

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

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

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

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INTRODUCTION

Fecal pellets in the sea are degraded by bacteria (Cho & Azam 1988) and possibly by zooplankton such as copepods (Paffenhöfer & Strickland 1970). Copepods degrade pellets through coprophagy (ingestion of pellets), coprorhexy (fragmentation of pellets), and coprochaly (loosening of pellets) (Paffenhöfer & Strickland 1970, Lampitt et al. 1990, Noji et al. 1991). Coprophagy is the best documented of the degradation processes. Paffenhöfer & Strickland (1970) observed

Calanus helgolandicus ingesting fecal pellets even in the presence of algal food. This ability was also shown for *C. helgolandicus* nauplii (Green et al. 1992). Further, grazing rates of fecal pellets have been obtained for many other species of copepods; for example, *Acartia omorii* ingests fecal pellets at high rates (Viitasalo et al. 1999), and *Eucalanus pileatus* consumes nauplii fecal pellets at the same rate as algae of similar size (Paffenhöfer & Knowles 1979). Coprorhexy was suggested by Lampitt et al. (1990), who observed copepods to be highly adept at breaking up their own fecal

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

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

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

MATERIALS AND METHODS

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

Zooplankton. *Calanus helgolandicus* females were collected in October 2004 and August 2005 in the north-eastern North Sea and kept in culture in filtered (0.2 μm) seawater (~33‰) at 14°C, reared on a mixed diet of *Rhodomonas salina*, *Thalassiosira weissflogii*,

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

Grazing experiments. Fecal pellet clearance rates and ingestion rates of adult females were determined in both the presence and absence of alternative food (Table 1). Incubations were run for 24 h, in a 12:12 h light:dark cycle, and on a plankton wheel rotating at ~1 rpm. This rotation speed was chosen to keep the pellets suspended and to have minimum effect on the copepods. Ploug et al. (in press) found sinking velocities of 28 \pm 3 m d^{-1} for similar sized pellets produced by *Temora longicornis* feeding on *Rhodomonas salina*. At a rotation speed of 1 rpm, the experimental bottles (8 cm diameter) were in each position (upright, sideways, and upside down) for ~15 s. The pellets sank 0.5 \pm 0.005 cm during 15 s; therefore, only pellets near the side of the vessel at the beginning of the experiment had their motion affected during incubation. When an alternative food source was present, it was always at a concentration of 3 \times 10⁶ cells l^{-1} to ensure the presence of alternative food during the whole incubation. A wide range of pellet concentrations was used to determine the functional response in pellet clearance to pellet concentration (Table 1). Only pellet concentrations <2 pellets ml^{-1} represent pellet concentrations found *in situ*. The pellets offered as food were distinguished from the pellets produced during the experiments due to a visually recognizable size difference. However, when offering *Pseudocalanus elongatus* fecal pellets together with an alternative food

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

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

^aSame pellet concentration both with and without algae

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

Behavior experiments. Grazing behavior was investigated by filming adult females feeding on a range of fecal pellet concentrations in the presence and absence of alternative food (Table 1). Filming was conducted in a cylindrical aquarium (*Calanus helgolandicus*: 10 cm diameter, 7.9 cm depth; *Oithona similis* and *Pseudocalanus elongatus*: 7.9 cm diameter, 7.9 cm depth). The aquarium was placed on a rolling table that was rotated at ~1 rpm to keep fecal pellets suspended. The rotation of the aquarium opposed the sinking velocity of the pellets, and they were not near the side of the rotating aquarium. The filming equipment consisted of a CCD video camera (Mintron MTV-1802CB) equipped with a 105 mm lens (Nikon Micro Nikkor 1:2.8) and connected to a video cassette recorder, a time-code generator, and a monitor. Infrared illumination was provided from behind by a light-emitting diode (LED), which was collimated through a condenser. Females were acclimated to the fecal pellet concentration in the aquarium for 1 h prior to filming. Alternative food was added to the aquarium after 40 min of filming, and the females were again

acclimated for 1 h prior to filming (Table 1). Fecal pellet concentrations were measured before and after each period of filming (40 min). The average fecal pellet concentration during filming was used as the experimental concentration. One female at a time was kept in focus and followed for at least 1 min if possible. The video tapes were analyzed frame by frame, and the fraction of time a female spent filtering, sinking, or jumping was recorded for approximately 1 min per female. The behavior of 4 females was analyzed at each fecal pellet concentration, in the absence and presence of alternative food. Periods when females were close to the aquarium wall were excluded from the analysis. The numbers of rejected and ingested fecal pellets observed during a total of 30 min of filming with a female in focus were recorded for each pellet concentration in the presence and absence of alternative food (half-hour analysis, see Tables 2 & 3).

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

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

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

RESULTS

Clearance and ingestion

Calanus helgolandicus

The clearance of fecal pellets was variable with no clear trend and was independent of pellet concentration both with and without the alternative food (*Thalassiosira weissflogii*; Fig. 1A). Maximum pellet clearance (mean \pm SD) was 172 ± 125 ml female⁻¹ d⁻¹ in the presence of *T. weissflogii* and 205 ± 198 ml female⁻¹ d⁻¹ when pellets were offered alone (Fig. 1A). The inges-

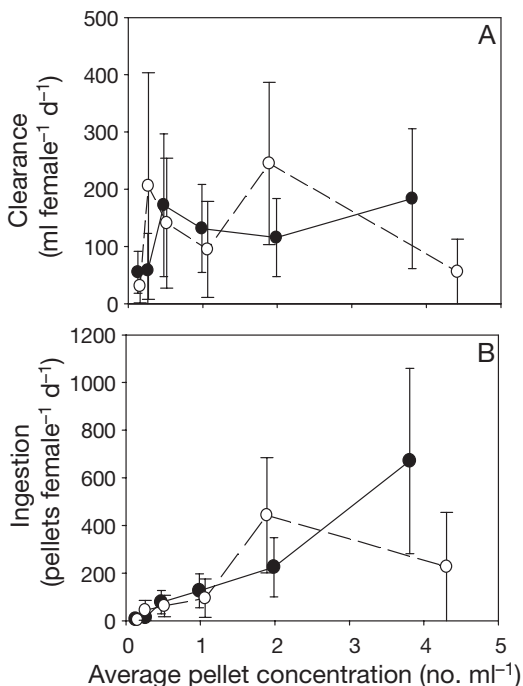


Fig. 1. *Calanus helgolandicus*. Average (A) clearance and (B) ingestion rates of fecal pellets with (●) or without (○) *Thalassiosira weissflogii* as an alternative food source. Fecal pellets were produced by *Acartia tonsa* adults feeding on *Rhodomonas salina*. Fecal pellet clearance and ingestion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

tion rate increased with increasing pellet concentration in the presence of alternative food to a level of 671 ± 389 pellets female⁻¹ d⁻¹ (Fig. 1B), equivalent to $43 \pm 32 \times 10^6$ μm^3 pellet material female⁻¹ d⁻¹. When offered only pellets, however, the pellet ingestion reached a maximum (443 ± 242 pellets female⁻¹ d⁻¹) at food concentrations of ~ 2 pellets ml⁻¹, whereupon it decreased again (Fig. 1B); the maximum ingestion was $23 \pm 16 \times 10^6$ μm^3 female⁻¹ d⁻¹. Clearance and ingestion of fecal pellets by *C. helgolandicus* were statistically independent of the presence or absence of *T. weissflogii* (clearance maximum: $p = 0.757$, Student's *t*-test; ingestion rate $p = 0.287$, ANCOVA; Fig. 1).

Pseudocalanus elongatus

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

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

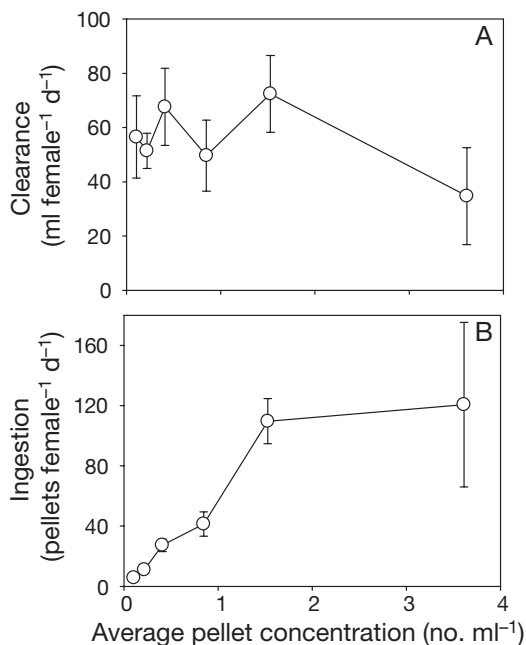


Fig. 2. *Pseudocalanus elongatus*. (A) Clearance and (B) ingestion rates of fecal pellets offered as sole food source. Fecal pellets were produced by *Acartia tonsa* adults feeding on *Rhodomonas salina*. Fecal pellet clearance and ingestion rates were estimated according to Frost (1972). Error bars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

Oithona similis

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

The ingestion rates of fecal pellets by *Oithona similis* increased with increasing pellet concentration in the presence of alternative food (Fig. 3C,D). The maximum ingestion in the presence of an alternative food source was $0.9 \pm 1.2 \times 10^6 \mu\text{m}^3 \text{ female}^{-1} \text{ d}^{-1}$ for the monoculture and $1.1 \pm 1.3 \times 10^6 \mu\text{m}^3 \text{ female}^{-1} \text{ d}^{-1}$ for the natural phytoplankton $<20 \mu\text{m}$ at concentrations of 7 and 2.8 pellets ml^{-1} , respectively (Fig. 3C,D). When pellets

were offered as the sole food, high variation in ingestion rates was observed. The maximum pellet ingestion was found at 6 pellets ml^{-1} , at which $0.6 \pm 1 \times 10^6 \mu\text{m}^3 \text{ female}^{-1} \text{ d}^{-1}$ was ingested (Fig. 3C). No significant difference in the ingestion rates was found between the different treatments ($p > 0.1$, ANCOVA), although higher ingestions were observed at concentrations <4 pellets ml^{-1} when an alternative food source was present (Fig. 3C,D).

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

Feeding behavior

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

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

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

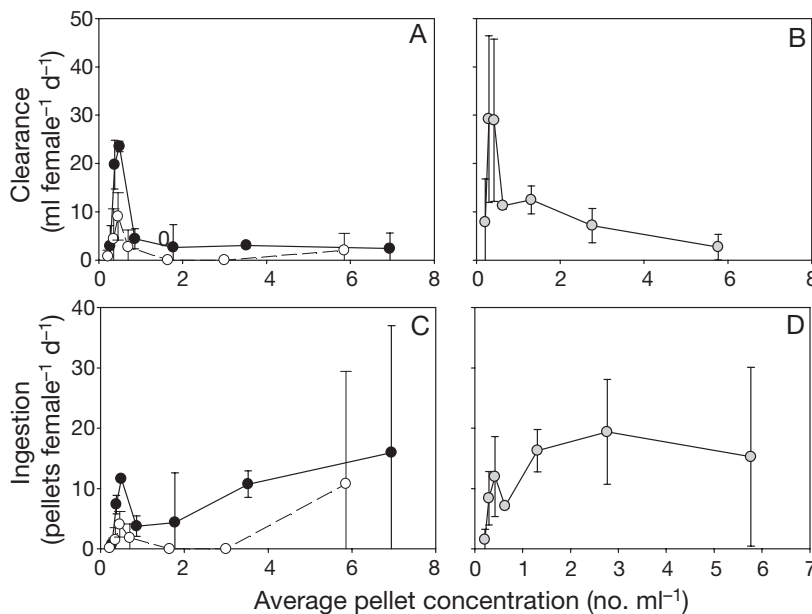


Fig. 3. *Oithona similis*. (A,B) Clearance rate and (C,D) ingestion rate of fecal pellets by *O. similis* are measured with fecal pellets as the sole food (O), offered together with *Rhodomonas salina* (●), and as pellets offered with a natural plankton community ($<20 \mu\text{m}$) from The Sound (Øresund,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

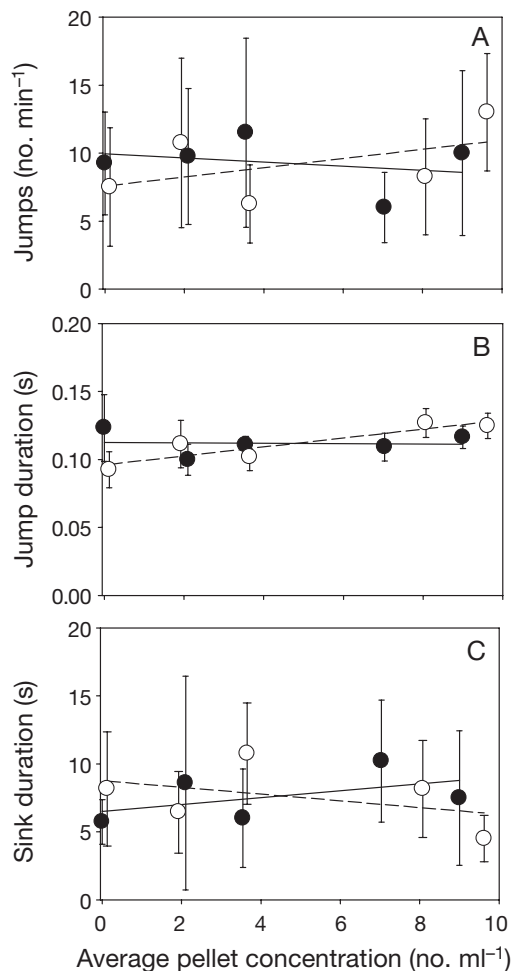


Fig. 4. *Oithona similis*. Female feeding behavior as a function of fecal pellet concentration in the absence (O, dashed lines) and presence (●, solid lines) of an alternative food source (*Rhodomonas salina*). Average (A) number of jumps per minute, (B) jump duration in seconds, and (C) sink duration.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

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

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

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

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

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

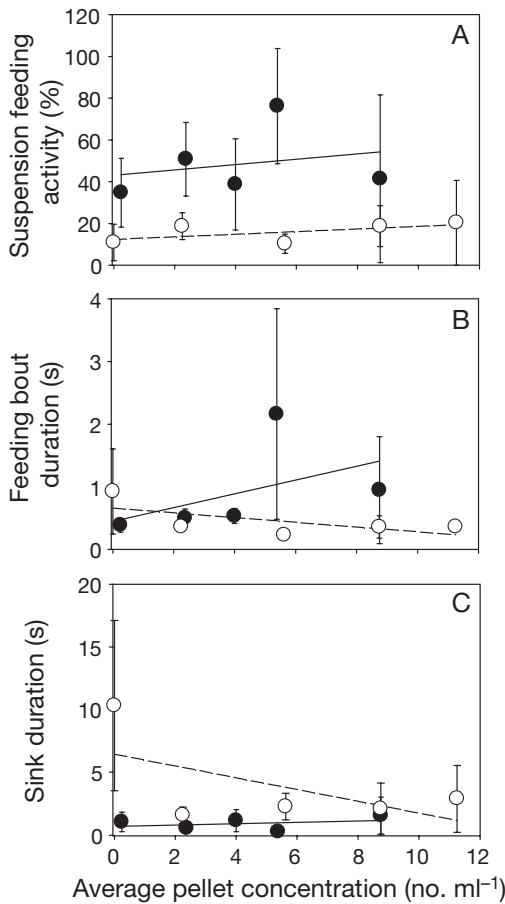


Fig. 5. *Calanus helgolandicus*. Female feeding behavior as a function of fecal pellet concentration in the absence (O, dashed lines) and presence (●, solid lines) of an alternative food source (*Thalassiosira weissflogii*). Average (A) suspension feeding activity as a percentage of total activity, (B)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

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

The presence of *Thalassiosira weissflogii* induced a significantly higher suspension feeding activity by *Pseudocalanus elongatus* ($p = 0.002$, ANCOVA; Fig. 6A), although this difference was most pro-

nounced at low pellet concentrations of <2 pellets ml^{-1} . No significant difference was observed in feeding bout duration in the presence of alternative food ($p = 0.9$, ANCOVA; Fig. 6B). Sink duration increased significantly when pellets were offered as the sole food ($p < 0.001$, ANCOVA; Fig. 6C). A difference in the number of pellet encounters was observed depending on the presence or absence of phytoplankton (Table 3).

Fecal pellet encounter and ingestion rates

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

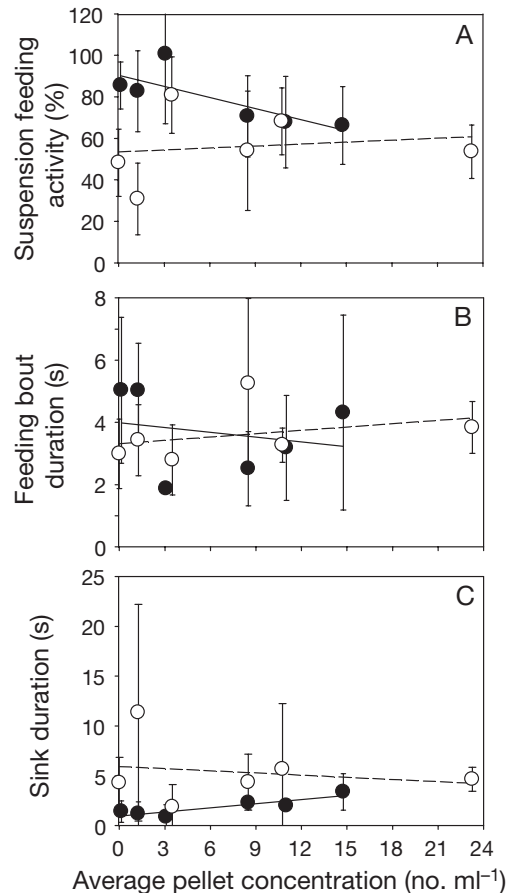


Fig. 6. *Pseudocalanus elongatus*. Female feeding behavior as a function of fecal pellet concentration in the absence (O, dashed lines) and presence (●, solid lines) of an alternative food source (*Thalassiosira weissflogii*). Average (A) suspension feeding activity as a percentage of total activity,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

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

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

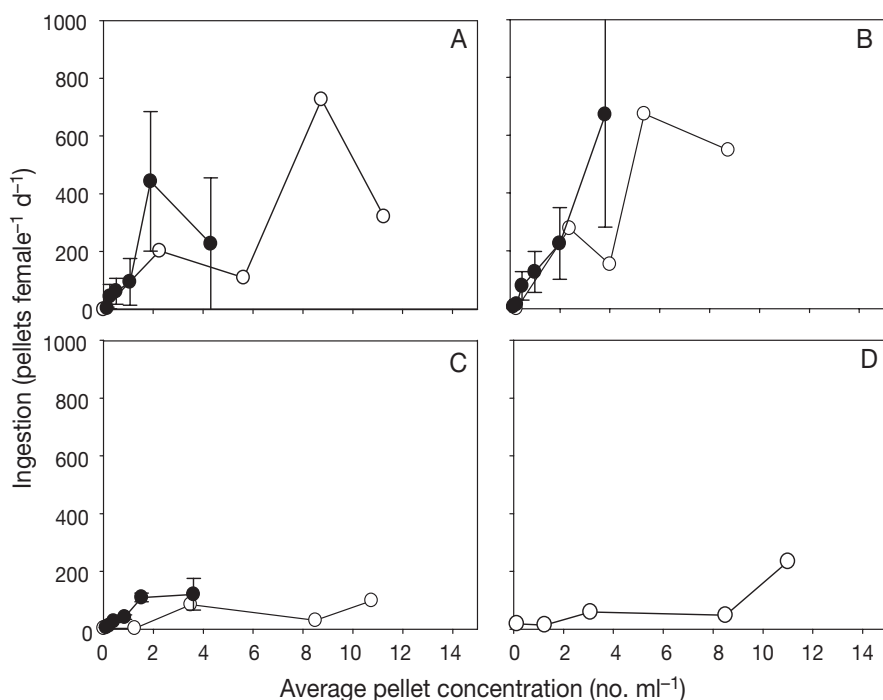


Fig. 7. (A,B) *Calanus helgolandicus* and (C,D) *Pseudocalanus elongatus*. Comparison between the ingestion rates of fecal pellets obtained from grazing experiments (●) and visual observations (○). Ingestion rates by *C. helgolandicus* and *P. elongatus* (B,D) with or (A,C) without an alternative food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

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

Ingestion rates were estimated from the observed ingestions during the video recordings, assuming a

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

Thus, visual observation revealed that the increased clearance of fecal pellets in the presence of alternative food was caused by an increased suspension feeding activity by the calanoid copepods. The presence of alternative food induced unintentional

ingestion of small pellet fragments along with the algae. Further, intact fecal pellets were avoided by both calanoid species. No feeding response was observed for *Oithona similis* during 7 h of filming, indicating that fecal pellets were generally not viewed as attractive food particles by any of the copepods.

DISCUSSION

Fecal pellet degradation by calanoid copepods

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

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

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

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

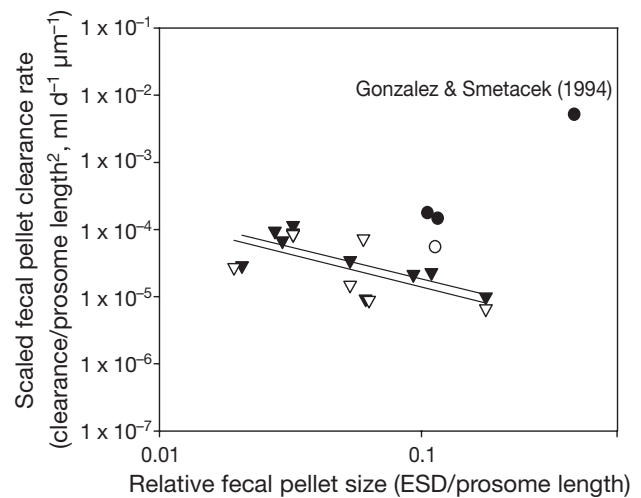


Fig. 8. Copepod fecal pellet clearance rate (normalized by copepod prosome length squared) as a function of relative pellet size (ESD/copepod prosome length) for calanoid copepods (∇ , \blacktriangledown) and *Oithona similis* (O, ●), in the absence (open symbols) or presence (closed symbols) of alternative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

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

mon feeding behaviors for calanoid copepods than coprophagy.

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

Fecal pellet degradation by *Oithona similis*

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

The obtained average fecal pellet removal rates for *Oithona similis* are low compared to removal rates reported by González & Smetacek (1994). At similar pellet concentrations, we found that the removal of pellets by *O. similis* was lower than the rates found by González & Smetacek (1994) by a factor of 5000 when offering calanoid pellets with an algal monoculture, and lower by a factor of 500 when offered with a natural plankton assemblage. In contrast, Reigstad et al. (2005) observed a complete lack of fecal pellet removal by *O. similis* when offering the same pellet type as used by González & Smetacek (1994). In the study by

González & Smetacek (1994), *O. similis* presumably grazed large pellets lying on the bottom of standing experimental bottles. This contradicts the results of several studies of grazing behavior, which show that *O. similis* is an ambush feeder that perceives its prey by detecting the hydromechanical disturbance generated when the prey is sinking or swimming (Paffenhöfer 1993, Kiørboe & Visser 1999, Svensen & Kiørboe 2000). Accordingly, the pellets must be sinking in order to be detected. To detect pellets on the bottom of a bottle, chemosensory perception is needed. Chemosensory perception of small prey such as fecal pellets requires a feeding current (Andrews 1983), and since *O. similis* lacks the ability to suspension feed (Svensen & Kiørboe 2000), the detection of pellets lying on the bottom is unlikely. We therefore question the fact that the high removal rate of pellets lying on the bottom of the bottles was due to ingestion by *O. similis*.

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

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

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

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