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Ingestion of microbially-synthesized organic aggregates and egestion of fecal pellets by marine harpacticoid copepods

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Abstract

Bacteria convert dissolved organic matter (DOM) into detrituslike particles and clump small particles into larger ones, which may then become available to higher consumers. Microbial aggregates produced in the laboratory from DOM and particles <100 µm (both sources derived from freshly collected North Sea macroalgae), were ingested by the epibenthic harpacticoid copepods *Paramphiascella vararensis* and *Tisbe holothuriae* in short-term laboratory experiments. The production of fecal pellets was used as an indicator of aggregate consumption. Results showed that between 1-3 pellets copepod^{-1}h^{-1} were produced by *P. vararensis*, independently of algal aggregate source and age. In contrast, *T. holothuriae* produced between 5-13 pellets copepod^{-1}h^{-1} depending on the type of algal aggregate source. Microscopical examination of ageing aggregates and pellets confirmed the presence of a rich bacterial flora as well as some protozoans that may provide nutrients for copepods. Enriched fecal material may also be disaggregated and transformed by microbial action into smaller particles and DOM ("fecal pellet loop"), demonstrating the various pathways for carbon flow within detritus-based nearshore food webs.

Introduction

Amorphous detritus particles, also termed "aggregates" or "marine snow", are frequently observed in natural waters. These aggregates originate from various sources, and may be significant as a substrate for microbial growth and as food for zooplankton and other metazoans (RILEY 1963, ALLDREDGE and SILVER 1988). Recent studies have demonstrated that the dissolved and fine particulate organic matter originating from coastal macrophytes is rapidly converted into macroscopic aggregates by microbial activity (ROBERTSON et al. 1982, BIDDANDA 1985).

In this study, we show that microbially synthesized aggregates derived from the dissolved and particulate fraction of several North Sea macroalgal species are

consumed by some marine harpacticoid copepods (Crustacea: Copepoda) in the laboratory. In short-term experiments, we have monitored the production of fecal pellets by these aggregate-fed copepods as an index of the ingestion of the aggregate material.

Material and methods

Preparation of aggregates: From the Helgoland rocky intertidal zone, green, brown and red macroalgae (*Ulva lactuca, Ulva* sp., *Fucus serratus, Laminaria* sp., *Delesseria sanguinea* and *Plocamium cartilagineum)* were freshly collected, ground in seawater with a mortar and pestle, and sieved through 100 µm mesh nylon gauze (see BIDDANDA 1985). Algal dissolved organic matter and particles <100 µm were aerated in 0.45 µm filtered seawater for 1 to 8 days at 15 °C until macroscopic aggregates were formed. Differently aged aggregates were offered as food to the copepods.

Copepods used: Healthy adult and late copepodid stages of the harpacticoid copepods *Paramphiascella vararensis* and *Tisbe holothuriae* from the first author's laboratory stock cultures were used in the feeding experiments (see RIEPER 1978). The sizes of adult *T. holothuriae* are about 930 µm for ♀♀ and 639 µm for ♂♂; adult *P. vararensis* average 780 µm for ♀♀ and 685 µm for ♂♂ (lenght without furcal setae).

Experimental conditions: Feeding experiments were performed at 18-20 °C in the laboratory in covered glass vessels containing 25-40 ml 0.45 µm filtered, autoclaved seawater, 30 ‰ S. For each treatment, 2-3 replicates were set up. Each replicate contained 5-20 copepods. The duration of the feeding experiments was 3-6 h. Depending on the density of the aggregates, the copepods received 0.5-5.0 ml suspension per vessel (about 0.1 mg dry weight). The food offered was in excess, i.e., some aggregates always remained at the end of the feeding period. Control vessels contained only copepods that had been fasting 12-18 h prior to start; another set of vessels contained copepods fed on laboratory fish food mixture (RIEPER 1978). At the end of an experiment, copepods were removed and fecal pellets were fixed, counted and measured under a dissecting microscope.

C:N analyses: C:N analyses were performed (with an Elemental Analyzer Model 1106, Carlo Erba Instruments) on the algal aggregates from *Ulva* sp., *Fucus serratus* and *Plocamium cartilagineum* 2 days and 8 days old, and on the fish food mixture.

Microbial colonization of fecal pellets: To examine the microbial colonization of fecal pellets with time, a stock culture of healthy *Tisbe holothuriae* consisting of a total of 20 ♂ and ♀ adults and late copepodid stages was kept in 40 ml filtered seawater with autoclaved fish food mixture in excess. (Total sterility was not the objective, but just to remove larger particles and outside contaminants). After 29 h at 20 °C, the fecal pellets produced were removed and transferred to watch glasses containing 5 ml 0.45 µm filtered seawater, and left at 20 °C. After intervals of 1, 2, 4, 7, 14, and 21 d, the pellets were fixed with formalin and examined, first with light microscopy and then DAPI-stained (PORTER and FEIG 1980, COLEMAN 1980).

Results and discussion

With increasing age, the algal aggregates formed by microbial action became

larger, and after 24 h were visible to the unaided eye. Microscopical examination of the aggregates offered to the copepods revealed many short rods present after 2 d; after 8 d longer rods and filaments were observed, occasionally with a few diatoms and microflagellates. The C:N ratios of 2 day old aggregates of *Plocamium, Fucus* and *Ulva* sp. were 5.9, 7.7 and 8.1, respectively. These values decreased after 8 d to 5.0, 7.0 and 5.8, respectively. The C:N ratio of fresh fish food mixture was 7.1.

Independently of the algal source and age of the aggregates ingested, *P. vararensis* produced 1-3 fecal pellets per individual h^{-1} at 18 °C (Fig. 1). In contrast,

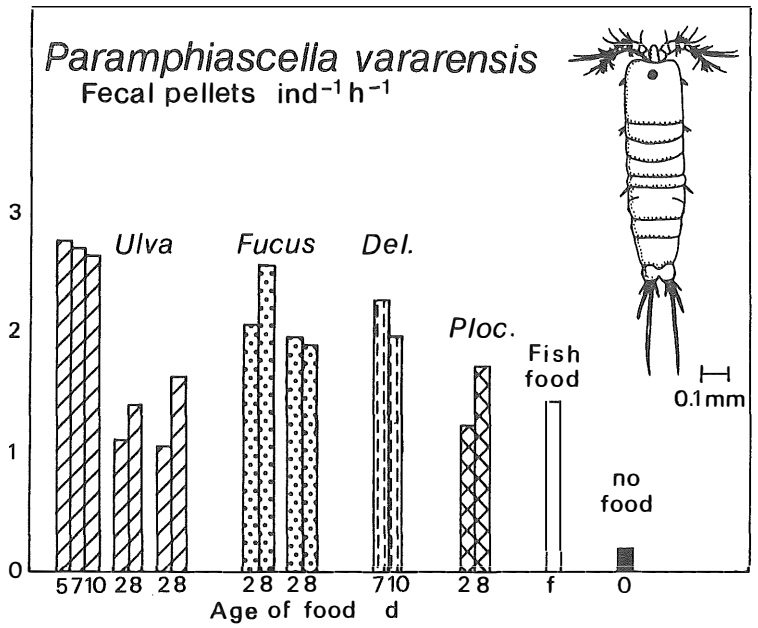

Fig. 1. *Paramphiascella vararensis:* number of fecal pellets produced per individual during short-term feeding experiments with macroalgal aggregates and fish food mixture at 18 °C.
Abbreviations: Del. = *Delesseria,* Ploc. = *Plocamium,* f = fresh.

during a single experiment with 7 day old aggregates only, *T. holothuriae* produced much greater numbers of pellets: with *Laminaria,* 4.8; *Fucus serratus,* 9.4; *Ulva lactuca,* 11.7; *Delesseria sanguinea,* 12.9; fish food mixture, <1 ind^{-1} h^{-1} (20 °C). The average sizes of the pellets produced during the feeding experiments are given in Table 1.

Microscopical examination of pellets produced by *T. holothuriae* fed with fish food mixture showed only very slight bacterial colonization of the pellet surface with some rods and cocci after 24 h. From 2-7 d, increasing numbers of large spirillae and curved rods appeared in the water but pellet colonization was still sparse. After 14-21 d, the dominant bacteria in the water were 4-6 µm long rods with fimbriae-like projections or slime strands. These completely colonized the

Table 1. Comparison of average sizes of fecal pellets produced by 2 harpacticoid copepod species fed with algal aggregates and fish food mixture.

Harpacticoid copepod species	Food source	Number of copepods	Number of fecal pellets	Average pellet size length [µm]	width [µm]
Paramphiascella vararensis	*Fucus serratus* aggregates	30	20	153	42
	Ulva sp. agg.	30	20	148	48
	Plocamium cartilagineum agg.	30	20	156	44
	Fish food mix.	ca 100	20	182	52
Tisbe holothuriae	Mixture of red algae + *Fucus* sp.	52	10	148	40
	Fish food mix.	10	20	109	39

pellet surface, and often appeared attached by their tips only. The pellet membrane was apparently still intact. Sickle-shaped bacteria were also common in the water but not on the pellets. The apparent bacteria-free zones surrounding the colonized pellets are as yet unexplained; this may be an artifact.

Although few harpacticoid species are known to feed directly on macroalgae (HICKS and COULL 1983), aggregates derived from macroalgal DOM and particulate matter were ingested both by *P. vararensis* and *T. holothuriae*. This finding agrees with earlier results of NAGEL et al. (1973) who showed that brackish water harpacticoids were able to grow on detritus from the green algae *Chara* and *Enteromorpha*. Detritus from other sources may also be utilized by harpacticoids (SCHUSTER et al. 1975, USTACH 1982) as well as the microbes associated with it (HEINLE et al. 1977). Microscopical examination of the algal aggregates in this study clearly showed that rapid microbial enrichment took place. Indeed, the dense bacterial colonization of aggregates visible after 2 days may partly account for the fact that these were ingested by *P. vararensis* as readily as aggregates aged 8 days. The decreasing C:N ratio of ageing aggregates is a commonly observed phenomenon in decaying macrophyte material (HARRISON and MANN 1975). This may be due not only to increased microbial protein but also to adsorption of dissolved nitrogen from the surrounding seawater onto microbial mucus (BIDDANDA and RIEMANN, ms. submitted). The C:N ratios for the algal aggregates are comparable to those for ageing macroalgal material in North Sea field studies (RIEPER-KIRCHNER 1989).

The relatively slow colonization of *T. holothuriae* pellets in the laboratory is in contrast to other observations on fecal material from copepods (FERRANTE and PARKER 1977, HONJO and ROMAN 1978, TURNER 1979, MARTENS and KRAUSE 1990) as well as from shrimps (JOHANNES and SATOMI 1966) and tunicates (POMEROY and DEIBEL 1980). This may be due partly to the lack of turbulence and the absence of ciliates and metazoans which may disrupt fecal pel-

lets (LAMPITT et al. 1990) and facilitate microbial colonization. Of interest are the large rod-shaped bacteria with fimbriae or slime strands seen on ageing fecal pellets in this study. Rod-shaped bacteria have been frequently associated with fecal material, in particular with the pellet membrane (FERRANTE and PARKER 1977, TURNER 1979, GONZÁLEZ and BIDDANDA 1990). The possible presence and role of internal bacteria within pellets as suggested by GOWING and SILVER (1983) was not examined.

Once the pellet membrane has been disrupted, dissolved contents may be released into the environment (JOHANNES and SATOMI 1966, HONJO and ROMAN 1978, ROY and POULET 1990) supporting growth of free bacteria and enhancing nutrient regeneration (GONZÁLEZ and BIDDANDA 1990). The flocculent, aggregate-like fecal material which remains, resembling marine snow, may be further colonized and broken down by bacteria, or provide food for other marine animals (coprophagy: JOHANNES and SATOMI 1966, FRANKENBERG et al. 1967, PFAFFENHÖFER and KNOWLES 1979), becoming feces again.

Thus the cycle is completed, which began with the formation of aggregates by bacterial action, their consumption by harpacticoid copepods, egestion as fecal pellets, and their return to the sea in the form of small aggregates and DOM (JOHNSON 1974, BIDDANDA and POMEROY 1988). The bacterial colonization of the pellet membrane, release of nutrients from pellet material, the formation of new aggregates and their consumption and egestion again constitute a "fecal pellet loop". These smaller pathways of carbon flow are all part of a much larger system. Epibenthic harpacticoid copepods, feeding on microbes and aggregates, are an important link in the detritus food web, being themselves consumed by fish larvae and smaller fish, which provide food for higher trophic levels (GEE 1989, ELLIS and COULL 1989, GRAINGER and HSIAO 1990). The "aggregate pathway" by which microbes mediate the incorporation of photosynthetically fixed energy into metazoan biomass, and the "fecal pellet loop" that returns part of this energy to the "aggregate pathway" in the sea, merits greater attention.

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References

ALLDREDGE, A.L. and M.W. SILVER, 1988. Characteristics, dynamics and significance of marine snow. Prog. Oceanog. 20, 41-82.

BIDDANDA, B.A., 1985. Microbial synthesis of macroparticulate matter. Mar. Ecol. Prog. Ser. 20, 241-251.

BIDDANDA, B.A. and L.R. POMEROY, 1988. Microbial aggregation and degradation of phytoplankton-derived detritus in seawater. I. Microbial succession. Mar. Ecol. Prog. Ser. 42, 79-88.

COLEMAN, A.W., 1980. Enhanced detection of bacteria in natural environments by fluorochrome staining of DNA. Limnol. Oceanogr. 25 (5), 948-951.

ELLIS, M.J. and B.C. COULL, 1989. Fish predation on meiobenthos: field experiments with juvenile spot *Leiostomus xanthurus* Lacépède. J. Exp. Mar. Biol. Ecol. 130, 19-32.

FERRANTE, J.G. and J.I. PARKER, 1977. Transport of diatom frustules by copepod fecal pellets to the sediments of Lake Michigan. Limnol. Oceanogr. 22 (1), 92-98.

FRANKENBERG, D., S.L. COLES and R.E. JOHANNES, 1967. The potential trophic significance of *Callianassa major* fecal pellets. Limnol. Oceanogr. 12 (1), 113-120.

GEE, J.M., 1989. An ecological and economic review of meiofauna as food for fish. Zool. J. Linnean Soc. 96, 243-261.

GONZALEZ, H. and B. BIDDANDA, 1990. Microbial transformation of metazoan (*Idotea granulosa*) faeces. Mar. Biol. 106, 285-295.

GOWING, M.M. and M.W. SILVER, 1983. Origins and microenvironments of bacteria mediating fecal pellet decomposition in the sea. Mar. Biol. 73, 7-16.

GRAINGER, E.H. and S.I.C. HSIAO, 1990. Trophic relationships of the sea ice meiofauna in Frobisher Bay, Arctic Canada. Polar Biol. 10, 283-292.

HARRISON, P.G. and K.H. MANN, 1975. Detritus formation from eelgrass (*Zostera marina* L.): the relative effects of fragmentation, leaching, and decay. Limnol. Oceanogr. 20, 924-934.

HEINLE, D.R., R.P. HARRIS, J.F. USTACH and D.A. FLEMER, 1977. Detritus as food for estuarine copepods. Mar. Biol. 40, 341-353.

HICKS, G.R.F. and B.C. COULL, 1983. The ecology of marine meiobenthic harpacticoid copepods. Ocean. Mar. Biol. Ann. Rev. 21, 67-175.

HONJO, S. and M.R. ROMAN, 1978. Marine copepod fecal pellets: production, preservation and sedimentation. J. Mar. Res. 36, 45-57.

JOHANNES, R.E. and M. SATOMI, 1966. Composition and nutritive value of fecal pellets of a marine crustacean. Limnol. Oceanogr. 11, 191-197.

JOHNSON, R.G., 1974. Particulate matter at the sediment-water interface in coastal environments. J. Mar. Res. 32 (2), 313-330.

LAMPITT, R.S., T. NOJI and B. v. BODUNGEN, 1990. What happens to zooplankton feacal pellets? Implications for material flux. Mar. Biol. 104, 15-23.

MARTENS, P. and M. KRAUSE, 1990. The fate of fecal pellets in the North Sea. Helgoländer wiss. Meeresunters. 44, 9-19.

NAGEL, H.-D., P. SPITTLER, J.-A. v. OERTZEN and R. KREUTZMANN, 1973. Benthogener Phytodetritus eutrophierter Küstengewässer als Energiequelle für Sekundärproduzenten. Wiss. Z. Univ. Rostock, Math.-Nat. Reihe 22, H. 10, 1129-1133.

PFAFFENHÖFER, G.-A. and S.C. KNOWLES, 1979. Ecological implications of fecal pellet size, production and consumption by copepods. J. Mar. Res. 37, 35-49.

POMEROY, L.R. and D. DEIBEL, 1980. Aggregation of organic matter by pelagic tunicates. Limnol. Oceanogr. 25 (4), 643-652.

PORTER, K.G. and Y.S. FEIG, 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 25 (5), 943-948.

RIEPER, M., 1978. Bacteria as food for marine harpacticoid copepods. Mar. Biol. 45, 337-345.

RIEPER-KIRCHNER, M., 1989. Microbial degradation of North Sea macroalgae: field and laboratory studies. Bot. Mar. 32, 241-252.

RILEY, G.A., 1963. Organic aggregates in seawater and the dynamics of their formation and utilization. Limnol. Oceanogr. 8 (4), 372-381.

ROBERTSON, M.L., A.L. MILLS and J.C. ZIEMAN, 1982. Microbial synthesis of detritus-like particulates from dissolved organic carbon released by tropical seagrasses. Mar. Ecol. Prog. Ser. 7, 279-285.

ROY, S. and S.A. POULET, 1990. Laboratory study of the chemical composition of aging copepod fecal material. J. Exp. Mar. Biol. Ecol. 135, 3-18.

SCHUSTER, E., P. SPITTLER and J.-A. v. OERTZEN, 1975. Untersuchungen über die trophische Bedeutung von Sedimentdetritus eutropher Küstengewässer. Wiss. Z. Univ. Rostock, Math.-Nat. Reihe 24, H. 6, 753-758.

TURNER, J.T., 1979. Microbial attachment to copepod fecal pellets and its possible ecological significance. Trans. Amer. Micros. Soc. 98 (1), 131-135.

USTACH, J.F., 1982. Algae, bacteria and detritus as food for the harpacticoid copepod, *Heteropsyllus pseudonunni* Coull and Palmer. J. Exp. Mar. Biol. Ecol. 64, 203-214.