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Characterization and site description of *Solemya borealis* (Bivalvia; Solemyidae), another bivalve-bacteria symbiosis*

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Abstract. *Solemya borealis* Totten was collected from anoxic sediments in Buzzards Bay, Massachusetts in April and July 1989 and examined for the presence of symbiotic, chemoautotrophic bacteria. In addition, sediment cores collected at the same site were analyzed throughout the year, to provide a detailed description of the *S. borealis* habitat. Here we present structural, enzymatic, biochemical, and stable isotope data which suggests that *S. borealis*, like the related species *Solemya velum* Say and *Solemya reidi* Bernard, contains high concentrations of symbiotic chemoautotrophic bacteria in gill bacteriocytes which play a significant role in nutrition. Transmission electron microscopy revealed the presence of rod-shaped cells, which resemble Gram-negative bacteria, within gill epithelial cells. Ribulose-1,5-bisphosphate carboxylase activity in cell-free extracts of *S. borealis* gill tissue was comparable with that found in other invertebrate-chemoautotroph symbioses. Very negative $\delta^{34}S$ ratios (−32.6 to −15.7‰) suggest the utilization of porewater sulfides as both an energy and a sulfur source for the symbionts. Carbon and nitrogen stable isotope ratios were extremely negative ($\delta^{13}C = -32$ to −34.6‰, $\delta^{15}N = -9.7$ to −8.6‰), similar to those of other bivalve-chemoautotroph symbioses. High concentrations of *cis*-vaccenic acid, a fatty acid previously found in other invertebrate-chemoautotroph symbioses, were found in all the major lipid classes of the gills of *S. borealis*. The stable isotope ratios and lipid composition of *S. borealis* suggest that most of this bivalve's nutritional requirements are supplied by bacterial endosymbionts. High levels of taurine in the free amino acid pool of *S. borealis* suggest the existence of unusual amino acid metabolic pathways which may be the result of endosymbiont activity. The *S. borealis* specimens were found in relatively shallow water sediments dominated by silts and clays. The sediments contain high concentrations of organic carbon and nitrogen, exhibit limited oxygen penetration, and have high rates of ammonium and sulfide input from the anaerobic microbial community. Sediment C and N stable isotope ratios reflect the input of algal-derived nutrients to the sediments ($\delta^{13}C = -20.7$ to −20.9‰, $\delta^{15}N = +7.7$ to +20.8‰). Sediment $\delta^{34}S$ ratios ranged from −18.7 to −25.1‰ demonstrating the presence of sulfur produced by bacterial dissimilatory sulfate reduction processes.

Introduction

During the last decade, symbiotic associations involving sulfur-oxidizing chemoautotrophic bacteria and marine invertebrates have been described for four phyla from a diverse range of habitats (Cavanaugh et al. 1981, Felbeck 1983, Giere et al. 1984, Southward et al. 1986). In general, these symbioses involve endosymbiotic chemoautotrophic bacteria contained within specific hypertrophied host tissues such as the gills of bivalves and the trophosome of vestimentiferan and pogonophoran worms. The endosymbionts are often intracellular. Physiological and biochemical studies of these symbioses have shown that in many of the habitats, such as hydrothermal vents, reducing sediments, and sewage outfall areas, a key feature is simultaneous access to both oxygen and reduced inorganic sulfur compounds. Energy present in the sulfur compounds appears to be exploited by the bacteria via oxidative pathways, and the ATP and NAD(P)H (nicotinamide adenine dinucleotide phosphate, reduced form) produced is used to drive the reductive reactions of carbon fixation (see review of Fisher 1990). In many invertebrate-chemoautotroph symbioses the digestive system of the host is small or absent entirely (Reid 1980, Cavanaugh 1985), suggesting that the host may obtain much of its nutritional requirements from the bacterial endosymbionts.

The protobranch mollusc family Solemyidae, is a small, remarkably uniform family of marine bivalves that is essentially worldwide in distribution. At least five species of Solemyids contain endosymbionts (Cavanaugh

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1983, Felbeck 1983, Reid and Brand 1987, Kuznetsov et al. 1990) including two of the better characterized marine invertebrate-chemoautotroph symbioses, *Solemya velum* and *S. reidi*. *S. reidi* is found along the Pacific coast of the U.S. and Canada and is gutless (Reid 1980) whereas *S. velum* occurs along the Atlantic coast and has a very small gut (Yonge 1939). Both species have large fleshy gills and harbor high concentrations of symbiotic, Gram-negative, sulfur-oxidizing chemoautotrophic bacteria within specialized gill bacteriocytes (Cavanaugh 1983, Felbeck 1983). The symbionts appear to be of importance in the nutrition of these solemyids. In *S. velum*, stable isotope analyses suggest that the symbionts can provide almost all of the C and N requirements of the host at some sites (Conway et al. 1989), whereas *S. reidi* may also be able to meet its carbon needs through autotrophy (Anderson et al. 1987).

We recently discovered two specimens of *Solemya borealis* in sediment samples from Buzzards Bay, Massachusetts, providing us with the opportunity to examine this species for the presence of bacterial symbionts. *S. borealis*, is an Atlantic coast species which ranges from Nova Scotia to Connecticut, and which was apparently fairly common in the early 1900s in bays, and offshore in depths of 15 m in Portland Harbor, Maine, to 550 m or more on Georges Bank (Morse 1913, 1919). Collections during the past 50 years have been rare judging from museum specimens. Morse (1913) describes *S. borealis* as resembling an enlarged *S. velum*, having large fleshy gills. *S. borealis* and *S. velum* belong in the subgenus *Petrasma* (Dall 1908) and are characterized by having an elongated oval shell with the dorsal and ventral margins more-or-less parallel, the ligament internal, largely or entirely posterior to the umbos, and supported by chondrophores. Morphologically the species are similar except for size and color; *S. borealis* specimens may reach 8.5 to 10 cm in length and have a heavy dark brown to almost black periostracum, whereas adults of *S. velum* are ca. 2.5 cm in length and lighter in color with a yellow-brown periostracum. *S. borealis* is considered to be gutless (Bernard personal communication, in Reid 1980). The morphological similarities between *S. borealis* and other solemyids known to contain endosymbiotic chemoautotrophic bacteria led us to hypothesize that a bacterial symbiosis may exist in *S. borealis*.

In this paper we provide microscopic, enzymatic, stable isotope, and biochemical evidence to support this hypothesis indicating that *Solemya borealis*, in common with *S. velum* and *S. reidi*, harbors chemoautotrophic bacterial symbionts. In addition, we characterize the sedimentary habitat where the specimens were found in order to provide information regarding the ecological niche of *S. borealis*.

Materials and methods

Solemya borealis and sediment collection

Single specimens of *Solemya borealis* Totten were collected from two sites in Buzzards Bay, Stn R (Sanders 1960) at 41°29.27'N; 70°53.34'W and an inshore site at 41°32.29'N; 70°52.77'W during April and July 1989, respectively, with a box corer (50 × 50 × 70 cm) deployed from the Woods Hole Oceanographic Institution R. V. "Asterias". Sediment samples were obtained concurrently during the April and July 1989 cruises and during three additional cruises to the same sites in September and October 1988, and February 1989. Sediment sub-cores were taken from the box at each station, for measurement of microbial SO_4^{2-} reduction rates, sulfur pools, and sediment characteristics. Both sites are in relatively shallow water (13 to 18 m) where infauna are subjected to large seasonal temperature variations (0.4 to 21.6 °C). Interestingly, although the benthic macrofauna at Stn R have been extensively documented (Sanders 1958, 1960), *S. borealis* has not previously been recorded at this site. The April *S. borealis* specimen was preserved whole and deposited in the Museum of Comparative Zoology mollusc collection at Harvard University, Cambridge, Massachusetts, USA (catalogue no. 302158). The July specimen was dissected into gills, foot tissue, and remaining soft-parts, and constituent tissues were subdivided for biochemical and microscopical studies.

Symbiosis characterization

Microscopy

Pieces of freshly dissected gill tissue from *Solemya borealis* were fixed and stored at 4 °C in 2% glutaraldehyde in 0.1 *M* phosphate buffer solution (pH 7.2). Tissues were post-fixed in 1% osmium tetroxide, dehydrated through an ethanol series, and embedded in Araldite 6005. Semithin sections (1 µm), stained with methylene blue were examined with a Zeiss Axioskop and photographed using differential interference contrast (DIC) and a Zeiss MC100 camera system. Thin sections were stained with lead citrate and uranyl acetate and viewed with a Hitachi 7000 transmission electron microscope operating at 60 kV.

Ribulose-1,5-bisphosphate carboxylase (RuBPcase) activity

RuBPcase activity was determined using the methods of Beudeker et al. (1980) and Williams et al. (1988). Ca. 0.1 g gill and foot sections previously frozen in liquid nitrogen and stored at −80 °C were homogenized on ice in 5 ml complete assay buffer (100 m*M* Tris/HCl, 20 m*M* $MgCl_2 \cdot 6\,H_2O$, 5 m*M* $NaHCO_3$, 5 m*M* dithiothreitol adjusted to pH 8.2 with NaOH) and passed through a French Press to thoroughly disrupt the cells. A cell-free extract (CFE) was obtained by centrifuging the tissue homogenate at 12 000 × *g* for 20 min. The CFE (300 µl) was pre-incubated for 20 min at 30 °C to allow for CO_2 activation of the enzyme, then 20 µl of $NaH^{14}CO_3$ were added and the sample was mixed. 20 µl samples were removed and added to scintillation vials containing 0.3 ml hyamine hydroxide and 10 ml Packard Ultima Gold scintillation fluid for determination of specific activity. The assay was started by the addition of RuBP to a final concentration of 1.0 m*M*. 50 µl samples were removed every minute for 6 min and added to scintillation vials containing 400 µl of glacial acetic acid at 60 °C and left for 20 min to remove any unfixed CO_2. Samples were counted in a United Technologies Packard Minaxib Tricarb 4000 series scintillation counter and corrected for quenching. The protein content of the CFE was determined using the Biuret protein assay, and the RuBPcase activity was calculated as nmol CO_2 fixed (mg CFE protein)$^{-1}$ min^{-1}. RuBPcase activity of fresh spinach was determined as a positive control, while background fixation rates were determined in controls without RuBP.

Stable isotope ratios

Stable isotope ratios were determined in the laboratories of Dr. B. Fry (Ecosystems Center, Marine Biological Laboratory, Woods

Hole, Massachusetts) and R. Michener (Boston University, Massachusetts) essentially as outlined by Conway et al. (1989). Sections of *Solemya borealis* gill and foot tissue were dried prior to analysis. $\delta^{13}C$ and $\delta^{15}N$ values of pooled sediment fractions (0 to 10 cm) were determined after the sediments were mixed, washed with distilled water, and dried. $\delta^{15}N$ ratios of porewater NH_4^+ were measured in pooled sediments (0 to 16 cm) from both sites after extracting triplicate cores (4.5 cm i.d.) in 2 *N* KCl (2:1, v/v) and steam distillation of the filtered extract (0.22 µm, Millipore). $\delta^{34}S$ values of the top 2 to 10 cm of the sediments were determined both before and after washing with distilled water (to remove sulfates). All isotope values are reported relative to Pee Dee Belemnite (PDB), atmospheric N_2, and Canyon Diablo Troilite, respectively, using the standard delta notation:

$$\delta X = [R_{sample}/R_{std} - 1] \times 10^3, \quad (1)$$

where $X = {}^{13}C$, ${}^{15}N$, or ${}^{34}S$, and $R = {}^{13}C:{}^{12}C$, ${}^{15}N:{}^{14}N$, or ${}^{34}S:{}^{32}S$.

Lipid analyses

Lipids were analyzed in tissue samples frozen in liquid nitrogen and stored at −70 °C as described by Conway and McDowell Capuzzo (1991). Lipids were extracted by successive sonication with isopropyl alcohol, chloroform:methanol (1:1), and chloroform:methanol (3:1) after the addition of two internal recovery standards. Half the lipid extract was used for total fatty acid determination and was saponified by heating with 0.5 *N* KOH in methanol. Fatty acids were converted to fatty acid methyl esters (FAMEs) by reaction with 10% BCl_3-methanol and separated from other lipid groups by fractionation on a silica gel column. The remaining lipid extract was separated into lipid classes by thin layer chromatography (TLC) on Whatman LK5D Linear K Silica Gel TLC plates. The fatty acids of each lipid class were saponified and free fatty acids converted to FAMEs as described above. All FAME samples were analyzed by gas chromatography after coinjection of an J & W Scientific Durabond DB-5 30 m fused silica column (0.32 mm i.d., 0.25 mm film thickness) and a J & W Scientific Durabond DB-Wax 30 m fused silica column (0.25 mm i.d., 0.25 mm film thickness). FAME identifications were confirmed by electron-impact gas chromatography-mass spectrometry (GC-MS).

Amino acid analyses

Total hydrolyzable amino acids (THAA) and free amino acids (FAA) were analyzed in gill and foot tissues using a modification of the phenylisothiocyanate (PITC) derivitization technique (Conway and McDowell Capuzzo 1992). For THAA analyses, tissues were hydrolyzed under nitrogen for 24 to 36 h in sealed ampules containing 6 *N* HCl. For FAA determination, tissues were extracted with 5% trichloroacetic acid in ethanol:water (50:50). Amino acids were derivitized with PITC solution (ethanol:water:triethylamine:PITC = 7:1:2:1) and separated by gradient elution high pressure liquid chromatography on a C_{18} reverse phase column at 48 °C.

Sediment analyses

Analyses were determined on triplicate subcores (6.5 cm i.d.) from box cores taken at each station during five cruises between September 1988 and July 1989. Subcores were subdivided into 2 cm depth fractions over a depth range of 0 to 16 cm.

Nutrient pools

Dissolved and total available ammonium, organic nitrogen and carbon, porosity, and dry density were determined at 2 cm depth intervals over the top 16 cm. Porewater for dissolved ammonium was collected by centrifugation; total available ammonium was determined after extracting fresh sediment (10 cm^3) with 20 ml of 2 *N* KCl (pH 2) for 18 h. Ammonium concentrations of the porewater and KCl extracts were measured using the Indophenol method (Scheiner 1976) after filtration (0.45 µm, Millipore). Corrections were made for porosity. Total carbon and nitrogen were determined using a Perkin Elmer CHN analyzer on dried sediment which had been treated with 2 *N* HCl to remove particulate carbonate. Sediment porosity and dry density were determined by drying known volumes of fresh sediment to constant weight at 60 °C.

Sulfate reduction rates and reduced sulfur pools

Sediment sulfur pools were fractionated into total sulfur, acid volatile sulfur (AVS; contains hydrogen sulfide and ferrous sulfide), and chromium reducible sulphur (CRS; contains elemental sulfur and iron pyrite). Total sulfur was determined using a Leco SC132 sulfur analyzer. AVS and CRS were determined as part of the sulfate reduction experiments outlined below. Rates of reduced sulfur input through microbial sulfate reduction were determined using direct injection of tracer levels of anoxic ${}^{35}S\text{-}SO_4$ (carrier free, New England Nuclear) into sediments subcores (4.5 cm i.d.) collected from the boxcorer and held and incubated at *in situ* temperature (Jørgensen 1977). Activity was stopped by freezing and cores were held at −40 °C until analysis. The AVS pool and its ${}^{35}S$ content were analyzed by anaerobic acidification in 1 *N* HCl of sediments during active distillation (argon passed over hot copper) of 2 cm core fractions and collection of liberated H_2S in traps containing ZnCl (10%) and excess ZnOH. The residual sediments were then digested anaerobically for 2 h with hot (860 °C) acidic reduced chromium (Zhabina and Volkov 1976, Howes et al. 1984) with subsequent H_2S release trapped as above.

Subsamples (0.6 ml) of the zinc traps were analyzed for H_2S concentration using a modification of the procedure of Cline (1969) to yield estimates of AVS and CRS pools. The radioactivity of the trap samples was determined using a Minaxib Tri-Carb 4000 liquid Scintillation Counter after the addition of 10 ml Aquasol II. These analyses were carried out on four cores over 24 h time course experiments during each of the five cruises. SO_4^{2-} concentrations were determined on porewater from 2 cm depth fractions of each of three parallel cores using a modification of the turbidimetric assay of Tabatabai (1974).

Results

Symbiosis characterization

Solemya borealis description

Both specimens of *Solemya borealis* were 6.5 cm in length and 2.5 cm in width and had a heavy dark brown to almost black periostracum (Fig. 1). These specimens were considerably larger than *S. velum* (~2 cm in length and 0.7 cm in width for adults) which occurs in similar sediments along Cape Cod, but were similar in size to adults of the west coast species *S. reidi* (Reid 1980, Anderson et al. 1987). The dissected specimen lacked an observable digestive system and had extremely hypertrophied gills which were red/purple in color with white flecks at the edges; these characteristics are typical of the other *Solemya* species which have been found to contain endosymbiotic bacteria (Cavanaugh 1983, 1985). The foot was large and pinkish in color. The periostracum of *S. borealis*, like that of *S. velum*, repelled water as described by Morse (1913).

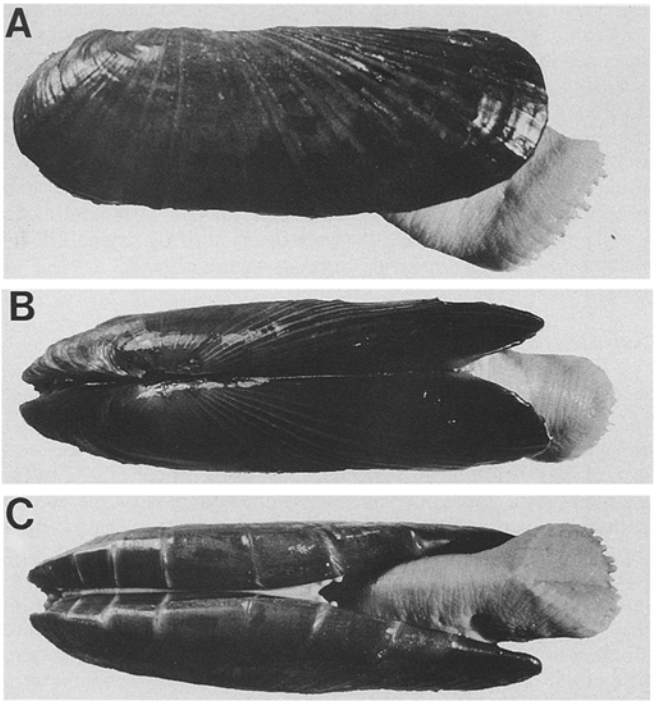

Fig. 1. *Solemya borealis.* (A) Side view showing partially extended foot; (B) dorsal view showing posterior umbos; (C) ventral view showing infolded periostracum. Length of valves = 6.5 cm

Electron microscopy – endosymbiont description

Numerous subcellular inclusions resembling procaryotic cells based on their size and ultrastructure, were observed in the gills of *Solemya borealis* (Fig. 2). The symbionts (as they will be referred to), are rod-shaped, quite variable in length (up to 15 μm) and width (0.5 to 3.0 μm), and have cell envelopes characteristic of Gram-negative bacteria. They appeared intracellular in gill epithelial cells termed bacteriocytes (Cavanaugh 1983), contained within vacuoles bounded by a peribacterial membrane [following the nomenclature of legume-*Rhizobium* symbioses (Verma and Long 1983)] which is presumed to be host derived. Within bacteriocytes, the symbionts were concentrated in the apical region while both myelin- and lipid-like inclusions were observed in the basal regions of the cell, near the host blood space.

The organization of cell types within the gills of *Solemya borealis* paralleled that of the *S. velum* and *S. reidi* symbioses (Cavanaugh 1983, 1985, Gustafson and Reid 1988). Bacteriocytes, separated by thin goblet-shaped "intercalary cells", were confined to the region of the gill filament just distal to the ciliated edge of the gill (Fig. 2). The apical surface of intercalary cells comprised the microvillar surface of the gills and covered the outer surface of the bacteriocytes. As in other solemyid symbioses (Cavanaugh 1983, 1985), symbionts were confined to bacteriocytes; intercalary and ciliated cells appeared symbiont-free. Mitochondria, abundant in both ciliated and intercalary cells, were observed much less frequently in bacteriocytes.

Carbon fixation

RuBP-dependent CO_2 fixation activity was detected in the cell-free extract (CFE) of the gills of *Solemya borealis*, where the symbionts are located, while CO_2 fixation in CFE of the foot tissue was not apparent (Fig. 3). Carbon fixation rates in the CFE of the gills of *S. borealis* were between 7.0 and 7.9 nmol CO_2 fixed (mg CFE protein)$^{-1}$ min^{-1} based on the slope of CO_2 fixation vs activity (Fig. 3).

Stable isotope ratios

The stable isotope ratios measured in *Solemya borealis* gills (containing endosymbionts) and foot tissue (no endosymbionts) were extremely negative ($\delta^{13}C = -32$ to -34.6‰; $\delta^{15}N = -8.6$ to -9.7‰, $\delta^{34}S = -15.7$ to -32.6‰; Table 1 a). Sediment total C and N stable isotope values at both sites were more positive ($\delta^{13}C = -20.7$ to -20.9‰, $\delta^{15}N = +7.6$ to 7.7‰; Table 1 b) reflecting algal nutrient input to the sediments. In addition, KCl-extracted ammonium values were highly enriched in ^{14}N ($\delta^{15}N = +8.7$ to $+20.8$‰), although values were quite variable (Table 1 b). Sediment $\delta^{34}S$ values were very negative, particularly in washed sediments, where seawater sulfate was removed (Table 1 b) reflecting bacterial dissimilatory sulfate reduction.

Lipid composition

The fatty acid composition of the gills of *Solemya borealis* is shown in Table 2 and Fig. 4 a. Two different sections of the gill tissue were extracted and analyzed. The most obvious characteristics of the lipids of *S. borealis* were the high concentrations of *cis*-vaccenic acid [18:1ω7, 2.6–15.0 μg (mg dry wt)$^{-1}$]. Analysis of the fatty acid composition of the lipid classes of *S. borealis* demonstrated that, as in *S. velum* (Conway and McDowell Capuzzo 1991), the *cis*-vaccenic acid found in *S. borealis* occurred in all the major lipid classes (Table 3, Fig. 4 b).

In *Solemya borealis*, only low concentrations of polyunsaturated fatty acids (PUFAs) were found, and these predominantly belonged to the ω6 series of PUFAs as opposed to the ω3 PUFAs typically found in most marine bivalves (cf. Joseph 1982). Levels of plant-derived sterols were negligible.

Amino acid composition

The total hydrolyzable amino acid (THAA) and free amino acid (FAA) composition of *Solemya borealis* (Fig. 5, Table 4) very closely resembled that of *S. velum* (Conway and McDowell Capuzzo 1992). Essential amino acids (EAAs)[1] are present in the THAAs of *S. borealis*

[1] Essential amino acids are those amino acids that cannot be synthesized by most metazoans including molluscs. These include arginine, histidine, valine, threonine, phenylalanine, tryptophan, methionine, valine, leucine, and isoleucine (Lehninger 1975, Bishop et al. 1983). Although it is not known whether *Solemya borealis* can synthesize these amino acids it is unlikely that this species differs from most metazoans studied, and we assume that these amino acids are essential dietary requirements for *S. borealis* throughout the following discussion

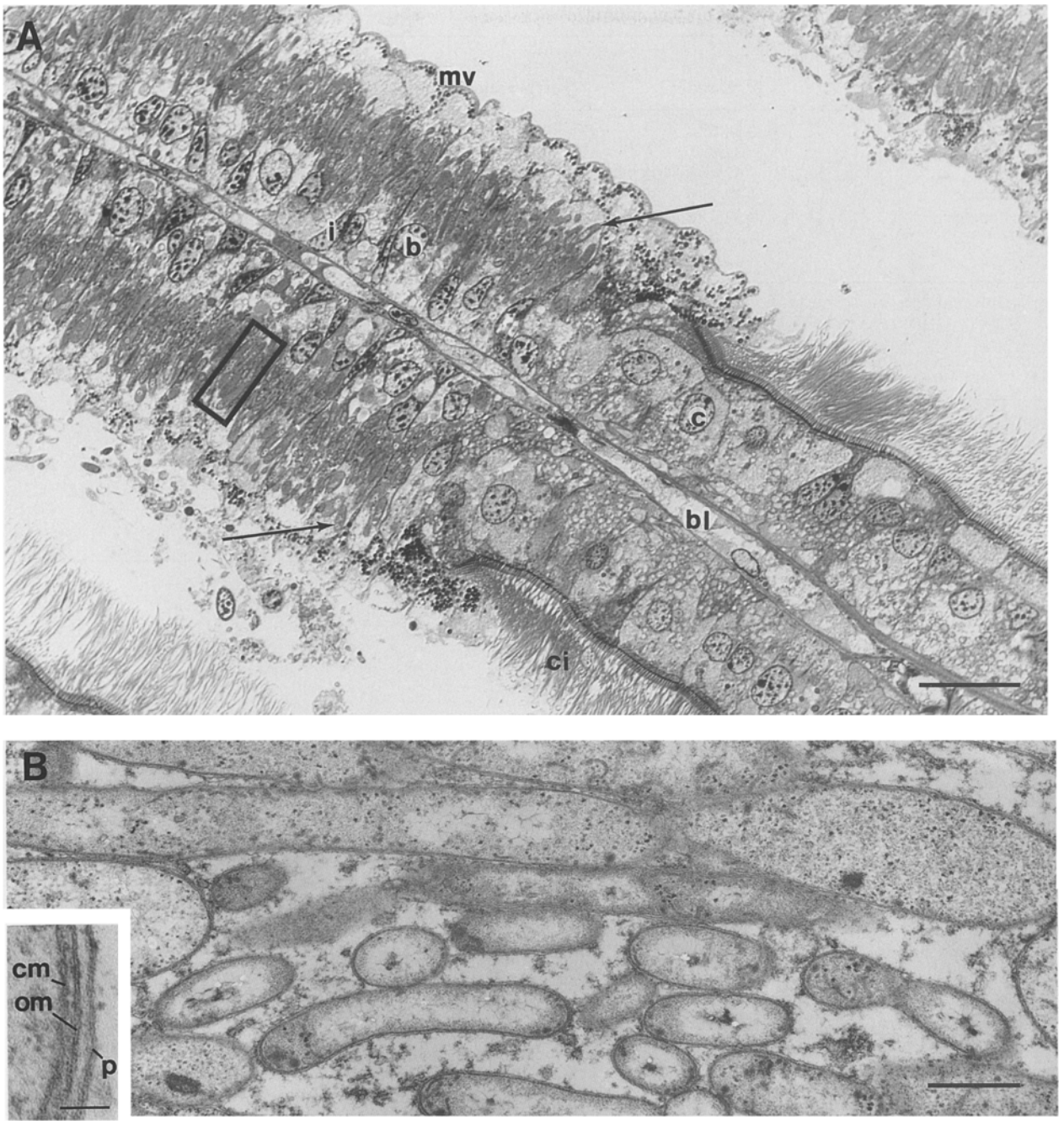

Fig. 2. *Solemya borealis.* (A) Transverse section of gill filaments showing intracellular rod-shaped bacteria (arrows, rectangle). Bacteriocytes are confined to the region proximal to the ciliated edge of the gill, and are flanked by symbiont-free intercalary cells which appear to comprise the microvillar surface of the gill filament. Light micrograph. Scale bar = 20 µm. b: bacteriocyte nucleus; c: ciliated cell nucleus; i: intercalary cell nucleus; bl: blood space; ci: cilia; mv: microvilli. (B) Higher magnification of symbionts showing cell ultrastructure typical of Gram-negative bacteria. Scale bar = 1 µm. Inset: Detail of symbiont cell envelope and peribacterial membrane. Scale bar = 0.05 µm. p: peribacterial membrane; cm: cell membrane; om: outer membrane

and account for 26 to 40% of the THAA pool. There were large amounts of taurine in the THAA fraction of the foot of *S. borealis* (Table 4, Fig. 5) similar to the foot tissue of *S. velum* (Conway and McDowell Capuzzo 1992). Taurine accounted for most of the FAA present in *S. borealis* (66 to 71%, Fig. 4) and occurred in high concentrations (100 to 200 µmol g wet wt^{-1}; Table 4), although concentrations were not as high as in *S. velum* (Conway and McDowell Capuzzo 1992).

Sediment characteristics

The sediments of the *Solemya borealis* sites in Buzzards Bay were predominantly composed of fine silts and clays (Fig. 6a) and contained significant levels of organic carbon and nitrogen which increased with depth from 0 to 16 cm (Fig. 6b); porosity decreased from 0.82 to 0.73 ml cm^{-3} over a similar depth range (data not shown). Sediment total sulfur content increased with depth from

Table 1. *Solemya borealis.* (a) Stable isotope ratios in comparison with *S. velum* and symbiont-free marine bivalves and (b) sediment values

(a) $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ ratios of animals tissues

Species	$\delta^{13}C$ ratios	$\delta^{15}N$ ratios	$\delta^{34}S$ ratios
Solemya borealis (gill)	−34.6‰	−9.7‰	−15.7‰
Solemya borealis (foot)	−32‰	−8.6‰	−32.6‰
Solemya velum (gill)[a]	−32.4 to −33.9‰	+0.4 to −9.8‰	−26.7 to −28.2‰
Solemya velum (foot)[a]	−30.9 to −32.1‰	+4.4 to −4.6‰	−29.2 to −31.1‰
Solemya velum "bacteria"[a]	−32.3 to −33.6‰	−7.9 to −8.6‰	
Mya arenaria[a]	−17.2 to −17.8‰	+8.3 to +8.5‰	
Tellina agilis[a]	−14.2 to −15.6‰	+6.3 to +8.2‰	

(b) Sediment isotope ratios:

Sample	$\delta^{13}C$ ratios	$\delta^{15}N$ ratios	$\delta^{34}S$ ratios[b]	
			'Unwashed'	'Washed'
Total sediment C, N, and S (2–10 cm)				
Inshore stn	−20.7‰, 20.9‰	+7.7‰	−18.9‰, −20.1‰	−25.1‰
Stn R	−20.9‰	+7.6‰, +7.7‰	−18.7‰, −19.2‰	−24.5‰, −24.6‰
KCl-extracted NH_4^+ (0–16 cm)				
Inshore stn		+8.7 to +13.6‰		
Stn R		+8.9 to +20.8‰		

[a] Values from Conway et al. 1989

[b] Unwashed values include contaminating seawater sulfate

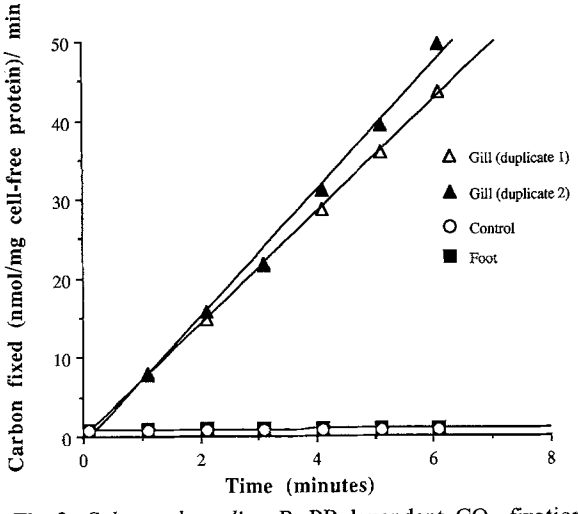

Fig. 3. *Solemya borealis.* RuBP-dependent CO_2 fixation by the gills in comparison with foot tissue (which contains no endosymbionts) and controls to which no RuBP had been added

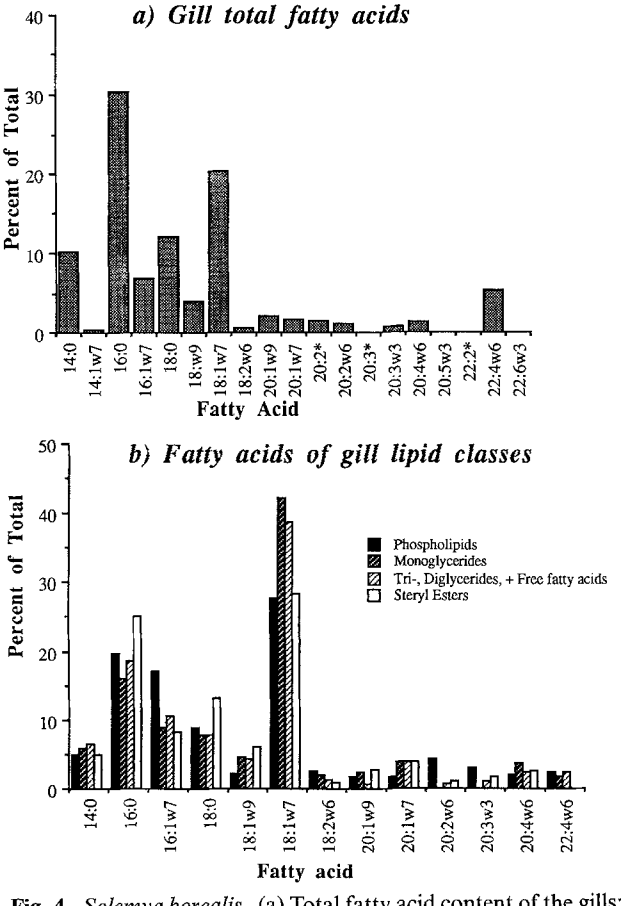

Fig. 4. *Solemya borealis.* (a) Total fatty acid content of the gills; (b) fatty acid content of the major lipid classes. Concentrations presented as percent of the total fatty acids found. (*) Denotes position of double bonds unknown

42 μmol cm^{-3} over the top 2 cm, to 163 μmol cm^{-3} in the 14 to 16 cm depth section (Fig. 6c). Over 50% of the sediment sulfur content is accounted for by the chromium reduced sulfur species (CRS) which include elemental sulfur and pyrites (Fig. 6c). Acid volatile sulfur species (AVS; includes H_2S and ferrous sulfide) also increased with depth from 0.1 μmol cm^{-3} at 0 to 2 cm to 3.4 μmol cm^{-3} at 14 to 16 cm, with a maximum of 5.0 μmol cm^{-3} at 8 to 10 cm (Fig. 6d). Porewater sulfate levels decreased with depth from a value of 21.1 μmol cm^{-3} in the top

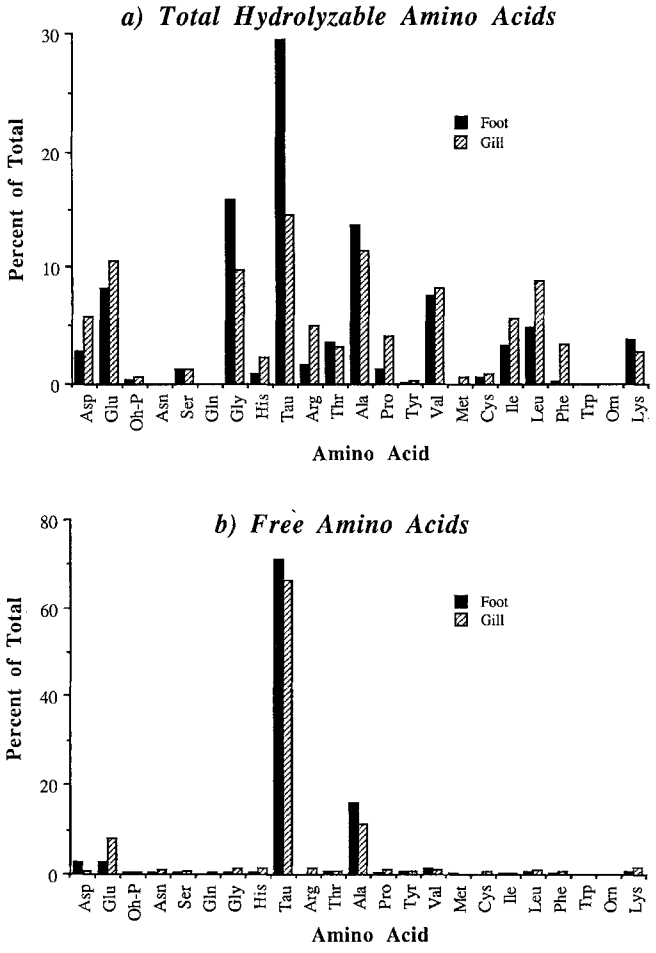

Fig. 5. *Solemya borealis.* (a) Total hydrolyzable and (b) free amino acid composition of the foot and gill. Concentrations presented as a molar percentage of the total amino acids measured

Table 2. *Solemya borealis.* Fatty acid composition of gills (ng mg dry wt^{-1}). (*) Denotes position of double bonds unknown. (**) Lipid is below the detection limit of 1 ng mg^{-1}

Fatty acid	Extraction 1 (ng mg dry wt^{-1})	Extraction 2 (ng mg dry wt^{-1})	Mean (ng mg dry wt^{-1})
14:0	4 510	4 340	4 420
14:1ω5	23	200	110
a-15:0	25	23	24
15:0	58	59	58
16:0	13 070	13 160	13 110
16:1ω7	4 820	1 040	2 930
a-17:0	53	0	27
17:0	190	64	130
18:0	5 550	4 910	5 230
i-18:0	0**	0	0
18:1ω9	1 800	1 620	1 710
18:1ω7	14 980	2 570	8 770
18:2ω6	480	19	250
18:3ω3	80	0	38
18:3ω6	0	0	0
18:4ω3	200	0	99
20:0	73	10	41
20:1*	0	120	58
20:1ω9	1 710	110	910
20:1ω7	1 290	220	750
20:2*	140	0	700
20:2ω6	810	120	460
20:3*	360	0	180
20:3ω3	590	150	370
20:4ω6	1 130	0	560
20:5ω3	0	0	0
22:0	28	0	14
22:1ω9	60	0	30
22:2ω6	0	0	0
22:2*	0	0	0
22:3ω3	0	0	0
22:4ω6	1 900	2 560	2 230
22:6ω3	36	0	18
24:0	38	0	19
Total	55 250	31 250	43 250

2 cm to 17 μmol cm^{-3} at 14 to 16 cm (Fig. 6d). The AVS and CRS pools and the decrease in porewater sulfate is a result of sulfate reduction by sulfur reducing bacteria. Surface sulfate reduction activity (pooled 0 to 16 cm fractions) showed a strong seasonal trend ranging from 266 μmol m^{-2} h in summer to 29 μmol m^{-2} h during the winter (Fig. 6e). This variability is most likely due to temperature changes, although secondary effects resulting from changes in organic matter availability cannot be ruled out.

Porewater ammonium concentrations increased from 0.06 to 0.14 μmol cm^{-3} over the 16 cm depth range analyzed (Fig. 6f) compared to 1 nmol ml^{-1} in the overlying water column (Howes unpublished data). Many of the invertebrate-bacteria symbioses described to date inhabit similar fine-grained, reducing sediments (Dando et al. 1985, Dando and Southward 1986). The predominant reduced inorganic compounds available for bacterial chemoautotrophy appear to be hydrogen sulfide and ferrous sulfides (AVS pool) and ammonium. At present, it is not possible to ascertain the extent to which FeS may be available for utilization.

Discussion

The results presented here suggest that: (1) the gills of the protobranch bivalve *Solemya borealis* contain intracellular symbiotic bacteria; (2) the symbiotic bacteria are autotrophic and may use sediment sulfides to fuel chemoautotrophic carbon fixation; and (3) the symbiotic bacteria provide a major nutritional source for the bivalve host.

Solemya borealis contains intracellular symbiotic bacteria

A symbiotic association appears to exist between *Solemya borealis* and procaryotic cells on the basis of electron microscope observations. At the ultrastructural level, the subcellular inclusions observed in the ctenidia resemble Gram-negative bacteria. The intracellular arrangement of the bacteria, which are contained within membrane-

Table 3. *Solemya borealis*. Fatty acid composition of the major lipid classes. (Fatty acids of each lipid class presented as a percentage of the total fatty acids of that class)

Fatty acid	Phospholipids (%)	Monoglycerides (%)	Diglycerides, triglycerides + FFAs (%)	Steryl esters + methyl esters (%)
14:0	4.9	5.8	6.4	5.0
16:0	19.7	16.1	18.7	25.1
16:1ω7	17.1	8.8	10.6	8.2
18:0	8.9	7.8	7.9	13.2
18:1ω9	2.1	4.5	4.4	6.1
18:1ω7	27.7	42.1	38.8	28.4
18:2ω6	2.5	1.9	1.2	0.9
20:1ω9	1.7	2.3	0.6	2.8
20:1ω7	1.8	4.0	4.0	4.0
20:2ω6	4.3	0.0	0.7	1.1
20:3ω3	3.0	0.0	1.0	1.7
20:4ω6	2.0	3.6	2.4	2.6
22:2ω6	2.3	1.8	2.4	0.0
Total	97.9	98.9	99.0	99.2
Sterols	Free sterols (ng/mg dry wt)	Percent of total	Steryl esters (ng/mg dry wt)	Percent of total
Cholesterol	1722	78.4	939	97.5
Other sterols	475	21.6	24	2.5

Table 4. *Solemya borealis*. Amino acid composition (μmol g^{-1} wet wt)

Amino acid	Total hydrolyzable amino acids		Free amino acids	
	Foot	Gill	Foot	Gill
Aspartic acid	38	71	7	1
Glutamic acid	110	130	7	13
Hydroxyproline	5	8	1	1
Asparagine[a]	0[c]	0	1	1
Serine[b]	18	16	1	1
Glutamine[a]	0	0	0	1
Glycine	210	120	1	2
Histidine	12	28	1	2
Taurine	400	180	190	100
Arginine	23	61	0	2
Threonine[b]	48	39	2	1
Alanine	180	140	43	17
Proline	18	49	1	1
Tyrosine	1	3	2	1
Valine	100	99	3	1
Methionine	0	9	1	0
Cystine/Cysteine	8	11	0	1
Isoleucine	46	69	1	1
Leucine	66	110	2	2
Phenylalanine	4	43	1	1
Tryptophan[b]	0	0	0	0
Lysine	53	34	2	2
% Essential amino acids	26	41	4.8	8
Total (μmol g^{-1} wet wt)	1340	1210	267	160

[a] Converted to aspartic and glutamic acid during acid hydrolysis
[b] Yields of these amino acids are reduced during acid hydrolysis
[c] Below detection limits

bound vacuoles, closely parallels that of *S. velum* and other bivalve-chemoautotroph symbioses (Cavanaugh 1985), as well as other associations involving intracellular bacteria, e.g. *Rhizobium*-legume symbioses (Verma and Long 1983). The high densities of bacteria found, and their confinement to certain cell types, suggest that the association is quite specific while the presence of myelin-like inclusions in the bacteriocytes suggests possible host digestion of symbionts. Overall, however, the bacteria appear "healthy", suggesting that the symbiosis is a reasonably stable relationship. The positioning of the symbionts in the apical region of bacteriocytes suggests that the nutrients necessary for chemoautotrophy (O_2, CO_2, sulfides, perhaps NH_4^+) may be transported across the gill surfaces.

While it appears that bacteria live as intracellular symbionts of *Solemya borealis*, it is not clear whether more than one type of symbiont is involved. On the basis of ultrastructure, it has been suggested that some bivalve-chemoautotroph symbioses harbor two or more symbiont types (Southward 1986, Soyer et al. 1987). However, the considerable variation in size and ultrastructure of the *S. borealis* symbionts parallels the pattern observed in *S. velum* (Cavanaugh 1983, Cavanaugh et al. 1988). Phylogenetic analyses of ribosomal RNA sequences suggest that only one type of symbiont exists in *S. velum* and other bivalve-chemoautotroph symbioses (Stahl et al. 1984, Distel et al. 1988). It is possible that the bacterial pleomorphism observed in *S. borealis* represents varying developmental stages of the symbionts rather than different symbiont types; alternatively, the apparent size differences may be sectioning artifacts.

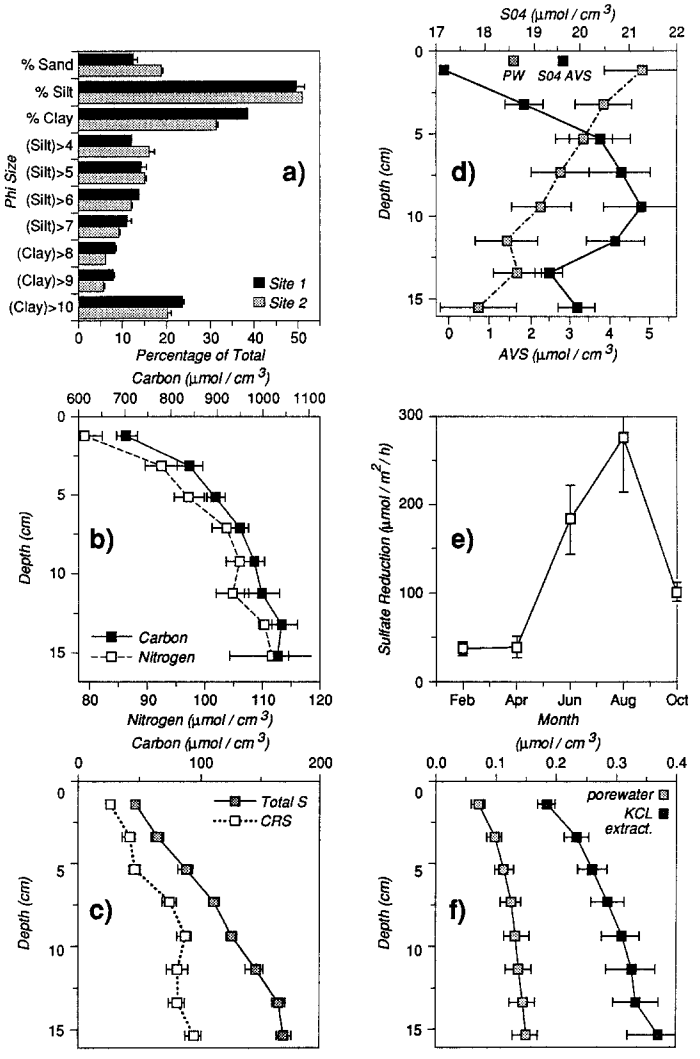

Fig. 6. Sediment characteristics at the two *Solemya borealis* sites. Analyses are the mean of triplicate sub-cores from each station during five cruises. (a) Sediment grain size (Phi scale; Site 1: Stn R, Site 2: inshore site); (b) organic carbon and nitrogen content (µmol cm^{-3}) of the top 16 cm measured at 2 cm depth intervals; (c) total sulfur (S) and chromium reducible sulfur (CRS) content (µmol cm^{-3}) of the top 16 cm measured at 2 cm depth intervals; (d) pore water sulfate (PW) and acid volatile sulfur (AVS) content (µmol cm^{-3}) of the top 16 cm measured at 2 cm depth intervals; (e) sulfate reduction rates (µmol m^{-2} h^{-1}) over an 8 mo period averaged over the top 16 cm; (f) KCL-extractable and porewater ammonium profiles (µmol cm^{-3}) of the top 16 cm measured at 2 cm depth intervals

The symbionts are autotrophic and may use sediment sulfides to fuel chemoautotrophic carbon fixation

RuBPcase activity is a strong indicator for the presence of autotrophic symbionts in an animal, as it is only found in autotrophic organisms such as plants, algae, photo- and chemoautotrophs (see review of Miziorko and Lorimer 1983). Carbon fixation rates in the gills of *Solemya borealis* are comparable with those of the bacteria-containing tissues of other invertebrate-chemoautotroph symbioses (Cavanaugh 1983, Williams et al. 1988). No RuBPcase activity was detected in the foot tissue of *S. borealis* which contained no endosymbionts. The results of the RuBPcase assays demonstrate that the putative bacterial symbionts of *S. borealis* are autotrophic.

Although we did not have the opportunity to examine *Solemya borealis* for sulfur oxidation potential, the highly negative $\delta^{34}S$ ratios found (−15.7‰ for gill tissue and −32.6‰ for foot tissue; Table 1) strongly suggest that isotopically light porewater sulfides, produced as a result of bacterial dissimilatory sulfate reduction processes, are utilized by the symbiosis. Dissimilatory sulfur reduction in marine sediments occurs with a large isotope fractionation effect (−30 to −70‰, Goldhaber and Kaplan 1975, Fry et al. 1986) and results in sediment sulfides with very negative sulfur isotope ratios (−15 to −30‰). The sulfur isotope ratios resulting from bacterial sulfur reduction are quite distinct from those of seawater sulfate (which has $\delta^{34}S$ ratios of ca. +20‰, Hartmann and Nielson 1969). The $\delta^{34}S$ ratios in the reducing sediments of the *S. borealis* habitats were in the −18 to −25‰ range (Table 1 b), with washed sediments (SO_4^{2-}-removed) having the most negative $\delta^{34}S$ values. These sediment $\delta^{34}S$ ratios are almost certainly a result of bacterial sulfate reduction. It is probable that the sulfides produced by sulfate-reducing bacteria are utilized by the symbionts of *S. borealis* as: (1) an energy source to fuel carbon fixation, and (2) a sulfur source. Sulfur incorporated by the bacterial symbionts may ultimately be utilized by the bivalve host. In the closely related species *S. velum* and *S. reidi*, which house symbionts similar in ultrastructure to the symbionts of *S. borealis*, considerable evidence exists to suggest the utilization of reduced sulfides as energy and sulfur sources (Cavanaugh 1983, Chen et al. 1987, Conway et al. 1989, Anderson et al. 1987).

The difference in $\delta^{34}S$ ratios between gill and foot tissue is intriguing and is seen to a lesser extent in *Solemya velum* (Conway et al. 1989). The differences are probably due to some as yet unknown internal isotope fractionation process. Similarly, the large differences in $\delta^{15}N$ isotope values between the available nitrogen pool (Table 1) and *S. borealis* tissues also suggests the presence of unknown isotope fractionation events.

The symbiotic bacteria provide a major nutritional source for the bivalve host

Analysis of the C, N, and S isotope ratios and the lipid composition of *Solemya borealis* suggests the utilization of endosymbiont nutrients by the host. Stable isotope ratios are often used to identify trophic pathways and processes because a few well-characterized reactions are responsible for the stable isotope compositions of most organic matter, and therefore the stable isotope ratios of an organism may provide information about its diet (Fry and Sherr 1984, Rounick and Winterbourn 1986, Spiro et al. 1986). This is important in the study of invertebrate-bacteria symbioses, as the fixation of carbon during bacterial chemoautotrophy can result in more negative or-

ganic carbon $\delta^{13}C$ ratios than photosynthetic carbon fixation processes (Degens 1969, Ruby et al. 1987). Thus, organisms utilizing bacterial carbon may have more negative $\delta^{13}C$ ratios than those utilizing phototrophically-fixed carbon sources.

The very negative carbon stable isotope ratios in *Solemya borealis* suggest that much of the bivalve's carbon is derived from bacterial chemoautotrophy; marine bivalves utilizing photosynthetically-derived carbon generally have $\delta^{13}C$ ratios similar to those of marine phytoplankton (−18 to −24‰, Gearing et al. 1984 and references therein). Uptake of dissolved organic matter is unlikely to be a major trophic mechanism in *S. borealis*, as the available dissolved nutrients are likely to be predominantly phytoplankton-derived, yet *S. borealis* does not have a phytoplankton-based isotope signature. The unusually low $\delta^{15}N$ isotope values found in gill and foot tissue of *S. borealis* (−8.6 to −9.7‰) are similar to those found in *S. velum* (4.4 to −9.8‰, Conway et al. 1989; Table 1, this paper) and suggest that the clam may be utilizing nitrogen derived from the endosymbionts, as non-symbiont containing bivalves generally have $\delta^{15}N$ values similar to that of seawater nitrate (~6 to 10‰). It is conceivable that the very negative nitrogen isotope values are due to the utilization of non-limiting supplies of pore water ammonium (concentrations of 0.06 to 0.14 μmol cm^{-3} were found over the 16 cm depth range analyzed) as has been proposed for the *S. velum* symbiosis (Conway et al. 1989), as large isotope fractionation effects can occur during uptake of non-limiting substrates. The large difference in $\delta^{15}N$ ratios between *S. borealis* tissues and both total sediment nitrogen and KCl-extracted NH_4^+ suggests the presence of unusual nitrogen fractionation events during uptake or metabolism of nitrogenous compounds such as NH_4^+ by the symbiosis.

The lipid composition of marine bivalves is usually influenced by dietary sources (Pollero et al. 1979, Moreno et al. 1980, Piretti et al. 1987) particularly with respect to polyunsaturated fatty acid (PUFA) and sterol compositions, as many of these lipids cannot be synthesized by animals de novo (Lehninger 1975, Goad 1976). Consequently, the lipids of invertebrate-bacteria symbioses may reflect the utilization of endosymbiont nutrients if endosymbionts are important in the nutrition of these species (Conway and McDowell Capuzzo, 1990, 1991 a).

Cis-vaccenic acid is abundant in the gills of *Solemya borealis*. *Cis*-vaccenic acid is also the most common lipid in *S. velum* (Conway and McDowell Capuzzo 1991 a), the *Inanidrilus* (=*Phallodrilus*) *leukodermatus* invertebrate-chemoautotroph symbiosis (Giere et al. 1991) and in many Gram-negative sulfur-oxidizing bacteria (Fulco 1983, McCaffrey et al. 1989). *Cis*-vaccenic acid is predominantly a bacterial fatty acid which is synthesized using the bacterial anaerobic monounsaturated synthesis pathway (Goldfine 1972, Fulco 1983). The high concentrations of this lipid in *S. borealis* suggest the incorporation of bacterial lipids by the host. The presence of high concentrations of *cis*-vaccenic acid in marine invertebrates may be diagnostic for the presence of chemoautotrophic bacterial symbionts.

The extreme variability between the *cis*-vaccenic acid (18:1ω7) composition of two different gill segments of *Solemya borealis* is unusual (Table 2). The endosymbionts of many bivalve-bacteria symbioses are often localized in specific sections of the gills, so the variability in lipid composition of the gills of *S. borealis* may reflect differences in the bacterial content of the gill sections examined. The occurrence of *cis*-vaccenic acid in all the lipid classes of *S. borealis* suggests that this lipid is probably involved in a variety of physiological processes in this species, and may be important in both membrane structure maintenance and energy production.

The low levels of plant derived PUFAs and sterols in *Solemya borealis* suggest that marine algae are not an important nutritional source for this species. Low levels of plant-derived lipids have also been noted in other invertebrate-chemoautotroph symbioses (Conway and McDowell Capuzzo 1991, Giere et al. 1991). The source of the small amounts of 20:4ω6 and 22:4ω6 PUFAs in *S. borealis* is unknown, but possibilities include in vivo synthesis by the host or the endosymbionts, ingestion of small amounts of marine algae, or epithelial uptake of dissolved organic matter. The high levels of *cis*-vaccenic acid and low levels of PUFAs found in *S. borealis* provide further evidence to suggest that the diet of this bivalve is based primarily on bacterial chemosynthesis.

The lack of a typical protobranch gut in *Solemya borealis*, and the likelihood that bacterial endosymbiotic bacteria are the main nutritional source for this species, suggest that a large proportion of the EAAs found in *S. borealis* are synthesized de novo by the symbiosis, either directly by the bacteria, or using carbon and nitrogen derived from the bacteria. In both *S. borealis* and *S. velum* (Conway and McDowell Capuzzo 1992) the foot tissue FAA pool contains the highest levels of taurine of the tissues examined. While only one specimen of *S. borealis* was examined, and amino acid profiles may vary among individuals, the close similarities between the amino acids of *S. borealis* and *S. velum* suggest that metabolic processes in the two congeneric species are similar, particularly with respect to taurine metabolism. Overall, the biochemical compositions of *S. borealis* and *S. velum* are strikingly similar, suggesting strong similarities between the types of bacterial symbiont present and host metabolic processes in these two related species.

The appearance of myelin-like inclusions in the basal region of bacteriocytes (Fig. 2) of *Solemya borealis* suggests that, in addition to the possible direct translocation of soluble organic compounds (Fisher and Childress 1986) and utilization of lipids (Conway and McDowell Capuzzo 1991) observed in other solemyids, the symbionts of *S. borealis* may be autolyzing or being actively digested. Farming of chemoautotrophic symbionts has been noted in other similar symbioses (Fiala-Médioni et al. 1986). Subsequent transfer of nutrients such as lipids and amino acids to the host may then occur via the adjacent blood space. The symbionts may thus be actively "farmed".

The detailed analysis of the sediments where *Solemya borealis* was found allows us to estimate the concentrations of reduced sulfur sources available for use by the

chemoautotrophic symbionts of this bivalve. The input of reduced sulfur to the sediments (0.86 mol S m^{-2} yr^{-1} for the top 16 cm of the sediments) from *in situ* microbial sulfate reduction and the highly negative $\delta^{34}S$ ratios of *S. borealis* support the potential use of sediment sulfide as the main energy source for the *S. borealis* symbiosis, as has been found for *S. velum* (Cavanaugh 1983, 1985, Chen et al. 1987) and *S. reidi* (Anderson et al. 1987).

Using available data on the sulfur cycle of the sediments inhabited by *Solemya borealis* and literature values for conversion efficiencies and respiration rates of congeneric species, it is possible to speculate upon the scales of bivalve sulfide requirements vs sulfide availability. Since whole clam O_2 consumption rates were not determined for *S. borealis*, the metabolic rates of *S. reidi*, a related species of similar size, were used in the calculations. Anderson et al. (1987) measured O_2 consumption rates of *S. reidi* under a variety of O_2 and sulfide concentrations. Under conditions of high O_2 availability, rates of 2 to 4.3 μmol O_2 g wet wt^{-1} h^{-1} were found in *S. reidi* (Anderson et al. 1987). The tissue of the *S. borealis* we measured weighed 2.3 g, which suggests an approximate respiration rate of 4.6 to 9.9 μmol O_2 $ind.^{-1}$ h^{-1}, assuming similar metabolic pathways in these related species. Using a respiratory quotient of 1.0 (molar ratio of CO_2 produced to oxygen consumed), and assuming the O_2 uptake primarily represents respiration and not sulfide oxidation, the organic carbon required to support this respiration rate would be 4.6 to 9.9 μmol $ind.^{-1}$ h^{-1}.

It is possible to estimate the symbiont sulfide oxidation requirement required to support this estimated carbon oxidation rate using the approach of Kelly and Keunen (1984). They calculate that for each mole of reduced sulfur oxidized by sulfur-oxidizing bacterial symbionts, 0.2 mol of carbon is fixed by the bacteria. Therefore, under steady state conditions the minimum symbiont sulfur oxidation rates required to support the symbiosis respiration rates would be 23 to 50 μmol S_2^- $ind.^{-1}h^{-1}$. This speculative sulfide reduction estimate is highly conservative, as we are assuming 100% carbon translocation and trophic efficiencies. If these values are less, the required sulfide oxidation rates needed to support the estimated respiratory flux would be increased. The calculated sulfide uptake requirement of *Solemya borealis* can be compared to sediment sources of reduced sulfur. The measured reduced S input in summer was 220 μmol m^{-2} h^{-1} (0 to 16 cm) vs a sedimentary pool of AVS of 1 to 5 μmol cm^{-3}.

It is likely that *Solemya borealis* is satisfying its sulfide requirement by "mining" the sediments, as the minimum sulfur demand of just one animal would appear to be between 11 and 23% of the daily sulfur input over the top 16 cm. The large potential bacterial sulfur requirement and the relatively low concentration of soluble sulfides suggest that either large volumes of porewater are being extracted, or more likely, that soluble, partially-oxidized sulfur species or "particulate" sulfides (most likely iron sulfides) are being utilized. The mechanism for such uptake is as yet unclear, but, given the potentially large sulfide demand of this organism even at low densities, its effect on sediment sulfur cycling may be significant. The seeming disparity between soluble sulfide availability and the sulfide demand needed to support bivalve-bacteria symbioses has been observed by other researchers (Dando et al. 1985, Dando and Southward 1986).

To summarize, the microscopical, enzymatic, stable isotope, and biochemical data presented here provides strong evidence for the existence of bacterial endosymbionts in the gills of *Solemya borealis*. The gills of *S. borealis* contain numerous subcellular inclusions which resemble procaryotic cells similar to the symbionts described in *S. velum* (Cavanaugh et al. 1983, 1985) and in *S. reidi* (Felbeck 1983, Anderson et al. 1987). High levels of RuBPcase activity are found only in the symbiont-containing gills of *S. borealis*, suggesting that the symbionts are autotrophic, while the very negative $\delta^{34}S$ ratios of *S. borealis* suggest that porewater sulfides may fuel bacterial chemosynthesis. The very negative $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotope ratios of *S. borealis* indicate that almost all of the clam's carbon, nitrogen, and sulfur is provided by a bacterial diet, while the lipid composition of this species demonstrates possible utilization of bacterial lipids. *S. borealis* is remarkably similar to *S. velum* with respect to stable isotope ratios and biochemical composition (Conway et al. 1989, Conway and McDowell Capuzzo 1991, 1992). When the morphology of the clam is considered (absence of typical digestive system, large gills containing endosymbiotic bacteria), it seems most likely that the endosymbionts provide the dominant food source for *S. borealis*. This conclusion is consistent with the availability of reduced sulfur species in sediments colonized by *S. borealis*, although uptake from reduced sulfur pools other than soluble S^{2-} may be important.

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Literature cited

Anderson, A. E., Childress, J. J., Favuzzi, J. A. (1987). Net uptake of CO_2 driven by sulphide and thiosulphate oxidation in the bacterial symbiont-containing clam *Solemya reidi*. J. exp. Biol. 133: 1–31

Beudeker, R. F., Cannon, G. C., Keunen, J. C., Shively, J. M. (1980). Relations between D-ribulose 1,5-bisphosphate carboxylase, carboxysomes and CO_2 fixing capacity in the obligate, chemolithoautotroph *Thiobacillus neapolitanus* grown under different limitations in the chemostat. Archs Microbiol. 124: 185–189

Bishop, S. H., Ellis, L. L., Burcham, J. M: (1983). Amino acid metabolism in molluscs. In: Hochachka, P. W. (ed.) The Mollusca, Vol. 1. Metabolic biochemistry and molecular biomechanics. Academic Press, New York, p. 244–327

Cavanaugh, C. M. (1983). Symbiosis of chemoautotrophic bacteria and marine invertebrates from sulphide-rich habitats. Nature, Lond. 302: 58–61

Cavanaugh, C. M. (1985). Symbiosis of chemoautotrophic bacteria and marine invertebrates from deep-sea hydrothermal vents and

reducing sediments. In: Jones, M. L. (ed.) Hydrothermal vents of the eastern Pacific: an overview. Bull. biol. Soc. Wash. 6: 373–388

Cavanaugh, C. M., Abbott, M. S., Veenhuis, M. (1988). Immunochemical localization of ribulose-1,5-bisphosphate carboxylase in the symbiont-containing gills of *Solemya velum* (Bivalvia: Mollusca). Proc. natn. Acad. Sci. U.S.A. 85: 7786–7789

Cavanaugh, C. M., Gardiner, S. L., Jones, M. L., Jannasch, H. W., Waterbury, J. B. (1981). Procaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: possible chemoautotrophic symbionts. Science, New York 213: 340–342

Chen, C., Rabourdin, B., Hammen, C. S. (1987). The effect of hydrogen sulfide on the metabolism of *Solemya velum* and enzymes of sulfide oxidation in gill tissue. Comp. Biochem. Physiol. 88B: 949–952

Cline, J. D. (1969). Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14: 454–458

Conway, N., McDowell Capuzzo, J. (1990). The use of biochemical indicators in the study of trophic interactions in animal-bacteria symbioses: *Solemya velum*, a case study. In: Trophic relationships in the marine environment. Proc. 24th Eur. mar. Biol. Symp.: 553–564 [Barnes, M., Gibson, R. N. (eds.) Aberdeen University Press, Aberdeen]

Conway, N., McDowell Capuzzo, J. (1991). Incorporation and utilization of bacterial lipids by the *Solemya velum* symbiosis. Mar. Biol. 108: 277–291

Conway, N., McDowell Capuzzo, J. (1992). Excess taurine in the *Solemya velum* symbiosis, possible sources and functions. Comp. Biochem. Physiol. B (in press)

Conway, N., McDowell Capuzzo, J., Fry, B. (1989). The role of endosymbiotic bacteria in the nutrition of *Solemya velum*: evidence from a stable isotope analysis of endosymbionts and host. Limnol. Oceanogr. 34: 149–155

Dall, W. H. (1908). A revision of the Solenomyacidae. Nautilus 22(1): 1–2

Dando, P. R., Southward, A. J. (1986). Chemoautotrophy in bivalve molluscs of the genus *Thyasira*. J. mar. biol. Ass. U.K. 66: 915–929

Dando, P. R., Southward, A. J., Southward, E. C., Terwilliger, N. B., Terwilliger, R. C. (1985). Sulphur-oxidizing bacteria and haemoglobin in gills of the bivalve mollusc *Myrtea spinifera*. Mar. Ecol. Prog. Ser. 23: 85–98

Degens, E. T. (1969). Biogeochemistry of stable carbon isotopes. In: Eglinton, E., Murphy, M. J. T. (eds.) Organic geochemistry. Springer-Verlag, Berlin, p. 304–329

Distel, D. L., Lane, D. J., Olsen, G. J., Ciovannoni, S. J., Pace, B., Pace, N. R., Stahl, D. A., Felbeck, H. (1988). Sulfur-oxidizing bacterial endosymbionts: analysis of phylogeny and specificity by 16S rRNA sequences. J. Bact. 170: 2506–2510

Felbeck, H. (1983). Sulfide oxidation and carbon fixation by the gutless clam *Solemya reidi*: an animal-bacterial symbiosis. J. Comp. Physiol. (Sect. B) 152: 3–11

Fiala-Médioni, A., Alayse, A. M., Cahet, G. (1986). Evidence of in situ uptake and incorporation of bicarbonate and amino acids by a hydrothermal vent mussel. J. exp. mar. Biol. Ecol. 96: 191–198

Fisher, C. R. (1990). Chemoautotrophic and methanotrophic symbioses in marine invertebrates. Rev. aquat. Sciences 2: 399–443

Fisher, C. R. Childress, J. J. (1986). Translocation of fixed carbon from symbiotic bacteria to host tissues in the gutless bivalve *Solemya reidi*. Mar. Biol. 93: 59–68

Fry, B., Cox, J., Gest, H., Hayes, J. M. (1986). Discrimination between ^{34}S and ^{32}S during bacterial metabolism of inorganic sulfur compounds. J. Bact. 165: 328–330

Fry, B., Sherr, E. B. (1984). $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. mar. Sci. Univ. Tex. 27: 13–47

Fulco, A. J. (1983). Fatty acid metabolism in bacteria. Prog. Lipid Res. 22: 133–160

Gearing, J. N., Gearing, P. J., Rudnick, D. T., Requejo, A. G., Hutchins, M. J. (1984). Isotope variability of organic carbon in a phytoplankton-based temperature estuary. Geochim. cosmochim. Acta 48: 1089–1098

Giere, O., Conway, N., Gastrock, G., Schmidt, C. (1991) Regulation of the gutless annelid ecology by endosymbiotic bacteria. Mar. Ecol. Prog. Ser. 68: 287–299

Giero, O., Felbeck, H., Dawson, R., Liebezeit, G. (1984). The gutless marine oligochaete *Phallodrilus leukodermatus* Giere, a tubificid of structural, ecological and physiological significance. Hydrobiologica 115: 83–89

Goad, L. J. (1976). The steroids of marine algae and invertebrate animals. In: Malins, D. C., Sargent, J. R. (eds.) Biochemical and biophysical perspectives in marine biology, Vol. 3. Academic Press, New York, p. 213–318

Goldfine, H. (1972). Comparative aspects of bacterial lipids. Adv. microb. Physiol. 8: 1–58

Goldhaber, M. B., Kaplan, I. R. (1975). Controls and consequences of sulfate reduction rates in recent marine sediments. Soil Sci. 119: 42–55

Gustafson, R. G., Reid, R. G. B. (1988). Association of bacteria with larvae of the gutless protobranch bivalve *Solemya reidi* (Cryptodonta: Solemyidae). Mar. Biol. 97: 389–401

Hartmann, U. M., Nielson, H. (1969). $\delta^{34}S$ Werte in rezenten Meeressedimenten und ihre Deutung am Beispiel einiger Sedimentprofile aus der westlichen Ostsee. Geol. Rdsch. 58: 621–655

Howes, B. L., Dacey, J. W. H., King, G. M. (1984). Carbon flow through oxygen and sulfate reduction pathways in salt marsh sediments. Limnol. Oceanog. 29: 1037–1051

Joseph, J. D. (1982). Lipid composition of marine and estuarine invertebrates. Part II: Mollusca, Prog. Lipid. Res. 21: 109–153

Jørgensen, B. B. (1977). The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnol. Oceanogr. 22: 814–832

Kelley, D. P., Kuenen, J. G. (1984). Ecology of the colourless sulphur bacteria. In: Codd, G. A. (ed.) Aspects of the microbial nutrition and ecology. Academic Press, New York, p. 211–240

Kuznetsov, A. P., Ohta, S., Endow, K. (1990). Morphofunctional consequences of bacterial symbiotrophy in *Solemya* (*Petrasma*) *pusilla* (Protobranchia, Bivalvia) from the Sagami Bay (Central Japan). Izv. Akad. Nauk SSSR 6: 895–903

Lehninger, A. L. (1975). Biochemistry, 2nd edn. Worth Publishers Inc., New York

McCaffrey, M. A., Farrington, J. W., Repeta, D. J. (1989). Geochemical implications of the lipid composition of *Thioploca* sp. from the Peru upwelling region –15 °S. Org. Geochem. 14: 61–68

Miziorko, H. M., Lorimer, G. H. (1983). Ribulose-1,5-bisphosphate carboxylase-oxygenase. A. Rev. Biochem. 52: 507–535

Moreno, J., Pollero, A. E., Moreno, V. J., Brenner, R. R. (1980). Lipids and fatty acids of the mussel (*Mytilus platensis* d'Orbigny) from South Atlantic Waters. J. exp. mar. Biol. Ecol. 48: 263–276

Morse, E. S. (1913). Observation of living *Solenomya velum* and *borealis*. Biol. Bull. mar. biol. Lab., Woods Hole 25: 261–281

Morse, E. S. (1919). Observations on living lamellibranchs of New England. Proc. Boston. Soc. nat. Hist. 35(5): 139–196

Piretti, M. V., Tioli, F., Pagliuca, G. (1987). Investigation of the seasonal variations of sterol and fatty acid constituents in the bivalve molluscs *Venus gallina* and *Scapharca inaequivalvis* (Bruguiére). Comp. Biochem. Physiol. 88B: 1201–1208

Pollero, R. J., Re, M. E., Brenner, R. R. (1979). Seasonal changes of the lipids of the mollusc *Chlamys technelcha*. Comp. Biochem. Physiol. 64A: 257–263

Reid, R. G. B. (1980). Aspects of the biology of a gutless species of *Solemya* (Bivalvia: Protobranchia). Can. J. Zool. 58: 386–393

Reid, R. G. B., Brand, D. G. (1987). Observations on Australian Solemyidae. J. malac. Soc. Aust. 8: 41–50

Rounick, J. S. Winterbourn, M. J. (1986). Stable carbon isotopes and carbon flow in ecosystems. BioSci. 236: 171–177

Ruby, E. G., Jannasch, H. W., Deuser, W. G. (1987). Fractionation of stable carbon isotopes during chemoautotrophic growth of sulfur-oxidizing bacteria. Appl. envirl Microbiol. 53: 1940–1943

Sanders, H. L. (1958). Benthic studies in Buzzards Bay. I. Animal-sediment relationships. Limnol. Oceanogr. 3: 245–258

Sanders, H. L. (1960). Benthic studies in Buzzards Bay. III. The structure of the soft-bottom community. Limnol. Oceanogr. 5: 138–153

Scheiner, D. (1976). Determination of ammonia and Kjeldahl nitrogen by indophenol method. Wat. Res. 10: 31–36

Southward, A. J., Southward, E. C., Dando, P. R., Barrett, R. L., Ling. R. (1986). Chemoautotrophic function of bacterial symbionts in small pogonophora. J. mar. biol. Ass. U.K. 66: 415–437

Southward, E. C. (1986). Gill symbionts in thyasirids and other bivalve molluscs. J. mar. biol. Ass. U.K. 66: 889–914

Soyer, J., Soyer-Gobillard, M.-O., Thiriot-Quiévreux, C., Bouvy, M., Cahet, G. (1987). Chemoautotrophic bacterial endosymbionts in *Spisula subtruncata* (Bivalvia, Mactridae). Ultrastructure, metabolic significance and evolutionary implications. Symbioses 3: 301–314

Spiro, B., Greenwood, P. B. Southward, A. J., Dando, P. R. (1986). $^{13}C/^{12}C$ ratios in marine invertebrates from reducing sediments: confirmation of nutritional importance of chemoautotrophic endosymbiotic bacteria. Mar. Ecol. Prog. Ser. 28: 233–240

Stahl, D. A., Lane, D. J., Olsen, G. J., Pace, N. R. (1984). Analysis of hydrothermal vent associated symbionts by ribosomal RNA sequences. Science, New York 224: 409–411

Tabatabai, M. A. (1974). Determination of SO_4 in water samples. Sulphur Inst. J. 10: 11–14

Verma, D. P. S., Long, S. (1983). The molecular biology of *Rhizobium*-legume symbiosis. Int. Rev. Cytol. (Suppl.) 14: 211–245

Williams, C. A., Nelson, D. C., Farah, B. A., Jannasch, H. W., Shively, J. M: (1988). Ribulose bisphosphate carboxylase activity of the procaryotic symbiont of a hydrothermal vent tube worm: kinetics, activity and gene hybridization. Fedn eur. microbiol. Soc. (FEMS) Lett. 50: 107–112

Yonge, C. M. (1939). The protobranchiate mollusca: a functional interpretation of their structure and evolution. Phil. Trans. R. Soc. (Ser. B): 230–279

Zhabina, N. N., Volkov, I. I. (1976). A method of determination of various sulfur compounds in sea sediments and rocks. In: Krumbein, W. E. (ed.) Environmental biogeochemistry and geomicrobiology, Vol. 3. Methods, metals and assessment. Ann Arbor Science Publ. Ann Arbor