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Bacterivory: A Novel Feeding Mode for Asteroid Larvae

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Planktotrophic larvae that occur beneath the annual sea ice in the Antarctic assimilate organic solutes and preferentially ingest bacteria, whereas they actively exclude phytoplankton. In regions where phytoplankton biomass is temporally limited by light or nutrient concentrations, the growth and development of planktotrophic larvae may not be directly coupled to phytoplankton production.

ECHINODERM LARVAE HAVE A COSMOPOLITAN distribution (*1*). They can ingest phytoplankton, bacteria, and detritus (*2–4*) as well as directly absorb dissolved organic solutes (*5, 6*). However, laboratory studies show that microalgae are the preferred food source for larvae of some tropical and temperate echinoderm species (*2, 3*). Food availability influences larval survivorship, and it has been suggested that low phytoplankton biomass, typical of some oceanic areas, may be insufficient to support the rapid growth and development of planktotrophic echinoderm larvae (*7, 8*). The tendency for increased brooding and lecithotrophic development at high latitudes (*1, 9*) has been attributed to the short growing season that results in low phytoplankton production and the slow developmental rates of the larvae (*10*). These conditions are thus unfavorable for planktotrophic larvae if they depend on phytoplankton as a primary food source. Echinoderms are a major component of the benthos in the Antarctic (*11*). Their planktonic larvae were first described almost 80 years ago (*12*), yet their reproduction and development have been examined only recently (*13*). In this report we describe the nutrition and feeding behavior of the larvae of the antarctic asteroid *Porania antarctica*. These larvae assimilated amino acids and glucose and ingested exclusively bacteria; they actively excluded as a food source all of the algal species that we tested. Field-collected larvae of other species also ingested bacteria as a source of food. These results suggest that the development of feeding larvae in the light-limited environment under the annual sea ice (*14, 15*) is not coupled directly to phytoplankton production.

Experiments were carried out in McMurdo Sound, Antarctica, during the 1985 austral spring (*16*). Ambient concentrations of amino acids and glucose but not of thymidine (TdR) were directly assimilated by *P. antarctica* (*17*). Assimilation rates of the amino acid mixture and glucose were significantly greater than that of leucine and glutamic acid (Table 1). These larvae were not treated with antibiotics (*6*). However, (i) the absence of direct [^{3}H]TdR uptake by the larvae (or gut-associated bacteria) and (ii) the negligible amount of filter-retained radioactivity (0.22 μm) (Table 1) imply that the washing procedure with sterile, filtered seawater removed whatever bacteria that may have adhered to the surface of the larvae (*18*). Thus the larvae mediated the uptake of these radioactive organic solutes.

Although particulate material was routinely observed within the gut of bipinnaria and brachiolaria larvae of *P. antarctica* that were fed bacterized algal cultures, the material did not exhibit chlorophyll a autofluorescence when examined by epifluorescence microscopy, and chlorophyll a was not detected in acetone extracts of the larvae. The ingestion of bacteria (bacterivory) and algae from natural populations and from bacterized cultures representing three classes of algae was assessed by dual labeling with [^{3}H]TdR and ^{14}C-labeled bicarbonate (*19*). Larvae of *P. antarctica* ingested ^{3}H-labeled particles exclusively. Particles labeled with ^{14}C were not ingested even at algal concentrations up to 35 μg of chlorophyll a per liter (Table 2). Clearance rates for *P. antarctica* that were reared in the laboratory and fed bacterized algal cultures were <0.01 μl per larva per hour and 43 to 70 μl per larva per hour for ^{14}C-labeled and ^{3}H-labeled particles, respectively. When *P. antarctica* fed on natural planktonic populations, clear-

Table 1. Incorporation of ^{3}H-labeled organic solutes by larvae of *P. antarctica*. L-Leucine (2, 3, 4, 5-^{3}H), L-glutamic acid (3, 4-^{3}H), the amino acid mixture (algal protein hydrolysate contained 8% L-alanine, 7% L-arginine, 8% L-aspartic acid, 12.5% L-glutamic acid, 4% glycine, 1.5% L-histidine, 5% L-isoleucine, 14.0% L-leucine, 6% L-lysine, 8% L-phenylalanine, 5% L-proline, 4% L-serine, 5% L-threonine, 4% L-tyrosine, and 8% L-valine), and D-glucose (6-^{3}H) were added at about 1.0 μCi ml^{-1} and thymidine (methyl-^{3}H) at about 0.1 μCi ml^{-1} final activity. Details of the experiment are in (*17*). The in situ concentrations of amino acids and glucose were not measured; however, previous studies (*30, 31*) have reported 0.1 to 5.0 μmol of dissolved free amino acids per liter. Glucose concentrations of 0.01 to 1.0 μmol liter^{-1} have been reported in the Sargasso Sea [R. E. Vaccaro, S. E. Hick, H. W. Jannasch, F. L. Carey, *Limnol. Oceanogr.* **13**, 356 (1968)]. Assimilation rates were calculated on the assumption that amino acid and glucose concentrations were 1.0 μmol liter^{-1} and 0.01 μmol liter^{-1}, respectively. Since minimum concentrations were used in these calculations, assimilation rates are conservative estimates. NS, not significant; radioactivity in larvae was not significantly greater than background radiation.

Substrate	Solute assimilation		Filter retained†
	Disintegrations per minute per larva*	Picogram per larva per hour	Disintegrations per minute per milliliter
L-Leucine	121,299 ± 15.6%, n = 8	31.5 ± 4.9	59
L-Glutamic acid	13,430 ± 13.5%, n = 9	19.8 ± 2.6	48
Amino acid mixture	100,783 ± 7.8%, n = 8	720 ± 56	52
D-Glucose	123,420 ± 13.4%, n = 9	238 ± 31	49
Thymidine	53 ± 18.2%, n = 9	NS	58

*Radioactivity accumulated by *P. antarctica* during a 4-hour incubation. Each value is the mean ± coefficient of variation. Background radioactivity was about 45 dpm. †Radioactivity in the 0.22-μm to 64-μm filtrate of the larval suspension. This represents potential bacterial contamination within the incubation container. Details in (*17*).

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ance rates were 228 μl per larva per hour for ^{3}H-labeled particles and were <0.01 μl per larva per hour for ^{14}C-labeled ones (Table 2). Similar high ingestion rates of ^{3}H-labeled particles were found for field-collected echinoderm, polychaete, and nemertean larvae (Table 3) that fed on natural plankton populations (*20*). Since [^{3}H]TdR was not taken up directly by *P. antarctica* (Table 1), the ^{3}H activity within these larvae was derived from ingesting ^{3}H-labeled particles rather than by bacteria within their gut assimilating [^{3}H]TdR. During short incubations, [^{3}H]TdR is assimilated primarily by bacteria (*18, 21*), and ^{14}C-labeled bicarbonate is taken up by autotrophic algae when there is sufficient light. The techniques used in these experiments had the sensitivity to detect the ^{14}C-uptake by a single algal cell (Table 2); thus the absence of ^{14}C in the larvae suggests that only bacteria were ingested (*22*). Similar experiments in which identical techniques were used in temperate and tropical regions typically showed significant and often preferential ingestion of ^{14}C-labeled particles by copepods, ciliates, and heterotrophic dinoflagellates (*23*).

Table 2. Clearance rates for larvae of *P. antarctica* fed bacterized algal cultures and natural plankton population labeled with [^{3}H]TdR and ^{14}C-bicarbonate [details of the experiment in (*19*)]. Radioactivity of the prey was calculated by dividing the particulate ^{3}H and ^{14}C activity by the bacterial and algal abundances, respectively. Each value is the mean ± standard deviation; $n = 8$ (clearance rates) or $n = 4$ (radioactivity of prey). NS, radioactivity in larvae was not significantly greater than background radiation. ND, not determined.

Food source	Clearance rates (μl per larva per hour)		Radioactivity of prey	
	^{3}H	^{14}C	^{3}H*	^{14}C†
Isochrysis galbana	50 ± 8.0	<0.01	3.3 ± 1.1	3.5 ± 0.09
Dunaliella tertiolecta	43 ± 6.8	NS	3.0 ± 0.29	1.7 ± 0.18
Phaeodactylum tricornutum	70.2 ± 16	NS	3.9 ± 0.91	4.2 ± 0.64
Natural population	228 ± 49	<0.01	5.3 ± 1.1	ND

*10^{-3} disintegrations per minute per cell. †Disintegrations per minute per cell.

Temperate and tropical echinoderm larvae can feed on a broad-sized spectrum of suspended particles. Although 4- to 6-μm particles are captured most efficiently, those <2 μm can be ingested when they are abundant (*2–4*). In nature, most free-living bacteria are <1.0 μm. However, bacteria often occur in aggregates of ≥3 μm (*24*) that can be more efficiently grazed by echinoderm larvae such as those studied here. Echinoderm larvae can preferentially ingest specific species of algae from mixed algal cultures and exclude particles that are too large or are unsuitable as food (*3*). Generally, particles are carried into the buccal cavity by water currents propagated by cilia; the particles are then transported into the gut by cilia within the circumoral field. In *P. antarctica*, the ciliary action may have been inadequate to transport larger algal cells into the gut. This pattern of preferential ingestion is unexpected, considering previous nutritional studies (*2, 3, 8*).

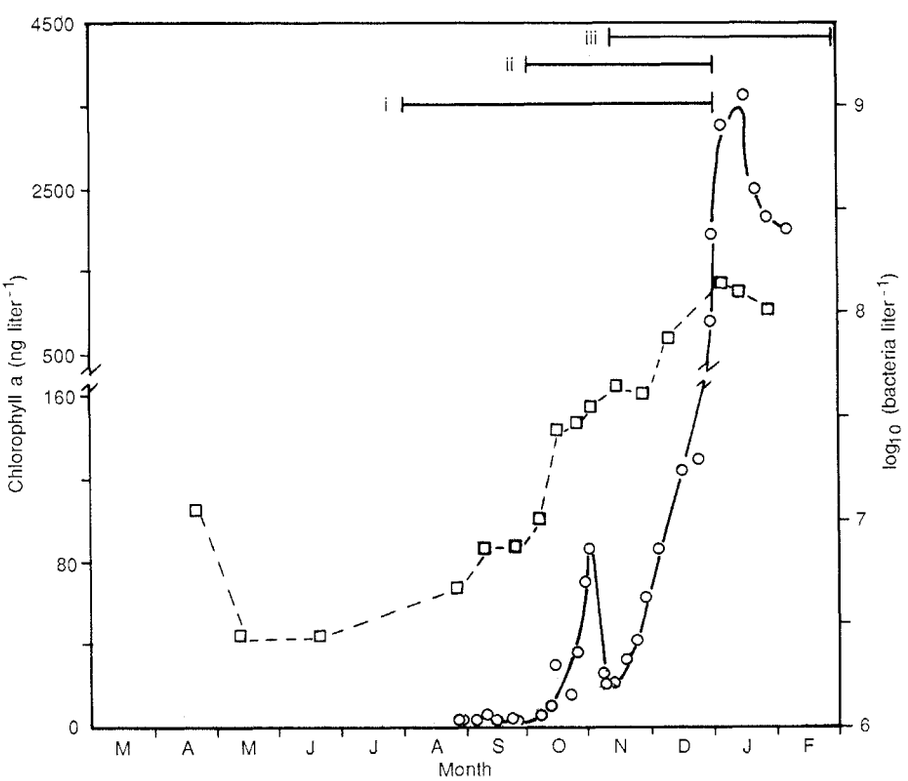

Fig. 1. Seasonal pattern of chlorophyll a, ○; bacteria, □; and the presence of the feeding stages of asteroid larvae—(i) *O. validus* and *O. meridionalis*, (ii) *P. antarctica*, and (iii) *S. neumayeri* in the plankton. Nemertean larvae were found in the plankton year-round. Chlorophyll a and bacterial data were collected during the 1985 (January and February) and 1985–1986 (August–January) field seasons for stations 10 to 20 km north of Cape Armitage. In April, May, and June 1985 samples of bacteria were collected by scuba divers near Cape Armitage by enclosing water samples in plastic bottles. These bacterial samples were preserved in 2% Formalin for up to 6 months before counting.

Growth and development of echinoderm and other larvae appear to be limited by the availability of food in some temperate coastal and tropical regions; typical chlorophyll a concentrations of 0.5 to 4 μg liter^{-1} appear to be inadequate to maintain development (*2, 7, 8*). At high latitudes, where the abundance of phytoplankton is highly seasonal, nonplanktotrophic larval development prevails (*9, 10*). Moreover, the slow developmental rates of species with feeding larvae can uncouple the planktonic feeding stage from the shortened phytoplankton bloom (*10, 12, 13*). In McMurdo Sound, the concentration of chlorophyll a is typically 10 to 100 ng liter^{-1} when *P. antarctica*, *Odontaster* spp., and feeding larvae of other echinoderm are in the water column (Fig. 1). These planktotrophic larvae develop when phytoplankton concentrations are up to about two orders of magnitude less than the amount that is considered limiting (*7, 8, 25*). In late December, when fewer feeding larvae are present (Fig. 1), large concentrations (1 to 6 μg of chlorophyll a per liter) of the prymnesiid *Phaeocystis* sp. are delivered from offshore waters into McMurdo Sound (*14, 15*). However, *Phaeocystis* is not a suitable food for many invertebrates (*26*). Since larvae feed most efficiently on nanoplankton-sized particles (*3*), proper size distributions as well as concentrations of phytoplankton are critical for their successful development. In McMurdo Sound, 70 to 80% of the chlorophyll a is >10 μm (*27*). In contrast, nanoplankton represent 80 to 95% of the phytoplankton biomass in most oligotrophic and oceanic environments (*28*).

Table 3. Ingestion rates for echinoderm, polychaete, and nemertean larvae collected from beneath the annual sea ice in McMurdo Sound and fed natural populations labeled with [^{3}H]TdR and ^{14}C-bicarbonate [details in (*19, 20*)]. NS, radioactivity in larvae was not significantly greater than background radiation.

Larvae	Clearance rate (μl per larva per hour)	
	^{3}H	^{14}C
Echinoderm		
Sterechinus neumayeri; echinopluteus	828 ± 281* $n = 4$	NS
Odontaster sp.; bipinnaria	593 (548–638)† $n = 2$	NS
Polychaete		
Unidentified post-trochophore	94.2 ± 2* $n = 4$	NS
Nemertean		
Parbolasia corrugatus	1087 (943–1231)† $n = 2$	NS

*Mean ± standard deviation. †Average and range.

Since (i) prolonging a planktonic phase of development typically leads to increased mortality and (ii) the persistently low phytoplankton concentrations of the appropriate sizes would significantly reduce rates of development (*29*), these echinoderm larvae ingest bacteria and assimilate organic solutes as primary nutritional sources. Unlike phytoplankton, bacterial abundances were relatively high (10^6 to 10^8 liter^{-1}) (Fig. 1) and constant throughout the year studied, and dissolved amino acid concentrations were 0.1 to 5.0 μmol liter^{-1} (*30, 31*) when larvae in the feeding stage were in the plankton. Thus these larvae have apparently evolved to use temporally conservative nutritional resources, such as bacteria.

The relatively constant environmental conditions and physiological adaptations of shallow-water benthic organisms in the Antarctic are similar to those in the deep sea (*31, 32*). Planktotrophic echinoderm larvae in McMurdo Sound during the austral winter and spring and in the deep sea would be exposed to similar nutritional environments (*33*); therefore, similar nutritional modes may be expected for these larvae.

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16. Adult *P. antarctica* collected on the western side of McMurdo Sound were induced to spawn by repeated injections with 1-methyladenine [M. Stevens, *J. Exp. Cell. Res.* **59**, 481 (1970)]. Embryos and larvae were reared in 4-liter containers provided with 5-μm of filtered seawater at about −1.0°C; they were fed with bacterized cultures of *Isochrysis galbana* and *Phaeodactylum tricornutum*. All experiments were done with brachiolarian larvae.
17. Larvae reared as described in (*16*) were serially transferred through three washes of sterile filtered seawater (SFSW) (0.22-μm membrane filter). After 3 to 6 hours of incubation in SFSW the larvae were transferred through an additional three SFSW washes and then placed into replicate sterile incubation tubes. Larvae were incubated at −1.0°C in the dark with ^{3}H-labeled amino acids, glucose, or thymidine (ICN Radiochemical) at a final activity of about 0.1 to 1 μCi per milliliter. The addition of 8 to 20 nmol of organic substrate per liter of radioisotope carrier solution did not significantly alter ambient concentrations. After about 4 hours, larvae were collected on 64-μm nylon screening and the initial filtrates were retained. The larvae were rinsed five times with seawater, resuspended, transferred through three serial washes of SFSW, and placed in liquid scintillation vials. Larvae were digested for 12 hours in 0.3 ml of 0.2*N* perchloric acid and radioactivity was counted by using Biofluor (New England Nuclear) as a scintillant. Larval controls killed with mercuric chloride (1% final concentration) did not show net uptake. The influx of a radioactive substrate is equivalent to net uptake (*6*) [G. L. Stephens, M. J. Volk, S. H. Wright, P. S. Blacklund, *Biol. Bull. (Woods Hole, Mass.)* **154**, 335 (1978); D. T. Manahan and D. J. Crisp, *Am. Zool.* **22**, 635 (1982)]. To detect bacterial uptake of the organic substrates within the larval suspension, portions of the 64-μm filtrate were collected on 0.22-μm membrane filters, and the radioactivity was counted as above.
18. During short incubations only heterotrophic bacteria take up [^{3}H]TdR [J. A. Fuhrman and F. Azam, *Appl. Environ. Microbiol.* **39**, 1085 (1980); *Mar. Biol.* **66**, 109 (1982); J. T. Hollibaugh, J. A. Fuhrman, F. Azam, *Limnol. Oceanogr.* **25**, 172 (1980)].
19. The ingestion of algae and bacteria was measured by the method of M. R. Roman and P. Rublee [*Mar. Biol.* **65**, 303 (1980)] as modified by E. J. Lessard and E. Swift, in (*23*). Plankton samples collected from McMurdo Sound by vertically towing a 20-μm aperture plankton net (diameter, 0.5 m) were filtered through a 103-μm aperture screen and were combined with an equal volume of unmodified seawater. Bacterized cultures of *Dunaliella tertiolecta, I. galbana*, and *P. tricornutum* were grown in half-strength F-enriched [R. R. L. Guillard and J. H. Ryther, *Can. J. Microbiol.* **8**, 229 (1962)] 35 parts per thousand seawater at 10°C and 100 μeinstein m^{-2} sec^{-1}. Algal cultures and natural samples were inoculated with both [^{3}H]TdR and ^{14}C-labeled bicarbonate (final activities of 0.1 and 1.0 μCi per milliliter, respectively), larvae [reared as described in (*16*)] were added, and after 2 to 3 hours at 50 μeinstein m^{-2} sec^{-1} and −1.0°C, larvae were collected, rinsed, and isolated into scintillation vials, as described in (*17*). Portions of filtrate were collected on 1.0-μm and 0.22-μm membrane filters to assess uptake by the particulate material. Radioactivity in the larvae and on the filters was counted as described in (*17*). Controls killed with mercuric chloride did not show net uptake. The concentration of chlorophyll a was measured fluorometrically. Bacterial and algal abundances were measured from Formalin-preserved samples by acridine orange staining [J. E. Hobbie, R. J. Daley, S. Jaspers, *Appl. Environ. Microbiol.* **33**, 1225 (1977)] and in a Palmer Maloney counting chamber, respectively. Algal abundance was not measured in natural samples. Ingestion rates expressed as microliters per larva per hour were calculated by using the three-compartment model of N. H. Daro [*Helgol. Wiss. Meeresunters.* **31**, 241 (1978)] where:

$$\text{Water dpm } q1 \xrightarrow{\lambda 1} \text{Phytoplankton or bacterial dpm } q2 \xrightarrow{\lambda 2} \text{Larvae dpm } q3$$

If $q1 >> q2 + q3$ and incubations are short to minimize isotope recycling, the larval specific ingestion rate can be calculated from

$$\lambda 2 = \frac{2 \times q3}{q2 \times \text{time}}$$

20. Ingestion rates of echinoderm, nemertean, and polychaete larvae collected from the plankton in McMurdo Sound and fed natural prey were measured during 4-hour incubations, according to the methods in (*19*). These larvae are typically found throughout the water column and at the ice-seawater interface (I. Bosch, unpublished data).
21. Eukaryotic algae incorporate [^{3}H]TdR only during longer incubations and at higher TdR concentrations [R. B. Rivkin, *J. Phycol.* **22**, 193 (1986)].
22. Larvae actively excluded ^{14}C-labeled particles. On the basis of clearance rates for ^{3}H-labeled particles (Table 2) and the measured particulate ^{14}C activity, larvae would have incorporated 75 to 500 dpm of ^{14}C if they ingested bacteria and algae without preference. However, the ^{14}C activity of *P. antarctica* did not differ significantly from that of killed controls. A net counting rate of 2 dpm is significant when these techniques are used [R. B. Rivkin and H. H. Seliger, *Limnol. Oceanogr.* **26**, 780 (1981)].
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25. Field-collected *Odontaster* sp. and *Sterechinus neumayeri* did not ingest phytoplankton at naturally occurring concentrations (Table 3). During these experiments (mid-November), concentrations of phytoplankton of the appropriate size may have been below the threshold for encounter [B. W. Frost, *Limnol. Oceanogr.* **20**, 263 (1975)].
26. *Phaeocystis* produces acrylic acid, and although it is apparently ingested by *Euphausia superba* [J. M. Sieburth, *Science* **132**, 676 (1960)], it is avoided by many other invertebrates and fish and is considered inedible [H. W. Harvey, *The Chemistry and Fertility of Sea Water* (Cambridge Univ. Press, Cambridge, 1963); A. D. Boney, *Oceanogr. Mar. Biol. Annu. Rev.* **8**, 251 (1970)]. *Phaeocystis* forms large gelatinous colonies (2 to 5 mm) and is too large to be grazed by ciliary feeders such as enchinoderm larvae. The fate of this ungrazed algal biomass is uncertain. In the late austral summer and autumn, *Phaeocystis* and ice algae may sink into the benthos and be either consumed by the benthic fauna or slowly remineralized [D. C. White, G. A. Smith, G. R. Stanton,

Antarct. J. U.S. **19**, 125 (1984)]. Thus there may be a steady flux of dissolved organic matter (DOM) from the benthos into the water column. This DOM may be used directly by the larvae or by bacteria.
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29. The development rates for *P. antarctica* were about 35% slower in cultures that were not fed bacterized algal cultures. *Odontaster validus* and *Sterechinus neumayeri* were also reared from egg through metamorphosis on bacterized algal cultures, and although they appear to ingest algae [I. Bosch, unpublished data], they also probably ingested bacteria, as was the case for field-collected specimens.
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33. Echinoderms with probable planktotrophic development are found in the deep sea [P. A. Tyler, A. Grant, S. L. Pain, J. D. Gage, *Nature (London)* **300**, 747 (1982); P. A. Tyler and J. D. Gage, *Deep Sea Res.* **31**, 387 (1984)]. The timing of reproduction of the deep-sea echinoid *Echinus affinis* may be phased to euphotic-zone primary productions. Particulate material delivered to the deep sea in the form of aggregates would be too large for direct ingestion by the larvae. These particulates may be substrate for bacterial growth. Bacterial abundances of 10^6 to 10^8 per liter and dissolved organic carbon concentrations of about 40 μg liter^{-1} in both the deep sea [H. Seki, E. Wood, I. Koike, A. Hattori, *Mar. Biol.* **26**, 1 (1974); P. M. Williams, A. F. Carlucci, R. Olson, *Oceanol. Acta* **3**, 471 (1980)] and McMurdo Sound (Fig. 1) [R. E. Hodson *et al.*, *Mar. Biol.* **61**, 89 (1981)] are similar. Because of the absence of viable phytoplankton and the small input (5 to 10 g m^{-2} year^{-1}) of particulate organic carbon [G. T. Rowe and N. Staresinic, in *The Deep Sea Ecology and Exploitation* (Royal Swedish Academy of Science, Stockholm, 1979), pp. 19–23], feeding larvae would probably use bacteria and DOM as nutritional sources.
34. We thank R. Britton, B. Marinovic, M. Moline, M. Putt, and M. Voytek for assistance in the field and D. Garrison, J. McClintock, R. Newell, and R. Olsen for comments on the manuscript. This work was supported by the National Science Foundation, Division of Polar Programs, grants DPP-8314607 (R.B.R.) and DPP-8317082 (J.S.P.) and by the University of Maryland, Center for Environmental and Estuarine Studies, contribution 1710.

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Oceanic Dimethylsulfide: Production During Zooplankton Grazing on Phytoplankton

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About half the biogenic sulfur flux to the earth's atmosphere each year arises from the oceans. Dimethylsulfide (DMS), which constitutes about 90% of this marine sulfur flux, is presumed to originate from the decomposition of dimethylsulfoniopropionate produced by marine organisms, particularly phytoplankton. The rate of DMS release by phytoplankton is greatly increased when the phytoplankton are subjected to grazing by zooplankton. DMS production associated with such grazing may be the major mechanism of DMS production in many marine settings.

DIMETHYLSULFIDE (DMS) IS UBIQUITOUS in the surface waters of the ocean and appears always to be present in concentrations far in excess of the concentrations expected at atmospheric equilibrium (*1*). This large concentration gradient between the oceans and the atmosphere drives almost half the biogenic sulfur flux to the earth's atmosphere (*2*). DMS constitutes about 90% of the flux of biogenic sulfur from the ocean to the atmosphere. As a result, considerable attention has been focused on the distribution and dynamics of DMS in ocean water in an effort to understand mechanisms controlling its flux to the atmosphere (*3, 4*).

The DMS in seawater appears to originate from algae, most likely produced by the decomposition of dimethylsulfoniopropionate (DMSP), a tertiary sulfonium compound analogous to the quaternary ammonium compounds (for example, glycinebetaine and proline) that are widespread in marine organisms (*5*). As with these ammonium compounds, DMSP may be involved in regulating cellular osmotic pressure in algae (*6, 7*). Decomposition of DMSP appears to occur mainly by an enzymatically catalyzed elimination reaction, yielding DMS and acrylic acid (*8*) (Fig. 1). First discovered in the marine alga *Polysiphonia fastigiata* by Challenger (*8*), DMSP has subsequently been documented in a wide range of marine algae (*9*). Many marine algae produce DMS in the normal course of metabolism (*7, 8*). Exact mechanisms and factors controlling DMSP decomposition in the ocean remain unknown.

Sieburth (*10*) reported that decomposition of DMSP originating in the alga *Phaeocystis* resulted in the accumulation of acrylic acid in the guts of penguins. We reasoned that, somewhere in the food chain, the decomposition of DMSP must also have resulted in loss of DMS to the water column. Furthermore, this process might occur throughout the marine food web. There is widespread evidence that zooplankton and other filter-feeding invertebrates ingest DMSP-containing plants with no adverse effects (*11*).

We investigated the production of DMS during grazing by the marine copepods *Labidocera aestiva* and *Centropages hamatus* on the dinoflagellate *Gymnodinium nelsoni*. Feeding experiments were conducted in silylated 1-liter glass bottles with silicone rubber stoppers containing 750 ml of filtered (5 μm) seawater. Four treatments were examined: with no organisms, with phytoplankton alone, with phytoplankton and zooplankton, and with zooplankton alone. We monitored DMS in the headspace of the bottles (*12*) to minimize disturbance to the plankton suspensions. Phytoplankton densities were set to 500 cells per milliliter, zooplankton to 30 to 40 animals per liter. These densities are higher than oceanic densities but occur in certain coastal situations. We selected these densities to ensure that measurements could be made over a 24-hour period. At the end of the experiment, the densities of phytoplankton and zooplankton were determined by direct counting.

The results of these experiments demonstrate that ingestion of phytoplankton by zooplankton releases DMS into the water column (Fig. 2). Using the weighted mean of linear least squares fits to the individual runs, we found that the rate of DMS production in bottles with zooplankton and phytoplankton averages 24 times that in bottles with phytoplankton alone. Bottles containing zooplankton alone and seawater without organisms showed no significant DMS production.

The weighted least squares slope for DMS concentration versus time in the phytoplankton bottles indicates a DMS production rate for the alga of 23×10^{-15} ($\pm 16 \times 10^{-15}$, 95% confidence) mol per cell per day. We know of only one other published estimate of DMS production by phytoplankton: *Hymenomonas carterae*, 1.3×10^{-15} mol per cell per day (at 35 parts per thousand) (*7*). The volume of cells of *G. nelsoni* ($\sim 2.4 \times 10^{-11}$ liter) is about 30 times that of *H. carterae* ($\sim 8 \times 10^{-13}$ liter), which may in part account for the difference in cell-specific rates of DMS production.

We estimated intracellular concentrations of DMSP by filtering known quantities of phytoplankton onto glass-fiber filters and treating the filters with base (*13*). The concentration of DMSP in *G. nelsoni* was about 280 mmol per liter of cell volume. In *H.*

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