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Metabolic activity and bioluminescence of oceanic faecal pellets and sediment trap particles

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Zooplankton faecal pellets are important in the vertical transport and exchange of elements in the oceanic ecosystem[1,2]. Although microbial, and in particular bacterial, processes have been implicated in the decomposition of aquatic faecal pellets[3–7], few quantitative data exist on the metabolic activities and growth characteristics of the associated microorganisms. As part of the Vertex (Vertical Transport and Exchange) programme, we studied these aspects using field-collected faecal pellets of known source and age. Our results, reported here, show that microbial biomass and metabolic activity are highest at the time of egestion, and decline with subsequent incubation. An independent observation from these experiments was the detection of bioluminescence in freshly excreted faecal pellets and in materials collected from sediment traps. These field data support an existing model for the ecological role of bacterial light emission in the mesopelagic zone of the ocean.

In the present study, freshly produced copepod faecal pellets were incubated in sterile filtered seawater in temperature-controlled conditions. Changes in ATP biomass, RNA and DNA synthesis rates (as measures of total microbial production and cell division) and bioluminescence were monitored with time. The data (Fig. 1) show that rates of RNA and DNA synthesis were highest at the time of egestion and decreased with subsequent ageing. Since nucleic acid synthesis rates are positively correlated with cellular biosynthesis[8–10], it can be concluded that both growth and cell division decreased with time. After 48 h, the RNA synthesis rate was 22% of the initial rate and the DNA synthesis rate had decreased to less than 3% of the original value. Two additional experiments, performed over 96 h, confirmed that microbial rate processes decreased with faecal pellet age. Total microbial biomass (as determined from faecal pellet ATP concentrations) was more variable among experiments. However, in no case was there evidence for an overall increase in the total microbial population during the incubation periods (up to 120 h). ATP values for fresh copepod faecal pellets in these experiments ranged from 70 to 1,156 ng per mg dry weight.

Although low temperatures have been related to decreases in metabolic activity and rates of faecal pellet degradation[5,11], there was no apparent response to temperature changes in our experiments. Pellets, aged at a constant temperature of 19° C, showed reductions in rates of RNA and DNA synthesis similar to those of faecal pellets aged as described in Fig. 1 legend.

The decreases we observed in the above experiments were unexpected as the present scenario for faecal pellet breakdown requires that the associated microbial populations exhibit rapid growth and division. Indeed, Honjo and Roman[4] observed that degradation of the surface membrane of laboratory-produced copepod pellets began within 3 h and was complete after 24 h. The implication that faecal pellets are colonized and degraded by external microbial populations is derived from time-course electron microscopic studies of pellet surfaces[3–5]. In our study, however, the high biomass, metabolic activity and luminescence (see below) associated with field-collected faecal pellets at the time of egestion indicated the presence and importance of gut-derived microflora. Furthermore, we were unable to detect any

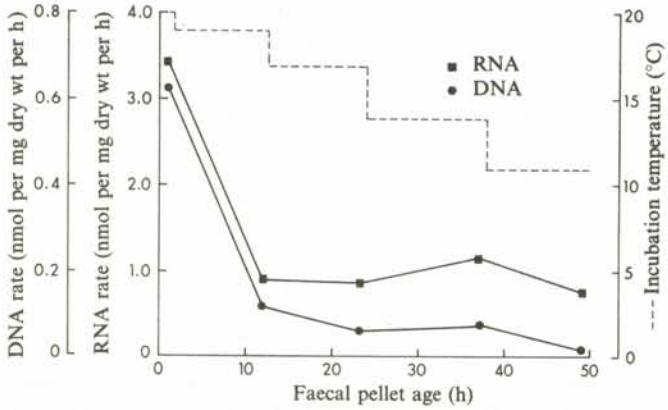


Fig. 1 Changes in rates of RNA and DNA synthesis as a function of faecal pellet age. Fresh faecal pellets from net-collected copepods were obtained by the method of La Rosa[29] as modified by Small *et al.*[13]. Known volumes of pellets, retained on a 68 μm Nitex net, were rinsed with distilled water, dried and weighed using a Cahn electrobalance. Faecal pellet material was also diluted 20-fold with 64 μm Nitex-filtered seawater and replicate 1-ml and 0.1-ml aliquots were incubated in small glass vials. The glass vials were moved through a temperature gradient with time (20–11 °C) to simulate the *in situ* conditions that sinking pellets would encounter. The rate at which the vials were rotated was determined by combining pellet sinking rate data[13] with the temperature regime of the water column. At selected times during the experiment, a 1-ml vial was incubated with 20 μCi ml^{-1} (final concentration) of [2-^{3}H]adenine (15.5 Ci $mmol^{-1}$; NEN) for 1 h, after which determinations of rates of RNA and DNA synthesis[30,31] and ATP[32] were made. Vials containing the 0.1-ml aliquots were filtered onto a combusted glass fibre filter and analysed for organic carbon, using a Perkin-Elmer CN analyser, throughout the incubation period. Faecal pellets were also aged in 0.22-μm Millipore-filtered seawater to assess contamination from planktonic bacteria. No differences in nucleic acid synthesis rates or ATP concentrations were observed between pellets incubated in 68 μm and in 0.22 μm-filtered seawater.

major differences between faecal pellets incubated in sterile filtered seawater and in unfiltered seawater. Recent transmission electron microscopic observations of thin sections from copepod faecal pellet interiors[7] also suggest that internal microbial communities have a major role in faecal pellet breakdown.

Oceanic zooplankton, grown in conditions of food limitation, are known to have higher assimilation efficiencies than coastal plankton or laboratory cultures maintained with high food concentrations[12]. Major differences between faecal pellets produced by the two groups have been noted[13]. Microbiological activity may similarly depend on the quality, compaction and composition of the egested pellets. Carbon analyses of our source-term pellets revealed that between 12 and 27% of the faecal pellet mass was organic carbon, which is in agreement with published data[4,5,14]. However, 50–100% of this organic carbon was living carbon as estimated from our ATP data (using an average C:ATP ratio of 250 (refs 15, 16)). It seems that reduced carbon sources may be insufficient to support the active growth and division of chemoheterotrophic microbial populations and that these organisms substantially reduce their metabolic activity soon after the pellets are voided. This is consistent with the previous observation that faecal pellets produced by euphausiids feeding on natural food sources can remain intact for weeks at temperatures as high as 18 °C (ref. 17).

In vivo bioluminescence, detected with a shipboard photometer, occurred in 70% of all samples surveyed including sediment trap-collected particulate matter, freshly collected zooplankton faecal pellets, crustacean molts, live animals (such as euphausiids, copepods, amphipods, etc.), and particulate detritus collected with plankton nets and water samplers. Long-term kinetics (s to min) revealed two distinct patterns of light emission, possibly reflecting various sources of luminescence. Erratic, luminous flashes predominated in the live animal and

Table 1 Vertical distribution of bioluminescence and ATP from particle interceptor traps (VERTEX II)

Depth (m)	Total sediment trap bioluminescence (10^5 photons cm^{-2} s^{-1})	Total sediment-trap ATP (μg)	Specific bioluminescence (photons cm^{-2} s^{-1} per pg ATP)
30	17.2	1.30 (0.296)	1.32
120	3.39 (0.024)	2.09 (0.172)	0.167
200	2.96 (0.041)	2.52 (0.235)	0.117
400	1.21 (0.022)	1.04 (0.094)	0.116
700	1.30 (0.023)	0.742 (0.161)	0.175
800	1.11 (0.009)	0.833 (0.067)	0.133
900	1.36 (0.020)	0.083 (0.025)	1.64
1,400	1.33 (0.027)	0.068 (0.002)	19.6
1,900	1.52 (0.008)	0.008 (0.010)	18.6

Free-floating MULTITRAP design particle traps[26] were deployed approximately 100 km off the western coast of Mexico (17°8′ N, 109°0′ W) from 27 October to 17 November 1981. The light emission (integrated over 1 min) from a known volume of sediment-trap material, was recorded using an SAI model 2000 ATP photometer set at maximum sensitivity. Mean values ($n = 3$) scaled to total trap volume are reported (±1 s.d.). The phototube detector was calibrated using a standard light source (Optronic Laboratories, Inc. lamp 245 C) and a US National Bureau of Standards traceable photodiode (United Detector Technology silicon photodiode, PIN 10DSB). The spectrum of light from the lamp was regulated by a Melles Griot wide-band filter (03F1B003) with peak transmission at 440 nm and full-width half maximum of 85 nm. The specific bioluminescence measured from a luminescent bacterial culture, *Photobacterium leiognathi* strain 721 (ref. 27), at maximum light intensity ($A_{660} = 0.54$) was 2.22×10^3 photons per cm^2 per s per pg ATP. Total sediment-trap ATP biomass was measured according to the methods of Karl and Knauer[28].

net plankton samples, whereas sediment-trap particles and faecal pellets characteristically showed a continuous and steady light output. The former response has been reported for certain species of dinoflagellates, copepods, euphausiids and planktonic larvae. The duration of these individual flash events is generally of the order of tens (or hundreds) of milliseconds[18–20]. On the other hand, light emission from luminescent bacteria is continuous, even in single cells, and exhibits neither flashes nor oscillations[21]. Our observations suggest that enteric marine luminous bacteria are responsible for the light emission from faecal pellets, and possibly from materials collected in the sediment traps also. Recent data from a subsequent Vertex field experiment, near the same site, confirm this hypothesis. Luminescent bacteria have been isolated from sediment-trap materials and source-term faecal pellets and the *in situ* light emission has been shown experimentally to be of bacterial origin (C.C.A. and D.M.K., in preparation).

The bioluminescence of particulate matter collected in sediment traps was greatest at 30 m, diminished rapidly to less than one-third of this value by 120 m, and gradually decreased to a constant level with depth (Table 1). Interestingly, when light emission per unit biomass is examined (that is, 'biomass-specific' luminescence), the depth profile exhibits a distinct increase below 800 m (Table 1); this may have been a direct effect of the endogenous control of *in vivo* bioluminescence resulting from specific changes in environmental conditions such as oxygen or nutrient concentrations[22,23]. The increase may also have been caused by an overall shift in the species composition favouring the succession of luminous bacteria at depth. A more likely explanation, however, is a direct relationship to the production and downward vertical flux of particulate matter. The observed increase in biomass-specific luminescence below 800 m correlates with the zone of enhanced biological activity and new particle production found just beneath the O_2 minimum (0.1–0.25 ml O_2 per litre). At 800 m, microbial rate processes associated with sediment trap-collected particles were maximal (D.M.K., unpublished) and within the water column, there was a substantial increase in the standing stock of nauplii and in total plankton dry weight (G. Knauer and M. Tuel, personal communication). Furthermore, stimulated *in situ* bioluminescence measured in the water column at the same station also showed increasing (but sporadic) activity below 700 m (ref. 24). These supplementary observations suggest that freshly produced pellets, at 800 m, may account for the greater particle bioluminescence at 900 m and below. It is conceivable that *in situ* measurements of bioluminescence, especially those which are associated with sinking particles, may be used for detecting the input of fresh organic detritus at intermediate oceanic depths.

The fact that copepod faecal pellets, as well as other particulate materials, are luminescent suggests an important relationship between feeding and light in the trophic structure of the mesopelagic realm. It also provides field support for the hypothesis that the functional importance of luminescence in marine bacteria[22,25] is related to the dispersion and propagation of bacterial species. If faecal pellet microbial populations are generally inactive once the pellets are voided into the water, as suggested by this work, then bacterial decomposition would be insufficient for remineralization to occur during descent of the pellets. Slow decomposition combined with rapid settling rates would result in the loss of large quantities of carbon and energy-rich particles to the benthos. Direct feeding on these sinking bioluminescent particles by organisms from higher trophic levels may ensure against such loss and facilitate the propagation of bacterial species.

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1. Turner, J. T. & Ferrante, J. G. *Bioscience* **29,** 670–677 (1979).
2. Urrere, M. A. & Knauer, G. A. *J. Plankton Res.* **3,** 369–387 (1981).
3. Ferrante, J. G. & Parker, J. I. *Limnol. Oceanogr.* **22,** 92-98 (1977).
4. Honjo, S. & Roman, M. R. *J. mar. Res.* **36,** 45–57 (1978).
5. Turner, J. T. *Trans. Am. microsc. Soc.* **98,** 131–135 (1979).
6. Azam, F., Cooper, N. & Jacobsen, T. *EOS, Trans. Am. geophys. Un.* **63,** 107 (1982).
7. Gowing, M. M. & Silver, M. W. *Mar. Biol.* **73,** 7–16 (1982).
8. Maaløe, O. & Kjeldgaard, N. O. *Control of Macromolecular Synthesis* (Benjamin, New York, 1966).
9. Pritchard, R. H., Barth, P. T. & Collins, J. in *19th Symp. Soc. gen. Microbiol.* 263–297 (Cambridge University Press, 1969)
10. Nierlich, D. P. *A. Rev. Microbiol.* **32,** 393–432 (1978).
11. Eppley, R. W. *Fish. Bull. (U.S.A.)* **70,** 1063–1085 (1972).
12. Gaudy, R. *Mar. Biol.* **25,** 125–141 (1974).
13. Small, L. F., Fowler, S. W. & Unlu, M. Y. *Mar. Biol.* **51,** 233–241 (1979).
14. Johannes, R. E. & Satomi, M. *Limnol. Oceanogr.* **11,** 191–197 (1966).
15. Holm-Hansen, O. & Booth, C. R. *Limnol. Oceanogr.* **11,** 510–519 (1966).
16. Karl, D. M. *Microbiol. Rev.* **44,** 739–796 (1980).
17. Small, L. F. & Fowler, S. W. *Mar. Biol.* **18,** 284–290 (1973).
18 Harvey, E. N. *Bioluminescence* (Academic, New York, 1952).
19. Eckert, R. in *Bioluminescence in Progress* (eds Johnson, F. H. & Haneda, Y.) 269–300 (Princeton University Press, Princeton, New Jersey, 1966).
20. LaPota, D. & Losee, J. R. *EOS, Trans. Am. geophys. Un.* **63,** 944 (1982).
21. Haas, E. *Biophys. J.* **31,** 301–312 (1980).
22. Hastings, J. W. & Nealson, K. H. *A. Rev. Microbiol.* **31,** 549–595 (1977).

23. Nealson, K. H. *Archs Microbiol.* **112,** 73–79 (1977).
24. Broenkow, W. W., Lewitus, A. J., Yarbrough, M. A. & Krenz, R. T. *Nature* **302,** 329–331 (1983).
25. Robison, B. H., Ruby, E. G. & Morin, J. G. *Abstr. A. Meet. West. Soc. Nat.* **55** (1977).
26. Knauer, G. A., Martin, J. H. & Bruland, K. W. *Deep-Sea Res.* **26,** 97–108 (1979).
27. Reichelt, J. L. & Baumann, P. *Int. J. syst. Bact.* **25,** 208–209 (1975).
28. Karl, D. M. & Knauer, G. A. *Deep-Sea Res.* (in the press).
29. La Rosa, J. *Deep-Sea Res.* **23,** 995–997 (1976).
30. Karl, D. M. *Appl. envir. Microbiol.* **42,** 802–810 (1981).
31. Karl, D. M., Winn, C. W. & Wong, D. C. L. *Mar. Biol.* **64,** 1–12 (1981).
32. Karl, D. M. & Craven, D. B. *Appl. envir. Microbiol.* **40,** 549–561 (1980).