomonas* pellets seem generally to degrade faster than *Skeletonema* pellets. However, these differences were also not significant (p < 0.1). There was no clear tendency for differences in the decay rate of non-pellet fecal matter (<10 µm fraction), probably because of a continuous supply of additional matter to the fraction of broken fecal pellets.
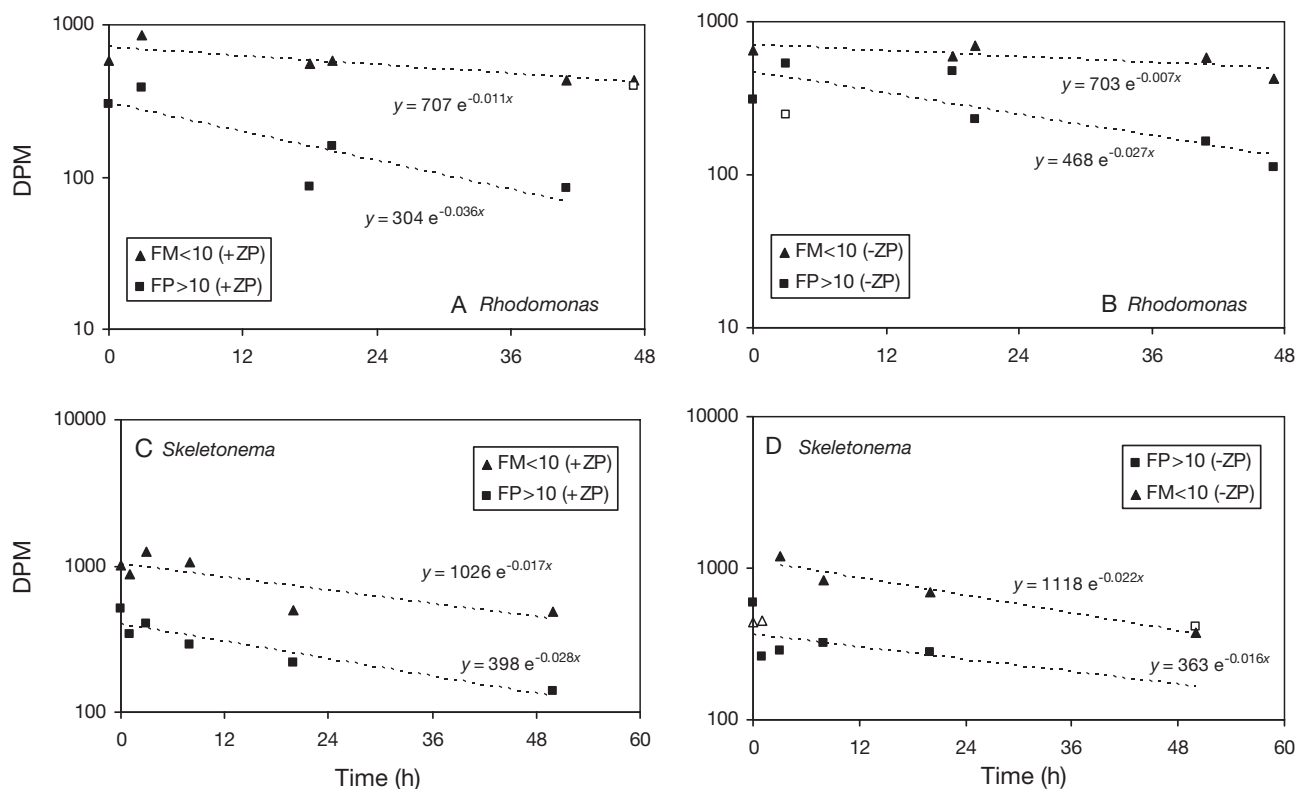


Fig. 3. Degradation of radioactive fecal matter produced from (A,B) *Rhodomonas salina* and (C,D) *Skeletonema costatum* by *Acartia tonsa* in terms of radioactive tracer (DPM). The degradation was followed in the presence (+ZP; A,C) and absence (–ZP; B,D) of starved unlabeled copepods. The fecal matter was divided into 2 size fractions: FP > 10 µm containing fecal pellets and FM < 10 µm containing non-pellet-sized egested matter. The fitted exponential decay rate constant, yielding the degradation rate, is shown for the solid data points (open symbols are outliers)

## DISCUSSION

### Egestion of fecal material

Similar differences in fecal pellet production between copepod fed on *Rhodomonas* and *Skeletonema* in the enclosure experiments (Table 3) were found in the tracer experiment. Assuming identical ingestion rates in the enclosure experiment as found in the tracer experiment, newly voided labeled pellets amounted to between 6 and 7% of *Rhodomonas* ingestion and 10 to 12% of *Skeletonema* ingestion in both experiments. The tracer experiment also revealed that fecal pellets comprised only one third of total feces loss for both types of food. The remaining two thirds consisted of small amorphous particles with no membrane. We examined whether these particles could possibly have resulted from a subsequent breakup of pellets due to mechanical handling of the egested material. We did not, however, find any difference in the number of intact pellets before and after filtration. Similarly, direct microscopic examination of particles released over a 30 min period by *Rhodomonas*-fed copepods in Petri dishes with GF/C-filtered water demonstrated that a substantial part of the defecation consisted of small dispersed particles. Finally, good agreement in the fecal pellet estimates from the 2 independent methods suggests that these small particles are indeed egested separately. To our knowledge, there have been no previous descriptions of fecal material egested from copepods in the form of small particles unbound by a peritrophic membrane. The formation of fecal pellets in calanoid copepods takes place in the posterior part of the midgut, while digestion takes place in the anterior part. In the case of fast gut clearance (e.g. when food is in excess, Kiørboe & Tiselius 1987), some of the fecal material could be egested without a surrounding membrane. This material may have been overlooked in the presence of large discrete fecal pellets, or even if recognized, misinterpreted as a product of fecal pellet break-down. Our results are consistent with recent bioenergetic studies on *Acartia*, in which production of fecal pellets was compared with ingestion (Besiktepe & Dam 2002, Xu & Wang 2003). Here, the defecation rates appeared low relative to ingestion. For instance, in Besiktepe & Dam (2002), the production of fecal pellets from *Acartia tonsa* fed on 5 different diets comprised less than 10% of the ingestion for 3 of the food items. This does not agree with the assimilation efficiencies, which rarely exceed 66% of ingestion (Kiørboe et al. 1985, Støttrup & Jensen 1989). Total egestion, relative to ingestion, was 18% for the *Rhodomonas* diet and 27% for the *Skeletonema* diet in the present study. These numbers agree with most reported assimilation efficiencies for species of *Acartia*

(Mauchline 1998). There is, therefore, indirect evidence supporting the present results, which show that up to 66% of the defecation from this genus may actually be egested as small particles not surrounded by a peritrophic membrane. As a consequence, less than half of the fecal material is egested as distinct pellet. Release of non-pellet debris for other pelagic non-copepod crustaceans has been reported for the shrimp *Palaemonates pugio* which, in addition to pellets, releases more than 50% of its fecal material in the form of DOC (Johannes & Satomi 1967).

### Ingestion and digestion based on chl *a* removal and Pheo production

The concentration of food in the experiments was high and *Acartia tonsa* was assumed to be well above saturation for the diet of both *Rhodomonas* and *Skeletonema* (Støttrup & Jensen 1990, Besiktepe & Dam 2002). The difference in the change of chl *a* between the enclosures, with and without zooplankton, can thus be interpreted as the rate of grazing under conditions of non-limiting feed. The net decrease in chl *a* concentration, expressed in terms of carbon per copepod, revealed grazing rates for *Rhodomonas* and *Skeletonema* that are in close agreement with other studies of *A. tonsa* feeding on chain-forming diatoms or small flagellates (Støttrup & Jensen 1990). Estimating the rate of grazing by means of chl *a* removal is based on an assumption that chl *a* is degraded in the gut of copepods. Another common method for estimating feeding is the gut fluorescence method, implying that chlorophyll pigments are not destroyed and that chl *a* is converted into pheopigments during copepod digestion (Shuman & Lorenzen 1975). However, it has now been recognized that pigments are, to some extent, destroyed during digestion (Dagg et al. 1989, Peterson et al. 1990, Head & Harris 1996, Goericke et al. 1999). Likewise, it cannot be excluded that some of the ingested chl *a* passes intact. It is, therefore, possible that ingestion rates based on changes in ambient chl *a* concentration represent underestimates.

The decrease in chl *a* and the increase in Pheo in the enclosure with copepods feeding on *Rhodomonas* revealed that 86% of the de-natured chl *a* is recovered as Pheo throughout passage through the gut. The difference between these 2 rates most likely expresses the degree to which chl *a* is degraded into non-fluorescent compounds. Assuming that this loss of pigment is entirely due to break-down of pigments during copepod digestion, a pigment destruction efficiency of 14% for copepods feeding on *Rhodomonas* is obtained. When *Skeletonema* was used as a diet, 63% of the chl *a* was recovered as pheopigments, which corre-

sponds to a destruction efficiency of 37%. This is in accordance with the 33 to 35% pigment break-down efficiency found by Shuman & Lorenzen (1975), Helling & Baars (1985) and Kiørboe & Tiselius (1987). The values are, however, somewhat lower than the measurements of Conover et al. (1986), who reported that 90 to 99% of the plant pigments were degraded to colorless residues during gut passage. Kiørboe & Tiselius (1987), who used the same species of copepod and food items as the current study, suspected that their number was too high due to a possible loss of pigments from feces, which might have escaped during their measurements. Using the same method as presented in this study but on *Calanus pacificus*, Dagg et al. (1997) obtained an average pigment destruction of 20%. However, because the data points were quite variable, this number is not significantly different from our findings.

The question as to whether intact algal chl *a* actually passes the gut can be clarified by comparing the grazing estimates from the 2 types of experiments. The ingestion rate for copepods feeding on *Rhodomonas*, obtained from the isotope experiment, fits well with the level found in the enclosure experiment indicating that most of the chl *a* in the case of *Rhodomonas* is either degraded into pheopigments or colorless compounds. The total removal of labeled material, when *Skeletonema* was used as food, suggests an ingestion rate that is 3 times the net loss of chl *a* in the enclosures. The rate of defecation was, however, quite similar in both types of experiment and suggests no difference in specific grazing activity. Assuming identical ingestion rates in the 2 experiments, a substantial part of the diatom chl *a* seems to pass the gut undigested. The literature on this subject does not always clearly distinguish between intact chl *a* and chl *a*-derived pigments when analyzing pigments from guts or fecal pellets of copepods. Indeed, Bathmann & Liebezeit (1986) found that fecal pellets, produced by copepods during a diatom bloom, contained high levels of chl *a* and relatively low levels of pheopigments. Scanning electron microscopic examination of their pellets showed that they contained a large amount of intact diatom cells, which explains the presence of non-degraded chl *a* in the pellets. The absence of recognizable debris in pellets from copepods, which have been grazing on small naked flagellates (Voss 1991), supports our finding that most chl *a* is degraded when *Rhodomonas* is used as food. Inefficient feeding (see next subsection) may imply a selective loss of tissues containing pigment which are not retained on a GF/C filter (Roy et al. 1989) and so the difference between our 2 grazing estimates can only be taken as a rough estimate of how much of the ingested chl *a* passes through the gut unconverted.

## Sloppy feeding

Another part of feeding that is not related to ingestion and digestion is the breakage of algal cells handled by the mouthparts of copepods (sloppy feeding, Lampert 1978). A measure of sloppy feeding was obtained by the difference between the removal of intact phytoplankton cells and the amount of labeled material finally retained by copepods (Fig. 2). The greater removal of labeled phytoplankton cells in comparison to the net reduction of total labeled material is likely due to inefficient ingestion. There was a 21% difference between the slope of the regression lines representing the 2 fractions of *Rhodomonas* consumption and a more noticeable difference of 40% for the *Skeletonema* feeding. This indicates that approximately 20% of the grazed *Rhodomonas* cells and 40% of the grazed *Skeletonema* cells were immediately lost as small particles or dissolved compounds to the surroundings. It is perhaps not surprising that sloppy feeding is more apparent on chains of *Skeletonema* versus single algal cells, which is probably attributed to differences in morphology between the 2 phytoplankton species. Indeed, loss caused by sloppy grazing on mainly large cells is well known (Conover et al. 1986, Roy et al. 1989). For example, inefficient feeding by *Acartia tonsa* on large phytoplankton has been reported by Møller & Nielsen (2001). Based on a bacterial response, these latter authors state that 50 to 70% of grazed net-plankton cells are released immediately into the surrounding water as labile DOC, but sloppy feeding seemed negligible with copepods feeding on small single cells such as *R. baltica.*

## Retention and egg production

The measured egg productions (i.e. 0.3 eggs female$^{-1}$ h$^{-1}$ for the *Skeletonema* diet and 0.6 eggs female$^{-1}$ h$^{-1}$ for the *Rhodomonas* diet) are within the range typical for *Acartia tonsa*. Maximum recorded egg production for this species is approx. 2 eggs per female$^{-1}$ h$^{-1}$ in both cultures (Kiørboe et al. 1985) and under natural conditions (Hazzard & Kneppel 2003). A less efficient production of eggs when *Acartia* feeds on diatoms has previously been reported. Applying a carbon content of 45.7 ng C egg$^{-1}$ (Kiørboe et al. 1985), carbon egg production was 25.6 ng C female$^{-1}$ h$^{-1}$ for copepods feeding on *Rhodomonas* and 11.4 ng C female$^{-1}$ h$^{-1}$ when *Skeletonema* was used as food. Adult copepods exhibit almost no somatic growth and so it is expected that egg production will reflect the assimilated energy. A retention of assimilated carbon of 19.3 and 9.1 ng C copepod$^{-1}$ h$^{-1}$ for the *Rhodomonas* diet and *Skeletonema* diet, respectively, were obtained

from the radiotracer experiments, and are a bit lower than the estimated egg-production rates. However, the rates still reflect the same trend with twice as high assimilation efficiencies when copepods feed on *Rhodomonas* versus *Skeletonema*. Apart from other methodological reasons, the lower retention rate may result from a presence of males in the experiment because males are expected to respire or excrete a larger fraction of the assimilated energy than females. Comparing the retention with ingestion of labeled phytoplankton reveals an overall gross efficiency of 29% when feeding on *Rhodomonas* but only of 4% when feeding on *Skeletonema*. *Skeletonema* is obviously a less nutritious diet than *Rhodomonas* and is likely the reason for the higher egg hatching viability for *Rhodomonas* (34%) than *Skeletonema* (25%). The copepods, however, may partly compensate for the lower nutritious value of *Skeletonema* by increasing their maximal rate of grazing.

Although *Acartia* willingly ingest *Skeletonema costatum*, some diatoms have recently been proven to be a less optimal food source for copepods. The lipid content of the diatoms seems to be 1 important reason (cf. Hazzard & Kneppel 2003), but also the production of certain unsaturated aldehydes, serving as a chemical defense when diatoms are disrupted by grazing, may harm the development of copepods (Pohnert et al. 2002). The noticeable passage of intact chl *a* through the gut is seen in this study when *Skeletonema* is

offered as food and also suggests *Skeletonema* cells are partly indigestible. This may be the result of a siliceous skeleton that reduces assimilation efficiency and consequently increases the relative rate of egestion. Higher carbon to nitrogen ratios in *Skeletonema* (5.0) than *Rhodomonas* (4.7, Table 2) also suggests that the former is of a lower food, which will tend to lower the retention in the gut of the ingested *Skeletonema* cells in comparison to the *Rhodomonas* cells. In summary, the high sloppy feeding in combination with the low assimilation efficiency makes the yield for the copepod of every grazed *Skeletonema* cell in terms of carbon about 10 times lower than that of *Rhodomonas*.

### Degradation of fecal matter

Hansen et al. (1996) found faster rates of degradation for flagellate-based fecal pellets from copepods than for diatom-based fecal pellets and suggest that the latter are less suitable for bacterial growth. They used the diatom *Thalassiosira weissflogii* and the nanoflagellate *Rhodomonas baltica* for food. However, at approximately the same temperature (18°C versus 16 to 18°C) and food concentration as in the present study, they found a more than 10 times slower rate of degradation for pellets produced on a diet of *Thalassiosira* than our findings on the *Skeletonema*-based pellets. For the *Rhodomonas*-based pellets, there is a better agreement between the 2 studies. There seems to be a strong proportionality between the surface:volume ratios of the fecal pellets and the degradation rates found in the 2 studies (Fig. 4). Although there is a mismatch between the volume calculation and the specification of the length and width of the pellets based on *T. weissflogii* in Hansen et al. (1996), the data suggest a close dependence of fecal matter degradation with the surface:volume ratio. Neglecting the data point on the *Thalassiosira*-based pellets, a strong exponential relationship was achieved (Fig. 4).

The significantly slower decay rates of the non-pellet fraction than of intact pellets suggest that the degradation of fecal pellets was not associated with simultaneous microbial decomposition. Bacterial production (data not shown) almost doubled in the enclosure with copepods, but far from an extent that would match the degradation rate of fecal pellets found in the tracer experiment. Fecal pellets, when copepods are absent, are likely to first disintegrate into tiny particles and then to be released into the surrounding water before being decomposed by bacteria. Such fragmentation is probably the main reason for the slower resulting decay rate of the non-pellet fraction.

Although the differences were not statistically significant, degradation of fecal pellets in the presence of
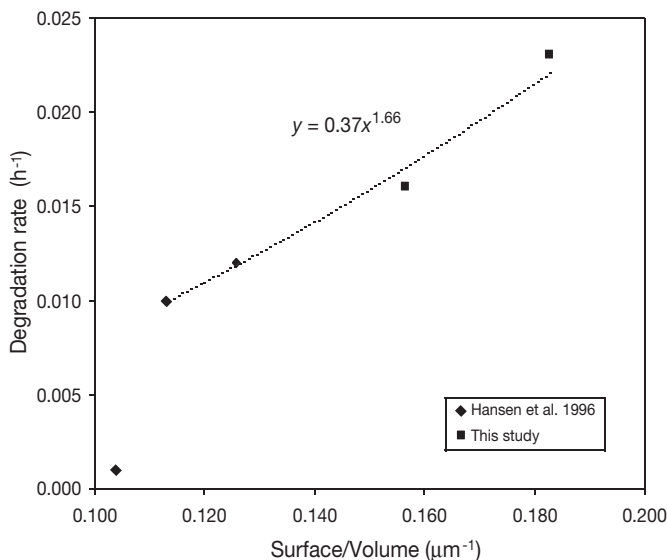


Fig. 4. Degradation rates versus surface:volume ratio of fecal pellets produced by *Acartia tonsa* on different unialgal diets: the diatoms *Skeletonema costatum* and *Thalassiosira weissflogii*, the nanoflagellates *Rhodomonas salina* and *R. baltica*, and the dinoflagellate *Heterocapsa triquetra*. Data are from this study and Hansen et al. (1996). The formula is given for the power curve fitted to the data without *T. weissflogii*

copepods was almost 50% higher (Fig. 3). This is likely due to 'tearing' during feeding and ingestion of the pellets by the animals. Re-egestion of this material into new fecal pellets may, however, have masked the influence of coprophagy in the present study. The behavior of breaking up their own pellets has previously been reported in several studies (cf. Turner 2002), but the precise effect on the degradation rate seems to vary a lot depending on feeding conditions and species composition. Increasing evidence from many costal waters indicates that fecal pellets from copepods are recycled rapidly in the water column but have often been explained as coprophagy.

## Ecological implications

Sinking rates of fecal pellets of ~5 m d$^{-1}$ seem to be low compared to other studies (cf. Turner 2002 and references therein) but so is the size of the pellets (cf. Mauchline 1998 and references therein). Converting our measure of pellet volume to an equivalent spherical diameter and using Stokes' Law, a density of 1.097 g cm$^{-3}$ for *Skeletonema* pellets and of 1.113 g cm$^{-3}$ for the *Rhodomonas* pellets can be calculated. Whilst assuming a cylindrical shape as comparable to a sphere, the estimated densities are within the range presented by Besiktepe & Dam (2002). Considering a mixed layer of 15 m, a sinking velocity of 5 m d$^{-1}$ corresponds to a 33% loss of the fecal pellets across the pycnocline per day. When a degradation rate of around 50% per day is also considered, then 40% of fecal pellets produced will leave the mixed layer as sinking material; the larger part (60%) will be degraded within the mixed layer. Taking the production of non-pellet fecal-material into account, an even larger part of the egested material is expected to degrade before it sinks out of the euphotic zone, because these small particles presumably sink very slowly. If this material comprises two thirds of the total egestion, up to 90% of the fecal material from copepods such as *Acartia* will be decomposed in the mixed layer. By comparing the 'potential fecal pellet production' determined from the abundance of copepods, mainly *Acartia* spp., Viitasalo et al. (1999) calculated that, in the Baltic, >99% of the fecal matter was recycled in the upper water column. The explanation for such high retention rates is that egested matter released from copepods is in the form of dispersed non-pellet bound particles.

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