Mar Biol (2009) 156:469–477
DOI 10.1007/s00227-008-1100-2

ORIGINAL PAPER

Increased production of faecal pellets by the benthic harpacticoid *Paramphiascella fulvofasciata*: importance of the food source

Marleen De Troch · Clio Cnudde · Wim Vyverman · Ann Vanreusel

Received: 18 July 2008 / Accepted: 24 November 2008 / Published online: 17 December 2008

Abstract The re-use of faecal pellets in the water column before sinking to the seafloor is known as an important pathway in marine food webs. Especially planktonic copepods seems to be actively use their faecal pellets. Since benthic copepods (order Harpacticoida) live in the vicinity of their pellets, it remains unclear how important these pellets are for their feeding ecology. In the present study a laboratory experiment was conducted to analyse the importance of faecal pellets for the feeding ecology of the harpacticoid *Paramphiascella fulvofasciata* and its grazing pressure on two diatom species (*Seminavis robusta*, *Navicula phyllepta*). By quantifying the amount and volume of the produced faecel pellets in different treatments, it was tested to what extent the food source and the lack of faecal pellets would influence the production of faecal pellets. We found that the grazing pressure of *P. fulvofasciata* depended on the diatom density since only a top-down effect could be found on the smaller *Navicula* cells during its initial exponential growth phase. The grazer had a negative effect on the diatom growth and controlled the cell density to about 4,000 cells/cm^2. In spite of the fact that the addition of faecal pellets did not show a significant positive effect on the assimilation of diatoms, the removal of faecal pellets strongly promoted the pellet production. Especially when grazing on *Navicula* the harpacticoid *P. fulvofasciata* produced significantly more and smaller faecal pellets when the pellets were removed. This outcome illustrates the need for faecal pellets of this harpacticoid copepod when grazing on the diatom *Navicula*. Apart from its selection for smaller diatom cells, it was suggested that the colonisation of heterotrophic bacteria enriched these pellets. This study is the first to indicate that trophic upgrading occurs on faecal pellets and not only on the initial autotrophic food sources per se.

Communicated by X. Irigoien.

M. De Troch (✉) · C. Cnudde · A. Vanreusel
Biology Department, Marine Biology, Ghent University, Campus Sterre, Krijgslaan 281-S8, 9000 Ghent, Belgium
e-mail: marleen.detroch@UGent.be

W. Vyverman
Biology Department, Protistology and Aquatic Ecology, Ghent University, Campus Sterre, Krijgslaan 281-S8, 9000 Ghent, Belgium

Introduction

Copepod faecal pellets can not be considered as waste products or a lost of energy. These pellets have long been thought to be an important component of the downward flux of particles to the bottom of marine and freshwater ecosystems. The most recent papers, however, conclude that the contribution of zooplankton faecal pellets to total material flux is highly variable (between <1 and ≤99%, see review of Turner 2002) and mainly large and fast-sinking particles (e.g. faecal pellets of macrozooplankton and fish) reach the bottom. The pellets produced by microzooplankton and small mesozooplankton are mostly recycled and reworked in the water column by microbial decomposition and coprophagy and have no chance to reach the benthos. The recycling of this faecal material within the euphotic zone rather than being exported (e.g. Viitasalo et al. 1999; Wexels Riser et al. 2007) underlines its role in studies of biogeochemical cycles and trophic energy transfer in the ocean (Turner 2002). A recent study by Jansen and Bathmann (2007) even illustrated that some diatoms remain viable within copepod faecal pellets.

So far, the re-use of faecal pellets and factors controlling the production of faecal pellets has intensively been studied

for planktonic copepods belonging to the order Calanoida. Several mechanisms of the breakdown and recycling of faecal material were described. Active reworking of faecal material by copepods has been defined as coprorhexy (fragmentation to smaller particles), coprochaly (loosening resulting into an increased volume of the faecal particles) and finally coprophagy (ingestion of faecal material) (Gauld 1957; Noji et al. 1991; Reigstad et al. 2005). Although the mechanisms for retention of faecal pellets in overlaying waters become well-known the main reasons for pellet recycling remain less clear. Despite of the fact that copepods are characterised by a relatively high assimilation efficiency ranging between 20 and 80% (e.g. Thor et al. 2007; Liu et al. 2006; Wotton 2001) a large amount of the food remains unused in the faecal pellets.

Based on this amount of information pointing at the importance of faecal pellets, one can wonder if faecal pellets are important in benthic communities as well since benthic copepods (mainly belonging to the order Harpacticoida) live in very close vicinity of (fresh) faecal material on the bottom. Feller (1980) observed coprophagy in lab cultures of *Huntemannia jadensis* and Walker (1979) saw *Amphiascoides* sp. feeding on its faecal material during short periods of food depletion or lack of fresh food. However, Cowey and Corner (1966) warned that such a feeding strategy, as they reported for the planktonic copepod *Calanus finmarchicus*, would not be energetic endurable for longer periods.

A side outcome of a previous experiment suggested a positive effect of faecal pellets on the assimilation of diatoms by the benthic harpacticoids *Nitocra spinipes* and *Paramphiascella fulvofasciata* (De Troch et al. 2005). The copepod feeding-faecal pellet production interactions were however, not analysed in detail. How determinant is the food source for faecal pellet production? How vital are faecal pellets for the survival of benthic copepods?

The present study aims to analyse the need for faecal pellets for the benthic harpacticoid *P. fulvofasciata*. We tested experimentally if the food source and the lack of faecal pellets would influence the production of faecal pellets. Systematic removal of faecal pellets can encourage the faecal pellet production or even accelarate it. As such, the results will provide more insight in the role of faecal pellets in the ecology of benthic copepod communities.

Materials and methods

Laboratory conditions

Laboratory stock cultures of the copepod species *Paramphiascella fulvofasciata* [family Miraciidae (former family Diosaccidae), initially collected from a subtidal area of Helgoland, Germany] were maintained in 1 L glass beakers, with artificial seawater (ca. 32 psu, Instant Ocean® salt, Aquarium Systems, France) (see also De Troch et al. 2005, 2006a, b). They were regularly provided with a mixture of benthic diatom cultures, composed of epipelic pennate species *Navicula phyllepta*, *Seminavis robusta* and *Cylindrotheca closterium*. Specimens of the copepod reaches up to 0.83 mm in size (body length) and a carbon content of 1.3 ± 0.2 µgC.

Cultures of the epipelic pennate diatom *Seminavis robusta* and *Navicula phyllepta* have been repeatedly proved their efficiency in serving as food source for various harpacticoid copepods in laboratory experiments (e.g. De Troch et al. 2006a, b, 2007). In the present investigation, we used a monoclonal culture of *Seminavis robusta* and *Navicula phyllepta*, further referred to as *Seminavis* and *Navicula*, respectively. The *Seminavis* clones from which the lineage was started were isolated from a sample collected in November 2000 from the 'Veerse Meer', a brackish water lake in Zeeland, The Netherlands, and their principal life-cycle traits were studied in detail (Chepurnov et al. 2002). The clone involved in the current experimental work is referred to as strain 84A in the diatom culture collection of the Laboratory of Protistology and Aquatic Ecology, Ghent University, Belgium. By the time of setting the experiment, the cells were measured to be 55.89 ± 0.94 µm in length.

Monoclonal *Navicula* cultures were isolated from sediment from intertidal mudflats in the IJzer estuary (Nieuwpoort, Belgium) where it was identified as an abundant species. It was grown under the same lab conditions as *Seminavis*. At the start of the experiment, the *Navicula* cells were on average 16.33 ± 0.26 µm in length.

Both diatoms were grown in f2 culture medium (Guillard 1975) which was based on sterilized artificial seawater (32 psu).

Both copepods and diatoms were kept and the experiments were performed at 17 ± 1°C and under a 12:12 h light–dark regime and 25–50 µmol photons m^{-2} s^{-1}.

Labelling technique

To trace diatom assimilation by *P. fulvofasciata*, the *Navicula* diatom cells were labelled with the stable isotope ^{13}C by adding 5 ml of a solution with $NaH^{13}CO_3$ (336 mg in 100 ml milliQ H_2O) per 100 ml of the culture medium. Diatom cells underwent multiple mitotic divisions to reach high densities and high levels of labelling. This labelling technique resulted in an increase in $\delta^{13}C$ from −17.9 to 4,681‰.

Prior to the feeding experiment, the labelled medium was replaced by artificial seawater. To estimate the density of diatom cells in the cultures, the cells were homogeneously

suspended by shaking the bottles in which the cultures were grown and subsequently 50 µl of the cell suspension was transferred into a well of a 96-well plate. In an hour, after all the cells confidently settled to the bottom of the well, their number was counted under a Zeiss Axiovert 135 inverted microscope (Zeiss Gruppe, Jena, Germany) and the values obtained allowed to estimate the densities in the experimental vessels.

Single specimens of copepods were picked up by micropipette from the original stock cultures and placed into Petri dishes containing artificial sea water where they were starved of food overnight. After starving, the copepods were transferred into the different treatments (see "Experimental design"). Only non-gravid adults were selected for this experiment in order to avoid any nauplii in the experimental units.

To detect $^{13}C/^{12}C$ ratios in the tissue of the harpacticoids, a minimum of 15 µg C per species was analysed per replicate corresponding to 20 adults of *Paramphiascella fulvofasciata* in one experimental unit. At the start of the experiment, triplicate samples of 20 individuals of *P. fulvofasciata* were put in the freezer for natural isotopic signature measurements (T_0 values). They had not been feeding on the labeled diatoms but on a mixture of unlabeled food and as such they are typically depleted in the ^{13}C stable isotope.

Experimental design

Diatom assimilation

The effect of faecal pellets on the diatom assimilation by *P. fulvofasciata* was tested by means of two treatments (four replicates each) with ^{13}C enriched *Navicula* cells:

- 'copepod + fp' treatment, with 20 adults of *P. fulvofasciata*, 1×10^6 ^{13}C enriched *Navicula* cells and the faecal pellets produced during the experiment;
- 'copepod + extra fp' treatment, with 20 adults of *P. fulvofasciata*, 1×10^6 ^{13}C enriched *Navicula* cells, the faecal pellets produced during the experiment and 200 extra faecal pellets of *P. fulvofasciata* individuals fed on unlabelled diatoms (*Seminavis* and *Navicula)*.

Diatom growth and faecal pellet production

To analyse the grazing pressure of *P. fulvofasciata* on the diatoms and to study its faecal pellet production, the experimental design consisted of four treatments (four replicates each):

- 'control' treatment with only diatom cells, without any copepods or faecal pellets;
- 'copepod + fp' treatment with diatom cells, three adults of *P. fulvofasciata* and their faecal pellets (fp);
- 'copepod − fp' treatment with diatom cells and three adults of *P. fulvofasciata*. Faecal pellets were removed every 24 h;
- 'copepod + extra fp' treatment with diatom cells, three adults of *P. fulvofasciata*, the faecal pellets produced during the experiment but also 50 additional faecal pellets of *P. fulvofasciata* individuals (fed on the unlabelled diatoms *Seminavis* and *Navicula)* were added to the experimental unit at the start of the experiment.

These four treatments were applied in three experiments:

- Experiment (1): the diatom *Seminavis* was used with an initial density of 47 cells/cm^2 and in the 'copepod + extra fp' treatment 50 pellets of *P. fulvofasciata* were added.
- Experiment (2): the diatom *Navicula* was used with an initial density of 960 cells/cm^2 and in the 'copepod + extra fp' treatment 100 pellets of *P. fulvofasciata* (fed on the unlabelled diatoms *Seminavis* and *Navicula*) were added.
- Experiment (3): the diatom *Navicula* was used with an initial density of 1,946 cells/cm^2 and in the 'copepod + extra fp' treatment 100 pellets of other copepod species (from our cultures, fed on the unlabelled diatoms *Seminavis* and *Navicula*) were added (allochtonous pellets).

All treatments were conducted in small Petri dishes (polystyrene, diameter = 5.2 cm, surface area = 21.2 cm^2, 20 ml) that were placed at random on a shelf under the same controlled conditions as diatoms and copepods were cultured.

Analytic techniques and data treatment

Diatom assimilation

After 96 h of grazing, the survival rate was estimated and the experiment was terminated by freezing of the experimental units. The further processing consisted of thawing the frozen petriplates and sorting of the copepods within two hours after thawing to prevent or minimise leakage of label (see Mourelatos et al. 1992; Moens et al. 1999). Subsequently, the specimens were washed several times in deionised water and placed in tin capsules (8×5 mm, pressed, standard weight) by means of a needle. The material collected was desiccated overnight in an oven at 60°C. Measurements of $\delta^{13}C$ values and copepod biomass (total carbon) were made using a continuous flow isotope ratio mass spectrometer (type Europa Integra) at the UC Davis Stable Isotope Facility (University of California, USA). The data are represented as $\Delta\delta$ ^{13}C values, calculated as the

difference between $\delta^{13}C$ values at the end of the experiment and the initial T_o values (see before): $\Delta\delta = \delta_{\text{modified}} - \delta_{T0\ \text{values}}$.

Diatom growth and faecal pellet production

Every day around the same time, the number of diatom cells was estimated under an inverted microscope (Zeiss Gruppe, Jena, Germany). The number of faecal pellets were counted daily under a binocular, for the treatments 'copepod + fp', 'copepopd − fp' and 'copepod + extra fp'. In the treatment 'copepod − fp' faecal pellets were counted prior to daily removal. In the 'copepod + extra fp' treatment the number of faecal pellets that were added at the start of the experiment (i.e. 50 pellets in experiment 1, 100 pellets in experiments 2 and 3) were taken into account as they were subtracted from the total count. In addition, some pellets were picked out at random and placed in a glycerine slide. These slides had a parafilm ring in order to avoid squeezing of the pellets when the slide was closed with a coverglass. A microscope (Leitz Dialux 20), Sanyo CCD video camera and an image analysis system (Quantimet 500 software) were used to estimate the length and width of the pellets in order to calculate the volume of the pellets.

Statistical analyses

Differences among the various treatments were tested by means of one-way and two-way analyses of variance (ANOVA) with Statistica sofware (Statistica 7.0). A posteriori comparisons were carried out with the Tukey test using 95% confidence limits.

Repeated measures ANOVA (RM-ANOVA) were used when data were represented in a cumulative way. This repeated-measures analysis can proceed only if it can be assumed that there is no interaction among times and treatments. Therefore, sphericity was tested by means of the Mauchley's test. In case of violations, corrections as proposed by Greenhouse and Geisser (1959) and Huynh and Feldt (1970) were applied.

Prior to all ANOVA's, the Cochran's C-test was used to check the assumption of homoscedasticity.

Results

Diatom assimilation

After 96 h, all harpacticoids were still alive and showed a measurable assimilation of *Navicula* cells (Fig. 1). The $\delta^{13}C$ values of *P. fulvofasciata* at the end of the experiment were far above its natural abundance value at the start of the experiment which was −19.6 ‰.

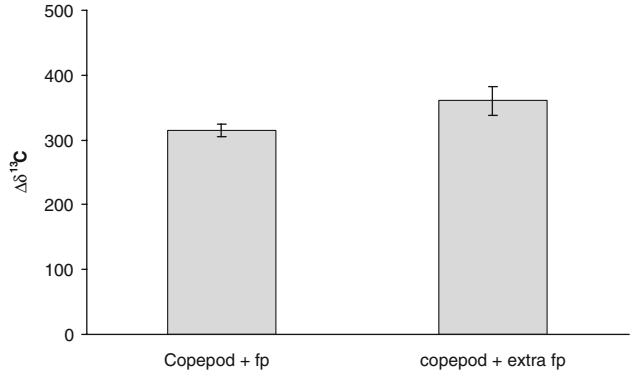

Fig. 1 Assimilation of ^{13}C enriched *Navicula phyllepta* cells (mean ± standard error) by *Paramphiascella fulvofasciata* expressed as specific uptake ($\Delta\delta^{13}C$) for the treatments 'copepod + fp' and 'copepod + extra fp'

There was no significant difference (one-way ANOVA, $P > 0.05$) in the specific uptake of *Navicula* cells by *P. fulvofasciata* (expressed as $\Delta\delta\ ^{13}C$, Fig. 1) when more faecal pellets were present in the experimental unit. The average specific uptake was 360 ± 23 and 315 ± 10 for 'copepod +fp' and 'copepod + extra fp', respectively.

Diatom growth

The growth of *Seminavis* (Fig. 2a) and *Navicula* (Fig. 2b) was followed over time and different growth phases were distinguished: an initial lag phase, an exponential growth phase and a stationary phase followed by a final decline of the diatom culture. For both diatoms, the lag phase lasts for 100 h (about 4 days) followed by an exponential growth for 200 h (until 300 h). For *Navicula* the stationary phase is shorter (72 h) than for *Seminavis* (120 h). The cell density in this phase is however, much higher for *Navicula* (61111 ± 3119 cells/cm^2) than for *Seminavis* (9,606 ± 484 cells/cm^2). The decline starts after 382 h and 430 h for *Navicula* and *Seminavis*, respectively.

The impact of the grazer and its faecal pellets on these growth curves was followed during the lag phase and the initial exponential growth phase (Fig. 2c–e). Only in experiment 3 there was a significantly lower cell density of *Navicula* when the grazer *P. fulvofasciata* was present (one-way ANOVA, $P < 0.05$). This effect was only clear after 48 h. Removal or addition of faecal pellets didn't show any significant effect on the diatom growth. In experiment 1, the treatment with extra faecal pellets resulted in an either decreased (at 110 h) or increased (at 86 and 134 h) diatom growth indicating a worse or a better growth of *Seminavis* cells or more or less assimilation of diatom cells by *P. fulvofasciata*, respectively.

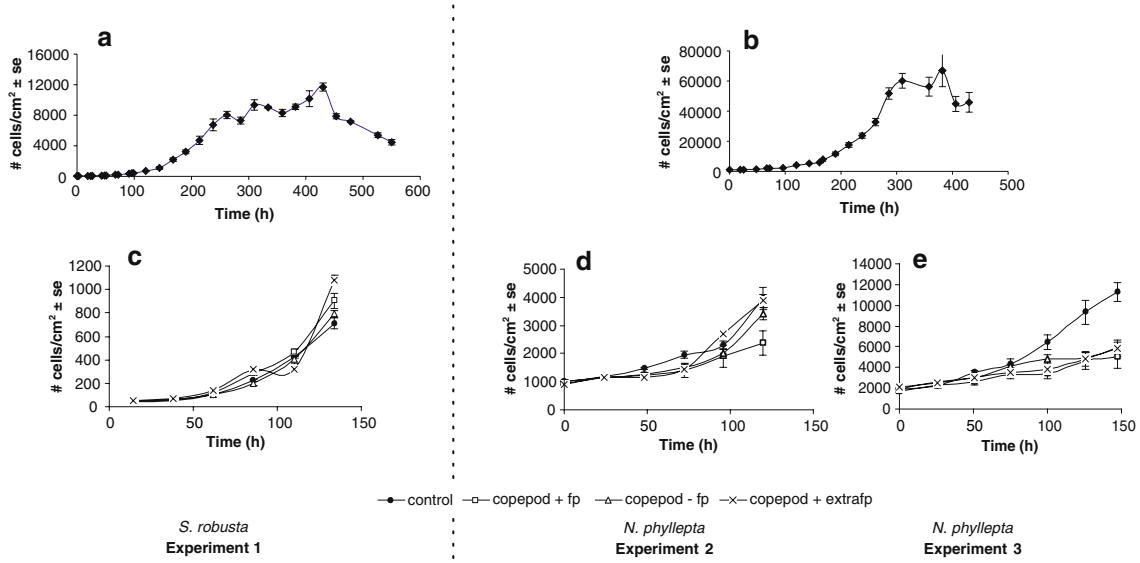

Fig. 2 Diatom growth (average number of cells ± standard error) with or without the grazer *Paramphiascella fulvofasciata*: **a** *Seminavis robusta* without the grazer, **b** *Navicula phyllepta* without the grazer, **c** *Seminavis robusta* with the grazer and **d**, **e** *Navicula phyllepta* with the grazer. The effect of the grazer was tested in different treatments: with faecal pellets ('copepod + fp'), treatment where faecal pellets were removed ('copepod − fp') and a treatment where extra faecal pellets were added ('copepod + extra fp'). In **e** these extra faecal pellets were produced by other species (allochtonous pellets)

Faecal pellet production

Independent of the treatment, the daily faecal pellet production by *P. fulvofasciata* when feeding on *Seminavis* cells increased significantly from day 1 onwards followed by a stabilisation (Fig. 3a, exp. 1, two-way ANOVA, effect of time: $P < 0.001$). When grazing on *Navicula* cells (exps. 2 and 3, Fig. 3d, g), there was a significant increase in daily faecal pellet production from day 3 onwards (Fig. 3d, exp. 2, two-way ANOVA, effect of time: $P < 0.01$) while the daily faecal pellet production in experiment 3 showed a slight decline after 4 or 5 days (Fig. 3g, two-way ANOVA, effect of time: $P < 0.001$).

The effect of the treatments on the daily faecal pellet production was only significant when *P. fulvofasciata* was fed with *Navicula* (exps. 2 and 3). In both cases, a significant higher daily production of faecal pellets was observed in the treatment with daily removal of the faecal pellets ('copepod − fp') (Fig. 3f, i; two-way ANOVA, effect of treatment: $P < 0.001$ and $P < 0.01$, Tukey HSD: $P < 0.001$). For both experiment 2 and 3, this higher production of faecal pellets was significant from day 5 onwards (two-way ANOVA, effect of time × treatment: $P < 0.001$, Tukey HSD: $P < 0.01$).

In terms of cumulative faecal pellet production, the addition of extra faecal pellets ('copepod + extra fp') of *P. fulvofasciata* (exps. 1 and 2, Fig. 3b, e) or other copepod species (exp. 3, Fig. 3h) showed only a clear effect in experiment 1 (*Seminavis* as food source). There were less freshly produced faecal pellets in the treatment where faecal pellets were left untouched in the experimental unit ('copepod + fp') than in the treatment where extra faecal pellets were added (Fig. 3b, two-way ANOVA, effect of treatment, $P < 0.05$). This indicates that more faecal pellets were produced by *P. fulvofasciata* in the latter series (Fig. 3c). In the experiments 2 and 3 (*Navicula*) the amount of faecal pellets were in step with each other.

In all experiments there was a high significant increase in the cumulative amount of faecal pellets with time (Fig. 3b, e, h; RM-ANOVA, effect of time: $P < 0.001$). In addition, there was also an interaction effect of time × treatment (RM-ANOVA, time × treatment: $P < 0.1$). In experiment 2 and 3, it was observed that the total amount of faecal pellets in the experimental unit reached an asymptote after 4 days (Fig. 3e, h). In addition, the initial higher food density applied in experiment 3 resulted in a higher total number of produced faecal pellets.

Faecal pellet volume

Besides an effect on the number of faecal pellets produced, the removal of faecal pellets also affected the volume of the freshly produced faecal pellets (Fig. 4). With *Seminavis* as main food source (experiment 1) there was no significant effect of the absence of faecal pellets on the volume of the produced pellets (one-way ANOVA, $P > 0.05$). When grazing on *Navicula* on the other hand the produced faecal pellets were significantly smaller (one-way ANOVA, $P < 0.05$).

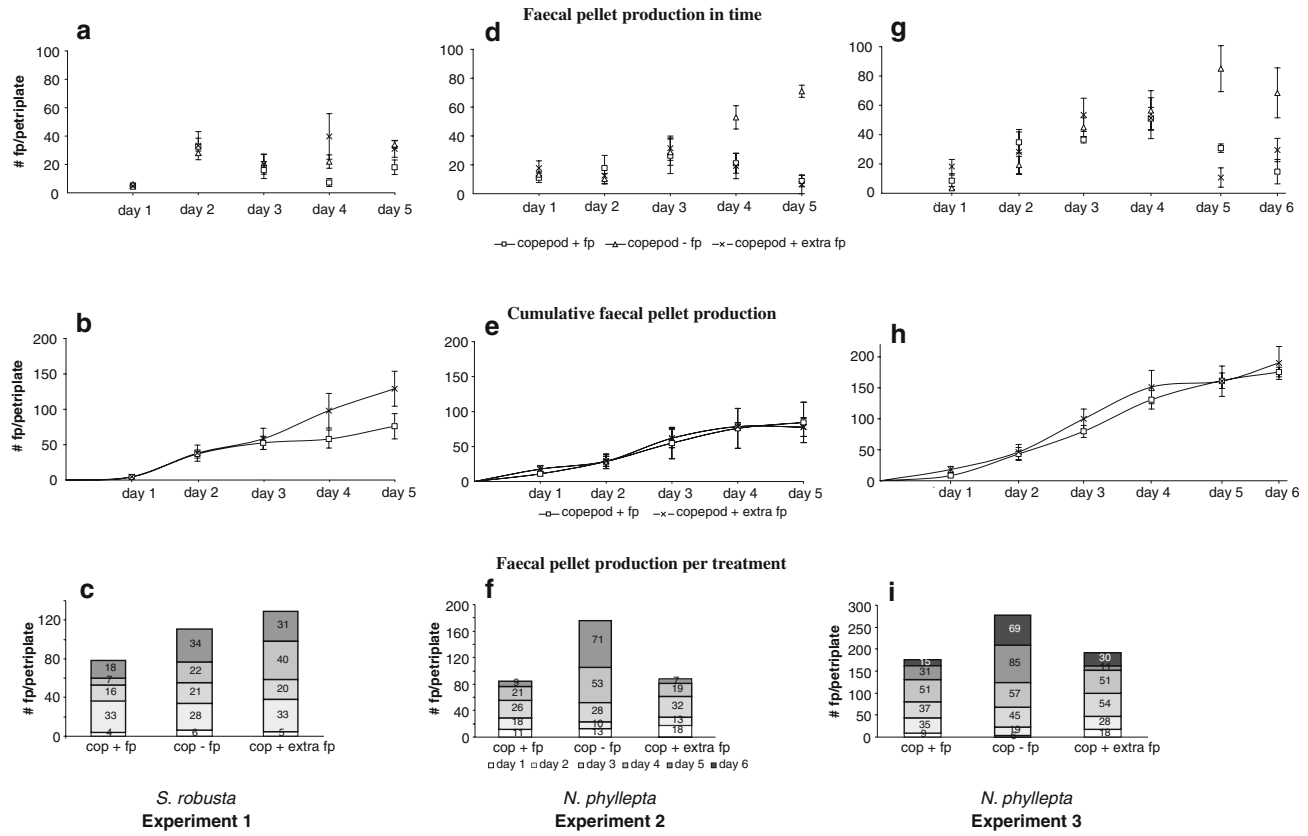

Fig. 3 Faecal pellet production by *Paramphiascella fulvofasciata* in experiment 1 (**a**–**c**), 2(**d**–**f**) and 3 (**g**–**i**) expressed as faecal pellet production in time (**a**, **d**, **g**), cumulative faecal pellet production (**b**, **e**, **h**) and daily faecal pellet production per treatment (**c**, **f**, **i**). Production is always expressed as the average number of faecal pellets (fp) per petriplate (± standard error)

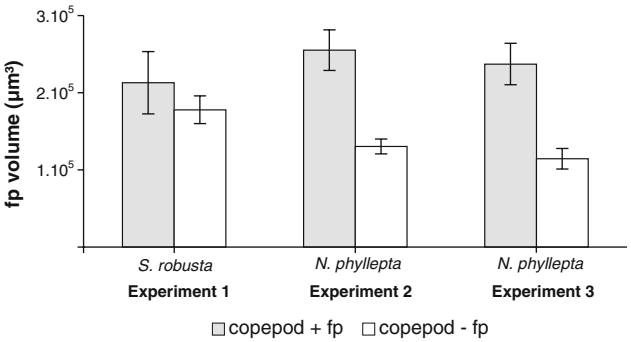

Fig. 4 Average volume of the faecal pellets (in μm^3, ± standard error) of *Paramphiascella fulvofasciata* feeding on *Seminavis robusta* (exp. 1) and *Navicula phyllepta* (exps. 2, 3), respectively. The effect of the daily removal of faecal pellets was tested by comparing two treatments ('copepod + fp' vs. 'copepod − fp')

Discussion

Grazing of *P. fulvofasciata* and its effect on diatom growth

The present study showed that the harpacticoid copepod *P. fulvofasciata* is an active grazer on diatoms, both on *Seminavis* and *Navicula* and potentially also on the associated film (see Decho and Fleeger 1988). In spite of the low copepod density (three individuals) used per experimental unit here, we found an effect of grazing on the growth of *Navicula*. This grazing activity had only a clear impact on the growth of the diatom *Navicula* when the initial diatom culture contained a high density of cells (>1,000 cells/cm^2). Independent of the presence or absence of faecal pellets, the grazer had a negative effect on the diatom growth and controlled the cell density to about 4,000 cells/cm^2, a clear top-down control on *Navicula*.

This outcome suggests that the grazing pressure of this copepod grazer is mainly clear during the initial exponential growth phase (<10,000 cells/cm^2) since this effect is governed by the diatom cell density. Moreover, this controlling effect of the grazer may unable the diatom culture to enter the later exponential phase and the stationary phase. Previous experiments (De Troch et al. 2007) showed that the grazing efficiency of *P. fulvofasciata* was diatom concentration-dependent with lower assimilation at lower diatom densities. From their study on a surface-deposit feeder, Karrh and Miller (1994) stated that if grazers are limited by food availability, grazing rates should increase with increasing food concentration. The ingestion of

T. weissflogii by the planktonic copepod *Eucalanus pileatus* increased asymptotically with increasing diatom concentration attaining its maximum near 1 mm^3 l^{-1} (Paffenhöffer and Van Sant 1985).

Hutchins et al. (1995) studied the effect of copepod grazing on fractionation of diatom cellular Fe and did not found a strong correlation between diatom growth phase and copepod assimilation, although there was a tendency toward higher cytoplasmic content (and consequently higher grazer assimilation efficiencies) in stationary phase cultures, as observed for other elements by Reinfelder and Fisher (1991).

In addition to the cell density, the cell size might be important to estimate the effect of the grazer on the diatom growth. In this experiment, *Seminavis* cells were about 3.5 times larger than the *Navicula* cells. In view of the size of its mouthparts, *P. fulvofasciata* is known to be selective for smaller cells (De Troch et al. 2006a) and assimilates typically more smaller cells.

From our data it can not be estimated whether the diatom species itself is a crucial factor for this grazing effect. In spite of the fact that *Seminavis* and *Navicula* are closely related species (both belonging to the family *Naviculaceae*) it remains unclear whether their food value for harpacticoid grazers differs. Araújo-Castro and Souza-Santos (2005) reported that the benthic diatom *Navicula* sp. was more favorable than a planktonic one to the copepod *Tisbe biminiensis* although the same study stated that these diatoms may be toxic in certain concentrations.

Faecal pellet production and volume

The assimilation of more *Navicula* cells in experiment 3 (with higher diatom densities) was also reflected in the production of more faecal pellets. In all experiments, independent of the diatom species or its density, there was a significant increase in the production of faecal pellets with time. Of course, the diatom density increased as well with time. The differences in initial diatom densities resulted in different total numbers of pellets that were counted at the stabilisation level (from day 4 onwards).

In addition, there was a clear increase in number of faecal pellets produced in the treatment where faecal pellets were removed on a daily basis. This outcome suggest that there is a high need for these faecal pellets in the vicinity of *P. fulvofasciata* when it is grazing on *Navicula* cells. However, the amount of carbon assimilated (expressed as $\Delta\delta^{13}C$) by *P. fulvofasciata* was not significantly different when more faecal pellets were present in the experimental unit. So, more faecal pellets didn't necessary promote the assimilation of fresh diatoms. The lack of faecal pellets did not affect the amount of diatoms that was grazed upon but it influenced the time that they spend in the copepod's gut. This accelerated production of faecal pellets was mainly found when feeding on *Navicula* (see further).

Wexels Riser et al. (2007) showed the importance of faecal pellet retention by planktonic copepods (Calanoida) since only 10% of the vertical particulate organic carbon flux consisted of copepod pellets. This is significantly less than what was produced by the copepod community. In conclusion, Wexels Riser et al. (2007) suggested that this retention of faecal pellets was partly caused by the zooplankton themselves. Several studies indicated that fragmentation of faecal pellets (coprorhexy) is probably the first step in recycling faecal pellets and obviously an important factor to reduce the vertical flux of pellets (Noji et al. 1991; Reigstad et al. 2005; Wexels Riser et al. 2007). However, in the present study the faecal pellets were not fragmented since only intact pellets were taken into account. We saw that older pellets had a more loosely structure as the experiment run but no active fragmentation by the grazer was observed. Here we studied benthic harpacticoid copepods that are in close contact with their faecal pellets at the bottom of the experimental unit while for planktonic species (like *Oithona* spp. and *Calanus* spp.) the sinking rates of the faecal pellets is a limiting factor. As the latter pellets sink through the water column a fraction of them will finally join the pellets produced by the benthic species on the substratum. As such, benthic copepods are not only in contact with their own faecal pellets but also the ones from other benthic and planktonic species. All the more reason why benthic copepods could become very specialised in the re-use of faecal pellets.

Mechanisms for retention of faecal pellets in overlaying waters become common knowledge but good explanations for pellet recycling should still be sought. These explanations might be different for benthic and planktonic copepods.

As pellets contain material that is difficult to digest, it is safe to assume that freshly-egested faecal pellets are of lower food quality than the foods that are available within the environment and that have recently been ingested. Freshly-produced pellets are thus best avoided by feeding animals although they make up a high proportion of marine deposited material (Wotton 2001).

On egestion, faecal pellets produce a pulse of dissolved organic matter (DOM) that leaches out and this probably attracts the first colonising bacteria. In addition, bacteria that have survived passage through the gut are packed together with usable substrates in the bound pellets and this is likely to be an ideal medium for their growth. The food value of faecal pellets increases as refractory substrates are broken down during conditioning, and valuable chemicals such as nitrogen are taken up from the water by attached and bound bacteria. As such, the pellets get enriched in nitrogen among others. A recent study by Miller and

Roman (2008) showed that the nitrogen excretion of the planktonic copepod *Acartia tonsa* increases when it consumes high densities of nitrogen-rich foods. The nitrogen excretion varies in order to create a homeostasis of the N/C ratio of the copepods.

On the other hand, elemental content alone is insufficient to predict food quality (Bec et al. 2006). Protozoa are known for their intermediary trophic role in transferring organic matter from small sized particles to grazers. Klein Breteler et al. (1999) were the first to suggest that heterotrophic protists could improve the biochemical constituents of poor quality algae for higher trophic levels, a phenomenon called 'trophic upgrading'. Several studies followed and tested whether the growth of protists on autotrophic picoplankton contributed to a better development of the copepod grazer (e.g. Tang and Taal 2005; Bec et al. 2006). The addition of essential fatty acids (mainly EPA and DHA) seemed to explain this trophic upgrading. The present study is novel since it is the first to indicate that trophic upgrading occurs on faecal pellets and not on the autotrophic food source per se.

So far, we have no solid evidence of the grazing of harpacticoid copepods on the bacteria associated with their pellets although we observed them frequently grazing on the pellets. Especially nauplius larvae are actively grazing and clinging on the pellets of the adults without breaking the pellets into pieces. These observations support the idea that copepods need faecal pellets in their vicinity. The other option, that they dislike their faecal pellets and consume more diatoms when the pellets are removed, becomes more unlikely.

The initial food source (*Navicula* vs. *Seminavis*) seems to rule the occurrence of trophic upgrading of faecal pellets. When *P. fulvofasciata* is feeding on *Navicula*, it is producing more faecal pellets that become available for colonisation by heterotrophic protists. The removal of faecal pellets catalyses this process. In addition, the faecal pellets produced on a diet of *Navicula* and after removal of faecal pellets were significantly smaller. The smaller volume of the pellets is intrinsic to a faster production of the pellets. These pellets spend less time in the gut and are consequently smaller in size. The harpacticoid copepod *P. fulvofasciata* produced more but smaller faecal pellets and thus had more potential for trophic upgrading when it grazed initially on *Navicula* cells. This outcome suggests that *Navicula* is a poorer food source for the tested copepod species than *Seminavis*.

Not only the initial food source seems to govern the process of trophic upgrading, also the age of the available faecal pellets matters. The faecal pellets that were added in experiment 1 (*Seminavis*, 'copepod + extra fp') were a bit older and this may explain the significant increase in production of faecal pellets in that series. The addition of rather fresh faecal pellets in experiment 2 and 3 (*Seminavis*, 'copepod + extra fp') did not result in any change of pellet production in comparison to the undisturbed series ('copepod + fp').

Further characterisation of the bacteria associated with faecal pellets will be the next step forward to unravel the feeding ecology of harpacticoid copepods and the role of trophic upgrading in benthic food webs.

Acknowledgments M. de Troch is a Postdoctoral Fellow of the Research Foundation - Flanders (FWO). This study was conducted within the frame of FWO research project G.0313.04. Additional support was provided by the Ghent University (BOF-GOA 01GZ0705). The authors acknowledge the support by the MarBEF Network of Excellence 'Marine Biodiversity and Ecosystem Functioning' which is funded by the Sustainable Development, Global Change and Ecosystems Programme of the European Community's Sixth Framework Programme (contract no. GOCE-CT-2003-505446). This publication is contribution MPS-08049 of MarBEF. Two anonymous referees provided valuable remarks in order to improve an earlier version of this paper.

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