Zooplankton Fecal Pellets Link Fossil Fuel and Phosphate Deposits

Abstract. *Fossil zooplankton fecal pellets found in thinly bedded marine and lacustrine black shales associated with phosphate, oil, and coal deposits, link the deposition of organic matter and biologically associated minerals with planktonic ecosystems. The black shales were probably formed in the anoxic basins of coastal marine waters, inland seas, and rift valley lakes where high productivity was supported by runoff, upwelling, and outwelling.*

Amorphous organic material from black shale associated with oil, coal, and phosphate deposits may provide a clue to the origin of these resources. We examined thinly bedded shale and phosphate nodule samples from five U.S. formations using palynological methods (*1, 2*). The deposits, from 11 to 300 million years old, represent diverse coastal, epicontinental sea, and lacustrine environments in which black shales are formed (*3*). We found that the acid-insoluble organic component, kerogen, contained fossilized zooplankton fecal pellets (*4*).

Samples of marine shale are from the Excello and Bandera Shale Members of the Sonora and Oologah Formations (Middle Pennsylvanian) of Oklahoma, Kansas, and Missouri; the Meade Peak Phosphatic Shale Member of the Phosphoria Formation (Lower Permian) in Idaho, Wyoming, Montana, and Colorado; and the Puente Formation (upper Miocene) in the Sante Fe Springs oil field in California. Lacustrine shale is from the Cumnock Formation (Upper Triassic) of the Deep River coalfield of North Carolina (*1, 2*).

In both thin sections and acid-insoluble residues of shale and nodule samples, the most abundant organic particles were cylindrical and subcylindrical pellets with average dimensions of 65 by 143 μm (Fig. 1, b to f). Particles identified as pellets have a length-to-width ratio of at least 2:1 and represent at least one in every 30 particles encountered in a scan of a microscope slide. The pellets constitute 2.5 to 62.6 percent by volume of the acid-insoluble organic component of the black shale samples and 2.0 to 8.2 percent by weight of the shale (Table 1). These are minimum estimates because of destruction in sample preparation and the counting of only intact pellets. The particles appear flattened because of compression and have thick centers and thin edges. Other microfossil remains are sparse, less than 1 percent by volume, and include algal tissue, acritarchs, spores and pollen, wood tracheids, fungal hyphae, and worm segments (*2*).

Chemical analyses of the shale and nodules (*5*) show high amounts of carbon, phosphorus, and nitrogen (Table 1). Organic carbon content ranges from 1.2 to 6.0 percent by weight; that of total nitrogen, from 0.09 to 0.29 percent; and that of total phosphorus, from 0.04 to 14.1 percent. Assuming that an average fresh fecal pellet contains 20 percent organic carbon, 4.5 percent nitrogen, and 1.7 percent phosphorus (*6*), we found that the intact pellet content accounted for 50 percent or more of these elements in 9 of our 15 analyses, making the pellets a possible major source of organic matter in the shales. The wide range of carbon-nitrogen-phosphorus ratios in the shale samples is consistent with the diverse ratios reported from samples of recent sediments receiving pellets as the primary source of these elements (*7*).

The textures, shapes, sizes, and length-to-width ratios of the fossil pellets are typical of modern zooplankton fecal pellets (Fig. 1a) such as those excreted by copepods, common members of planktonic ecosystems in lakes and oceans (*8*). Copepod pellets are smaller and more cylindrical than the coprolites of bottom-dwelling benthic polychaetes, molluscs, holothurians, and chironomids commonly identified in marine and lacustrine rocks (*9*). The fossil pellets closely resemble aged copepod fecal pellets that were produced by animals feeding on natural plankton and were partially eroded during natural sedimentation.

Zooplankton fecal pellets can contain intact and partially digested remains of algae, bacteria, and small zooplankton as well as detritus, minerals, and other particulate matter (*7, 8, 10*). Identifiable remains, shown in Fig. 1a, include algal and bacterial cells and cell walls, diatom frustules, cell membranes, chloroplasts, and exoskeletons of prey ingested by a

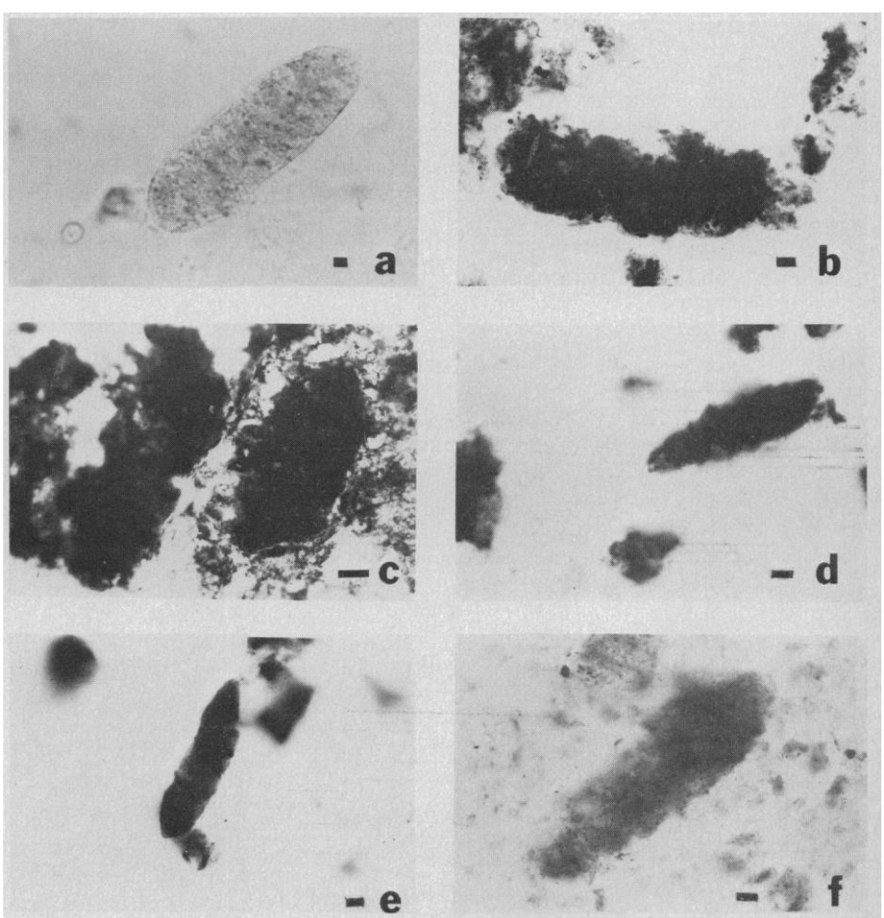

Fig. 1. Photomicrographs of a fresh copepod fecal pellet (a) showing partially digested algal remains surrounded by a peritrophic membrane. Fossil pellets are from the marine (b) Puente, (c) Phosphoria, (d) Excello, and (e) Bandera black shales and the lacustrine (f) Cumnock black shale; (b, d, e, and f) are from acid-insoluble shale residues, and (c) is from a thin section of shale. Scale bars are 10 μm.

0036-8075/81/0522-0931$00.50/0

freshwater copepod (*10*). Planktonic pellets can also contain clays and minerals of terrestrial origin and anthropogenic pollutants such as spilled oil, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and radionuclides (*7, 11, 12*).

Copepod fecal pellets are relatively large, protected, and rapidly sinking particles that can enhance the preservation and deposition of smaller particulate matter ingested by zooplankton. Copepod fecal pellets sink at rates of ten to several hundreds of meters per day depending on their size and content (*8*). The inclusion of heavier minerals and hard parts can ballast lighter organic components in the pellets (*12*). The pellets are compacted in the gut of the animal and are protected by a mucilaginous or chitinous peritrophic membrane, which also helps preserve the contents from biological, geochemical, or physical disruption and dissolution. Copepod pellets and fragments are the major particles that survive sedimentation and predominate in deep-sea sediment traps (*7*). They also preserve calcium carbonate skeletons of coccoliths to 5000 m, well below the compensation depth at which single carbonate skeletons would be dissolved (*12*). Pellets have been suggested as the source of carbonate grains in some Paleozoic sediments (*13*).

Preservation of pellets and of banding in sediments is best when benthic suspension and deposit feeders are excluded and microbial activity is reduced. This is achieved by inhospitable chemical environments in the water column above the sediments. Anoxia or hypersalinity will reduce or exclude benthic forms (*14*) and reduce microbial activity. These conditions must be maintained in permanent low-energy environments to limit biological activity that would otherwise destroy planktonic pellets and sediment banding even through brief periods of colonization and bioturbation. Suitable anoxic environments occur in hypersaline and deep tropical lake basins, in marine basins on shelves and in inland seas, and in marine-silled fjords, trenches, and canyons. Unsuitable environments are shallow nearshore areas subject to wave and storm disturbance, seasonally circulated and oxygenated lakes, oxygenated deep seas, and unproductive shelves. Suitable nonbasinal environments may also be found on shelves and slopes in highly productive upwelling regions, where high rates of deposition and burial occur and midwater oxygen minima intersect the sediment surface (*15*). These depositional environments are temporarily suitable although their permanence over geologic time is questionable.

We propose model environments for the deposition of the pellets on the basis of modern environments in which pellet-rich, thinly banded sediments are being deposited today. The key factors required in the models are a high rate of pellet deposition for long periods of time under conditions suitable for the preservation of both pellets and banding in the sediments. Pellet deposition is greatest when production rates are high and when large numbers of pellets are preserved after sinking through the water column. Preservation in the sediments is greatest in low-energy, anoxic, silled, or closed basins. Thick deposits of sediments that have pellets and thin banding indicate deposition in anoxic basins over long periods of time. This is contrary to models for phosphate, black shale, and epicontinental sea deposits which propose brief cataclysmic events or deposition in shallow aerobic systems (*3, 16*).

We propose a coastal upwelling model for the environment that produced the Puente Formation, with deposition in permanently anoxic basins with banded sediments analogous to those in basins off the coast of southern California and Mexico today (*13*). The Excello and Bandera shales were formed in epicontinental seas fed in part by outwelling from the rich coastal wetlands. This is supported by evidence of a shift from primarily humic organics and terrestrial microfossils in the eastern, landward extent of the Excello shale to increasing algal remains and hydrocarbons in its western, seaward extent (*17*). Our evidence suggests that the Cumnock Formation was formed in a deep, large anoxic lake system similar to the African rift lakes (*18*), where the water column is permanently stratified due to climatic thermal stability or hypersalinity. The Phosphoria Formation has been ascribed to the intersection of a midwater oxygen minimum caused by upwelling on a shelf or slope; however, we prefer to derive at least some of the nutrients from the phosphate-rich Precambrian Belt Series (*19*).

Pellet microfossils link the deposition of organic matter with that of elements such as phosphorus, nitrogen, and trace metals commonly associated with organisms. The diversity of particulate matter

Table 1. Zooplankton fecal pellet abundances and organic chemistry of selected samples of black shale associated with coal, oil, and phosphate deposits.

Sample	Pellet content		Organic chemistry (% by weight)*			Pellet contribution to sample (%)†		
	Percent volume of kerogen	Percent by weight	Organic carbon	Total nitrogen	Total phosphorus	Organic carbon	Total nitrogen	Total phosphorus
Puente (upper Miocene)								
Shale 1	26.8	3.6	3.5	0.20	0.11	20.6	81	55.6
Shale 2	22.9							
Cumnock (Upper Triassic)								
Shale 1	5.75	7.0	1.2	0.15	0.11	117	210	109
Phosphoria (Permian)								
Shale 1	62.6							
Shale 2	54.0							
Shale 3	10.0							
Shale 4	54.8							
Shale 5	31.3							
Shale 6	5.0							
Shale 7	30.0							
Shale 8‡	19.6	8.2	3.7	0.11	13.80	44.3	335	1.0
Shale 9	36.9							
Shale 10	2.5							
Shale 11	10.9							
Shale 12	13.6							
Shale 13	6.9							
Excello (Middle Pennsylvanian)								
Shale 1	33.4							
Nodule 1	21.5							
Bandera (Middle Pennsylvanian)								
Shale 1	9.0	3.5	6.0	0.29	0.04	11.7	54	149
Nodule 1	18.5	2.0	4.3	0.09	14.10	9.3	100	0.2

*The carbon-nitrogen-phosphorus ratios for the five chemically analyzed samples are 32:1.8:1, 10.9:1.4:1, 0.27:0.01:1, 150:7.3:1, and 0.30:0.01:1, respectively. †Values greater than 100 percent indicate that the pellet content of the shale can account for more of the organic component than actually remained in the shale. ‡A pisolitic, nodular shale.

in planktonic environments coupled with biological processes of selective feeding, digestion, and degradation during pellet formation and deposition, provide an array of organic compounds as diverse as the ecosystems that produced them. This chemical diversity of potential petroleum precursors can explain, in part, the variety of hydrocarbon compounds found in crude oils. The pellets are also a major source of phosphate that later is available to precipitate into nodules. The specific environments in which pellet-rich banded sediments are being produced today may provide depositional stratigraphic models for future fossil fuel and mineral resource discovery and for the simulation of hydrocarbon-producing food chains.

KAREN GLAUS PORTER
Department of Zoology and
Institute of Ecology,
University of Georgia, Athens 30602
ELEANORA IBERALL ROBBINS
U.S. Geological Survey,
Reston, Virginia 22092

References and Notes

1. Samples are from the Bandera Shale Member of the Oologah Formation and the Excello Shale Member of the Senora Formation collected from roadcuts east of Tulsa, Oklahoma; the Meade Peak Phosphatic Shale Member of the Phosphoria Formation collected from Mabie Canyon, Snowdrift Mountain, and Bloomington Canyon, Idaho, and Sublette Ridge and Coal Canyon, Wyoming; the Cumnock Formation collected from BMDH D-1 core hole from a depth of 156.7 m, north of Cumnock, North Carolina; and the Puente Formation collected from Union Oil Company well, Bell 107, in two samples between 3831 and 3957 m, Santa Fe Springs, California. For quantitative analysis, 10-g pieces of whole shale and nodules were treated at 22°C with 10 percent HCl for 24 hours, rinsed three times with distilled water, settled by gravity, and treated with 50 percent hydrofluoric acid for 24 hours. Samples were then rinsed five times with distilled water and passed through a 125-μm sieve and then a 25-μm sieve. Subsamples of the microfossil fraction were measured and counted at magnifications of 100 to 1000. Standard use of hot acid, centrifugation, hydroxide, and Shultze solution will destroy pellet remains. Thin sections of shale samples were examined qualitatively. The complete technique is described by E. I. Robbins and A. Traverse, in *Carolina Geologic Society Guidebook* (Savannah River Laboratory, Aiken, S.C., 1980), section B, p. 1.
2. Details of microfossil analyses, reconstructions of the paleoenvironments, and the economic importance of the formations are described by E. I. Robbins and K. G. Porter, in preparation.
3. H. A. Tourtelot, *Clays Clay Miner.* **27**, 313 (1979).
4. E. I. Robbins and K. G. Porter, *Palynology* **4**, 30 (1979).
5. Standard chemical analyses were performed by N. G. Skinner and Z. A. Hamlin, U.S. Geological Survey. The percent by weight of the intact pellet in the total sample was calculated by weighing the microfossil fraction (> 25 μm) and multiplying this weight by the intact pellet percent by volume.
6. R. E. Johannes and M. Satomi, *Limnol. Oceanogr.* **11**, 191 (1966).
7. S. Honjo, *J. Mar. Res.* **38**, 53 (1980).
8. J. T. Turner and J. C. Ferrante, *BioScience* **29**, 670 (1979); S. Honjo and M. R. Roman, *J. Mar. Res.* **36**, 45 (1978).
9. H. B. Moore, *J. Mar. Biol. Assoc. U.K.* **17**, 325 (1931); *ibid.*, p. 359; A. J. Iovino and W. H. Bradley, *Limnol. Oceanogr.* **14**, 898 (1969).
10. K. G. Porter, *Verh. Int. Ver. Theor. Angew. Limnol.* **19**, 2840 (1975); *Science* **192**, 1332 (1976); *Am. Sci.* **65**, 159 (1977); S. A. Poulet, *Limnol. Oceanogr.* **23**, 1126 (1978).
11. R. Conover, *J. Fish. Res. Board Can.* **28**, 1327 (1971); F. G. Prahl and R. Carpenter, *Geochim. Cosmochim. Acta* **43**, 1959 (1979); J. R. Syvitski and A. G. Lewis, *J. Sediment. Petrol.* **50**, 869 (1980).
12. S. Honjo, *J. Mar. Res.* **36**, 469 (1978).
13. H. B. Moore, in *Recent Marine Sediments*, D. D. Trask, Ed. (American Association of Petroleum Geologists, Tulsa, Okla., 1939), p. 516; D. E. Hattin, *J. Sediment. Petrol.* **45**, 1 (1975); A. Soutar, S. A. Kling, P. A. Crill, D. Duffrin, K. W. Bruland, *Nature (London)* **266**, 136 (1977); G. Knauer, J. H. Martin, K. W. Bruland, *Deep-Sea Res.* **26A**, 97 (1979); B. Hargrave, in *Marine Benthic Dynamics*, K. R. Tenore and B. C. Coull, Eds. (Belle W. Baruch Library in Marine Sciences, Columbia, S.C., No. 11, 1980), p. 243.
14. D. C. Rhoads and J. W. Morse, *Lethaia* **4**, 413 (1971).
15. G. J. Demaison and G. T. Moore, *Org. Geochem.* **2**, 9 (1980).
16. M. Brongersma-Sanders, *Mar. Geol.* **11**, 123 (1971); A. P. Bennison, *Tulsa Geol. Soc. Dig.* **37**, 1125 (1972); R. Zangrel and E. S. Richardson, Jr., *Fieldiana Geol. Mem.* **4**, 1 (1963).
17. M. M. Cassidy, *Bull. Am. Assoc. Pet. Geol.* **52**, 295 (1968); G. W. James and D. R. Baker, *Kans. State Geol. Surv. Bull.* **204**, 3 (1972).
18. P. E. Olsen, C. L. Remington, B. Cornet, K. S. Thomson, *Science* **201**, 729 (1978).
19. R. P. Sheldon, *U.S. Geol. Soc. Prof. Pap. 313-B* (1963), p. 1; R. A. Gulbrandsen, *ibid. 550-D* (1966), p. D-199; V. E. McKelvey, *Bull. U.S. Geol. Surv. 1252-D* (1967); E. K. Maughan, in *Proceedings of the International Conference of Comparative Geology of Phosphate and Oil Deposits* (Bureau de Recherches Geologiques et Minieres, Orleans, France, 1980), p. 63.
20. We thank V. E. McKelvey, G. L. Bain, L. E. Eames, and T. H. McCulloh for samples and C. W. Byers, R. R. Cohen, E. R. Force, S. Honjo, G. E. Hutchinson, T. M. Kehn, M. L. Pace, J. S. Patton, J. W. Porter, D. Rhoads, T. M. Schopf, S. Stanley, and G. A. Younse for their comments on the manuscript. Contribution No. 6 of the Lake Oglethorpe Limnological Association.

21 January 1981

Didemnins: Antiviral and Antitumor Depsipeptides from a Caribbean Tunicate

Abstract. *Extracts of samples of a Caribbean tunicate (ascidian, sea squirt) of the family Didemnidae inhibit in vitro at low concentrations the growth of DNA and RNA viruses as well as L1210 leukemic cells. The active compounds isolated from the tunicate, didemnins A, B, and C, are depsipeptides, and didemnin B (a derivative of didemnin A) is the component active at the lowest concentration in inhibiting viral replication in vitro and P388 leukemia in vivo.*

We have isolated from a Caribbean tunicate a new class of depsipeptides, including highly active antiviral and antitumor agents (*1*). Although these depsipeptides—termed didemnins after the name of the tunicate family from which they are isolated—are closely related to one another, they vary in activity, suggesting the possibility of further chemical modification. This discovery confirms our earlier observations (*2*, *3*) that the subphylum Tunicata or Urochordata (phylum Chordata) is of special interest both for the chemistry and for the bioactivity of the compounds tunicates contain (*4*, *5*).

The tunicate in our study was collected at a number of sites (including Colombian, Honduran, Mexican, Belizean, and Panamanian waters) during the *Alpha Helix* Caribbean Expedition 1978 (AHCE 1978) (*3*). It has been assigned (*6*) to the family Didemnidae and is a member of the *Tridemnum* genus. Repeated tests of methanol-toluene (3:1) extracts of the didemnid on shipboard against herpes simplex virus, type 1, grown in CV-1 cells (monkey kidney tissue) indicated that it inhibited the growth of the virus, over and above an underlying cytotoxicity to the CV-1 cells. This result suggested that compounds in the tunicate extract offered promise both as antiviral agents and,

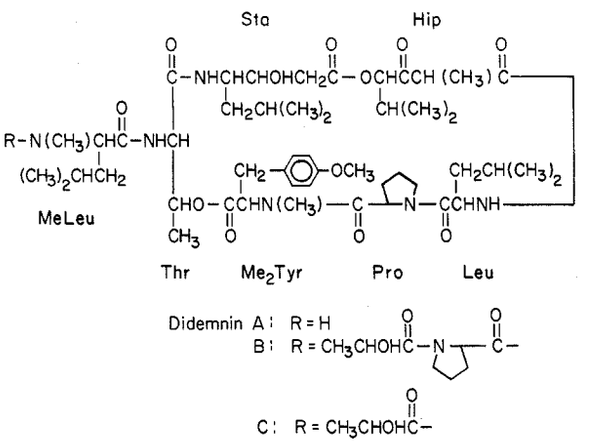

Fig. 1. Structures of didemnins.

0036-8075/81/0522-0933$00.50/0