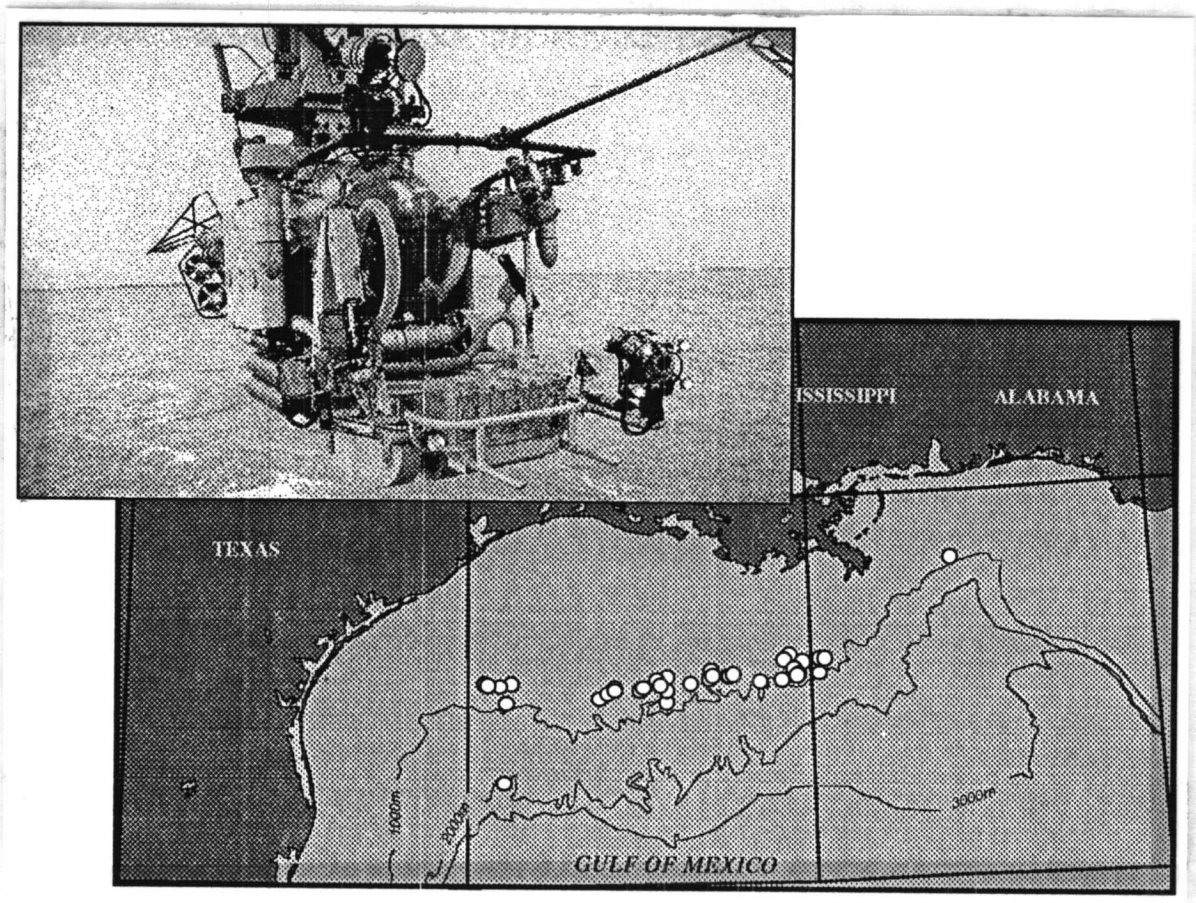
OCS Study
MMS 92-0035

Northern Gulf of Mexico

Chemosynthetic Ecosystems Study

Literature Review and Data Synthesis

Volume III: Appendix



MMS U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

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Editor

Ian R. MacDonald

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COVER PHOTOGRAPH

The foreground photograph shows the submersible *Johnson Sea-Link I* preparing for one of its many dives to study chemosynthetic ecosystems. The map depicts the locations of known chemosynthetic ecosystems in the northern Gulf of Mexico.

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Geology of a Continental Slope Oil Seep, Northern Gulf of Mexico

The American Association of Petroleum Geologists Bulletin
V. 72, No. 2 (February 1988), P. 105-114, 14 Figs., 1 Table

Geology of a Continental Slope Oil Seep, Northern Gulf of Mexico[1]

E. WILLIAM BEHRENS[2]

ABSTRACT

An oil and gas seep was documented by replicate sampling with piston corer, abundant high-resolution and sparse multichannel seismic reflection profiling, and chemical and isotopic analyses. The seep occurs on the upper continental slope over a salt ridge interpreted to split and plunge eastward, northeastward, and northward. The relatively shallow diapir over which the seepage occurs is manifested at the surface by a graben in strike section and by a half-graben in dip section. Faulting over the crest is commonly associated with loss of reflected energy or acoustic wipeouts. Most cores taken in wipeouts with prolonged bottom echoes contain oil and gas. The cores also commonly contain secondary carbonate derived from microbial degradation of hydrocarbons. The isotopic lightness of the carbonate and its negative correlation with porosity may be subtle indicators of seepage at sites where oil and gas are not obvious. The seepage demonstrates the existence of source rocks and maturation at this site.

INTRODUCTION

Of the four requirements for a hydrocarbon deposit (source organics, maturation, porosity, and entrapment), satisfaction of the first two is directly proven by observing hydrocarbons that have migrated to the surface—an oil seep. A seep is particularly significant in a frontier province of petroleum exploration. This paper reports on the geology of an oil seep located on the upper continental slope in the Green Canyon sector of the northern Gulf of Mexico (Figure 1). This is the first offshore oil seep in the northern Gulf of Mexico to be documented by a detailed geophysical survey, replicate sampling, and chemical and isotopic analyses of the hydrocarbons.

Direct evidence of oil migration is the occurrence of up to 8% extractable hydrocarbons in sediments cored at this locality. Of 16 piston cores taken in the area, at least nine contained liquid crude oil macroscopically visible either to the unaided eye or as bright fluorescence under ultraviolet light. The structural features of the continental slope off western Louisiana and Texas result primarily from salt tectonics, with diapiric structures underlying approximately one-half of the region (Martin, 1980).

Location

The 1,500 km^2 (440 nmi^2) area studied lies between latitudes 27°35′ and 28°00′N and longitudes 91°05′ and 91°25′W and includes water depths from about 200 to 1,000 m (660-3,280 ft) on the upper continental slope (Figure 1). The study area is west of the Mississippi fan and in the central slope (Holland, 1970), a province characterized by such an abundance of diapirs that sedimentary strata occur essentially as isolated or semi-isolated intraslope troughs or basins (Martin and Bouma, 1978). The oil-bearing cores were from a more restricted (70 km^2; 20 nmi^2) area within latitudes 27°43′ to 27°45′N and longitudes 91°12′ to 91°22′W in water depths from about 540 to 630 m (1,770-2,070 ft; Figures 1, 2).

METHODS

The area was crossed first (March, 1981) while collecting multichannel data for a regional intraslope basin study, which included piston coring. A core containing oil was taken during July 1982. After this discovery, a rectilinear grid of 36 north-south and 26 east-west lines was surveyed (Figure 3). High resolution (3.5 kHz) vertical reflection profiles were collected continuously on all cruises: 1,355 km (730 nmi) within the grid; 1,620 km (875 nmi) total. All navigation was done with LORAN-C.

The 3.5 kHz reflections were from as deep as 100 m (330 ft) and thus enabled mapping of not only bathymetry but also features such as faults, gas wipeouts, and extent of strata (Figure 2). These features were used to interpret deeper structures, especially diapirs, which could be verified at a few points with the multichannel data. Subbottom depth estimates are based on velocity analyses of multichannel data nearest the site discussed.

The target for the core in which oil was initially discovered was a fault escarpment. Thus, the 15 subsequent cores were aimed at faulted zones and 3.5 kHz reflection patterns commonly associated with faults. Cores were

[1]Manuscript received, May 2, 1985; accepted, August 14, 1987.

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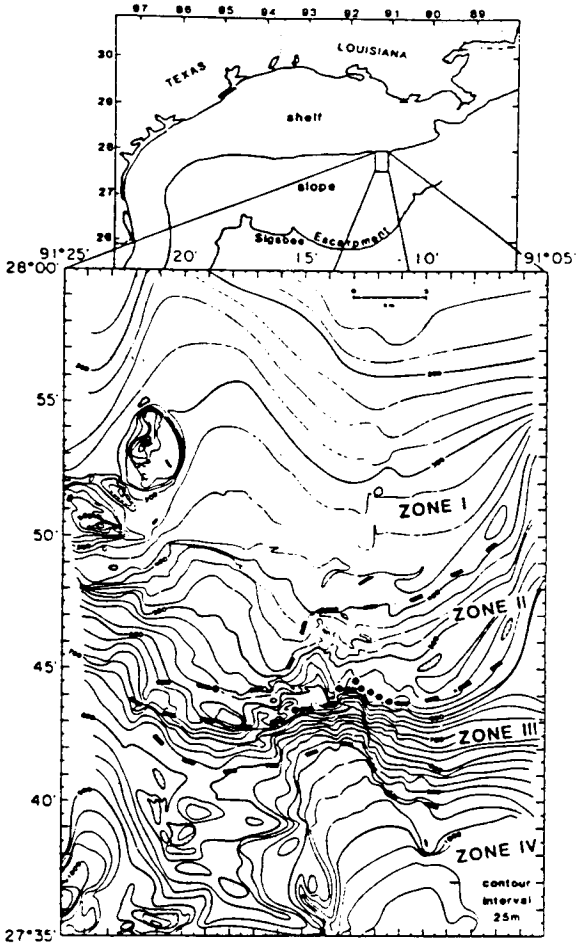

Figure 1—Location and index maps. Bathymetric zones I-IV (separated by dashed lines) are discussed in the text. Locations of cores with visible oil are shown as solid circles.

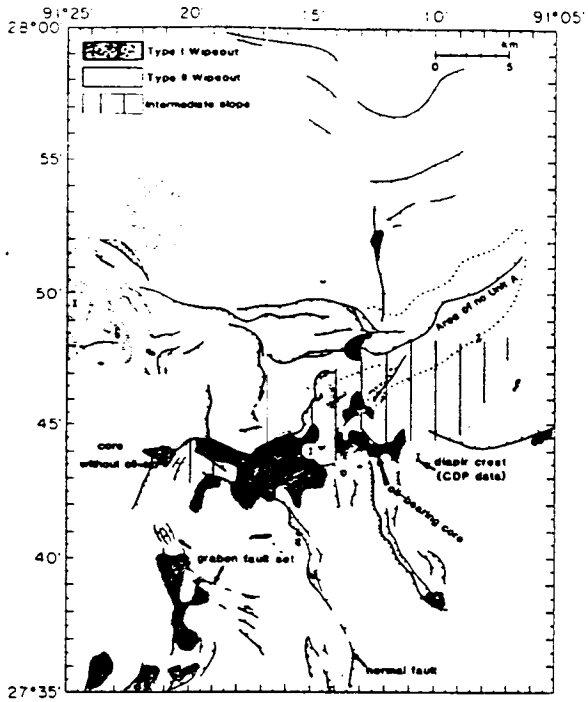

Figure 2—Geologic map. Type I wipeouts have weak or normal bottom echoes. Type II wipeouts have strong and prolonged bottom echoes. "G" is location of deep wipeout shown in Figure 10. Hatchures and small squares are on the downthrown sides of faults. Patterns and symbol meanings are indicated by labels on figure.

analyzed for bulk density, water content, and $CaCO_3$ content at 20-cm (8-in.) intervals where possible. Organic carbon, stable carbon isotope, and hydrocarbon and gas analyses were done on a variety of samples selected on the basis of visible oil or oil fluorescence. Chemical analyses have been reported by Anderson et al (1983) and Anderson (1984).

OBSERVATIONS AND INTERPRETATIONS

Faults and 3.5 kHz Reflection Characteristics

Normal faults are abundant. Recently active movement is suggested by spectacular seabottom fault escarpments of 70 m (230 ft) or more (Figures 4-6). Two fault-block types were mapped: single, normal fault-blocks and crestal grabens. Crestal grabens are defined descriptively as fault pairs or sets with a central downthrown zone and located in a region that is structurally elevated above adjacent areas or is upwarped from a region of monoclinally dipping beds. This structural style is, of course, typical of the zone of extension over a diapir.

The most widespread reflection pattern in this and many other parts of the northern Gulf of Mexico consists of multiple, parallel to subparallel, draping reflections through about 100 msec of record, representing 75-100 m (250-330 ft) or more of subbottom. In (and beyond) the study area, three stratigraphic units commonly can be identified within this section (Figure 7). Upper and lower [(a) and (c) respectively] mostly transparent units enclose a thicker unit (b) with multiple (20-30) internal parallel reflections. Unit (a) is generally 5-15 m (16-50 ft) thick; (b) is 35-120 m (115-400 ft), and (c) is 15-60 m (50-200 ft) in thickness. Unit (a) thins to zero over part of the study area (Figures 2, 7).

Sedimentation rates for upper slope regions (10-60 cm/1,000 years; 4-24 in./1,000 years) suggests the boundary between units (a) and (b) represents the time when the depositional system changed from that characteristic of low sea level glacial conditions to that characteristic of the present high sea level hemipelagic domain, or about 15,000-10,000 years B.P. Unit (c), by analogy, may represent an earlier period of relatively high sea level.

Many subbottom reflections within these units weaken or disappear completely. These lost reflections are termed wipeouts, of which two forms are recognized. In

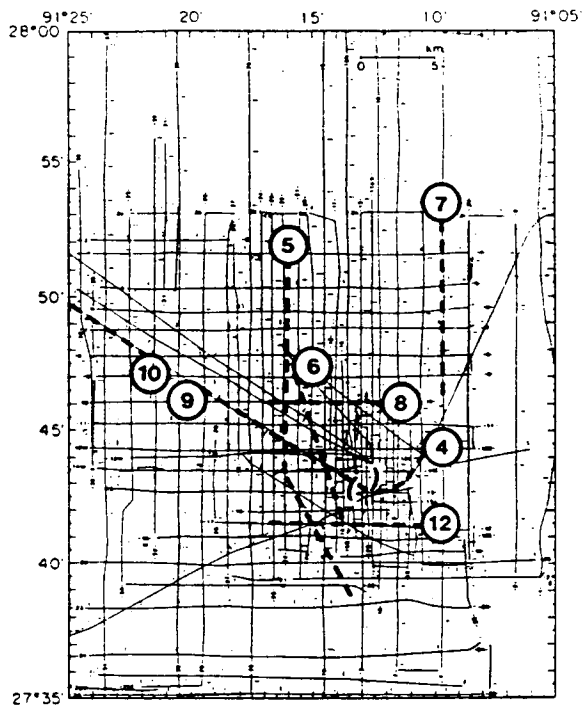

Figure 3—Track lines used to map bathymetry and geology. Heavier dashed lines with circled numbers are parts of tracks illustrated in other figures, with circled numbers being figure numbers. Lines in Figures 4 and 9 overlap slightly with extents indicated by parentheses at the end of the line opposite the figure number. Indiscernably small numbers are time annotations—usually every 15 min.

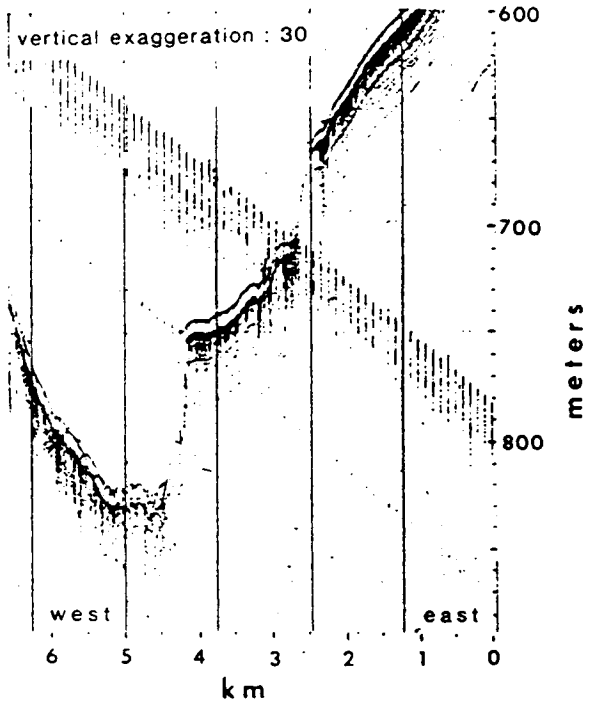

Figure 4—Fault escarpments of approximately 70 and 50 m (230 and 165 ft) suggest very active tectonism. 3.5 kHz reflection profile; see Figure 3 for location.

the more widespread type (I), the loss of subbottom reflections is accompanied either by no change or by weakening of the echo from the sediment-water interface (Figures 5, 6). In the second type of wipeout (II), the subbottom characteristics are the same as in type I, but the bottom echo is strong and prolonged (Figures 6, 7).

Loss of subbottom reflections must represent either a loss of reflectors or a loss of sound energy before it can be reflected. Loss of energy can result from scattering or absorption (conversion to heat energy). The presence of gas bubbles (even small quantities) in water and sediment has been shown to affect very high attenuations by both mechanisms (Silberman, 1957; Anderson, 1974), and correlations of gaseous sediment with reflected energy loss (wipeouts) have been reported (e.g., Addy and Worzel, 1979).

Prolonged bottom echoes have commonly been correlated to rough or coarse-grained sediment textures (e.g., Damuth, 1978; Addy et al, 1982). In type II wipeouts, loss of subbottom reflections may involve gas attenuation, but also may result from high reflectivity and scattering at or just below the water bottom.

In the study area, wipeouts almost always occur associated with faults and/or areas of high relief or irregular bathymetry. Wipeouts, especially type II, were the reflection characteristic targeted for coring. These targets were successfully struck at 14 of 16 sites, and 12 of 14 on-target cores contained gas.

Bathymetry and Structural Analysis

Bathymetrically, the study area can be divided into four east-trending zones, subparallel to depth contours (Figure 1). The shallowest, northern zone (I) is characterized by broad features and gentle slopes (1°) except for two high-relief banks in the western part. The second and third zones (II and III) have intermediate (2.5°) and high (5°) slopes, respectively. The southernmost zone (IV) is characterized by alternating troughs and broad uplifts.

Zone I.—Zone I is interpreted as an upperslope basin bounded by piercement diapirs on the west and, downslope, by a salt ridge, which swings northeastward forming a partial lateral boundary to the east. A common-depth-point (CDP) profile (Figure 9) shows the upslope (but basinward) dip reversal in the southern part of zone I and shows at least three well-developed seismic sequences in the upper 3 sec (2.8 km or 9,200 ft) of this basin. Bouma (1981) and Beard et al (1982) have interpreted such sequences as reflecting Pleistocene glacioeustatic cycles. Beard et al (1982) recognized eight Pleistocene seismic sequences at another point on the slope. Thus, the section seen here is probably Pleistocene.

The western diapirs have bathymetrically obvious relief of up to 200 m (651 ft). Piercement is indicated by

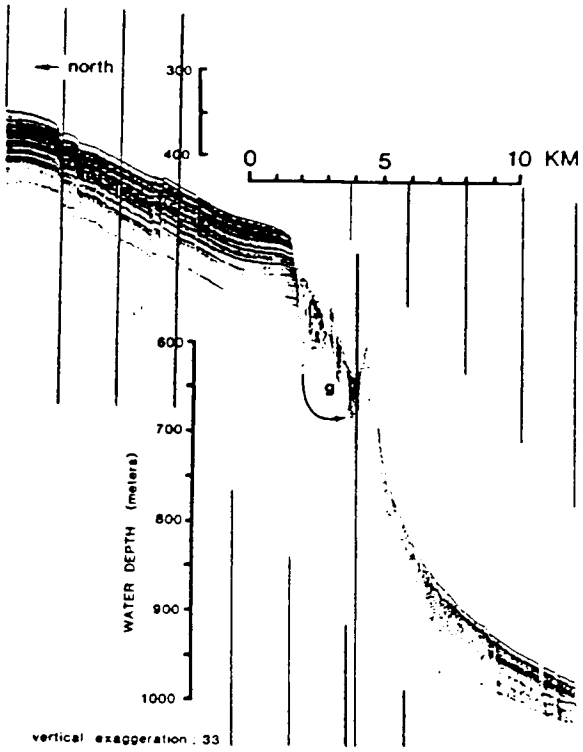

Figure 5—Type I wipeouts (g) in 3.5 kHz reflection profiles. Wipeout occurs in highly faulted zone of intermediate slope (bathymetric zone II) between flatter section updip (north) and steeper section downdip (south). Curved arrow shows suggested fault movement. See Figure 3 for location.

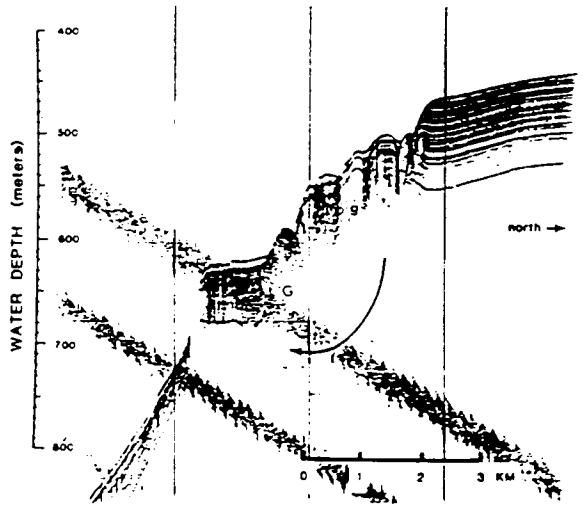

Figure 6—Types I (g) and II (G) wipeouts in a 3.5 kHz profile across the same features shown in Figure 5. Arrow shows suggested fault movement. See Figure 3 for location.

the pinching out of shallow stratigraphic units (a)-(c) against very prolonged bottom echoes, which suggest indurated sediments and/or a coarse lag of surface material. The CDP profile (Figure 10) shows salt or caprock at approximately 790 m (2,600 ft) subbottom and, possibly, a deep, gas wipeout adjacent to the diapir.

Eastward from these diapirs, the bathymetric contours show first, a broad southward-plunging trough and second, a rise across zone I (Figure 1). These contours may represent, respectively, salt withdrawal and a deep, salt pillow. A set of east-west faults (27°47′ to 27°49′N, 91°13′ to 91°17′W) appears to form a graben with small fault displacements (0-10 m; 0-33 ft) and with a minimum of associated uplift; however, this feature may be a trough between two slightly overlapping uplifts to the west and east. The eastern feature is a faulted, broad upwarp from which unit (a) is absent (Figures 2, 7). The erosional or nondepositional hiatus includes the uppermost one of about 20 reflections in unit (b), but all older units are unaffected. Thus, uplift occurred near the end of or after deposition of unit (b). The amount of uplift estimated by measuring the height of the crest above a uniform average slope for adjacent parts of the line is 57 m (190 ft). Using an age range for the top of unit (b) that represents a possible range of times when sedimentation changed from low to high sea level conditions (9,500 to 19,000 years B.P.), the rate of uplift ranges from 3 to 6 mm (0.12 to 0.24 in.) per year.

Zone II.—In zone II, average slopes increase to 1°-5°, but relief caused by faulting increases abruptly and is the highest of the four zones. Wipeouts are also most widespread, sometimes extending entirely across the zone (e.g., 91°15′ to 91°19′W; Figure 2). East of 91°15.5′W, this zone doubles in width by bifurcating, one ridge continuing more or less eastward and the other trending northeastward.

West of this widening, faults are almost all downthrown downslope, but northeastward, past the bifurcation, the faults become more symmetric and form a crestal graben (Figure 8). The ridge bearing this graben continues northeastward, becoming the upwarp without unit (a), and therein, faulting simplifies into one fault downthrown northward into the zone I basin. Faulting does the same in the eastward extension from the first bifurcation. At approximately 91°12.5′W, the northeastern ridge may bifurcate again with the northward extension being the broad ridge (salt pillow?) in zone I.

Zone III.—In Zone III, the slope increases abruptly to 2°-9°. The zone extends from 800-1,000 m (2,600-3,280 ft) water depth and has few faults. Zone III is interpreted as the downslope flank of a diapir ridge that forms the central structure of the study area.

The simplest form of this structure can be seen in the region between longitudes 91°16′ and 91°20′W. There, bathymetric zones I, II, and III can be considered as analogous to the flank-graben-flank sequence seen in Figure 8, but different in that the whole system is tilted downslope. The addition of regional dip to the diapiric uplift makes the flank slopes unsymmetric—flatter upslope, steeper downslope. Within the crestal zone (II), the downslope component of gravity enhances extension at the upslope side, but weakens or replaces extension with

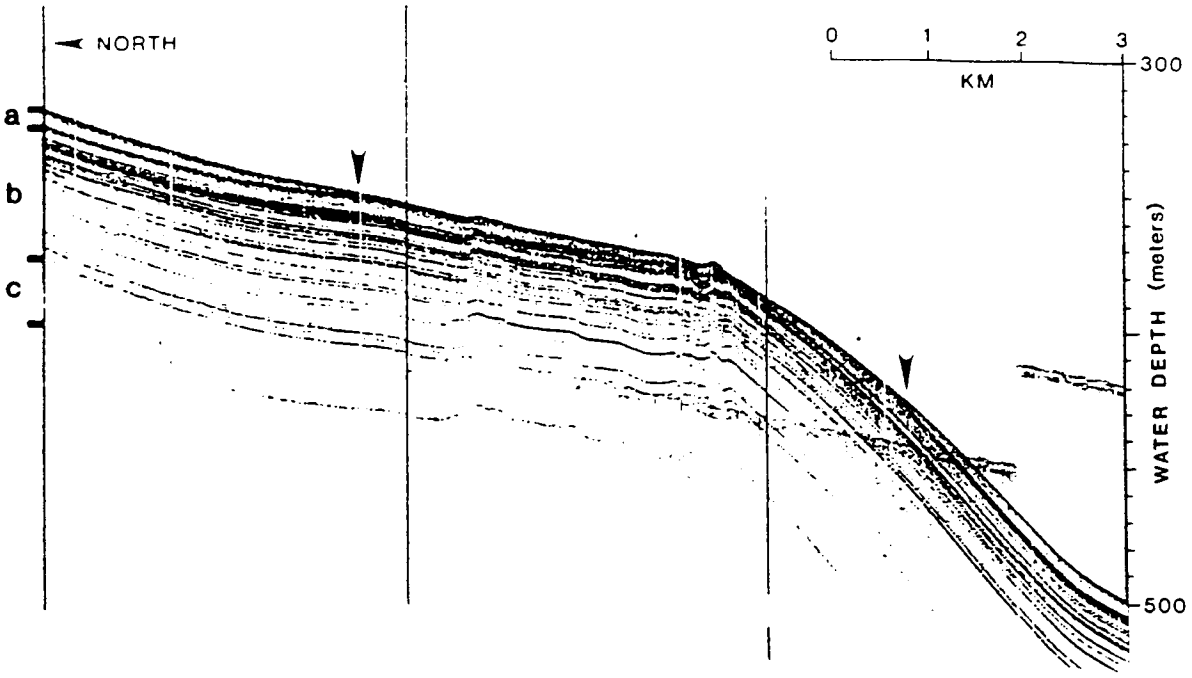

Figure 7—High resolution (3.5 kHz) profile showing stratigraphic units (a, b, and c) usually present. Unit (a) is missing between arrows. See Figure 3 for location.

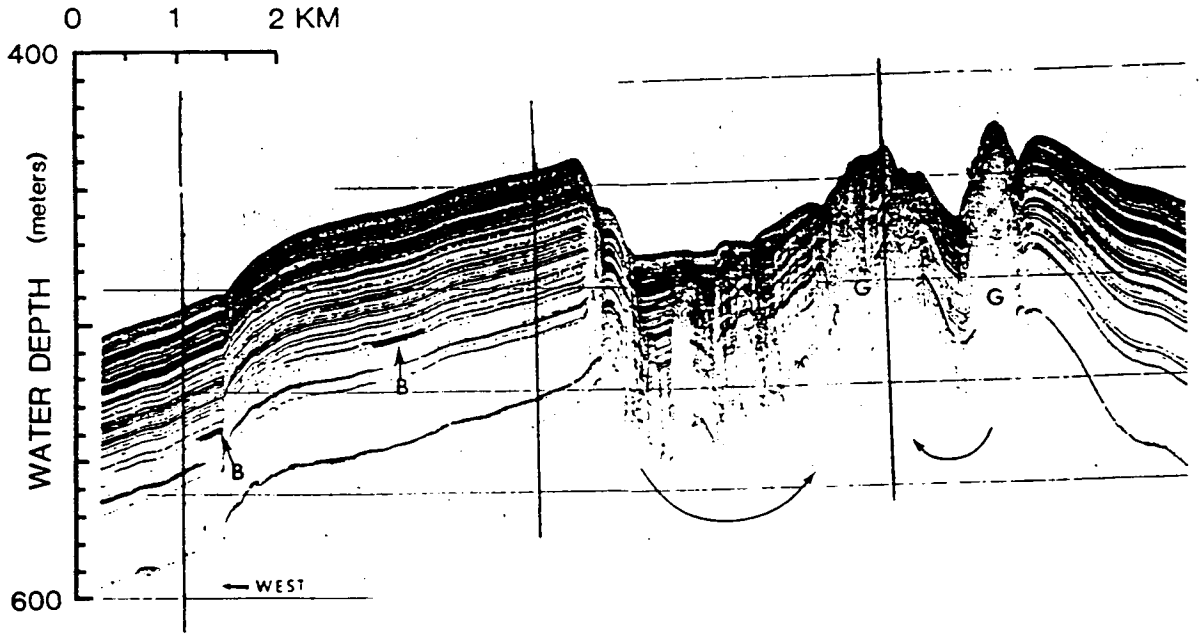

Figure 8—East-west (strike) 3.5 kHz profile showing graben faulting and type II (G) wipeouts in the ridge forming the northeastward expansion of bathymetric zone II. Extraordinarily high amplitude reflections (B) may represent secondary carbonate, gas, or sulfides. Arrows show suggested fault movements. See Figure 3 for location.

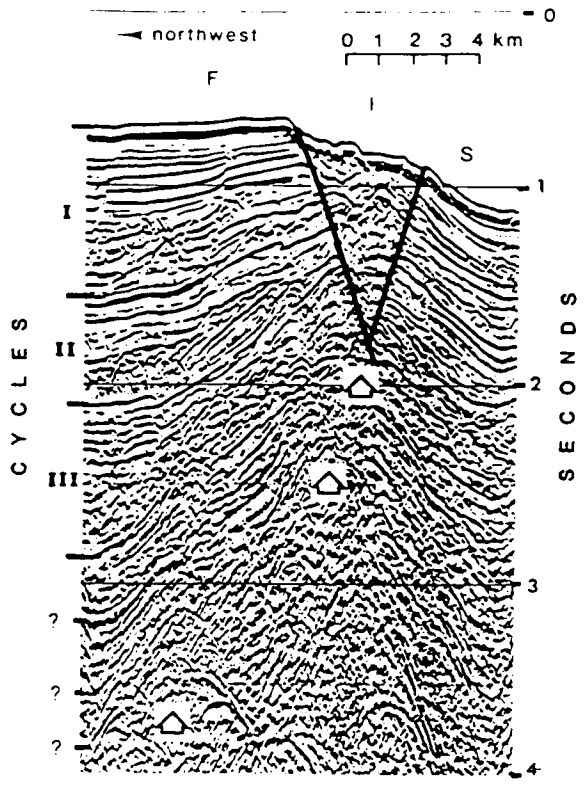

Figure 9—Multi-channel (12-fold) profile through central diapir ridge (CDR) showing sequence of flatter slope (F) updip, intermediate slope (I) over the diapir crest, and steeper slope (S) downdip. Cyclic sedimentary units (I, II, and III) consisting of alternating chaotic and strong subparallel reflections are shown in basin upslope (northwest) of diapir. Question marks indicate other possible cycle or sequence boundaries. Faulting within zone of intermediate slope is more intense and complex than elsewhere. Note deeper structural crests (arrows) beneath this zone seem to be farther upslope. See Figure 3 for location.

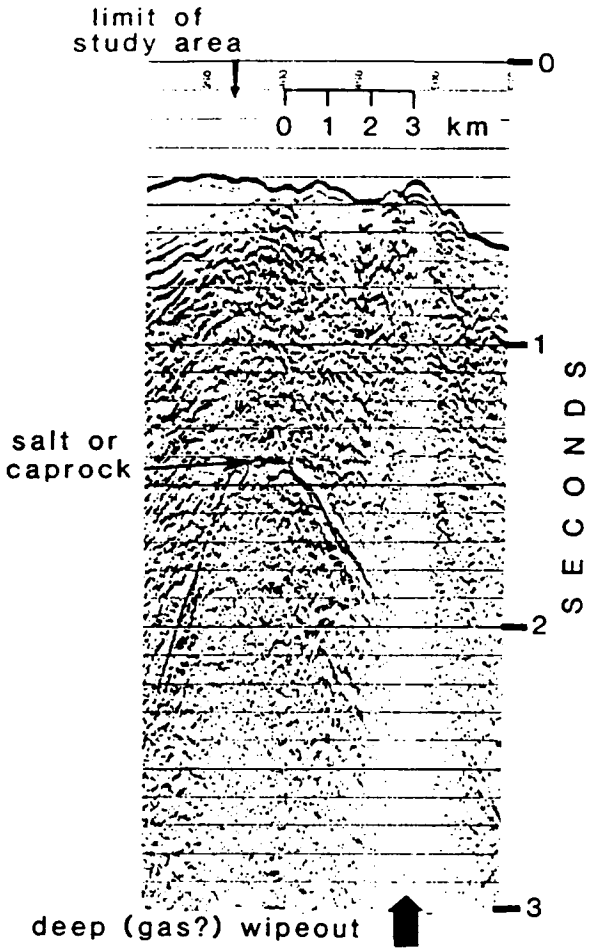

Figure 10—Multi-channel (12-fold) profile through the high-relief banks in northwest part of study area. Uplift is underlain by strong reflection (at approximately 1.4 sec) characteristic of salt or caprock. One pinnacle is underlain by a deep loss of seismic energy analogous, perhaps, to gas wipeouts in 3.5 kHz profiles. See Figure 3 for location.

compression at the downslope side; and the graben in strike-section becomes a half-graben in dip-section (Figures 6, 9).

Although the multichannel line (Figure 9) shows that uplift is centered under the faulted zone II, the profile shows no reflection strong enough to be interpreted as salt or caprock. However, the presence of salt is indicated by a downward increase in interstitial water salinity to >200 °/₀₀ (six times that of normal seawater) in a core taken within the zone (Figure 11).

Zone IV.—Zone IV, from west to east, consists of part of a trough, a rise, another trough, and part of another rise. These structures are interpreted as alternating zones of salt withdrawal and diapirism. Graben-type faulting and type I wipeouts on the subcentral rise support the diapir interpretation.

The trough east of this uplift is well defined by faults, (Figure 12) which, in fact, make it a graben. With a width of approximately 7 km (4 nmi), the trough is roughly twice as wide as the largest diapir-crest graben. The trough is also much longer, extending southeastward beyond the study area. This trough merges northward into the steep slope of zone III and projects into the narrower graben cresting the northeastward-trending arm of the bifurcating diapir ridge in zone II. The faults forming the boundaries of this trough are interpreted to result from differential vertical movements due, in turn, to salt flow at depth, and not to result from extension across an uplift. The trough shape may be fortuitous or may reflect glacierlike downslope movement of the salt, sliding by gravity over thinned, transitional crust at an early stage of basin development.

Diapir Network

The diapirs in this area may form a network of structures (Figure 13). The more separated diapirs occur in the

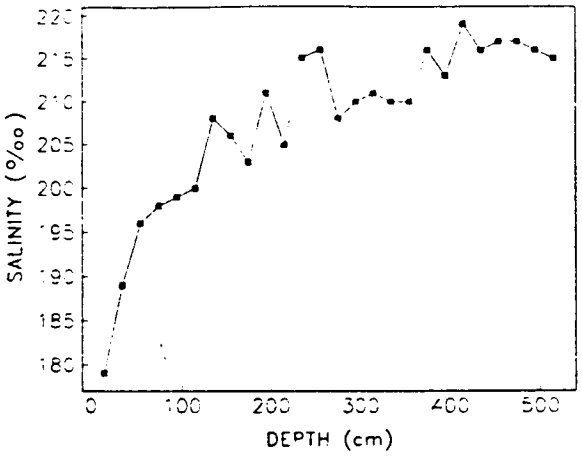

Figure 11—Salinity vs. depth in one oil-bearing core.

northwest and southwest of the study area (although connections to other diapirs at depths greater than about 5 sec cannot be ruled out entirely). The primary network consists of a central diapir ridge (CDR), which splits twice to form eastern, northeastern, and northern arms, each plunging away from the CDR. This splitting results in zone II changing from highly faulted to the west into a smooth swale between two less deformed anticlines to the east.

The CDP line crosses the CDR near the first bifurcation. The salt cannot be seen clearly, but the structure suggests that its top is no shallower than 824 m (approximately 1 sec or 2,700 ft) subbottom (Figure 9). At greater depths, structural crests seem displaced upslope. The CDP line also crosses the eastward and northeastward extensions of the CDR where minimum depths to diapir tops appear to be 980 and 1,360 m (3,200 and 4,460 ft) respectively. This line also shows that diapiric uplift crests slightly downslope of the bathymetric crest of each ridge. Furthermore, a normal fault near the bathymetric crest of each ridge has the downthrown side to the north, suggesting slightly greater extension upslope. These observations could be explained by slight downslope tilting, overturning, or thrusting of the diapirs.

At about 27°48'N and 91°12'W, the northeastern arm may split again forming a continuation to the east-northeast and a new arm under the broad, slightly faulted ridge extending due north to the shelf break (Figure 13).

Hydrocarbon Occurrence

The hydrocarbons are within a homogeneous to layered, clayey mud. Most of the mud is olive gray, but some with higher organic content is dark gray to black, and some surficial mud (top 1-3 m of core) is lighter in color and browner. A brown/gray color change commonly associated with the Holocene-Pleistocene boundary occurs in six cores at depths from 40 to 320 cm (16 to 126 in.). The radiocarbon age of this color change in other slope cores is 12,000 years B.P., indicating an average Holocene deposition rate of 13 cm (5 in.)/1,000 years at about 70% porosity, which is consistent with other estimates for slope sedimentation rates (Behrens, 1980). The oil occurs in both Pleistocene and Holocene strata.

The oil occurs in four modes. In large amounts, oil forms (1) an interconnecting network of veins with gum-like texture. In smaller amounts, oil may be (2) disseminated as tiny droplets, (3) disseminated as linings of gas vugs, or (4) lining high-angle fractures. The fractures have the attitudes of normal faults, but displacement is not apparent within the cores. Gas-liquid chromatography (GLC) analyses showed the oil is largely an unresolved, complex mixture with both the normal alkanes and aromatic hydrocarbon peaks being nearly absent from the chromatographs (Anderson et al, 1983). This

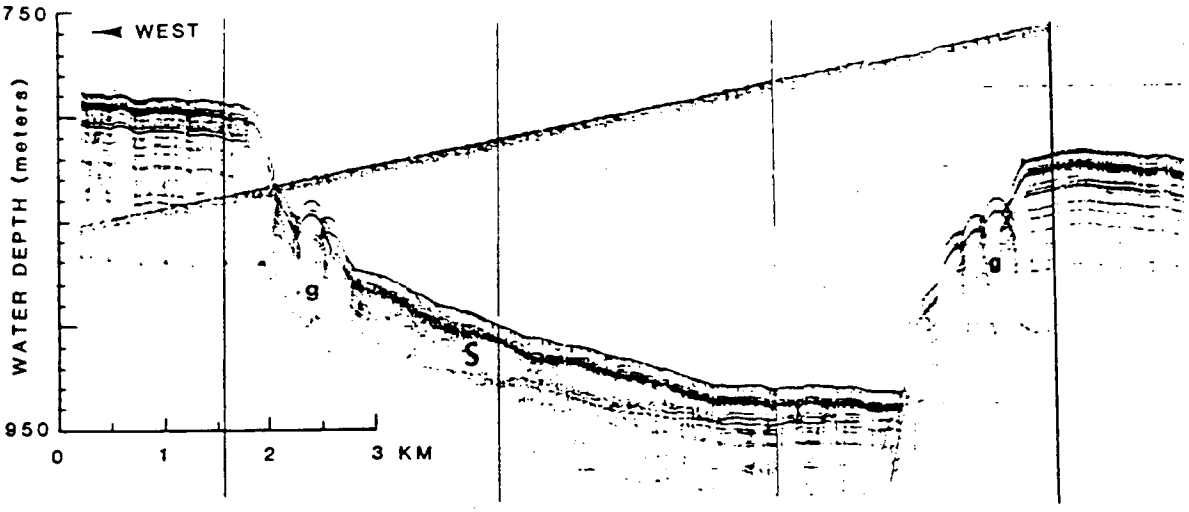

Figure 12—Transverse 3.5 kHz profile of major trough in bathymetric zone IV. Note slump (S) extending from the western margin as an acoustically opaque zone thinning eastward. See Figure 3 for location; (g) shows type I wipeout.

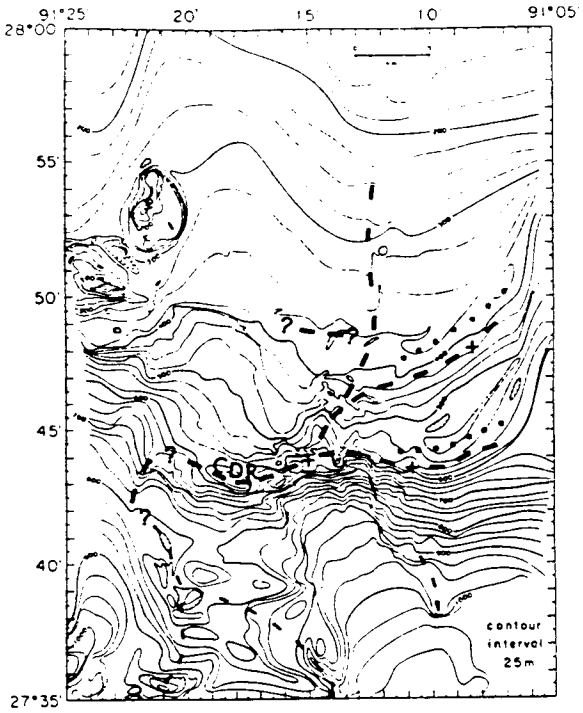

Figure 13—Distribution of diapir crests. Dashes are diapir crests interpreted from bathymetry and shallow structure except at multi-channel line crossings (+). Improbable diapir crest suggested by graben faults shown between question marks. Lines of squares show bathymetric crests.

finding indicates a high degree of microbial degradation of the oil and a consequent, significant production of CO_2.

The gases sampled were almost all methane dominated but had significant quantities of ethane, propane, and butanes. Isotopic characteristics indicated a major fraction of the gases had a petrogenic or thermal origin at depth, rather than coming from shallow, bacterial, methane generation (Anderson et al, 1983). Thus, the migration of oil and gas may be considered together.

Table 1 gives core locations and hydrocarbon occurrences. The high percentage of cores with oil and/or gas supports the association of the hydrocarbons with the targeted wipeouts in faulted areas. The occurrence of oil on fracture planes within the cores further indicates that migration is associated with this faulting.

Although sampling was not enough to define the full extent of oil and gas seepage, the seepage occurs repeatedly within the intensively faulted, intermediate slope zone that is interpreted as the crest of the CDR (Figure 13). More specifically, the seepage seems to be related to the faults at the downslope (7 cores) and upslope (2 cores) margins of this zone. These faults appear to extend to at least 1.7 sec depth (approximately 700 m [2,300 ft] subsurface; Figure 9). The young age of the faults indicated by the seabottom escarpments suggests that seepage is related to fault activity rather than just fault presence.

Two migration routes are conceivable: one lateral to the crestal faults from adjacent Pleistocene strata and one from deeper along fractures adjacent to the salt. Lateral migration seems unlikely because it would require an extraordinary thermal gradient one or two orders of magnitude higher than those usually occurring (2.55 °C/km; 0.14 °F/100 ft) on the Gulf Coast, to reach a temperature that would produce maturation within the Pleistocene. The high interstitial water salinity in one core (Figure 11) supports the second route.

Associations Related to Seepage

Eight of the 16 cores taken in this study had round or irregular carbonate nodules of obviously secondary origins. These nodules ranged from unlithified soft mud to rock hard enough and large enough to stop and bend over 600 kg (1,300 lb) of core pipe and lead weight. $\delta^{13}C$ for carbonate precipitated from the inorganic bicarbonate of seawater is usually near zero (± 4 ‰), but values for the nodules were as negative as -40. $\delta^{13}C$ values for extractable bitumen range from -26 to -28 and those for kerogenlike residue from -22 to -36. Values for gases range from -24 to -61. Thus, a likely source for the secondary carbonate is the hydrocarbons via CO_2 generated by microbial degradation.

Secondary $CaCO_3$ precipitation is obviously at the expense of porosity. Undisturbed continental slope muds from piston cores in this area commonly have porosity of approximately 80% at the core tops to approximately 70% at about 10 m (32 ft). Fifteen of 16 cores taken for this study had porosities below 70%, 13 had porosities below 65%, and 9 had below 60%. Plots of porosity vs. carbonate content for individual cores graphically show the negative slopes that reflect secondary carbonate (Figure 14). Interestingly, these negative correlations occur not only in plots for cores containing oil, but also in plots for cores having only gas and for some cores having neither oil nor gas (Figure 14). Thus, hydrocarbons may migrate into surficial sediment, be microbially oxidized with consequent carbonate precipitation and then migrate on (or be wholly consumed), leaving only the altered carbonate-porosity relationship (testable by ^{13}C analyses) as evidence of the event. The occurrence of these associations in cores without hydrocarbons implies that hydrocarbon seepage has been more widespread than the present distribution of oil indicates.

The secondary carbonate may also have produced an acoustic diagenesis, which led to the high degree of success in resampling the oil seep. That is, the association of prolonged bottom echoes with coarse gravels (e.g., algal-nodules of Addy et al, 1982) may indicate that the nodular texture of the secondary carbonate is the origin of this type of reflection in the study area.

An additional acoustic phenomenon also may be related to diagenesis associated with hydrocarbon seepage. Reflections of extraordinarily high amplitude (or brightness) but quite limited extent occur in many 3.5 kHz records (Figure 8). The reflections are usually within unit (b) and increase in abundance near faults. These

high-impedance contrasts may be from carbonate cementation but also could be from low-density gas pockets or high-density sulfides. Carbonate content probably is not the cause because bright spots did not occur at any of the sites where cores containing carbonate material were taken. Gas pockets larger than those that could resonate with the acoustic source would not produce much attenuation, but would have large density differences and thus, impedance contrasts with surrounding muds. However, sulfides are also known to occur as diagenetic precipitates associated with hydrocarbons (Sassen, 1980), and their high densities could certainly account for these anomalously strong reflections. A sulfide explanation has the added advantage of involving a substance which, once formed, is nonmigratory and thus could remain on a dipping bedding plane, which is the form of most bright spots (Figure 8).

SUMMARY

The cores studied demonstrate replicate sampling of crude oil and associated thermogenic gases in surficial sediments, which, in turn, indicate that the basic reservoir requirements of source organic material and its maturation are satisfied beneath the upper continental slope in the northern Gulf of Mexico. The presence of seep oil and gas correlated positively with location over the shallowest part of a set of diapiric ridges, the degree of extensional faulting over the diapir, and the very recent activity of the faults.

High-resolution (3.5 kHz) reflection profiles showing the surficial expression of the structure of this region reveal diapir distribution by a variety of features. Surface piercement is obvious as relatively shallow, high-relief areas with strong prolonged bottom echoes (without subbottom reflections) against which the normally well-stratified sediments pinch out. Moderately deep diapirs commonly have extensional fault systems (grabens or half-grabens) at their crests. Within these faulted zones, shallow reflections commonly disappear, probably due to the scattering and attenuating effects of gas. Deeper or low-relief diapirs may be reflected at the surface by broad, simple anticlinal structures wherein crestal faulting is minor.

On the continental slope the distinctive diapir-crest grabens are most obvious in sections parallel to the regional strike, and they appear similar to structures on the continental shelf or on land (e.g., Figure 8). In dip section, however, the force of gravity is asymmetric relative to the two diapir flanks, and favors the formation of a half-graben with normal faults downthrown downslope on both sides of the crestal zone. The oil seepage documented in this study occurs in fault zones at the updip and downdip margins of just such a half-graben.

The diapir under the seep is apparently part of a complex set of ridges. The seepage occurs over the shallowest ridge which extends approximately 13 km (7 nmi) eastward. This ridge apparently bifurcates and plunges eastward and northeastward, with the northeastern arm bifurcating again and continuing to plunge northeastward and northward. The seepage over the shallowest part of this ridge system suggests the possibility of upplunge as well as updip migration.

Simple normal faults over the deeper ridges eastward and northeastward of the seep are downthrown upslope. Thus, the diapirism has a downslope component possibly due to salt overturning or thrusting in that direction. Multichannel data show peaks of crestal structures slightly downslope from the bathymetric crests and deeper structural peaks located more upslope than shallower ones. This, in turn, supports a model of upslope depocenters acting something like rolling pins and squeezing salt downslope.

The occurrence of highly saline interstitial water in one oil-bearing core suggests the migration pathway was close to the salt core of the diapir rather than from strata laterally adjacent to the extensional crestal faulting.

Table 1. Cores Taken in Oil Seep Study Area

Core Number	Latitude N	Longitude W	Corrected Water Depth (m)	Core Length (cm)	Gas*	Oil
IG4701-1	27°44.10′	91°12.40′	614	485	P	P
IG4701-2	27°44.05′	91°16.32′	557	602	—	—
IG4702-1	27°35.31′	91°21.99′	937	378	P	—
IG4702-2	27°44.21′	91°18.60′	568	870	P	P
IG4702-3	27°43.38′	91°13.51′	684	1,015	—	—
IG4702-4	27°44.56′	91°13.32′	553	1,010	P	—
IG4702-5	27°44.43′	91°12.93′	567	870	P	P
IG4702-6	27°44.06′	91°13.70′	562	907	tr.	—
IG4702-7	27°44.02′	91°12.27′	554	906	P	P
IG4702-8	27°44.27′	91°12.58′	550	405	P	P
IG4702-9	27°44.21′	91°13.43′	567	240	—	P
IG4702-10	27°43.44′	91°15.35′	642	540	P	P
IG4702-11	27°44.09′	91°18.33′	574	435	tr.	—
IG4702-12	27°43.98′	91°18.10′	563	370	P	P
IG4702-13	27°43.60′	91°21.44′	689	510	—	tr.?
IG46-5	27°44.40′	91°12.70′	555	425	P	P

*P = present, tr. = trace.

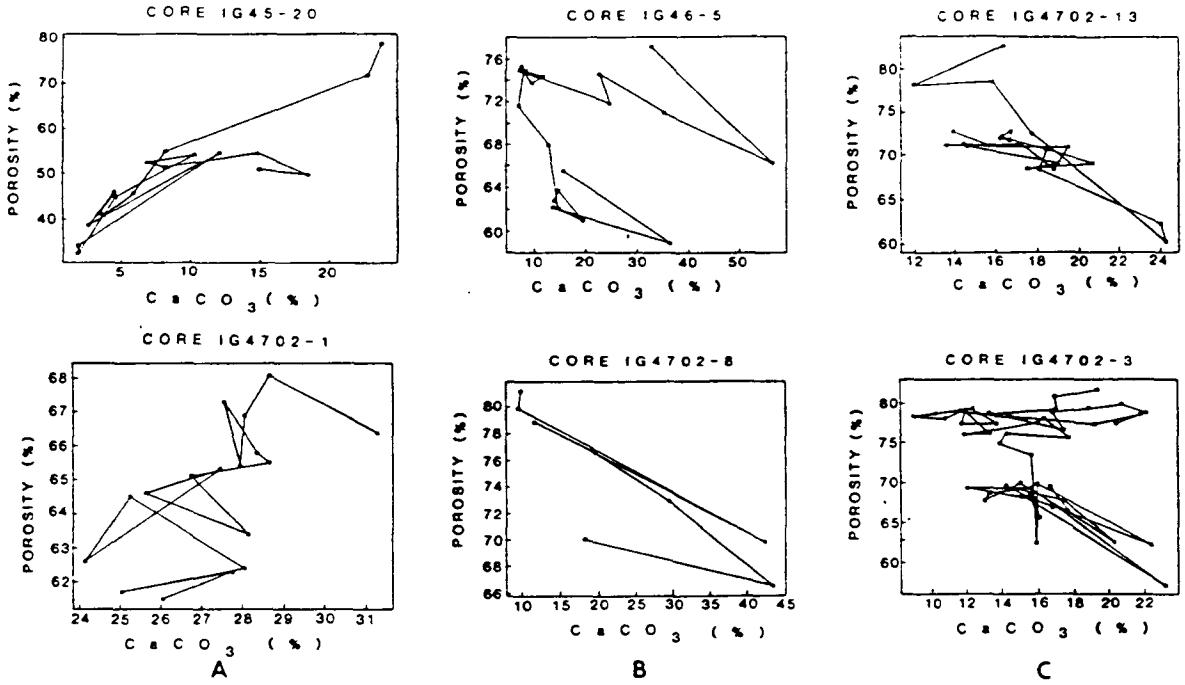

Figure 14—Porosity-carbonate relationships. Porosity is volume percent water of original wet sample; carbonate is weight of dry sample lost by acidification. Data points connected in succession downcore. (A) Core IG45-20 is from continental slope outside of study area; core IG4702-1 is within study area but not from above diapir crest where seepage was found. Major trend of positive correlations between porosity and carbonate is related to environmental controls in depositional environment (Behrens et al, 1984a, b). (B) Cores IG46-5 and IG4702-8 contain abundantly visible oil and gas. Negative correlations result from loss of porosity by diagenetic addition of carbonate derived from hydrocarbon biodegradation. In IG46-5, two negative trends with similar slopes but different positions within graph are stratigraphically separated and may represent Holocene and Pleistocene levels within core. (C) Cores with trace or no apparent hydrocarbons. IG4702-13 has no gas and a questionable trace of oil, but negative correlation of upper six samples suggested secondary carbonate precipitation in at least two samples. IG4702-3 has neither gas nor oil visible in core, but three samples from older strata (more compacted, lower porosity data points) show negative correlation characteristic of hydrocarbon-related diagenesis.

Isotopically light secondary carbonate obtained CO_2 from microbial degradation of the hydrocarbons. This carbonate probably affects the type of acoustic reflections and reduces porosity. The negative correlation between porosity and carbonate content may be a subtle indication that oil seepage has occurred even where no hydrocarbons appear in the sediment.

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Thermogenic Gas Hydrates in the Gulf of Mexico

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Thermogenic Gas Hydrates in the Gulf of Mexico

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Thermogenic Gas Hydrates in the Gulf of Mexico

Abstract. *Thermogenic gas hydrates were recovered from the upper few meters of bottom sediments in the northwestern Gulf of Mexico. The hydrates were associated with oil-stained cores at a water depth of 530 meters. The hydrates apparently occur sporadically in seismic "wipeout" zones of sediments in a region of the Gulf continental slope at least several hundred square kilometers in area.*

We report here the occurrence of thermogenic gas hydrates in ocean sediments. The hydrates were discovered fortuitously during geochemical surveys involving piston coring operations at 27°47′N and 91°30′W in 530 to 560 m of water in the northwestern Gulf of Mexico. Hydrates were observed dispersed in carbonate rubble in three cores and ranged in size from minute crystals to objects several centimeters in diameter. Although the cores were severely disrupted by gas expansion during the 10- to 15-minute interval between coring and retrieval on deck, the gas hydrates appeared to be distributed from the top of the core to a sediment depth of at least several meters. Samples of the hydrates were preserved in liquid nitrogen for laboratory analysis.

Gas hydrates are solid icelike clathrate structures in which gases are occluded in a crystalline water lattice under appropriate conditions of high pressure and low temperature. Hydrates can exist in two forms. Structure I gas hydrates have a symmetrical shape and exclude molecules larger than ethane. Structure II hydrates are slightly larger and can accommodate not only methane and ethane but also propane and isobutane. Molecules as large or larger than *n*-butane cannot be accommodated in either lattice structure (*1*). The stability zone of gas hydrates in marine sediments is generally found in continental slope areas where water depths are greater than 500 m and water temperatures at the bottom are near 0°C. Although a wide range of molecules (methane, ethane, propane, isobutane, CO_2, N_2, O_2, and H_2S) can form hydrates, methane and possibly CO_2 are the only gases found in sufficient quantities in deep-sea sediments to form gas hydrates. Under conditions of temperature and pressure appropriate for hydrate stability, gas concentrations must exceed solubility levels before hydrates can form. Therefore, methane hydrates can be found only in regions where there is significant biogenic methane production or where there is migration of thermogenic gases from deeper horizons. Thermogenic gases do not form hydrates at their site of production because the ambient temperatures are outside the stability zone of hydrates.

The presence of gas hydrates in marine sediments has long been suspected on the basis of laboratory stability studies and the existence, in some sediments, of a bottom-simulating reflector (BSR)—an anomalous acoustic reflector that approximately parallels the bottom topography, cutting across bedding planes and deepening with increasing water depth (*2*). The BSR is thought to represent the lower boundary of gas hydrate stability, below which gas hydrates decompose because of increased temperatures. The existence of hydrates has been inferred in many ocean areas on the basis of seismic records (*3*). Gas hydrates appear to be common in the continental margins of all the oceans. However, to our knowledge the only direct observations of gas hydrates in marine sediments have been in shallow cores from the Black Sea (*4*); at a subbottom depth of 238 m on leg 76 of the Deep Sea Drilling Project/International Phase of Ocean Drilling (DSDP/IPOD) in the Blake Outer Ridge of the Atlantic Ocean (*5*); and on DSDP/IPOD legs 66, 67, and 84 in the Middle America Trench off Mexico and Guatemala (*6*). Because hydrates are not stable at atmo-

Table 1. Data on the molecular and isotopic composition of hydrate gas and water obtained after decomposition in a pressure device consisting of a 23-cm³ sample holder, gauge block, and gas sampling port with septum (Parr Instrument). Two experiments were performed with separate hydrate samples taken from a depth in the core of 1.0 to 1.5 m. N.D., not determined.

Parameter	Experiment 1	Experiment 2
*Gas (all compositions in percent)**		
Methane	55.1 (−44.6)	67.5 (−44.8)
Ethane	2.6 (−29.3)	4.5
Propane	14.4 (−18.6)	14.9
Isobutane	4.4 } (−28.6)	4.2
n-Butane	0.2 }	0.2
Carbon dioxide	3.4 (18.5)	3.9 (13.3)
Nitrogen	N.D.	4.1
O_2 + argon	N.D.	<0.1
Methane/ethane + propane	3.2	3.5
Total components	83.3	99.2
δD, methane (per mil)	−189	
Water (ionic compositions in parts per thousand)†		
Salinity (refractive index)	9	9
Chlorinity	5.9	4.8
Na^+	3.5	3.0
Mg^{2+}	0.21	0.16
K^+	0.14	0.11
Ca^{2+}	0.20	0.16
Sr^{2+}	0.0054	N.D.

*Numbers in parentheses are carbon isotopic ($\delta^{13}C$) values in per mil. †Chlorinity was determined by Mohr titration and the cations by inductive coupled plasma.

spheric pressure (even at −20°C), only the sample from DSDP/IPOD leg 84 was successfully collected for laboratory study prior to the discovery reported here. The four previous samplings of gas hydrates yielded predominantly biogenic hydrocarbon gases (mainly methane) on decomposition.

The thermogenic nature of the hydrates collected in this study is indicated by (i) molecular compositions containing large amounts of ethane, propane, and isobutane, (ii) carbon isotopic compositions, and (iii) the presence of oil in the cores. Results of the molecular and isotopic analyses of gas and water obtained from the decomposing hydrate samples are summarized in Table 1 (*7*). The hydrates had a gas:fluid ratio of 70:1 on decomposition. The large amounts of propane and isobutane indicate that a structure II hydrate was present. Hydrocarbons larger than isobutane were detected at very low concentrations. The large amounts of ethane, propane, and isobutane and the heavy carbon isotopic ratio (−45 per mil relative to Pee Dee belemnite) of the hydrate gases are characteristic of thermogenic gases produced deep in the sedimentary column (*8*). The large amounts of nonmethane gases in the hydrate must stabilize the hydrate lattice, since the 6° to 8°C temperature of water at 530 m in this part of the Gulf of Mexico is outside the temperature limit of methane hydrate stability. Since the presence of thermogenic hydrates in shallow sediments implies that the hydrate gas has migrated upward from deep in the sedimentary column, thermogenic hydrates could exist as deep in the sediment column as their stability would allow.

The three cores that contained gas hydrates were also oil-stained. Results of chemical analysis of two of the hydrate cores are presented in Table 2. The cores contained as much as 12.1 percent hexane-extractable material. Column chromatography was used to separate the extractable organic matter into saturate, aromatic, and polar compound types (*9*). The oil was extensively biodegraded, with both the saturate and aromatic gas chromatograms being dominated by the unresolved complex mixture. Column chromatography of the extractable material indicated that most of the degraded oil was aromatic in nature (23.1 percent saturate, 44.6 percent aromatic, and 7.6 percent polar compounds for oil in the 0- to 5-cm section of core 165). The large amounts of calcium carbonate in the core may be the result of microbial oxidation of petroleum. Chlorinities in the interstitial waters in excess of seawater levels may be due to the presence of a salt diapir underlying the site. The erratic distribution of chlorinities may reflect the fact that hydrates exclude salts from the clathrate structure because of the size of their ionic radii.

Although the area where the three hydrate cores were collected is restricted

Table 2. Data on sediment and interstitial water in two hydrate-containing cores. N.D., not determined (some sections contained large amounts of oil, making certain analyses impossible).

Station	Depth (cm)	Extractables (%)	Organic carbon* (%)	$CaCO_3$ (%)	Sulfur (%)	Refractive index (per mil)	Chlorinity (per mil)	SCO_2 (milligrams of carbon per liter)
166	0 to 5	1.2	3.2	16.8	1.0	37	19.1	14.2
166	20 to 25	3.1	2.4	18.8	1.2	38	19.3	3.1
166	40 to 45	1.3	2.8	19.3	1.2	37	19.2	21.6
166	60 to 65	3.2	14.3	N.D.	0.6	N.D.	N.D.	N.D.
166	80 to 85	12.1	11.9	N.D.	0.8	N.D.	N.D.	N.D.
165	0 to 5	2.7	3.8	44.9	0.76	38	18.4	54.2
165	20 to 25	3.5	4.5	31.5	0.84	N.D.	N.D.	N.D.
165	40 to 45	2.1	3.0	37.7	0.88	N.D.	N.D.	N.D.
165	80 to 85	0.8	3.0	65.0	0.51	N.D.	N.D.	N.D.
165	140 to 145	1.8	2.8	30.8	1.21	55	30.9	25.9
165	160 to 165	0.4	1.1	16.9	1.20	72	41.2	29.2
165	180 to 185	0.2	1.1	19.3	1.37	73	41.8	19.7
165	200 to 205	0.6	1.6	8.2	1.37	64	35.6	47.0
165	220 to 225	0.3	1.4	15.7	1.55	72	41.5	36.1
165	240 to 245	0.5	1.7	7.2	1.68	60	35.2	37.5
165	260 to 265	0.4	1.4	14.2	1.66	61	34.6	30.4
165	280 to 285	0.3	1.3	10.0	1.87	72	39.3	31.8

*Organic carbon content was highly variable in many sections because of a separate oil phase.

in size (a few square kilometers), there is evidence that thermogenic gas hydrates may be widespread on the Gulf continental slope. Anderson *et al.* (*10*) recently reported oil-stained sediments containing large amounts of gas over a 20-km^2 area of the upper slope. Some of the sediment gases they collected contained large amounts of isobutane but little *n*-butane, suggesting that hydrates were originally present in these cores. Our report expands the area where oil, and probably hydrates, occur intermittently in surface sediments to ~250 km^2. The migration of thermogenic gas and oil to the surface in this area occurs along faults and fractures created by salt tectonics in the area. Since these processes are pervasive over large areas of the Gulf Coast, hydrates associated with thermogenic hydrocarbon seepage may be common along the continental slope.

Little seismic evidence for gas hydrates in the Gulf of Mexico has been reported. BSR's have not been reported for the northern Gulf of Mexico, although they have been reported along the Mexican Ridge systems (*2*). Sidner *et al.* (*11*) observed anomalous seismic features described as chaotic facies (gas-charged sediments). The gas hydrates sampled in this study were associated with chaotic facies or gas "wipeout" zones. Sections reported as chaotic facies may in reality be the top of a sediment section containing disseminated gas hydrates (*12*).

The discovery of thermogenic hydrates associated with oil-stained cores in the Green Canyon area of the Gulf of Mexico will necessitate more detailed chemical, geological, and biological studies of the area. The extent and distribution of hydrates, their seismic signature, and their possible association with active oil and gas seepage are only a few of the areas of interest suggested by this discovery. Many complicating processes in these cores need further study, such as (i) the response of the microbial ecosystem to seeping oil and gas and dissolving salt; (ii) the effect of the microbial processes on isotopic fractionation in the oil, methane, and carbon dioxide, and (iii) geochemistry associated with carbonate formation from degradation of the seeping oil. Because of the apparent widespread occurrence of oil in slope sediments from natural seepage, questions are also raised as to our ability to differentiate between natural seepage and petroleum pollution in the Gulf of Mexico and to determine baseline levels. The effect of solid hydrates and oil-stained sediments on the benthic ecology of an area is unknown. Gas hydrates may also represent a recoverable resource if they exist in significant quantities in the subsurface.

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$$\delta^{13}C = \frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{std}}{(^{13}C/^{12}C)_{std}} \times 1000$$

The δD value is reported relative to standard mean ocean water.
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Deep-Sea Hydrocarbon Seep Communities: Evidence for Energy and Nutritional Carbon Sources

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Mussels, clams, and tube worms collected in the vicinity of hydrocarbon seeps on the Louisiana slope contain mostly "dead" carbon, indicating that dietary carbon is largely derived from seeping oil and gas. Enzyme assays, elemental sulfur analysis, and carbon dioxide fixation studies demonstrate that vestimentiferan tube worms and three clam species contain intracellular, autotrophic sulfur bacterial symbionts. Carbon isotopic ratios of 246 individual animal tissues were used to differentiate heterotrophic ($\delta^{13}C = -14$ to -20 per mil), sulfur-based ($\delta^{13}C = -30$ to -42 per mil), and methane-based ($\delta^{13}C = <-40$ per mil) energy sources. Mussels with symbiotic methanotrophic bacteria reflect the carbon isotopic composition of the methane source. Isotopically light nitrogen and sulfur confirm the chemoautotrophic nature of the seep animals. Sulfur-based chemosynthetic animals contain isotopically light sulfur, whereas methane-based symbiotic mussels more closely reflect the heavier oceanic sulfate pool. The nitrogen requirement of some seep animals may be supported by nitrogen-fixing bacteria. Some grazing neogastropods have isotopic values characteristic of chemosynthetic animals, suggesting the transfer of carbon into the background deep-sea fauna.

WE REPORT HERE A STUDY OF THE energy and nutritional carbon sources of mussels, clams, and tube worms from hydrocarbon seep communities on the Louisiana continental slope (*1*). The organisms were collected in trawls near hydrocarbon seep sites in water depths between 400 and 920 m on R.V. *Gyre* cruises 86-G-1/2. The northern Gulf of Mexico slope is extensively faulted and fractured by salt tectonics, thus providing conduits for the upward migration of oil and gas (*2*). The taxa at these sites are similar to those of the hydrothermal vent sites of the Pacific (*3*), the cold seep sites of the Florida Escarpment (*4*), and the Oregon Subduction Zone (*5*). The Louisiana sites are distinct in that the vent taxa are living in a high hydrocarbon environment derived from deeper reservoired petroleum. These vent-type taxa use organic matter produced in situ by chemoautotrophic, sulfide-oxidizing bacteria and endosymbiotic chemoautotrophs (*6, 7*). Methane use has been demonstrated for the mussels from the Louisiana site (*8*) and from the Florida Escarpment (*4, 9*) and has been suggested for the animals at the Oregon Subduction Zone (*5*).

A variety of tests were used to determine the nature (and presence) of endosymbionts in these seep fauna. The mussel is the only animal with confirmed methanotrophic symbionts (*8*) and is the only one of these seep species that possesses methanol dehydrogenase, an enzyme characteristic of methylotrophy (Table 1). The mussel is also the only animal tested whose bacterial symbionts contain stacked internal membranes (typical of type I methanotrophs). Mussel gills lack the enzymes characteristic of sulfur oxidation [adenosine triphosphate (ATP) sulfurylase and adenosine-5′-phosphosulfate reductase], lack elemental sulfur, and have only trace activities of ribulose-bisphosphate carboxylase (an enzyme characteristic of autotrophic carbon fixation); these factors indicate that symbionts of mussel gills are not sulfur-oxidizing chemoautotrophs.

The other three bivalves and the two

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vestimentiferans appear to harbor sulfur-oxidizing chemoautolithotrophic symbionts (Table 1). The enzyme activities, the presence of elemental sulfur in the symbiont-containing tissue, and electron microscopy provide evidence that both vestimentiferans and the lucinid clam, *Pseudomiltha* sp., contain chemoautotrophic, sulfur bacterial symbionts. The evidence for the vesicomyid clams is not as conclusive since no tissue from *Calyptogena ponderosa* was frozen in liquid nitrogen for enzymatic analysis and the one *Vesicomya cordata* collected died before dissection. The absence of specific enzyme activities is thus of questionable significance. Nonetheless, the high level of elemental sulfur in the gills of *C. ponderosa* and the high levels of ATP sulfurylase in *V. cordata* gills suggest that sulfur-oxidizing symbionts were present. The sulfide oxidase activities in all animals assayed are at the level expected for invertebrates exposed to a sulfide environment (*10*).

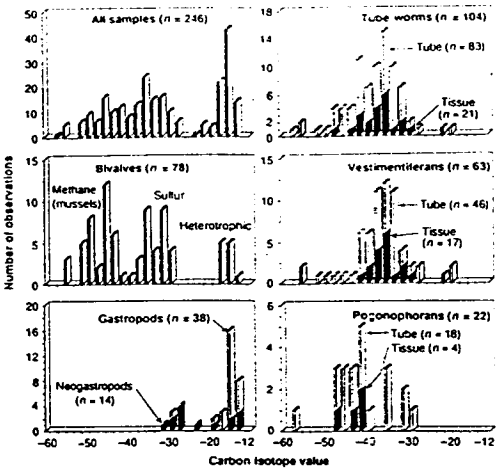

Fig. 1. Carbon isotopic values (per mil relative to Pee Dee belemnite standard) of 246 animals collected at seep sites on the Louisiana continental slope.

The seep fauna at the Louisiana site contain mostly "dead" carbon (Table 2). Several sources of dietary carbon are possible for the seep animals. First, carbon can be derived from particulate detritus fixed photosynthetically in the upper water column ($\Delta^{14}C = 100 \pm 20$ per mil; $\delta^{13}C = -18$ to -20 per mil; background fauna are in Table 3). Second, carbon can be derived from bacterial organic carbon synthesized chemoautotrophically from dissolved inorganic carbon (DIOC). The DIOC can be derived either from ambient bottom water ($\Delta^{14}C = -100$ per mil; $\delta^{13}C = -0$ per mil) (*11*) or from dead CO_2 ($\Delta^{14}C = -1000$ per mil). Dead DIOC can be derived from (i) seeping oil and gas, (ii) bacterial degradation of the seeping oil and gas, (iii) dissolu-

Table 1. Enzyme activities, elemental sulfur (S^0) content, stable carbon isotope ratio ($\delta^{13}C$), presence of symbiotic bacteria (S.B.), and methane consumption (CH_4) in individual Louisiana slope seep organisms. Assays were conducted on symbiont-containing tissues (bivalve gills and vestimentiferan trophosome). One unit of enzyme activity will convert 1 μmol of substrate to product. RuBP, ribulose-bisphosphate carboxylase; ATP, ATP sulfurylase; APS, adenosine-5′-phosphosulfate reductase; methanol, methanol dehydrogenase; sulfide, sulfide oxidase; ND, not detected; N, no; Y, yes; NT, not tested; WW, wet weight; and EM, electron microscopy.

Animal	Identification No.	Enzyme activity (unit/g WW/min)					S^0 (% WW)	$\delta^{13}C$	S.B. (EM)	CH_4*
		RuBP	ATP	APS	Methanol	Sulfide				
					Mollusca					
Lucinidae									Y	N
Pseudomiltha sp.	14-1	0.43	12.86	0.83	ND	2.1	0.02	−33.5		
	14-2	0.41	2.47	0.66	ND	1.94	0.06	−33.6		
	14-3	0.44	15.43	1.36	ND	2.04	ND	−32.5		
	14-14	NT	NT	NT		NT	0.5	−37.7		
Mytilidae									Y	Y
Undescribed	24-1	0.011	ND	ND	0.66	0.7	ND	−51.8		
	24-2	0.017	ND	ND	0.53	0.75	ND	−52.0		
	25-1	0.027	ND	ND	0.4	1.09	ND	−52.6		
Vesicomyidae									Y	NT
V. cordata†	18-1	0.003	29.58	ND	ND	NT	ND	−39.8		
C. ponderosa									Y	NT
	CAT-1	NT	NT	NT	NT	NT	0.4	−37.9		
	CAT-2	NT	NT	NT	NT	NT	8.3	−36.9		
	CAT-3	NT	NT	NT	NT	NT	ND	−39.1		
					Vestimentifera					
Lamellibrachiidae									Y	N
Lamellibrachia sp.	25-3	0.24	4.24	0.70	ND	1.77	4.5	−36.6		
	25-4	4.03	1.03	NT	ND	3.15	6.1	−36.8		
	25-5	4.97	0.51	0.78	ND	5.47	2.6	−37.4		
Undescribed family									Y	N
Undescribed	24-1	3.57	0.32	ND	ND	NT	0.1	−36.4		
	25-1	3.73	1.03	1.54	ND	5.32	NT	−39.9		
	25-2	5.40	1.90	ND	ND	2.98	1.9	−41.0		
	25-3	NT	NT	ND	NT	2.37	0.4	−37.0		

*Methane consumption from incubation of gill or trophosome tissue measured gas chromatographically (*8*). †The *V. cordata* died before dissection.

tion of ancient carbonate, or (iv) degradation of sedimentary organic matter. Dead carbon can also be derived from the direct use of methane by symbiotic bacteria and most likely results from direct use of methane by the mussels (*8*) and extensive biodegradation of the oil and gas by bacteria. Large amounts of isotopically light, authigenic carbonate and extensively biodegraded oil in sediments from the seep sites (*12*) indicate active CO_2 production. Although most reservoired gases in the Gulf of Mexico contain small amounts of CO_2 (*13*), this source of dead carbon is hypothesized to be minor relative to other CO_2 sources.

At the hydrothermal vent sites (Table 2), the principal source of dietary carbon for mussels and tube worms is DIOC (*6*). At the Florida Escarpment, where methane is apparently the major energy source for the mussels, the radiocarbon content of three tube worms and the mussels was older, although not predominantly dead (Table 2). In contrast to the hydrothermal vent and Florida Escarpment sites, many of the mussels, tube worms, and clams at the Louisiana site contain nearly dead carbon. The dead carbon in the mussels supports the metabolic and physiological studies that indicate there is a bacterial symbiosis between the mussel and methanotrophic bacteria. In contrast, the sulfur-based tube worms and clams must use dead DIOC derived from bacterial degradation of hydrocarbons. Thus much of their dietary carbon is derived ultimately from the sediments and not from the more recent DIOC of seawater. These observations are consistent with the hypothesis that oil and gas are the energy sources for these seep taxa.

The carbon isotopic content of these seep organisms reflects the isotopic fractionation that occurs during the synthesis of organic tissues (*6*) and the food source of the animals (*14*). Figure 1 presents the carbon isotopic compositions of 246 organisms from trawl samples collected on the Louisiana–Upper Texas slope (34 sites). The most striking feature of Fig. 1 is the three isotopically distinct groups of bivalves. The mussel tissues all have $\delta^{13}C$ values less than −40 per mil. The $\delta^{13}C$ values between −30 and −42 per mil represent clams with sulfur bacterial symbionts. These values are similar to those of the hydrothermal vent clams (*6, 15, 16*), which appear to derive their energy from hydrogen sulfide. We assume that clam $\delta^{13}C$ values typical of deep-sea fauna (−14 to −20 per mil) are heterotrophic.

The light carbon isotopic values of the mussels are characteristic of the methane symbiosis between the bacteria and the mussel (*8*). On Johnson *Sea-Link-I* dives 1877 and 1878, we collected mussels living in a bubbling gas stream at 630 m in Green Canyon (GC) Block 185. The mussels $\delta^{13}C$ (−40.6 per mil) closely reflected the composition of the methane (−41.2 per mil) used by the bacterial symbionts. Biogenic methane is characterized by $\delta^{13}C$ values less than −60 per mil with few, if any, longer chain hydrocarbons, whereas thermogenic gas contains higher hydrocarbon gases and $\delta^{13}C$ values heavier than −45 per mil (*17*). The mussels from GC-185 have thermogenic isotopic values. Most of the mussels collected from the trawls suggest an admixture of biogenic and thermogenic methane. The gill and mantle tissue from three mussels in our study have similar isotopic compositions, indicating a transfer of bacterial carbon from the symbionts in the gill to the mussel's other tissues.

Tube worm tissues and tubes from these sites show a range of $\delta^{13}C$ values from −20 to −58 per mil (Fig. 1). These values are atypical of the few previous reports from the hydrothermal vent (*6, 15, 16*) and other cold seep sites (*4, 5*). The vestimentiferans (*Riftia pachyptila*) from the hydrothermal vents all have $\delta^{13}C$ values near −10 per mil (*6, 15*; Table 2). One suggested explanation of the heavy values is that CO_2 limitation during growth precludes discrimination at the site of carbon fixation (*6, 15*). The other $\delta^{13}C$ values for tube worms from the Florida Escarpment and the Oregon Subduction Zone show lighter $\delta^{13}C$ values. Thus tube worm values heavier than −42 per mil are characteristic of sulfur-based endosymbionts. Values lighter than −42 per mil in the pogonophorans may indicate a contribution from methane endosymbionts. The three heavy values (−20 to −22 per mil) in the vestimentiferans from the Louisiana sites may reflect processes similar to those occurring at the hydrothermal vent sites. The wide range of values also reflect the multiple sampling sites, the patchiness of thermogenic hydrocarbon seepage, and perhaps a difference in the $\delta^{13}C$ of the DIOC utilized by the animals. All of the tissue and tube

Table 2. Stable carbon and radiocarbon measurements of seep and vent taxa from the deep sea. Numbers of individuals, without parentheses, refer to carbon isotopic measurements; numbers in parentheses refer to radiocarbon analyses. TR, values in this report.

Sample description	Number of individuals	$\Delta^{14}C$ (per mil)	$\delta^{13}C$ (per mil)	Reference
Louisiana hydrocarbon seep sites				
Clam tissue (*C. ponderosa*)	4		−31.2 to −35.3	(*1*)
Clam tissue (*C. ponderosa*)	3		−36.9 to −39.1	TR
Clam tissue (*Pseudomiltha* sp.)	17 (1)	−753	−30.9 to −37.7	TR
Mussel	38 (2)	−829, −840	−40.1 to −57.6	TR
Snail (neogastropod)	1		−31.5	(*1*)
Neogastropods	10 (2)	−210, −544	−14.6 to −32.8	TR
Tube worm (*Lamellibrachia*)				
Tissue	1		−27.0	(*1*)
Tube	1		−28.1	(*1*)
Tube worm (*Lamellibrachia*)*	37 (1)	−586	−29.8 to −57.2	TR
Tube worm (pogonophorans)*	22 (2)	−205, −749	−30.5 to −59.3	TR
Tube worm (Escarpia-like)*	24		−21.4 to −48.6	TR
Hydrothermal vent sites (Galápagos and 21°N)				
Clam tissue (*C. magnifica*)	2		−32.1, −32.7	(*15*)
Clam tissue (*C. magnifica*)†	4		−32.1 to −39.9	TR
Mussel tissue (*Bathymodiolus thermophilus*)	3 (3)	−270 to −228	−32.8 to −33.9	(*6*)
Mussel tissue (*B. thermophilus*)	1		−32.7 to −33.6	(*16*)
Mussel tissue (*B. thermophilus*)	24		−32.1 to −37.2	TR
Tube worm (vestimentiferan) tissue	1 (1)	−270	−10.9	(*6*)
Tube worm (vestimentiferan) tissue	1		−10.8 to −11.0	(*15*)
Tube worm tissue‡	4		−11.9 to −13.7	TR
Florida escarpment site				
Mussel tissue (mytilid)	10 (3)	−567 to −247§	−74.3 ± 2.0 (SD)	(*4*)
Gastropod tissue (trochid)	2		−59.9 ± 0.7 (SD)	(*4*)
Tube worm (vestimentiferan) tissue	3 (2)	−419, −424§	−42.7 ± 0.7 (SD)	(*4*)
Oregon subduction zone site				
Clam tissue (*Calyptogena* sp.)	1		−35.7	(*5*)
Clam gills (*Calyptogena* sp.)			−51.6	(*5*)
Clam tissue (*Solemya* sp.)	1		−31.0	(*5*)
Tube worm (*Lamellibrachia*)				
Tissue	1		−31.9	(*5*)
Segment			−26.7	(*5*)

*Includes both tubes and tissues of different individuals. †Values include isolated gills and remains. ‡Values include isolated trophosomes and vestimentum. §Values reported originally as percentage of modern. The conversion to $\Delta^{14}C$ assumed modern as 0 per mil.

Table 3. Stable carbon, nitrogen, and sulfur isotopic ratios and radiocarbon measurements of Louisiana slope seep organisms. Numbers in parentheses indicate the number of animals represented by the range. Locations are 9-square-nautical-mile Mineral Management Service lease areas and blocks (GC, Green Canyon; GB, Garden Banks; EB, Ewing Bank; and MC, Mississippi Canyon). All measurements are on animal soft tissue.

Animal	Location	$\delta^{13}C$ (per mil)	$\delta^{15}N$ (per mil)	$\delta^{34}S$ (per mil)	$\Delta^{14}C$ (per mil)
		Symbiont-containing animals (chemosynthetic)			
Bivalves					
Mussel (Mytilidae undescribed)	GC-272	−50.1 to −45.5 (9)	−12.9 to +3.0 (10)	+13.4, +7.5 (2)	−829 (1)
Clam (*C. ponderosa*)	GC-272, GC-234	−34.8, −35.0 (2)	+1.1 to +7.1 (3)	−0.1 to +2.1 (3)	
Clam (*V. cordata*)	GC-116	−36.3 (1)	−0.9 (1)		+254 (1)
Clam (*Pseudomiltha* sp.)	GC-79	−36.0 to −31.8 (3)	−3.5 to +6.1 (3)	−11.5 to +1.3 (4)	−753 (1)
Vestimentiferans					
Tube worm (Escarpia-like)	GC-272, GB-458	−40.9 to −30.4 (3)	+2.9, +5.4 (2)	−3.5 (1)	−205, −949(2)
Tube worm (*Lamellibrachia* sp.)	GC-33	−43.2 (1)	+2.7 (1)	−2.7 (1)	−586 (1)
		Heterotrophic deep-sea animals			
Neogastropods	MC-839, EB-1010, GC-33	−32.8 to −14.8 (5)	+2.8 to +13.0 (4)	0.0 to +18.7 (4)	−210, −544 (2)
Shrimp	GB-300	−19.5 to −18.6 (2)	+13.3 (1)	+13.3 (1)	+123 (1)
Clam (*Acesta*)	GC-272	−18.7 (1)	+8.9 (1)	+16.1 (1)	+96 (1)

pairs (more than 12 pairs) analyzed show similar carbon isotopic compositions (Fig. 1).

The sulfur isotopic content (Table 3) of the seep fauna also differentiates sulfur and methane energy sources. Most animals from food webs based on phytoplankton have sulfur isotopic compositions between 13 to 20 per mil, similar to the seawater sulfate pool (+20 per mil) (*18, 19*). Fry *et al.* (*20*) found that the fauna at hydrothermal vent sites had values between −5 to +5 per mil, similar to the sulfur-bearing minerals of the vents. Although the H_2S isotopic content of the Louisiana sites is unknown, the sulfide-based tube worms and clams have values between −12 to +2 per mil. Some neogastropods have values in this range, reflecting chemosynthetic dietary carbon and sulfur, whereas others have values characteristic of deep-sea heterotrophs. These values are consistent with Fig. 1, which shows that most of the gastropods contain heterotrophic carbon. However, some of the neogastropods show transfer of chemosynthetic carbon into the background slope fauna, which most likely results from the food source of these snails that often prey on bivalves. The methane-based mussels have sulfur values more characteristic of the heavier seawater sulfate.

In a manner similar to carbon and sulfur isotopes, nitrogen isotopes may indicate sources of nitrogen and food web relations. Hydrothermal vent communities (*21*) and seep communities of the Florida Escarpment (*4*) have unusually depleted nitrogen isotopic compositions compared to typical marine values (5 to 15 per mil) (*22*). Such ^{15}N-depleted values have been attributed to fractionations of source nitrogen either through assimilation of depleted nitrate (*21*) or ammonium (*4*). At the Louisiana sites, ^{15}N values range from similar to those previous studies (*4, 21*) to even more depleted values (−12 per mil). Such depleted ^{15}N values have been reported for laboratory algal cultures with high (millimolar) concentrations of ammonium or nitrate (*23*), ammonium-rich hot springs (*24*), and nitrate-rich lakes in Antarctica (*25*). In all of these cases, elevated inorganic nitrogen concentrations allow for marked discrimination in the uptake of ^{14}N rather than ^{15}N, with resulting cells being much depleted in ^{15}N. However, such unusual environments are not expected at the seep sites. Normal (micromolar) levels of nitrate have not been associated with large ^{15}N depletions or anomalous values (*22, 26, 27*).

An alternative source of nitrogen is fixation of nitrogen gas (N_2) associated with methane from the seeps. The N_2 of the vent gas, once fixed, may be the sole source of nitrogen for some of the organisms as indicated by their ^{15}N depletion. The N_2 gas isolated from a nearby oil well has a ^{15}N value of −2.9 per mil. Microorganisms fixing this gas would then have isotopic values near −6 per mil. Natural gases from other oil reservoirs have been reported to be as depleted as −14.6 per mil (*28*), so the N_2 gas associated with the seep area could be even more depleted.

The dietary carbon, nitrogen, and sulfur; energy sources; and trophic relations in the Louisiana seep ecosystems are complex. The wide isotopic ranges of organisms from these sites as opposed to other vent and cold seep sites suggest that either (i) the Louisiana seep communities are more complex and diverse or (ii) the fewer isotopic measurements at the other sites do not adequately reflect their diversity and complexity.

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Gulf of Mexico Hydrocarbon Seep Communities: Part IV—Descriptions of Known Chemosynthetic Communities

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Gulf of Mexico Hydrocarbon Seep Communities: Part IV— Descriptions of Known Chemosynthetic Communities

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ABSTRACT

The Geochemical and Environmental Research Group (GERG) at Texas A&M University has discovered vent-type, chemosynthetic communities associated with our recent findings of widespread hydrocarbon seepage, gas hydrates, and authigenic carbonate on the Texas/Louisiana continental slope. These vent-type taxa (clams, mussels and tubeworms) are unique in that they are associated with active gas and oil seepage and harbor endosymbiotic, chemoautotrophic bacteria, including a proven methanotroph.

INTRODUCTION

Deep water chemosynthetic organisms were discovered by Geochemical and Environmental Research Group (GERG) scientists in 1984 while trawling at previously discovered sites of oil seepage and gas hydrates (1,2) on the Louisiana continental slope. The chemosynthetic communities consist of assemblages of tubeworms, clams, mussels, bacterial mats and other associated organisms. The chemosynthetic communities associated with hydrocarbon seeps on the Louisiana/Texas continental slope are one of a series of functionally and taxonomically related assemblages in the deep-sea. These communities are characteristically associated with sources of hydrogen sulfide or methane in an oxygenated environment. The underlying geological processes supplying these reduced compounds vary from site to site.

In the Gulf of Mexico chemosynthetic organisms assume an applied importance because we are faced with the potential impact upon a fauna that is uniquely associated with exploitable hydrocarbon reserves. The U.S. Dept. of Interior's Minerals Management Service Notice to Lessees (NTL-88-11) requires "all operators of leases in water depths greater than 400 m ... to provide a consistent and comprehensive approach which will avoid damage to high density chemosynthetic communities". Thus, MMS is requiring "prior to approvals of Applications of Permit to Drill (APDs) and Pipeline Applications, the operators shall delineate all seafloor areas which would be disturbed by the proposed operations. Additionally, an analysis of geophysical information for these areas, as well as any other pertinent information available, shall be furnished which discusses the possibility of disturbing geological phenomena (such as hydrocarbon charged sediments, seismic wipe-out zones, anomalous mounds or knolls, gas vents, or oil seeps) that could support chemosynthetic organism". Thus, the existence of chemosynthetic organisms on the continental slope is of practical importance to the oil industry.

This paper summarizes the visual observations of chemosynthetic communities from submersible dives on the Johnson "*Sea-Link*" and U.S. Navy *NR-1* in 1986-1988. Chemosynthetic communities have been confirmed at sites (3) other than the ones summarized here based on the recovery of organisms from trawls.

BACKGROUND

Recent discoveries in the northern Gulf of Mexico are dramatically altering our understanding of the geological, chemical and biological processes which control the overall ecology of the continental slope. In the geological area, high resolution profiling has increasingly shown that salt tectonics and related processes dominate mesoscale topography and produce islands of hard substrate in a predominantly mud environment (4). Active and widespread geochemical systems involving hydrocarbons at or near the deep-sea sediment-water interface were first confirmed by the discovery of oil-stained cores and thermogenic hydrates by GERG (1). Trawling in these areas later discovered that

References and illustrations at end of paper.

a fauna utilizing chemosynthetic symbionts were associated with these systems (5,6). During our ongoing research on seep geology-geochemistry-biology, we have made a number of advances which are especially relevant as follows:

(1) Identified chemosynthetic organisms (either tubeworms, mussels and/or clams) at 17 northwestern Gulf of Mexico continental slope sites;
(2) Confirmed, based on enzyme activities, elemental sulfur content and electron microscopy; that tubeworms and clams from these sites contain chemoautotrophic, bacterial endosymbionts;
(3) Found a mussel that is capable of utilizing methane as its sole carbon and energy source [the first demonstrated symbiosis between a methanotrophic bacteria and an animal (6,7)];
(4) Identified shallow seismic "wipe-out" zones as high probability sites for chemosynthetic ecosystems;
(5) Shown that oil seepage is associated with all Gulf of Mexico slope chemosynthetic ecosystems located to date;
(6) Demonstrated that carbon, nitrogen and sulfur isotopes are useful in differentiating heterotrophic, sulfur-based and methane-based ecosystems;
(7) Identified the transfer of carbon from the chemosynthetic ecosystem to background heterotrophic organisms;
(8) Documented two sites of active liquid hydrocarbon seepage to the sea surface;
(9) Discovered at least 12 gas hydrate locations in the Gulf of Mexico; and
(10) Determined that shell beds are being produced in and around areas of petroleum seepage.

DESCRIPTION OF GULF OF MEXICO SITES

The following are visual descriptions of known chemosynthetic communities on the Gulf of Mexico continental slope.

Bush Hill - GC-184/185

Bush Hill occurs over a salt diapir that rises about 40 m above the surrounding sea floor to a minimum water depth of 540 m. The feature is located 210 km south-southwest of Grand Isle, LA at 27°47'N, 91°30.4'W. It lies in the Green Canyon offshore leasing area between blocks 184 and 185, approximately 3500 m from the drill template of what is currently the world's deepest oil-production platform. The sediment in this area consists of silty-clay and is of considerable thickness; however, much of the sedimentary facies of Bush Hill itself have been wiped out by rising gas and liquid and by *in situ* formation of authigenic carbonate and sulfides (Figures 1 and 2; 2,8).

The sediments of the depauperate periphery of Bush Hill are pale ochre in color, with an easily disturbed flocculent layer. Although the bottom in this region shows extensive ichno-traces, including burrows, shallow depressions and mounds, very few organisms are seen. Generally, as one progresses up the slope of the carbonate cap, the color of the sediment changes to a slate-grey, and the ichno-traces appear to become less frequent. Carbonate outcroppings can be seen, ranging from rubble to prominent boulders. Along the western side of the carbonate cap, the carbonate outcroppings form an escarpment, which rises about 15 m at its steepest margin. The larger boulders are topped by gorganians, which are in turn frequently encrusted with large ophiuroids. Large colonies of the scleractinian coral *Lophelia* sp. are also seen attached to the exposed portions of the boulders. Filigreed patches of bacteria can be observed on the sediments. Closer to regions of greater community density, the bacteria patches increase in area and are interspersed with the slender (3.5 mm) black tubes of a pogonophoran *Galathealinum* n. sp., family Polybranchiidae (E. Southward, pers. comm.). The most prominent feature of the dense area of the community are tube worm bushes, which occur both among the carbonate outcroppings and on soft sediments away from surficial rubble.

Two species of vestimentiferan tube worms have been collected from Bush Hill and other Louisiana Slope seep communities. These have been identified as *Lamellibrachia* sp., family Lamillibrachidae and *Escarpia*-like species, family Escarpiidae (M.L. Jones, pers. comm.). The escarpid, which can be distinguished in the 35 mm photographs by its distinctive flairing of the tube opening, is fewer in number and generally forms sparse clusters of recumbent individuals. The *Lamellibrachia* sp. forms bush-like clusters in numbers ranging from a few tens to many thousands of individuals. Although *Lamellibrachia* sp. is clearly dominant, mixed clusters of *Lamellibrachia* sp. and the escarpid do occur.

An undescribed mussel (Mytilidae), which is similar to members of the genus *Bathymodiolus* (R. D. Turner, pers. comm.) forms discrete beds on both soft sediments and among carbonate outcroppings. Mussels and tube worms have been observed together; however, the larger mussel beds usually contain only stunted tubeworms, if any. The beds are irregular in shape, often in close proximity to each other, and range in area from less than 1 m^2 to approximately 20 m^2.

Streams of bubbles, primarily methane, can be observed escaping from the substrate, both within the mussel beds and in their immediate vicinity. Some of these bubble streams are intermittent releases; others continued throughout the period of observation. Disturbance of the bottom in the vicinity of methane streams can cause the release of large oil globules, which float upward. Such releases of oil can also be observed in other locations, usually as a result of some disturbance of the bottom. A dense orange-colored mat of bacteria often covers the oily sediments.

A diverse assemblage of common slope fauna has been recorded in the still photographs and the video tapes taken at Bush Hill. Bathypelagic organisms include tunicates, squid and trichiurid fishes. The fish *Hoplostethus* sp. is frequently seen hovering over the tubeworm bushes. Other fishes (including *Chaunax picts*, *Urophycis cirratus* and *Peristenion greyae*) are frequently observed swimming near or resting on the bottom. Crustaceans include decapod crabs (*Geryon* sp., *Bathyplax typhla* and *Rochinia crassa*), shrimp and the giant isopod *Bathynomous gigas*.

Mussel and Tube Worm Communities in GC-234

Lease block GC-234 is currently known to contain two large communities of chemosynthetic organisms. The larger, in terms of areal cover, is a community that is dominated by a mixture of seep mussels and a lucinid clam (*Pseudomiltha sp.*). This community is located on the western side of GC-234 and extends across an area of level topography along the 980-m isobath. A second community, smaller in area than the bivalve community (but possibly more substantial in terms of biomass), comprises what is essentially a single, contiguous tube worm bush occupying an area of approximately 1600 m^2.

The mussel/lucinid community has a broad, linear form of about 300 by 75 m. The sediment on the periphery of the community is the tan and bioturbated silty clay that is typical of the mid-slope. Proceeding into the community, the sediment color changes abruptly to a slate-grey tone. Although a few low rocky outcroppings can be seen, surficial relief is quite uniform throughout the area. One soon notices a scattering of chalky and disarticulated lucinid shells, as well as a series of shallow depressions within which the sediments appear to be stained dark grey. These depressions are quite variable in shape, ranging from roughly circular areas 30 to 50 cm across to curvilinear features several meters in length. Most striking are branching, linear features that give the distinct impression of having been formed by the flowage of a dense liquid across the sediment surface. White and orange bacterial mats are common. Seep mussels appear first in widely separated clusters of 15 to 20 individuals. As the density of the community increases, these clusters join to form stringers up to 3 m in length and, in several places, densely packed beds that are irregular in shape and up to 5 m across. Areas of dead mussel shell are common. Although variable in density, the scatter of lucinid shells does not appear to be segregated from the mussels; however, living lucinids have not yet been observed or collected.

Streams of gas bubbles are commonly seen escaping from the substrate. Attempts to take sediment cores in this community were often frustrated by a layer of impenetrable material, probably carbonates, that underlays the soft surficial sediments at a depth of less than 10 cm. At the western end of the community the occurrence of bivalves and bacterial mats ceases altogether. The bottom then becomes quite flat, seemingly coarser in texture and appears to consist of a visually striking mixture of bone-white gypsum streaked with a light rust colored material, possibly elemental sulfur. The streaking patterns are large in area (20 to 30 m across) and vary from sunburst shapes to broad flowage patterns. Although fish and crabs are commonly seen among the mussels, few or none have been seen in this area.

The tube worm community occurs in an apparent graben fault in an area of variable relief on the northeastern side of the lease block. Rocky outcroppings are prominent and frequent, ranging to house-sized blocks. On the western end of the tube worm area, there is an extensive field of large (up to 2 m high) gorgonians arrayed in perfectly linear rows with 2 to 3 m separation between rows. There is a brief extent of sparse tube worm cover at the edges of the community, but once over the bush proper, the coverage is so dense that the bottom is rarely visible. The tube worms are uniformly straight, upright and at least 2 m in length. Although the individuals on the edges of the community were encrusted with hydroids, sponges, *Acesta bullisi* and even a gorgonian on one occasion, the tubes of individuals in the center of the community appeared quite clean and free from encrustation.

Gas venting can be observed at several points among the tube worms; however, only one small cluster of mussels was observed with certainty. Experience with the Johnson *Sea-Link* showed the sediments to be heavily oil-stained. Several push cores collected from this site contained thermogenic gas hydrates, so the occurrence of hydrates immediately below the surface is evidently common.

Mussel and Clam Communities in GC-272

This lease block contains an extensive chemosynthetic community that is dominated by seep mussels and the vesicomyid clams *Calyptogena ponderosa* and *Vesicomya cordata*. The occurrence of clams and mussels in this area is apparently correlated with the substrate; mussels predominate where the substrate is an exposed carbonate, while clams are restricted to areas of soft surficial sediments. Tube worms are uncommon, and are stunted and convoluted when they occur.

The northeastern corner of the lease block is characterized by slopes in excess of 30° and by a highly irregular micro-relief consisting of exposed carbonate. The carbonate varies in form from a low rubble, to prominent, spire-like boulders and broad, perforated plates. Gas venting is commonly observed. Several beds of seep mussels have been observed near the top of a rocky rise at a depth of 620 m. A very large (> 50 m^2) mussel bed can be found in a shallow depression that runs perpendicular to the contours of the rise. Background fauna are abundant and include numerous hag fish, holothuroids and nephropid lobsters. Attached epifauna include *Acesta bullisii* (attached both to tube worms and to rocks), scleractinian corals and brachiopods.

The slope of the rise is covered with soft sediment and is generally free from burrows or visible megafauna. Several broad terraces cross the slope; these are carpeted with dead and mostly disarticulated clam shells. Coming off the rise and onto an area of relatively flat terrain, the density of shells increases as does the frequency of articulated shells. The clams occur in diffuse clusters roughly 100 m in diameter. Several such clusters were observed during dives that explored the area south of the mussel beds. Living clams are comparatively uncommon and generally occur at the ends of curving trails, which form as the animals plow through the surficial sediments. Presence of these trails is the only reliable means for distinguishing living clams from dead, articulated shells. Attempts to obtain push cores from this area demonstrated that the soft surficial sediments are underlain with a layer of very densely packed clay.

Mussel and Tube Worm Communities in GB-388

The Garden Banks block - 388 chemosynthetic communities occur over a salt diapir at a water depth of 2200 to 2300 ft. The feature is located ~140 miles south of Louisiana at 27°37'N and 92°11'W. Currently this is the only seep community that has been verified by submersible observation in the Garden Bank lease area. However, a number of other areas of Garden Banks have been identified that contain oil-stained cores inferring that chemosynthetic communities may also be common in this deep water lease area.

Topography in the GB-388 seep area is undulating with several areas of rough terrain. Scattered throughout the area are large carbonate boulders, surface faults, and craters (blow-out pockets). Gas seeps are associated with these areas of rough terrain. Three types of gas seeps were observed: those associated with bacterial mats (sporadic gas bubbles with low volume releases); those found along surface faults (rapid bubbles with high volume gas releases); and those associated with craters (blow-out pockets with sporadic bubbles and low volume flow). A large wipe-out zone is present in the area. Piston coring has produced several cores with oil staining, gas pockets, and hydrogen sulfide. Sediment of the area consists of grey silty-clay and is of considerable thickness.

Bacterial mats are widely distributed on the sea floor with three distinct colors (white, purple and orange). Several mats contained all three colors in concentric rings with the orange mat occurring in the center.

A diverse faunal assemblage has been recorded from trawl samples, *NR-1* observations, still photographs and video tapes. Organisms previously recorded from GB-388 included two species of vestimentiferan tube worms (*Lamellibranchia sp.* and an Escarpia-like species). The black pogonophoran (*Galathealinum* n. sp.) has been collected from the trawls but has not been observed on the sea floor. Fish and other species observed or collected in trawls include *Gaza fisheri*, *Chaunax pictus*, *Urophycis cirratus*, and *Peristenion greyae*. Several crabs, starfish, shrimp, neogastropods, burrowing anemones, and other invertebrates have also been recorded. Large boulders were topped by coral and gorgonians, on which are large aphiuroids.

On the 1988 *NR-1* cruise a large bed of the undescribed mussel (Mytilidae) which was recored at Bush Hill was discovered at this site. A large bed of these mussels were found with tube worms located adjacent to the mussels. Gas seepage was present but was very limited at the mussel site.

Chemosynthetic Community in EB-376

The known East Breaks chemosynthetic communities occur on a topographic high in the northwest corner of the EB-376 lease block. This high between depths of 1800 and 1700 feet rises over 300 feet above the surrounding sea bottom and is associated with a strong seismic wipe-out zone. Stratified sediments are present on the margins of the topographic high. Sediments are topped by soft brown mud becoming grey-green in the deeper sections.

A search in the *NR-1* was conducted in water depths ranging between 1700 and 2050 feet. Bottom topography was mostly flat, featureless, mud bottom; however, several areas were observed with exposed authigenic carbonate boulders distributed in a linear zone oriented approximately east-west. The rocks were from one to several feet across and up to 6-8 feet high. In close proximity to the rocks a small gas seep was observed at a depth of 1728 ft.

Tube worms and clam shells were generally associated with the boulder field areas. No live clams or mussels were observed. Also associated with the large boulders were sea fans, corals, anemones, crabs and fish. The tube worms were normally single bushes, no extensive beds were observed. Scattered bacterial mats were also present.

Trawl samples taken across the top of this topographic high have recovered *Calyptogena* and *Vesicomya* shells, and vestimentiferan and pogonophoran tubes.

Other Communities

Other communities have been surveyed by the submersibles including sites in the Green Canyon block 29/31 areas which contained scattered tube worms and mussel beds (1987 *NR-1*); a tube worm and mussel community several thousand feet south of Bush Hill in GC-185 (1987 *NR-1*); a normal heterotrophic community in GC-195 (1988 *Pisces*); and a gas seep, bacterial community in GC-52 (1988 *Pisces*).

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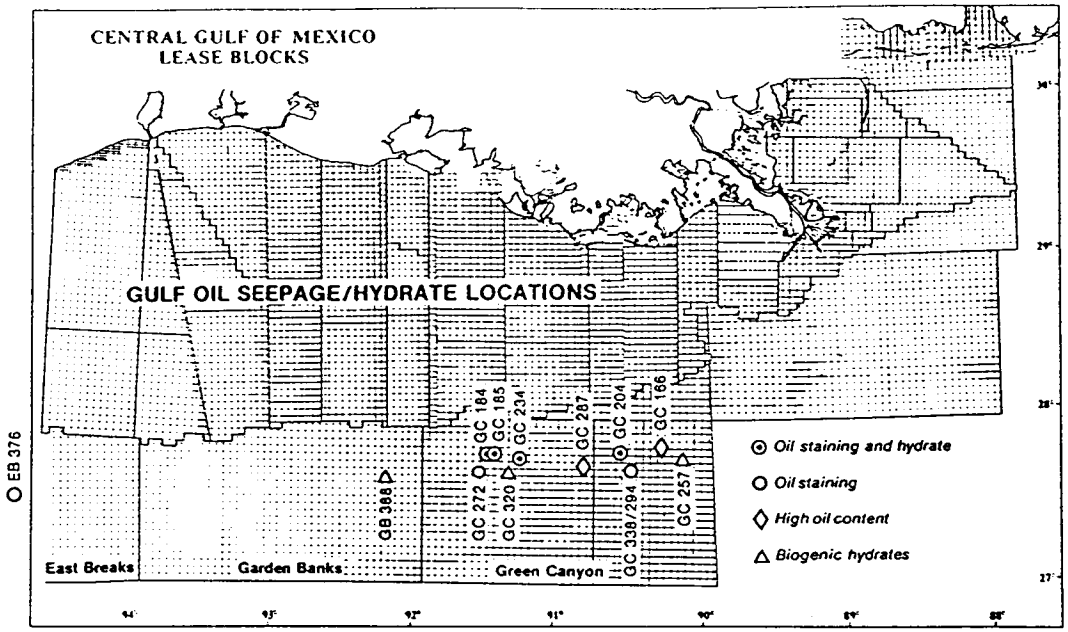

Fig. 1—Location of oil seepage and gas hydrates in the Gulf of Mexico. Most sites are associated with chemosynthetic ecosystems and all are associated with seismic wipe-out zones.

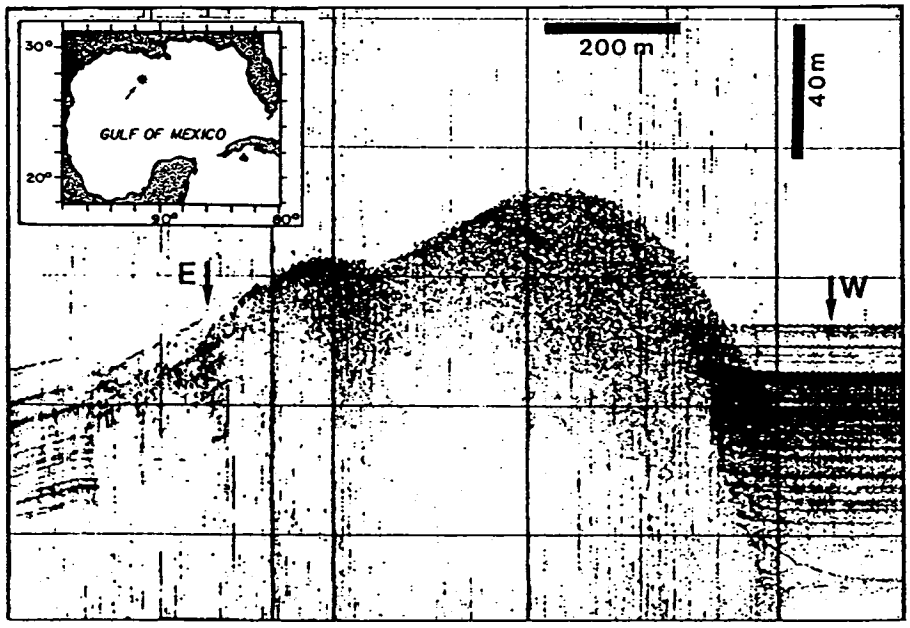

Fig. 2—Subbottom (3.5 kHz) profiler transect across Bush Hill in Green Canyon Lease Block 184/185. Notice the wipe-out nature of the mound.

Gulf of Mexico Hydrocarbon Seep Communities V. Biofacies and Shell Orientation of Autochthonous Shell Beds Below Storm Wave Base

Gulf of Mexico Hydrocarbon Seep Communities V. Biofacies and Shell Orientation of Autochthonous Shell Beds Below Storm Wave Base

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Clam and mussel assemblages associated with petroleum seepage on the Louisiana continental slope form the only substantial shell accumulations below storm wave base on the northwestern Gulf of Mexico shelf and slope. Four distinct biofacies are present at the seeps, dominated respectively by mussels, lucinid clams, vesicomyid clams and tubeworms. Each primary seep site is typically composed of a series of not necessarily contiguous, autochthonous beds dominated by one biofacies. Mussels and tubeworms often co-occur, but neither normally co-occur with lucinid or vesicomyid clams.

Lucinid and vesicomyid clam beds have the best chance of preservation. The vesicomyids produce a two-dimensional shell pavement underlain in some areas by subsurface lucinids: the lucinid beds are normally thicker, more massive shell beds. Taphonomic parameters differ significantly within topographically and sedimentologically equivalent areas, both on the surface and in the subsurface, even in adjacent samples. Local variability in taphonomic characteristics may be a general feature of autochthonous, spatially time-averaged assemblages. Despite essentially undisturbed accumulation in quiet water below storm wave base, concavity ratios rarely differ from 1:1 and frequency of articulation may be low. Dominantly concave-up valves previously reported in quiet water may result from man's fishing activities. Significant variability in shell orientation, frequency of articulation and concavity ratio between adjacent samples indicates that many individual stratigraphically-equivalent samples should be used in any taphofacies analysis of assemblages formed in low-energy environments. Lucinid beds which form below the sediment surface and vesicomyid beds which form on the sediment surface differed significantly in shell orientation and articulation frequency. Assemblages forming below storm wave base in low-energy environments may comprise a wide variety of taphofacies depending upon whether formation occurs primarily beneath the sediment surface or on the sediment surface, despite contemporaneous formation under similar environmental conditions.

INTRODUCTION

Benthic communities dependent upon chemoautotrophy were recently discovered associated with petroleum seepage on the Louisiana continental slope in the northern Gulf of Mexico (Kennicutt et al., 1985; Brooks et al., 1987). These assemblages, dominated by vesicomyid and lucinid clams, mytilid mussels and vestimentiferan tubeworms, are strikingly similar to those found at hydrothermal vents (Fustec et al., 1987), hypersaline seeps at the base of the Florida escarpment (Paull et al., 1984) and methane seeps at the Oregon (Kulm et al., 1986) and Japan (Juniper and Sibuet, 1987) subduction zones. Widespread shell accu-

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mulations are not forming today on the continental slope in the northwestern Gulf of Mexico (Davies et al., 1989). Petroleum seep-associated assemblages produce the only significant localized shell accumulations in this area.

Preserved individuals present in fossil assemblages are of three types (sensu Kidwell et al., 1986): autochthonous, parautochthonous, and allochthonous. The proportion of each type in an assemblage generally figures prominently in discussions of the assemblage's characteristics, although the nomenclature may vary (Powell et al., 1989b; Scott, 1970; Fagerstrom, 1964; Johnson, 1960). The term parautochthonous typically applies to individuals no longer in life position yet remaining within the habitat characteristic of the species. As such, assemblages with a dominantly parautochthonous component are of at least two distinctive types. One, usually above storm wave base, contains shells predominately redistributed over a relatively wide area by physical means; the other, usually below storm wave base, contains shells primarily redistributed over a much smaller area by biological means.

In the first case where physical processes are principally responsible for shell distributional patterns, redistribution can and may frequently be regional in extent—throughout a bay or over much of the inner continental shelf, for example. Although possibly far from where they die, these individuals remain within the species' characteristic habitat. A great bulk of recent continental shelf and bay assemblages contain individuals predominantly of this type (Powell et al., 1989b; Cummins et al., 1986a; Davies et al., 1989). In these assemblages, physical reworking and burial is the dominant process for shell accumulation and preservation, although bioturbation may be locally important. Few shells accumulate on the sediment surface because the rate of taphonomic loss is high and only deep infauna may remain in life position (Cummins et al., 1986b).

A second and significantly different type of assemblage forms in low-energy environments [following Brett and Baird (1986), we will define low-energy as persistent current velocities too low to transport fine sand and well below the velocity needed to move most shells]. In these assemblages, those shells no longer in life position have been redistributed only locally, perhaps during death (by predation) or through postmortem biological disturbance. Such individuals not only remain within-habitat but, in fact, remain within the locale in which they lived. In this sense, the assemblage as a whole is autochthonous (Powell et al., 1989b). It represents a collection of individuals which lived together or succeeded one another within the same locale. If formed and buried rapidly enough, such assemblages may occur above storm wave base (Norris, 1986), but most extensive accumulations can only be formed below storm wave base in low-energy environments. Most of these assemblages are not restricted to infaunal organisms, therefore they require (biological) carbonate production rates that exceed taphonomic loss rates at the sediment surface. Only then can shells accumulate at the sediment surface and be preserved.

Outer continental shelf/upper slope assemblages on soft sediments are commonly encountered in the fossil record (e.g., Fürsich, 1984; Jablonski and Bottjer, 1983; Jablonski et al., 1983; Norris, 1986). Most are composed of autochthonous individuals or are mixtures of autochthonous and parautochthonous individuals of local origin as just described, but recent analogs have not been studied. Petroleum seeps represent an important recent analog where shell beds are formed below storm wave base in a low-energy environment and potentially preserved by gradual burial. All individuals are of local origin, many are in life position. Norris (1986) called this type of assemblage a community bed. Most would be mixed autochthonous-parautochthonous assemblages as defined by Kidwell et al. (1986) and autochthonous TAZ (taphonomically-active zone) accumulations as discussed by Powell et al. (1989b). They fall outside the purview of Johnson's (1960) models and are not well differentiated by Fagerstrom's (1964) or Scott's (1970) system. For simplicity, we will use the term autochthonous for these shell beds hereafter.

Autochthonous assemblages in low-energy environments are commonly characterized by a suite of taphonomic parameters including the presence of articulated bivalves, preferred concave-up orientations for single valves on the sediment surface, and more vertically oriented shells at and below the surface (Kidwell et al., 1986; Emery, 1968; Clifton 1971; Grinnell, 1974; compare Wilson, 1986 for a deep water high energy alternative). As Emery (1968) and Powell et al. (1989b) pointed out however, all recent studies of low-energy depositional settings were conducted in areas potentially affected by commercial fishing and shellfishing activities which may have reoriented shells, particularly those on the sediment surface. Such is not the case at petroleum seeps where water depth and geographic isolation ensure that shell orientations result only from natural processes. Here we briefly describe the biofacies and geological setting of petroleum seeps on the Louisiana upper continental slope and then consider the orientational characteristics of shells in undisturbed, autochthonous assemblages formed in low-energy environments.

METHODS

In 1987 the research submersibles Johnson-Sea-Link and Navy NR-1 were deployed in 550 to 750 m of water for reconnaissance and photographic surveys in Green Canyon (GC) lease blocks 184, 272 and 234 on the Louisiana upper continental slope (Fig. 1). Still and video photographic surveys of chemoautotrophic organisms associated with petroleum seepage were utilized to construct biofacies maps. Shell orientation, articulation and fragmentation were determined on shells 3-cm long or larger by analyzing twenty-eight 35-mm photographs selected from these surveys. This size class also represents the most frequent size class used elsewhere in the literature on death assemblages (Powell et al., 1989b). Three terrains, slopes, terraces and flat plains, were distinguished (as described later). In each terrain, a series of sites was chosen along the video transects for data collection. Site selection was determined by shell abundance ($\geq$10 3-cm or larger shells per camera frame). In some cases, adjacent frames were examined as

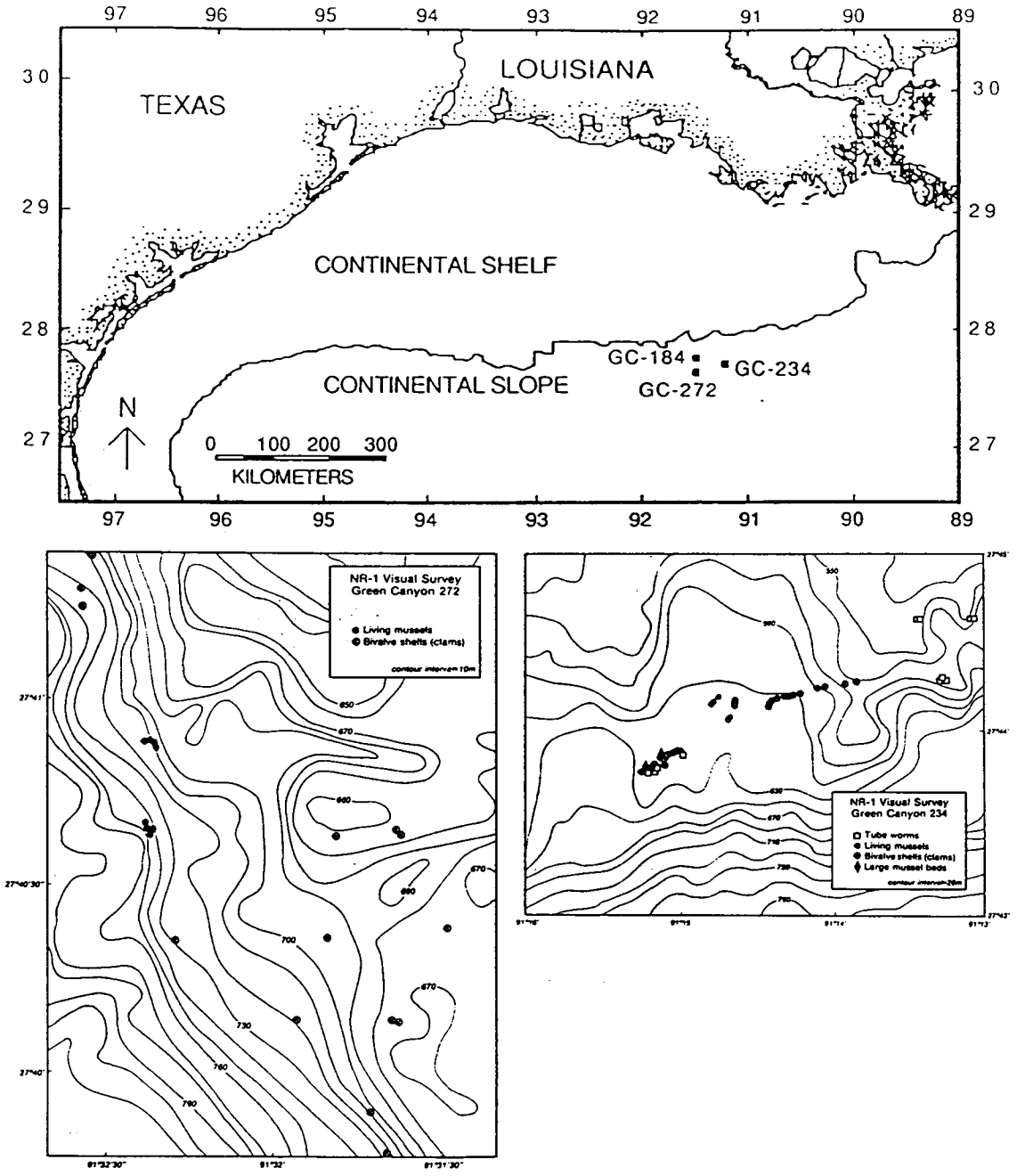


FIGURE 1—Location of seep faunas in Green Canyon lease blocks 272 and 234 on the Louisiana upper continental slope. Upper. Location in the Gulf of Mexico. Lower left. Location of sites in GC-272. Lower right. Location of sites in GC-234. Submersible transects provided extensive coverage above 760 m at GC-272 and 670 m at GC-234. Hence most large sites above these depths should have been observed.

well to investigate close-order variability. As a check on the accuracy of photographic analysis, samples were collected by submersible-operated grab during the Johnson-Sea-Link dives and by box core a few months later using the surface vessel R/V Gyre. The latter were also used to obtain shell orientational characteristics for the equivalent size classes below the sediment surface.

GEOLOGICAL SETTING

Hydrocarbon seepage is common in the Gulf of Mexico (Behrens, 1988; Kennicutt et al., 1988) and is controlled primarily by fault systems created by salt diapirism (Martin and Case, 1975; Behrens, 1988). The substrate on the Louisiana continental slope generally consists of 10 to 15 cm of dark gray flocculent terrigenous clay underlain by firm clay. In most areas, numerous burrows and mounds indicate considerable bioturbation. By contrast, in areas of active petroleum seepage, tar occurs throughout the sediment, the sediment smells strongly of hydrogen sulfide, liquid oil and gas (methane) hydrates are occasionally observed, and the number of worm burrows and mounds is markedly decreased, although bioturbation by epifaunal vesicomyid clams may be extensive (Rosman et al., 1987). Hence, a reduction in infaunal burrowing activity often associated with low oxygen environments (Bromley and Ekdale, 1984; Savrda and Bottjer, 1987) can also be produced at petroleum seeps without concomitantly low oxygen in the overlying water ($[O_2] \approx 3.0 - 5.2$ ml/l).

Authigenic carbonate is common at and below the sediment-water interface at petroleum seeps (Roberts et al., 1987; Behrens, 1988) and other methane seeps (e.g., the Oregon subduction zone—Kulm et al., 1986; Ritger et al., 1987; the North Sea—Hovland and Sommerville, 1985; Hovland et al., 1987). At GC-184, 272 and 234, carbonates visible on the sediment surface form large blocks or ledges 10 to 40-cm thick and up to several meters across. Most carbonate blocks were parallel to bedding but several blocks were observed that dip as much as 45°. As at the Florida escarpment (Paull and Neumann, 1987), dissolution has produced cavities and overhangs at the edges of many of the horizontal carbonate blocks. The authigenic carbonate formed below the sediment surface consists of sand to gravel-sized grains and elongate irregular tubes and columns. Tubes and columns were present either as discrete lithoclasts or partially cemented together with associated shell debris. At GC-234 and 184, a discrete horizon of partially-cemented authigenic carbonate and shell debris occurs 10 to 20 cm below the sediment surface and, in places, can extend down to at least 65 cm.

Local depressions (pockmarks) formed by gas expulsion through the sea floor observed at North Sea sites by Hovland et al. (1987) were occasionally observed at the Louisiana petroleum seeps. These features were much smaller than the large crater reported by Prior et al. (1989) southeast of our investigative area. Shell halos surrounded these depressions and central shell lags produced by gas expulsion were observed. The infrequent observation of pockmarks suggests that only a small percentage of the entire preserved fauna was affected by this process.

BIOFACIES DESCRIPTIONS AND SPATIAL DISTRIBUTION

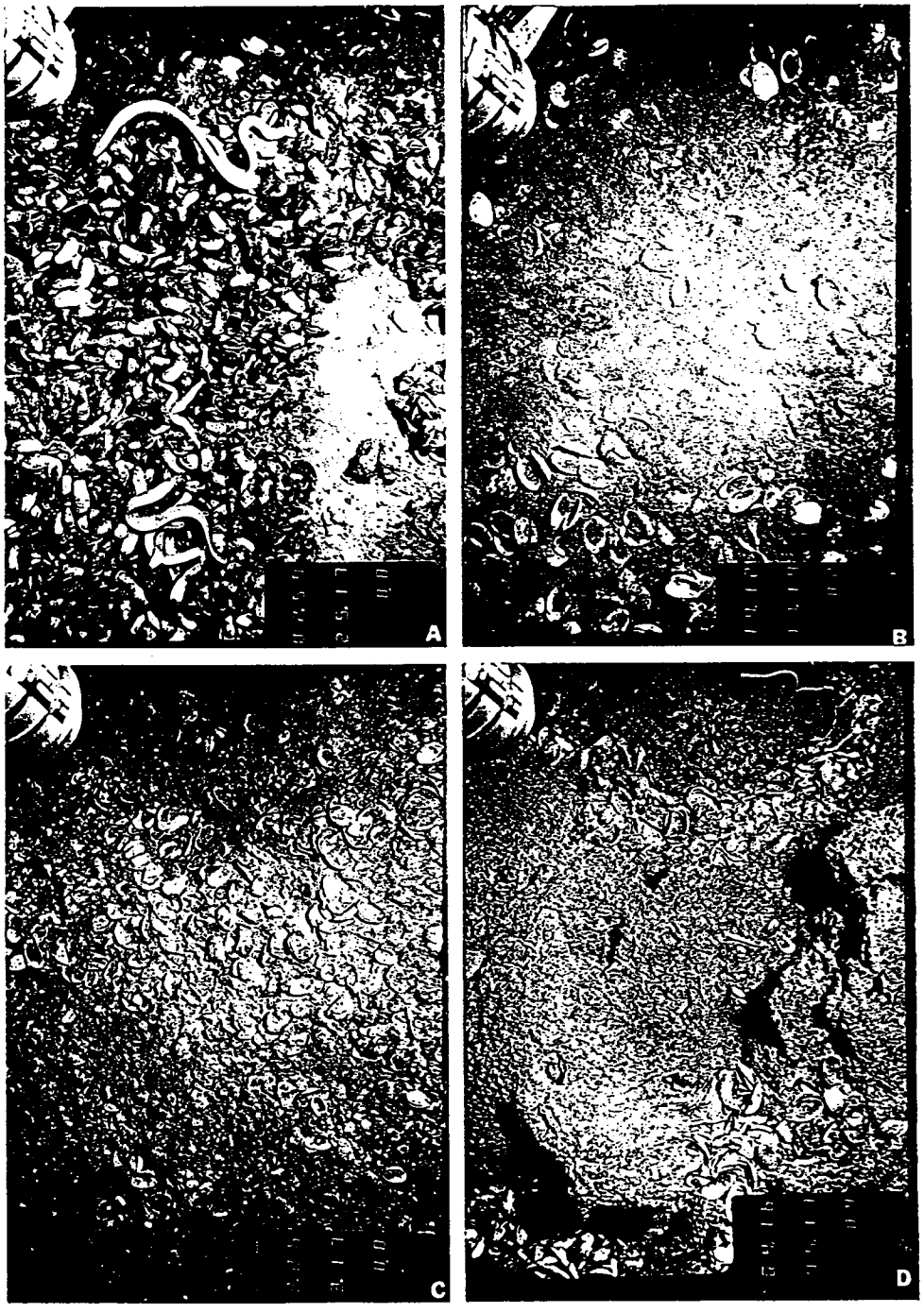
Four distinct biofacies are present at the petroleum seep sites dominated respectively by mussels, lucinid clams, vesicomyid clams and tubeworms. Orange and white bacterial mats occur sporadically in association with each biofacies. The clams and vestimentiferans contain endosymbiotic bacteria which ultimately derive energy from hydrogen sulfide oxidation. Mussels, like those at the Florida escarpment and possibly the Oregon subduction zone, contain endosymbiotic bacteria which utilize methane as an energy source (Childress et al., 1986; Cavanaugh et al., 1987; Kulm et al., 1986).

The mussel biofacies, dominated by the methanotrophic mytilid cf. *Bathymodiolus* sp. (Fig. 2A), occurs in areas of high methane concentration usually associated with fresh oil on or near the surface and brine seepage. Gas bubbles were frequently observed rising from live mussel aggregations and collected live mussels were often coated with oil. Live mussels generally occurred in dense, elongate patches with distinct boundaries and were commonly surrounded by or adjacent to smaller concentrations of dead mussels.

The two clam biofacies, dominated respectively by the vesicomyids *Calyptogena ponderosa* and *Vesicomya cordata* (Fig. 2B–D) and the lucinids *Lucinoma atlantis* and *Lucinoma* sp. (*Pseudomiltha* sp. of Brooks et al., 1987 and Powell et al., 1989a), generally occurred farther away from the influence of active seepage than the mussel biofacies. Sediments were often impregnated with tar. The infaunal lucinids typically were associated with subsurface carbonate lithification. Dead lucinids, although infrequently seen on the sediment surface, were distributed in box cores to a depth of at least 65 cm, so the surface expression of the lucinid biofacies obtained from photographic surveys probably represents an underestimate of its importance. Most of the epifaunal vesicomyids, in contrast, were collected in the top 5 cm of sediment. Whether this restricted distribution is due to recent colonization or a lower preservational potential than the lucinids is unknown.

The tubeworm biofacies is dominated by vestimentiferan tubeworms *Lamellibrachia* sp. and *Escarpia* sp. which occur as individual tubes, single bushes, or dense thickets of closely packed bushes. Limid bivalves (*Acesta bullisi*) up to 15 cm long were commonly attached to worm tubes and white gorgonians up to 1 m across were occasionally observed.

Each primary seep site is typically composed of a series of not necessarily contiguous, autochthonous beds dominated, usually, by only one type of biofacies. Mussels and tubeworms often co-occur (e.g., GC-184—Brooks et al., 1987; MacDonald et al., 1989), but neither normally co-occur with lucinid or vesicomyid clams. Living mussels were usually found at every mussel site, but living lucinid and vesicomyid clams were observed at only a fraction of


A
B
C
D

the sites where dead individuals were found. The discontinuous distribution of shells and the even more restricted distribution of living organisms is also characteristic of cold-water seeps at subduction zones (Sibuet et al., 1988; Okutani and Egawa, 1985), as well as most other death assemblages (Fürsich and Flessa, 1987; Powell et al., 1989b).

At GC-184, the seep sites occupy the top of a diapiric knoll, Bush Hill (Brooks et al., 1987; MacDonald et al., 1989), whereas at both GC-234 and GC-272, the individual seep sites are distributed discontinuously along a linear trend not necessarily associated with the surface topography (Fig. 1B, C). Some suspected ancient seeps are characterized by similar discontinuous, linear trends in shelly carbonate (Howe, 1987; Beauchamp, 1988). The densest concentration of organisms in GC-234 occurred on Behrens' (1988) "central diapir ridge" on a broad plateau (Fig. 1C). A large mussel bed, "Mussel Beach," was present at the southwestern end of the linear trend in GC-234. Lucinid beds covered a wide area north and east of the mussel bed. Large tubeworm thickets with occasional small pockets of mussels were observed at the opposite, northeastern end of the same linear trend, as well as at GC-184. At GC-272, vesicomyid clam beds covered extensive areas. Small mussel patches were widely scattered throughout the area. Vestimentiferan tubeworms were uncommon. Dense lucinid beds were sampled by box core at GC-184, Bush Hill, and GC-234 near Mussel Beach. Many lucinids were also collected in the subsurface at the vesicomyid sites in GC-272. The areal extent of the lucinid beds at these sites remains poorly described.

The spatial distribution of shells in the clam and mussel biofacies are distinctly different. At GC-234, Mussel Beach, mussel concentrations extended discontinuously over a 300 m long, 60 m wide area. Individual beds containing both live and dead mussels were typically elongate, sinuous features 20 to 200 cm wide and 2 to 5 m long that commonly bifurcated or crossed other beds. Smaller patches of densely-clustered live mussels punctuated the discontinuous distribution of dead mussels in the beds. Other mussel beds in the Mussel Beach area occurred as irregularly-shaped patches up to several meters across. Again, smaller patches of densely-clustered live mussels occurred within the main shell bed.

Vesicomyids typically produced significant accumulations only 1 to 5 cm deep, underlain by shell-poor mud. Lucinid beds, in contrast, often extended tens of centimeters deep (>65 cm at GC-234) but the surface expression was less conspicuous. Hence, in pure form, the vesicomyid bed is a two-dimensional lenticular accumulation or shell pavement whereas the lucinid bed is a three-dimensional shell bed. Where they co-occurred (e.g., GC-272), the vesicomyids contributed most of the surface-accumulated component to the three-dimensional bed. Lenticular and more massive columnar shell accumulations are also described from some ancient seeps (Gaillard et al., 1985; Gaillard and Rolin, 1986).

The vesicomyid beds in GC-272 have two distinct modes of spatial distribution. The most common distribution, on flat areas and sloping areas, is a scattering of shells over the sediment surface (Fig. 2B). The density of clams is generally lower than that of mussels but in places scattered shells grade into a pavement of shells covering the entire sediment surface (Fig. 2C). In contrast, densely packed, elongate accumulations of dead vesicomyids are found on small flat terraces 0.5 to 1 m wide at the base of broad 30°–45° slopes (Fig. 2D). The shells present the appearance of having been moved downslope and collected there. The slopes above contain sparse concentrations of live and dead clams.

The extent to which sediment degassing events, slumps and biological disturbance might contribute to downslope transport is unknown, but normal bottom circulation is certainly insufficient to move these shells (Sahl et al., 1987; Snedden et al., 1988; Halper et al., 1988). In over 1500 hr of submersible observation over several years in early to late spring, summer and early fall, no bottom current was observed strong enough to produce any shell movement or substantial sediment resuspension. Ripple marks were never observed and submersible tracks from previous years were readily identified still intact in subsequent years. Moreover, seep communities had relatively sharp boundaries (over ≤50 m). Sampling by submersible confirmed information obtained by camera transect that shells disappeared rapidly at seep boundaries. No shells, not even small individuals, were found abundantly outside the seep sites. Accordingly, shell movement by currents is unlikely and biological disturbance by epifaunal organisms such as crabs appears to be the only important mechanism redistributing shells. Such redistribution is only important locally within single seep sites.

TAPHONOMY

Surficial Accumulations

The preservational potential of the tubeworm biofacies is low because most of the dominant organisms, with the exception of *Acesta*, have no preservable hardparts. Live mussels grow prolifically and occur in dense concentrations, but a low percentage of dead mussels was observed. Mussel shells are more adversely affected by dissolution than clams: many live mussels were collected with deeply dissolved umbos and mussel shells are more easily broken than clams. Samples collected outside live mussel beds rarely yielded dead mussel shells and never in any quantity. Consequently the mussel biofacies is probably poorly preserved. In contrast, very few live clams were observed

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FIGURE 2—Photographs of representative seep biofacies. A. Mussel bed, cf. *Bathymodiolus* sp. with hagfish. B. Vesicomyid clams on flat terrain. C. Vesicomyid clams on slopes. D. Vesicomyid clam shells accumulating on a terrace at the base of a slope against carbonate blocks.

TABLE 1—Taphonomic condition of individuals from photographic surveys and box cores. Rows under each location represent analysis of consecutive camera frames. Significance levels given in the order breakage (B), articulation (A), inclination (I), concavity (C), refer to chi-square comparisons ($\alpha = 0.05$) between adjacent samples within a given location: – not significant, Letter (B, A, I, or C) significant. Data are the number of individuals (single or articulated valves) observed.

Site	Terrain	Significance (chi-square, $\alpha = 0.05$)	Breakage		Articulation		Inclination			Concavity	
			Broken	Whole	Single valve	Valves together	Horizontal	Inclined	Vertical	Concave-up	Concave-down
Variation in adjacent samples from a site											
Clams, mostly vesicomyid (all GC-272)											
3	slope	(----)	0	55	55	0	55	0	0	23	32
			0	80	80	0	80	0	0	30	50
6	slope	(B---)	11	34	33	1	44	0	1	19	25
			0	31	31	0	31	0	0	15	16
7	flat	(B---)	7	21	2	0	28	0	0	12	16
			0	48	48	0	48	0	0	28	20
8	flat	(BA-C)	0	53	47	6	51	0	2	37	14
			0	28	20	8	25	1	2	7	13
			9	40	40	0	46	3	0	22	27
10	slope	(----)	0	24	24	0	23	1	0	12	12
			0	11	11	0	11	0	0	5	6
11	terrace	(B---)	15	14	14	0	26	3	0	10	19
			0	7	7	0	7	0	0	5	2
			0	27	27	0	24	3	0	13	14
16		(-A--)	0	27	23	4	27	0	0	9	15
			0	33	21	12	33	0	0	13	14
			0	62	57	5	61	1	0	31	26
Mussels (GC-272)											
11	terrace	(---C)	0	14	14	0	13	0	1	9	4
			0	48	47	1	38	9	1	24	23
			0	9	9	1	9	0	0	1	8
Non-replicated sites											
Clams, mostly vesicomyid (all GC-272)											
2	terrace		1	24	24	0	25	0	0	6	19
5	flat		0	92	107	14	122	4	4	66	54
9	slope		0	36	36	0	36	0	0	12	24
13			0	22	22	0	21	0	1	16	5
14			0	22	22	0	22	0	0	11	11
15			2	18	18	0	25	0	0	6	14
Mussels (GC-272)											
10	slope		0	81	81	0	66	0	15	44	22
Clams (from box cores)											
GC-272			6	19	15	6	22	2	1	14	6
			8	17	10	7	25	0	0	6	12
			0	2	2	0	2	0	0	0	2
			1	7	5	2	4	0	4	3	0
GC-184			21	48	20	28	33	13	22	15	9
			3	3	3	0	2	3	0	3	2

on or under the sediment surface but there were abundant dead clams present over wide areas. Samples taken in areas with no living lucinids or vesicomyids frequently yielded shells of both types in abundance. The two clam biofacies are likely to be well preserved. Hence we concentrate our taphonomic analyses on the characteristics of clam beds.

Concavity (concave-up vs. concave-down), fragmentation (as defined by Powell et al., 1989b), articulation

TABLE 2—Results of chi-square comparisons within and between terrains and between mussels and clams. For clams only, under categories for variation within and between terrains only (*), parentheses indicate significant chi-square results that failed the more restricted nested ANOVA ($P > 0.05$) because most variation could be explained by differences in adjacent samples. Mussels were not tested using nested ANOVA.

	Breakage	Articulation	Inclination	Concavity
Variation within terrains (clams* only)				
Terrace	($P = 0.03$)	$P > 0.05$	$P > 0.05$	$P > 0.05$
Flat area	($P = 0.02$)	($P < 0.0001$)	$P = 0.004$	$P > 0.05$
Slope	($P < 0.0001$)	$P > 0.05$	$P > 0.05$	$P > 0.05$
Variation between terrains				
Clams*	($P < 0.0001$)	($P < 0.0001$)	($P < 0.0001$)	($P = 0.009$)
Mussels	$P > 0.05$	$P > 0.05$	$P < 0.0001$	$P = 0.04$
Variation between clams and mussels				
Terrace	$P < 0.0001$	$P > 0.05$	$P > 0.05$	$P > 0.05$
Slope	$P > 0.05$	$P > 0.05$	$P < 0.0001$	$P < 0.0001$
Total	$P < 0.004$	$P < 0.02$	$P < 0.0001$	$P = 0.02$
Variation between surficial, mostly vesicomyid (from photographic survey) and subsurface, mostly lucinid (from box core) clams	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P > 0.05$
Variation between lucinids restricted to the nearsurface (from box cores at GC-272) and surficial vesicomyids (from photographic survey)	$P < 0.0001$	$P < 0.0001$	$P = 0.002$	$P > 0.05$
Variation between lucinids restricted to the nearsurface (from box cores at GC-272) and lucinids from deeper, more massive beds (from box cores at GC-184)	$P > 0.05$	$P = 0.03$	$P < 0.0001$	$P > 0.05$
Variation between surface (from photographic survey), shallow subsurface at GC-272 (from box core) and more massive beds at GC-184 (from box core), disarticulated shells only			$P < 0.0001$	

(single vs. articulated valves), and inclination (horizontal, inclined, vertical) were recorded for three terrains: flat areas (slope less than 10°), slopes (slope 10°–45°), and terraces (0.5 to 1 m wide areas running parallel to slope contours). Where possible, we examined three scales of variation: 1) between adjacent samples, 2) within terrains, and 3) between terrains. Constraints with obtaining data from photographs certainly affected these analyses. Comparative use of these data in paleontology should take into account that whole shells were more easily seen than fragments and that live and dead articulated individuals were not always easily distinguished. Ground-truth collections on the photographic transects confirmed the rarity of live clams, so that articulation frequencies are accurate for clams. Live mussels were common, hence articulation frequencies are suspect for this species.

Clam (mostly vesicomyid) and mussel shells on the sediment surface were normally whole single valves, oriented horizontally. Mussel shells were more likely to be inclined or vertical in orientation (18% vs. 3% of clam shells). Clam fragments were more common than mussel fragments and mussel shells tended to be concave-up whereas clams tended to be evenly split between concave-up and concave-down (neither was significantly different from a 1:1 ratio, however) (Tables 1, 2). Most of these differences could be attributed to the poorer preservation of mussels. Most intact mussels were probably recently dead. Hence the original orientation (vertical to inclined) was preserved more frequently. Fewer recognizable fragments indicated that taphonomic processes rapidly reduce shell size below the resolution of our photographic analysis.

The distribution of living organisms probably changes frequently because the distribution of near-surface petroleum seepage is temporally variable (see also Juniper and Sibuet, 1987). Hence the living organisms are rarely distributed equivalently with the dead shells. Spatial time averaging occurs when noncontemporaneous individuals are added to the death assemblage at many locations in the same stratigraphic horizon (Powell et al., 1989b) and could result in spatially-variable taphonomic characteristics in areas otherwise equivalent in environmental character. The spatial scale of this variability may be important.

Paired samples from our study areas, essentially con-

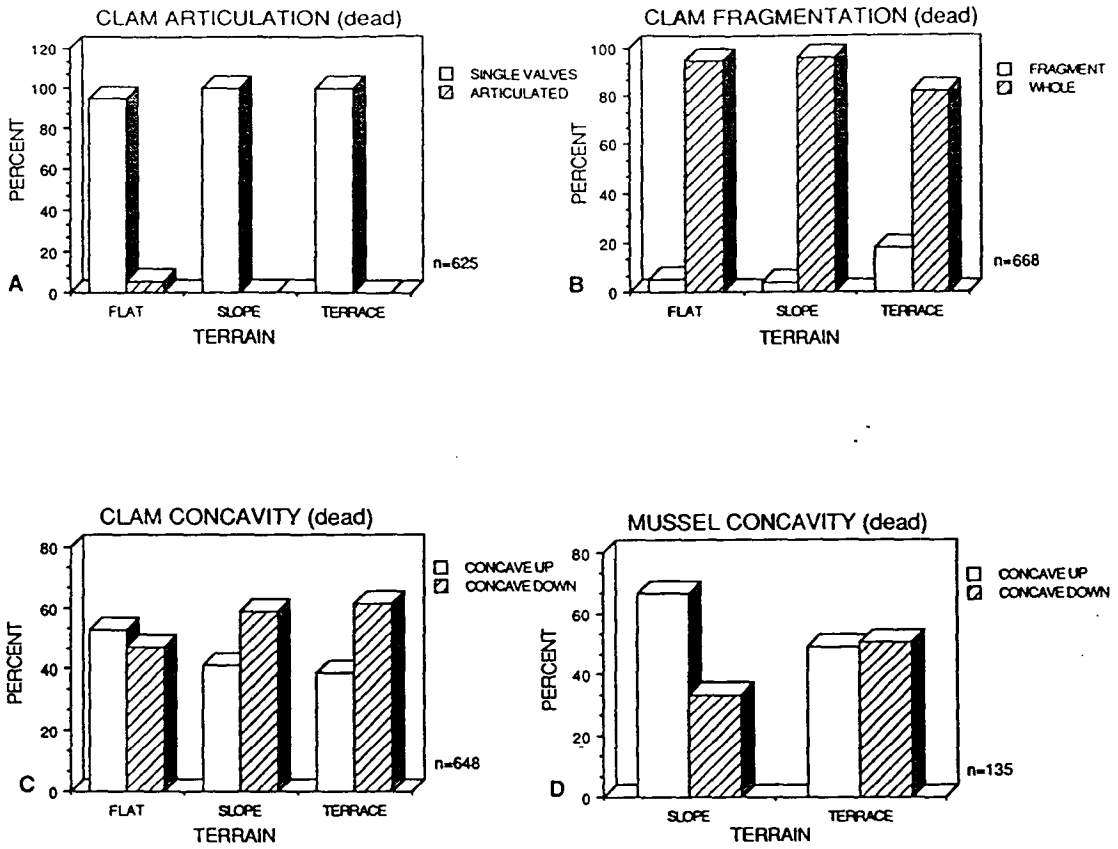

FIGURE 3—Shell taphonomy. A. Clam (mostly vesicomyid) articulation. B. Clam (mostly vesicomyid) fragmentation. C. Clam (mostly vesicomyid) concavity. D. Mussel concavity.

secutive camera frames, differed significantly in shell inclination no more frequently than expected by chance (Table 1) (chi-square followed by a binomial test, $\alpha = 0.05$). The number of fragments differed significantly in four of eight paired frames (chi-square, $\alpha = 0.05$) and shell concavity and articulation in two of eight paired frames, indicating substantially greater local variability. A series of sites was compared in each of the three terrains, slopes, terraces, and flat areas (Fig. 3A–D). Although chi-square indicated a number of significant differences between sites, a nested ANOVA demonstrated that this variability was not significantly greater than the local variability (camera frame to camera frame) present in the data (Table 2). The same result accrues from an examination of the variation between terrains. Variation between terrains was not significantly greater than the local variability within each site. Clam valves, for example, were mostly whole on both flat areas and slopes, but more fragmented clam valves were found on terraces (Fig. 3B). A higher degree of fragmentation on terraces could result from downslope transport of physical or biological origin, but adjacent camera frames were just as variable, so variation in the frequency of fragmentation between terrains could not be judged significant.

Four (sites 2, 3, 9, 13) of thirteen sites for clams and one (site 10) of two for mussels had concavity ratios significantly different from 1:1 (chi-square, $\alpha = 0.05$) (Table 1). In three cases, the number of concave-down valves predominated. In two cases, concave-up valves were most common. Slightly more concave-up valves (52.7%) were observed in the flat terrain, but this distribution was not significantly different from 1:1 (Fig. 3C). The percentage of concave-up valves decreased significantly in both the slope (41.3%) and terrace terrains (38.6%) (chi-square, $\alpha = 0.05$). Mussel concavity showed the same decreasing trend in concave-up percentages from slopes where the

ratio was significantly different from 1:1 (66.7%) to terraces where it was not (49.3%) (Fig. 3D) but the changes in mussel concavity were more dramatic. Because local variability was so high, the increase in the percentage of concave-down valves on slopes and terraces could be produced by the chanciness associated with sampling, but the same trend would be expected if downslope transport imparts a preferred orientation of concave-down. In any case, the relationship of preferred concave-up valves in low energy environments (Emery, 1968; Brett and Baird, 1986; Powell et al., 1989b) was not observed in any terrain, indicating that low energy environments need not always be characterized by a predominance of concave-up valves. In some cases, concave-down valves may be equally as common.

Overall, then, shell accumulations present on the sediment surface were characterized by spatial variability in their taphonomic attributes. The predominant scale of this variability was on the order of one camera frame, a scale of centimeters rather than meters or more.

Subsurface Accumulations

Box cores were necessary to examine the orientation of shells below the sediment surface, but photographic surveys permitted a much more intensive analysis of surface shells and avoided the possibility of reorientation of surface shells during sampling. Consequently, we compare the data obtained by box core with that obtained by photographic survey. Although box cores could not be collected at the same time as the photographic survey was conducted, observation by submersible does not indicate any reason why subsurface/surface shell orientations should not have been conservative over the time scale of sampling (several months) and the nearsurface data obtained by box core at GC-272 are internally consistent with the trend established between the photographic surveys of surficial accumulations and the box core data for deep shell beds obtained at GC-184, as discussed later.

Sites at GC-184, 234, and 272, including the vesicomyid-dominated sites at GC-272, were sampled by box core. Vesicomyids were generally restricted to the upper 5 cm of sediment. At GC-272, where the underlying sediment was heavily impregnated with tar, the lucinid component was restricted to the upper 5 cm of the sediment column, but lucinid shells were rarely found on the surface. At the lucinid beds in GC-184 and 234, lucinids extended to much deeper depths in the sediment.

Overall, the infaunal (mostly lucinid clams, by box core) and epifaunal (mostly vesicomyid clams, by photographic survey) assemblages differed significantly in most respects (Table 2). The fraction of vertical or inclined shells was significantly higher in subsurface beds (34.5% vs. 3.2%), although the fraction of concave-up shells did not differ significantly. Fragments were much more common (but this may be a sampling artifact; fragments were easier to see in box cores). Articulated shells were much more abundant (43.9% vs. 4.6%). Like the vesicomyid-dominated surface, the ratio of concave-up to concave-down shells in the lucinid-dominated subsurface did not differ significantly from 1:1 (chi-square, $\alpha = 0.05$).

Subsurface data from box cores indicates that infaunal, lucinid-dominated sites differed significantly among themselves in inclination ($P < 0.0001$)—one of three sites where the number of individuals exceeded 20 had many more inclined and vertical shells. The subsurface samples also differed significantly in concavity ($P = 0.06$—concave-down shells predominated at one site), but not in breakage frequency or articulation frequency (Table 2). Even at GC-272, where the shells were restricted to the 0 to 5 cm stratum, individual samples differed significantly in concavity ($P = 0.02$) and inclination ($P = 0.001$). Hence, like the surface (photographic) samples, subsurface samples differed substantially site to site.

A comparison between the vesicomyid-dominated assemblages obtained from photographic survey and the lucinid-dominated assemblages obtained from box cores at GC-272, where all shells were restricted to the 0 to 5 cm stratum, demonstrates that surface and subsurface shells differed considerably in condition and orientation even at this narrow scale. Inclined or vertical shells, articulated shells and fragments were more common below the surface in the 0 to 5 cm stratum than directly on the surface (Fig. 4A–D). Although the large site-to-site variation observed in both the photographic surveys and box cores precludes an unambiguous statistical comparison, few photographic samples contained as many inclined or articulated shells. Vesicomyids at petroleum seeps generally live umbo up, at a slight angle to the horizontal (Rosman et al., 1987) [vs. the vertical orientation of *Calyptogena soyoae* (Okutani and Egawa, 1985)]. The single living *Lucinoma atlantis* collected was positioned vertically. The collection, by box core, of individuals that died in place probably explains the differences between the surface and subsurface assemblages.

The deeper, more massive lucinid beds at GC-184 differed even more from the surface, photographic surveys. Inclined or vertical shells and articulated shells were even more common than in the 0 to 5 cm stratum at GC-272, despite both assemblages being lucinid dominated (Table 2). The collection of more individuals that died in place could again explain these differences. If so, then disarticulated shells should be inclined or vertical no more frequently below the surface than on the surface. This was not the case; subsurface disarticulated shells were more frequently inclined or vertical (chi-square, $P < 0.0001$), just as were the articulated shells. Disarticulated shells certainly had been disturbed during or after death. Bioturbation is thought to increase the inclination of shells (Salazar-Jimenez et al., 1982), but, except for the clams themselves, bioturbating infauna are rare at petroleum seeps. Perhaps, the activity of living, vertically-oriented bivalves gradually reorients subsurface shells. Subsurface space for new living individuals is certainly a limited commodity in most of these lucinid beds.

Overall, the subsurface lucinid-dominated samples comprised a mixture of individuals that died below the surface and those that died at the surface. The differences between

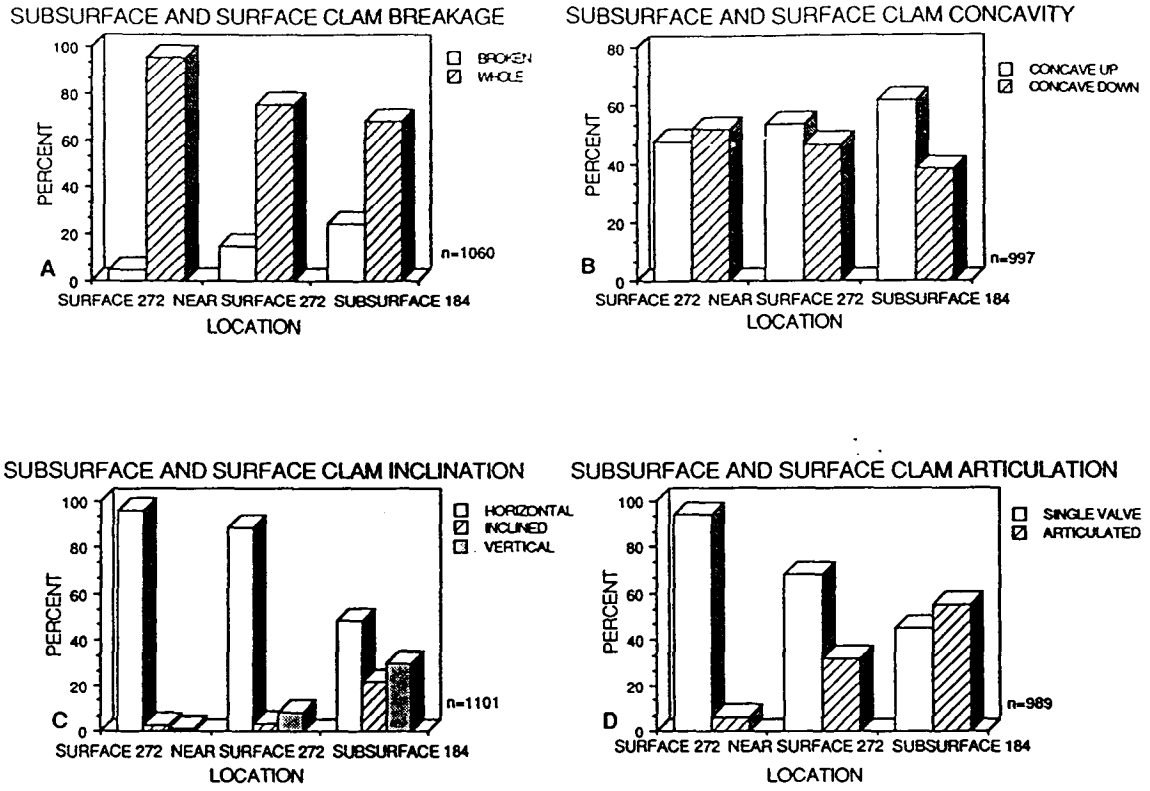

FIGURE 4—Shell taphonomy. Variation between surface (mostly vesicomyid) and nearsurface (mostly lucinid, GC-272) and deeper subsurface (mostly lucinid, GC-184) clams. A. Breakage frequency. B. Concavity. C. Inclination. D. Articulation.

these and the surficial vesicomyid-dominated samples observed photographically originates in 1) a greater fraction of shells in life position in the lucinid beds and 2) the differing impacts of a) infaunal bivalves on the disposition of subsurface shells and b) predators and other biological agents on the disposition of shells at the sediment surface. In the extreme comparison between the surficial vesicomyids and the deeper, more massive lucinid beds at GC-184, 55% of the lucinid bed fauna was inclined or vertically oriented or articulated and probably in life position, whereas less than 5% of the vesicomyids were in equivalent condition. Only 4.6% were articulated for instance.

CONCLUSIONS

Petroleum seep deposits are paleoecologically important because they are the only known setting in the Gulf of Mexico continental shelf and slope where autochthonous shell beds are formed. Four biofacies are common at petroleum seeps, dominated respectively by vesicomyid clams, lucinid clams, mussels, and tubeworms. The two clam biofacies have the highest preservational potential. They probably represent the extremes in the types of autochthonous beds currently forming on the low-energy continental slope. The vesicomyids at GC-272 produce a two-dimensional shell pavement that is underlain in some areas by a narrow band of subsurface lucinids, whereas the lucinid beds at GC-184 and 234 are thicker, more massive shell beds. The two differ significantly in many taphonomic attributes including shell articulation and inclination. The thicker lucinid-dominated beds contain many articulated shells, and many shells that are oriented vertically or inclined rather than horizontal. In contrast, surface shell lenses have few articulated shells. Most shells were horizontal. Whether the shell bed was formed predominantly by surface or subsurface accumulation could be estimated from the fraction of articulated and inclined or vertical shells. Spatial time averaging was probably important in both types of assemblages. Few live animals were observed, hence all shells were probably not contemporaneous at

stratigraphically-equivalent levels. The areal extent of the seep sites, as preserved, far exceeds the areal extent of the living communities present at any one time.

Site-to-site variability was the general rule; that is, taphonomic parameters differed significantly within topographically and sedimentologically equivalent areas, both on the surface and in the subsurface, and even in immediately adjacent samples. Local variability in taphonomic characteristics may be a general feature of autochthonous, spatially time-averaged assemblages (see also Fürsich, 1984; Brookfield and Brett, 1988). Site-to-site variability suggests the requirement of using many stratigraphically-equivalent samples in any taphofacies analysis.

Petroleum seep assemblages are probably typical of autochthonous assemblages forming in low-energy environments below storm wave base. The characteristics of seep assemblages demonstrate the following which may be generally true. 1) The disposition of shells on the sediment surface may differ significantly even from shells immediately (a few cm) below them within the sediment. The characteristics of the fossil assemblage will depend upon whether surficial or subsurface accumulation predominated. 2) Articulated shells need not be common in autochthonous assemblages. To the extent that shell accumulation occurs on the sediment surface rather than within the sediment, articulated shells may, in fact, be rare.[1] 3) Assemblages dominated by concave-up shells are not typical for low-energy environments, even where horizontally-oriented shells dominate the assemblage. A concavity ratio of 1:1 was the rule, trending, in fact, toward dominance by concave-down valves. Dominance of concave-up shells in surficial accumulations in shallow water, observed in studies of modern death assemblages, might be artifacts of man's fishing activities. It is essential in studying recent death assemblages to absolutely exclude the impact of man's activities on death assemblage composition before appropriate comparisons to the fossil record can be made.

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[1] An assemblage at Garden Banks lease block 425, dominated by the lucinid *Thyasira oleophila* Clarke 1989, consists solely of articulated shells in life position (our unpubl. data). Accordingly, articulation frequencies range from <5% to ~100% depending upon the conditions of accumulation.

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A Methanotrophic Marine Molluscan (Bivalvia, Mytilidae) Symbiosis: Mussels Fueled by Gas

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A Methanotrophic Marine Molluscan (Bivalvia, Mytilidae) Symbiosis: Mussels Fueled by Gas

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An undescribed mussel (family Mytilidae), which lives in the vicinity of hydrocarbon seeps in the Gulf of Mexico, consumes methane (the principal component of natural gas) at a high rate. The methane consumption is limited to the gills of these animals and is apparently due to the abundant intracellular bacteria found there. This demonstrates a methane-based symbiosis between an animal and intracellular bacteria. Methane consumption is dependent on the availability of oxygen and is inhibited by acetylene. The consumption of methane by these mussels is associated with a dramatic increase in oxygen consumption and carbon dioxide production. As the methane consumption of the bivalve can exceed its carbon dioxide production, the symbiosis may be able to entirely satisfy its carbon needs from methane uptake. The very light ($\delta^{13}C = -51$ to -57 per mil) stable carbon isotope ratios found in this animal support methane ($\delta^{13}C = -45$ per mil at this site) as the primary carbon source for both the mussels and their symbionts.

BACTERIAL ENDOSYMBIONTS USING reduced sulfur compounds as energy sources and fixing CO_2 by means of the Calvin-Benson cycle have been implicated as the major source of primary production around the deep-sea hydrothermal vents (*1–3*). These bacteria are found in the gills of the clams and within the trunk of the vestimentiferan tubeworms that live near the vents. Since the initial discovery, such symbioses have been found in a variety of other taxa (*2, 4*) as well as a variety of other habitats (*5–7*) characterized by the availability of both reduced sulfur compounds and O_2. Shortly after the initial discovery of these symbioses, investigators began looking for symbioses based on other reduced compounds found in some of these environments. We present here evidence of a methane-based symbiosis between an animal and intracellular bacteria. Methane is the principal component of natural gas.

Childress *et al.* have shown CH_4 consumption by the bacteria-containing tissue of the vent tubeworm *Riftia pachyptila* (*8*), but the intact animal does not take up CH_4 (*9*). Other researchers have suggested on the basis of the internal membranes seen in some pogonophoran and mussel symbionts that some symbioses consume CH_4 (*2, 10*); however, there has been no demonstration of CH_4 uptake by these symbioses. On the basis of their observations of unusually light stable carbon isotope ratios, Kulm *et al.* have suggested that the clams and worms of the Oregon subduction zone consume CH_4 (*11*). However, the absence of any supporting data on the Oregon organisms and the fact that the same species found elsewhere have sulfur-based symbioses (*2*) make this suggestion highly speculative at best. Arp *et al.* have also failed to demonstrate significant CH_4 uptake in the vent clam *Calyptogena magnifica* (*12*). Thus, the results described in this report were unexpected. The very first measurements and all subsequent ones have shown that these mussels consume CH_4 at a high rate. Whole animal experiments have now been conducted on nine individual mussels. In addition, 28 separate experiments have been carried out on excised gill tissue from eight different individual mussels. In every case where O_2 was not limiting and no inhibitor was being used, the rate of CH_4 consumption was high, generally approaching that of O_2 consumption.

Table 1. Gas exchange rates in tissue pieces from intact mussels of an undescribed species from a hydrocarbon seep off Louisiana. The tissue pieces were incubated in 20-ml glass syringes in membrane-filtered (0.45 μm) seawater. The whole animals were measured in a flowing stream of water. All gases were analyzed by gas chromatography (*9*). All measurements were made at 7.5°C. Gill protein content averaged 15.3% of wet weight by the Lowry method with bovine serum albumin as a standard. Numbers in parentheses are the 95% confidence intervals.

Conditions	Tissue	*n*	O_2 range (μmol/liter)	CH_4 range (μmol/liter)	Gas exchange rates (μmol/g wet weight per hour)		
					CO_2	O_2	CH_4
Aerobic	Gill	6	90–200		+1.44(±0.35)	−1.35(±0.39)	
Aerobic, CH_4	Gill	10	90–200	44–190	+2.09(±0.38)	−2.10(±0.22)	−1.36(±0.23)
Aerobic, CH_4, acetylene	Gill	3	100–210	58–169	+1.36(±0.20)	−1.33(±0.43)	−0.10(±0.11)
Hypoxic, CH_4	Gill	4	5–30	200–435	+1.05(±0.47)	−0.15(±0.08)	−0.17(±0.25)
Aerobic, CH_4	Foot	2	130–200	110–207	+0.45, +0.94	−0.86, −0.43	+0.05, −0.11
Aerobic, CH_4	Mantle	2	140–200	84–226	+0.65, +0.38	−0.54, −0.53	−0.14, +0.07
Aerobic	Whole animal	2	120–200		+0.40, +0.34	−0.29, −0.24	
Aerobic, CH_4	Whole animal	2	50–150	20–205	+0.50, +0.37	−1.19, −1.20	−0.74, −0.90

The mussels used in this study (*13*) were collected in two trawls near hydrocarbon seep sites on the Louisiana slope of the Gulf of Mexico (*6*) (27°41′N, 91°32′W) at bottom depths of 600 to 700 m. The same trawls also retrieved vestimentiferan tubeworms (*14*) and pogonophorans. Immediately after capture, the blood gas contents of seven vestimentiferans, which were collected in a clump at the first site, were analyzed by gas chromatography (*9*). Five of these animals contained H_2S (113, 42 and 27 μmol/liter and two with trace amounts), three contained CH_4 (142 and 110 μmol/liter and one with a trace amount), and two contained CO (24 and 16 μmol/liter). Thus, this appears to be a habitat with CH_4 as well as sulfide available at high concentrations. As confirmation, a piston core taken along the trawl track (27°40.5′N, 90°31.6′W) showed visible oil staining and H_2S. Methane concentrations in the sediments can be very high since gas hydrates are present in this region (*15*).

Our initial measurements of O_2 and CH_4 consumption by whole mussels showed extraordinarily rapid rates of initial CH_4 and O_2 consumption, making experimentation difficult. Therefore, we focused our efforts at sea on studying the metabolism of gill pieces (Fig. 1, A, B, and C, and Table 1). The gills were rapidly dissected free of the animals, rinsed in 0.45-μm membrane-filtered seawater (MFSW), cut into 0.4- to 0.5-g pieces, and kept at 7.5°C in MFSW until used. The gills' ciliary activity continued for more than 12 hours after dissection under these conditions; however, we completed all experiments within 7 hours. The separated pieces of gill were incubated at 7.5°C in 20-ml glass syringes closed with plastic valves. The syringes were filled with MFSW that had been partially decarbonated (total $[CO_2]$ ≈600 μmol/liter), adjusted to *p*H 8.3, and equilibrated with appropriate gases. Imme-

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diately after introducing the gill piece, a sample of water taken from the syringe was analyzed for CO_2, O_2, N_2, CH_4, and acetylene by gas chromatography (*9*). In each sampling a total of 2 ml was removed from each incubation syringe to provide the 0.5-ml sample for analysis and to flush the sampling syringe. Each incubation syringe was sampled four times at 40- to 50-minute intervals. The rates declined with time, apparently because of mixing problems associated with the high metabolic rates, since changing the incubation media restored the original rates. The rates presented in Table 1 are based on the average rates over the first two sampling intervals (that is, the first three analyses). Control samplings demonstrated that the seawater medium showed no significant consumption of CH_4 or other gases and that the consumption rates were insignificant when the gills were removed after 2 hours and the incubation was continued with the same water. Measurements were also made on gill pieces from *Pseudomiltha* sp., *Vesicomya cordata*, *Solemya reidi*, and *Calyptogena elongata*, as well as on trophosome preparations from two vestimentiferan tubeworms [*Lamellibrachia* sp. and an unidentified vestimentiferan belonging to an undescribed genus (*14*)]. All of these species have either been demonstrated to harbor symbiotic sulfur-based chemoautotrophic bacteria or to have close relatives for which this mode of life has been demonstrated (*2*, *4*). Although all of these other preparations from symbiont-containing tissues showed significant O_2 consumption and CO_2 production, none showed significant CH_4 consumption, supporting the contention that CH_4 consumption is occurring within the seep mussel gills and is not due to contaminating bacteria or an unknown artifact. Figure 1 shows the concentration changes observed in the syringes during experiments with tissues from the seep mussel and shows the typical range of conditions used and analytical precision achieved. This figure is not directly convertible to rates because the volumes in the syringes changed after each analysis.

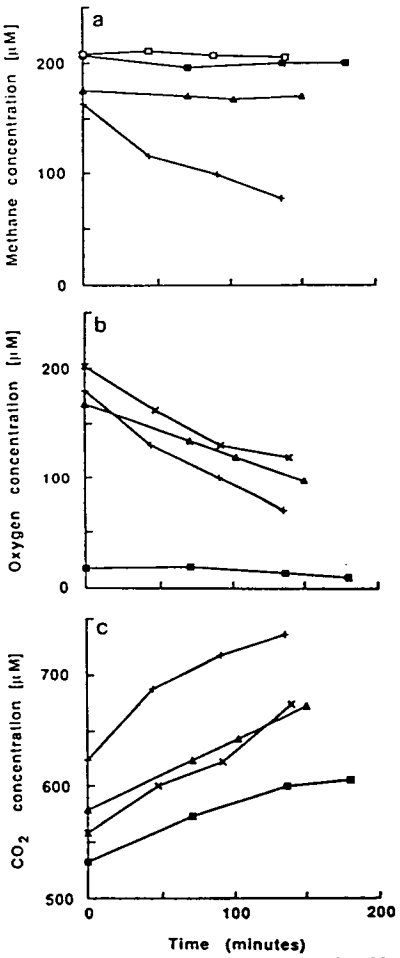

Fig. 1. (a) Changes in CH_4 concentration in a 20-ml syringe during the incubation of a piece of mussel flesh in membrane-filtered (0.45 μm) seawater. Two milliliters was withdrawn at each time period, and 0.5 ml was analyzed by gas chromatography (*9*). +, Mussel gill (0.445 g) under aerobic conditions with CH_4 present; ■, mussel gill (0.462 g) hypoxic in presence of CH_4; ▲, mussel gill (0.418 g) under aerobic conditions with CH_4 and 38 μmol/liter acetylene present; □, mussel foot (0.667 g) under aerobic conditions with CH_4. (b) Changes in O_2 concentration under same conditions as (a). Same symbols as in (a) with the addition of x, mussel gill (0.436 g) aerobic with no CH_4. (c) Changes in CO_2 concentration under same conditions as in (b) with the same symbols.

Table 1 and Fig. 1 show significant consumption of CH_4 by the mussel gills in the presence of O_2. The rates of O_2 consumption and CO_2 production are also significantly elevated (Mann-Whitney U test, $P = 0.01$ for CO_2 and $0.01 > P > 0.005$ for O_2) when CH_4 is being consumed. These data indicate that CH_4 is being oxidized by O_2 but that much of the carbon is also being retained within the organism, presumably as organic carbon. The observed CH_4 consumption is abolished by low O_2 or by acetylene (20 to 40 μmol/liter), as is typical of methanotrophic bacteria (*16*). Neither foot nor mantle tissue of the mussels show significant consumption of CH_4, indicating that it is limited to the bacteria-containing gills of the mussel and is not caused by bacteria living on the surface of the mussel tissues.

In whole animal experiments (Table 1), individuals were placed in chambers in a running stream of filtered (0.2 μm), ultraviolet-sterilized, antibiotic-treated (penicillin G and streptomycin sulfate at concentrations of 50 mg/liter each) seawater at 7.5°C and of controlled gas content. The gas concentrations in the seawater before entering and after leaving the chamber were measured by gas chromatography (*9*) and compared to those from a control chamber. The measurements support the gill studies, showing very high rates of CH_4 consumption, four- to fivefold stimulation of O_2 consumption in the presence of CH_4, and CH_4 stimulation of CO_2 production. Rates of CH_4 consumption were sustained for more than 24 hours, supporting the concept that these mussels are oxidizing CH_4. The fact that CH_4 consumption exceeds CO_2 production at these CH_4 levels (50 to 200 μmol/liter) suggests that this symbiosis can potentially derive its carbon needs entirely from methane consumption.

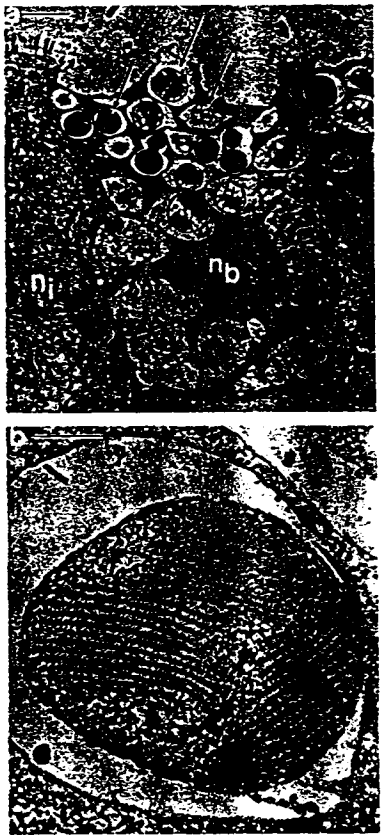

Fig. 2. (a) Electron micrograph of a cross section through a mussel gill filament. Central bacteriocyte is flanked by an intercalary cell with microvilli on the left, and an intercalary cell and bacteriocyte on the right. Some of the symbiotic bacteria are indicated by arrows; n_b: nucleus of bacteriocyte; n_i: nucleus of intercalary cell (×2550). Scale bar, 2 μm. (b) Electron micrograph of a bacterium within a mussel gill cell. Stacked internal membranes are typical of type I methanotrophs (×38,250). Scale bar, 0.2 μm.

The gill tissues of three mussels of this species had carbon isotope ratios ($\delta^{13}C$) of −51.8, −51.6, and −52.1 per mil, and the mantles of the same mussels had $\delta^{13}C$ values of −57.3, −52.1, and −52.3 per mil, respectively. Such extremely light numbers separate the seep mussels from the other (apparently sulfur-based) animal bacterial symbioses around these seeps, which have $\delta^{13}C$ values between −27 and −35 per mil (*6*). Since, as indicated above, the other symbioses tested do not appear to consume CH_4, the $\delta^{13}C$ values in the mussel may reflect the carbon isotopic composition of thermogenic CH_4 (−45 per mil) in its environment (*16*). The apparent homogeneity of the $\delta^{13}C$ values throughout the seep mussel tissues indicates that the carbon derived from CH_4 is distributed throughout the animal. Since CH_4 oxidation is taking place only in the gills, this implies the translocation of organic carbon derived from CH_4 from the gills to other tissues throughout the animal. The degree to which the $\delta^{13}C$ differs from that of the other organisms from the same environment also suggests that oxidation and incorporation of CH_4 carbon is a major nutritional input for this mussel. The stable carbon isotope data suggest that the $\delta^{13}C$ of animal tissue on the Louisiana slope may be useful for differentiating CH_4-based symbioses from sulfur-based symbioses. However, extrapolation of isotopic ranges between chemosynthetic systems (hydrothermal vent, subduction zones, brine seeps) is highly speculative, since a variety of processes can affect carbon isotope ratios, and CH_4 stable isotope ratios vary widely (*17*).

The question remains: what is the agent responsible for the oxidation? Optical and transmission electron microscopy (*18*) reveal the presence of abundant intracellular coccoid bacteria in vacuoles within the gills (Fig. 2a). Stacks of intracytoplasmic membranes, typical of type I methanotrophs (*16*), are visible in many of these bacteria (Fig. 2b). Type I methanotrophs have the ribulose monophosphate cycle and incorporate carbon from CH_4 into organic compounds (*16*). These symbionts are very close to the gill surface, which would facilitate CH_4 uptake from the seawater.

This symbiosis between a methanotrophic bacteria and an animal host is potentially able to derive a large fraction of its energetic and carbon needs from the consumption of the reduced single carbon compound CH_4 (*19*). This form of symbiosis may well be found in other vent and seep mussel species.

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13. This is an apparently undescribed species of mussel related to *Bathymodiolus*. Specimens have been provided to R. Turner, Museum of Comparative Zoology, Harvard University.
14. Specimens of these tubeworms have been provided to M. L. Jones, U.S. National Museum of Natural History, Smithsonian Institution, Washington, DC.
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18. Tissues were removed from living animals and fixed (on board ship) within hours of collection in 3% glutaraldehyde in 0.1*M* phosphate-buffered 0.35*M* sucrose (pH 7.35) and stored at 4°C until returned to the laboratory (2 weeks). The tissues were then postfixed in 1% osmium in buffered sucrose, dehydrated through a graded ethanol series, and embedded in Spurr's resin. Sections (80 to 90 Å in width) were stained with uranyl acetate and lead citrate, and then viewed with a Siemens 1A electron microscope.
19. Although many sulfur-fueled symbioses (lucinid clams excepted) are reputed to be rather repulsive food items because of their high sulfide contents, one of us (J.J.C.) has sampled this mussel and finds its flesh to be quite sweet and delicious.

20. Supported by the Biological Oceanography program of the National Science Foundation through grant OCE83-11257 to J.J.C. and by the Marine Chemistry Program of the National Science Foundation through grant OCE83-01538 to J.M.B. Additional support to J.J.C. came from BSRG S07 RR 07099-19 awarded by the Biomedical Research Support Grant Program, National Institutes of Health and to J.M.B. and M.C.K. from Texas A&M University's Sea Grant Program (No. 18931).

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Chemosynthetically Sustained Ecosystems in the Deep Sea

water (Corliss et al., 1979; Jannasch and Wirsen, 1979). The immediate vicinity of these "hydrothermal vents" was inhabited by dense populations of marine invertebrates: mussels, clams, and tube worms of unusual size and representing new species, genera, and families (reviewed by Grassle, 1986). It was quite clear from the first observations that these immense amounts of biomass could not be explained by a supply of photosynthetically produced food particles. Instead, the energy resource was theorized, and later found, to be terrestrial (chemical) rather than solar (reviewed by Jannasch, 1985).

It seems evident that physical energy (high temperature and pressure) is converted into potential chemical energy by geothermal processes within the earth's crust. The resulting highly reduced hot hydrothermal fluid is carried upward to the sea floor where the release of energy on contact with cold oxygenated seawater takes place chemically or, if mediated microbiologically, is accompanied by the fixation of CO_2 into organic carbon. Thus, in an unexpected manner the geophysical/geological exploration of plate tectonics resulted in the discovery of a major microbiological phenomenon: the chemolithotrophic sustenance of thriving communities of organisms in the deep sea—a timely contribution to the 100th anniversary of Winogradsky's observation on microbial autotrophy in the absence of light.

8.2 DEEP SEA SOURCES OF ENERGY

The tectonic plate movements at spreading centers involve an intermittent penetration of seawater several km into the earth's crust. Through heating by underlying magma chambers it may reach temperatures of 1200°C before ascending again and reaching the seafloor as hydrothermal fluid with temperatures of 350°–360°C. These "hot" hydrothermal vents have flow rates of 1–2 m/s. The instant precipitation of black polymetal sulfides gives them a characteristic appearance that has been termed "black smokers." The precipitation process also produces anhydrite ($CaSO_4$) which forms part of these chimney-like structures (Edmond and Von Damm, 1983). More important for the occurrence of rich animal populations are the "warm" vents with temperatures around 25°C and flow rates of 1–5 cm/s, where a premixing of hydrothermal fluid with ambient seawater takes place in the porous sub-seafloor lava beds prior to emission (Figure 8.1). An overview of the hydrothermal processes involved is given in a symposium volume edited by Rona et al. (1983).

"Hydrothermal fluid" is thermally altered seawater which is anoxic, acid, and highly reduced. Its chemical composition is the key for chemolithotrophic processes. Certain ions are lost during subsurface precipitations or exchange reactions; others are enriched through leaching processes. The chemical composition of most offshore vent emissions resembles that presented for the 21°N site (about 100 miles off the Pacific coast of northern Mexico) in Table 8.1. At the Guaymas Basin vent site (in the Gulf of California), on the other hand, the actual basalt lava emissions are overlayed by several hundred meters of sed-

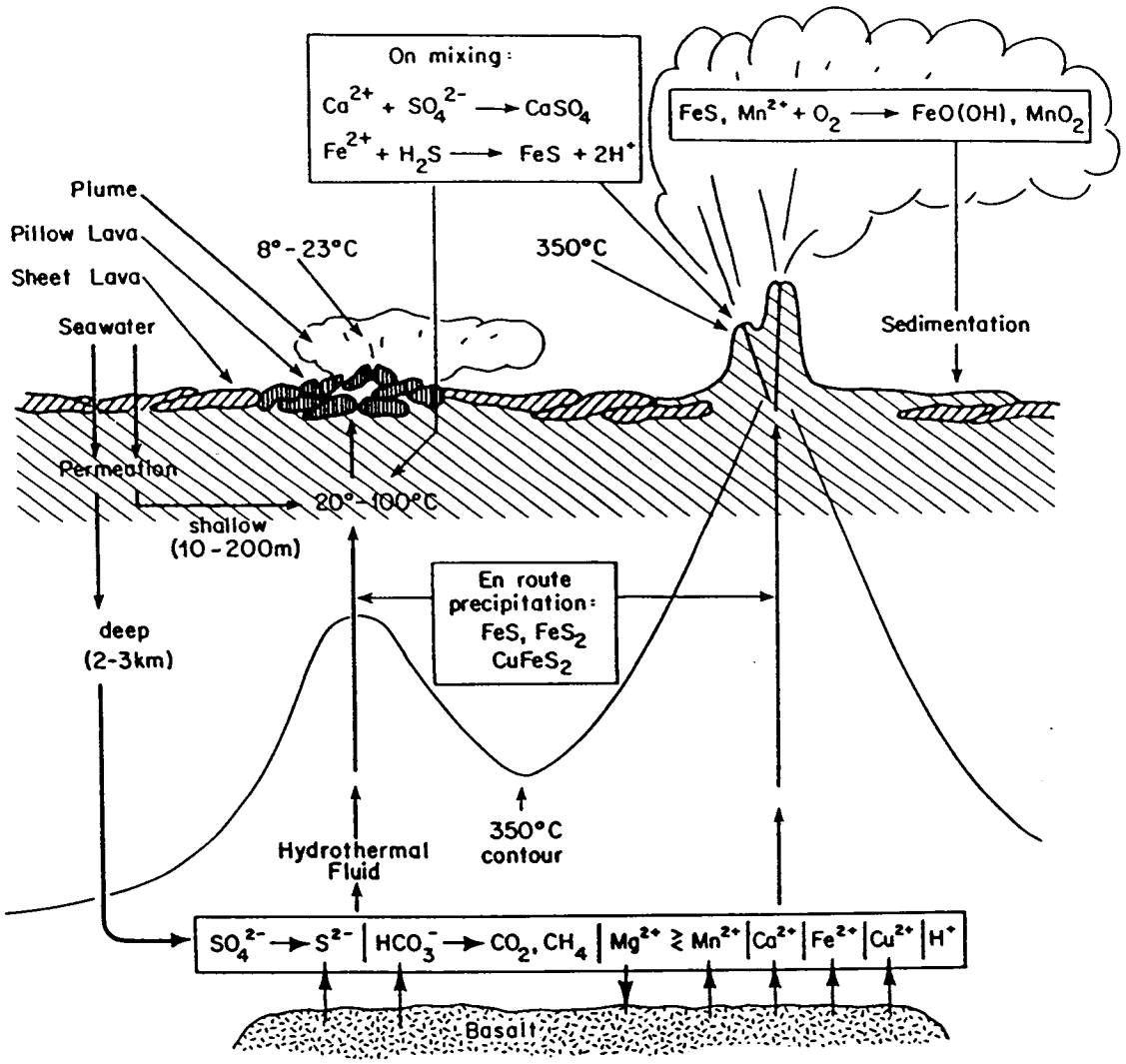

Figure 8.1 The major geochemical processes occurring within the oceanic crust and on the deep sea floor. As seawater penetrates several km into the crust, it is heated to 350–400°C, reacts with basaltic rocks and leaches various chemical species into solution. The highly reduced "hydrothermal fluid" rises and reaches the seafloor either directly (hot vents) or after mixing with cold, oxygenated seawater before emission (warm vents). On mixing, polymetal sulfides and calcium sulfate (anhydrite) precipitate either within subsurface lava conduits or as "chimneys" and suspended particulate matter in the "black smokers" (modified from Jannasch and Mottl, 1985).

iment which is penetrated by the hydrothermal fluid (data in Table 8.1). Geochemical processes result in the difference of ion composition in the sea floor emissions at these two sites (Lonsdale et al., 1980; Edmond and Von Damm, 1985).

Most of the heavy metal enrichment found in the offshore "bare lava" vent emissions is stripped during the passage of hydrothermal fluid through sediments at the Guaymas Basin site (Table 8.1). As a result, the typical 350°C black smokers are here transparent. Intrusion of magnesium indicates the degree of seawater mixing with the originally magnesium-free hydrothermal fluid. The calcium enrichment of hydrothermic fluid produces, at the point of mixing

Table 8.1 Chemical composition of hydrothermal emissions in the deep sea are compared with ambient sea water

Chemical	Unit of concentration	Guayamas Basin	21°N	Sea water
Fe	(μM)	56	1664	0.001
Mn	(μM)	139	960	0.001
Co	(nM)	5	213	0.03
Cu	(μM)	1	35	0.007
Zn	(μM)	4.2	106	0.01
Ag	(nM)	230	38	0.02
Pb	(nM)	265	308	0.01
Na	(mM)	489	432	464
K	(mM)	48.5	28	9.79
Li	(μM)	1054	891	26
Mg	(mM)	29	0	52.7
Ca	(μM)	29	15.6	10.2
Sr	(μM)	202	81	87
Ba	(μM)	12	7	0.14
Cl	(mM)	601	489	541
SO_4	(mM)	0	0	27.9
Si	(mM)	12.9	17.6	0.16
Al	(μM)	0.9	5.2	0.01
NH_3	(mM)	15.6	0	0
H_2S	(mM)	5.82	7.3	0
H_2	(mM)	8	50	0.001
CH_4	(mM)	1	1.6	0.001
pH		5.9	3.4	8.0
alk meq		10.6	−0.4	0.16

The samples were collected at vents in the Guaymas Basin (depth 2003 m) and the site at 21°N (depth 2610 m). (Data from Welhan and Craig, 1983; Edmond and Von Damm, 1985; Jannasch and Mottl, 1985).

with the sulfate-containing cold seawater, fast-growing cones of anhydrite (calcium sulfate) blackened by traces of metal sulfides. On cooling, anhydrite redissolves in seawater, a process that also applies to the above mentioned anhydrite constituent of hot vent chimneys (Edmond et al., 1982). The resulting porosity of the chimney walls becomes an important feature for the slow passage of sulfide-containing hydrothermal fluid and growth of microbial mats.

The amount of sulfide ions, as the most important electron donor for chemolithotrophy at deep sea vents, derives only in part from chemical reduction of the original sulfate content of the entrained seawater. Some of the geothermal sulfate reduction and metal sulfide deposition takes place during the downward movement of seawater into the earth's crust, and part of the sulfide content of the recirculating hydrothermal fluid stems from sulfur leaching of basalt at high temperature (Figure 8.1). The additional metal sulfide depositions from the ascending hydrothermal fluid in the Guaymas Basin sediments lowers the final sulfide concentration at this vent site.

At the same time, the organic constituents of these sediments, derived from the deposition of diatoms in the photosynthetically highly productive waters of the Gulf of California, provides the vent emissions of the Guaymas Basin site with a high ammonia content. This is, again, very different from the

"bare lava" vents of the 21°N site where the photosynthetic input is negligible and no ammonia is found in the hydrothermal fluid (Table 8.1).

The amounts of dissolved hydrogen and methane and other potential electron donors for chemosynthesis, are highly variable at every vent site studied so far and, therefore, undistinguishable for the two vent sites recorded in Table 8.1. Their background quantities in ambient seawater are equally nonconservative, i.e., subject to biological conversion. Methane, being organic, is not commonly considered a substrate for chemolithotrophy. Dealing with deep sea vent emissions, however, where the bulk of methane is of geothermic and not of biological origin (Welhan and Craig, 1983), one is tempted to treat it like an inorganic compound and a source of energy and carbon at the same time in a mixed chemolithomethylotrophy.

8.3 *IN SITU* CHEMOSYNTHETIC ACTIVITY

The first observations of dense bacterial suspensions in the plumes of "warm" vents (around 23°C) at the Galapagos Rift ocean spreading center (Corliss et al., 1979; Jannasch and Wirsen, 1979) provided evidence to support the assumed microbial base of the food chain leading to the rich animal communities. During the years following these initial observations, studies on chemolithotrophic *in situ* activities, physiology of microbial isolates, and measurement of the transfer of "chemosynthate" to the vent animals have been limited by the rare occasions to visit the deep sea sites by submersible vessels and by the relatively restricted operations that are possible on the deep sea floor.

Attempts to measure the total biomass of natural microbial populations have advanced during the last decade from plate counts and microscopic cell counts, using epifluorescence optics on acridine orange-stained cells, to ATP and total adenylate determinations. In addition, the ratio of GTP:ATP (GTP, guanosine 5'triphosphate) has been used as a determination of microbial growth activity at certain warm vent sites (Karl et al., 1980).

Such general growth or biomass assessments (Karl, 1980) do not estimate, however, the part played by chemolithotrophic organisms. Chemosynthesis was measured, therefore, in much the same manner that field studies of photosynthetic CO_2-fixation are conducted. Sets of six 120 ml syringes, precharged with ^{14}C-labeled bicarbonate, were filled (using the mechanical arm of the submersible) with the bacterial suspension from a warm vent (around 23°C). One set was incubated *in situ* in ambient water of temperature around 3°C; two other sets were incubated in the ship's laboratory at 3° and 23°C. Rates of CO_2 fixation of a typical experiment are presented in Figure 8.2. The hatched columns show data from syringes that were also supplemented with 1 mM thiosulfate. Placing a syringe holder within a vent plume for incubation at *in situ* temperature was not possible and may also be irrelevant in the absence of accurate temperature recording.

Three major characteristics of chemolithoautotrophic activity in warm vent emissions emerge from the data presented in Figure 8.2: 1) the effect of *in situ*

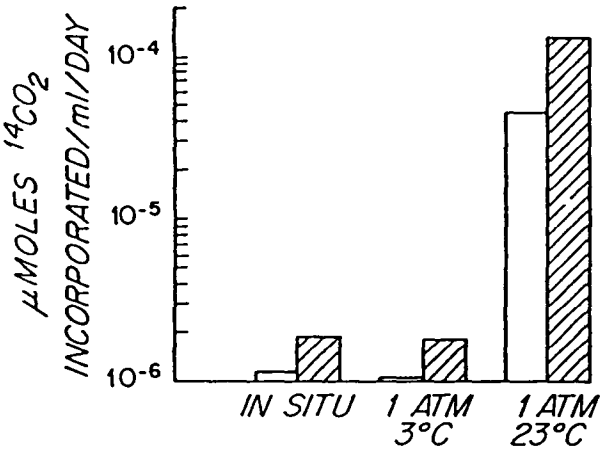

Figure 8.2 Rate of carbon dioxide incorporation by the natural population of bacteria suspended in "warm vent" water incubated *in situ* (260 atm, 3°C) and in the ship's laboratory at two temperatures. Hatched columns indicate a 1 mM thiosulfate supplement (from Jannasch, 1984).

pressure (around 250 atm) is negligible; 2) the response to temperature indicates that the organisms are mesophiles; 3) the addition of thiosulfate resulted in at least a doubling of the CO_2-fixation rate. A more detailed study (Tuttle et al., 1983; Wirsen et al., 1986) confirmed this general scheme. Experiments conducted at 1 atm under otherwise equal conditions resulted in similar data, i.e., within the range of error, as in the *in situ* experiments. The CO_2-fixation rate slowed abruptly when the samples were cooled from 23°C to ambient seawater temperature (2–3°C). In addition to thiosulfate, tetrathionate was also oxidized by the natural microbial warm vent populations. Experiments supplementing ammonia, nitrite, hydrogen, or methane have not been done. Only the latter two are present in the water issuing from bare lava vents. In separate experiments, uptake of the ^{14}C-label from acetate and glucose was found to be quite rapid (Tuttle et al., 1983; Wirsen et al., 1986).

One disturbing result of these studies was the early notion that the entire production of bacteria within a warm bare lava vent field would not be sufficient to support the growth of those massive invertebrate populations that are found outside of the areas where the bacterial cell suspensions occurred. The explanation emerged later when the assumption of a new type of endosymbiotic chemolithotrophy was substantiated (see below).

An unusual feature of the sediment-covered Guaymas Basin vent site is the massive occurrence of mats of essentially monocultures of *Beggiatoa* (Nelson et al., 1988). Chemoautotrophy was demonstrated in these mats by measuring ribulose bisphosphate carboxylase (RuBPC) and $^{14}CO_2$-uptake activities on freshly collected material; the results were similar to those obtained earlier with pure cultures of marine *Beggiatoa* (Nelson and Jannasch, 1983).

8.4 CHEMOLITHOTROPHIC ISOLATES

Isolates of thiobacilli-like organisms were readily obtained in large numbers. A representative list of strains in Table 8.2 is subdivided according to metabolic

characteristics such as acid formation as well as growth and CO_2 fixation in the presence and absence of an organic substrate (Ruby et al., 1981). The wide range of organisms from obligate chemolithotrophs, through the mixotrophs and facultative chemolithotrophs to the chemolithoheterotrophs—and certain true heterotrophs that are able to oxidize sulfur compounds with no measurable gain of energy—is not different from other marine habitats and from what is known of aerobic microbial sulfur oxidation in general (see Chapter 4, and Kuenen and Beudeker, 1982).

The acid-producing and slightly acidophilic obligate chemolithotrophs may only be able to grow competitively in dense mats on rock surfaces of subseafloor lava chambers. Many of the mixotrophs and facultative chemolithotrophs grow well at the pH of seawater (Figure 8.1) and may even raise the pH slightly by producing polythionates instead of sulfate. Their growth is favored by the occurrence of variable concentrations of dissolved organic compounds in the sulfide- and thiosulfate-containing vent water surrounding the rich animal communities. Gottschal et al. (1979) have emphasized the ecological significance of the metabolic flexibility of these facultative sulfide-oxidizing organisms from results of continuous culture studies on competitive population dynamics (discussed in detail in Chapter 4). This principle appears to be directly applicable to the microbial vent populations considering, of course, that no homogeneous mixing exists and that part of the various metabolic types are spatially separated. However, the result appears to be the same: the larger portion of the total population of aerobic sulfur-oxidizing bacteria consists of nonobligate chemolithotrophs.

Tests for low-temperature adaptation (the psychrophile character, shown by growth optima between 8° and 16°C) and high-temperature adaptation

Table 8.2 Growth of bacterial isolates (thiobacilli-like) from vent water samples on thiosulfate and yeast extract media[a]

		Growth[b]		$\mu g\ HCO_3^-$ fixed per 10^7 cells		
Strain	Final pH in T-ASW	Y-ASW	T-ASW	Y-ASW	T-ASW	TY-ASW
L-12	4.7	.7	67	IG[c]	3.1	3.2
RTPMB	4.7	.6	56	IG	2.7	3.1
TB-49	4.7	1.4	86	IG	6.1	3.7
SS-T	3.9	2.0	199	IG	3.1	3.6
NF-18	6.3	203	12	.1	2.5	.1
AG-33	6.5	218	7	.2	.6	.2
TB-66	5.7	106	4	.3	.6	.2
RTRG	6.2	ND[d]	6	.1	.7	.2
AG-25	8.4	351	0.4	0.1	IG	0.1
NF-13	8.8	115	.7	.2	IG	.2
TB5.5-9	8.7	245	.6	.1	.1	.1
TB-A	7.9	ND	ND	.1	.1	.1
K-12	7.8	ND	ND	.0	IG	.1

[a]T, thiosulfate (10 mM); ASW, artificial seawater; Y, yeast extract [0.01% wt (vol)]; initial pH was 7.0.
[b]Ratio of maximum cell density (as determined by epifluorescence direct counts) over that in the unsupplemented control
[c]IG, insufficient growth for determination
[d]ND, no data
Data from Ruby et al., 1981.

(thermophily, growth optima above 40°C) were negative: all isolates were mesophilic with optima between 28° and 35°C. Growth studies at various pressures relevant to deep-sea origin of the isolates revealed them to be barotolerant rather than barophilic, i.e., highest growth rates occurred at 1 atm while pressures of 200–300 atm lowered the rates no more than 20 percent. No growth occurred at pressures above 500–600 atm.

Two physiologically typical isolates, listed in Table 8.2, were studied in more detail: strains L-12 and NF-18. The obligately chemolithotrophic strain L-12 was found to belong to the genus *Thiomicrospira* (Ruby and Jannasch, 1982) and appeared not to be different enough from *T. pelophila* (Kuenen and Veldkamp, 1972) to create a new species. A typical microaerophile, it could be grown to unusually large colonies on agar plates when care was taken to reduce the oxygen concentration of the atmosphere. Also unusual was the high tolerance of this strain (*Thiomicrospira* L-12) for hydrogen sulfide (300 μM). Its growth response toward temperature and pressure fell within the ranges given above for natural populations of sulfur-oxidizing bacteria. During later studies, a *Thiomicrospira* strain was isolated from another vent site (21°N) that was indeed different from *T. pelophila*, mainly by its G+C mol percent content and its higher growth rate and optimal pH in the same thiosulfate medium. It was described as a new species, *T. crunogena* (Jannasch et al., 1985).

The second strain (NF-18 in Table 8.2) studied specifically represents a facultative or mixotrophic thiosulfate oxidizer. The stimulation of CO_2-fixation by thiosulfate is almost 10-fold (Figure 8.3) The addition of an organic substrate, such as glucose or yeast extract, eliminated or obscured any growth advantage due to thiosulfate oxidation (Ruby et al., 1981). By virtue of their catabolic flexibility and their ability to grow optimally at a large range of relatively high pH values (6.5–8.5), many organisms in this category are likely to constitute the major population of sulfur-oxidizing bacteria at the hydrothermal vents.

Attempts to isolate extremely thermoacidophilic, aerobic sulfur oxidizers of the *Sulfolobus*-type from the deep sea hydrothermal vents have so far been unsuccessful. It is quite possible that the gradient between the two zones, the hot anoxic hydrothermal fluid on the one side and the cold oxygenated mixture of hydrothermal fluid and ambient seawater on the other, is simply too steep to permit the establishment of a *Sulfolobus* population. Fluctuations at this interface may also be highly destructive to such populations. If such organisms exist, they do not seem to be extensive and might simply have escaped detection.

The first isolation of an anaerobic chemolithotroph (Jones et al., 1983) originated from the outside of a 21°N black smoker and turned out to be an extremely thermophilic methanogen. It grew optimally at a temperature of 86°C with a doubling time of 28 min, and was identified as a new species of the genus *Methanococcus*. Very similar organisms were isolated later from the hot Guaymas Basin sediments independently by W. J. Jones, Georgia Institute of Technology at Atlanta, and by Zhao et al. (1988). This extremely thermophilic methanogen appears to be a common constituent of the microbial hydrothermal vent population.

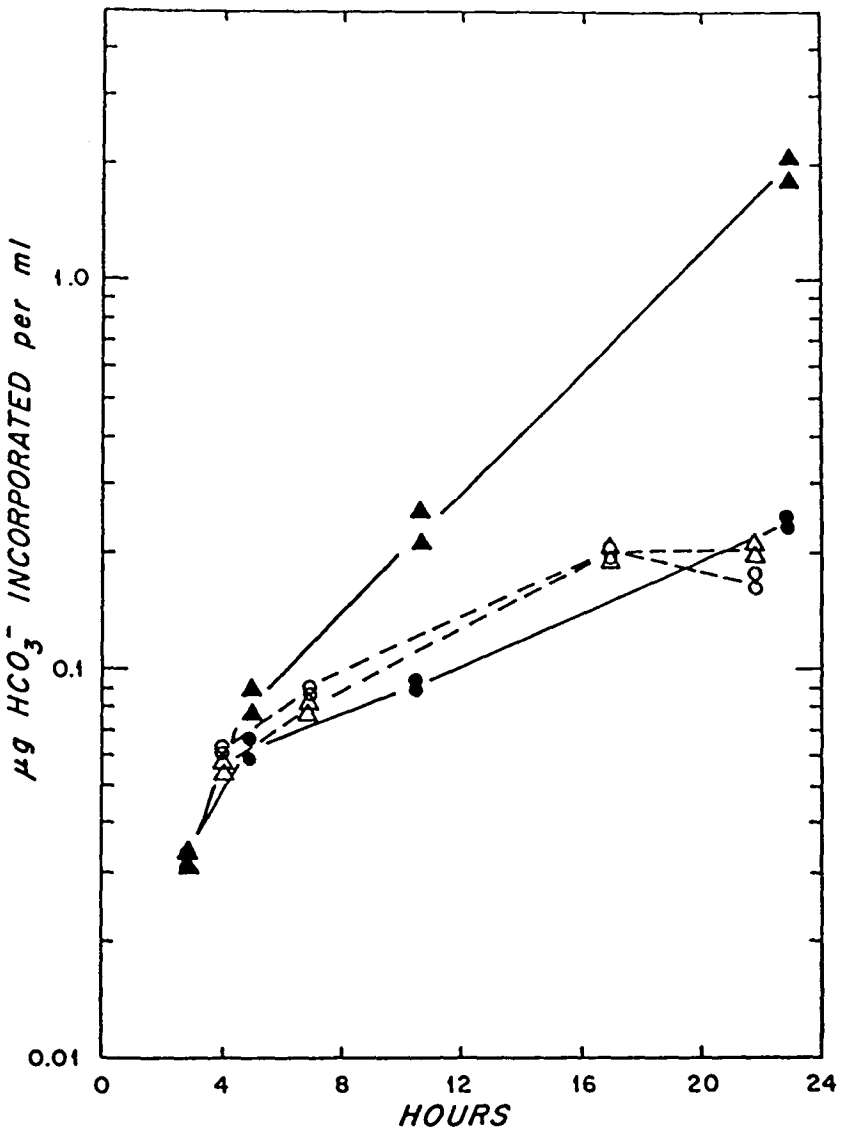

Figure 8.3 Incorporation of ^{14}C-labeled bicarbonate by the mixotrophic thiosulfate-oxidizer, strain N-18, in artificial seawater medium (ASW, dots) and in ASW plus 10 mM thiosulfate (filled triangles), as compared to the base-producing strain TB5.5-9 (see Table 8.2) in ASW (circles) and in ASW plus 10 mM thiosulfate (triangles) (from Ruby et al., 1981).

These archaebacteria were recently joined by another one: extremely thermophilic sulfate-reducing bacteria (Stetter et al., 1987). At least in its capability to use molecular hydrogen as a source of electrons, this organism might also be found as part of the lithotrophic microbial deep-sea vent population. So far the predominant group of extremely thermophilic organisms isolated from various vent sites is represented by fermentative and sulfur-reducing archaebacteria (Jannasch, 1988).

8.5 SYMBIOTIC CHEMOLITHOAUTOTROPHY

Three major species of invertebrates constitute the bulk of the rich animal populations observed in the immediate vicinity of the "bare lava" hydrothermal

vents: mussels (*Bathymodiolus thermophilus*, Kenk and Wilson, 1985), the "giant" white clams (*Calyptogena magnifica*, Boss and Turner, 1980, Figure 8.4), and the vestimentiferan tube worms (*Riftia pachyptila*, Jones, 1981, Figure 8.5). This is in contrast to the sediment-covered vents at certain ocean spreading or subduction zones (Lonsdale et al., 1980; Kulm et al., 1986) where the presence of photosynthetically provided organic substrates limit the significance of a primary chemosynthetic production of organic carbon and where a much larger variety of partly well-known deep sea invertebrates are observed.

Of the above three groups of animals, only some mussels occur within the dense microbial suspensions of the immediate warm vent plumes. The rest, as do most of the white clams and the vestimentiferan tube worms, occur outside the direct vent emissions in clear water where the concentration of particulate organic matter is much too low to serve as a food source. Subsequently it was found by electron microscopy and by enzymatic studies that the gill tissue of the bivalves contain procaryotic endosymbionts that are involved in the production of ATP through the oxidation of inorganic sulfur compounds and in the reduction of CO_2 to organic carbon (Cavanaugh et al., 1981; Felbeck, 1981). Specifically indicative of microbial metabolism is the presence of ribulose bisphosphate carboxylase (RuBPC; see Chapter 19) and adenosine 5'phosphosulfate reductase (APSR; see Chapter 14). While microbial symbionts may be absent from mussels or occur in variable quantity, the white clams appear to depend on their presence.

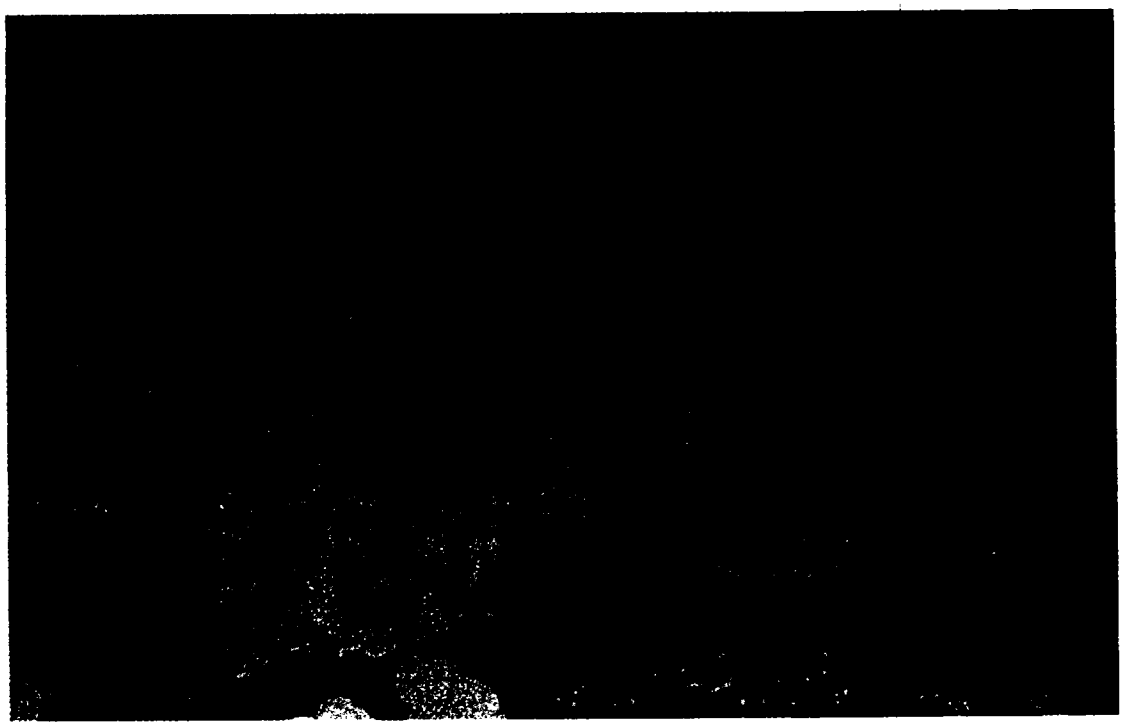

Figure 8.4 Populations of white clams (*Calyptogena magnifica*, Boss and Turner, 1980), 20–30 cm in length, located within cracks between boulders of "pillow lava" at the 21°N East Pacific Rise vent site (depth 2610 m, from H. W. Jannasch, 1984).

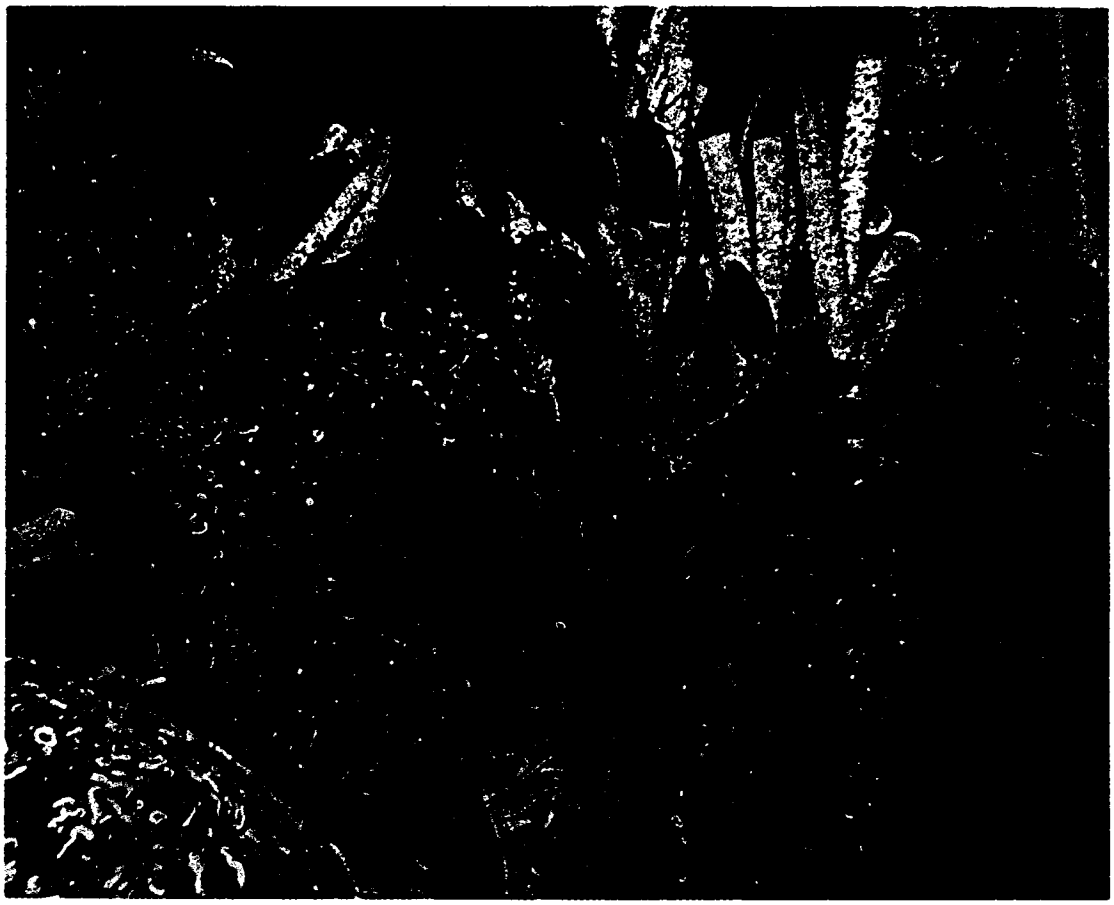

Figure 8.5 Populations of vestimentiferan tube worms (*Riftia pachyptila*, Jones, 1981), about 40 cm to 1 m in length, brachyuran crabs (*Bythograea thermydron*, Williams, 1980), limpets (Neomphalidae), and the small white housings of certain polychaetes (Serpulidae) attached to the rock surface. Also shown is one specimen of the so far undescribed "vent fish." Galapagos Rift vent site (depth 2550 m, photo J. M. Edmond).

Dependence on microbial symbionts is also true for the vestimentiferan tube worms (Figure 8.5) which reach lengths of more than 2 m and can occur at densities that appear to exceed the highest concentration of biomass per area known anywhere in the biosphere. The high biomass alone is astounding, especially considering that hydrogen sulfide, a potential metabolic poison, is serving as the major source of potential energy (electron donor). Detailed anatomical studies (Jones, 1981) revealed the absence of a mouth, stomach, and intestinal tract, in short: the absence of any ingestive and digestive system. Instead, the animals contain in their body cavity the so-called "trophosome" (Figure 8.6), a tissue consisting of coccoid procaryotic cells interspersed with the animals' blood vessels (Cavanaugh et al., 1981; Cavanaugh, 1983). A "chemoautotrophic potential" in these worms was described by Felback (1981) based on assays of enzymes catalyzing the synthesis of ATP via sulfur oxidation (rhodanese, APSR and ATP sulfurylase) as well as the Calvin cycle enzymes RuBPC and ribulose 5'phosphate kinase (APK) (Figure 8.7). ADP sulfurylase (ADPS) and phosphenolpyruvate carboxylase (PEPC) were also found (Felbeck,

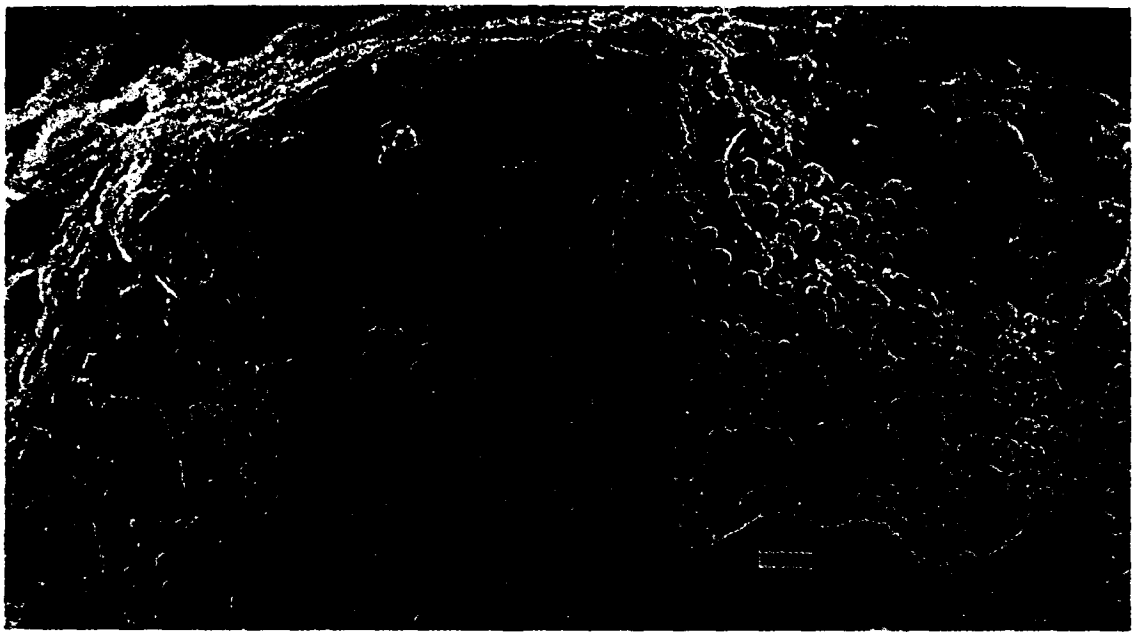

Figure 8.6 Trophosome "tissue" of *Riftia pachyptila* consisting of coccoid procaryotic cells, 3–5 μm in diameter, and blood vessels as eucaryotic animal tissue; pieces of elemental sulfur (bottom left center) form during contact with free oxygen (scale bar 10 μm, from Jannasch, 1984).

et al., 1981). None of these enzymes were detected within the worm tissue proper.

The trophosome may represent more than half of the wet weight of these worms. The necessary simultaneous transport of oxygen and hydrogen sulfide from the retractable plume of gill tissue (Figure 8.5) to the trophosome is carried out by an extracellular hemoglobin of the annelid-type blood system (Arp and Childress, 1981; Wittenberg et al., 1981; Terwilliger et al., 1983). Respiratory poisoning appears to be prevented by the presence of a sulfide-binding protein (Arp and Childress, 1983).

No chemolithotrophic symbiont has so far been cultured from any of the above-mentioned vent invertebrates. A study on concentrated and purified cell suspensions obtained from mussel gill tissue and from tube worm trophosomes by density centrifugation (Belkin et al., 1986) revealed that the symbionts of these two types of animals are distinctly different. While the symbionts of the mussel *Bathymodiolus thermophilus* use thiosulfate as the only electron donor and are clearly psychrophilic, i.e., they show an optimal CO_2-uptake activity at 16°C with a maximum just beyond 22°C (Figure 8.8), the trophosome symbionts of *Riftia pachyptila* oxidize hydrogen sulfide and are active at temperatures up to 35°C. In addition to differences in cell size, the mussel symbionts use free oxygen while the worm symbionts depend on its uptake from oxidized hemoglobin. From the analyses of 5S rRNA nucleotide sequences (Pace et al., 1985), and more recently from 16S rRNA sequences (D. L. Distel, personal communication), it became apparent that the procaryotic symbionts of all chem-

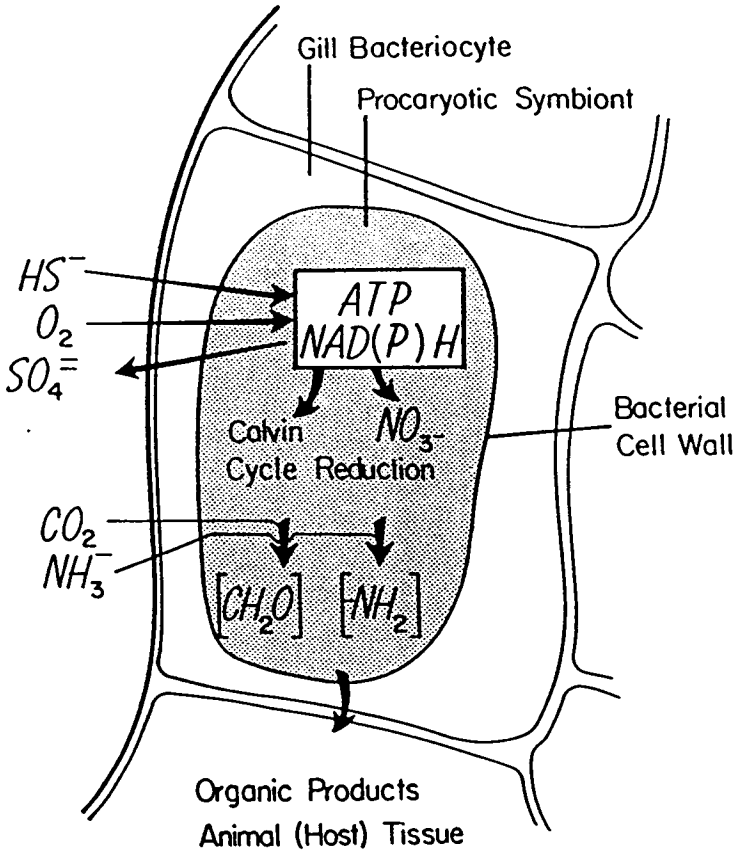

Figure 8.7 Scheme of metabolic processes in procaryotic symbionts within bacteriocytes in the gills of vent bivalves; the presence of the enzymes catalyzing ATP-production, the Calvin cycle, and nitrate reduction have been demonstrated (adapted from Felbeck et al., 1983).

olithoautotrophically existing marine invertebrates so far investigated belong in different groups.

8.6 TOTAL CHEMOSYNTHETIC PRODUCTION IN THE DEEP SEA

It has been a classical enigma for geochemists that the globe's heat flow balance could not be explained from conductivity calculations nor does the actual mineral composition of seawater come anywhere near the sum of weathering processes, river input, and dissolution/precipitation reactions (Edmond and Van Damm, 1983). These apparent anomalies can be resolved by the assumption that the total volume of the oceans' seawater (1.37×10^{21} liters) percolates through the earth's crust about once every 8 million years. While this hydrothermal cycling only amounts to about 1/200 of the oceans' river input, the mineral enrichment of the vent flow is 1000 times that of land runoff. These geochemical calculations lead to a total annual entrainment of 120 million tons of SO_4^{2-}-sulfur. Three-quarters of this amount is deposited as polymetal sul-

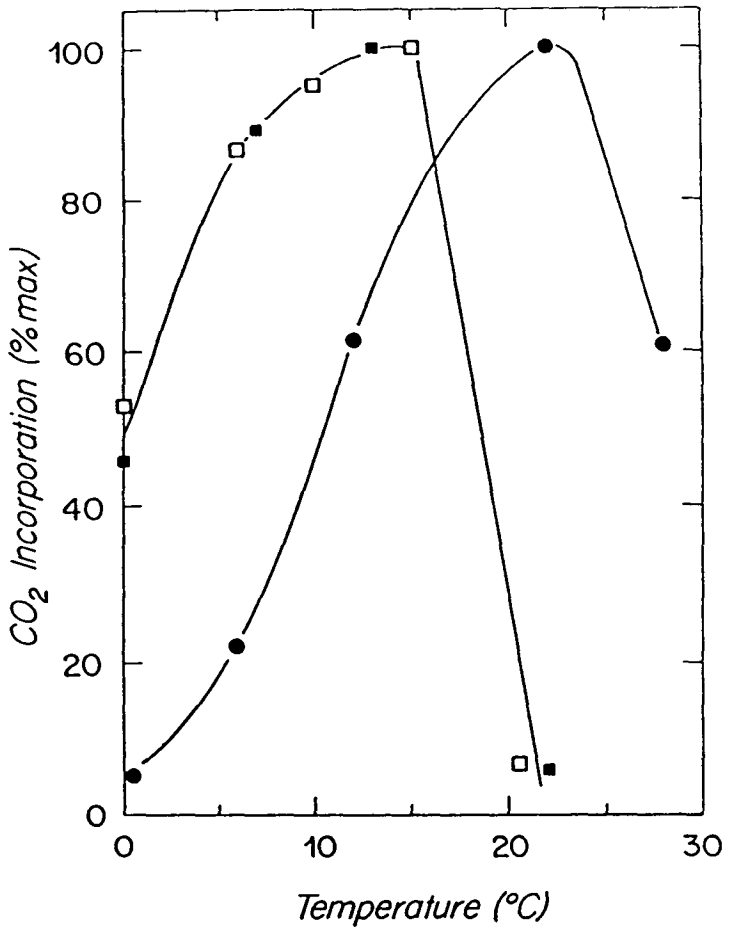

Figure 8.8 Incorporation of ^{14}C-labeled bicarbonate by homogenates of mussel (*Bathymodiolus thermophilus*) gill tissue and *Riftia pachyptila* trophosome at various temperatures. Open squares: gill homogenate incubated with 200 μM $Na_2S_2O_3$; filled squares: purified fraction of mussel gill homogenate incubated with 400 μM $Na_2S_2O_3$; dots: trophosome homogenate incubated with 600 μM Na_2S. Maximal incorporation rates in these three experiments were 6.0, 1.0, and 3.3 nmol CO_2 mg^{-1} protein h^{-1}, respectively (from Belkin et al., 1986).

fides on both sides of the spreading axis while the rest, 30 million tons of S^{2-}-sulfur, is emitted on the seafloor by hydrothermal venting (Edmond et al., 1982).

It is the general consensus of geochemists that most of the H_2S emission into oxygenated deep seawater takes place by diffusion through hydrothermal conduits or by slow mixing in warm vents. Only the smaller portion of hydrothermal fluid is emitted by the hot vents or black smokers, the chemosynthetic potential of the reduced inorganic compounds being lost by a quick dilution and subsequent slow chemical oxidation in the water column. On these grounds, it may be a generous but reasonable assumption that half of the available S^{2-}-sulfur at hydrothermal vents, i.e., 15 million tons, may be used for chemosynthesis. If the ensuing amount of organic carbon (in a 1:1 stoichiometric proportion of C:S) is compared to the annual photosynthetic production (global: 77.6 $\times$ 10^9 tons, oceanic: 18.7 $\times$ 10^9 tons, Woodwell et al.,

1978), it follows that the global chemosynthetic production, based on geothermally reduced sulfur, does not amount to more than 0.02 percent, and the oceanic production alone to about 0.1 percent of photosynthesis. However, this negligible proportion increases considerably, namely to 10 percent, if we consider the deep sea environment only where not more than about 1 percent of the surface-produced photosynthetic food materials arrive in the form of sedimenting particles.

Other reduced inorganic compounds in hydrothermal fluid are NH_3, H_2, CH_4, Fe^{2+}, and Mn^{2+} (Table 8.1). Their microbial oxidation has been demonstrated (Jannasch, 1985), but the largely qualitative data do not permit an estimation of their significance with respect to *in situ* chemosynthesis. The considerable concentrations of ammonia at the sediment-covered vents of the Guaymas Basin suggest a substantial rate of nitrification-based chemosynthesis which, however, has not yet been measured.

The concentrations of hydrogen and methane are highly variable in vent emissions, and their contributions to the *in situ* production of microbial cell material is equally difficult to determine as that of nitrification. Methylotrophy was first indicated by the characteristic morphology of methane-oxidizing bacteria occurring in microbial mats that cover surfaces within a vent plume (Jannasch and Wirsen, 1981). More recently, symbiotic methylotrophy has been suspected on the basis of ^{13}C-isotope depletion in the tissue of certain invertebrates (Kulm et al., 1986) and has been later demonstrated enzymatically (Childress et al., 1986; Schmaljohann and Flügel, 1987; Cavanaugh et al., 1987). In the latter case, however, the methane is not a product of geothermal activity but occurs in the neighborhood of bituminous deposits or results from the decomposition of organic matter.

Although the occurrence of extremely thermophilic methanogenic bacteria at hot vent environments (see above) suggests biogenic production of methane, studies on stable carbon isotope ratios indicate that most of the methane contained in the hydrothermal fluid is of geothermal origin (Welhan and Craig, 1983). This abiogenic origin may define it as an inorganic compound and, in turn, its microbial oxidation a chemolithotrophic gain of energy. The coupling to autotrophy is insignificant, however (Whittenbury and Dalton, 1981).

Microbial iron oxidation can neither occur at the high pH of oxygenated seawater nor in the acidic but oxygen-free hydrothermal fluid. It may occur at certain limited localities in acidified microbial mats (Jannasch and Wirsen, 1981). The successful isolation of manganese-oxidizing bacteria on the other hand, and observations of their *in vitro* activity render their *in situ* growth more likely (Ehrlich, 1983).

8.7 DISCUSSION

The term "primary production" can be used for the aerobic chemolithotrophic fixation of CO_2 in the deep sea, keeping in mind that free oxygen is a product

of photosynthesis (Jannasch and Wirsen, 1979). This qualification is not necessary in the case of anaerobic chemosynthesis. The same applies when contrasting the "terrestrial" (chemical) source of energy for the support of life at the deep sea vents with the "solar" energy for the support of life at the continental and sea surface (Figure 8.9). In this context the term "source of energy" is applied to an electron donor or reductant, taking into account that a suitable electron acceptor or oxidant is present for the liberation of the potential energy. The suitability of the electron donor/acceptor couple determines the amount of energy released.

Anaerobic chemosynthesis at the vents, e.g., methanogenesis, can truly be termed a primary production of organic carbon based on terrestrial energy because both the electron donor, H_2, and the electron acceptor, CO_2, are of geothermal origin. However, its actual contribution to organic carbon production might be small in comparison to the aerobic chemolithotrophic production in accordance with the yield of free energy of the oxidations involved:

$$H_2 + \tfrac{1}{4}CO_2 \rightarrow \tfrac{1}{4}CH_4 + \tfrac{1}{2}H_2O \qquad -8.3 \text{ kcal}$$

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \qquad -190.4 \text{ kcal}$$

Thermodynamically free oxygen plays the key role in the efficiency of chemosynthesis at the deep sea vents. It is derived from photosynthesis and is commonly present in deep seawater at half-air-saturation values. Its store in the world's ocean is enormous when compared to the amounts consumed by deep sea chemosynthesis. It has been theorized, therefore, that a catastrophic darkening of the earth's surface and the resulting temporary obstruction of

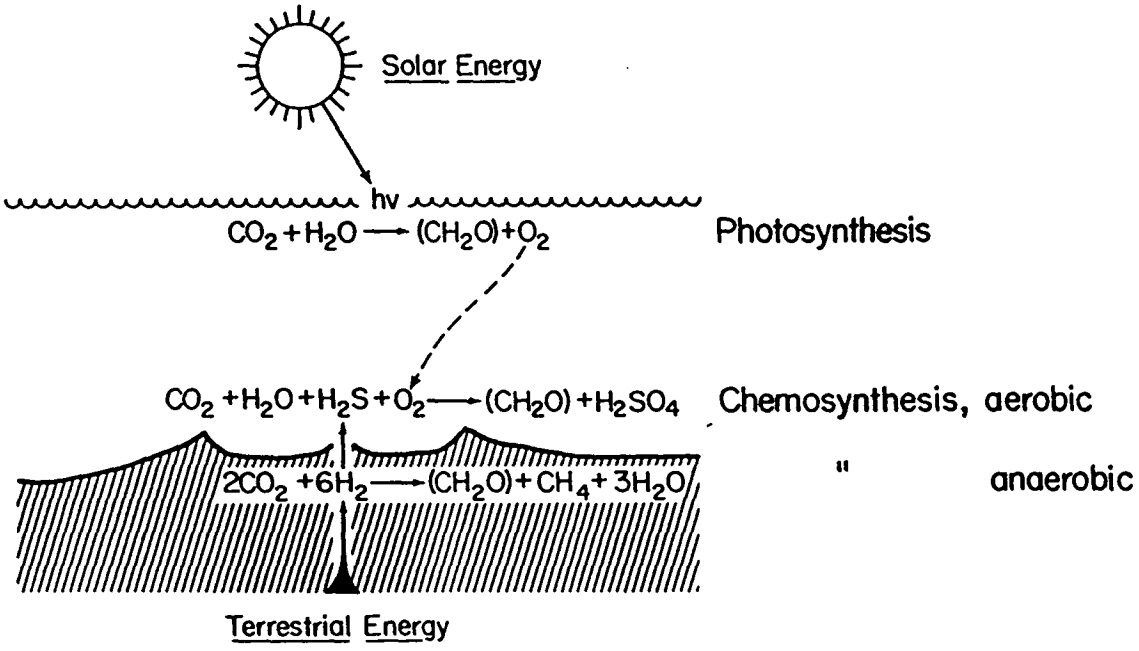

Figure 8.9 Scheme of energy supply for photo- and chemosynthesis and the role of free oxygen at deep sea hydrothermal vents.

photosynthesis would not necessarily affect the light-independent vent ecosystems and would hence give them highest survival chances (Jannasch and Mottl, 1985). Anaerobic chemosynthesis is, of course, entirely independent of the photosynthetically produced oxygen, but is limited to procaryotic ecosystems. It should be pointed out that the reaction between the two most prevalent constituents of hydrothermal fluid, HS^- and CO_2, is endergonic:

$$HS^- + 2HCO_3^- + H^+ \rightarrow 2\text{“}CH_2O\text{”} + SO_4^{2-} \qquad +4.6 \text{ kcal}$$

Any speculation or calculation of a production of free energy by this reaction at high temperatures and pressures rests on the rather independent problem of the existence and functioning of biological systems under these conditions (Jaenicke, 1987).

The predominantly symbiotic chemosynthesis, based upon the oxidations of H_2S as well as CH_4, has now also been found in anoxic marine sediments of nontectonic origin in shallow waters. The detour via the discovery of deep-sea vents was apparently necessary to detect this metabolic way of life in certain animals that have been known for a long time (Cavanaugh, 1983; Schmaljohann and Flügel, 1987; Southward, 1982; Giere et al., 1987). No symbiotic nitrification has yet been demonstrated.

The most surprising characteristic of the chemolithotrophic sustenance of the copious deep-sea communities is its efficiency. Considering the point source of the geothermally provided energy for bacterial growth in the normal food chain, filter feeding on bacterial cells from the quickly dispersing vent plumes appears highly wasteful. Evolution overcame this problem by transferring the chemosynthetic production of organic carbon to a site within the animal where the electron donor as well as the acceptor are made available with the aid of the respiratory system. This symbiotic association combines the metabolic versatility of the procaryotes with the genetic and differentiative capabilities of the eucaryotes and takes advantage of a most direct and efficient transfer of the chemosynthate to the animal tissue. These transfer processes and the biochemical interactions between the microbial and animal metabolisms are presently the focus of research in this area.

In 1965 the eminent ecologist G. E. Hutchinson, as cited by Grassle (1986), stated—with an admiringly intuitive foresight over a decade before the deep sea hydrothermal vent ecosystems were discovered: "The internal heat of a planet, mostly of radioactive origin, in theory would provide an alternative to incoming radiation though we have little precedent as to how an organism could use it."

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Chemoautotrophic and Methanotrophic Symbioses in Marine Invertebrates

Chemoautotrophic and Methanotrophic Symbioses in Marine Invertebrates*

Charles R. Fisher

I. INTRODUCTION

Chemolithoautotrophic bacteria are chemotrophic, they obtain energy from chemical sources; lithotrophic, their chemical energy source is inorganic; and autotrophic, their source of cellular carbon is inorganic. Strictly speaking methanotrophic and methylotrophic bacteria are not autotrophic because their cellular carbon source can be organic (a variety of reduced C-1 compounds) although they are often grouped with the chemolithoautotrophic bacteria. The chemolithoautotrophic (chemoautotrophic for short) and methanotrophic symbioses reviewed here are associations between bacterial symbionts and marine invertebrate hosts. It appears that the symbionts in all of the described chemoautotrophic associations use some form of reduced sulfur as an energy source, although there is no a priori reason not to suppose that other types of chemoautotrophic symbionts may yet be discovered (for example symbionts that use hydrogen, reduced iron or ammonia as an energy source are plausible). It must also be noted that since no confirmed, chemoautotrophic or methanotrophic symbionts have yet been cultured (although several methylotrophic and autotrophic bacteria have been isolated from high dilutions of homogenates of symbiont-containing invertebrate tissues),[1,2,3] details of their metabolic capabilities are not currently known and even the well studied symbionts may only be facultative chemoautotrophic sulfur oxidizers, with polytrophic capabilities.

When the hydrothermal vent communities were first discovered in 1977 biologists were astounded by the density of biomass associated with the active vents.[4] Although early investigators postulated that these communities might be nourished by high concentrations of surface-derived particulate matter entrained in the vent plume,[5] it soon became apparent that the source of the organic carbon in the vent animals was of local, chemosynthetic, origin.[6,7] Perhaps the most unique animal found in this ecosystem was the giant tube-worm, *Riftia pachytila*.[8] Its large size and gutless condition inspired investigators to look for non-conventional sources of nutrition for this animal. In 1981 Felbeck[9] reported on the presence of high activities of several enzymes of the Calvin-Benson cycle and bacterial sulfur metabolism in the trophosome of *R. pachyptila*, and Cavanaugh et al.[10] reported that this tissue was packed with large numbers of intracellular prokaryotic cells. These two papers represented the first demonstration of a chemoautotrophic bacterial symbiosis with a marine invertebrate.

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In the eight years since the first reports of chemoautotrophic symbioses, a large portion of the literature has been concerned with the demonstration of other chemoautotrophic and methanotrophic symbioses with marine invertebrates. Section II of this review critically evaluates the various types of evidence currently being used in the demonstration of chemoautotrophic symbioses with the aim of giving the reader the background necessary to separate the "wheat from the chaff" in this rapidly growing body of literature.

In Section III the marine invertebrate groups containing species that are symbiotic with chemoautotrophic bacteria are reviewed. In this section a representative selection of the literature on these animals is reviewed, although I have concentrated on ecological, morphological, ultrastructural, and physiological studies. Details of sulfur metabolism in these hosts and symbionts have recently been reviewed[11] and are only briefly covered here.

In 1986 the first documented methanotrophic symbiosis was reported,[12] although the existence of this type of symbiosis had been postulated virtually since the discovery of sulfur based chemoautotrophic symbiosis.[13] Literature on the three well-documented methylotrophic (and in two cases methanotrophic) symbioses are reviewed in Section IV. Readers interested in this type of symbiosis are also refered to Section III.E.1.e. for discussion of some of the other postulated methanotrophic symbioses.

As noted, it was the gutless condition of *Riftia pachyptila* that first caused investigators to look for the presence of symbiotic chemoautotrophs in this animal. The assumption made was that autotrophic symbionts would contribute to the hosts' nutritional carbon needs. Despite the fact that this assumption is basic to most models of the functioning of these symbioses, there is little literature that directly addresses this question. In the last section of this review, that literature, as well as other work (and lines of reasoning) that indirectly support nutritional ties between the host and symbionts are discussed.

II. DEMONSTRATION OF CHEMOAUTOTROPHIC SYMBIOSES

The first conclusive demonstration of a symbiosis between chemoautotrophic bacteria and an invertebrate host was in *Riftia pachyptila*, where Felbeck[9] found activities of a number of enzymes diagnostic of chemoautotrophic bacteria in the trophosome tissue of the giant tube-worm, and Cavanaugh et al.[10]

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demonstrated appreciable quantities of lipopolysaccharide (a compound characteristic of the outer cell wall of gram negative bacteria) and identified numerous gram negative bacteria in the same tissue. Since then a large portion of the literature on this subject has been concerned with demonstrating that similar associations exist in other animals. A variety of different types of evidence have been used to demonstrate the presence of chemoautotrophic symbionts in a given host. Each type of evidence can have its own pitfalls and so must be examined critically. Furthermore, no one type of evidence is in itself sufficient to demonstrate the existence of a chemoautotrophic symbiosis, and therefore the results of at least two approaches (and often more) must be reviewed before definitive conclusions can be reached.

A. Microscopy

The demonstration of the presence of bacteria in significant numbers, intimately associated with a particular host species, is essential to the demonstration of a chemoautotrophic symbiosis. Because of the relatively small size of most chemoautotrophic symbionts, truly convincing evidence (for the presence of prokaryotes) can only be found in electron micrographs, where the prokaryotic nature of the symbionts (including the gram negative cell wall) can be visualized. Once the symbionts have been seen and their location in the host tissue identified by electron microscopy the symbionts can sometimes be visualized by light microscopy, although examination of light micrographs alone can be misleading (i.e., Reference 14). A technique for visualizing chemoautotrophs in homogenates of host tissue using the light microscope has recently been described.[15] Although this technique may be useful for "field" screening of possible symbioses, it does not distinguish between bacteria contaminating the surface of a tissue and true symbionts. Demonstration of the presence of prokaryotic cells within a host's tissues is of course not alone sufficient to demonstrate a chemoautotrophic symbiosis since it does not address the nature of the bacteria.

A variety of other information can also be derived from the examination of electron micrographs, such as morphological indications of the taxonomic affinities of the symbionts,[12,16-19] specific location of the symbionts (see Section III), and sometimes information on the fate of the symbionts within a host cell (see Section V).

B. Enzyme Activities

Activities of a variety of enzymes have been used to implicate chemoautotrophic bacteria as the symbionts in some associations. Some of these enzymes are only found in autotrophic organisms and are therefore strong evidence for chemoautotrophy, once photoautotrophy has been ruled out. Others are also found in some heterotrophic organisms and are therefore indicative of chemoautotrophic bacteria only when found in abnormally high activities. The level of activity is especially relevant for any enzyme when there is a possibility of the material being contaminated by free living bacteria or algae. This possibility becomes almost a probability when examining bivalve gills because of the large microvillar and ciliated surface areas bathed by the ambient sea water. The problem is compounded by the fact that the reducing environments where chemoautotrophic symbioses are found are also well suited to free-living chemoautotrophic microrganisms. Thus, it is not only the presence of an enzyme activity that is of import, but also the level of activity of that enzyme.

1. Enzymes of the Calvin Cycle

Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBPC/O, EC 4.1.1.39) catalyses the first reaction in the Calvin-Benson cycle, the carboxylation of RuBP. It is only found in autotrophic organisms (chemoautotrophic bacteria and photoautotrophs), carbon monoxide-oxidizing bacteria, and at low activities in some methylotrophic bacteria.[20] Thus, when this enzyme is demonstrated at substantial activities in an organism from the deep-sea (where photoautotrophy can be ruled out) it is convincing evidence for chemoautotrophy. I emphasize substantial activities because of the potential for contaminating bacteria on the surface of an animal's tissues or within an animal's GI tract. An example of moderate RuBPC/O activities found in a symbiont-containing bivalve which are not to be attributable to the major symbiont is an undescribed species of mussel collected near hydrocarbon seeps on the Louisiana Slope.[21] The only intracellular symbiont seen in electron micrographs is a methanotroph and the methanotrophic nature of this symbiosis is well documented.[12,21] RuBPC/O activity was found in the gills of freshly collected specimens of this mussel but is apparently not attributable to the methanotrophic symbiont since the RuBPC/O activity does not persist when the mussel is maintained in captivity (even for only 48 h) although the methanotroph does.[21] Whether this is due to contaminating bacteria on the surface of the seep mussels' gills or a second symbiont is yet to be established, but it nonetheless demonstrates the danger of drawing conclusions from low or moderate enzyme activities. Another enzyme of the Calvin-Benson cycle, ribulose-5-phosphate kinase (EC 2.7.1.19), has also been demonstrated in the tissues of some chemoautotrophic symbioses[9,22] and is subject to the same considerations as RuBPC/O as a diagnostic tool.

Cavanaugh et al.[23] have recently used immunological methods to demonstrate that the enzyme RuBPC/O is localized inside the symbionts in the gills of the bivalve *Solemya velum*. This study provides some of the most definitive evidence to date for a symbioses with chemoautotrophic bacteria, because it combines evidence for the presence of a diagnostic enzyme with the demonstration of its localization in prokaryotic symbionts. Immunological and molecular methods will undoubtedly prove useful in the demonstration, and the study, of these associations.

2. Enzymes of Sulfur Metabolism

The source of electrons used to fuel chemoautotrophy and therefore, the type of bacterial symbiont can often also be inferred from enzyme activities. The enzyme adenosine-5'-phosphosulphate reductase (APS reductase, EC 1.8.99.2) catalyzes the reaction of AMP and sulfite to produce adenosine phosphosulfate in some autotrophic sulfur bacteria,[24,25] and can catalyze the reverse reaction in some sulfate reducing bacteria.[26] Activity of this enzyme has not been demonstrated in any other types of organisms and is therefore diagnostic for the presence of sulfur bacteria. Unfortunately APS reductase has only been demonstrated in two species of free-living sulfur-oxidizing chemoautotrophs and sulfite oxidation is catalyzed by other enzymes in other chemoautotrophs.[27] Therefore its absence is not evidence against chemoautotrophy, and in fact it has only rarely been demonstrated in chemoautotrophic symbioses. However, when it is found at appreciable activities along with RuBPC/O, it is strong evidence for a sulfur based chemoautotrophic symbiosis.

ATP sulfurylase (EC 2.7.7.4) catalyzes the reversible reaction of adenosine-phosphosulfate and pyrophosphate to yield ATP and sulfate. ATP sulfurylase is found in a variety of organisms[28,29] but has only been found in high activities in chemoautotrophic sulfur bacteria[30] and chemoautotrophic symbioses. Appreciable activities of this enzyme have been demonstrated in symbiont containing tissues of all chemoautotrophic symbioses tested.[21,22,31-35] In studies with paired symbiont-containing and symbiont-free tissues from the same animals, ATP sulfurylase has only been detected in the symbiont containing tissues.[31,36] No activity of this enzyme was found in the gills of the methanotrophic symbiont-containing hydrocarbon seep mussel even though it is present in the closely related hydrothermal vent mussel, *Bathymodiolus thermophilus*.[21] The enzyme is quite stable and activity does not decrease even after several years at −70°C.[21] The assay is a straightforward, spectrophotometric assay with a large extinction coefficient and therefore a strong signal.[9] Therefore, even though this enzyme is not diagnostic for chemoautotrophic bacteria, it has proven to be a good indicator for chemoautotrophic symbioses.

Sulfate adenylyl transferase (ADP) (EC 2.7.7.5) catalyzes basically the same reaction as ATP sulfurylase but yields ADP instead of ATP. It is subject to the same considerations as ATP sulfurylase as a diagnostic tool for chemoautotrophic symbioses. Although Felbeck was unable to demonstrate activity of this enzyme in trophosome of *R. pachypitila*[9] or in the gills of *Calyptogena magnifica*, *Bathymodiolus thermophilus* and *Solemya reidi* (pers. comm. H. Felbeck, Scripps Institute of Oceanography) other workers have demonstrated activity of this enzyme in the gills of some lucinid and thyasirid bivalves and in smaller pogonophorans.[32,33,37] However, these same investigators[37] found low activities of this enzyme in symbiont-free tissues of one of the same bivalves, which compromises the usefulness of this enzyme as a diagnostic tool.

Rhodanese (EC 2.8.8.1) has been proposed to catalyze the cleaving of thiosulfate into sulfite and sulfur in thiobacillus.[27] It can also catalyze other reactions and is found in a variety of other organisms at appreciable activities.[38] Rhodanese is therefore of questional significance as an indicator of chemoautotrophic symbionts. In fact Cavanaugh[36] found similar levels of activity of this enzyme in foot tissue (without symbionts) and gill tissue (with chemoautotrophic symbionts) of the bivalve *Solemya velum*.

Sulfide oxidase catalyzes the first step in the oxidation of hydrogen sulfide in a variety of animals. Oxidation of sulfide can also proceed non-enzymatically when free oxygen is present. Sulfide oxidase has been demonstrated in both symbiont-containing and symbiont-free tissues of several species of animals with chemoautotrophic symbionts as well as in the tissues of some animals from sulfide rich environments which lack symbionts.[11,34,39-41] The role of sulfide oxidation in animals from reducing environments has recently been extensively reviewed,[11] and is only briefly reviewed here. In animal tissues (without symbionts) it has been proposed to serve a protective function, by oxidizing toxic sulfide species to thiosulfate.[11,40,41] In animals with symbionts its role appears much more complex. In some chemoautotrophic symbioses it is found in the symbionts as one would expect.[40] Sulfide oxidation enzymes located on the peripheral tissues of symbiotic species from high sulfide environments appear to serve the same type of protective function as similar enzymes in nonsymbiotic species.[40] However the recent demonstration of sulfide oxidation coupled to ATP production in mitochondria from *Solemya reidi* indicates that animal tissues may derive energetic benefits from this oxidation as well.[42] Furthermore, carbon fixation by the symbionts of two *Solemya* species and *Bathymodiolus thermophilus* is stimulated by thiosulfate (and not sulfide in the case of *B. thermophilus*), and relatively high concentrations of thiosulfate have been demonstrated in the blood of two species.[36,43-45] Taken together these data support the possibility that thiosulfate is produced in the animal tissues of these symbioses (via sulfide oxidase) and transported in this nontoxic form to the symbionts where it serves as electron donor, fueling chemoautotrophic carbon fixation. In conclusion, sulfide oxidase activity is a good indicator of an animal's exposure to a reducing environment containing elevated sulfide levels, but is not a reliable indicator of chemoautotrophic symbioses.

3. Enzymes of Inorganic Nitrogen Metabolism

The ability to assimilate inorganic nitrogen sources such as nitrite or nitrate is restricted to autotrophic organisms.[46] Nitrate reductase (EC 1.6.6.1) catalyzes the reduction of nitrate to nitrite and nitrite reductase (EC 1.7.7.1) catalyzes the formation of ammonia from nitrite. Activity of either of these enzymes is indicative of the presence of either chemoautotrophic or photoautotrophic organisms. Therefore, like the enzymes of the Calvin-Benson cycle, significant activity in habitats

where photoautotrophy can be ruled out is a strong indicator of chemoautotrophy. If this activity is in a tissue where the possibility of contamination by free-living autotrophs is negligible then this is strong evidence for the presence of symbionts. Activity of both of these enzymes of nitrogen metabolism has been demonstrated in the tissues of some animals with chemoautotrophic symbionts.[9,22,47]

The ability to fix molecular nitrogen is limited to prokaryotic organisms. The enzyme responsible for nitrogen fixation, nitrogenase (EC 1.18.6.1) has not yet been demonstrated in any chemoautotrophic symbiosis. However, the negative $\delta^{15}N$ values found in some chemoautotrophic and methanotrophic symbioses are suggestive of molecular nitrogen as the nitrogen source for these symbioses (see Section II.C.3.), and work is currently underway in several laboratories investigating this possibility. Demonstration of nitrogenase activity would be strong evidence of a symbiosis with nitrogen-fixing prokaryotes.

C. Lipopolysaccharide

Lipopolysaccharide (LPS) is a component of the outer cell wall of gram negative bacteria and occurs naturally only in these bacteria.[48] The presence of appreciable levels of this compound in the tissues of a marine invertebrate is therefore good evidence for the occurrence of symbiotic gram negative bacteria. Assaying for LPS with the *Limulus* amoebocyte lysate test[48,49] is a straight forward spectrophotometric procedure, which was used by Cavanaugh et al.[10] in their original demonstration of prokaryotic symbionts in *Riftia pachyptila*. It should be remembered that this test only demonstrates the presence of gram negative bacteria and does not address their nature (chemoautotrophic or otherwise), therefore additional tests such as enzyme assays are necessary before one can conclude that the bacteria are in fact chemoautotrophic. Furthermore, the amount of LPS detected in association with a specific tissue should also be noted (especially in the case of tissues such as gills which are in constant contact with the external environment) so that one can distinguish between large numbers of bacteria such as one finds in chemoautotrophic symbioses, and activity due to contamination of the surface of a tissue. Since most marine bacteria, and all chemoautotrophic symbionts so far investigated appear to be gram negative, and since LPS accounts for a relatively constant proportion of gram negative bacterial cell walls, this assay has potential not only in the demonstration of symbioses, but also for quantitation of symbiont biomass. In fact, recent work has demonstrated that this assay is proving quite versatile in that regard with a number of different types of marine invertebrate-bacterial symbioses (personal communication C. Cary, Scripps Institute of Oceanography).

D. Stable Isotope Ratios

1. Carbon

The stable carbon isotope composition ($\delta^{13}C‰$) of animal tissues has often been used to examine trophic relationships between organisms since the isotopic fractionation of carbon between an animal and its food source is relatively small, usually about $-1‰$.[50-52] Chemoautotrophic symbioses are well suited for investigations of this sort because these symbioses utilize novel sources of carbon (for metazoans), with distinctive stable carbon isotope composition. Stable carbon isotope ratios have been used to suggest whether a marine animal's carbon source is photoautotrophic (algae usually range from -12 to $-24‰$ with exceptions as high as $-6‰$),[53,54] chemoautotrophic ($\delta^{13}C = -23$ to $-47‰$),[6,7,22,34,55-57] or methanotrophic ($\delta^{13}C < -40‰$),[34,58-61] although this distinction does not hold for an organism utilizing a very heavy source of thermogenic methane, or a light source of carbon dioxide. Stable carbon isotope values have also been used as evidence of carbon transfer between symbiont and host (see Section V). This tool has already provided a wealth of information on chemoautotrophic and methanotrophic symbioses, but care must be taken in the interpretation of these data because many factors affect the stable isotope composition of animal tissues, particularly in autotrophic symbioses.

The stable carbon isotope composition of a sample is usually expressed relative to the PeeDee belemnite (PDB) standard[62] where

$$\delta^{13}C = \{[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}] - 1\} \times 10^3 \ (‰)$$

Thus a negative number reflects a sample enriched in ^{12}C relative to the PDB standard and a positive number, a sample enriched in ^{13}C relative to the standard.

The reason that $\delta^{13}C$ measurements have proven to be useful in the study of chemoautotrophic symbioses is because the symbionts preferentially incorporate isotopically light carbon during chemoautotrophic growth. This is due mainly to significant fractionation of stable carbon isotopes by sulfur-oxidizing chemoautotrophic bacteria during the fixation of inorganic carbon into cellular organic carbon. Ruby et al.[56] have recently reported large stable carbon isotope fractionation by cultures of two species of sulfur-oxidizing bacteria during chemoautotrophic growth. In their experiments the $\delta^{13}C$ of the cellular carbon in a strain of *Thiomicrospira* isolated from the Galapagos hydrothermal vents and *Thiobacillus neapolitanus* was depleted by 24.6 and 25.1‰, respectively, from their inorganic carbon source. This is the only direct measure of carbon isotope fractionation during chemoautotrophic growth by sulfur-oxidizing bacteria although there is some evidence that other chemoautotrophic bacteria may fractionate carbon by as much as 30‰ or more.[63] Further studies on isotopic fractionation by free-living chemoautotrophs are needed and will contribute to our understanding of the phenomenon in chemoautotrophic symbioses.

Although stable isotope data are very useful in the study of chemoautotrophic symbioses, there are a number of consid-

erations which should be kept in mind when evaluating this type of data. These considerations fall into three basic categories, which are interrelated: the carbon source $\delta^{13}C$, sources of isotopic fractionation, and carbon limitation.

The $\delta^{13}C$ of total dissolved inorganic carbon (ΣCO_2) in sea water is typically around 0‰ but varies slightly in different waters and in the same bodies of water at different times.[64] Furthermore, there are quite significant differences in the $\delta^{13}C$ of ΣCO_2 in the pore water of marine sediments. Spiro et al.[55] measured the $\delta^{13}C$ of pore water ΣCO_2 in the sediments of the Bay of Biscay (1700 m) and found it to be -17.8 ± 3.3‰. Presley and Kaplan[65] report similar values for the ΣCO_2 of near-shore sulfate-reducing sediments off California. It is probable that similar depleted values will be found in pore waters of the sediments in many of the environments where chemoautotrophic symbioses occur, particularly when those pore waters contain dissolved methane (see Section III.E.1.e.). This variation of $\delta^{13}C$ of inorganic carbon in different environments will be reflected in the $\delta^{13}C$ of the tissues of chemoautotrophic organisms and symbioses from these environments.

In order to determine the isotopic fractionation between an inorganic carbon source and the organic carbon of an animal's tissue it is necessary to know what the inorganic carbon source is (CO_2 or HCO_3^-) as well as the $\delta^{13}C$ of that inorganic carbon source. The $\delta^{13}C$ values of the two probable inorganic carbon sources for chemoautotrophy, CO_2 and HCO_3^-, are not equal in a given body of water. This variation ranges from 9.2 to 6.8‰ over the temperature range of 0 to 30°C, with CO_2 being the more negative, due to discrimination during equilibration between CO_2 and HCO_3^-.[66] The species of inorganic carbon taken up by invertebrates with chemoautotrophic symbionts has not been determined and may well vary in different species. It is well documented that CO_2 is the species fixed by RuBPC/O in the Calvin cycle, but as that enzyme is found within the cytoplasm of a bacterial cell (which in the case of most of the symbioses discussed here is located inside a vacuole within a host cell) the inorganic carbon must first be taken up through at least two membranes. If CO_2 is the species taken up from sea water then diffusion across the unstirred layer of water immediately adjacent to the site of uptake would play a role in discrimination against ^{13}C, which could be as great as -11‰ (see Smith and Walker[54] for an extensive treatment of this subject). Furthermore local depletion of CO_2 would affect the isotopic equilibrium between CO_2 and HCO_3^-.[54] If HCO_3^- is the species used then diffusive limitation might still affect the isotopic composition of the bicarbonate reaching the site of uptake where one would expect a further discrimination since this charged species would presumably be actively transported across the host cell membrane.[47] This effect is compounded in symbiotic associations where the inorganic carbon must often pass through several membranes and diffuse through appreciable cytoplasm before reaching the site of fixation. Therefore, even if the carbon species taken up by the association, and the $\delta^{13}C$ of that species is known, there are other intermediate steps during incorporation of the inorganic carbon that can affect the final $\delta^{13}C$ of the animal's tissues.

Once the inorganic carbon reaches the site of fixation there are additional factors which will affect the level of discrimination at this key point. Different carbon fixing enzymes discriminate to different degrees, and the primary carbon fixing enzyme must be known for adequate interpretation of isotopic data. It has been suggested that the initial carbon fixation step is not by RuBPC/O in both *Riftia pachyptila* and *Lucinoma aequizonata*, but rather that inorganic carbon is incorporated by the animal (not the symbionts) into four carbon compounds which are ultimately decarboxylated and the CO_2 refixed by the symbionts through the Calvin cycle in a manner analagous to C-4 plants.[67,68]

Temperature can also affect isotopic discrimination at the level of carbon fixation. Higher temperatures reduce the discrimination of RuBPC/O against ^{13}C.[69,70] This point should be considered when comparing animals from different habitats, especially when hydrothermal vent organisms are compared to their other deep-water relatives.

Enzymatic isotopic discrimination is also directly affected by substrate availability. In fact discrimination will only occur if a substrate is not limiting, because under conditions of substrate limitation the majority of that substrate will be consumed (subject of course to the K_m of the enzyme for that substrate). This has been confirmed for RuBPC/O and phosphoenol-pyruvate carboxylase (PEP carboxylase) by Berry and Troughton[71] who found no isotopic descrimination by either C-3 or C-4 plants when grown under conditions of carbon limitation (in closed containers).

One result of all of the above variables is that similar $\delta^{13}C$ values can be found in different species due to dissimilar isotopic discrimination events. One example of this is the two very different symbioses, the alvinellid polychaete *Alvinella pompejana* ($\delta^{13}C = -9.6$ to -11.2‰) and *Riftia pachyptila* ($\delta^{13}C = -9.0$ to -13.3‰), both from hydrothermal vents.[72-74] The relatively heavy values for *Riftia pachyptila* have been attributed to substrate limitation of the intracellular symbionts for inorganic carbon,[73] a situation which is unlikely for the epibiotic symbionts of the alvenellids. Another example is certainly the methanotrophic mytilid from the Louisiana Slope ($\delta^{13}C = -40.1$ to -57.6‰),[34] the pogonophores, *Siboglinum atlanticum* and *S. ekmani*, from Norwegian Fjords and the Bay of Biscay ($\delta^{13}C = -44.6$ to -45.8‰),[55] and the vestimentiferan with chemoautotrophic symbionts, *Escarpia laminata*, from the Florida Escarpment site ($\delta^{13}C = -42$ to -47‰).[57,75]

Another result of the many factors affecting the stable isotopic composition of an animal is that one can expect to find a range of $\delta^{13}C$ values in similar or identical chemoautotrophic symbioses from different environments. A dramatic example of this are the $\delta^{13}C$ values reported for vestimentiferans (with chemoautotrophic sulfide oxidizing symbionts) from different

environments. Hydrothermal vent vestimentiferan $\delta^{13}C$ values range from -9.0 to $-15.0‰$[6,34,74,76] and the $\delta^{13}C$ values reported for vestimentiferans from the Louisiana Slope and the Florida Escarpment are all less than $-27‰$.[34,75] In fact, with the organisms from the Louisiana slope, where the most $\delta^{13}C$ determinations have been made, there is a relatively wide range of $\delta^{13}C$ values within a given species. $\delta^{13}C$ of soft tissues (not tubes or shells) of the lucinid *Pseudomiltha* sp. ranged from -30.9 to $-37.7‰$, of the methanotrophic mussel from -40.1 to -57.6, and of the vestimentiferan *Lamellibrachia sp.* from -27.0 to $-43.2‰$.[34] This variation in $\delta^{13}C$ within a species may reflect a larger habitat diversity (with respect to both sulfide concentrations and the $\delta^{13}C$ of inorganic carbon) at the Louisiana Slope than in other habitats where these symbioses are found, but is also a reflection of the larger number of $\delta^{13}C$ determinations from this site.

Yet another possible pitfall in the use of isotopic evidence for the demonstration of a chemoautotrophic symbiosis is that an animal which feeds on a chemosynthetic organic carbon source will have a tissue $\delta^{13}C$ which reflects that carbon source, even though that animal is not itself chemoautotrophic. One example is the polynoid scale worm, *Branchipolynoe symmytilida*, which is commensal with the hydrothermal vent mussel, *Bathymodiolus thermophilus*. The polychaete apparently does not contain symbiotic chemoautotrophs but its tissue $\delta^{13}C$ (-33.0 to $-37.8‰$) reflects the $\delta^{13}C$ of its host mussel (-32.4 to $-36.0‰$).[45] Other apparent examples of this are the predatory neogastropod from the Louisiana Slope, with tissue $\delta^{13}C$ as low as $-32.8‰$ in individuals collected from the vicinity of active seeps,[77] and a trochid gastropod from the Florida Escarpment site ($\delta^{13}C = -59.7‰$).[75]

In conclusion, evidence based on stable carbon isotopic composition while suggestive, is by itself insufficient for the demonstration of a chemoautotrophic symbiosis, and all possible sources of fractionation must be considered when making physiological and biochemical conclusions or comparisons based on this type of data alone.

2. Nitrogen Isotopes

Like carbon, stable nitrogen isotope ratios ($\delta^{15}N‰$) have been used to indicate sources of nitrogen and trophic relationships between organisms. The stable nitrogen isotope composition of a sample is conventionally expressed relative to air, where

$$\delta^{15}N = \{[(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{air}] - 1\} \times 10^3 (‰)$$

Typical marine values in biological samples range from 5 to 15‰. Substantially lower $\delta^{15}N$ values have been reported for animals with chemoautotrophic and methanotrophic symbionts. Reported $\delta^{15}N$ values for the hydrothermal-vent species *Riftia pachyptila*, *Calyptogena magnifica*, and *Bathymodiolus thermophilus* are 0.04 to 5.2‰, 2.1 to 4.9‰, and -8.7 to $+6.8‰$, respectively.[45,74,78,79] The $\delta^{15}N$ values of chemosynthetic and methanotrophic symbioses from the Florida escarpment range from -9.34 to $-2.72‰$[75] and similar animals from the Louisiana Slope are also quite depleted in ^{15}N (-12.9 to $7.1‰$).[34] These low $\delta^{15}N$ values are possibly due to assimilation of isotopically light nitrate or ammonium by the symbionts or, in the case of the very negative values, perhaps fixation of N_2;[34,45,74,79,81] however, N_2 fixation by a chemoautotrophic or methanotrophic symbiont has not yet been demonstrated. Whatever the source of the low $\delta^{15}N$ values, the correlation of these values with chemoautotrophic and methanotrophic symbioses indicates that stable nitrogen isotopes may also be useful indicators of these symbioses, subject to many of the same types of constraints outlined earlier for carbon isotopes.

3. Stable Sulfur Isotopes

The isotopic sulfur composition ($\delta^{34}S$) of reduced sulfur in an animal's tissues is related to the source of the sulfur. The stable sulfur isotope composition of a sample is conventionally expressed relative to sulfur in Canyon Diable troilite[82] (ctd standard), where

$$\delta^{34}S = \{[(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{ctd\ standard}] - 1\} \times 10^3 (‰)$$

Organic sulfur derived from assimilatory reduction of sea-water sulfate reflects the $\delta^{34}S$ of the sulfate, as there is little discrimination associated with this process.[83,84] Similarly, there is apparently little discrimination of sulfur isotopes associated with heterotrophic processes.[85,86] The largest reported biological isotopic discrimination is associated with microbial reduction of sulfate to sulfide and can be on the order of $-50‰$, although the magnitude of discrimination associated with this process is affected by a number of variables (see Chambers and Trudinger[84] for review). On the other hand there is very little fractionation associated with abiotic sulfide production (thermal decomposition of organic matter). The $\delta^{34}S$ of sulfide is therefore quite variable in the marine environment. Since the $\delta^{34}S$ of the tissues of animals with chemoautotrophic symbionts seems to reflect the $\delta^{34}S$ of their sulfide source,[85,86] this measurement can be used to infer the origin of the sulfide oxidized by the symbionts.[34,57] This observation is especially clear cut when the $\delta^{34}S$ of the elemental sulfur (derived solely from symbiont sulfide oxidation) in the animals tissue is analyzed.[86] However, animals with symbionts are not the only animals that can oxidize sulfide[11] and animals without symbionts from high sulfide environments may also have $\delta^{34}S$ values that reflect the $\delta^{34}S$ of sulfide from that environment.[86,87] Therefore, because of the variability of the end members in the marine environment,

and the fact that animals without symbionts can also oxidize sulfide, $\delta^{34}S$ values of animal tissues are of little use in the demonstration of chemoautotrophic symbioses.

E. Elemental Sulfur

The presence of elemental sulfur has been demonstrated either analytically or inferred visually for a large number of animals which contain chemoautotrophic symbionts.[11,32,34,37,44,74,79,88,89] Elemental sulfur has also been found associated with a variety of free-living sulfide oxidizing chemoautotrophic bacteria.[90-92] It has been suggested that elemental sulfur may serve as a reserve energy source for the symbionts in some chemoautotrophic symbioses.[88] Regardless of whether elemental sulfur serves an energy storage function, is a result of sulfide detoxification, or is a byproduct of sulfide oxidation by the symbionts, its presence has been demonstrated or inferred in so many associations that it must be considered a good indicator for chemoautotrophic symbioses. However, the presence of elemental sulfur is not sufficient for the demonstration of a chemoautotrophic symbioses because it has also been found in the body wall of some "thiobiotic" worms.[93] The absence of elemental sulfur should not be considered negative evidence for a chemoautotrophic symbiosis because its presence is variable and dependent on the physiological state of symbionts.[11,34,37,74,79,88,91,94,95]

F. Animal and Tissue Physiology

As investigations of these symbioses progress, physiological studies are providing some of the most conclusive demonstrations of functioning chemoautotrophic symbioses. Examples of this type of work are experiments that demonstrate stimulation of carbon fixation, in live animals or tissues, by a reduced sulfur compound,[43,96,97] experiments which demonstrate net uptake of inorganic carbon in the presence of reduced sulfur compounds,[44] and experiments which demonstrate growth of symbiont-containing animals provided with only bacterial carbon and energy sources.[98] These studies are reviewed in the appropriate sections on the different host groups.

G. Host Anatomy and Habitat

The gross anatomy of the host is not direct evidence for or against the presence of a chemoautotrophic symbiosis, but the host anatomy (i.e., lack of a gut, hypertrophied gills, reduction in particulate feeding ability, etc.) often suggests the presence of some kind of a nutritive symbiosis. Similarily, the hosts are often limited to habitats where the substrates necessary for chemoautotrophy are present, suggesting a dependence on these substrates. These arguments are detailed in the section on current evidence for nutritional exchange (Section V) and are only mentioned here because the same arguments have been used to support the existence of some chemoautotrophic symbioses.

III. HOSTS

The taxonomic range of invertebrate hosts harboring chemoautotrophic bacterial symbionts is proving to be quite wide. All members of two phyla (Vestimentifera and Pogonophora) are thought to harbor chemoautotrophic symbionts, as are all members of three families of the Mollusca (Lucinidae, Solemyidae, and Vesicomyidae) and one sub-family of the Annelida (Phallodrilinae). Chemoautotrophic symbioses have also been demonstrated in two other phyla of worms and three additional molluscan families. The wide variety of hosts to chemoautotrophic symbionts all have one feature in common: they must provide their symbionts with the substrates necessary for chemosynthesis, an electron donor, an electron acceptor, and a source of carbon. For most of the symbioses I review here the electron donor is a reduced sulfur species, either hydrogen sulfide or thiosulfate, the electron acceptor is oxygen and the carbon source is carbon dioxide or bicarbonate. Because sulfide is both toxic to aerobic respiration and rapidly disappears in the presence of oxygen, the animal's role in providing their symbionts with both reduced sulfur species and oxygen is often quite complex. In the following section, I review the pertinent facts concerning the current evidence for chemoautotrophic symbioses in the different groups of invertebrates, the host's habits and morphologies, the ultrastructure of the symbioses and symbionts, and the physiology of the symbioses.

A. Vestimentifera

As mentioned, the first described symbiotic association between chemoautotrophic symbionts and an animal host was *Riftia pachyptila*, the giant hydrothermal vent tube-worm. Although when this animal was first described it was placed in the subphylum Obturata in the phylum Pogonophora,[8] the Vestimentifera are currently considered by Jones to constitute a phylum.[99,100] Similarly, Mañé-Garzón and Montero[101] have proposed that the obturate pogonophores deserved their own phylum and proposed the phylum Mesoneurophora, although Phylum Vestimentifera apparently has precedence.[102] Southward on the other hand continues to consider the Obturata a sub-phylum of the Pogonophora, based on anatomical similarities and new developmental evidence.[103,104] I follow the taxonomy of Jones[99] in this review.

The Vestimentifera are all found in habitats characterized by active venting or seepage of pore waters (Table 1). The reasons for this restricted habitat may be explained by the animal's anatomy. Unlike bivalves or the smaller Pogonophora and annilids, the symbionts in the Vestimentifera are situated deep in the interior of a relatively large animal with no close connection to the outside environment. Therefore, the supply of all nutrients to the endosymbiotic bacteria must be by way

Table 1
Vestimentifera; Collection Sites and Evidence of Chemoautotrophic Symbionts

Species	Location	Enzymes	$\delta^{13}C$ (‰)	EM	°S	Ref.
Vestimentifera						
Axonobranchia						
Riftiida						
Riftiidae						
Riftia pachyptila Jones	Assorted H. T. V.	Ru, R, Ar, As	−9.0 to −13.3	+	+	9, 10, 74, 105
Basibranchia						
Lamellibrachiida						
Escarpiidae						
E. laminata Jones	Florida Escarpment	Ru, As	−42 to −47		+	55, 75
Escarpia spicata Jones	San Diego Trough	Ru, As			+	17, 31
Undescribed genus	Louisiana Slope	Ru, As, Ar	−30.4 to −41	+	+	34
Lamellibrachiidae						
Lamellibrachia barhami Webb	San Diego Trough	Ru, As			+	31, 36
	Oregon Sub. Zone		−31.9			59
L. luymesi van der Land and Nørrevang	Guyana Shelf					106
L. victori Mañé-Garzón and Montero	Coast of Uruguay					101*
L. sp. (may be *L. luymesi*)	Louisiana Slope	Ru, As, Ar	−27 to −37.4	+	+	34
Tevniida						
Ridgeiidae						
Ridgeia phaeophiale Jones	Endeavour Seg. of JFR			+		107, 108
R. piscesae Jones	Axial Seamount, Explorer and Southern JFR			+		107, 108
R. sp. ("small")	Explorer Ridge			+		107
R. sp. ("ring")	Southern JFR			+		107, 108
Tevniidae						
Tevnia jerichonana Jones	East Pacific Rise, 13°N		−9 to −15			76
Oasisia alvinae Jones	East Pacific Rise, 13°N, 21°N					99

Note: Abbreviations: HTV-hydrothermal vents; JFR-Juan de Fuca Ridge; Ru-Ribulose BisPhosphate carboxylase/oxygenase; R-Ribulose 5' Kinase; As-ATP sulfurylase; Ar-APS reductase. Em-symbionts visualized by electron microscopy °S - Appreciable elemental sulfur present in trophosome

* Authors indicate that symbionts are not bacteria but algae or algal spores, based only on light micrographs (?)

Taxonomy according to Jones[99,100]

of the blood, which contains an abundant hemoglobin that binds both oxygen and sulfide simultaneously and reversibly.[109-112] Also unlike most other animals with endosymbiotic chemoautotrophs, the Vestimentifera are presumably immobile once they have settled. The vestimentiferans must, therefore, be exposed to both sulfide and oxygen simultaneously. As sulfide and oxygen do not persist when both are present in a static environment the tubeworms must live in an environment where sulfide and oxygen are constantly being supplied, or form a living bridge between reduced and oxic environments. *Riftia pachyptila*, which is only found in areas with actively venting hydrothermal fluid, lives with its plume exposed to mixed vent and ambient water,[74,113] and apparently takes up both sulfide and oxygen across its plume surface.[112,114] This would seem to be the case with other hydrothermal-vent vestimentiferans considering their habitats, but may not be the case with some of the seep vestimentiferans. Investigators working with vestimentiferans associated with hydrocarbon seeps on the Louisiana Slope of the Gulf of Mexico[77] have been unable to detect sulfide around their plumes at most sites and the highest concentration of sulfide detected near their plumes was 3 μM.[115] It is possible that these worms are taking up sulfide across their tube walls since they are often buried by over 1 m of reduced sediments.

The general body plan of the Vestimentifera, as typified by *Riftia pachyptila*, is shown in Figure 1. The following anatomical description of the Vestimentifera is taken from Jones.[99,100,105] Vestimentifera have no mouth, gut, or anus. There are four easily recognized body regions in the Vestimentifera: at the anterior end of the worm is the obturacular region which consists of a central supporting structure, the obturaculum, and the branchial plume, which is highly vascularized and functions as a gas-exchange organ. Posterior to the plume is a muscular region, termed the vestimental region, that serves both to anchor the animal in its tube when the plume is extended and to secrete the tube as the animal grows. The worm's brain is located in the anterior part of the vestimental region and the paired genital pores of both sexes are located

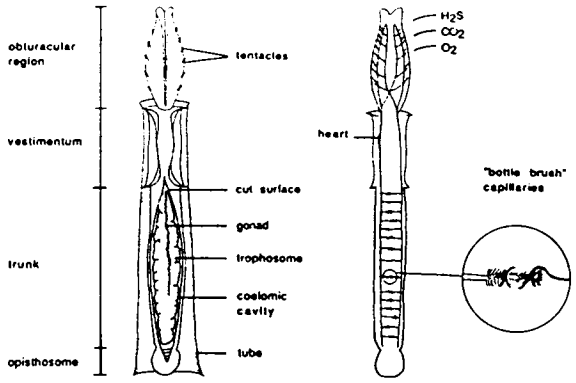

FIGURE 1. Schematic drawings of *Riftia pachyptila* showing major body regions and gross vascularization. Insert at right shows "bottle brush" arrangement of capillaries found in each lobule of the trophosome. The heart is located on the dorsal vessel. (Drawings by R. E. Kochevar.)

on the dorsal surface in this region. Behind the vestimentum is a region which makes up the bulk of the worm, the trunk. The trunk tapers from the anterior to the posterior end of the worm in all described species of Vestimentifera except *R. pachyptila*. Inside the trunk is a pair of coelomic cavities separated by medial mesenteries. Between mesenteries, and running the length of the trunk, are gonads and the trophosome. The term trophosome was originally suggested because it was thought to nourish the gonads[116] and has proved to be even more appropriate than the original authors imagined. In *R. pachyptila* the trophosome accounts for 15.3 ± 4.9% (SE, n = 11) of the animal's wet weight.[111] It is now known that the trophosome consists primarily of symbiont-containing bacteriocytes, associated cells, and blood vessels, the ultrastructure of which is detailed next (Figure 2). At the posterior end of the worm is a short region, termed the opisthosome, which apparently serves to anchor the base of the worm to its tube and, in *R. pachyptila*, to secrete basal partitions inside the tube. The tube of *R. pachyptila* is composed of a chitin proteolglycan/protein complex[117] and surrounds the animal up to the top of the vestimentum. The worm is able to withdraw completely into the tube at will.

The ultrastructure of the trophosome of *Riftia pachyptila* has been described by several investigators. Jones[100] described the circulatory system and details the vascularization of the trophosome, which is summarized in the following. Both Bosch and Grassé[118,119] and Hand[94] describe the ultrastructure of the lobules of the trophosome with emphasis on the disposition and ultrastructure of the symbionts and the possible roles of the outer, symbiont-free cells of the trophosome lobules. The following discussion draws heavily on their observations as well as my own, and while it deals specifically with *R. pachyptila* the points are generally true for the other vestimentiferans which have been examined[106-108] (and personal observation of two species from the Louisiana Slope).

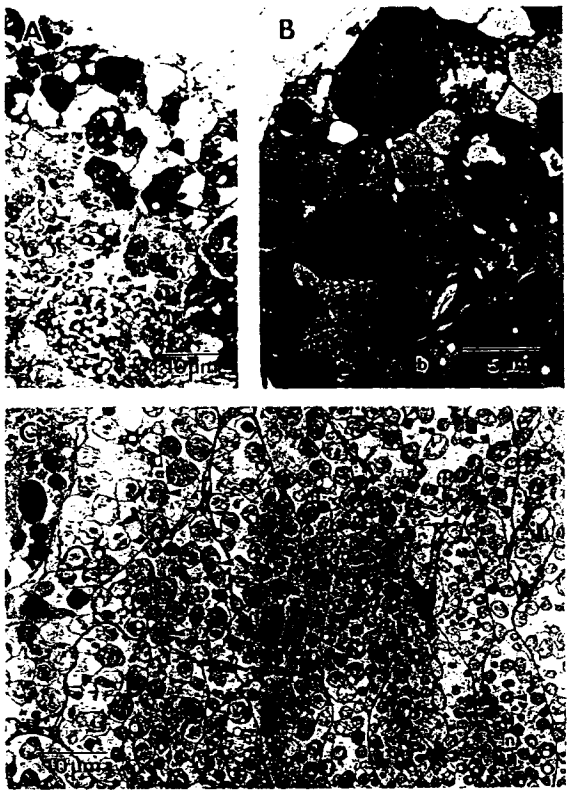

FIGURE 2. Vestimentiferan trophosome. (A) *Riftia pachyptila*. Low magnification electron micrograph of a section of a trophosome lobule. Inner portion of lobule in lower left of micrograph. Presumed site of extracted elemental sulfur visable as empty vacuoles in many of the symbiotic bacteria. Several symbionts indicated by arrows. "Trophotheca" comprises the upper right half of the micrograph. (B) *Riftia pachyptila*. Higher magnification micrograph of trophochrome cell, with a few bacteria (b) visable in lower right corners of micrograph. (C) Unidentified hydrocarbon-seep Escarpid. Low magnification electron micrograph of a portion of a trophosome lobule. Although fixation was not excellent, the size gradient of symbionts from the middle of a lobule (in lower right quadrant) to the edge of the lobule is evident. A trophochrome cell is visable in the upper left corner. Several bacteria are indicated by arrows, and a bacteriocyte nucleus by n. (Figures (2A) and (B) from Hand.[94])

Trophosome tissue is a fragile tissue of gelatinous consistency which is composed of numerous well vascularized lobules, each about 0.15 mm in diameter.[94,100] As noted, the chemosynthetic substrates (H_2S, O_2, and CO_2) required by the symbionts in the trophosome must be transported through the host. The most likely avenue for this is through the animal's vascular circulatory system (Figure 1).[111] The vascular hemo-

globin binds sulfide and oxygen with high affinity simultaneously and reversibly.[109,110,112] Sulfide- and oxygen-laden blood, fresh from the branchial plume passes into the trunk region via the ventral vessel. Blood passes from the ventral vessel through the afferent trophosomal vessels which ramify over the surface of the trophosomal lobules. From these vessels numerous capillaries arise which carry the blood into the center of the lobules where a single efferent trophosomal vessel runs the length of each lobule. Efferent vessels from different lobules come together and eventually join to either a blood plexus in the mesentery between the mesenterial and dorsal vessels or directly to the mesenterial vessel itself. The mesenterial vessel in turn is connected to the dorsal vessel where the blood flows back to the plume of the worm. The result of this is a high degree of vascularization throughout the trophosome so that the bacteria in the trophosome are all in a position to be well-supplied with nutrients and substrates transported by the blood. In fact, vestimentiferan blood appears to contribute to the creation of an environment in the trophosome with large pools of sulfide present at low activity, an environment that allows quite high rates of chemoautotrophic carbon fixation.[97] Conversely, the products of either bacterial translocation or digestion can be readily transported to other host tissues from the trophosome, via the vascular blood.

As noted, sulfide is rarely detectable (<3 μM) around the plumes of the hydrocarbon seep vestimentiferans *in situ*.[115] The highest level of sulfide that has been detected in the blood of freshly collected seep vestimentiferans is 147 μM,[12] which corresponds to free (unbound) sulfide concentrations in the blood below 1 μM.[97] They could, therefore, acquire sulfide across their plume from very low environmental concentrations (in an equilibrium situation at low sulfide concentrations, free sulfide in their blood will approximate the environmental concentration) or take it up across their tube and body wall. If sulfide were taken up across the tube and body wall of these species, it would be bound by the coelomic fluid which has properties similar to the vascular blood.[112] Childress et al.[111] have demonstrated that sulfide, ΣCO_2 and pH are in equilibrium in the two fluids (vascular blood and coelomic fluid), suggesting ready exchange between the two fluid compartments. Sulfide in the coelomic fluid could, therefore, be transfered to the vascular blood for transport to the symbionts in the trophosome. In this proposed scenario the role of the vascular blood would be basically the same as the role described, with the only difference being the site of uptake of sulfide.

Trophosome lobules have a complex but well-defined structure.[94,108,118,119] The central portion of a lobule is occupied by bacteriocytes (cells which contain bacteria). The appearance of the bacterial symbionts and the bacteriocytes changes from the interior to the exterior of a lobule. Hand[94] noted an increase in average size of the symbionts of *R. pachyptila* outwards from the interior of a lobule. This is true not only for *Riftia pachyptila*, but also for several species of vestimentiferans from the Juan De Fuca Ridge[108] and for two species of vestimentiferans from the Louisiana Slope (personal observation, Figure 2). DeBurgh[107] also notes an anterior to posterior size gradient of symbionts within the trophosome of several species from the Juan de Fuca Ridge hydrothermnal vents, and Powell and Somero[40] found anterior-posterior gradients of enzymatic activity in *R. pachyptila* trophosome. Distel et al.[120] analyzed the 16S RNA of two individual *R. pachyptila* and concluded that one species of symbiont accounted for >90% of the 16s RNA in each (<10% of a unique 16S RNA would not be detectable by their methods), and that the two individuals contained the same species of symbiont. The symbionts within a single species of vestimentiferan can range in diameter from 1 to 9 μm.[107] The possibility that the size gradients within lobules are due to some interaction with the host (and not unique to a specific symbiont) is suggested by the morphological dissimilarity of the symbionts in different vestimentiferan species (Figure 3). Based on morphology, there are three different symbionts in the Juan de Fuca worms (one of which is similar to the symbiont in *Riftia pachyptila*),[108] and a fourth symbiont in Louisiana Slope vestimentiferans (personal observation, Figure 3). In all of these associations there is the

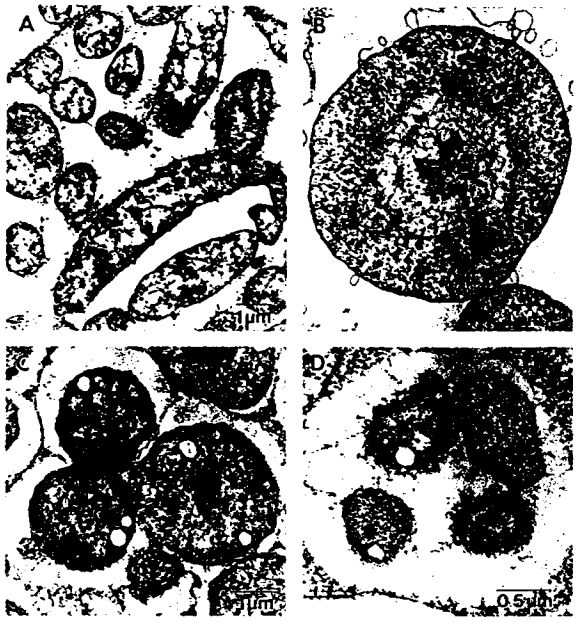

FIGURE 3. Vestimentiferan symbionts. (A) *Ridgea phaeophiale*. "small symbiont" of de Burgh et al.[108] (B) *Ridgea piscesae* Typical symbiont form. Ranges in size from 0.6 to 10 μm in diameter. *Riftia pachyptila* symbionts. (C) Large symbionts from periphery of lobule. (D) Small symbionts from central region of same lobule. (Micrographs in Figures 3(A) and (B) were prepared by M. de Burgh and C. Singla.)

same basic spatial arrangement of small and large symbionts within a single lobule. Whether this is due to a metabolic gradient as suggested by Hand[94] or "developmental stages" of the symbionts as suggested by Bosch and Grassé,[119] the presence of this phenomenon in diverse vestimentiferans and their symbionts suggest that it is due to a considerable degree of integration between the symbiotic partners.

Other aspects of the "developmental stages" have been noted. Hand[94] observed that the amount of elemental sulfur deposits in the symbionts decreases with distance from the center of the lobule, and Bosch and Grassé[119] note the degeneration of the symbionts towards the margin of a lobule. The French authors go a step further and describe four stages in the "evolution" of the bacteriocytes from infection (in the center of a lobule) to the eventual digestion of the entire bacteriocyte towards the margin of the lobule. In the first stage the bacteria divide in cells in the center of a lobule. In the stage two bacteriocytes both the bacteria and the bacteriocytes grow and are apparently "healthy." In the third stage, bacterial lysis occurs and there are numerous degenerate bacteria and pseudomyelinic bodies in the bacteria. In the fourth stage all that remains of the bacteria are "myelin-like bodies" (concentric membranes inside a vacuole), and the bacteriocytes, themselves, degrade. This last stage occurs just inside the perifery of the lobule. DeBurgh et al.[108] report observations of bacterial degradation similar to those reported by Bosch and Grassé[118,119] but question their interpretation, suggesting that bacterial digestion may not be quantitatively important to the host and may only represent "cellular housekeeping" within the trophosome.

The external surface of the lobule is covered by cells that do not contain bacteria. This is termed the "trophotheca" by Bosch and Grassé[118,119] and the outermost layer of cells are called "trophochrome cells" by Hand[94] (Figure 2). The inner layer of the trophotheca consists of metabolically active, non-bacteriocyte cells, which have been suggested to function in the assimilation of the products of bacterial degeneration (digestion). The outer layer (trophochrome cells) is tightly packed with at least three types of membrane-bound inclusions that can be recognized with TEM (Figure 2b). The function of these cells and the identity of the inclusions have not been determined. Hand notes the similarity of one type of inclusion to mucus droplets found in goblet cells of intestinal epithelia, and speculates that "the highly organized crystalline arrays of material" in the most osmophilic granules may be proteinaceous. Bosch and Grassé suggest that these cells may serve an "excretory function" and that the granules are probably lysosomes and contain waste products. Considering the probable function of the trophosome, i.e., to supply nutritive carbon to the rest of the animal, I would suggest the possibility that the membrane-bound inclusions in these cells (which are immediately adjacent to cells in which bacteria are apparently being digested) may also contain products of bacterial digestion or translocation which are subsequently mobilized by the host.

It is interesting to note that the functional morphology of the trophosome has caused two investigators to suggest that the blood flow is from the interior of a lobule outward (the opposite of what Jones suggests). Bosch and Grassé[119] suggest that the trophotheca is in contact with efferent blood vessels, presumably carrying the products of bacterial digestion to the animal. Hand[94] suggests that a gradient of access to sulfur compounds may be the cause of the variation in the occurrence of elemental sulfur deposits in the symbionts. This suggests higher concentrations of sulfide in the interior of a lobule (adjacent to the efferent vessels) than in the exterior of a lobule, a situation which one would expect to occur only if the blood flow is from inside to out. These apparent contradictions are a reflection of the fact that the study of this unique tissue has only just begun and further work is required before its functional morphology can be inferred with a high degree of confidence.

Symbiotic chemoautotrophic bacteria have been demonstrated in all of the Vestimentifera which have been examined to date for their presence. Considering the worms' anatomy, blood properties, and the level of integration between the symbiont and the hosts in the studied species, it seems safe to assume that all vestimentifera will be found to harbor chemosynthetic symbionts in their trophosome. Table 1 presents the taxonomy of the described Vestimentifera (following Jones[99]) along with the location from which they have been collected and current evidence for the presence of symbiotic chemoautotrophs in these animals. In addition to the species shown in the table there are several other undescribed species, (personal communication, M. L. Jones, Smithsonian Institute). The bacterial volume in the trophosome of *Riftia pachyptila* has been calculated to be between 15 and 35% of the total volume of the trophosome.[40] The levels of RuBPC/O found in vestimentiferan trophosome are the highest levels measured in any chemoautotrophic symbiosis.[9,34,74] Similarly, the amount of elemental sulfur in trophosome tissue is often very high. Brooks et al.[34] reports as much as 6.1% of the wet weight of *Lamellibrachia* sp. trophosome is elemental sulfur and levels as high as 10.3% of wet weight have been measured in samples of *R. pachyptila* trophosome.[74]

In addition to the evidence summarized in Table 1, a variety of experiments have been conducted with trophosome homogenates (containing intact symbionts) that confirm the chemoautotrophic nature of the symbionts in *Riftia pachyptila* and the vestimentiferans from the Louisiana slope. Fisher and Childress[121] documented sulfide consumption by the trophosome preparations from *R. pachyptila*. Powell and Somero[40] have found ATP production to be stimulated by sulfide in cell-free trophosome preparations. Belkin et al.[43] demonstrated that sulfide, but not thiosulfate, will stimulate carbon fixation in *R. pachyptila* trophosome homogenates, a fact that has been confirmed in our laboratory (Fisher and Childress). Sulfide stimulation of autotrophic carbon fixation by several orders of

magnitude has recently been demonstrated with trophosome preparations from another vestimentiferan (an undescribed species of escarpiid) using homogenates prepared microaerobically and incubated in dilute vestimentiferan blood.[97]

The $\delta^{13}C$ values reported for vestimentifans vary considerably in different species, as well as within a single species. Values ranging from -27 to $-43.2‰$ for different individuals of *Lamellibrachia sp.* and -30.4 to $-41.0‰$ for individuals in an undescribed genus of escarpiid have been reported in animals collected from hydrocarbon seep areas in the Gulf of Mexico.[34] Individuals of *Escarpia laminata* from the Florida Escarpment cold seep communities range in $\delta^{13}C$ from -42 to $-47‰$.[57,75] This variation may reflect a variation in the end member $\delta^{13}C$ of their inorganic carbon source, different ages, and/or different growth rates, among other possibilities. Whatever the cause of the variation between individuals of these species, these values are in the range we have come to expect from chemoautotrophic symbioses. The organic carbon in *Riftia pachyptila* on the other hand is consistantly much heavier than other chemoautotrophic associations. Rau[7] published the first $\delta^{13}C$ values for this animal and others have since confirmed the range of -9 to $-13.3‰$ for tissues of *R. pachyptila*.[34,74] The reason for these heavy values in *Riftia pachyptila* has never been adequately demonstrated but there are two plausible and related theories which could account for this. One hypothesis was put forward by Felbeck,[9,67] who determined that the first products of inorganic carbon fixation by intact animals were the C-4 compounds succinate and malate which are produced in the plume. He also found appreciable decarboxylation of malate in the trophosome. Based on these data and the published $\delta^{13}C$ values, he (cautiously) postulated that *Riftia pachyptila* may function like a C-4 plant, with the primary fixation of inorganic carbon occuring at the site of uptake, into C-4 compounds that are then transported via the circulatory system to the trophosome where they are decarboxylated and the CO_2 is refixed by the Calvin-Benson cycle. If the bacteria fix all of the inorganic carbon that reaches the trophosome, then the only isotopic discrimination of carbon should occur before and during the initial fixation step in the plume, and like a C-4 plant, the stable carbon isotopic signature would be heavier than if carboxylation by RuBPC/O were the primary fixation step. A second hypothesis which would also account for the heavier $\delta^{13}C$ values is that *Riftia pachyptila* symbionts are carbon limited and, therefore, fix virtually all of the inorganic carbon supplied to them.[7,73,76] This would result in little, or no, isotopic discrimination at this carbon fixation step. This hypothesis is supported by the high bacterial volumes in *Riftia pachyptila*, its large size, its high chemoautotrophic potential (as evidenced by RuBPC/O levels), the levels of inorganic carbon measured in its blood, and the K_m of its symbionts for CO_2.[76]

Two recent developmental studies have found a transient digestive tract in juvenile vestimentiferans.[104,122] An anterior ciliated opening leading to a presumptive gut and out through a posterior basal opening (anus) was observed by both groups in the earliest developmental stages found. Neither group found any "symbionts" in these early developmental stages, and Jones and Gardiner[122] suggest that the future symbionts are acquired at this stage from the free-living bacterial population of the surrounding environment. Intermediate stages with bacteria present in the gut epithelium were observed by both groups.

Cary et al.[123] found no visual evidence of symbionts in TEM sections of freshly released, positively bouyant, eggs of *R. pachyptila*. Although this study and the developmental studies described suggest strongly that the symbionts are not transmitted directly from generation to generation, they do not rule out the possibility of transmittal of the symbionts in a cryptic form as has been suggested for the bivalve *Solemya reidi*.[124] TEM (morphological) and 16S rRNA analysis of the symbionts indicate that associations with vestimentiferans are very specific.[119,120] Considering the highly-integrated obligate nature of these symbioses, and the transient nature of hydrothermal vents, it would be surprising if each generation must rely on the *de novo* acquisition of symbionts from the surrounding environment. Indirect transmission of symbionts would require that the appropriate free-living (future symbiont) bacteria precede the larval vestimentiferans to each new vent site, a very inefficient means to assure colonization of these ephemeral sites.

B. Pogonophora

As mentioned previously, the taxonomy of this group is currently in dispute. While Jones considers the Pogonophora and Vestimentifera to be two separate phyla, Southward continues to follow the original division of the Pogonophora into two subphyla; the Perviata or "small" pogonophora and the Obturata or large vestimentiferan worms. The vestimentiferan worms are discussed in the previous section and the small perviate pogonophores are covered here.

The small pogonophores are anatomically quite distinct from their larger relatives. They are quite thin, ranging from 0.1 to 3 mm in diameter and from 50 to over 500 mm in length.[125] Over 100 species of small pogonophora have been described.[126,127] They inhabit tubes partially buried in sediments or in rotting wood, and have been collected at depths ranging from 20 to 9950 m.[125] Due to their small size and lack of a mouth and gut these worms were previously thought to depend on dissolved organic matter for nutrition.[128,129] After the demonstration of chemoautotrophic symbioses in the closely related vestimentifera, the nutrition of this group was re-examined and chemoautotrophic bacterial symbionts were discovered in the post-annular region of several species. In gross anatomy they are more complex than the vestimentiferans and their trunk is subdivided into several distinct regions (Figure 4A). The symbionts are housed in cells (bacteriocytes) in a tissue homologous to the vestimentiferan trophosome (Figure 4).[130] The amount

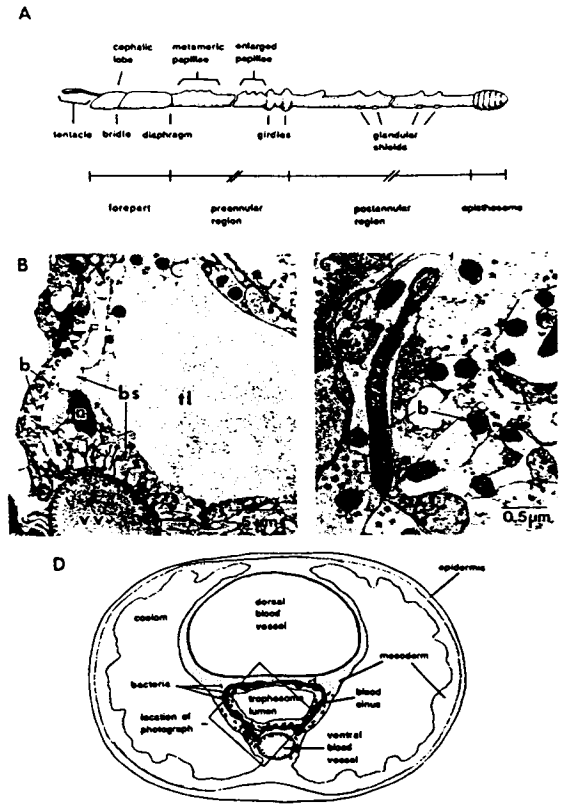

FIGURE 4. Small Pogonophorans. (A) Diagramatic representation of a typical small pogonophoran, redrawn from Southward.[130] Note the occurrence of symbionts is restricted to the post annular region. (B) *Siboglinum fiordicum*. Low magnification micrograph of a portion of a section through the postannular region. Position of micrograph is indicated in (D) b, bacteria; bs, blood spaces, n, nucleus of bacteriocyte; tl, trophosome lumen; vv, ventral vessel. (C) *Siboglinum fiordicum*. Higher magnification electron micrograph of symbionts *in situ* in vacuoles. Sections through two of the bacterial symbionts indicated by b. (D) Schematic drawing of a cross-section through the postannular region of a pogonophoran, redrawn form Southward.[130] (Drawings by R. E. Kochevar. Micrographs complements of Dr. E. Southward.)

of trophosomal tissue in the pogonophores is much reduced in comparison to the vestimentiferans. Bacteriocytes are found only in the post-annular region of the trunk of the worms, and the volume occupied by trophosome tissue is estimated to be about 10% of the total volume of that region in *Siboglinum fiordicum* (Figure 4).[130] In other species the relative volume of the trophosome can be even more reduced, particularly in males with developed testes.[130] Even within the pogonophoran trophosome, symbiotic bacteria can be relatively rare (Figure 4).[130] Estimates of the volume occupied by the symbionts in pogonophores ranges from 0.3 to "less than 1.0%" of the whole animal.[130,131] The blood of all species of small Pogonophora so far examined contains hemoglobin with a very high affinity for oxygen,[132,133] which has been postulated to function in transporting oxygen to the symbionts in the deeply buried posterior end of the worms.

Symbiotic bacteria have now been visualized in eight species of small pogonophorans (Table 2). Southward[130] presents numerous electron micrographs and an extensive discussion of the symbionts of small Pogonophora. The following descriptions are taken almost entirely from that work. The symbionts in all eight species so far examined are found in host cells (bacteriocytes) and in all but one of these species the symbionts are clearly enclosed within host cell membranes or vacuoles.[16,130] Incidence of bacteria within the bacteriocytes ranges from "rare to abundant." The symbionts found enclosed in host membranes in six species of small pogonophore are all morphologically similar (the other species with symbionts in vacuoles, *S. poseidoni*, contains methanotrophic symbionts and is discussed next). The symbionts in these six species are Gram-negative thin rods, varying in diameter from 0.15 to 0.3 µm and from 2.0 to 4.8 µm in maximum length (Figure 4D). The cytoplasm in these symbionts is moderately electron-dense and sometimes contains small ribosomes or electron-lucent vacuoles. No host membrane could be seen around the symbionts in *Sclerolinum brattstromi*. The symbionts in *S. brattstromi* are thicker (0.9 to 1.2 µm in diameter) and about 4 µm in maximum length. Occasional intracytoplasmic membranes are sometimes seen in these symbionts.

Conclusive demonstration of the chemoautotrophic nature of the symbionts in these worms was somewhat problematic due to the small size of the animals and the relatively low abundance of the symbionts within the worms. For example, individuals of two of the pogonophores studied by Southward et al.,[33] *Sibloglinum fiordicum* and *S. atlanticum*, ranged in wet weight from 0.43 to 2.1 mg and 3.7 to 16.6 mg, respectively. However, RuBPC/O and APS reductase (at varying activities) have now been demonstrated in the post annular region of five species of small pogonophores (Table 2).[13,33,131] Southward et al.[33] also reported increased rates of carbon fixation in whole animals and isolated tissues of *S. fiordicum* in the presence of sulfide and thiosulfate when compared to controls incubated in seawater without added sulfide. However, as pointed out by the authors, the levels of stimulation were low and should be viewed with caution. The chemoautotrophic nature of some pogonophoran associations is also supported by their stable carbon isotopic content. $\delta^{13}C$ values for *S. atlanticum*, *S. fiordicum* and *S. ekmani* range from -35.5 to $-45.8‰$ in areas where the values for dissolved organic and inorganic carbon are both about $-18‰$.[13,33,55]

The small pogonophoran, *Siboglinum poseidoni*, harbors methanotrophic symbionts. The symbionts in this species contain stacked internal membranes like those commonly found

Table 2
Pogonophora With Indications of Chemoautotrophic or Methanotrophic Symbionts

Species	Enzymes[a]	EM	$\delta^{13}C$ (‰)	Ref.
Diplobrachia capillaris (Southward)		+		130
Galathealinum sp.			−30.5 to −59.3	34
Oligobrachia gracilis (Southward)	Ru(−), S(+)	+		13, 33
Sclerolinum brattstromi (Webb)	Ru(+), S(+)	+		33, 130
Siboglinum angustum (Southward & Brattegard)		+		130
S. atlanticum (Southward & Southward)	Ru(+), S(−)	+	−43.8 to −45.3	13, 33, 55
S. ekmani (Jägersten)	Ru(+), S(+)	+	−45.3	13, 33
S. fiordicum (Webb)	Ru(++), S(++)	+	−35.5	13, 33, 131
S. poseidoni (Frügel & Langhof)	MMO[b]	+	−73.6 to −74.4	13, 60, 134

Note: Abreviations: EM - symbionts visualized by electron microscopy; Ru- Ribulose bisphosphate carboxylase/oxygenase; S- Enzymes involved in oxidation of sulfur compounds (including ATP sulfurylase and APS reductase); MMO- Methane monooxygenase.

[a] Relative activities are indicated: low (−), medium(+), high(++).
[b] Homogenates oxidize ^{14}C-Methane to $^{14}CO_2$.

in type I methanotrophs.[16] Both intact animals and homogenates of the postannular region oxidize ^{14}C-methane to ^{14}C-organic carbon compounds and $^{14}CO_2$.[134] The tissues of *S. poseidoni* were also very depleted in ^{13}C (δ^{13}C from −73.6 to −74.4‰).[60] These pogonophores will normally die within three weeks of culture in seawater, but have been maintained for up to three months in seawater under an atmosphere of air and methane.[134]

Another "small" pogonophore has been collected from hydrocarbon seep areas in the Gulf of Mexico.[77,115] This pogonophore is thin, like the other species discussed, but pieces of individuals in excess of 80-cm-long have been collected by otter trawl (personal observations). It has been identified as a new species of *Galathealinum* in the perviate family Polybrachiidae (personal communication, E. Southward, Mar. Biol. Assoc. U.K.). The reported δ^{13}C values of from −42 to −48‰ for tissue from this species suggest that it contains symbionts which are either chemoautotrophic or methanotrophic.[34] There are reports in the literature of tissue closely resembling pogonophoran trophosome in two other genera of small pogonophorans, *Polybranchia* and *Lamellisabella*.[126] Investigators currently working on the small pogonophora have suggested that trophosome tissue and the symbiotic habit are likely to be universal features of the phylum.[125,130]

C. Annelida

Two groups of annelids, one genus of polychaete and two genera of oligochaete, have been described which harbor symbiotic chemoautotrophic symbionts. In both groups the symbionts are extracellular, either on the epidermis or in subcuticular regions.

1. Polychaetae

The polychaete family Alvinellidae[135] contains two genera, both of which are found solely at hydrothermal vents. Alvinellids of the genus *Paralvinella* do not harbor symbiotic bacteria although the insides of the tubes of *P. grasslei* are often heavily colonized with (apparently) chemoautotrophic bacteria.[136] The "Pompeii worms", *Alvinella pompejana* and *A. caudata*, both have numerous morphological forms of bacteria associated with their epidermis (Figure 5). *A. pompejana* (=*A. pompejana*, forme *hirsuta*) and *A. caudata* (=*A. pompejana*, forme *caudata*) were thought to represent different ontogenic stages of the same animal when first described,[137] but are now considered two distinct species.[135] The two species build organic tubes on zinc sulfide chimneys ("white smokers"), in waters ranging from 20 to 40°C.

The bacteria associated with the two species of *Alvinella* are never intracellular although they are occasionally subcuticullar.[138] "Cluster-like" associations of rod-shaped, coccoid, and filamentous bacteria are located in the intersegmentary spaces of both species (Figure 5). Filamentous bacteria are associated with the numerous epidermic expansions along the dorsal portion of *Alvinella pompejana*. *A. caudata* is devoid of epidermic expansions, but its posterior parapods are modified and covered with a high density of filamentous bacteria. Additionally, a variety of morphological forms of bacteria (rod-shaped, prosthecaded, spiraled, and unsheathed filamentous) are distributed singly over much of the rest of the tegument in both species.[19,72,139]

Enzyme assays indicate the presence of RuBPC/O both on the "skin" of both species of *Alvinella* and in homogenates of tube material,[140] and *in situ* ^{14}C-bicarbonate incubations

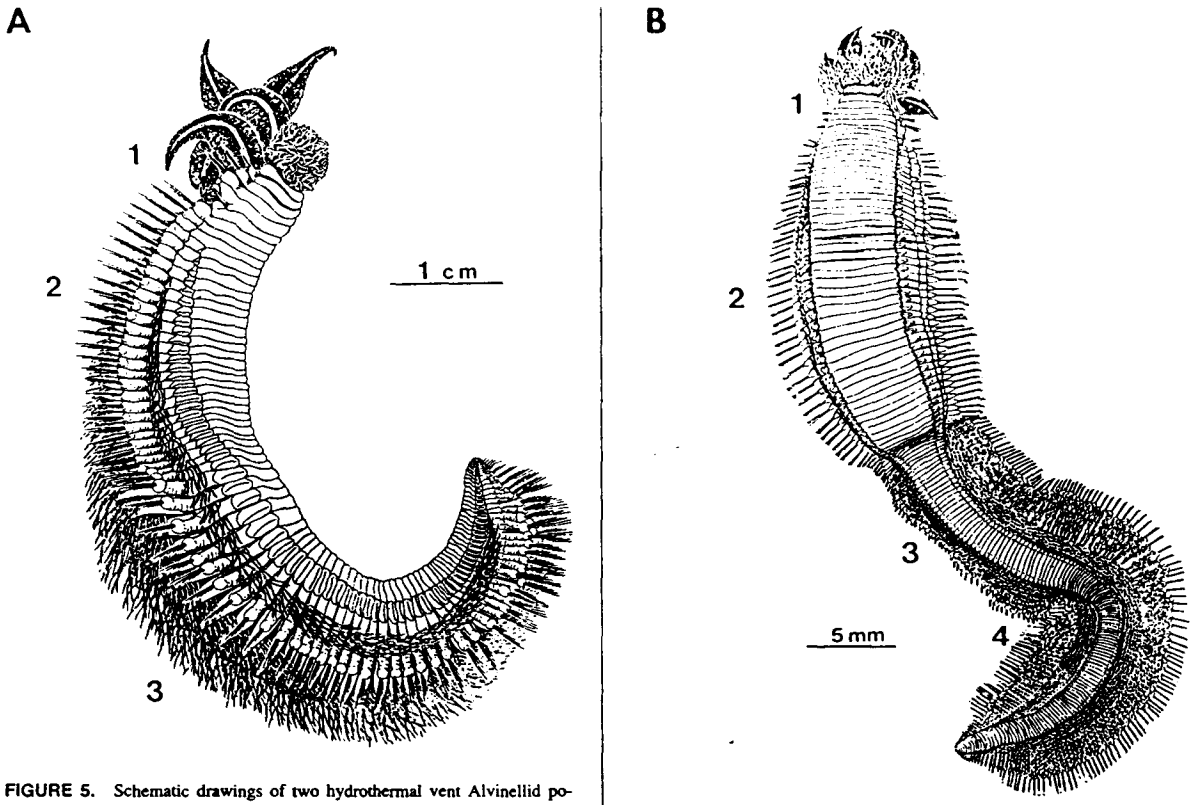

FIGURE 5. Schematic drawings of two hydrothermal vent Alvinellid polychaetes. (A) *Alvinella pompejana*. Few bacterial aggregates are present on the most anterior portion of the worms (1) but density of the aggregates increases on the intersegmental spaces in the area indicated by (2). Aggregates are abundant on the epidermal expansions on the dorsal portion of the posterior half of the worm (area indicated by 3). (B) *Alvinella caudata*. Density of bacterial aggregates in the intersegmentary spaces of areas (1) and (2) are similar to *A. pompejana* but increase in density in area (3) of *A. caudata*. *A. caudata* is devoid of epidermal expansions but the posterior parapodia in area (4) are modified and covered with filamentous bacteria. (Figures 5 A and B are from Desbruyères and Laubier.[137])

indicate that at least the "cluster-like" associations of bacteria have some chemoautotrophic activity.[141] The same two studies also indicate that some of the associated bacteria are facultatively heterotrophic, based on phosphoenolpyruvate carboxylase activity[140] and uptake of ^{3}H-thymidine without associated ^{14}C-bicarbonate incorporation (growth without autotrophy).[141]

The nature of the interactions in these epibiotic associations is unclear. The $\delta^{13}C$ value measured in *A. caudata* tissue ($-11.2‰$) is similar to those found in *R. pachyptila* tissue, and the authors suggest that this implies a common nutritional source of carbon.[72] However, as discussed, the $\delta^{13}C$ values of hydrothermal vent vestimentiferans are anomolous among chemoautotrophic symbioses and probably are due to factors related to the supply of carbon to the intracellular symbionts in the interior of the worms' bodies. Whether the similar values in the two distinctly different symbioses are due to similar causes, or the coincidental result of different causes, remains an open question.

The worm's anatomy suggests a mixotrophic existence. Both species of *Alvinella* have a functional mouth and gut, and gut content analyses indicate the ingestion of filamentous bacteria and mineral particles.[136] On the other hand, there are a variety of ultrastructural features which may be specializations for exchange of metabolites between the epibionts and their host. These include disorganized collagen fibers and a highly vascularized underlying epithelium in some of the heavily colonized intersegmentary zones, as well as thin filamentous structures linking some bacteria to the worm's cuticle.[19] However, *in situ* labeling studies revealed no difference in the labeling pattern between the two species of *Alvinella* and *Paralvinella grasslei* (a species without symbionts) after exposure to ^{14}C-bicarbonate.[141] Elucidation of the nutrition of the two species of *Alvinella* awaits further experimentation.

Several authors have suggested that the associated bacteria may also serve a "detoxification" function, either with respect to heavy metals, or sulfide.[19,141] Whatever the nature of the

interactions between the partners in this symbiosis, the morphological adaptations of the worms to their epibionts, the abundance of the associated bacteria, and the specificity of the different types of bacteria to the various regions of the worms all indicate a significant functional interaction.

2. Oligachaeta

The oligochaete sub-family Phallodrilinae contains two mouthless and gutless genera whose taxonomic descriptions include symbioses with chemoautotrophic bacteria. These worms all live interstitially in mildly reducing sediments. The gutless condition of *Phallodrilus leukodermatus* (= *Inanidrilus leukodermatus*) was described by Giere in 1979.[142] Simultaneously five other gutless species in the Phallodrilinae were described.[143] Two years later the presence of subcutaneous symbiotic bacteria was demonstrated in *P. leukodermatus*.[144] Since then similar symbioses in a number of other gutless oligochaetes have been described.[145-148] On the basis of these discoveries and the descriptions of 22 new species, Erséus[147] re-evaluated the taxonomic status of the gutless Phallodrilinae and proposed that the 38 species be divided into two genera, the pre-existing *Inanidrilus* and a new genus, *Olavius*, thus leaving only species with guts in the other genera of the Phallodrilinae. The descriptions of both gutless genera include the presence of symbiotic bacteria ("Body wall densely granular and chalky white due to presence of subcuticular symbiotic bacteria.")[147]

Inanidrilus leukodermatus remains one of the most intensively studied of the gutless oligochaetes although *Olavius* (= *Phallodrilus*) *planus* has also been investigated by a number of authors.[146,149,150] The symbionts in both species (and presumably in all species of the two genera) are found in a subcuticular space between the cuticle and the epidermis (Figure 6A). The thickness of the bacterial-containing layer varies along the length of the individuals. In both species there two kinds of (usually) extracellular gram negative bacteria (Figure 6B). The large ones are generally oval in shape and measure about 2.1 × 3.3 μm with some as long as 4.6 μm. The smaller rod-shaped symbionts are about 0.5 × 1.7 μm and have a thicker, multilayered cell envelope than the larger symbionts.[149,150] Distribution of the two symbionts over the worms is non-random. The larger symbionts are abundant in the post-clitellar region, becoming more rare towards the anterior end of the worm and are completely lacking in the most anterior segments (segments I to VII). The smaller bacteria are present in every segment of the worm and are scattered between the larger symbionts when they co-occur.[149,150] The symbionts are especially dense and abundant in "genital pads" of the clitellar region. Histological studies indicate that the symbionts are directly transmitted between generations. Giere and Langheld[150] conclude that the symbionts of *I. leukodermatus* are transmitted from the parental body to its eggs by external intrusion during oviposition. The source of the transmitted symbionts is apparently the bacteria in the genital pads.

FIGURE 6. *Phallodrilus leukodermatus*. (A) Cross-section through the body wall, showing location of two types of bacteria in the subcuticular space. c u, cuticle; e b, endocytosed bacteria; l b, large bacteria; l m longitudinal muscle; n epidermal cell nucleus; s b, small bacteria. (B) Higher magnification electron micrograph showing a single large and several small bacterial symbionts. (C) Higher magnification electron micrograph of a lysosome from an epidermal cell. (Micrographs compliments of O. Giere.)

A variety of results indicate that the symbionts are chemoautotrophic. The enzymes RuBPC/O, ATP sulfurylase, sulfite oxidase and nitrate reductase have been demonstrated in tissues of *Inanidrilus leukodermatus* and *Olavius planus*.[146] Both RuBP carboxylase and nitrate reductase are characteristic of autotrophic organisms. More recently slight thiosulfate stimulation of CO_2 fixation has been demonstrated in *I. leukodermatus*, although the high unstimulated carbon fixation rates (29 μm g^{-1} h^{-1}) mitigate interpretation of the results.[151] X-ray microanalysis indicates that the symbionts contain sub-

stantial amounts of sulfur reserves and biochemical analyses indicate that the bacterial reserve, polyhydroxybutyrate (PHB), may account for as much as 10% of the dry weight of the worms.[149,150] When carbon fixation rates of "white" and "pale" worms were compared, much higher rates were recorded for the "white" worms, worms which the authors indicate were replete with sulfur and PHB reserves.[151] These results, combined with the worms' vertical distribution and their mouthless and gutless condition, provide strong evidence for the chemoautotrophic nature of these symbioses.

D. Other Worms

A variety of gutless worms which live interstitially in reducing sediments have been re-examined by scientists since the demonstration of chemoautotrophic symbionts in the Vestimentifera. As a result, worms from two additional phyla have been suggested to contain chemoautotrophic symbionts. A nematode, *Astomonema jenneri* (Phylum Nematoda), and a turbellarian, *Paracatenula urania* (Phylum Platyhelminthes) contain abundant symbiotic bacteria in their cells, which may be chemoautotrophic.[152] The catenulid turbullarian, *Paracatenula urania*, contains abundant Gram-negative bacteria, about 2 to 4 μm wide and up to 10 μm in length, in vacuoles in "gut rudiment" cells. The Nematode, *Astomonema jenneri*, also contains abundant Gram-negative procaryotic cells inside the cells of its "gut rudiment". However, there are two morphological types of symbionts in *A. jenneri*: small, relatively uncommon, rod shaped bacteria (0.1 to 0.5 μm in length), and larger oval bacteria (about 1 by 3 μm in length). No host derived membrane could be seen by the authors around either of the symbionts in *A. jenneri*. The symbionts pack the "gut rudiment" cells in which they are found, and account for an estimated 25 to 50% of the body mass of the nematode. Ott et al.[152] suggest that the symbionts of both the nematode and catenulid are probably chemoautotrophic, based on the symbiont morphology, the gutless condition and habitat of the host, and lack of an epidermis specialized for uptake of DOM or POM in either host. While I agree that a chemoautotrophic symbiosis does seem likely in these two worms (and will likely be discovered in other members of the sulfide meiofauna as well), demonstration of the chemoautotrophic nature of these symbioses awaits further study.

E. Mollusca

Chemoautotrophic symbioses are proving to be widespread in the phylum Mollusca. Chemoautotrophic or methylotrophic symbionts have been clearly demonstrated in numerous species of bivalves from five different families and in one species of gastropod. A listing of the bivalve species which have been suggested to contain chemoautotrophic symbionts and the current evidence supporting these symbioses is summarized in Table 3. There are many similarities in the various associations between mollusks and chemoautotrophic symbionts although the behavioral, physiological, and morphological adaptations differ in the various families. In all families the symbionts are located in the gills, in the subfilamental tissue or its analog. The symbionts are intracellular in all families but the Thyasiridae. The bacteria are not found in ciliated cells but rather in or associated with more or less specialized cells termed bacteriocytes, which often alternate with intercalary cells (which do not contain bacteria) in the subfilamental tissue. When intracellular, the symbionts are found in vacuoles within the cells, not free in the host cytoplasm. The number of symbionts per vacuole varies in the different species. These symbiotic associations are found in a wide variety of habitats with considerable variation in habitat chemistry. These mollusks are mobile, and although some of symbiont-containing species are found in dynamic environments where sulfide and oxygen are both present (such as active vents or seeps) most of the species must bridge the interface between sulfide and oxygen with some kind of a behavioral adaptation. These behavioral responses for the acquisition of both sulfide and oxygen vary from family to family, and of course in the different environments.

1. Bivalvia

a. LUCINIDAE

About half of the described bivalve species with chemoautotrophic symbionts are in the family Lucinidae (Table 3). All members of this family which have been investigated for the presence of symbiotic chemoautotrophs have been shown to contain them, and many investigators, including myself, believe this type of symbiosis is inherent in the family.[103,154,159] It has been suggested that the symbiotic habit may have been the most important factor in the evolution of the super-family Lucinacea and the family Lucinidae.[159,161] Lucinids are characterized by a greatly reduced gut, reduced labial palps, absence of an incurrent siphon, and large fleshy gills,[167] all characteristics presumably associated with their symbiotic habit.

Lucinids have been collected from a wide range of habitats with depths ranging from the intertidal to the deep-sea. They have been collected from both tropical and temperate oceans, from sediments which range from strongly reducing to only slightly so.[37,95,154,168] Collection of lucinids and thyasirids from habitats where the free sulfide concentration is less than 1 μM dramatically expands the expected range of chemoautotrophic symbioses.[37,95,103]

Lucinids do not have an incurrent siphon, and ventilatory water enters the animal through an anterior "feeding tube" formed through the substrate by the extensible foot (Figure 7).[167] When actively ventilating this is the route for the acquisition of oxygen. Several methods have been proposed whereby the clams could acquire sulfide. Reid[161] has proposed that by ceasing ventilation, the lucinids may allow sulfide to accumulate in their burrows which could then be taken up after diffusion directly into the mantle cavity through the anterior gape. Most (if not all) lucinid species also produce with their

Table 3
Bivalves With Chemoautotrophic Symbionts

Species	EM	Enzymes	$\delta^{13}C$ (‰)	°S	Ref.
Lucinidae					
Anodontia philippiana (Reeve)	+	Ru			153, 154
Codakia costata (Orbigny)	+	Ru			153, 154
C. orbicularis (Linné)		Ru, As, R	−23.2 to −28.3		22
C. (Ctena) orbiculata (Montagu)		Ru			154
Linga (Lucina) pennsylvanica (Linné)		Ru			22
Loripes lucinalis (Lamarck)	+	Ru, Ar, As			155, 156
Lucina nassula (Conrad)		Ru	−23.0		22
L. radians (Conrad)		Ru			154
Lucinella divaricata (Linné)	+	Ru, Ar, As			156, 158
Lucinoma aequizonata[a] (Stearns)	+	Ru, As, R		+	31, 36, 88, 157
L. annulata (Reeve)	+				157
L. atlantis (McLean)			−31.2 to −33.0		77
L. borealis (Linné)	+	Ru, Ar	−24.1 to −29	+	55, 89
Myrtea spinifera (Montagu)	+	Ru, Ar, As	−23.4 to −24.2	+	37, 55
Parvilucina multilineata (Tuomey & Holmes)	+	Ru			153, 154
P. tenuisculpta (Carpenter)	+	Ru, As,		+	31, 159
Pseudomiltha (Lucina) floridana (Conrad)	+	Ru, Ar		+	47, 88, 157
Pseudomiltha sp.	+	Ru, Ar, As	−30.9 to −37.7	+	34
Solemyidae					
Solemya reidi (Bernard)	+	Ru, As, Ar, R	−30	+	31, 35, 44, 160
S. velesiana (Iredale)	+				161
S. velum (Say)	+	Ru	−30.9 to −33.9		36, 162
S. sp.			−31		59
Thyasiridae					
Thyasira equalis (Verrill & Bush)	+	Ru			32, 163
T. flexuosa (Montagu)	+	Ru, Ar, As	−29.3	+	32, 55, 159, 163
T. gouldi (Philippi)	+		+32, 163		
T. sarsi (Philippi)	+	Ru, Ar, As	−28.2 to −31	+	32, 55, 163
Vesicomyidae					
Calyptogena elongata (Dall)	+			+	88
C. laubieri (Okutani & Métiver)	+			+	164, 165
C. magnifica (Boss & Turner)	+	Ru, As	−32.1 to −51.6	+	7, 31, 36, 59, 79, 166
C. phaseoliformis (Métiver et al)	+		−37.8 to −40.1	+	61, 164, 165
C. ponderosa (Boss)	+		−31.2 to −39.1	+	34
Vesicomya cordata (Boss)	+	Ru, As	−39.8		34
V. gigas (Dall)[b]	+	Ru, As			31, 36
Mytilidae					
Bathymodiolus thermophilus (Kenk & Wilson)	+	Ru, As	−30.5 to −37.1	−	6, 21, 45

Note: Abreviations; Ar = APS Reductase, As = ATP Sulfurylase, R = Ribulose 5' kinase, Ru = Ribulose bisphophate carboxylase/oxygenase, EM = Symbionts visualized by electron microscopy, °S = appreciable elemental sulfur present in gills.

[a] Incorrectly identified as *L. annulata* in References 31, 36, 88.
[b] Incorrectly identified as *Calyptogena pacifica* in References 31, 36 (personal communication R. Turner, Harvard Mus. of Comp. Zool.)

extensible foot at least one, and often many, small tubes which extend down into the substrate from the ventral edge of their shell (Figure 7).[169] Turner[170] has hypothesized that these tubes may be the result of the clam using its extensible foot to obtain sulfide from interstitial water in the underlying sediments. Cary et al.[95] demonstrated the existence of small "pockets" of sulfide rich sediments in the muds colonized by *Lucinoma aequizonata* (the muds contain generally low concentrations of sulfide) and hypothesized that the clams may use their foot to "search out and tap" this sulfide source. *Parvilucina tenuisculpta* has a posterior bellows arrangement which Reid and Brand[159] have suggested could serve to ventilate the suprabranchial chamber and thus provide sulfide to the symbionts in the gills. Dando et al.[37] have noted that some lucinids live in environments where free sulfide concentrations are very low (<1 μM), but acid labile forms are more abundant (~700 μM/

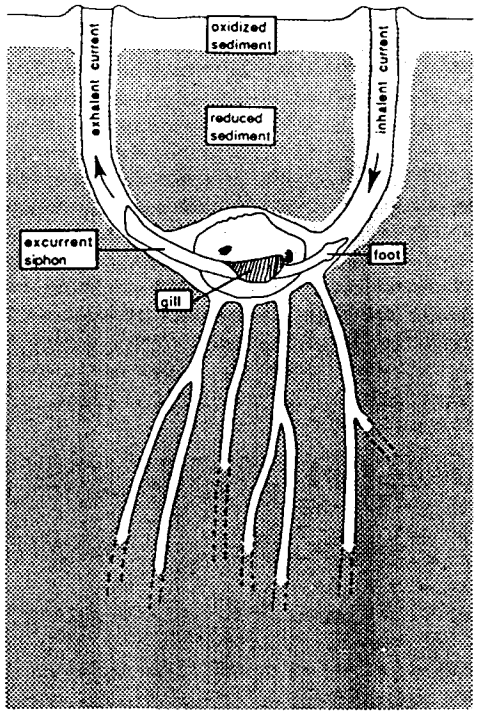

FIGURE 7. Diagram of a "typical" lucinid burrow drawn from Stanley's X-rays.[169] Permanence of exhalent tube as well as the extent of excavation below the burrow is variable between species. (Drawing by R. E. Kochevar.)

dm^3 of sediment), and suggested that the clams might either enrich sulfide production in their immediate environment through the production of pseudofeces, or utilize products such as thiosulfate which result from the partial oxidation of the iron sulfides in the sediments in contact with their feeding tube. Additionally, Reid and Brand[159] suggest that lucinids may use the sediment bound, acid-labile sulfides, by ingesting the sediment into their guts where soluble sulfides are released in the acidic environment. Which one (or combination) of these routes for the acquisition of reduced sulfur compounds is actually employed by the lucinids remains to be experimentally verified.

The symbionts of the lucinids are located in vacuoles in bacteriocytes, found in the subfilamental region of the gills. The number of bacteria per vacuole varies between species of lucinids. There is normally one bacterium per vacuole (occasionally two) in *Lucinoma borealis* and *Myrtea spinifera*.[37,163] Giere[153] reports one or a few bacteria per vacuole in *Anodontia philippiana* and three species of *Lucina* from Bermuda, and Distel and Felbeck[157] report "tens" of bacteria per vacuole in two species of *Lucinoma* from California. Lucinid gills consist of a single large demibranch on each side of the clam. In *Lucinoma aequizonata* and *Myrtea spinifera* the gills comprise about 35 and 23%, respectively, of the wet weight of the clam soft tissues.[37,157] Lucinid gills range in color from pink through red, and brown to a creamy beige.[153,157,163] The ultrastructure of the gills and bacteriocytes of a variety of lucinids has been described, based primarily on transmission electron micrographs of the gill filaments.[37,47,153,159,163] However, Distel and Felbeck[157] present a much more complete, three dimensional description of the structure of the gills of three species of lucinids. This proposed three dimensional structure seems compatible with all other published micrographs of lucinid gills, and therefore the following description (and Figure 8) is taken primarily from that work.[157] The outer surface of each demibranch is covered by a ciliated, ctenidial filament zone (CFZ) which has a structure typical of eulamellibranch clams. Immediately below the CFZ is a zone termed the transition zone by Distel and Felbeck.[157] This zone is at most a few cells thick, and consists of cells which are structurally intermediate between the overlying CFZ and the bacteriocyte zone. Cells in the transition zone are not ciliated and do not contain symbionts. The cells of the bacteriocyte zone are arranged in tubular stacks, termed bacteriocyte cylinders (Figure 8D). These cylinders are continuous through the gill lamellae and allow the free passage of sea water between the mantle cavity and the interlamellar space, thus allowing ventilation of all bacteriocytes. Bacteriocytes are the dominant cells in this zone. The size of the bacteriocytes varies between species. Bacteriocytes in four species of lucinids from Bermuda are between 12 to 20 μm in height and 6 to 14 μm in width.[153] Distel and Felbeck[157] report bacteriocytes from *Lucinoma aequizonata, L. annulata* and *Lucina floridana* ranging from 20 to 40 μm in diameter and Fisher and Hand[47] report that the bacteriocytes in *L. floridana* range up to 60 μm in diameter. The symbionts are located in vacuoles in the external-most portion of the bacteriocytes (closest to the central bacteriocyte channel), with the nuclei displaced towards the opposite end of the cells.[153,157,163] Myelin-like figures are also visible in many bacteriocytes suggesting lysosomal digestion of senescent symbionts.[47,153,157,158,163] Squeezed between the bulging bacteriocytes are intercalary cells (termed "normal" cells by Giere[153]). Intercalary cells do not contain symbionts and are covered with microvilli on their external surface. Several authors report that the bacteriocytes themselves are also covered by microvilli on their external surface and are directly exposed to sea water with either no[153] or only partial coverage of the bacteriocytes by extensions of intercalary cells.[37,163] Other workers disagree and report that the surface of the bacteriocytes facing the bacteriocyte channels are covered by thin extensions of the intercalary cells from which the microvilli arise, and therefore the bacteriocytes are never directly exposed to sea water.[47,157] Since two conflicting reports concern the same species,[47,153] this difference in opinion concerning microvilli and exposure of the bacteriocytes to sea

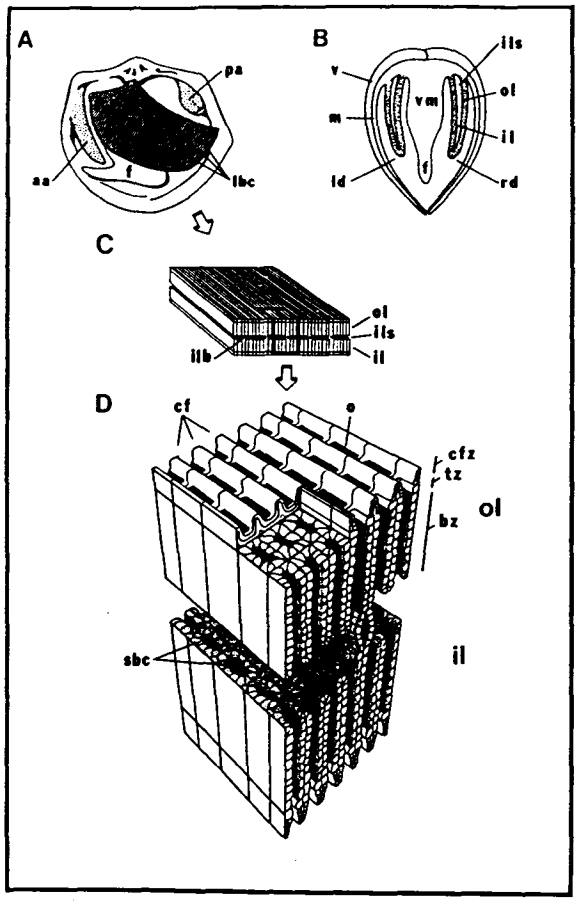

FIGURE 8. *Lucinoma aequizonata*. Structure of the gills. (A) Clam with left valve removed. (B) Transverse section through entire clam at median point of anterior-posterior axis. (C) Enlargement of block of gill tissue removed from region indicated by box in (A). (D) Enlargement of block of gill tissue removed from region indicated by box in (C). aa, anterior adductor; bz, bacteriocyte zone; cf, ctenidial filaments; cfz, ctenidial filament zone; f, foot; il, inner lamella; ilb, interlamellar bridge; ils, interlamellar space; lbc, large bacteriocyte channel; ld, left demibranch; m, mantle; o, ostia; ol, outer lamella; pa, posterior adductor; rd, right demibranch; sbc, small bacteriocyte channel; tz, transition zone; v, valve; vm, visceral mass. Figure 8 is from Distel and Felbeck.[157]

water may be due to fixation artifacts (such as shrinking of intercalary cells) as well as representing true differences between species. The proposed three dimensional structure, as well as the generally recognized gill structure (with the ciliated cells on the external surface of the lamellae) has been suggested to function in maintaining a favorable, low oxygen tension around the bacteria.[47,153,157]

A variety of different sizes and shapes of bacterial symbionts with cell wall ultrastructure characteristic of Gram-negative bacteria have been reported in the Lucinidae (Table 4). In general the symbionts are fairly large and pleomorphic. A number of different types of particles and inclusions have been reported in the symbionts of the Lucinidae. Elemental sulfur (often represented by empty vesicles in TEM sections) is often reported in invaginations of the plasma membrane of the symbionts.[37,88,157,159,163] Electron dense particles ranging from 40 to 80 nm in diameter are also reported in many symbionts and various authors suggest these may be either glycogen particles or clumped ribosomes[37,163] or carboxysomes.[88,153] Other larger granules visible in the bacterial cytoplasm are suggested to contain bacterial storage products such as polyphosphates.[37,153,163]

b. MYTILIDAE

There are apparently four different species of mussel currently known to contain symbiotic chemoautotrophic or methylotrophic symbionts. As of this writing only one of these has been described taxonomically, *Bathymodiolus thermophilus*, the hydrothermal vent mussel.[171] Another mussel has recently been discovered at the Mariana back arc basin[172] and preliminary evidence indicates that it may harbor chemoautotrophic symbionts (personal observation). Two other species of mussels, one found at the Florida Escarpment Site,[58] and another from hydrocarbon seeps in the Gulf of Mexico,[77] both contain methylotrophic symbionts.[12,18] These two species are distinct (personal communication, R. Turner, Harvard Museum of Comp. Zool.), and the details of their methylotrophic symbioses are covered in a later section.

Bathymodiolus thermophilus was described from material collected at the Galápagos Rift, and published ultrastructural studies of the gills of mussels collected at 13°N on the East Pacific Rise indicate that these are probably the same species, with ultrastructurally similar symbionts.[21,173,174] Stable carbon isotope values,[6,45] histological studies,[21,173,174] enzyme analysis,[21,31,45] thiosulfate stimulation of ATP production[40] and carbon fixation[43,175] all indicate that the symbionts in this mussel are chemoautotropohic. However, the levels of autotrophic enzyme activities, stimulated ATP production and stimulated carbon fixation are quite low when compared to other vent invertebrates with symbiotic chemoautotrophs (see References 21 and 45). Reconciliation of these low activities with the abundance of symbionts in the mussel gills must await further experimentation. Another anomalous fact concerning this chemoautotrophic symbiosis is the lack of elemental sulfur in the gills of all individuals investigated (n = 27, collected from a variety of *in situ* conditions).[45] Although levels of elemental sulfur can vary from undetectable to over 3% of the gill wet weight within individuals of a given species of bivalve with chemoautotrophic symbionts, to my knowledge this is the only association of this sort in which elemental sulfur has not been demonstrated, despite numerous attempts.

At hydrothermal vents where *B. thermophilus* is found it is widely distributed both spatially and with respect to water

Table 4
Bacterial Symbionts of the Lucinidae

Species	Shape	Length	Width	Diameter	Ref.
Anodontia philippiana	oval	1.3—3.2	1.0—1.1		153
	rod	5.5—6.0	0.4—0.65		
Codakia orbicularis	rod	1—2			22
Lucina costata	rod	1.8—4.4	0.5—0.8		153.
L. floridana	rod and sphere	4—7	1—2	3—4	88
	rod	4—6	1—1.5		47
L. multilineata	oval	2.2—5.4	1.1—2.3		153
	rod	8.9	0.9		
L. radians	rod	1.8—6.4	0.5—3.1		153
Lucinella divaricata	oval			0.4—1.7	158
*Lucinoma aequizonata**	rod and sphere	4—7	1—2	3—4	88*
	rod	4—10	2—5		157
L. annulata	rod to oval	4—10	2—5		157
L. borealis	oval			≤4.0	163
Myrtea spinifera	rod	4—9	0.4—2.8		37
Parvilucina tenuisculpata	oval	~3	~0.5		159

Note: All dimensions are given in μm.

* Incorrectly identified as *L. annulata* in Reference 88.

chemistry, although it has never been found outside the vent environment.[45,176,177] These mussels have been collected from among tube-worms where water temperatures can reach 14°C, from peripheral areas in a vent field where both temperature and sulfide anomalies were undetectable, and from a range of intermediate environments.[45,178] Their distribution within the vent field indicates that, unlike *Riftia pachyptila* and *Calyptogena magnifica* which both require rather specific conditions of vent flow to flourish,[74,79] *B. thermophilus* is able to survive under a wide range of conditions.[45] Their ability to tolerate this wide range of conditions has been explained by their ability to filter feed, an ability first attributed to the mussels based on anatomical studies and gut content analysis,[173,179] and later verified experimentally.[180] Another possible source of nutrition for these mussels is uptake of dissolved organics, and Fiala-Medioni et al.[175] have demonstrated uptake of labeled amino acids from relatively low environmental concentrations. Evidence for differential incorporation of organic carbon from the three sources available to the mussels (symbiotic, particulate, and dissolved) is found in a comparison of the stable carbon isotopic composition of the animal tissues of mussels collected from different sites within the Rose Garden hydrothermal vent field, where mussels collected from more peripheral clumps had a significantly heavier $\delta^{13}C$ than mussels collected from central sites.[45] Despite their multiple nutritional strategies, these mussels are never found outside the vent field, and when collected from very peripheral environments are in a poorer nutritional state than similar mussels collected from "warmer" more central areas in the same vent site.[45,178]

Evidence for multiple "strategies" of nitrogen assimilation is also found in stable isotopic studies of the mussels from the Rose Garden hydrothermal vent field.[45] Mussels collected from the central (warmer) sites were significantly lighter (average $\delta^{15}N$ of $-3.9‰$) than mussels from a nearby peripheral clump (averaged $+3.5‰$). The $\delta^{15}N$ values of the possible organic and inorganic nitrogen sources available to the mussels at the hydrothermal vents are not currently known, but the authors conclude that the negative $\delta^{15}N$ values in mussel tissues is probably due to incorporation of nitrogen derived from nitrogen gas either assimilated by free living bacteria on which they feed or directly by the symbionts.

Detailed ultrastructural studies of the gills of both *Bathymodiolus thermophilus* and the hydrocarbon seep mussel have been published.[21,174] The gills of both species are composed of numerous filaments with abundant ciliation on the lateral and frontal edges. The filaments are held in place by ciliary tufts on their lateral faces. The bulk of the lateral faces of both species are covered by large bacteriocytes (containing the symbionts) which alternate with symbiont-free intercalary cells (Figure 9). The bacteria are found in vacuoles in the apical portion of the bacteriocytes in both species. The seep-mussel bacteriocytes house fewer bacteria, with only one to three bacteria visible per vacuole in cross section. *B. thermophilus* bacteriocytes are larger and contain hundreds of bacteria per cell, with many (>20) bacteria often visible in a single vacuole in cross section (Figure 9). Additionally, the vacuoles in *B. thermophilus* bacteriocytes are often interconnected and may in fact be a single large vacuole.

B. thermophilus symbionts are small cocci or short rods ranging from 0.3 to 0.75 μM in diameter, with a cell wall ultrastructure typical of gram negative bacteria (Figure 9).[21,174] The symbionts in the hydrocarbon-seep mussel and the major

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RuBPC/O is restricted to the symbionts in *S. velum*, a fact which autoradiographic analysis of short term incubations of *S. reidi* in $NaH^{14}CO_3$ indicate is most likely the case for that species as well.[35] Transfer to symbiont-fixed carbon to host tissues has been demonstrated by Fisher and Childress,[35] using two different C-14 tracer techniques. The kinetics of the carbon transfer indicates that the symbionts translocate a portion of the carbon they fix to their host in a manner analogous to the well-studied algal-invertebrate associations.[35] The possibility that digestion of symbionts also contributes to the nutrition of the host cannot be ruled out and is in fact suggested by the presence of "myelin-like" structures in the gill cells, as similar structures in other symbioses have been suggested to be lysosomes containing the remnants of bacterial digestion.[174] The $\delta^{13}C$ values of paired gill and non-gill tissue from four *S. reidi* were very close although the gills were consistently about 1‰ more negative than the non-gill tissue (average 32‰) (personal communication, J. M. Brooks, Texas A & M University). Similar $\delta^{13}C$ values have been reported for gill and foot tissue

several ways: the incurrent tube is semi-permanent (unlike Lucinids which construct new ones every few days); their burrows have no excurrent tube to the surface; and they construct a more elaborate network of narrow tunnels radiating out from their living chamber than do the lucinids.[32,167] Due to similarities in habit and habitat to those of the lucinids, the same hypotheses have been advanced to explain how these bivalves acquire reduced sulfur species to fuel chemoautotrophy (see section on Lucinidae preceding). Southward[163] suggests that these clams may take up reduced sulfur species across their vermiform foot from the underlying sediments (as suggested by others for lucinids),[95,170] although she notes that the symbionts' proximity to the external environment suggests that direct uptake of such compounds by the symbionts is likely.

As mentioned, the thyasirid symbionts do not appear to be intracellular. Although some authors disagree[159,191] Southward's micrographs are quite convincing (Figure 13A,B).[163] Both Herry and Le Pennec[191] and Reid and Brand[159] state that the symbionts are located in a single large

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Table 5
Collection Sites of Living Vesicomyidae

Collection site	Species	Collection depth	Ref.
Hydrothermal vents			
Galapagos Rift	*Calyptogena magnifica*	2400—2700 m	79, 193
21°N East Pacific Rise	*C. magnifica*	2600 m	177
Guaymas Basin	*Vesicomya gigas*[a]	2667 m	195
Juan de Fuca ridge	*C. pacifica*		196
Subduction zones			
Oregon	*C. magnifica*	2036 m	59
Nankai, Japan and Kurile trenches	*C. phaseoliformis*	5600—6000 m	197, 205
Nankai, Tenryu Canyon	*C. nautilei, C. laubieri, C. kaikoi*	3780—4020 m	198, 201, 205
Cold seeps			
Gulf of Mexico, hydrocarbon seeps	*C. ponderosa, V. cordata*	600—940 m	77, 200
Florida escarpment, saline seeps	*C. sp.*	3266 m	58
Other sites			
San Diego fault vents	*V. gigas*[a]	1750 m	31, 202
Santa Barbara Channel, CA	*C. elongata*	500 m	88, 193
Southern CA to Alaska	*C. pacifica*	55—1244 m	193, 199
Sagami Bay, Japan	*C. soyoae*	750—1100 m	203
Caribbean Sea	*C. ponderosa*	421—1767 m	193
66 miles off Colombia	*C. modioloforma*	42—641 m	193
British Columbia, Canada to N. CA	*C. kilmeri*	549—1464 m	193
Sagami Bay, and Coast of Japan	*Akebiconcha kawamurai*[b]	≥200 m	193, 204

[a] R. Turner, Harvard, pers. comm., previously identified as *C. pacifica*.[31,195,202]

[b] Taxonomy in dispute, may be a synonym of *C.*

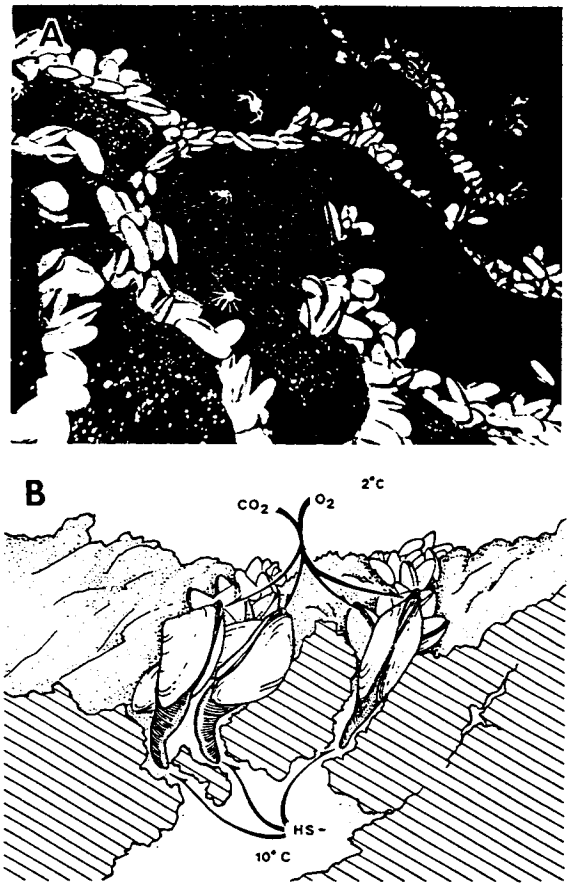

FIGURE 14. *Calyptogena magnifica.* (A) *In situ* photograph from the "Clam Acres" ste at 21°N on the East Pacific Rise. (A. Arp photographer.) (B) Schematic representation of *C. magnifica in situ.* (From Arp et al.[206])

Colonies of vesicomyids were also observed and sampled in the Japan subduction zone at depths up to 5960 m. These colonies were oval in outline, up to 60 cm in the longest dimension, and rough density estimates[201] suggest some colonies ("nurseries with relatively small individuals") may contain as many as 1000 to 2000 individuals/m^2. Reports of the orientation of the clams *in situ* are similar to the reported orientation of the vesicomyids on the Louisiana slope and *C. elongata*,[200,201] however their densities are considerably higher. Positive temperature anomalies of 0.2 to 0.6°C within the sediments indicate that these colonies may be the site of localized channeling of hydrothermal fluids to the surface, an observation which may explain the high densities of clams at these sites.

C. elongata is another vesicomyid which apparently uses a similar method for the simultaneous acquisition of sulfide and oxygen. We have collected *C. elongata* by trawling from reducing sediments at the interface between deep anoxic waters and overlying oxic waters (550 to 570 m) in the Santa Barbara Channel. These small elongate vesicomyids orient themselves vertically in the substrate with the anterior two thirds of their shell buried in the reducing mud. These vesicomyids are also able to bridge the oxygen sulfide interface by extending their foot into the substrate while positioning their incurrent siphon in the overlying sea-water. Again, evidence of their orientation *in situ* is provided from examination of their shells (and by their behavior in the laboratory; personal observation). No periostracum remains on the portion of the shell which is buried in the substrate but it is clearly evident on the posterior one third of the shells of freshly collected living specimens (personal observation). Thus, in all of these vesicomyids sulfide and oxygen are apparently acquired simultaneously, sulfide through the foot and oxygen across the gill.

Methanotrophic symbioses have been suggested for several species of vesicomyids based on measurements of tissue $\delta^{13}C$.[59,61] However, based on our present general knowledge of vesicomyid physiology and ecology, information concerning the specific habitats involved and current information about the species in question, there is no reason to postulate a new type of association in a family of clams highly evolved for chemoautotrophic (sulfur-oxidizing) symbiosis. As discussed in Section II. C., stable carbon isotope values of animal tissues can be misleading, due to the variety of pathways which may lead to the same isotopic "signature." The first suggestion of a methanotrophic symbiosis in a vesicomyid was made by Kulm et al.[59] for *Calyptogena magnifica* collected from the Oregon subduction zone, based on a $\delta^{13}C$ value of $-51.6‰$ for gill tissue and $-35.7‰$ for periostracum. The blood of this species accumulates sulfide and does not bind methane.[206] This is important because in the absence of a methane accumulating and transport system in the clam, high concentrations of methane would be required to support the dense populations of symbionts in the gills. Enzyme activities in the gills of *C. magnifica* collected from other sites indicate the presence of chemoautotrophic sulfur-oxidizing symbionts, and the gills also contain appreciable amounts of elemental sulfur.[79] According to Kulm et al.[59] the pore waters in the sediments around the clams show "nutrient patterns characteristic of microbial sulfate reduction" (which would include elevated sulfide concentrations) and only slightly elevated methane (the highest measurement was 418 nl/l or 0.02 μM in bottom waters). They also calculate that as much as 30% of the total CO_2 (ΣCO_2) in interstitial waters results from microbial decomposition of methane. This implies a ΣCO_2 significantly depleted in ^{13}C and when combined with the well known discrimination against ^{13}C by chemoautotrophic mircoorganisms, provides a more reasonable (and well documented) explanation for the measured *C. magnifica* tissue $\delta^{13}C$ values: symbiotic sulfur oxidizing

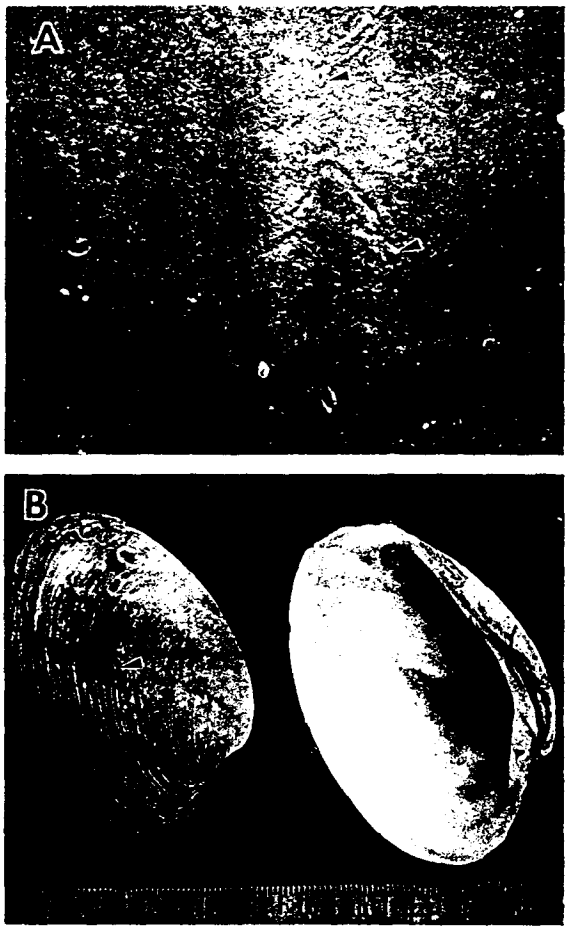

FIGURE 15. (A) Vesicomyid trails on the Louisiana Slope in the Gulf of Mexico at approximately 1000 m depth. (Photograph by I. MacDonald.) (B) *Calyptogena ponderosa*. Shell with living tissue freshly removed. Note dark line on inside of valve which disappears within 2d after exposure to air. Arrow indicates position of orange line on external surface of shell. Note encrusting organisms in area of exhalent opening. (Photograph by G. Boland.)

bacteria using isotopically light pore water CO_2 as a carbon source.

Methanotrophic symbioses have also been suggested for the vesicomyids discovered on the Japan Subduction Zone.[61,201,205,208] Once again this was based on the presence of methane in the sediments and overlying waters, and the stable carbon isotopic composition of the clam tissues (-37.8 to -40.1‰ for soft tissues and -1.7 to -6.4‰ for shells).[61] There is also evidence for a sulfide based symbiosis in these clams given in the same papers. Boulègue et al.[61] report the presence of elemental sulfur in the gills and show micrographs of symbionts which are morphologically similar to those found in documented sulfur-based vesicomyids. These features were also noticed in a more in-depth ultrastructural study of the gills and symbionts of these vesicomyids,[164] and those authors conclude that a sulfur based symbiosis is much more likely. Boulègue et al.[61] also calculate that the $\delta^{13}C$ of ΣCO_2 is between -10 and -30‰ in the sediments below the clams, but are apparently unaware of the discrimination against ^{13}C by chemoautotrophs[56] because they site this as evidence against CO_2 being the carbon source for the clams, when in fact it supports that probability. Finally, similar $\delta^{13}C$ values have been reported for *C. magnifica* (-31.4 to -34.4‰), *C. ponderosa* (-31.2 to -39.1‰) and *V. cordata* (-36.3 to -39.8‰) all of which contain chemoautotrophic sulfur-oxidizing symbionts.[34,77,79]

Vesicomyid gill lamellae are composed of numerous filaments which are connected by inter-filamental bridges and are ciliated on their external surfaces. Adjacent filaments are separated distally and their lateral surfaces are composed entirely of bacteriocytes with occasional intercalary cells. A complete description of the ultrastructure of the gills of *C. magnifica* and the Japanese subduction zone bivalves *C. laubieri* and *C. phaseoliformis* can be found in Fiala-Médioni and Métivier[166] and Fiala-Médioni and Le Pennec,[164] respectively. The bacteriocytes are covered with microvilli on their external face and are literally packed with bacteria, with numbers of bacteria per host vacuole ranging from one to several. A diagrammatic representation of a bacteriocyte is shown in Figure 16. This general arrangement of symbiotic bacteria in vacuoles located throughout the bacteriocytes with a basal nucleus and few other organelles other than abundant lysosomes seems to hold for

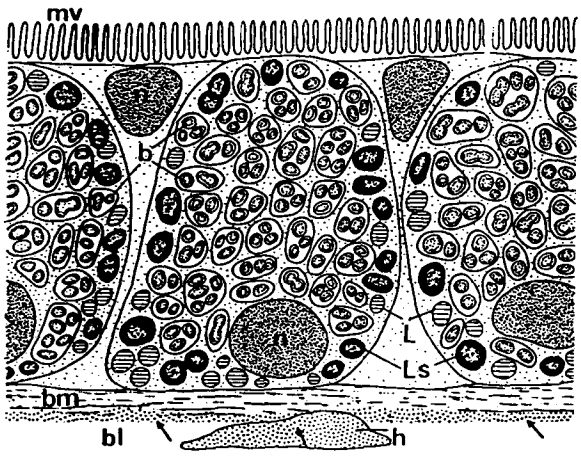

FIGURE 16. Schematic representation of typical vesicomyid bacteriocyte. b, bacteria; bl, blood lacuna; bm, basal membrane; h, hemocyte; L, lipids; Ls, lysosomes; mv, microvilli, n, nucleus. From Fiala-Médioni and Le Pennec.[209]

all of the vesicomyids examined[209] (pers. obs of *C. ponderosa* and *V. cordata*). Fiala-Médioni and Le Pennec[209] suggest that this ultrastructure represents a more advanced (highly integrated) symbiosis than that found in hydrothermal vent mytilids, citing mainly the relative absence of cellular organelles and preponderance of bacteria in vesicomyid bacteriocytes, and the difference in particulate feeding ability between the two groups for this hypothesis.

The bacteria in all vesicomyid species examined are morphologically similar. The symbionts are small (≤1 μm), polymorphic, contain elemental sulfur deposits, and are abundant throughout the cytoplasm of the host cell (Figure 17).[17,36,88,164,166] Division stages of the symbionts are often apparent.[61,164,166]

2. Gastropoda

A chemoautotrophic symbioses has recently been described in a gastropod collected from hydrothermal vents at the spreading center of the Mariana Back-Arc Basin.[210] The gastropod, *Alviniconcha hessleri*, was the dominant organism in the warm water of the flourishing hydrothermal-vent community at the "Snail Pits" site at 3670 m.[172,211] The shell of this gastropod ranges up to about 5 cm in length and is covered with spirally arranged periostracal hairs.[211] The hypertrophied gills account for about 40% of the body wet weight and harbor abundant symbiotic bacteria. The symbionts are intracellular and in the published micrographs appear to be normally enclosed singly in host vacuoles, although vacuoles with more than one symbiont can be seen at the base of the bacteriocytes. The outer surface of the bacteriocytes are covered with microvilli and intercalary cells are not evident in the published micrographs.[210] The dominant symbionts are rod-shaped Gram-negative bacteria, up to about 5 μm in length and apparently

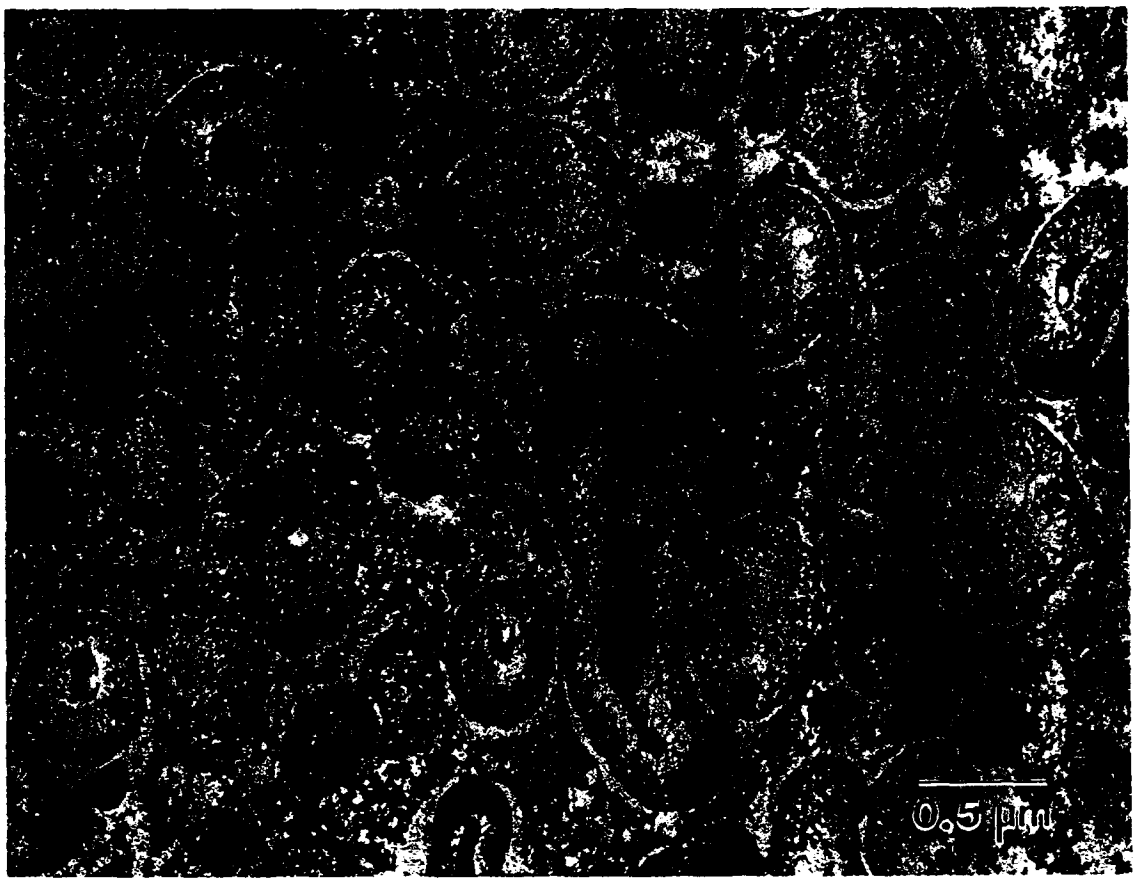

FIGURE 17. *Calyptogena ponderosa*. Transmission electron micrograph of section through a gill bacteriocyte showing many polymorphic symbiotic bacteria.

contain elemental sulfur.[210,212] Enzyme activities, elemental sulfur levels, and the bacterial morphology all support the contention that the symbionts are chemoautotrophic.[210] Those authors[210] found no activity of methanol dehydrogenase in the frozen samples of gill tissue. However Ohta et al.[212] recently reported on the presence of a second morphological type of symbiont in the snail's gills which resembles the symbionts found in the described methanotrophic symbioses.[12,16,18] The second morphological type of symbiont is relatively rare, and even if it is methanotrophic, may not be abundant enough for methanol dehydrogenase to be detected in gill homogenates.

Bacterial colonization of, and endocytosis of the bacteria by the gill of an archaeogastropod limpet, *Leptodrilus fucensis*[213] from the Juan de Fuca Ridge hydrothermal vents was described in 1984.[214] Anatomical studies suggest a functional gut, worn radula, and the ability to feed on suspended particles as well.[215] There is no evidence at this time, other than their habitat, that the epibionts are chemoautotrophic. De Burgh and Singla[214] postulate that this species might represent an intermediate stage in the establishment of a symbiotic association, and it is mentioned here because of these possible evolutionary implications.

IV. METHANOTROPHIC SYMBIOSES

Methanotrophic (or methylotrophic) symbioses have now been well documented in three species of marine invertebrates: an undescribed mussel found associated with hydrocarbon (and methane) seeps on the Louisiana Slope;[12,21] a different undescribed mussel collected from the vicinity of hypersaline seeps at the base of the Florida Escarpment,[18,75] and a recently described pogonophoran, *Siboglinum poseidoni*, collected from the central Skagerrak.[16,134] Location and ultrastructure of the symbionts in their host tissues are described in the preceding sections dealing with the host groups. As previously pointed out the ultrastructure of the symbionts in these symbioses is unique (Figure 10) and provides independent supporting evidence of the methanotrophic nature of the symbionts. The same types of evidence used to demonstrate a chemoautotrophic symbiosis can be applied to the demonstration of a methanotrophic (or methylotrophic) symbiosis, and like chemoautotrophic symbioses a single line of evidence is not sufficient to conclude that a given symbiosis is methanotrophic. The distinction made here between methylotrophic and methanotrophic symbionts stem from the fact that the enzyme which catalyses the oxidation of methane (methane mono-oxygenase) is unstable when frozen,[216] and therefore demonstration of methanotrophy (versus just methylotrophy) requires living material or fresh tissue. Since neither enzyme assays or other relevant experiments have yet been conducted using fresh tissue, or live Florida Escarpment mussels, one can only conclude that this mussel is methlotrophic,[18] although the abundant methane in its environment and its stable carbon isotopic content would suggest it is methanotrophic as well.[75]

The first enzyme in the oxidation of methane by methanotrophic organisms is methane mono-oxygenase (EC 1.14.13.25). As noted above this enzyme is very labile[216] and it has not been directly demonstrated in any of the methanotrophic symbioses (only frozen tissues have been assayed). However, activity of methane mono-oxygenase can be inferred in two of the described symbioses because oxidation of methane by isolated tissues and whole animals has been documented.[12,21,134] Oxidation of methane has been demonstrated using two different methods. Consumption of methane (with concomitant increase in oxygen consumption and CO_2 production) from sea water around isolated gill pieces and live mussels has been documented for the mussel from the Louisiana Slope using gas chromatographic analysis of the dissolved (unlabeled) gases.[12] Oxidation of ^{14}C-methane to $^{14}CO_2$ and ^{14}C-organic carbon has been demonstrated in gill tissue from the same mussel[21] and for both whole animals and homogenized tissue from the pogonophoran.[134] When oxidation of methane cannot be documented (such as when only frozen and preserved tissues are available for study)[18] then activity of methanol dehydrogenase can be assayed. Methanol dehydrogenase (EC 1.1.1.1) catalyzes the second step in the oxidation of methane, the oxidation of methanol to formaldehyde, and is found in all methanotrophic and methylotrophic organisms.[217] This enzyme is stable when frozen and activity of this enzyme indicates the presence of methylotrophic symbionts[18] and supports the presence of methanotrophic symbionts.[21]

There are two main pathways of carbon assimilation in methane oxidizing organisms. Type I organisms assimilate carbon at the oxidation level of formaldehyde by the ribulose monophosphate pathway and Type II organisms use the serine cycle.[217] Cavanaugh et al.[18] found high levels of activity of hexulose phosphate synthase (a key enzyme in the ribulose monophosphate pathway), and variable or no activity of two enzymes of the serine pathway in the Florida seep mytilid. This supports the contention that the major symbiont in the Florida seep mussel is a Type I methanotroph, a fact which is further supported by its morphology. Interestingly, morphology typical of Type I methanotrophs (stacked internal membranes) has been documented in all three of the documented methanotrophic symbionts.[12,18,134]

In methanotrophic symbioses the distinctive carbon isotope "signature" is a reflection of the isotopically light carbon source (methane) utilized by these organisms. Both biotic (biogenic) and abiotic (thermogenic) processes that produce methane discriminate heavily against ^{13}C which results in distinctive isotopic "signatures" for methane. Thermogenic methane $\delta^{13}C$ values are typical $> -50‰$ and biogenic methane (produced by methanogens) $\delta^{13}C$ values are typically $< -50‰$.[219,220] There has been little published on isotopic fractionation of carbon from methane into cell carbon by free-living methanotrophic and methylotrophic organisms. The few numbers in the literature[221,222] indicate that this fractionation is relatively small

(−2.4 to −5.6‰) and the $\delta^{13}C$ of microorganisms using methane as a carbon source therefore basically reflects the source methane $\delta^{13}C$. This appears to hold true for all of the currently described methanotrophic symbioses.[12,34,60,75] The $\delta^{13}C$ values for the Florida seep mussel (−74.3 ± 2.0‰, n = 10) and the pogonophoran (−73.6 and −74.4‰) reflect the biogenic methane in their environments.[60,75] When the methane in a bubbling gas stream and the mussels living adjacent to it were both analyzed, the mussel $\delta^{13}C$ (−40.6‰) very closely reflected the $\delta^{13}C$ of that methane stream (−41.2‰).[34]

The ability of the Louisiana Slope mussel to grow with methane as sole carbon and energy source has recently been demonstrated. Cary et al.[98] used a laser diffraction apparatus to measure shell growth in small (<2 cm) mussels. They report growth rates increasing from zero to up to 17.2 μm/d in response to methane.

Methanotrophic symbioses have been suggested in several other hosts from two other habitats based solely on the stable carbon isotope values in the animals tissues and the presence of slightly elevated methane in the environment.[59,61,201,208] The case for the vesicomyid clams is discussed in Section III.E.1.e. of this review. A vestimentiferan (*Lamellibrachia barhami*) from the Oregon subduction zone has also been suggested to contain methanotrophic symbionts based on its $\delta^{13}C$ value of −31.9‰.[59] Arguments against the proposed methanotrophic symbiosis and an alternative explanation for the $\delta^{13}C$ values in this worm are essentially the same as those given for the vesicomyid clam from the same environment. Additionally, $\delta^{13}C$ values ranging from −27 to −47‰ have now been reported in the two species of vestimentiferans (one of which is in the same genus) from the Louisiana Slope, and *Escarpia laminata* from the Florida escarpment, all of which harbor confirmed chemoautotrophic sulfur-oxidizing symbionts,[34,57,75,97] and all of which come from environments with elevated methane concentrations.[12,34,75] Furthermore this same species has been collected from the San Diego Trough and shown to harbor chemoautotrophic (sulfide oxidizing) symbionts.[31,36] Clearly the stable carbon isotope values alone are not sufficient to demonstrate a methanotrophic symbiosis, especially in Vestimentiferan, a phylum of worms highly adapted for sulfide-oxidizing symbionts.

V. EVIDENCE FOR NUTRITIONAL EXCHANGE BETWEEN SYMBIONT AND HOST

Ever since the discovery of chemoautotrophic symbioses in the major sessile hydrothermal vent fauna, investigators in the field have hypothesized that the symbiotic bacteria are contributing to the nutrition of their hosts. A large amount of indirect evidence has been compiled over the last ten years which supports that hypothesis for a variety of symbioses. However, very little direct evidence of nutritional transfer from symbiont to host has been published and in most cases the details of nutritional interactions within the symbioses are vague at best. Indirect evidence supporting the hypothesis that the symbionts contribute to the nutrition of their host falls into four categories: host anatomy, host habitat, stable isotope contents of animal tissue, and ultrastructural studies of the symbiont-containing tissues.

Many of the hosts of chemoautotrophic symbionts have lost the ability to feed by conventional means. Adult vestimentiferans, pogonophorans, a variety of other interstitial worms, and several species of bivalves have no gut, mouth, or anus.[99,125,147,152,181,184] Most of the other bivalves with chemoautotrophic symbionts have substantially reduced guts, labial palps, and associated structures for particulate feeding.[103,159,170] Before the discovery of chemoautotrophic symbioses, experimental evidence suggested that the smaller pogonophorans could potentially live on DOM alone, due to their small size and large surface to volume ratio.[128,129] However, this mode of nutrition was clearly inadequate for the larger gutless vestimentiferans and gutless clams like *Solemya reidi*. The discovery of chemoautotrophic symbionts in these gutless species (and other species with reduced particulate feeding ability) provided an attractive hypothesis for the nutrition of the intact symbioses, but anatomical considerations are only circumstantial evidence for this hypothesis.

Another line of reasoning supporting the hosts' reliance on their symbionts for nutrition is the distribution of the hosts. They are only found in reducing environments which can support symbiont chemoautotrophy. Since these environments are often quite hostile (with elevated levels of hydrogen sulfide) there must be an overwhelming reason to occupy these habitats. Studies on *Bathymodiolus thermophilus* (the hydrothermal-vent mussel) indicate that the host condition is worse in mussels collected from peripheral vent environments (compared to mussels collected from central vent environments) and deteriorates in animals experimentally removed from contact with vent water.[45,178]

The stable carbon and nitrogen isotopic contents of chemoautotrophic symbioses provide some very compelling circumstantial evidence for nutritional carbon and nitrogen transfer from symbiont to host. This topic has recently been reviewed by Southward[103] and I only briefly cover it here. When paired samples of symbiont-containing and symbiont-free tissues from a particular host are compared, inferences can be drawn concerning nutritional exchange between partners. This type of analysis is possible because of the distinctive "isotopic signatures" of chemoautotrophic and methanotrophic organisms (see Section III.C). This approach has been used to infer nutritional interactions, at different levels, in a number of chemoautotrophic and methanotrophic symbiotic associations, including vestimentiferans,[7,34,74] pogonophorans,[13,55] and a variety of bivalves.[7,12,34,45,55,79,95,162] However, it is not appropriate to use this technique for quantitative analysis of the input of

symbiont derived organics (cf. References 95 and 162), because free-living chemoautotrophs or methanotrophs found in the hosts environment would have similar isotopic "signatures" to the symbionts, as would dissolved organic carbon derived from free-living methanotrophs and chemoautotrophs. It is, therefore, impossible to distinguish between these three sources of organic carbon and nitrogen by this technique. This is not a minor consideration because the possibility of free-living chemoautotrophs becomes a probability in most of the environments where chemoautotrophic and methanotrophic symbioses are found. However, one can sometimes determine minimum values for the input of non-chemoautotrophic carbon using stable carbon isotopes. Comparison of the $\delta^{13}C$ values of isolated symbionts and symbiont-free host tissues led Cary et al.[95] to conclude that at least 25% of the organic carbon in *Lucinoma aequizonata* is not provided by the symbionts.

Host anatomy, distribution, and stable isotopic composition of host tissues, all suggest that the symbionts are contributing to the nutrition of their host but do not suggest what the mechanism of this interaction might be. There are two obvious ways whereby organic compounds synthesized by the symbionts could be utilized by the host. Either the symbionts could translocate a portion of the carbon and nitrogen they incorporate across their cell walls and into the host in a manner analogous to many algal-invertebrate associations[223] or the host could digest its symbionts. If a host receives only translocated organic carbon compounds from its symbionts (and does not assimilate digested symbionts) then that host may also need additional feeding strategies to procure essential amino acids, essential fatty acids, and other compounds which it cannot synthesize for itself. This is because it is unlikely that a symbiont which is permeable to all of these compounds could survive. Depending on the specific compounds translocated from the symbionts, the host might also require a source of bulk nutritional nitrogen.[224,225] Ultrastructural studies suggest that digestion of the symbionts occurs in a variety of chemoautotrophic symbioses. Both Bosch and Grassé[119] and Hand[94] report "development stages" of bacteria within lobules of the trophosome in *Riftia pachyptila*. These stages include degeneration of the symbionts towards the periphery of the lobules and digestion of the symbionts in lysosomes, with resultant "myelin-like bodies" (concentric membranes inside of a vacuole, which are the remains of digested bacteria according to Bosche and Grassé[119]). Fiala-Médioni et al.[174] report visible digestion of symbionts in lysosomes in the hydrothermal vent mussel, *Bathymodiolus thermophilus* (Figure 9D). Degenerate symbionts, abundant lysosomes, and myelin like bodies have been reported, or are visible in the published micrographs, of many other intracellular chemoautotrophic symbioses and are reviewed in previous sections.

Giere and Langheld[150] describe a functionally similar pattern for the extracellular, subcuticular symbionts of the oligochaete *Inanidrilus* (= *Phallodrilus*) *leukodermatus*. Actively dividing, apparently healthy symbionts are found in a peripheral, subcutaneous "zone of bacteria" (Figure 6). Immediately below this zone the host epidermal cells tend to encircle the symbionts with long strands of cytoplasm. In an inner "zone of lysis" the cytoplasm of the host epidermal cells often contains the remains of degenerate, progressively lysed bacteria, and no healthy bacteria are found in this zone. Similarly, Southward[163] describes phagocytosis of the extracellular symbionts of some Thyasiridae by the underlying gill cells. Although the ultrastructural evidence of bacterial digestion is compelling for a variety of species, it must be emphasized that these observations are static and no quantitative conclusions can be drawn from micrographs, concerning the flow of organic compounds from symbiont to host. This is especially true in the case of vestimentiferans, where dividing symbionts are extremely rare (in electron micrographs), and one must wonder how the "digested" symbionts are replaced and what the time course is of these phenomena (see Hand[94] for further discussion). It is possible that limited digestion of symbionts may function in providing needed essential compounds and that their bulk organic carbon needs are derived from translocated organic compounds.

As indicated previously there is very little direct experimental evidence of transfer of organic carbon from symbiont to host. To my knowledge two such studies have been completed. Fisher and Childress[35] reported translocation of organic carbon from symbiont to host in the gutless bivalve *Solemya reidi*. The authors used two techniques to follow the flow of ^{14}C labeled inorganic carbon into the symbionts, and the subsequent transfer of ^{14}C labeled organic compounds to host tissues. Based on the time course of transfer of labeled carbon within the association, Fisher and Childress[35] conclude that the symbionts are translocating to their host about 40% of the carbon they fix in the form of soluble organic compounds. They do not, however, rule out the eventual digestion of senescent symbionts as an additional source of nutrition for the host clam.

Distel and Felbeck[68] examined the release of carbon fixation products by freshly isolated and "purified" symbionts from the lucinid clam *Lucinoma aequizonata*. Less than 5% of the fixed carbon was found in the incubation media after incubations ranging from 7.7 to 60 min. Similar data have been published for algae freshly isolated from invertebrate hosts. However, when the algae are incubated under appropriate conditions (such as in the presence of host homogenate and an energy source for the symbionts) a substantial increase (often up to about 60%) in translocated products is documented.[223,226] Therefore, the results of Distel and Felbeck[68] must be considered inconclusive with respect to the magnitude of translocation in this association.

Enzyme analyses and carbon fixation studies clearly demonstrate that many of these associations have the potential for high rates of chemoautotrophic carbon fixation. Cary et al.[98] have demonstrated shell growth of a mussel with symbiotic

methanotrophs using methane as sole carbon and energy source, and the recent demonstration of *net* inorganic carbon uptake by *Solemya reidi*,[44] indicates that *S. reidi* has at least the potential for being fully autotrophic with respect to carbon. Whatever the mechanism and details of the nutritional interactions in chemoautotrophic symbioses, the various lines of reasoning and evidence presented above support a tight nutritional reliance of most of these hosts upon their symbionts.

The study of chemoautotrophic and methanotrophic symbioses is still in its infancy, although great strides have been made in the understanding of these associations in the last ten years. These symbioses have caught the interest and kindled the imagination of hundreds of investigators all over the world. The amount of literature published on these symbioses over this short period of time testifies to the intellectual efforts currently aimed at unraveling questions posed by these associations. There is no doubt in my mind that in the interval between the writing and publication of this review, both new sites and associations will be discovered that lead to new insights into these symbioses.

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Vent-type Taxa in a Hydrocarbon Seep Region on the Louisiana Slope

Vent-type taxa in a hydrocarbon seep region on the Louisiana slope

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Recent discoveries on the northern Gulf of Mexico continental slope have altered our understanding of biological and chemical processes occurring in the deep ocean. A biological community of hydrothermal vent-type organisms was recently discovered at the base of the Florida Escarpment[1], where the fauna are apparently nourished by hydrogen sulphide-rich hypersaline water seeping out onto the sea floor. Dense colonies of deep living chemosynthetic benthic organisms were first discovered during investigations of warm water anomalies along the axis of the Galapagos Rift in the Pacific Ocean in 1977[2-4], and this first discovery of clusters of clams, tube worms and other filter feeders in the immediate proximity of warm water vents has been followed by the discovery of a number of other hydrothermal vent sites, for example Guaymas Basin, East Pacific Rise at 21° N. The dense population assemblages at these sites are apparently restricted to small areas of the ocean floor where hydrogen sulphide-rich water is escaping from spreading centres, but the Florida Escarpment discovery indicates that these communities can also exist on passive margins. Here we report the discovery of dense biological communities associated with regions of oil and gas seepage on the Louisiana continental slope. These communities of large epi- and infaunal organisms are similar to those associated with the vents of the Pacific and the hypersaline brine seeps of the Florida Escarpment. Carbon isotope analyses suggest that these communities are chemosynthetic and derive their energy from hydrogen sulphide and/or hydrocarbons. Similar communities may be widely distributed on the sea floor in other oil-producing regions of the ocean.

In July 1984, we reported the discovery of thermogenic gas hydrates in marine sediments from the Louisiana slope[5]. These hydrates were associated with oil-stained cores similar to those found at a nearby location[6]. Based on the widespread occurrence of oil-stained cores (containing up to 15% extractable organic matter) in the Green Canyon lease area (~27°-28° N and 90°-92° W) and the flux of hydrocarbons into the overlying waters, two benthic trawl samples were taken to determine the effect of these hydrocarbons on the benthic communities (see Fig. 1 for locations). Populations of hydrothermal vent type organisms were recovered in both trawls including bivalves, gastropods and vestimentiferan tube worms.

Our first trawl was taken near 27°40′ N and 91°32′ W at water depths between 600 and 700 m just south of an area known to contain oil-stained sediments and thermogenic gas hydrates[5]. The site was chosen because of a large seismic 'wipeout' zone where the stratifications of the sediments are masked (Fig. 2) and the presence of a persistent oil slick at the surface (presumably caused by sea bottom oil seepage). Coring of these seismic wipeout zones in the Green Canyon area have consistently recovered oil-stained, gas charged and/or gas hydrate-containing sediments. The 10-m semi-balloon otter trawl was on the sea bottom for ~60-min over a 3.5-nautical mile-long track. This track crossed a ~2.0-n. mile-wide wipeout zone. The first trawl contained 800 kg of bivalve and gastropod shells, including both living and disarticulated bivalves ranging from 5 to 10 cm in length. The haul contained 30% living bivalves, 60% disarticulated bivalves and 10% living gastropods. Organisms collected in our first trawl included the bivalves *Calyptogena ponderosa*, *Vesicomya cordata*, *Lucinoma atlantis* and an unidentified neogastropod. Both *Calyptogena* and *Vesicomya* contain haemoglobin.

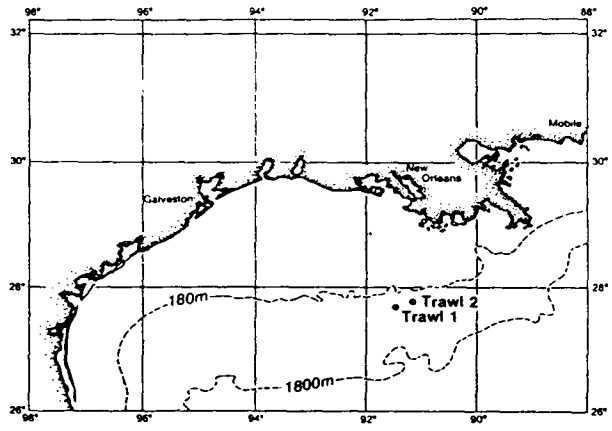

Fig. 1 Trawl locations on the Gulf of Mexico slope.

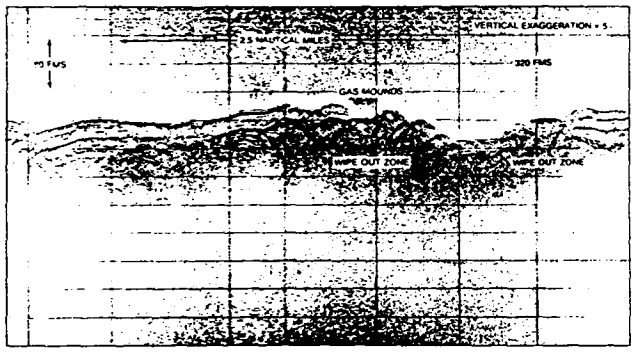

Fig. 2 Acoustical record (3.5 kHz) along the track of trawl one. Wipe-out zones represent areas of oil, hydrate and/or gas-charged sediments. The wipeout zones are thought to be the locations of the chemosynthetic ecosystems.

The second trawl (~27°45′ N and 91°14′ W) was taken in the region where Anderson *et al.*[6] previously reported oil-stained cores. Seismic data and additional cores obtained in this region confirmed the occurrence of hydrocarbon seepage. The trawl covered a distance of ~2.5 n. miles and crossed a second seismic wipeout zone. The trawl contained an entanglement of vestimentiferan tube worms (*Lamellibrachia* sp.) with various gastropods and bivalves. The vestimentiferans recovered in the trawl were ~2.0 m long and up to 1 cm in diameter. The organisms in this second trawl included *Acesta bullisi*, *L. atlantis*, *Gaza fischeri* and the same unidentified neogastropod as retrieved in the first trawl. Both trawls also contained significant numbers of crabs, shrimp and fish that have yet to be identified. The tube worms and gastropods recovered from these trawl sites are taxonomically different from those reported for the Florida Escarpment and the Galapagos Rift. However, one of the vesicomyid clams (*C. ponderosa*) collected at the first trawl site belongs to the same genus as the clams found at the Florida Escarpment (*Calyptogena* sp.), Galapagos Rift (*Calyptogena magnifica*) and Guaymas Basin (*Calyptogena pacifica*).

Carbon isotope analyses were performed on selected organisms to determine their food source (Table 1). Stable carbon isotopes are useful in delineating the flow of carbon through ecosystems as there is considerable evidence for minimal carbon isotope fractionation along marine food chains[7-10]. Organisms that feed on photosynthetically derived carbon from marine algae have carbon isotope values ranging from −19 to −21‰ (Table 1 and ref. 11). Tissue from mussels recovered at the

Table 1 Carbon isotope values ($\delta^{13}C$ in ‰ relative to PDB) for organisms obtained from trawls on the Gulf of Mexico continental slope.

Organisms	Description	Station	Depth (m)	$\delta^{13}C$ (‰)	Position		Comment
Geryon quinquedens	Crab	E-1	390	−17.2	28°24′ N	85°58′ W	
Bembrops gobioides	Fish	E-1	390	−17.8	28°24′ N	85°58′ W	
Synaphobranchus brevidorsalis	Eel	E-3	840	−18.1	28°11′ N	86°26′ W	
S. brevidorsalis	Eel	E-4	1,225	−19.2	28°07′ N	86°36′ W	
Bathypterois guardrifilis	Fish	E-4	1,225	−18.6	28°07′ N	86°36′ W	
Synaphobranchus oregoni	Eel	E-4	1,225	−19.5	28°07′ N	86°36′ W	
Nematocarcinus rotundus	Shrimp	E-4	1,225	−18.2	28°07′ N	86°36′ W	
Acanthephyra eximia	Shrimp	E-4	1,225	−18.3	28°07′ N	86°36′ W	
G. quinquedens	Crab	E-4	1,225	−19.3	28°07′ N	86°36′ W	
S. oregoni	Eel	C-1	345	−19.6	28°03′ N	90°15′ W	
G. quinquedens	Crab	C-4	1,390	−17.4	27°28′ N	89°44′ W	
Bathygadus macrops	Fish	W-2	550	−17.5	27°25′ N	93°19′ W	
Monomitopus sp.	Fish	W-3	790	−18.1	27°08′ N	93°24′ W	
Dicrolene sp.	Fish	W-3	790	−18.3	27°08′ N	93°24′ W	
Halosaurus guentheri	Fish	W-3	790	−17.5	27°08′ N	93°24′ W	
Stereomastis sculpta	Shrimp	W-4	1,390	−17.0	26°44′ N	93°19′ W	
*Calyptogena ponderosa**	Clam	42	600	−35.4 −35.3	27°40′ N	91°32′ W	Seep area
*Lucinoma atlantis**	Clam	42	600	−31.2 −33.0	27°40′ N	91°32′ W	Seep area
Unidentified neogastropod	Snail	42	600	−31.5	27°40′ N	91°32′ W	Seep area
Lamellibrachia sp.	Tube worm flesh	43	600	−27.0	27°45′ N	91°14′ W	Seep area
Lamellibrachia sp.	Worm tube	43	600	−28.1	27°45′ N	91°14′ W	Seep area
Nezumia aequalis	Fish†	42	600	−17.6	27°40′ N	91°32′ W	Seep area
Monomitopus sp.	Fish	42	600	−17.9	27°40′ N	91°32′ W	Seep area
Chaunax pictus	Fish	42	600	−17.9	27°40′ N	91°32′ W	Seep area
Coryphaenoides colon	Fish	43	600	−17.2	27°45′ N	91°14′ W	Seep area

Carbon isotopes samples were prepared in a Craig-type combustion system with CO_2 determination on a Finnigan MAT 251 isotope ratio mass spectrometer. Depths are approximate as many areas of the slope are steep.

* $\delta^{13}C$ values were determined on two individuals.

† Fish were not necessarily collected in the immediate vicinity of the seeps but could have been collected at other areas during the trawl.

Pacific vents have $\delta^{13}C$ values near −33‰ (refs 12–14). The vent communities of the Pacific are based on chemoautotrophic bacteria that gain energy from the oxidation of hydrogen sulphide[15–18]. In turn, the associated filter-feeding organisms feed on these isotopically light bacteria. Internal symbiotic bacteria are found in clams, mussels and vestimentiferans from the hydrothermal vents[19] and are probably present in the gills of the bivalves as well as the vestimentiferan worms at this site.

Carbon isotope analysis of freeze-dried mantle and foot tissue of bivalves from the first trawl had $\delta^{13}C$ values of −31 to −35‰. This suggests that the food source of these animals results, at least in part, from chemosynthesis rather than terrestrial or marine photosynthetic organic carbon. These isotope values provide supporting evidence that the food source of the bivalves is sulphur- or hydrocarbon-oxidizing bacteria in this hydrocarbon/sulphide-rich environment ($\delta^{13}C$ of the oil and methane is −26.5 and −45‰, respectively). The bivalves smelled strongly of hydrogen sulphide during dissection. The vestimentiferan worms and their tubes collected in the second trawl have $\delta^{13}C$ values of −27 and −28‰, respectively. In comparison, tube worms (*Riftia pachyptila*) sampled from the Galapagos Rift had considerably heavier isotope values (−11‰; ref. 12). One explanation for these heavier isotope values is that internal symbiotic chemosynthesis in tube worms limits the supply of CO_2, thus reducing isotope fractionation. The oil-seep tube worms must also have a mechanism of carbon assimilation that reduces isotope fractionation relative to the bivalves.

This report significantly expands the geographical area in which one would expect to find dense hydrothermal vent-type taxa in the deep ocean. It also suggests that oil and gas seeping to the surface from deeper hydrocarbon reservoirs can support, by chemosynthesis, vent-type organisms in the deep ocean. Hydrocarbon seepage occurs in many shelf and slope regions, thus making it probable that these communities are more widely distributed than previous discoveries have suggested. As these sites are studied further, several new species may be found (R. Turner and M. Jones, personal communication).

This discovery is distinct from those in Pacific hydrothermal vents and Florida Escarpment communities in several ways. First, the source of reduced compounds (possibly hydrogen sulphide, hydrocarbons and/or ammonium) is not a point source. The seepage is diffuse and occurs over a wide area in the seismic wipeout zones. It is impossible to determine from trawl catches whether the organisms are living in the seep area or along its perimeter. Second, the biological assemblages are associated with seeping hydrocarbons that have significant toxicities (particularly aromatic hydrocarbons). Third, these chemosynthetic communities are not at abyssal depths. Fourth, water temperatures are not elevated. The high productivity at the hydrothermal vent communities seems to be sustained by high bacterial turnover rates in the high-temperature vent plumes[18]. It is not known how hydrocarbons effect bacterial production rates. Finally, the shallower seep taxa reported here may serve as a food source for various deep-sea organisms. Further study is needed to elucidate: (1) how and to what extent the taxa adapt themselves to high hydrocarbon environments; (2) how widespread geographically these organisms are on the Louisiana slope, as well as other oil-producing regions; and (3) how significant a biomass contribution they make to the deep sea.

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Gulf of Mexico Hydrocarbon Seep Communities
I. Regional Distribution of Hydrocarbon Seepage and Associated Fauna

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Gulf of Mexico hydrocarbon seep communities—I. Regional distribution of hydrocarbon seepage and associated fauna

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Abstract— A series of otter trawls demonstrate that communities based on chemosynthesis are broadly distributed across the northwestern Gulf of Mexico continental slope in hydrocarbon seep areas. Thirty-nine trawls were taken at 33 locations reported to exhibit transparent or chaotic seismic "wipe-out" zones. The sites, in water depths from 180 to 900 m, span an area from offshore the Mississippi River delta to the upper Texas continental slope. Endosymbiont-containing organisms or their remains (either tube worms, mussels and/or clams) were recovered at 21 sites on the northern Gulf of Mexico slope. Tube worms, clams and mussels known to be associated with symbionts were retrieved at 18, 12 and 5 sites, respectively. Carbon isotope analysis of selected animal tissues confirmed the chemosynthetic association. Animals containing isotopically light chemosynthetic carbon were collected at 21 sites. Piston cores at each site were used to determine the presence of mature hydrocarbons. Nine of 30 piston cores were visibly oil-stained. Trawl collections at locations where visibly oil-stained cores were recovered contained at least one species of chemosynthetic-associated organism and generally represented the most abundant catches of endosymbiont-containing animals. The chemical environment (oil and gas seepage) necessary to support chemosynthetic-based ecosystems is widespread on the northern Gulf of Mexico continental slope.

INTRODUCTION

RECENT discoveries of hydrocarbon seepage, gas hydrates and chemosynthetic communities has caused a re-evaluation of our understanding of chemical, biological, and geological processes occurring on continental slopes (ANDERSON *et al.*, 1983; BROOKS *et al.*, 1984, 1986a,b, 1987a,b; KENNICUTT *et al.*, 1985; CHILDRESS *et al.*, 1986). Gas seepage has been reported and/or sampled on the continental shelf in the Gulf of Mexico by several authors (DUNLAP *et al.*, 1960; BERNARD *et al.*, 1976; SACKETT, 1977), although reports of offshore oil seepage are sparse. Oil seepage has only recently been extensively documented on the Gulf of Mexico slope (ANDERSON *et al.*, 1983; BROOKS *et al.*, 1984; 1986a,b; 1987a,b; KENNICUTT *et al.*, 1988b). Several cores containing as much as 4% oil, by weight, were recovered in 1983 (ANDERSON *et al.*, 1983). Subsequent to this thermogenic gas hydrates were also discovered on the Gulf of Mexico continental slope (BROOKS *et al.*, 1984, 1986b). Ten additional Gulf of Mexico sites containing biogenic or thermogenic gas hydrates have been identified (BROOKS *et al.*, 1986b). Thermogenic gas hydrates are often associated with sediments that contain as much as 15% oil by weight. Oil-stained sediments have been recovered in more than 100 cores across the Gulf of Mexico in water depths ranging from 200 to 3000 m (unpublished data). At one location this massive oil

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seepage has been directly linked to oil reservoired at ~2500–3000 m beneath the sea floor (KENNICUTT *et al.*, 1988b).

To determine the hydrocarbon exposure of benthic fauna in these seep areas, otter trawls were taken to obtain biological samples for analysis (KENNICUTT *et al.*, 1985; BROOKS *et al.*, 1986a). These trawls collected tube worms and large quantities of bivalves that are known to contain endosymbiotic bacteria. Fifteen trawls taken during two separate research cruises in 1984–1985 confirmed the presence of bivalves and tube worms on the Gulf of Mexico continental slope. Subsequent taxonomic, enzymatic, and isotopic analyses suggested that these communities were similar to those found at hydrothermal vents in the Pacific and that the primary mode of nutrition was chemosynthesis by endosymbiotic bacteria (KENNICUTT *et al.*, 1985; CHILDRESS *et al.*, 1986; BROOKS *et al.*, 1987b). The majority of the bivalves (*Calytogena ponderosa*, *Vesicomya cordata*, and *Psuedomiltha* sp.) and vestimentiferans (*Lamellibrachia* sp. and an unidentified *Escarpia*-like species) are primarily if not exclusively deriving their energy from the bacterial oxidation of H_2S to elemental sulfur and sulfate. In contrast, laboratory incubations with ^{14}C labeled substrates, enzymatic analyses, and carbon isotopic compositions confirmed that a mussel (*Bivalvia, Myltilidae*) at these sites could derive its carbon, energy and nutritional needs from a symbiotic relationship with methane-oxidizing bacteria in it's gills (CHILDRESS *et al.*, 1986; BROOKS *et al.*, 1987b; FISHER *et al.*, 1987). This was the first confirmed report of a molluscan symbiosis based on methane.

The studies described above have documented the presence of chemosynthetic-based communities at a few, relatively restricted, areas on the Gulf of Mexico continental slope. To determine if these processes are broadly distributed in the northern Gulf of Mexico, an extensive program of piston coring and otter trawling was undertaken. The results of this regional study are reported here.

Table 1. Location of trawls taken on the northern Gulf of Mexico continental slope

	On bottom			Off bottom		
Site	Time	Latitude	Longitude	Time	Latitude	Longitude
1	1426	27°36.0′	94°45.6′	1600	27°39.2′	94°49.1′
2	0945	27°38.2′	94°25.0′	1057	27°40.1′	94°23.6′
3	1818	27°34.9′	93°35.9′	1924	27°37.4′	93°34.9′
4	1243	27°22.1′	93°31.1′	1422	27°24.9′	93°27.8′
5	0000	27°27.5′	93°10.6′	0111	27°27.6′	93°06.6′
6	1754	27°45.0′	92°59.3′	1809	27°44.4′	92°59.6′
7	0523	27°30.1′	93°00.5′	0625	27°30.0′	93°03.7′
7	0658	27°29.6′	93°01.1′	0824	27°31.6′	93°04.0′
8	1417	27°53.7′	92°52.9′	1502	27°51.6′	92°52.6′
9	2209	27°50.6′	92°31.3′	2318	27°53.8′	92°31.3′
10	1625	27°41.2′	92°11.4′	1738	27°44.1′	92°09.5′
11	0941	27°36.4′	92°11.3′	1044	27°38.6′	92°11.3′
11	1246	27°36.3′	92°12.3′	1450	27°36.9′	92°17.5′
11	2316	27°35.7′	92°12.0′	0030	27°38.4′	92°09.5′
12	2145	27°33.5′	91°49.2′	2330	27°38.0′	91°49.1′
13	1520	27°39.5′	91°31.8′	1630	27°42.2′	91°32.4′
13	1908	27°39.6′	91°31.9′	2017	27°42.2′	91°31.8′
15	0741	27°46.0′	91°30.3′	0850	27°47.3′	91°30.4′
16	1432	27°41.2′	91°30.5′	1606	27°45.0′	91°29.8′
17	0414	27°43.4′	91°16.8′	0520	27°44.2′	91°13.6′
17	0915	27°43.2′	91°15.7′	1034	27°43.2′	91°19.4′
18	0147	27°39.6′	90°50.5′	0243	27°39.6′	90°47.3′
19	2013	27°39.0′	90°28.9′	2124	27°41.9′	90°29.3′
20	1422	27°52.4′	90°33.2′	1557	27°56.2′	90°32.8′
20	2021	27°53.4′	90°12.0′	2113	27°55.7′	90°12.0′
21	1120	27°56.9′	90°30.4′	1207	27°55.9′	90°32.6′
21	1133	27°57.3′	90°30.0′	1242	28°00.4′	90°29.9′
22	0833	27°57.0′	90°23.4′	0929	27°59.1′	90°23.4′
23	1205	27°47.0′	90°16.9′	1341	27°46.5′	90°12.5′
24	0151	27°55.2′	90°11.5′	0252	27°57.3′	90°11.3′
25	0643	27°53.2′	90°11.7′	0745	27°55.6′	90°12.1′
26	2142	27°57.3′	89°56.2′	2302	27°56.0′	89°59.9′
27	1237	27°56.9′	89°57.7′	1445	27°57.6′	89°57.3′
28	0858	28°06.4′	89°58.8′	0955	28°04.2′	89°58.8′
29	1810	27°55.5′	89°54.4′	1924	27°58.7′	89°54.2′
30	1258	28°07.1′	89°45.5′	1418	28°07.1′	89°49.3′
31	2009	28°29.9′	89°47.2′	2120	28°29.8′	89°43.8′
32	0445	28°40.3′	89°04.3′	0542	28°42.4′	89°04.2′
33	1115	28°51.4′	88°31.5′	1218	28°53.8′	88°31.4′

extracted (hexane) for 12 h. The extracts were weighed and analysed by total scanning fluorescence. Sediment extracts of the bottom two sections were analysed for aliphatic, high molecular weight, hydrocarbons by capillary gas chromatography with flame ionization detection. Internal standards were added before Soxhlet extraction to provide quantitative determinations of hydrocarbon concentrations.

Gas chromatographic and fluorescence analytical techniques are described in detail elsewhere and will only be briefly described here (Brooks *et al.*, 1986c; Kennicutt *et al.*, 1987a). The sediment extract is analysed on fused silica columns (50-m × 0.3-mm ID, BP1/QC2 SGE Ltd.) with flame ionization detection. The detector is calibrated with authentic standards and internal standards are used to correct for experimental losses.

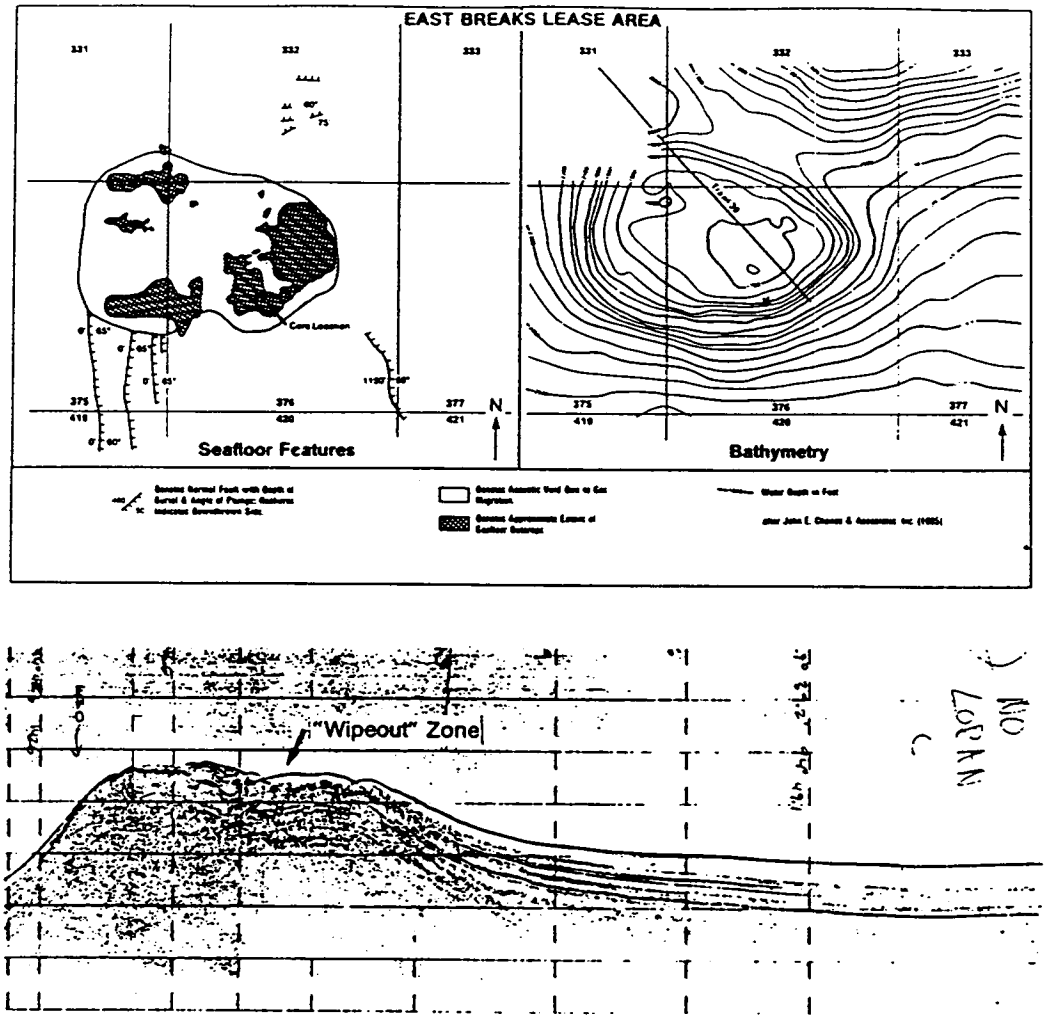

Fig. 1. Shallow seafloor features, bathymetry and 3.5 kHz sub-bottom profile for the trawl line at EB-376.

The same extract is also analysed for its fluorescence excitation-emission spectrum on a Perkin-Elmer 650-40 fluorometer.

Tissues for carbon isotopic analysis were either freeze-dried or oven-dried at 40°C. Tissues samples were acidified to eliminate carbonate and combusted to CO_2 in either a Craig-type combustion system or in Pyrex tubes containing cupric oxide (SACKETT *et al.*, 1970; SOFER, 1980; BOUTTON *et al.*, 1983). The carbon isotopic composition of the CO_2 was determined with a triple collector Finnigan MAT-251 Isotope Ratio Mass Spectrometer. Values are reported relative to PeeDee Belemnite (PDB) in the standard per mil (‰) notation.

Table 2. Location of piston cores taken on the northern Gulf of Mexico slope

Site	Latitude	Longitude	Water depth (m)	Penetration (m)
1	27°36.9′	94°46.1′	512	3.8
2	27°40.0′	94°23.3′	420	4.2
3	27°37.0′	93°34.2′	402	5.0
4	27°22.9′	93°30.8′	620	4.4
5	27°27.6′	93°10.4′	540	4.7
6	No piston core taken			
7	27°29.8′	93°01.5′	650	4.7
7	27°30.8′	93°02.9′	695	4.8
8	27°53.5′	92°52.9′	180	5.4
9	27°49.9′	92°30.1′	320	4.0
10	27°41.7′	92°10.9′	410	4.4
11	27°36.7′	92°11.1′	665	4.4
11	No piston core taken			
11	No piston core taken			
12	27°35.9′	91°49.4′	549	4.0
13	27°40.5′	91°31.6′	680	3.4
13	No piston core taken			
15	27°46.8′	91°30.4′	530	4.4
16	No piston core taken			
17	27°43.9′	91°13.9′	540	4.2
17	No piston core taken			
18	27°39.6′	90°49.8′	805	5.7
19	27°39.2′	90°28.9′	895	5.0
20	27°54.2′	90°32.5′	512	4.4
20	No piston core taken			
21	27°58.4′	90°30.1′	393	4.7
21	No piston core taken			
22	No piston core taken			
23	27°46.9′	90°16.5′	658	4.8
24	27°56.4′	90°11.9′	555	5.0
25	27°53.[illegible]	90°12.0′	600	4.4
26	27°56.4′	89°59.4′	610	4.8
27	27°58.3′	89°57.7′	512	3.8
28	28°06.1′	89°58.8′	402	4.7
29	27°57.5′	89°53.9′	750	4.8
30	28°07.2′	89°47.6′	594	4.2
31	28°30.0′	89°49.3′	512	4.5
32	28°41.4′	89°04.4′	570	4.4
33	38°53.3′	88°31.4′	805	4.5

RESULTS AND DISCUSSION

Seismic "wipe-out" zones, oil-staining, gas pockets, and a H_2S odor were observed at 24, 9, 12 and 18 sites, respectively (Fig. 2). Tube worms, clams and mussels known to contain endosymbionts were recovered at 18, 12 and 5 sites, respectively.

Extractable organic matter

Methylene chloride extractable organic matter can have a biological (lipids) as well as thermogenic (petroleum) origin. In general, Gulf of Mexico marine sediments contain less than 50 ppm of extractable organic matter of biological origin, though this value can be quite variable (KENNICUTT *et al.*, 1987a, 1988a and references therein). Extractable organic matter content is elevated in sediments that contain petroleum. Total sediment

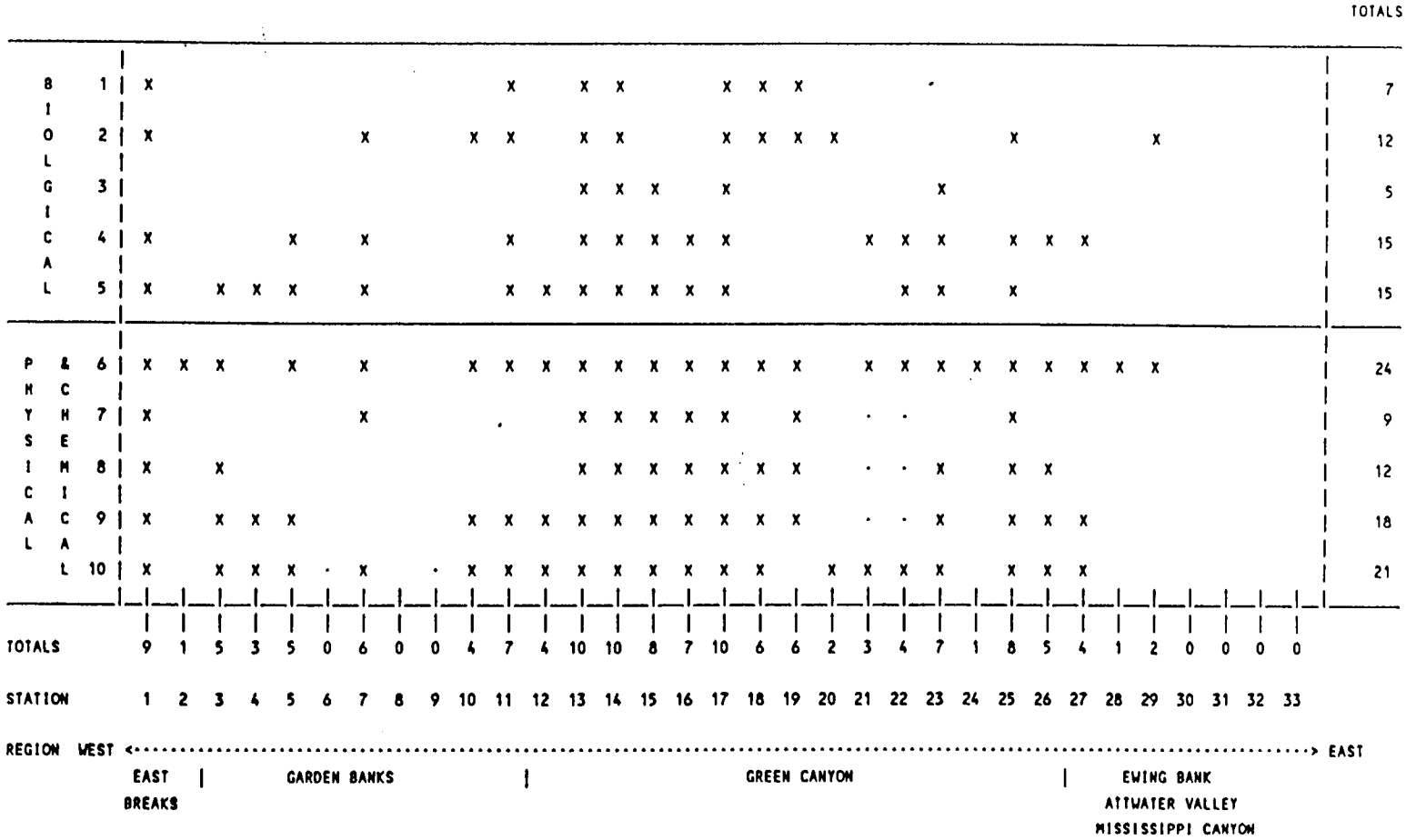

Fig. 2. Summary of the occurrence of biological, physical and chemical parameters associated with hydrocarbon seepage and chemosynthetic communities: 1, *Calyptogena ponderosa*; 2, *Vesicomya cordata*; 3, unidentified mussel; 4, vestimentiferan tube worms; 5, pogonophoran tube worms; 6, seismic "wipe-out" zone; 7, oil-stained core; 8, gas pockets in core; 9, H_2S odor in core; and 10, isotopically light tissue carbon.

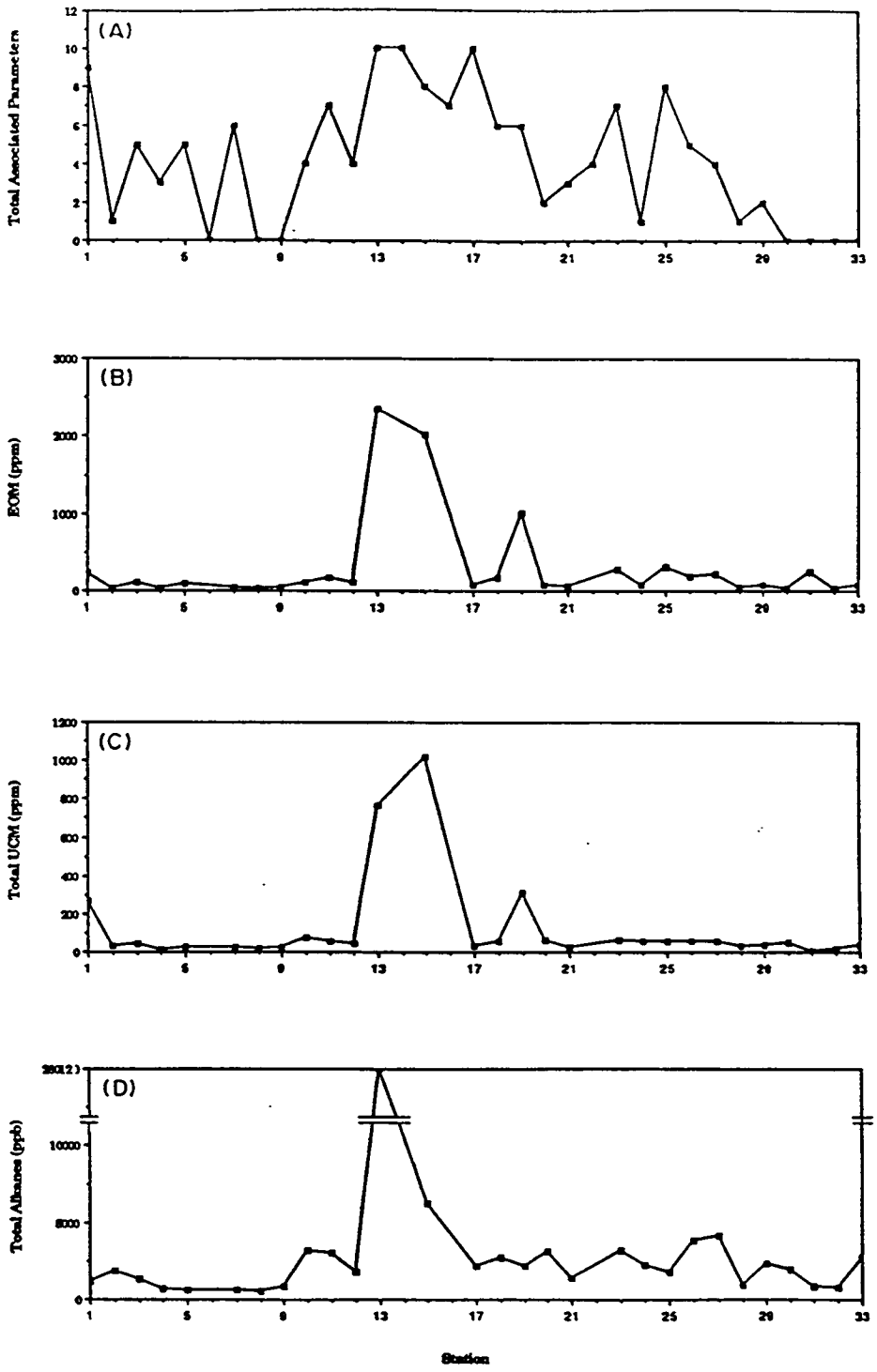

Fig. 3. Geographical distributions of : (A) chemosynthetic community associated parameters (see Fig. 5 for total); (B) extractable organic matter, EOM; (C) total unresolved complex mixture (UCM); and (D) total *n*-alkane concentrations (15–32 carbons).

extractable organic matter ranged from 21 to 5800 ppm with many sediments containing large amounts of material attributable to petroleum (Fig. 3).

Gas chromatography

Representative gas chromatograms for sediment extracts containing high concentrations and trace amounts of thermogenic hydrocargons are shown in Fig. 4A and B, respectively. Sediments containing petroleum hydrocarbons are characterized by a complex mixture of compounds that are not resolved gas chromatographically under the given analytical conditions (UCM), C_{15} to C_{32} normal alkanes, and isoprenoids. This mature hydrocarbon pattern is overprinted with odd carbon number normal alkanes with 23 or more carbons which are presumably derived from terrigenous organic matter (FARRINGTON and MEYERS, 1975; TULLOCH, 1976; FARRINGTON and TRIPP, 1977; GIGER and SCHAFFNER, 1977; GIGER *et al.*, 1980; KENNICUTT *et al.*, 1987b). Samples containing high levels of petroleum are often extensively biodegraded and contain only an unresolved complex mixture (Fig. 4C, D). High concentrations of *n*-alkanes in the C_{15} to C_{20} range suggest an upward-migration source since hydrocarbons in this molecular weight range do not generally survive transport through the environment (i.e. pollution, KENNICUTT *et al.*, 1987c). This interpretation is supported by the depth of occurrence of the hydrocarbons in the sediments as well as a general increase in concentration with increasing depth within the core. Deep penetration (>2 m) of the sediment column insures that the sample is below the pollution horizon, thus the petroleum hydrocarbons detected are migrating upward from deep in the subsurface and are not being deposited from the overlying water column (KENNICUTT *et al.*, 1988b). Total unresolved complex mixture concentrations varied from 8 to 1033 ppm, with most locations exceeding the low level biological background of 5–10 ppm (Fig. 3). These concentrations suggest that petroleum hydrocarbons are present at most, if not all, of the locations. Total *N*-alkane (n-C_{15} to n-C_{32}) concentrations ranged from 504 to 31,350 ppb and were also indicative of petroleum (Fig. 3).

Total scanning fluorescence

Fluorescence is selectively sensitive to compounds with conjugated double bonds, i.e. aromatic hydrocarbons. A total scanning fluorescence spectrum provides a semi-quantitative estimate of total aromatic compounds (fluorescence intensity) as well as an estimate of the ring-number distribution of the fluorescent compounds. In general, the fluorescence excitation-emission maximum increases in wavelength with an increasing number of aromatic rings. Sediment extracts containing petroleum contain three-ring and larger aromatic compounds that emit fluoresced light at high wavelengths (>350 nm). Fluorescence spectra are not extensively altered by biodegradation of water washing, though severe degradation alters aromatic compound distributions (KENNICUTT *et al.*, 1988b; KENNICUTT, 1988). Aromatic compounds, in general, are moderately resistant to degradative removal and this resistance increases with an increase in the number of aromatic rings. Fluorescence analyses confirm the presence of aromatic hydrocarbons related to petroleum at all of the sites sampled.

Oil seepage evaluation

A ranking system base on total scanning fluorescence and gas chromatographic analysis has been devised to evaluate the amount of oil seepage at a location (BROOKS *et*

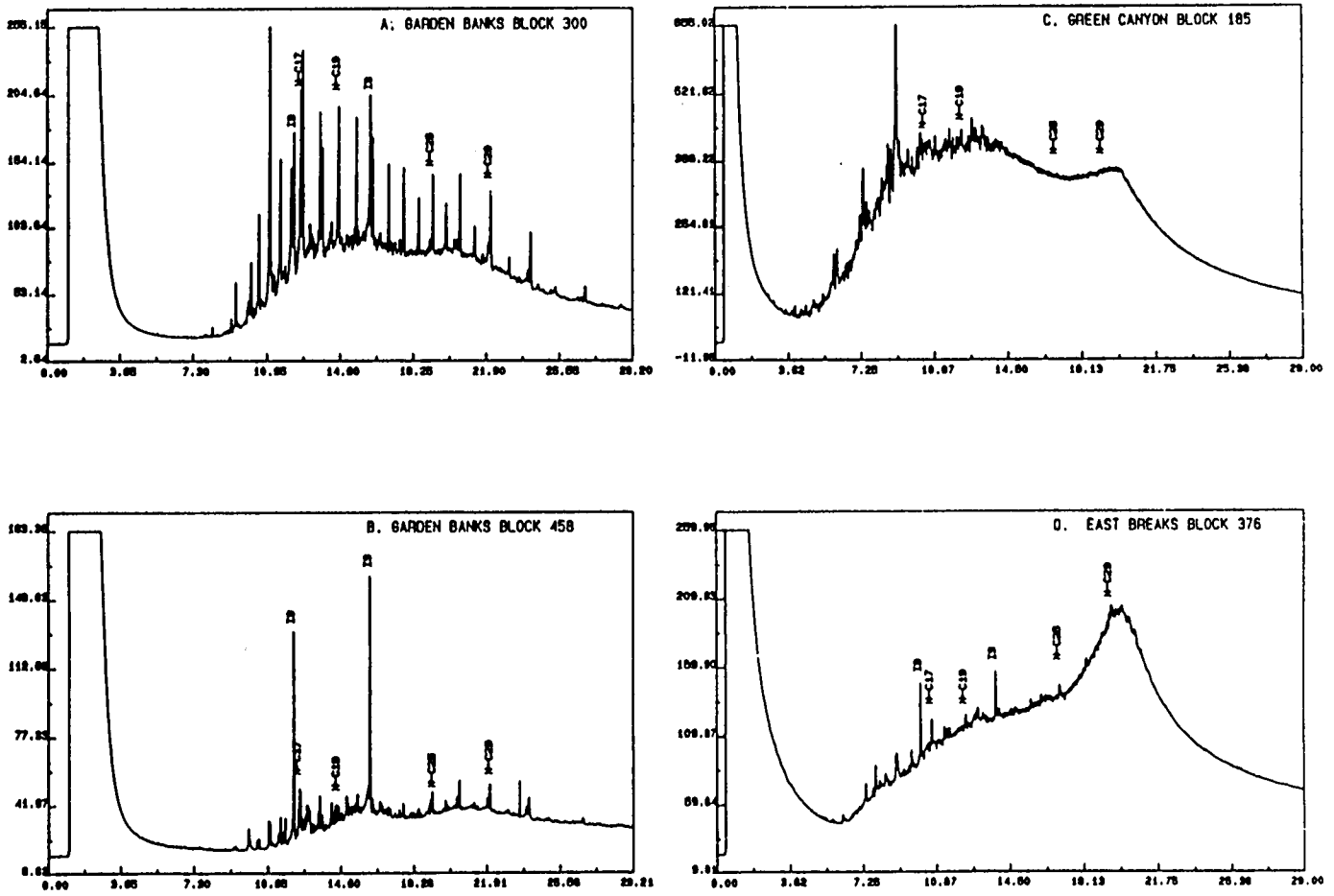

Fig. 4. Representative gas chromatograms of sediment extracts from selected sites. See text for details.

al., 1986c). In this ranking system, zero represents no seepage while 15 is indicative of substantial macroseepage. Oil seepage is evaluated from the fluorescence intensity, the ratio (RI) of fluorescence at 360/270 to 320/270 ($Em\lambda/Ex\lambda$) and the amount and composition of the gas chromatographic signature of the sediment extract. The presence of oil in the gas chromatogram of an extract is based on the fact that oils contain a complete suite of *n*-alkanes, pristane, and phytane whereas recent organic matter of a biological origin contains only a relatively few specific aliphatic compounds (primarily odd carbon number normal alkanes with 23 to 31 carbons; KENNICUTT *et al.*, 1987b).

The presence of oil is evaluated on a scale of 0–15 that is derived from the sum of the three parameters discussed above (each based on a 0–5 scale). The amount of oil seepage is evaluated as: 12–15, very high; 8–12, medium/high; 4–8, low and 0–4, very low. Based on this evaluation scheme, significant oil seepage was present at most of the locations sampled (Fig. 5).

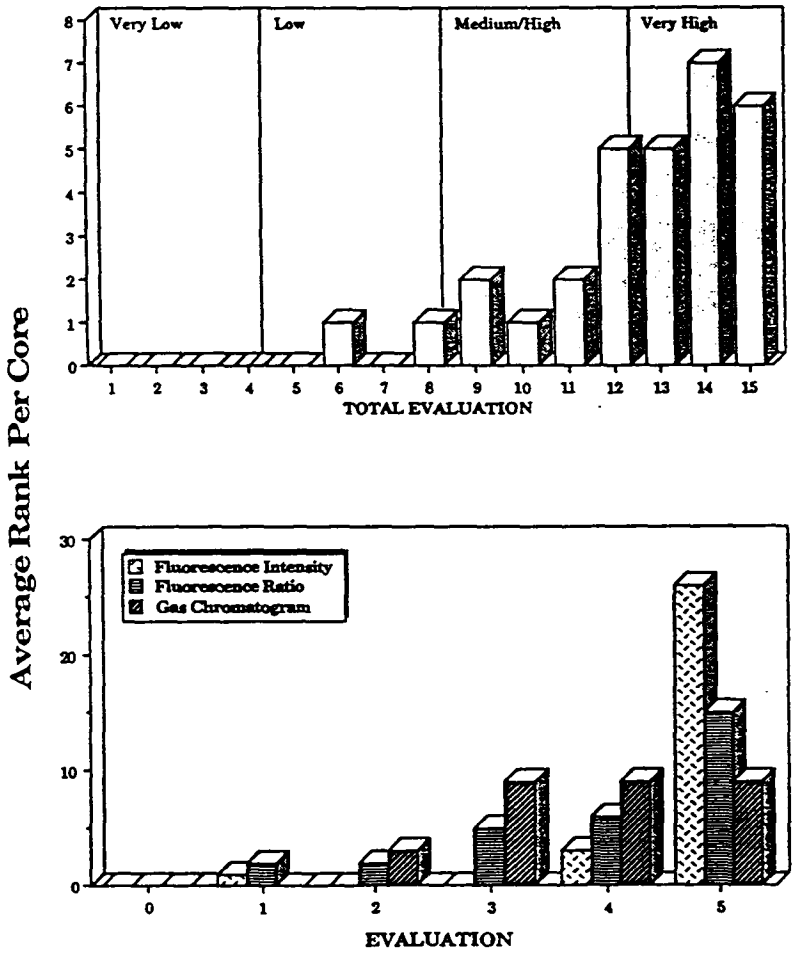

Fig. 5. Summary of the oil seepage evaluations calculated for piston cores retrieved on the northern Gulf of Mexico continental slope.

Carbon isotope analyses

Stable carbon isotope analyses of tissues excised from organisms recovered from trawl catches can be used to determine an organism primary nutritional mode, i.e. chemosynthetic or heterotrophic (Brooks *et al.*, 1987b). Carbon isotope data are only briefly dicussed here as an indication of chemosynthesis while more detailed discussions of isotope data are presented elsewhere (Brooks *et al.*, 1987b). Twenty-one trawls contained organism tissues with isotopically light carbon indicating the presence of chemosynthetic biomass:

Green Canyon blocks: 29, 31, 40, 72/116, 79, 166, 184, 185, 233/234, 272, 273/279, 287, and 398 (13 sites).
Garden Banks blocks: 300, 359, 388, 458/459, 499/ 500, 581 (6 sites).
Ewing Bank block: 1010 (1 site).
East Breaks block: 376 (1 site).

The carbon isotope composition of the organisms analysed ranged from –14 to –58‰. Background heterotrophic organisms are generally in the –14 to –22‰ range (Kennicutt *et al.*, 1985; Childress *et al.*, 1986; Brooks *et al.*, 1987b). Tube worm tissues (vestimentiferans and pogonophorans) ranged from –20 to –58‰. Pogonophoran tubes and tissues had $\delta^{13}C$ values (–30 to –58‰) indicative of chemosynthetic carbon. Vestimentiferan tissue $\delta^{13}C$ values ranged from –20 to –56‰ with three values >–28‰ (Fig. 6).

Based on the oil seepage evaluation described above, no chemosynthetic-based organisms were recovered in areas which had a combined ranking of 10 or less, i.e. Mississippi Canyon—44/445, 282; East Break—339, 878; and Garden Banks—581. In contrast, at 15 of 18 areas with a seepage evaluation of 13 or more, endosymbiont-containing organisms were retrieved. At all locations evaluated at 15, two or more chemosynthetic associated species were present. These data strongly suggest a direct coupling between the chemical environment induced by hydrocarbon seepage (H_2S, CH_4 and oil) and chemosynthetic processes on the northern Gulf of Mexico continental slope. These environments are closely linked to the massive seepage of oil and gas and the resulting anaerobic, H_2S-rich sedimentary conditions. The natural seepage of hydrocarbons may represent a significant source of hydrocarbons to the deep oceans and

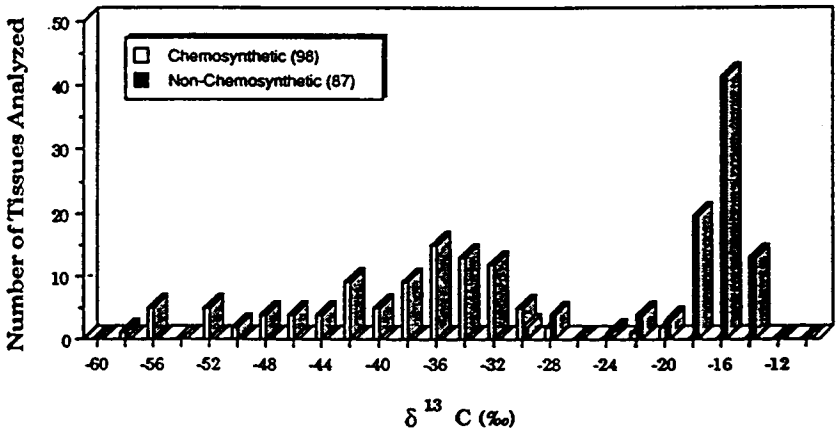

Fig. 6. Summary of the carbon isotopic analyses of tissues from selected organisms recovered from the trawls.

chemosynthetic biomass appears to be an important component of the slope ecology in the areas studied.

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Leakage of Deep, Reservoired Petroleum to The Near Surface on the Gulf of Mexico Continental Slope

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ABSTRACT

Kennicutt, M.C., II, Brooks, J.M. and Denoux, G.J., 1988. Leakage of deep, reservoired petroleum to the near surface on the Gulf of Mexico continental slope. Mar. Chem., 24: 39–59.

Reservoired oils, shallow sediment cores (2 m), sea slicks and tar balls were collected in the Green Canyon Lease area of the northern Gulf of Mexico continental slope. The gaseous and liquid hydrocarbons associated with near surface sediments and water have migrated from deep (2000–3000 m) subsurface reservoirs and/or source rocks. This conclusion is based on molecular (GC/FID, GC/FPD, GC/MS) and carbon isotopic evidence. Visual observations at two locations on the continental slope confirm the presence of massive amounts of active liquid as well as gas seepage. Hydrate gas recovered in sediment cores originates from deep, oil-associated gas. This gas has migrated to shallow sediments with little or no isotopic fractionation. In contrast, near surface hydrocarbon liquids (shallow bitumens and sea slicks) are depleted in aliphatics, 4-ring or larger aromatics, naphthalene, C_1-naphthalenes and C_2-naphthalenes as compared to the reservoired fluids.

These near-surface fluids are extensively altered by the concurrent processes of migration, dissolution and microbial degradation. However, the distributions of highly alkylated ($> C_2$) naphthalenes, phenanthrenes and dibenzothiophenes, triterpanes, steranes and triaromatized steranes are similar to the precursor reservoired oil. This study documents, for the first time, a direct link between natural seepage in a deep water marine setting and sea slick and tar ball formation. This and other studies suggest that the natural seepage of oil and gas can be a significant process in the deep ocean.

INTRODUCTION

It has long been speculated that natural petroleum seepage is a significant contributor of hydrocarbons to the marine environment. However, direct observations of oil seepage in strictly marine settings have been limited (Geyer, 1980). The magnitude, occurrence, and significance of natural seepage to the marine environment is difficult to assess (NAS, 1975; 1985). Gas seepage has been widely documented in the ocean (Dunlap et al., 1960; Bernard et al., 1976; Sackett, 1977; Cline and Holmes, 1977) but reports of liquid hydrocarbon seepage are few (Wilson et al., 1973; Geyer, 1980; Jeffrey, 1980). However,

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recent discoveries in the Gulf of Mexico have shown that natural liquid hydrocarbon seepage is a widespread phenomenon on the continental slope and may represent a significant hydrocarbon input to the deep sea (Anderson et al., 1983; Brooks et al., 1984; 1985; 1986a,b; 1987).

As an exploration tool for locating petroleum reservoirs, natural seepage has been historically important (Link, 1952; Philp and Crisp, 1982; Brooks et al., 1986a). Many of today's major petroleum provinces were first discovered by drilling beneath surface oil seeps (Degolyer, 1940; Hunt, 1979). Modern surface geochemical exploration techniques are based on the premise that deep, reservoired petroleum creates near-surface manifestations that can be detected (Faber and Stahl, 1984; Brooks et al., 1986a). This near-surface expression must be recognizable above the ambient in situ biological and/or inorganic background. The most direct of these surface prospecting methods rely on detecting some fraction of the reservoired hydrocarbons (Stahl, 1977; Horvitz, 1972; 1978; 1985; Philp and Crisp, 1982; Brooks et al., 1986a). The recognition of thermogenic hydrocarbons from the deep subsurface in near surface sediments is hindered by the overprinting of recent biologically generated compounds (Bernard et al., 1978; Brooks et al., 1979). These recent inputs can be the same as the upward migrated thermogenic hydrocarbons (i.e., methane and n-alkanes) or similar in composition and structure (Rice, 1975; Fuex, 1977; Rice and Claypool, 1981; Hunt et al., 1980; Whelan et al., 1980). Near-surface hydrocarbon expressions are further attenuated by alteration processes such as microbial activity. Petroleum can also be physically or chemically altered or fractionated during migration (Thompson, 1987a,b).

Few reports have traced the movement of petroleum from a deep reservoir to the near surface. In general, these studies have been limited to the gaseous components (Stahl, 1975; 1977; Coleman et al., 1977; Hunt, 1979; Fuex, 1980; 1981; Hunt et al., 1981; Reitsema et al., 1981; Stahl et al., 1981; Leythauser et al., 1982; Stepanova et al., 1982). The continental slope region of the Gulf of Mexico has recently been found to be the site of active gaseous and liquid petroleum seepage, authigenic carbonate precipitation (mediated by microbial activity), hydrate formation and chemosynthetic communities (Anderson et al., 1983; Brooks et al., 1984; 1985; 1986b; 1987; Kennicutt et al., 1985). As such this area presents an unique opportunity to trace the movement of hydrocarbons from the reservoir (2000–3000 m), to the near-surface sediments (water depth $\sim$ 600 m), into the water column and finally to slick and tar ball formation at the air/sea interface.

Seepage to the near-surface on the Gulf of Mexico slope is predominantly controlled by fault systems created by salt tectonics that provide a nearly-direct vertical conduit to shallow sediments (Martin and Case, 1975). To trace the movement of these hydrocarbons the following chemical analyses were chosen: gas chromatography with flame ionization (FID) and flame photometric detection (FPD), stable carbon isotopic composition, total scanning fluorescence, and specific molecular distributions by gas chromatography/mass spectrometry (i.e., triterpanes and steranes). These fingerprinting techniques are

suitable for the detection of compositional features which are unique and fairlystable during migration and degradation. Other parameters that are less resistant to change were also monitored, including gas molecular and isotopic compositions and alkane distributions. This study evaluates bulk and molecular parameters as indicators of deeper reservoired petroleum, determines the extent and type of fractionation that accompanies movement of hydrocarbons through several thousand meters of sediment and subsequently through the water column, establishes a link between natural seepage and sea slick or tar ball formation and determines which of the measured parameters are least susceptible to alteration and thus more clearly reflect their source.

METHODS

Materials and sampling

Oils and gases reservoired at 2000–3000 m in the Green Canyon (GC) area were provided by Conoco, Inc. Previous studies have identified the GC-184 and 190/234 areas as sites of oil seepage and thermogenic gas hydrates (Brooks et al., 1984; 1986b). The GC lease area is the site of a number of active seeps as identified by visibly oil-stained cores (Anderson et al., 1983; Brooks et al., 1984; 1986b; 1987). The location of oil seepage is confirmed by coring in shallow seismic wipe-out zones identified from 3.5 kHz data (Brooks et al., 1984; 1986b). Figure 1 shows a shallow hazard survey from GC-184 (after McClellan Engineers, personal communication, 1985). Oil-stained cores are preferentially recovered in these seismic wipe-out or transparent zones.

Sediments for this study were retrieved by piston coring. The core utilized for this study was taken at 27°44.2′N, 91°11.9′W. Core data from this and nearby areas can be found in Brooks et al. (1984; 1986b). Surface slick samples were obtained at 27°43.1′N, 91°08.8′W by skimming surface water into precleaned 2-l glass bottles and by adsorption onto a metallic screen. During Cruise 85-G-5 (May 1985, R/V "Gyre") oil droplets were observed bursting at the sea surface in both the GC-184/185 and 190/234 areas. The sea was calm during this period allowing observation of the seepage. At these sites small oil droplets would rise to the sea surface, forming circular slicks ~0.4 m in diameter. Oil droplets were observed bursting at the surface every few seconds within a few hundred meters of the drifting ship. Fresh tar balls in the area were collected by manually retrieving them from the sea surface. Hydrate samples recovered during piston coring were immediately stored in liquid nitrogen (Brooks et al., 1986b).

Gas analyses

Hydrates were allowed to thermally decompose in pressure vessels at room temperature. Headspace samples were then removed by syringe for molecular and isotopic analyses (Brooks et al., 1986b). Reservoir gases were collected in stainless steel pressure vessels. Gases were analyzed for molecular composition

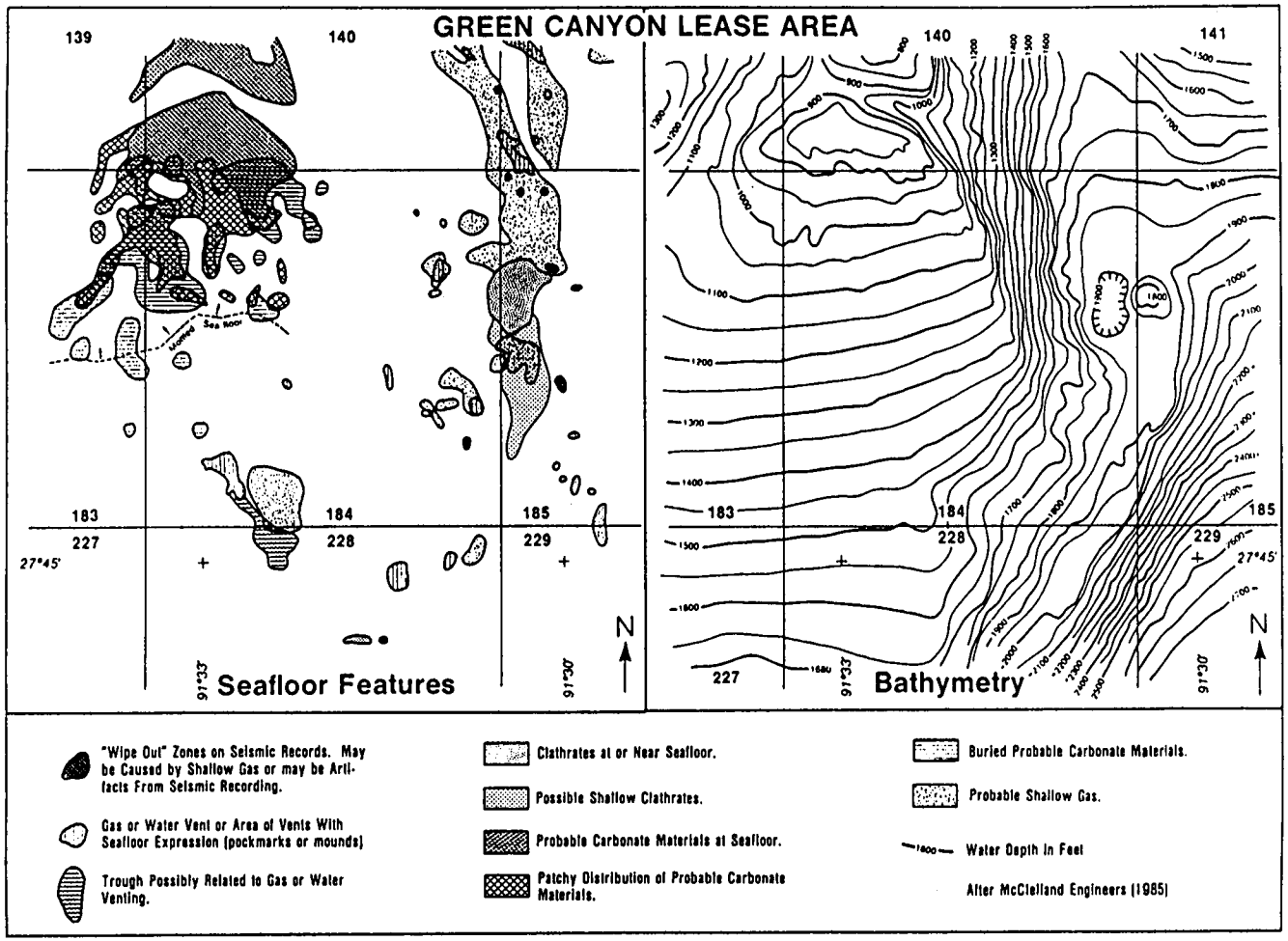

Fig. 1. Shallow hazard survey of GC-184 area.

by gas chromatography (Brooks et al., 1986b). Headspace and adsorbed gas analyses were similar to those performed by Bernard et al. (1978) and Faber and Stahl (1983), respectively.

Extraction and fractionation

Hydrocarbons were recovered from sediment and sea slick samples by extraction with CH_2Cl_2. Sediment samples were dried with Na_2SO_4 and Soxhlet extracted with CH_2Cl_2 for 1–3 h onboard ship. Slick-containing water was acidified with HCl to pH 2 and solvent extracted three times with CH_2Cl_2 (10:1 solvent:sample, v:v). All glassware was precleaned with solvents and combusted at 450°C for 4 h when appropriate. The collected extracts were concentrated by rotoevaporation. Extracts were then fractionated by alumina/silica gel column chromatography for further molecular and isotopic analyses. An aliphatic fraction was eluted with 150 ml of hexane and an aromatic fraction was obtained by elution with benzene (150 ml). Fractions were rotoevaporated and further concentrated with a purified air stream.

Gas chromatography

A variety of gas chromatographic columns were used for the various analyses. Whole oils were analyzed on a high performance cross-linked methyl silicon fused silica capillary column with splitless injection (film thickness 0.52 μm; i.d. = 0.31 mm; length = 50 m). The column was initially held at – 20°C for 10 min and then ramped at 8°C min^{-1} to 300°C (15 min hold time). The injection port and detector were both held at 300°C. Column chromatography fractions were also analyzed by capillary gas chromatography with flame ionization detection (GC-FID) on a column similar to that used for whole oil analysis except that a smaller i.d. (0.20 mm) and a shorter length (25 m) were used. GC-FID conditions for aliphatic and aromatic fraction analyses were an initial temperature of 60°C with no hold time and then programming at 12°C min^{-1} to 300°C (9 min hold time). The injection port and detector were again held at 300°C. The aromatic fractions were also analyzed by GC-FPD for sulfur aromatic compound distributions using a cross-linked methyl silicon liquid phase (film thickness = 1.05 μm; i.d. = 0.32 mm; column length = 50 m). The temperature program was identical to that used for the aliphatic fraction GC-FID analysis. The detector was held at 250°C and the injection port at 300°C. The gas chromatographs were either Hewlett-Packard Model 5880 or 5790.

Gas chromatography/mass spectrometry (GC/MS)

GC/MS was performed with a Hewlett-Packard 5996 GC/MS system linked to a HP 1000 computer for data storage and processing. The GC/MS was operated in the electron impact mode using 70-eV electrons. The injection port, interface, and source temperatures were 300, 250 and 270°C, respectively.

Extract fractions were analyzed for various molecular level distributions. Aliphatic fractions from column chromatography were molecular sieved (5 Å) to remove straight chain alkanes. The aliphatic fraction was then analyzed in the selected ion mode for triterpanes (m/z = 191), steranes (m/z = 217), monoaromatized steranes (m/z = 239, 253) and demethylated triterpanes (m/z = 177). Aromatic fractions were analyzed, with no further preparation, for triaromatized steranes (m/z = 231, 245), naphthalenes (m/z = 128, 142, 156, 170) and phenanthrenes (m/z = 178, 192, 206, 220). The column used for GC/MS was identical to that used for the whole oil analysis. The column was held at 50°C for 10 min and then programmed as follows: 10°C min^{-1} to 200°C (15 min hold time); 5°C min^{-1} to 250°C (24 min hold time); 2°C min^{-1} to 280°C (24 min hold time); and 1°C min^{-1} to 281°C (6 min hold time).

Other procedures

Samples to be analyzed for stable carbon isotopes ($\delta^{13}C$) were processed by standard methods using both Craig-type and closed-vessel combustion techniques (Sackett et al., 1970; Schoell et al., 1983). Carbon dioxide was analyzed on a Finnigan MAT-251 isotope ratio mass spectrometer. The isotopic composition is reported in the usual δ-notation (versus the Pee Dee Belemnite standard).

The total scanning fluorescence method is described in detail elsewhere (Brooks et al., 1983; Kennicutt and Brooks, 1983; Kennicutt et al., 1986). Briefly, the aromatic fraction recovered from liquid chromatography was quantitatively diluted in hexane. The sample was then introduced, via a cuvette, into a 650-40 Perkin-Elmer microprocessor-controlled spectrofluorometer. The excitation monochromator was set at a given wavelength and the emission monochromator was stepped from 200 to 500 nm. Resolution was 10 nm and the excitation wavelengths were varied from 200 to 500 nm.

RESULTS AND DISCUSSION

Gases

The reservoired gases from Green Canyon area (Table I) are characteristic of mature, oil-associated gas (Schoell, 1983). The gas occluded in hydrates from the Green Canyon area (Table I; Brooks et al., 1986b) are very similar in carbon isotope composition to the reservoired gas, suggesting that the hydrate gas is derived from the deep reservoired gas. Brooks et al. (1986b), in reporting the compositions of Green Canyon hydrates, were unable to explain isotopically-heavy CO_2 in the hydrate lattice while large quantities of isotopically-light authigenic carbonate also occur. The authigenic carbonate in these shallow sediments results from bacterial degradation of oil or gas, producing CO_2 which is then precipitated as calcium carbonate (Brooks et al., 1984; 1986b). Reservoir gas data indicates that the hydrate CO_2 originates from the reservoir with little

TABLE I

Gas compositions (%) of reservoired and sediment gas from the Green Canyon (carbon isotopic compositions in parentheses)

	GC-184[a] 6603–6652	GC-184[a] 6807–6860	GC-184[a] 7264–7294	GC-185[b] hydrate	GC-183[c] free	GC-183[d] adsorbed
Methane	84.9(−46.4)[e]	89.0(−46.1)	75.4(−45.8)	67.5(−44.8)	91.2–99.8 (−56 to −85)	83–87 (−43 to −50)
Ethane	7.3(−30.3)	6.8(−30.0)	6.3(−31.8)	4.5(−29.3)	0.1–1.7	7.2–8.6
Propane	3.5(−28.7)	2.9(−27.8)	2.5(−27.0)	14.9(−18.6)	0.01–7.8	3.8–5.2
i-Butane	0.3(−34.4)	0.4	< 0.1	4.2 (−28.6)	< 0.1	0.6–1.0
n-Butane	0.8	< 0.1	< 0.1	0.2	< 0.1–0.2	0.8–1.4
CO_2	2.9(+11.5)	1.1(+7.6)	0.2	3.9(+13.3)		

[a] Reservoired gas from the specified depths (in feet).
[b] Gas from hydrate decomposition (Brooks et al., 1984).
[c] Free (headspace) gas from 12-m piston core; values represent ranges of compositions; methane concentrations in the sediment range from 43 to 24 500 ppb by weight; one surface value was not included in the ranges because it exhibited oxidation effects; the methane isotopic values were mainly in the −75 to −85‰ range with the heavier values occurring in the sulfate reducing zone in the upper portion of the core; high methane concentrations in the lower portion of the core are a result of production in the sulfate-free zone. This data is taken from Faber et al. (1987).
[d] Adsorbed gas data is from the same core as the free gas analyses; methane concentrations range from 410 to 1537 ppb by weight; an extensive treatment of this data is available in Faber et al. (1987).
[e] Carbon isotopic compositions in ‰ vs. PDB.

or no contribution from CO_2 derived from oil degradation (Table I). The cores contain isotopically-light pore fluid CO_2, suggesting that little isotopic exchange occurs between pore fluid gas and gas occluded in the hydrates (Brooks et al., 1984). Molecular compositional differences between hydrate and reservoired gases can be explained by exclusion of gases larger than the hydrate cage structure. Little n-butane is detected in the hydrate gas because this molecule is too large. The anomalous isotopic composition of the propane in the gas hydrate sample may be an analytical artifact; other hydrate samples from the Green Canyon area have propane isotopic ratios characteristic of reservoir propane (Brooks et al., 1984).

Faber et al. (1987) have reported headspace and adsorbed gas molecular and isotopic compositions from an area (GC-183) adjacent to the reservoired gases reported here. The average and range of molecular and isotopic compositions from a 12-m piston core in this block are presented in Table I. Faber et al. (1987) discuss these data in detail and suggest that the adsorbed gas is more characteristic of the upward migrating gas than the free (headspace) gas. The free gas apparently originates from shallow microbial gas production. Adsorbed gas

data from the GC-184 area suggests upward migration of gas from deeper, more mature sources (Faber et al., 1987).

Aliphatic hydrocarbon distributions

Whole oils reservoired in the Green Canyon area have a range of compositions (Fig. 2). These oils are generally depleted in normal alkanes, suggesting that they are biodegraded to varying degrees. In conflict with this is the substantial amount of C_3 to C_{10} hydrocarbons, which should be depleted at the level of degradation inferred from the loss of the higher n-alkanes. In particular, Oil III in Fig. 2 is substantially enriched in gasoline range hydrocarbons. These may represent admixtures of degraded oil and lighter condensate/oil. These oils represent the range of chemical compositions of known production in the Green Canyon area and thus potential original fluid compositions for seepage. Oil IV (Fig. 2) is used for comparisons in Figs. 3–12.

Sediment extracts from GC-184/185 and 190/234 seep areas show a wide range in the extent of biodegradation. Exhaustive degradation (the complete loss of alkanes, isoprenoids and selected aromatics and biomarkers) occurs in many sediments containing high concentrations of oil (> 500 ppm). In other nearby areas containing only microseepage (< 500 ppm), the complete suite of

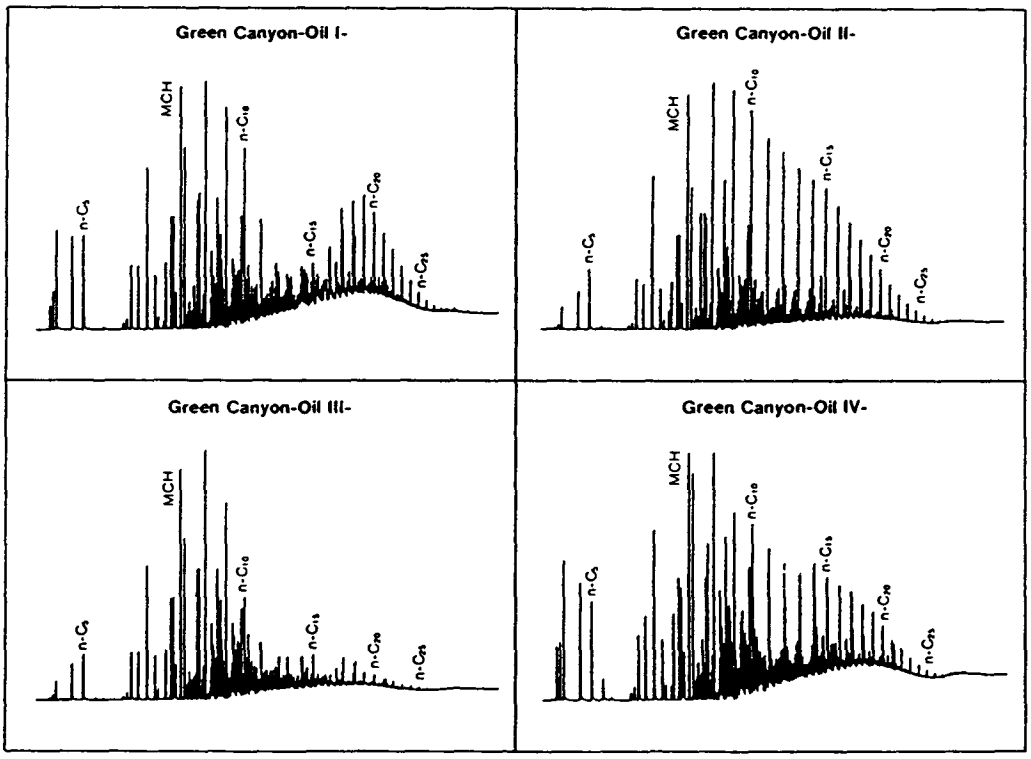

Fig. 2. Capillary gas chromatograms of four reservoired oils from the Green Canyon area (MCH = methyl cyclohexane).

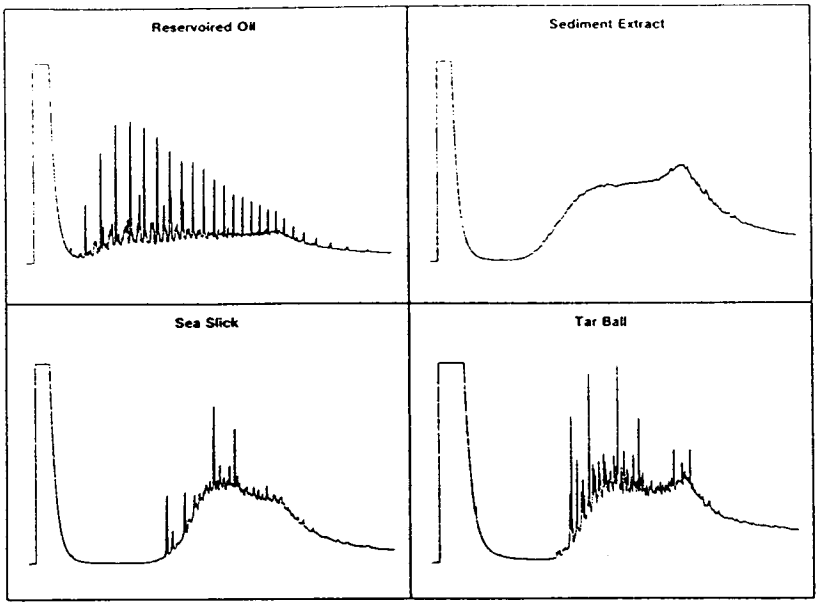

Fig. 3. Capillary gas chromatograms of a reservoired oil, sediment extract, sea surface slick and tar ball.

n-alkanes and isoprenoids are generally present. The sea slick and tar ball are less degraded than the sediment extract presented in Fig. 3. The resolved peaks observed in the slick and tar ball gas chromatograms are primarily aromatic compounds, not aliphatics. The sea slick and tar ball have been extensively altered by biodegradation (Kennicutt and Brooks, 1983; Kennicutt, 1987, and references therein).

Aromatic hydrocarbons

The distribution of naphthalenes in the reservoired oil is very similar to that in the sediment extract, somewhat similar to that in the sea slick but rather dissimilar to that in the tar ball (Fig. 4). The tar ball has been highly altered and few naphthalenes are present. The isomeric distributions of the C_2- and C_3-naphthalenes are similar for the oil and sediment extract. The amount of naphthalene compared to the total naphthalenes decreases from the oil to the extract to the slick. A similar loss of lower molecular weight compounds has been shown to be due to microbial degradation and/or dissolution (Romeu, 1986; Kennicutt, 1987; Philp and Lewis, 1987).

The distribution of phenanthrene and its alkylated homologs is also similar in all four fluids (Fig. 5). Although few naphthalenes were present in the tar ball, significant amounts of phenanthrenes were found. The higher alkylated homologs are more concentrated in the sediment extract, the sea slick and the tar ball than in the reservoired oil (Table II). This enhancement is again due to the selective loss of lower molecular weight compounds by degradation or

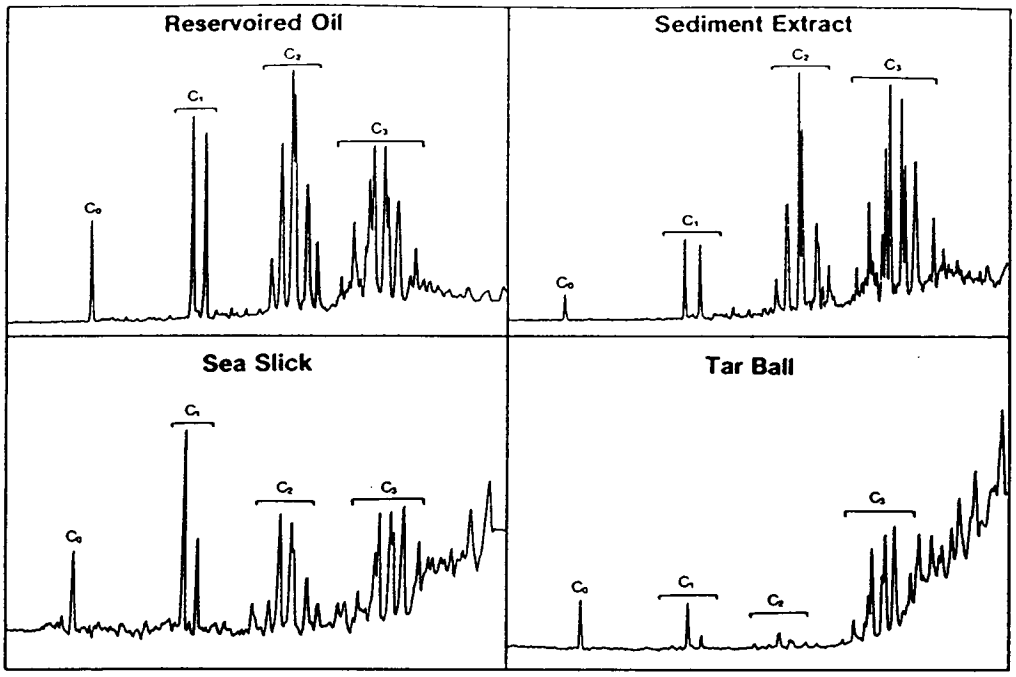

Fig. 4. The distribution of naphthalene and C_1-, C_2- and C_3-naphthalene in the four hydrocarbon fluids studied (m/z = 128 + 142 + 156 + 170).

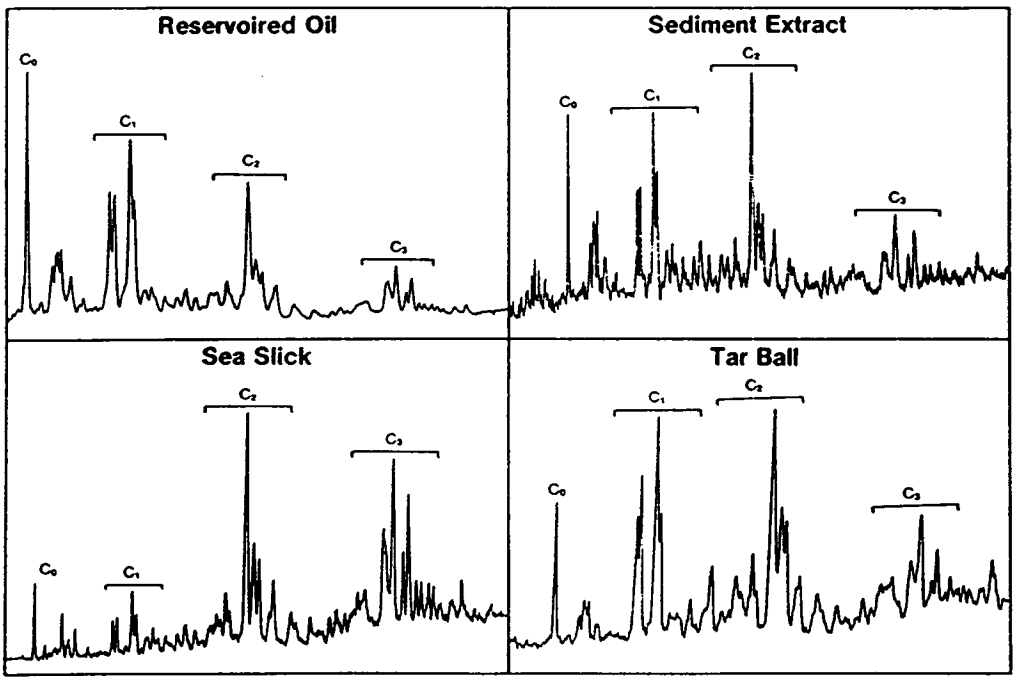

Fig. 5. The distribution of phenanthrene in the four hydrocarbon fluids studied (m/z = 178 + 192 + 206 + 220).

TABLE II

Relative compositions of selected aromatics (%)

	Reservoired oil	Sediment extract	Surface slick	Tar ball
Naphthalenes[a]				
C_0	4.2	1.8	7.3	9.2
C_1	18.0	10.1	24.1	11.0
C_2	39.1	35.0	29.3	7.6
C_3	38.7	53.2	39.4	72.2
Phenanthrenes[a]				
C_0	13.6	7.4	13.1	9.2
C_1	36.1	25.4	15.4	30.4
C_2	33.5	39.4	39.3	39.0
C_3	16.8	27.7	32.1	21.4
Dibenzothiophenes[b]				
C_0	1.8	0.2	0.0	0.7
C_1	16.2	9.7	4.1	15.3
C_2	46.8	43.7	37.0	53.3
C_3	35.3	46.4	58.9	30.6
ΣNaphthalenes				
ΣPhenanthrenes	3.5	0.04	0.15	4.0
Phenanthrenes C_3/C_0	1.2	3.7	2.5	2.3
Naphthalenes C_3/C_0	9.2	29.6	5.4	7.9

[a] Compositions are calculated from the integrated areas of their respective molecular ions.
[b] Compositions are calculated from the integrated areas obtained by GC/FPD analysis.

water solubilization. Degradation is known to be isomer specific within a given degree of alkylation (Fedorak and Westlake, 1981; Solanas et al., 1984; Volkman et al., 1984; Romeu, 1986), but minimal changes in the phenanthrene isomer distributions are apparent. Solubility differences between isomers of the same degree of alkylation would be minor, thus maintaining isomeric ratios to a large extent. The distribution of alkyl phenanthrenes suggests that the increase of higher over lower molecular weight components is due to differential solution and not degradation. It should also be noted that the largest increase occurs between the reservoired oil and the near-surface sediment (Table II).

Alkyldibenzothiophene distributions were similar for all four hydrocarbon mixtures (Fig. 6). There was again a preference for higher over lower molecular weight components within the sediment extract, slick and tar ball as compared to the reservoired oil.

In general, the wavelengths of maximum fluorescence increase with increasing ring number (Brooks et al., 1983; 1986a; Kennicutt and Brooks, 1983; Kennicutt et al., 1986). The reservoired oil has significantly more fluorescence due to more highly condensed aromatic compounds than the extract, slick or tar ball (Figs. 7 and 8). In this case, the near-surface residues appear to be

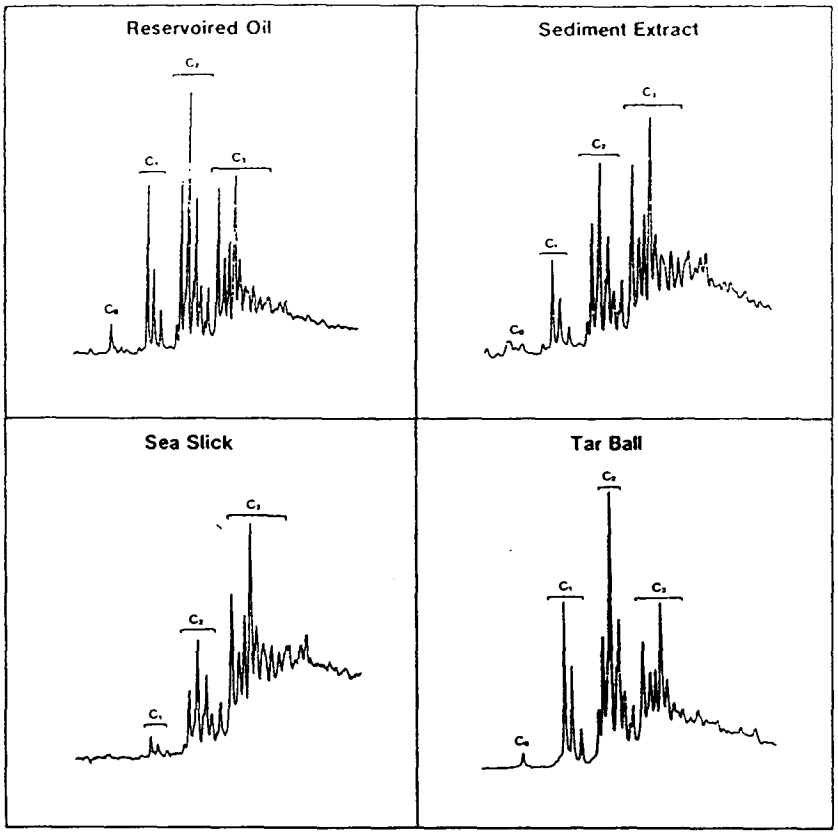

Fig. 6. The distribution of dibenzothiophene and C_1-, C_2-, and C_3-dibenzothrophenes in the four hydrocarbon fluids studied.

enhanced in lower molecular weight compounds over the reservoired fluid. This is in contrast to the specific compound class analyses afforded by GC/MS and GC-FPD, where within a homologous series, the higher molecular weight compounds are enhanced over lower ones. Changes in alkylation cause only minor shifts in fluorescence spectra (Brooks et al., 1986a; Kennicutt et al., 1986).

Three processes determine the composition of the near surface fluids. Two of these, dissolution and degradation, tend to deplete the fluids in lower molecular weight components. Degradation greatly depletes the normal and branched alkanes in the fluids. The third, migration, tends to preferentially enhance lower over higher molecular weight compounds which have less mobility. The end product is a fluid depleted in larger ring number aromatic compounds, as evidenced by fluorescence analyses, as well as in the less alkylated compounds within a homologous series, as evidenced by GC/MS and GC-FPD analyses (Table II).

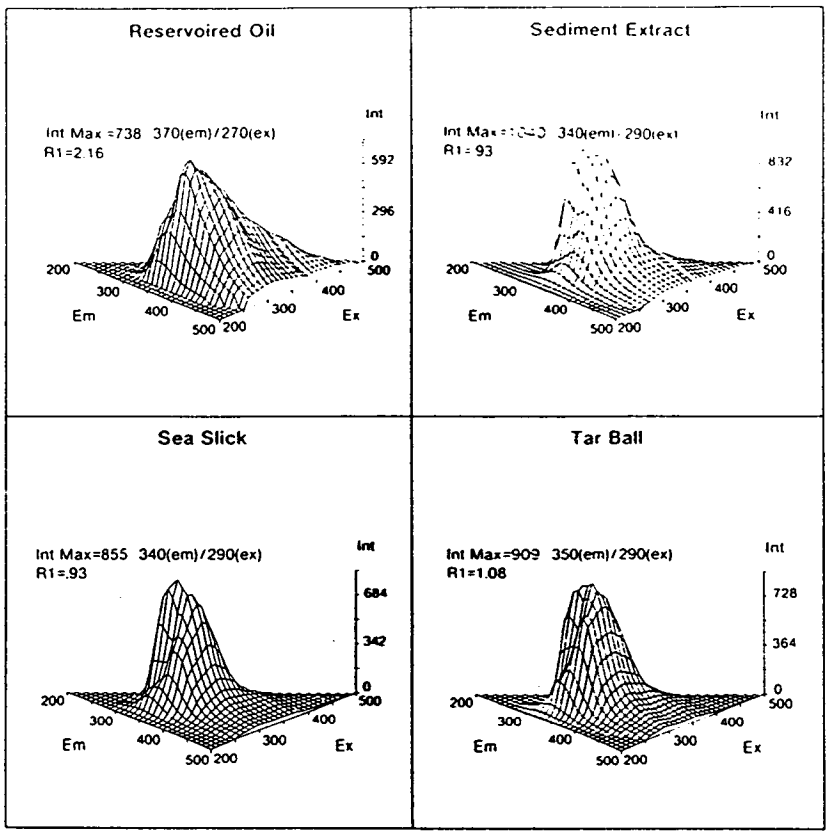

Fig. 7. Total scanning fluorescence spectra of the four hydrocarbon fluids studied (three dimensional presentation).

Carbon isotopic compositions – C15⁺

The carbon isotopic composition of the aliphatic and aromatic fractions of reservoired oils, sediment extracts, a sea slick and a tar ball are summarized in Table III and Fig. 9. The carbon isotopic composition of the sediment extracts, sea slick and the tar ball fall within the range of reservoired Gulf of Mexico oils. The range of oils presently known in the GC area is relatively restricted in its carbon isotopic composition. The larger range of sediment extract carbon isotopic composition may be due to mixing with carbon isotopically light, sedimentary indigenous lipid material or alteration in the near-surface sediments. However, carbon isotopic compositions are in general relatively resistant to microbial alteration. The range of carbon isotopic values is larger than would be expected given known production in the area, though a majority of the sediment extracts carbon isotopic compositions are similar to the reservoired oils. The sea slick and tar ball compositions fall close to the ranges for sediment extracts but would appear to be isotopically different from the known

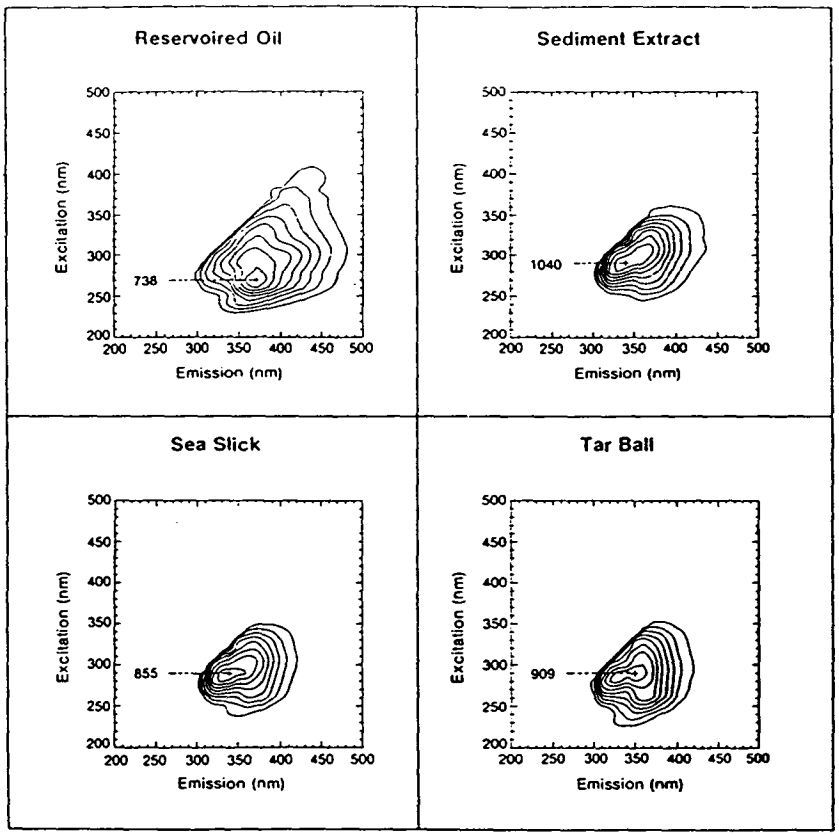

Fig. 8. The fluorescence spectra of the four hydrocarbon fluids studied (contour presentation).

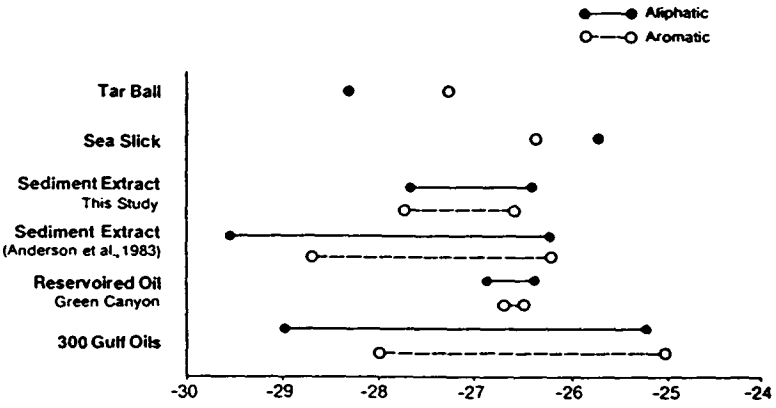

Fig. 9. A summary of the carbon isotopic composition of the aliphatic and aromatic fractions of hydrocarbons from the study area.

TABLE III

Summary of the carbon isotopic compositions of high molecular weight hydrocarbons at a seep zone in the Green Canyon lease area

Sample	Aliphatics[a]	Aromatics[a]
Reservoired oil[b]		
GC-184 (9026)	−26.4	−26.5
GC-184 (9050)	−26.5	−26.5
GC-184 (6458)	−26.9	−26.6
GC-52 (4500)	−26.9	−26.7
Average	−26.7(±0.3)	−26.6(±0.1)
Sediment extracts[c]		
0–20	−26.4	−26.6
21–40	−27.7	−27.7
0–20	−26.8	−26.6
21–40	−26.8	−26.6
41–60	−26.6	−26.6
61–80	−26.5	−26.5
81–100	−26.7	−26.6
141–160	−26.7	−26.7
161–180	–	−26.7
141–160	−26.8	−26.6
Average, this study	−26.8(±0.4)	−26.7(±0.3)
Average, Anderson et al., (1983) (n = 19)	−27.0(±1.0)	−26.9(±0.8)
Sea Slick	−25.7	−26.4
Tar Ball	−28.3	−27.3

[a] $\delta^{13}C$ values vs. the PDB standard deviation are given (‰); NBS-22 = −29.8‰.
[b] Subsurface depths (feet) are given in parentheses.
[c] Depths in cm.

reservoired oil in the GC area. The reason for this difference, given the high degree of similarity of numerous molecular level analyses, is not clear. It is possible that the sea slick and tar ball contain some input of non-Gulf of Mexico oil that was present at the sea surface. However, this component would not appear to be important based on other analyses.

Selected biomarker distributions

Biological marker distributions are similar between the reservoired oil and the three near surface hydrocarbon mixtures (Figs. 10–12; Table IV). Sediment extracts often have triterpane and sterane distributions that are reduced in intensity, though the concentration ratios are similar to those in reservoired oil. The tar ball is similar to the reservoired oil in all biomarker distributions except for the ratio of C_{30} to C_{29} hopanes.

Triterpane distributions are typical of mature Gulf of Mexico oils, with C_{29} and C_{30} being the predominate hopanes. The ratios Ts/Tm (C_{27} steranes) and $20R/(20S + 20R)$ (S and R isomers) for C_{31-34} hopanes are similar for all four

TABLE IV

Relative compositions of selected biomarkers[a]

	Reservoired oil	Sediment[b] extract	Surface slick	Tar ball
Triterpanes				
Ts/Tm	0.88	–	0.95	0.87
Hopane C_{30}/C_{29}	1.25	–	1.12	0.87
$S/(R + S)$				
C_{31}	0.58	–	0.56	0.58
C_{32}	0.63	–	0.62	0.62
C_{33}	0.59	–	0.61	0.62
C_{34}	0.67	–	0.76	0.68
Hopane/moretane				
C_{29}/C_{29}	11.05	–	10.10	11.67
C_{30}/C_{30}	12.38	–	8.44	6.32
Steranes[c]				
$S/R + S$				
C_{29}	0.48	–	0.53	0.52
C_{28}	0.69	–	0.65	0.64
C_{27}	0.59	–	0.66	0.60
$\alpha\alpha/\beta\beta$				
C_{29}	0.61	–	0.66	0.77
C_{28}	0.98	–	1.01	1.20
C_{27}	0.62	–	0.68	0.80
Triaromatized steranes				
$C_{20} + C_{21}/C_{26}$–C_{28}	0.23	0.21	0.15	0.27
$C_{20}/C_{20} + C_{27}$	0.56	0.48	0.38	0.61
$C_{20}/C_{20} + C_{28}$	0.67	0.59	0.50	0.69

[a] All ratios are calculated on integrated areas from their respective fragment ions (triterpanes, m/z = 191; steranes, m/z = 217; triaromatized steranes, m/z = 231).
[b] The sediment extract triterpane and sterane traces were highly altered.
[c] C_{27} and C_{28} steranes contain coeluting diasteranes.

hydrocarbon fluids (Table IV). As mentioned, the sea slick and tar ball are depleted in C_{30}- relative to C_{29}-hopane as compared to the reservoired oil. The slick and tar ball are also depleted in the C_{30}-hopane relative to moretane, as compared to the reservoired oils, suggesting preferential loss of the C_{30}-hopane. The reason for these subtle differences are not clear and may simply reflect the range in the properties of reservoired oils in the area.

Sterane distributions are also similar for all four hydrocarbon fluids (Fig. 10; Table IV). One observation is the depletion of $\beta\beta$ steranes over $\alpha\alpha$ steranes in the near surface fluids compared to the reservoired oil. This depletion is present in all three sets (i.e., $C_{27,28,29}$) of sterane peaks. This feature may reflect variability in the reservoired oils. Triaromatized sterane distributions are very similar for all four hydrocarbon mixtures (Fig. 11). A slight depletion

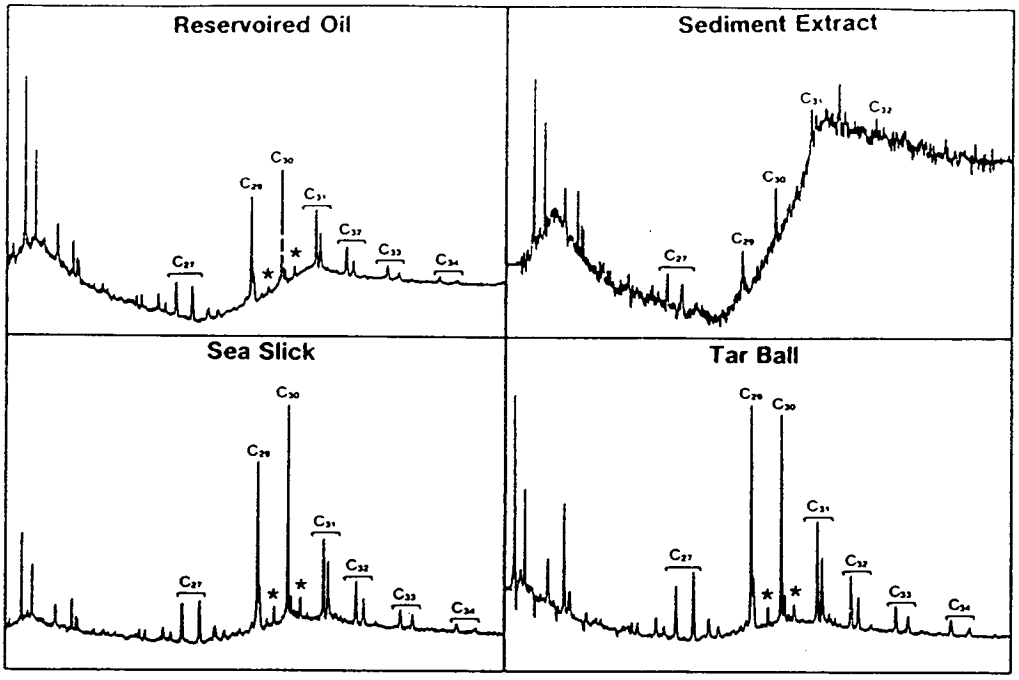

Fig. 10. The distribution of triterpanes in the four hydrocarbon fluids studied (m/z = 191).

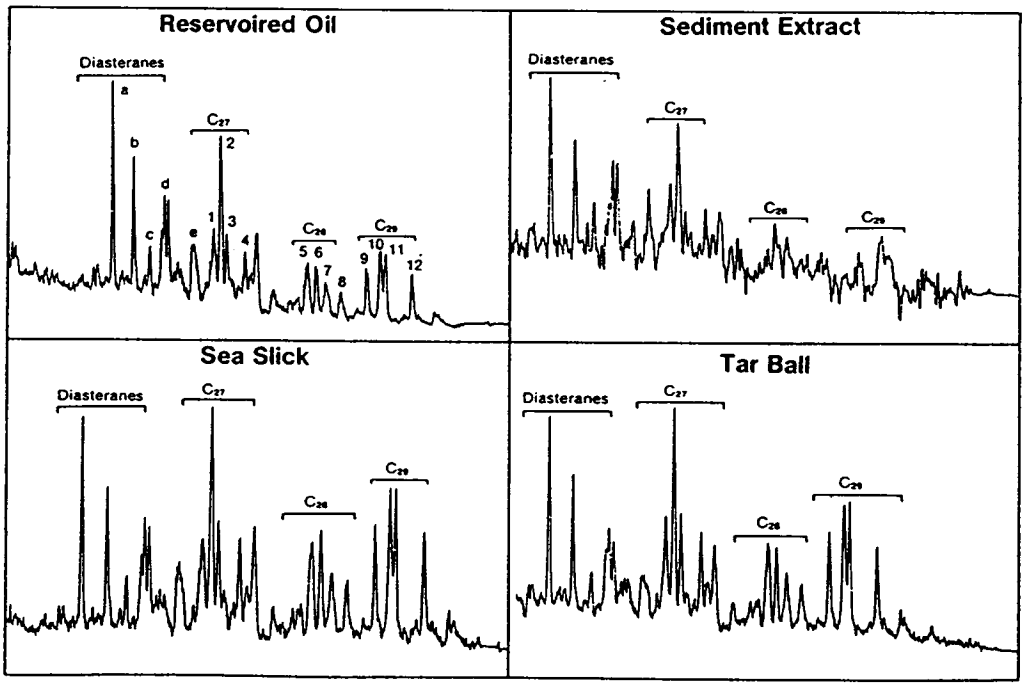

Fig. 11. The distribution of regular and rearranged steranes in the four hydrocarbon fluids studied (m/z = 217).

in lower molecular weight compounds is observed in the sediment extract and sea slick for triaromatized steranes over the reservoired oil.

CONCLUSION

Oil and gas generated deep in subsurface strata have migrated up faults to the near surface on the Gulf of Mexico continental slope. In the area studied, the amount of oil in the sediments is sufficient for globules to leave the sediment, rise to the seawater surface and form slicks and tar balls. Conditions are such that gas hydrates have formed in the sediments. Extensive alteration of oil and gas has directly led to the authigenic formation of isotopically light carbonate through the biologically mediated formation of excess CO_2. Deeply reservoired CO_2 ($\delta^{13}C = +7.6, +11.1$) is present in the hydrate lattice and interstitial waters. The hydrate gases have undergone little or no isotopic fractionation during migration through several thousand meters of sediment.

Liquid hydrocarbons are subject to changes during migration as well as, after their arrival at the near surface. Many characteristics of the molecular distribution patterns are similar between the reservoired oils and the near surface fluids, including the compositions of the higher alkylated ($> C_2$) aromatic compounds, triterpanes, steranes and aromatized steranes. These similarities directly link sediment extracts, sea slicks and tar ball formation with a common source in the reservoired oils. Migration tends to concentrate

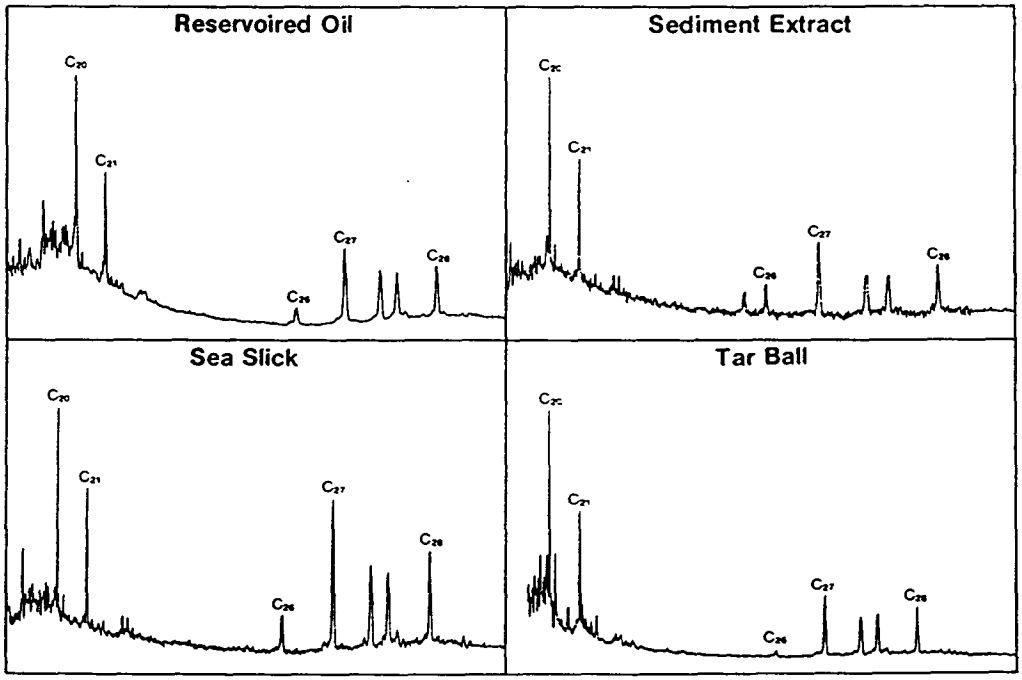

Fig. 12. The distribution of triaromatized steranes in the four hydrocarbon fluids studied ($m/z = 231$).

lower molecular weight compounds, whereas degradation and dissolution concentrate higher molecular weight compounds in the residue. The end-product fluid is depleted in larger ring aromatics as well as parent and shorter chain alkylated compounds within a homologous series. Alteration of the fluid reaching the sea surface can occur in the sediment as well as in the water column, though this will be a function of the residence time of the fluid at a particular location. The relative locations of the hydrocarbon fluids do not necessarily reflect an orderly or sequential relationship.

This and other studies suggest that natural liquid and gaseous hydrocarbon seepage is a wide-spread phenomenon in the marine environment.

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High-molecular Weight Hydrocarbons in the Gulf of Mexico Continental Slope Sediment

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High molecular weight hydrocarbons in Gulf of Mexico continental slope sediments

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Abstract—Sediments on the Gulf of Mexico continental slope contain a mixture of terrigenous, petroleum and planktonic hydrocarbons. The relative amount of these three inputs varies as a function of location, water depth, and time of sampling. The hydrocarbon concentrations measured are generally lower than those previously reported for shelf and coastal Gulf of Mexico sediments. The influence of land-derived material decreases from the central to the western to the eastern Gulf of Mexico. Petroleum inputs are measurable at all sites sampled. Natural seepage was considered to be a significant source of hydrocarbons to slope sediments. Hydrocarbon concentrations vary by 1–2 orders of magnitude along a given isobath due to changes in sediment texture and hydrocarbon inputs. Variability along an isobath is as great if not greater than that seen over a depth range of 300–3000 m along a single transect. In general, the highest aliphatic hydrocarbon concentrations are associated with the more clayish/organic-rich sediments. Aromatic hydrocarbons are below gas chromatographic detection limits at all sites (<5 ppb), but their presence is inferred from spectrofluorescence analyses, confirming the presence of petroleum-related hydrocarbons at all sites.

INTRODUCTION

THE OUTER continental shelf and slope in many areas of the world contain potential oil and gas reserves that only recently have become accessible. New technology, as well as new applications of old technology, has made the exploitation of mineral reserves in water depths exceeding 300 m economically feasible (FEDDERSON, 1982; HEDBERG, 1983; ANONYMOUS, 1983). The energy industry is rapidly moving into deeper and deeper water in the search for oil and gas to meet the world's energy needs (HUNT, 1983; SHANKS, 1983).

The onset of deep-water drilling activities may have, as has been suggested for shallow marine waters, an effect on marine hydrocarbon concentrations. A large number of reports have monitored the effects of petroleum development on the continental shelf (e.g. MIDDLEDITCH *et al.*, 1977, 1978, 1979; LYTLE and LYTLE, 1979; MENZIE, 1983; GASSMANN and POCKLINGTON, 1984; RICHARDSON, 1984), but little or no information is available at sites in water deeper than 350 m. In view of the potential hydrocarbon reserves in the deep (>300 m) Gulf of Mexico and the technological advances in deep-water drilling operations, the U.S. Department of Interior's Minerals Management Service deemed it important to develop a basic knowledge of deep Gulf fauna, their environment and ecological processes in advance of impending petroleum development.

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The program included the characterization of the present levels of hydrocarbon contamination in the sediments and selected biota in anticipation of petroleum resource development beyond the shelf slope break. The concentrations, distributions, and sources of hydrocarbons in Gulf of Mexico continental slope sediments are reported here for the first time.

METHODS

Sample locations and collection

Sediment samples for high molecular weight hydrocarbon analyses were collected during five cruises between 1983 and 1985. Locations of the sampling stations are shown in Fig. 1. Either three or six replicate samples were taken at each station using a 30 × 30 cm stainless steel box core. Undisturbed, uncontaminated replicate samples were taken from the top 10 cm of sediment immediately after removal of the overlying seawater. Subsamples for hydrocarbon analyses were stored frozen (–20°C) in precombusted glass jars. Each individual core sample from cruises I and II was analysed. Sediments from cruises III, IV, and V were pooled as one representative sample for each station.

Precleaning of all equipment and glassware included extensive washings with micro cleaning solution and triple rinsing with distilled water, acetone, and methylene chloride and/or combustion at 400°C for 4 h. All solvents were nanograde purity (Burdick & Jackson). Cleaning procedures were tested by collecting the final rinses and subjecting them to the entire analytical scheme. Blanks were reduced to negligible levels for all parameters monitored.

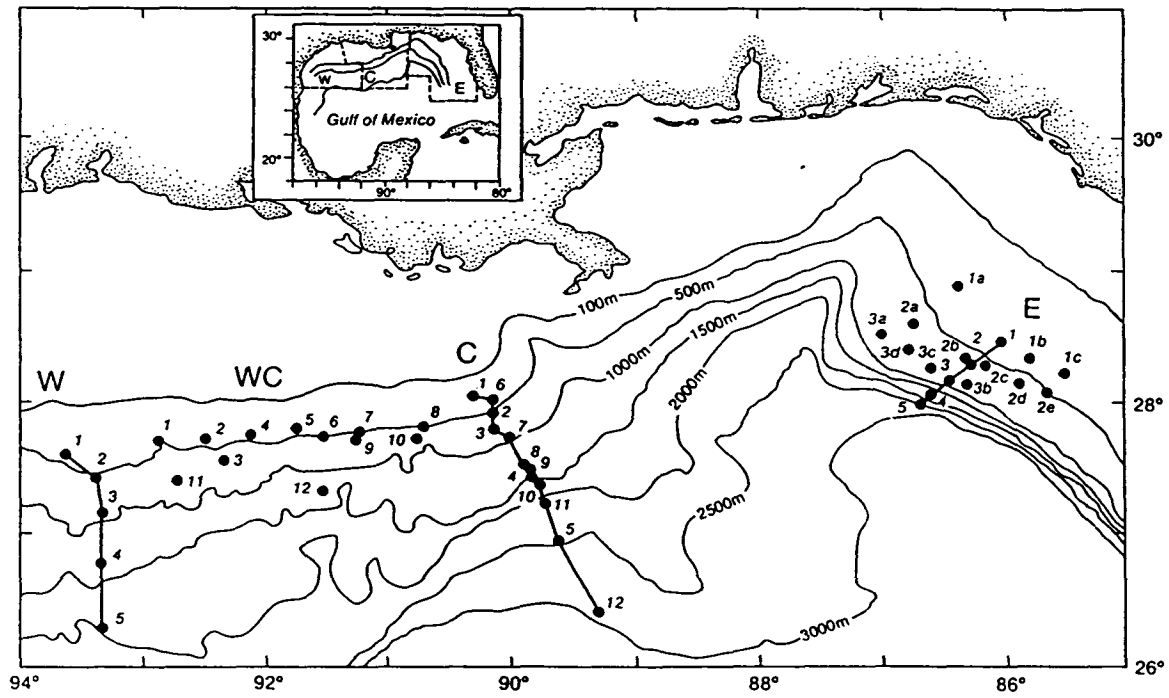

Fig. 1. Sampling locations on the Gulf of Mexico continental slope.

Digestion, extraction and separation

The digestion and extraction procedures followed, with slight variations, those of FARRINGTON *et al.* (1973) and DUNN (1976). Fifty grams of homogenized, freeze-dried sample were mixed with 90% ethanol or methanol (150 ml), hexane (60 ml), and KOH (10 g). The mixture was refluxed at 80°C for 3 h. The digested material was filtered and extracted three times with hexane. The combined extracts were washed three times with distilled water (500 ml each), dried with anhydrous Na_2SO_4 (3 g), and treated with activated copper to remove sulfur. After sulfur removal, the extracts were roto-evaporated to near dryness and transferred to clean, precombusted vials using small volumes of hexane. At all times care was exercised to ensure that the extracts did not go to complete dryness to prevent loss of the more volatile sample components.

The extracts, dissolved in 0.5 ml of hexane, were fractionated into saturated (f1) and aromatic/ester (f2) fractions using alumina/silica gel (80–100 mesh) columns (10 g each). Silica gel and alumina were activated at 150 and 350°C, respectively, for 16 h and then partially deactivated with 5% distilled water (w/w) (FARRINGTON *et al.*, 1973). The columns were eluted with 100 ml of hexane (f1) and 100 ml of benzene:hexane (50:50) (f2). After collection, each fraction was roto-evaporated, transferred to a precombusted vial, and dried. The aliphatic fractions were weighed to 0.1 μg on a Cahn Electrobalance by dissolving the sample in 100 μl of methylene chloride, withdrawing a 10 μl aliquot, and applying it to a pre-weighed filter pad.

The benzene:hexane fractions were further purified using Sephadex LH-20 columns (25–100 mesh) after RAMOS and PROHASKA (1981). The columns were calibrated by eluting a mixture of azulene and perylene of sufficient concentration to be visible under u.v. light. Aromatic standards were also run to confirm the fraction to be collected as per RAMOS and PROHASKA (1981). Samples, dissolved in 1 ml of the eluting solvent (cyclohexane:methanol:methylene chloride, 6:4:3) were applied to the top of the column. The purified f2 fractions were roto-evaporated, transferred to a vial, and weighed as described for the hexane fractions.

All samples were spiked with known amounts of internal standards to correct for experimental losses and incomplete extraction. Internal standards (IS) included *n*-decylbenzene, *n*-tetradecylbenzene (aliphatic), 1,1-binaphthyl, and 9,10-dihydroanthracene (aromatic). The internal standards were sufficiently resolved by gas chromatography from all sample components and were added at concentrations similar to the sample components of interest.

Gas chromatography (GC)–gas chromatography/mass spectrometry (GC/MS)

Fractions f1 and f2 were quantified by fused silica capillary GC using a Hewlett–Packard gas chromatograph (Model 5880) in a splitless capillary mode. Fused silica capillary columns coated with a bonded phase (BPI/QC2; SGE, Ltd) were used to obtain separation of the extract components. Columns were 50 m long with 0.25 mm i.d. Helium was used as a carrier gas at a flow rate of 2–3 ml min^{-1} and as a make-up gas between the capillary column and the flame ionization detector (FID). Temperatures were set at 300°C and 350°C for the injector and detector, respectively. Typical instrumental parameters were: initial temperature, 80°C (0 min); rate, +6°C min^{-1}; final temperature, 300°C (20 min).

Compounds in the gas chromatograms were identified and quantified by comparing the retention times and detector responses with the corresponding authentic standard.

Unresolved complex mixture (UCM) concentrations were calculated based on the average *n*-alkane response over the volatility range covered and the integrated area above the baseline.

GC/MS was used to confirm the identify of the sample components and to identify, when possible, any unknown compound with the use of mass spectral libraries and standards. The GC/MS analyses were conducted with a Hewlett–Packard 5995 GC/MS system coupled with a Hewlett–Packard 1000 data system. Typical operating conditions for the mass spectrometer were: source temperature, 300°C; electron energy, 70 eV; and scan rate, 215 amu s^{-1}. GC/MS columns and oven conditions were the same as those established for quantitative gas chromatographic analyses. Helium was used as carrier gas at a flow rate of 2 ml min^{-1}. A splitless injection technique was used and the total column effluent was routed directly into the ion source of the mass spectrometer.

RESULTS AND DISCUSSION

Sediments on the Gulf of Mexico slope contain a mixture of terrestrial, petrogenic and planktonic sourced hydrocarbons. Molecular level alkane distributions are similar at all locations sampled whereas the quantitative importance of the three major inputs varies with location, time of sampling and water depth. However, hydrocarbon concentrations are relatively uniform across the slope given the large geographical area. Extractable organic matter, aliphatic hydrocarbon concentrations and the aliphatic unresolved complex mixture range from 4.0 to 94.2, 0.1 to 5.2, and 0.7 to 81.4 μg g^{-1} dry weight of sediment, respectively (Table 1). These concentrations are generally lower than previously reported for Gulf of Mexico sediments (Table 2).

Individual hydrocarbon compounds are present at concentrations ranging from <0.01 to 0.5 μg g^{-1}. In general, the qualitative molecular level alkane distribution is similar at all sites sampled. The dominant *n*-alkane in the 15–22 carbon range is variable, whereas the normal alkanes with 23–32 carbons are consistently dominated by *n*-C_{29} or *n*-C_{31}. Alkane distributions for the Central Transect during cruise I are typical for all locations sampled (Fig. 2).

Table 1. *The averages and ranges (values in parentheses) for selected hydrocarbon parameters in Gulf of Mexico continental slope sediments* (μg g^{-1} *dry weight of sediment*)

Cruise	Location (transect)	Extractable organic matter	Aliphatic hydrocarbons	Aliphatic UCM
I	Central	28.4 (13.9–61.3)	1.6 (1.3–2.0)	23.3 (19.3–29.8)
II	Central	21.7 (18.0–25.2)	1.7 (1.6–1.8)	8.9 (6.0–14.0)
	Western	26.0 (14.0–55.2)	1.1 (0.8–1.3)	11.1 (5.2–11.4)
	Eastern	8.6 (7.6–10.9)	0.7 (0.5–1.0)	5.4 (3.2–7.3)
III	Central	18.1 (4.0–44.4)	1.4 (0.6–4.6)	9.7 (4.4–17.4)
IV	West/central	30.0 (17.7–94.2)	0.9 (0.4–5.2)	16.8 (4.2–81.4)
V	Eastern	7.2 (4.7–13.4)	0.2 (0.1–0.4)	2.0 (0.5–5.0)

Table 2. Summary of Gulf of Mexico sediment hydrocarbon analyses (concentrations are averages, ranges in parentheses)

Location	Total HC ($\mu g\ g^{-1}$)	Saturated HC ($\mu g\ g^{-1}$)	Predominant Source	References
Texas/Louisiana—coastal*	(20–190)		B/(P)	SMITH, Jr (1952)
Texas/Louisiana—coastal*	Low concentrations		B	STEVENS *et al.* (1956)
Gulf of Mexico—coastal†	Biogenic waxes		B	BRAY and EVANS (1961)
Florida (Bay)—sandy sediments†	4.4	2.0	B	PALACAS *et al.* (1972)
—muddy sediments†	86.0	30.0		
N.E. Coast—sandy sediments†	5.8 (0.2–19.9)	1.14 (0.1–3.8)	B/(P)	PALACAS *et al.* (1976)
STOCS—coastal† (Before, during and after drilling activities)	1.14 (0.22–5.6)	0.2 (0.1–0.5)	B/(P)	PARKER *et al.* (1976)
Texas/Louisina—coastal banks†	0.02–0.80		B	PARKER (1978)
MAFLA—nearshore Florida (<40 m)†	1.90	0.86 (0.29–1.60)	B	BOEHM (1979)
—>40 m Florida†	1.39	0.83 (0.29–1.89)	B/(P)	
—Mississippi/Alabama Shelf†	1.61	1.1 (0.28–2.89)	B/P	
Freeport, Texas—coastal†	7.15 (0.9–45)	0.71 (0.1–2.4)	B	SLOWEY (1980)
Texas Shelf†	1.7 (1.4–2.0)	0.5 (0.4–0.5)	B	LYTLE and LYTLE (1979)
Florida coastal (<60 m)†	3.1		B/P	GEARING *et al.* (1976)
W. of Mississippi R.—coastal (<60 m)†	11.7		B/P	GEARING *et al.* (1976)
Texas/Louisiana—coastal†	36.5 (5.71–87)	21.4 (3.1–50)	B/P	NULTON *et al.* (1981)

* Method, gravimetry.
† Method, GC, GC/MS.
B = biogenic; P = petrogenic.

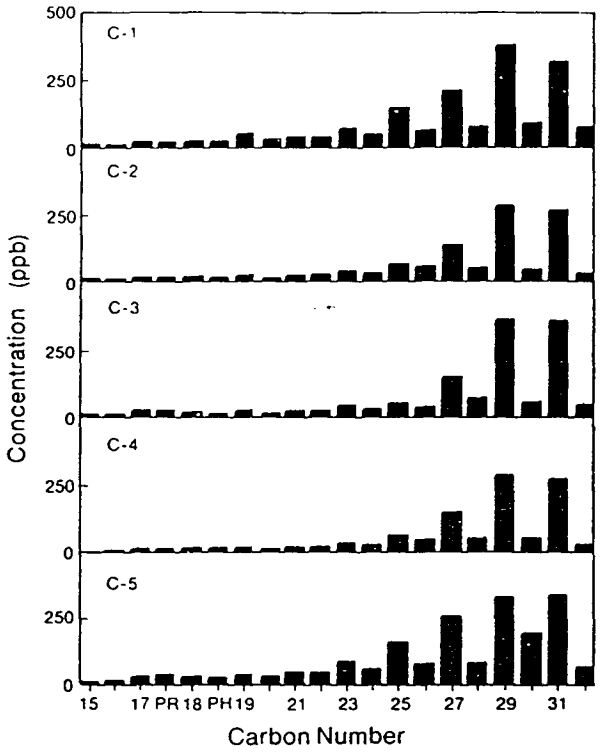

Fig. 2. Molecular level alkane distributions for sediment from the central transect during cruise I.

Hydrocarbon sources

Molecular level and bulk parameters can be used to estimate the relative importance of hydrocarbon sources at a given location. These parameters are based on the premise that hydrocarbon sources have unique fingerprints, i.e. certain recognizable suites of compounds. In nature however, few unique end-members occur. To better understand the dynamics of hydrocarbons in Gulf of Mexico slope sediments several diagnostic parameters were monitored:

Source	Indicator compound	Abbreviation
Planktonic/petroleum	Σn-$C_{15,17,19}$; pristane	PL-1
Petroleum/(planktonic?)	Σn-$C_{16,18,20}$; phytane	RE-Lo
Land/(petroleum)	Σn-$C_{25,27,29,31}$	TERR
Petroleum/(biogenic)	Σn-$C_{24,26,28,30}$	PE-Hi
Petroleum/(biogenic?)/ Recycled	Unresolved complex mixture	UCM

To use these indicators certain assumptions are made and need to be understood for the proper evaluation of the observed distributions. Plankton generally produce a simple mixture of hydrocarbons dominated by n-$C_{15,17,19}$ and pristane, so the presence of these compounds can be useful as a planktonic indicator (PL-1) (CLARK and BLUMER, 1967; BLUMER *et al.*, 1970; GOUTX and SALIOT, 1980; SALIOT, 1981). Petroleum also contains these compounds but usually contains nearly equal amounts of n-$C_{16,18,20}$ and phytane as well (FARRINGTON and TRIPP, 1977; FARRINGTON *et al.*, 1973; NAS, 1975, 1985; BOEHM,

1979; BROOKS, 1979). Thus a low molecular weight petroleum indicator (PE-Lo) can be used to assess the petroleum contribution to the planktonic indicator. In this case, we assume the contribution of petroleum to each indicator is equal, therefore the planktonic component can be inferred as PL-1 minus PE-Lo. This assumption is based on an extensive evaluation of over 400 Gulf of Mexico oils to be presented elsewhere. Gulf oils are typically mature to very mature with equal amounts of odd and even normal alkanes. Gulf of Mexico oils are generally dominated by alkanes between C_5 and C_{15} accompanied by a rapid decrease with higher molecular weight. Alkanes with >25 carbons are generally only a minor component of the oils.

Straight chain biowaxes with 25, 27, 29 and 31 carbons have been used extensively as an indicator of terrestrial or land-derived input (GEARING *et al.*, 1976; FARRINGTON and TRIPP, 1977; GIGER and SHAFNER, 1977; GIGER *et al.*, 1980; WAKEHAM and FARRINGTON, 1980). As such, the sum of these four normal alkanes can be used to indicate the terrestrial (TERR) hydrocarbon component. As with the planktonic indicator, these normal alkanes can also have a source in petroleum. Again, in general Gulf of Mexico petroleums contain near equal amounts of n-$C_{24,26,28,30}$ (PE-Hi). No evidence is known for immature petroleum reservoired in the Gulf which would contain a significant odd carbon preference. Immature extracts can be obtained from deeply buried sediments but this material would tend to remain *in situ*. As in the planktonic indicator, the terrestrial component can be estimated by subtracting the PE-Hi from the TERR concentration. Plants themselves also contain significant amounts of indigenous even carbon alkanes. Thus, this type of indicator provides a maximum petroleum indicator and a minimum terrestrial indicator over this molecular weight range. It should again be noted that most Gulf oils contain relatively small amounts of >25 carbon n-alkanes. The mixing of recent terrestrially derived hydrocarbons with mature oil would maintain a high CPI due to the mass balance. Thus the petroleum indicator is divided into a low and high molecular weight indicator to minimize the high estimates of petroleum due to biologically (terrestrial) produced n-alkanes.

These parameters, with the previously stated assumptions, can be used to assess the dynamics of hydrocarbons on the slope as a function of water depth, location, and time of sampling. In general, water depth will be considered with location and time of sampling discussions. These indicator parameters are also evaluated in terms of other paramenters such as: the UCM, an indicator of petroleum input; carbon preference index, an indicator of the relative amounts of odd and even normal alkanes; and bulk sediment parameters such as carbon isotopic compositions in order to more fully understand the observed distributions.

Areal distribution

Sampling during cruise II was undertaken to assess the distribution of sediment hydrocarbons on transects from the central, western and eastern Gulf of Mexico continental slope (Fig. 1). Extractable organic matter (EOM) is a composite of both biologically produced and petroleum-related lipid material. In general, EOM is lowest on the eastern transect and nearly equal on the western and central transects, with the exception of Sta. W1 (Fig. 3). The aliphatic UCM, a petroleum indicator, is similar for all three transects though slightly elevated in central transect sediments (Table 1). The elevated EOM at Sta. W1 corresponds to an increased UCM (i.e. petrogenic compo-

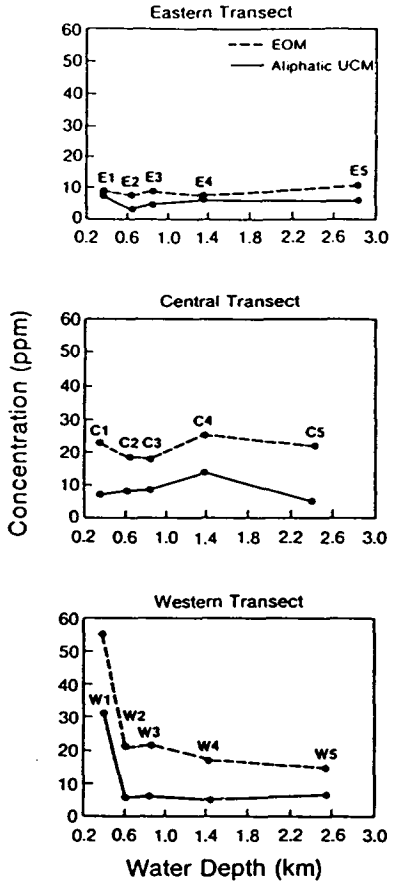

Fig. 3. Variation in extractable organic matter and the aliphatic unresolved complex mixture along transects in the eastern, central, and western Gulf of Mexico continental slope.

nent). The UCM which is used to indicate petroleum does not delineate the source of the petroleum, i.e. seepage, the water column or recycled organic matter.

The influence of land-derived material decreases from the central to the western to the eastern transect (Figs 4 and 5). Terrestrial hydrocarbon concentrations, as indicated by the Σn-$C_{25,27,29,31}$ (TERR), are relatively uniform with water depth on the central and western transects, whereas terrestrial content increases with water depth on the eastern transect. The influence of the land and/or river-derived material, as suggested by the predominance of odd *n*-alkanes from C_{23} to C_{31}, is readily apparent at all three locations and accounts for a majority of the GC-resolvable alkanes.

Plankton-derived hydrocarbons are low compared to the terrigenous and petroleum hydrocarbons and are often difficult to discern at the central and western transects (Figs 4 and 5). In general, the planktonic input is higher at the shallower stations of these two transects. The low planktonic hydrocarbon concentrations in the western and central transects may be due to the high sedimentation rate and/or dilution with terrestrial material. On the eastern transect, the planktonic input is discernible and relatively constant with depth. In general planktonic inputs accounted for <10% of the GC resolvable alkanes. Sediment biogenic hydrocarbons on the slope are dominated by the

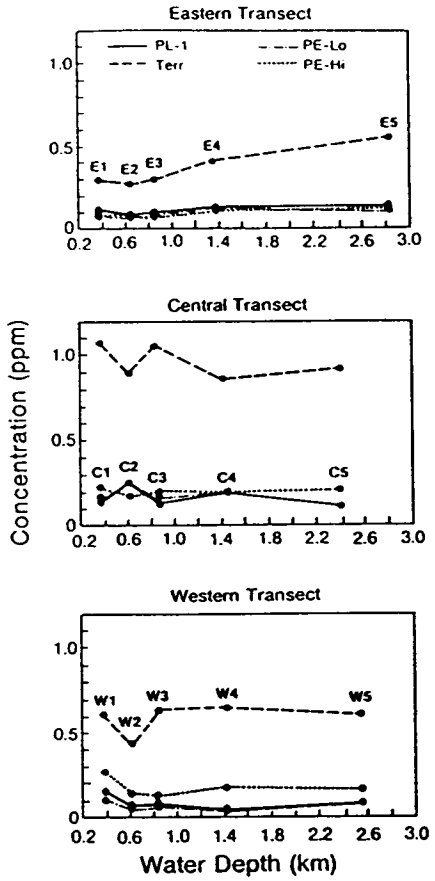

Fig. 4. Variation in hydrocarbon source parameters along transects in the eastern, central, and western Gulf of Mexico continental slope (for definition of abbreviations see the text).

more microbially resistant terrestrial components and the degree of dominance was a function of proximity to the Mississippi River and the topography of the slope.

Petroleum inputs, measured both by alkane parameters and the UCM, are present at all sites (Figs 3–5). In general less petroleum is indicated at the eastern than the western, with the highest values at the central transect. Petroleum hydrocarbons (a maximum estimate) are observed at low concentrations at all locations. In an effort to determine if the petroleum hydrocarbons detected are sourced in transported particles or due to upward migration, the petroleum indicators are compared to terrestrial and planktonic indicator distributions (Fig. 6). The general relationships might suggest a dual source for petroleum hydrocarbons. Low molecular weight hydrocarbons (PE-Lo) tend to increase with an increased terrestrial input on the eastern transect, but do not on the central and western transects. The higher molecular weight petroleum indicator (PE-Hi) strongly correlates with the terrestrial indicator (TERR). In this case, this simply reflects the large biogenic contribution to PE-Hi indicator. From this cross-plot, the ratio of the two parameters corresponds to a carbon preference index of ~4.3 indicating that a majority of the PE-Hi in these samples is biogenic in origin. Pre-industrial revolution sediments show a similar CPI of 4.9 (WADE and QUINN, 1979). Compared with Stas W2–W5, Sta. W1 contains significantly elevated petroleum hydrocarbon concentrations (see Fig. 7) as determined by the UCM which is an estimate of the amount of petroleum hydrocarbons.

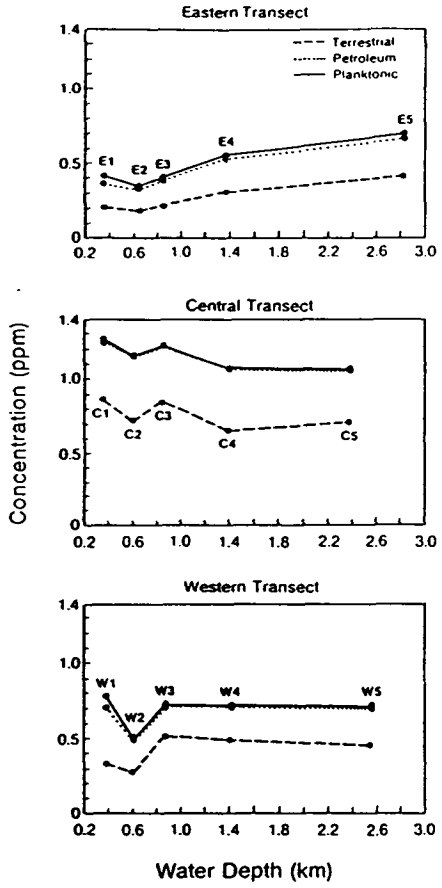

Fig. 5. Variation in planktonic, terrestrial, and petroleum hydrocarbons along three transects presented as a *cumulative* concentration.

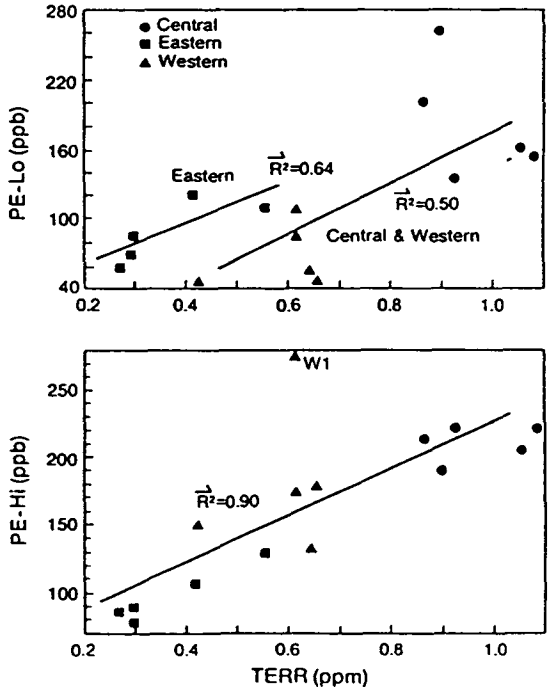

Fig. 6. The relationship between alkane petroleum indicators and a terrestrial indicator.

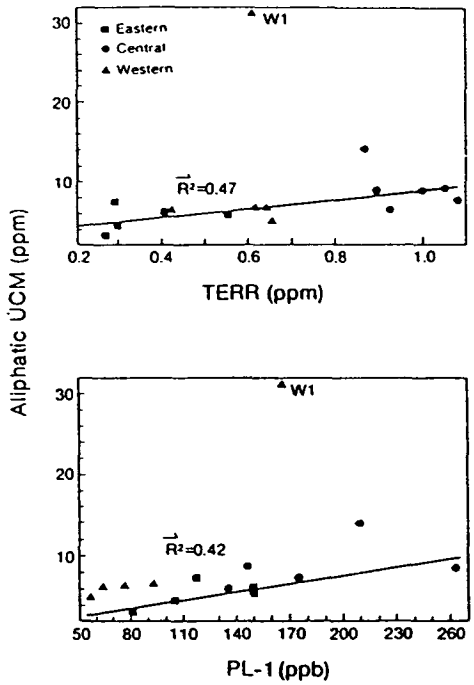

Fig. 7. The relationship between the aliphatic unresolved complex mixture and the planktonic and terrestrial alkane indicator.

The UCM varies independently of the planktonic or terrestrial input (Fig. 7). This may suggest an indigenous source such as upward migration from deeper reservoirs. However, any attempt to correlate petroleum and terrestrial inputs assumes that the relative amount of petroleum to terrestrial hydrocarbons transported to the location is constant with time, which may or may not be true. Extensive natural hydrocarbon seepage documented on the Gulf of Mexico continental slope further supports natural seepage as a major petroleum hydrocarbon input to Gulf of Mexico slope sediments (ANDERSON *et al.*, 1983; BROOKS *et al.*, 1984, 1987). Piston coring on the Gulf slope have shown that petroleum hydrocarbons increase in concentration with depth in areas of known seepage (i.e. separate phase oil droplets in the sediment) and that the bitumens match isotopically and compositionally the deep reservoired fluids (KENNICUTT *et al.*, 1986; LACERDA *et al.*, 1986). It is also evident that some fraction of the petroleum hydrocarbons are transported to the slope by river/land-derived particles. The UCM can be due to recycled material and cannot necessarily be distinguished as due to a single source.

Temporal variations

Cruise I, November 1983; cruise II, April 1984; and cruise III, November 1984 all sampled the central transect in an attempt to document changes between different sampling times. The distribution of EOM and aliphatic UCM during these three samplings is shown in Fig. 8. On an average, the aliphatic UCM was highest on cruise I primarily due to the elevated levels measured at Sta. C1 (Fig. 8, Table 1). UCM concentrations during cruise II and at the shallower stations of cruise III (<1500 m) were similar. During cruise III the UCM was higher than cruise II by a factor of 1.5–2.2 at stations deeper than 1500 m. Molecular level indicators are similar along the central

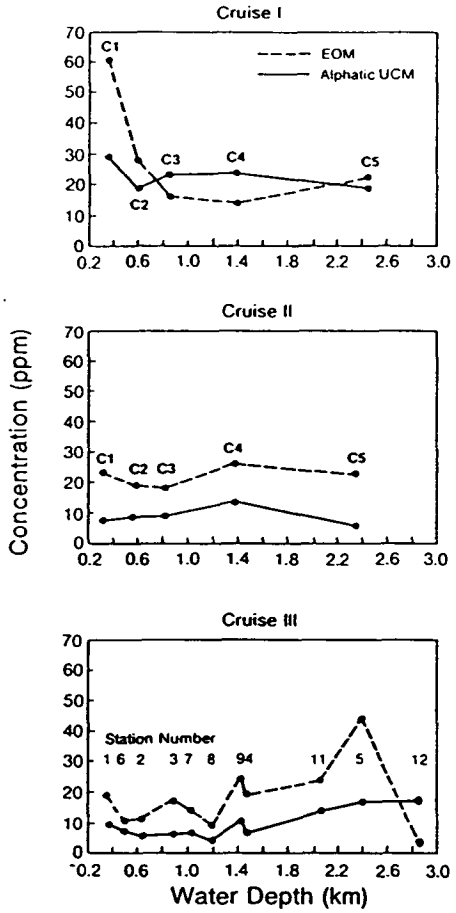

Fig. 8. Variations in extractable organic matter and the aliphatic unresolved complex mixture during three samplings of the central transect.

transect during cruises I and II (Figs 9 and 10). Variability with depth was observed during cruise III sampling. Shallower stations during cruise III had a decreased hydrocarbon content possibly due to dilution with inorganic material. Terrestrially sourced hydrocarbon concentrations are reduced over the entire cruise III transect as compared to cruises I and II. The deepest stations (>1500 m) on cruise III have elevated levels of petroleum hydrocarbons. This is substantiated by the hydrocarbon source parameters previously discussed (Figs 9 and 10). Examination of carbon preference index distributions and gas chromatograms suggest the presence of relatively fresh petroleum hydrocarbons probably from oil seepage at the deepest stations (Fig. 11). Station C7 also has the lowest CPI of this transect suggesting anomalously high petroleum hydrocarbons. The maintenance of a high CPI with the presence of mature petroleum can be explained by the mixing of an oil substantially depleted in $>C_{25}$ alkanes (typical Gulf oil) with a sediment dominated by odd carbon $>C_{25}$ terrestrially derived hydrocarbons. Recycled organic matter tends to be signicantly reworked by evaporation, dissolution and microbial degradation leading to a depletion in aliphatic hydrocarbon. These differences between samplings most likely represent the patchiness associated with hydrocarbon distributions and do not reflect a temporal change (i.e. flux).

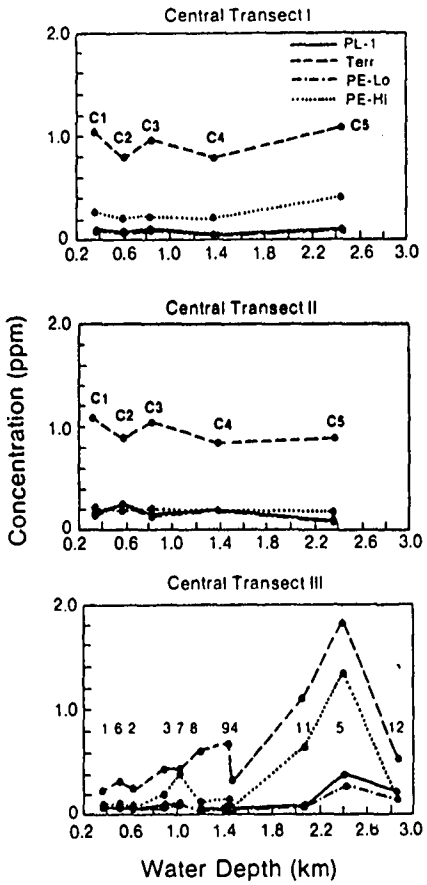

Fig. 9. Variations in hydrocarbon source parameters during three samplings of the central transect.

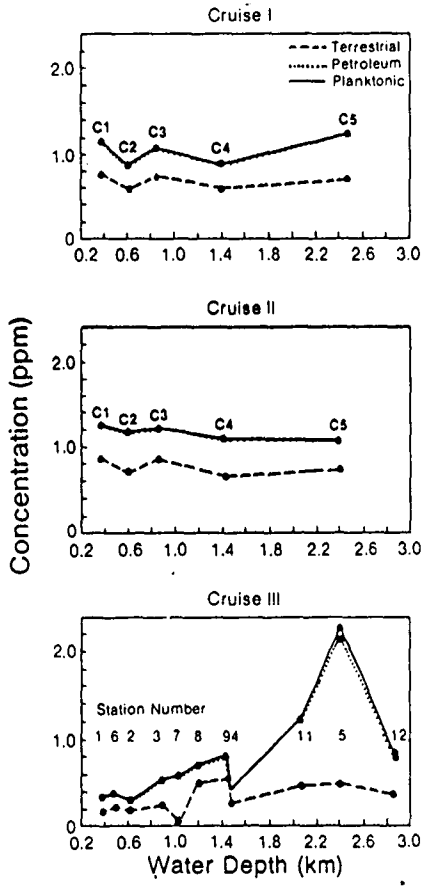

Fig. 10. Variation in plankton, terrestrial and petroleum hydrocarbons during three samplings of the central transect presented as a *cumulative* concentration.

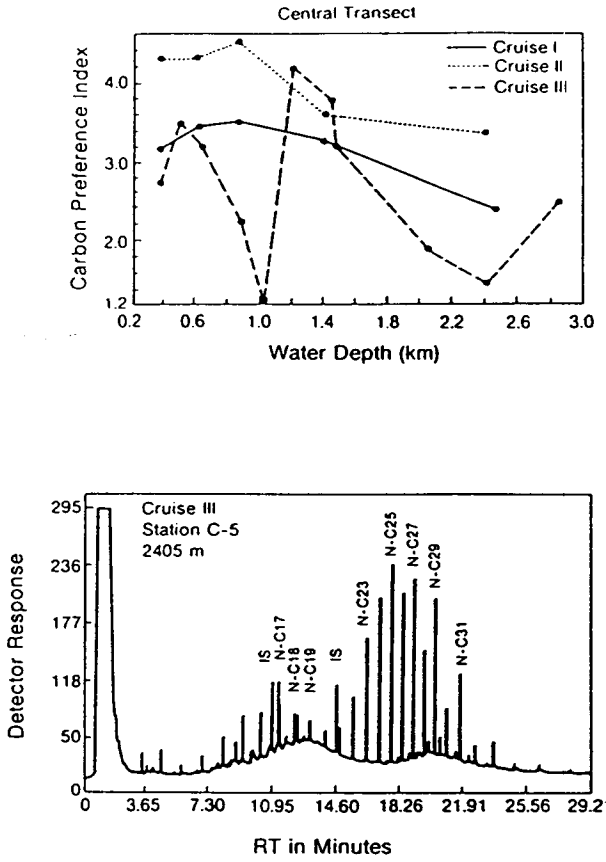

Fig. 11. Variation in the carbon preference index as a function of depth along the central transect during cruise III and a representative fused silica gas chromatogram of the aliphatic hydrocarbons from Sta. C-5, cruise III.

Variability along isobaths

Cruise V in the eastern Gulf of Mexico occupied stations along three isobaths to assess lateral variation in the measured parameters. Hydrocarbon parameters are summarized in Table 3. Bulk and molecular level parameters are low compared with previous samplings and represent some of the lowest values measured during this study (Table 1); as such the variability observed along this transect is probably a maximum. These sampling sites were chosen to contrast sediment texture which will also contribute to the observed variability of hydrocarbon parameters. The aliphatic UCM and total EOM vary by factors of 1.7–7.6 at a given depth. Molecular level indicators (i.e. individual component sums) vary by a factor of 2.0–7.6 along a given isobath. These data suggest that, at these low concentrations, hydrocarbons are as variable along isobaths as they are with water depth. These data also emphasize the patchy nature of hydrocarbon distributions. Bulk sediment parameters such as percent sand, varied by as much as a factor of 3 along an isobath, illustrating variations in sediment texture as well.

Samples along isobaths in the central and western Gulf were also taken. Stations from cruises I, II, III and IV at ~250 m are compared in Table 4. The variability in hydrocarbon parameters reflecting terrestrial input show the greatest variation, as much

Table 3. Variability in hydrocarbon parameters along isobaths—eastern Gulf of Mexico

	Variable ranges		
Depth (m)	342–383	619–630	819–859
Parameter	$n = 4$	$n = 6$	$n = 5$
Total EOM	5.8–13.4	4.7–9.9	4.9–8.2
(ppm)	(9.7)*	(6.8)	(5.8)
Aliphatic UCM	0.7–5.0	0.5–3.8	0.7–3.1
(ppm)	(3.0)	(1.8)	(1.7)
PL-1†	11.5–94.1	8.1–59.1	6.9–44.8
(ppb)	(54.3)	(29.9)	(23.4)
TERR†	36.0–74.0	55.9–120	23.4–148
(ppb)	(55.9)	(78.6)	(122)
PE-Lo†	13.3–101	13.5–39.8	11.0–27.3
(ppb)	(56.2)	(21.1)	(18.8)
PE-Hi†	14.4–30.3	20.4–53.1	17.5–77.6
(ppb)	(22.3)	(33.1)	(48.6)
Terrigenous‡	21.6–49.8	24.0–66.7	5.9–99.5
(ppb)	(33.6)	(45.4)	(72.8)
Petroleum‡	33.5–118	34.6–67.9	28.5–104
(ppb)	(78.5)	(54.3)	(67.4)
Planktonic‡	0.0–2.8	0.0–20.1	0.0–17.5
(ppb)	(0.7)	(9.5)	(7.3)

* Average.

† PL-1 = Σn-$C_{15,17,19}$ and pristane; TERR = Σn-$C_{25,27,29,31}$; PE-Lo = Σn-$C_{16,18,20}$ and phytane; PE-Hi = Σn-$C_{24,26,28,30}$.

‡ Terrigenous = (TERR) – (PE-Hi); petroleum = (PE-Lo) + (PE-Hi); planktonic = (PL-1) – (PE-Lo).

Table 4. Variability in hydrocarbon parameters along isobaths—Western central Gulf of Mexico

Depth (m)	298–371*	547–550†	748–759‡
Parameter	$n = 6$	$n = 3$	$n = 3$
Total EOM	15.9–61.3	17.4–23.9	17.0–57.9
(ppm)	(34.5)	(20.6)	(30.9)
Aliphatic UCM	6.0–31.4	6.9–7.9	5.6–11.9
(ppm)	(15.6)	(7.6)	(8.4)
PL-1	36.3–175	47.4–65.3	50.0–68.0
(ppb)	(122)	(56.4)	(59.9)
TERR	93.4–1080	109–2731	169–181
(ppb)	(546)	(201)	(176)
PE-Lo	36.2–155	39.1–52.5	43.4–48.9
(ppb)	(100)	(43.7)	(45.2)
PE-Hi	70.9–280	80.6–122	48.0–96.6
(ppb)	(172)	(99.4)	(64.8)
Terrigenous	22.5–861	29.2–252	83.9–131
(ppb)	(375)	(101)	(112)
Petroleum	123–388	119–162	91.4–140
(ppb)	(272)	(143)	(110)
Planktonic	0.1–57.7	4.0–26.2	6.6–19.1
(ppb)	(21.5)	(12.7)	(14.7)

* Cruises I, II and III, Stas C1 and W1; cruise III, Sta. C6; and cruise IV, Sta. WC1.

† Stations WC2, WC4, WC8.

‡ Stations WC3, WC9, WC10.

as 40-fold. The plankton indicators are also highly variable, most likely due to dilution with terrestrially sourced material. Bulk parameters such as clay content vary by a factor of 2 and sand content varies from 0.5 to 36.6% at these six locations. These variations again reflect the substantial influence of river/terrestrial-derived material. Three samples from cruise IV along the 550 and ~750 m isobath are relatively uniform. The lateral extent covered is relatively small as compared to the 350 m isobath sampling. Bulk parameters are also uniform at these locations.

Seep to non-seep comparison

Stations occupied in known seep areas of the west/central Gulf of Mexico were compared to control stations. In general the petroleum indicators are elevated by a factor of two to three at the seep vs the non-seep sites (Table 5). Most of the petroleum is in the form of EOM and aliphatic UCM. This suggests that the petroleum is substantially biodegraded. Variability in hydrocarbon concentrations at seep/non-seep areas is of the same order of magnitude as along isobaths with varying sediment type. Previous samplings have retrieved sediments with total EOM as high as 150,000 ppm as contrasted to the average of 60.3 ppm for the two "seep" sites sampled in this study. This again emphasizes the patchy nature of hydrocarbon distributions and in particular the non-uniform distribution of petroleum seepage in any given area. The extremes of petroleum hydrocarbon input to slope sediments are not represented in this set of samples, though previous work documented that the samples are in an area of active, natural oil seepage.

Topographic features

One set of paired stations (WC-11 and WC-12) were taken to compare bottom topography effects. The sediment sample at a topographic high is elevated in petroleum hydrocarbons (Table 6). This difference cannot be ascribed simply to topographic

Table 5. Comparison of sediment hydrocarbon parameters at seep and non-seep locations on the west/central Gulf of Mexico continental slope

Parameter	Seep* $n = 2$	Non-seep† $n = 3$	Ratio‡
Total EOM (ppm)	26.3–94.2 (60.3)	17.4–23.9 (20.6)	2.9
Aliphatic UCM (ppm)	6.8–46.2 (26.5)	6.9–7.9 (7.6)	3.5
PL-1 (ppb)	153–272 (212)	47.4–65.3 (56.4)	3.8
TERR (ppb)	147–237 (192)	110–273 (201)	0.95
PE-Lo (ppb)	92.7–219 (156)	39.1–52.5 (43.7)	3.6
PE-Hi (ppb)	99.9–119 (110)	80.6–122 (99.4)	1.1
Terrigenous (ppb)	46.8–117 (82.1)	29.2–151 (102)	0.8
Petroleum (ppb)	193–457 (266)	120–174 (144)	1.9
Planktonic (ppb)	52.1–60.0 (56.1)	4.0–26.2 (12.7)	4.4

* Stations WC6, WC7.
† Stations WC2, WC4, WC8.
‡ Ratio of seep parameter:non-seep parameter.

Table 6. Comparison of sediment hydrocarbon parameters at two different topographic settings

	WC-11 Topo-Hi	WC-12 Topo-Low
Depth (m)	1226	1236
Total EOM (ppm)	18.9	17.1
Aliphatic UCM (ppm)	81.4	4.2
PL-1 (ppb)	266	67.5
TERR (ppb)	3070	183
PE-Lo (ppb)	248	45.7
PE-Hi (ppb)	852	44.9
Terrigenous (ppb)	2230	138
Planktonic (ppb)	18.4	21.8
Petroleum (ppb)	1100	90.6

differences and more likely suggests that an additional input of hydrocarbons has occurred at Sta. WC-11. More detailed studies will need to be performed to understand the relationship between hydrocarbons and topographic expressions.

Relationship to bulk parameters

In general, the highest aliphatic hydrocarbon concentrations were associated with the more clayish/organic carbon-rich sediments. To understand more fully the sedimentological relationships, the three primary hydrocarbon sources are considered individually since their distribution is controlled by different factors. The data must also be considered in the context of the sampling design, i.e. areal, temporal and water depth dependencies. The terrigenous or land-derived component tends to increase with clay content within a given sampling period (Fig. 12). When the data are considered as a complete set,

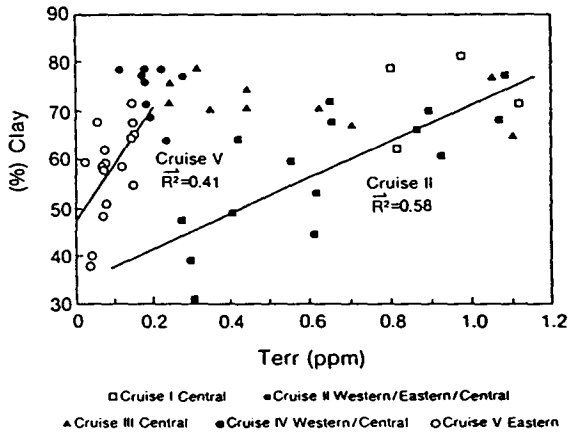

Fig. 12. The relationship between a terrestrial hydrocarbon indicator and sediment clay content.

no trend is apparent. This may be due to a changing clay to terrigenous organic matter ratio with time and location. Among the samples, the cruise III central transect and the west/central samplings correlated least with grain size. This may be due in part to a substantial petroleum input to the TERR indicator that is independent of the Mississippi river or a variable terrestrial input. It is also probable that the distance the material is transported and the composition of the transported material varies with time. The largest range in clay content was observed during cruise II when the western, central and eastern transects were sampled (Fig. 13). In this case, TERR generally increases with clay content and decreases with sand content. Within a given transect, the correlation does not exist. In general, the relative importance of riverine material between geographical areas can be estimated, though variability within a given area (i.e. along a transect) can be substantial. Petroleum indicators were generally independent of grain size, though as previously mentioned some component of the petroleum is apparently related to river-associated particles (Fig. 14). Phytoplankton-derived hydrocarbons did not correlate with grain size.

Aromatic hydrocarbons

Sediment aromatic hydrocarbons are below the GC/FID detection limit (~5 ppb) at all locations sampled. This low level of individual aromatic compounds is consistent with the low level of aliphatic hydrocarbons. The aromatics, though a significant fraction of the total weight of a petroleum, are generally on an individual compound basis, an order of magnitude less concentrated than the *n*-alkanes. The presence of aromatic hydrocarbons at low concentrations was inferred from total scanning fluorescence analyses supporting the conclusion that a low level chronic petroleum input is present at all locations

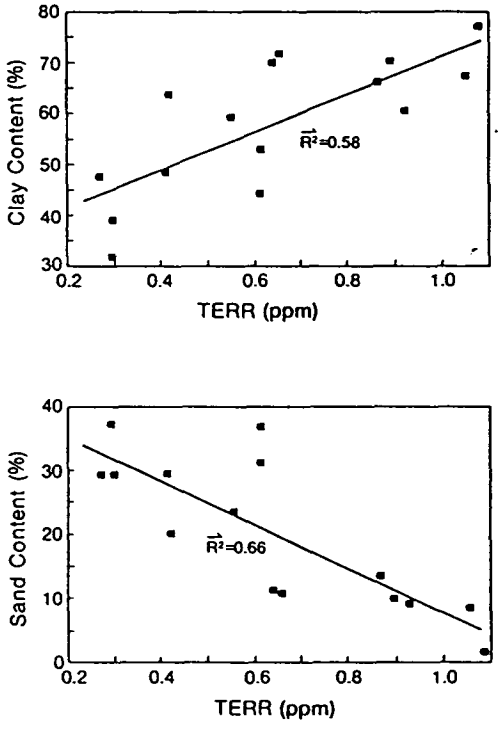

Fig. 13. The relationship between a terrestrial hydrocarbon indicator and the clay and sand content of sediments from cruise II.

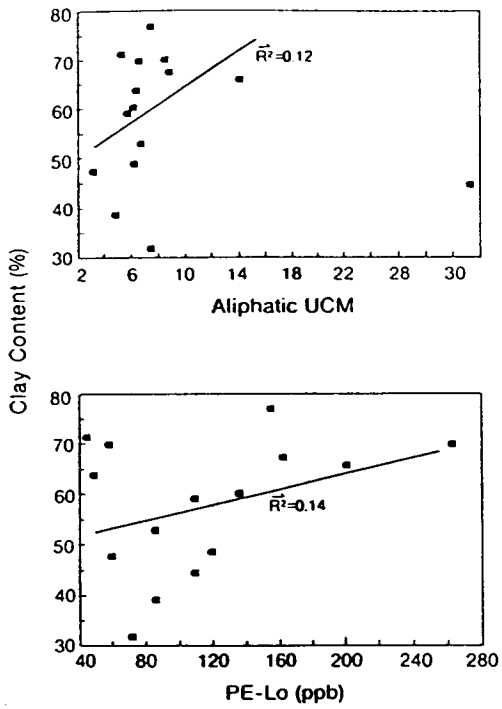

Fig. 14. The relationship between two petroleum hydrocarbon indicators and the clay content of sediments from cruise II.

sampled. This petroleum input could be due to sedimentation from the overlying water column, transport of recycled organic matter from shallow water sediments (i.e. turbidity flows) or petroleum seepage.

Carbon isotopic composition of sedimentary organic matter

The carbon isotopic composition of sedimentary organic matter for all five cruises is summarized in Fig. 15. Isotopic data confirms the previously inferred influence of river/land-derived material on the Gulf of Mexico slope. Though there are numerous complicating factors, in general a more negative carbon isotopic composition suggests greater land influence. Terrestrially sourced organic matter $\delta^{13}C$ varies from ~–25 to –28‰ and planktonic-derived carbon varies from ~–16 to –21‰. The average $\delta^{13}C$ of sedimentary organic matter becomes increasingly positive from the central to the west central to the eastern sampling sites (Fig. 15). This trend infers a decreased influence of terrestrial material at the eastern sites. Further discussion of the carbon isotopic data will be presented elsewhere.

CONCLUSIONS

Gulf of Mexico slope sediments contain a mixture of terrigenous, petroleum, and planktonic hydrocarbons. The influence of river/land-derived material is widespread and is probably delivered to the slope by secondary sediment movement such as a slumping and slope failure. Petroleum hydrocarbons were detected at all locations and have a dual

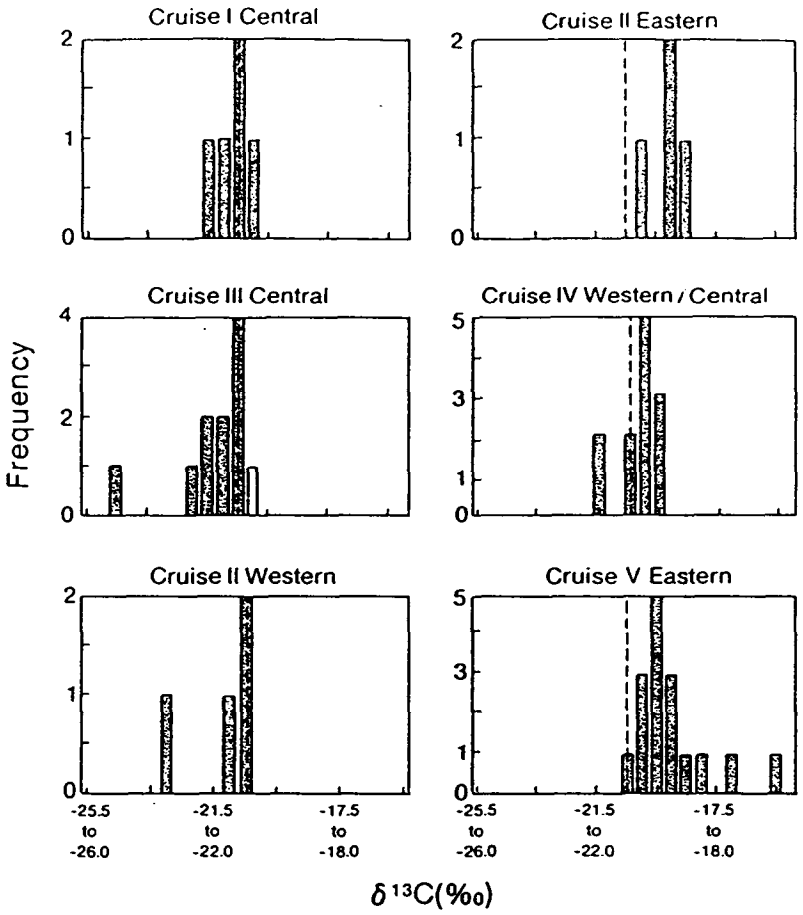

Fig. 15. Summary of the carbon isotopic composition of sedimentary organic matter.

source in natural seepage and river-associated transport. Other studies have suggested that natural seepage is much more widespread on the Gulf of Mexico slope than previously thought and probably represents a significant if not a major input of petroleum hydrocarbon to Gulf slope sediments (BROOKS *et al.*, 1985, 1987; LACERDA *et al.*, 1986; KENNICUTT *et al.*, 1986). In general the concentration of hydrocarbon in slope sediments was lower than previous reports for shelf and coastal sediments but no regular decrease with increasing water depth was apparent below 300 m. Hydrocarbon distributions in general are patchy on the slope and this may be due in part to the non-uniform distribution of natural seepage on the slope. Variability in hydrocarbon concentrations were as much as 1–2 orders of magnitude along an isobath due to changes in sediment texture and hydrocarbon inputs. Hydrocarbons were preferentially associated with clayish, organic-rich sediments, again suggesting a linkage with river-derived material. Aromatic hydrocarbon concentrations were very low at all locations but their presence was confirmed by fluorescence.

Large areas of the Gulf of Mexico slope may be exposed to high levels of natural petroleum seepage. The implications of this as far as the adaption of biota to high hydrocarbon levels and one's ability to discern changes in hydrocarbon levels after oil development has begun may be far reaching.

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Gulf of Mexico Chemosynthetic Communities II. Spatial Distribution of Seep Organisms and Hydrocarbons at Bush Hill

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Marine Biology

Gulf of Mexico hydrocarbon seep communities

II. Spatial distribution of seep organisms and hydrocarbons at Bush Hill

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Abstract

Sediment and water samples were collected by submersible in September 1986 at 16 locations on the carbonate cap overlying a conical diapir, which was formed by the upward migration of oil and gas through a subsurface fault on the continental slope off Louisiana, USA (27°47'N; 91°30.4'W). The biological community at the site was photographed quantitatively with still and video cameras. Rigorous spatial sampling indices were maintained so that variation in chemical parameters and in the abundance of photographed organisms could be estimated within the bounds of the study site. Concentrations of extractable organic material (EOM) ranged from 0.24 to 119.26‰ in the sediment samples, while methane concentrations in the water samples were from 0.037 to 66.474 μM. The visible biological community was predominantly composed of the chemosynthetic tube worms (Vestimentifera) *Lamellibrachia* sp. and *Escarpia* sp., and an undescribed, methane-oxidizing mussel (Mytilidae: *Bathymodiolus*-like), as well as diverse non-chemosynthetic organisms. The ranked abundance of tube worms was significantly correlated ($p<0.05$) with the concentration of EOM in the sediment samples, while the abundance of mussels was significantly correlated ($p<0.05$) with the concentration of methane in the water samples. Tube worms and mussels both occurred in dense clusters; however, the clusters of mussels had a more restricted distribution within the study site than did clusters of tube worms. Both organisms were most abundant in the vicinity of the subsurface fault.

Introduction

Deep-sea communities of tube worms (Riftiidae) and bivalves (Vesicomyidae and Mytilidae) were first discovered at hydrothermal vents at the Galápagos Rift (Corliss et al. 1979) and the East Pacific Rise (Rise Project Group 1980). These animal assemblages were shown to be dependent upon chemolithotrophic processes mediated by internal symbionts (Cavanaugh et al. 1981, Felbeck 1981). Subsequent exploration increased the known geographic range of similar communities and documented their occurrence at cold seeps as well as hydrothermal vents (Paull et al. 1984, Kennicutt et al. 1985, Suess et al. 1985, Laubier et al. 1986). Primary food sources for the symbionts of both tube worms from hydrothermal vents and those from hydrocarbon seeps (Lamellibrachidae and Escarpidae) are reduced sulfur compounds (Felbeck 1981, Brooks et al. 1987b). An undescribed seep mussel harbors methanotrophic bacteria in its gills and oxidizes dissolved methane (Childress et al. 1986).

Ecological descriptions of vent and seep communities have been based primarily upon interpretations of still and video photographs, which have provided information concerning spatial distribution and temporal variation of abundance (Hecker 1985, Hessler et al. 1985, Tunnicliffe et al. 1985, Fustec et al. 1987, Juniper and Sibuet 1987, Rosman et al. 1987). Despite the geographic and taxonomic diversity of the communities described, they share several significant characteristics. Although the depth range was large (500 to 6 000 m), all were found below the photic zone. All apparently occurred at a gradient between reducing and oxidizing environments, either where reduced compounds, particularly sulfides and methane, were issuing into oxygenated waters or where anoxia occurred in the benthic substrate. The density of individuals and the diversity of the communities greatly exceeded those of surrounding benthic areas. Distribution of organisms within the communities was spatially heterogeneous. Frequently, the transition between the chemosynthetic community and the surrounding environment was abrupt. Patterns observed in the distribution of vent and seep fauna have been attributed to supposed spatial and temporal variations in the supply of sulfides and methane. However, investigations of the distribution of these compounds within the communities have been limited (Johnson et al. 1986).

Thermogenic hydrocarbons are widespread in surface sediments on the upper continental slope of the Gulf of Mexico (Anderson et al. 1983, Brooks et al. 1984–1987a, Kennicutt et al. 1987a, b, 1988). Studies of the biota associated with several of these hydrocarbon seeps indicate that the seeps can support communities with substantially greater biomass and diversity than is typical of the slope benthos (Kennicutt et al. 1985, Brooks et al. 1987a, Rosman et al. 1987). Transport of biological production from hydrocarbon seeps to the surrounding benthos may contribute significantly to the ecology of the continental slope. However, documentation and quantification of such a contribution will be difficult; the true extent of hydrocarbon seepage is not known, the mechanisms for transfer are unclear, and the spatial patterns characteristic of seep communities are not well characterized.

Distribution and abundance of an assemblage of organisms dependent on seeping hydrocarbons should reflect the pattern of seepage and the quantity of hydrocarbons present. A useful spatial description of such a community should give its areal extent, the distributions and relative abundances of the dominant organisms, and the environmental variables that correlate with these distributions (Fustec et al. 1987, Juniper and Sibuet 1987). The present study describes the chemosynthetic community at a site of natural hydrocarbon seepage on the Louisiana Slope (Brooks et al. 1984, 1986, 1987a). The analyses of sediments and water associated with the distribution of the dominant megafauna within the community are quantified; variations in faunal abundance are compared to local concentrations in hydrocarbons and to geological features.

Materials and methods

Site description and field methods

The site, known as Bush Hill, lies over a salt diapir that rises about 40 m above the surrounding sea floor to a minimum water depth of 540 m. The feature is located 210 km south southwest of Grand Isle, Louisiana, at 27°47′N; 91°30.4′W. It lies in the Green Canyon offshore leasing area between Blocks 184 and 185, approximately 2 000 m from the drill template of what is currently the world's deepest oil-production platform (Anonymous 1987). The sediment in this area consists of silty-clay and is of considerable thickness (>1 000 m). However, much of the sedimentary stratification of Bush Hill itself (Fig. 1) has been eliminated by rising gas and liquid and by in situ formation of authigenic carbonate and sulfides, thereby creating a seismic wipe-out zone (Brooks et al. 1986, Behrens 1988).

Bush Hill and its immediate surroundings were explored during four dives of a research submersible in September 1986. A series of sub-bottom profiles of the study area were obtained with a 3.5 kHz precision depth recorder. The submersible carried a series of sediment punch-cores, each constructed of 25 mm (i.d.) PVC pipe and fitted with a handle for operation by the submersible's mechanical arm. A length of flexible tubing for collection of water samples was run from the end of the arm to an intake port in the aft dive-compartment.

Color images were recorded by a high-resolution video camera (MOS Model 3000) mounted on a pan-and-tilt unit and a 35 mm still camera (Benthos Model 372) mounted vertically. Both cameras were equipped with ranging devices. The video camera carried a pair of lasers, mounted in parallel with 10 cm separation. Their beams appeared as red dots in the video record; the distance between the dots provided a 10 cm scale for measuring photographed subjects and the field of view. On the 35 mm camera, a short-range altimeter recorded the distance from the bottom in each exposure. This distance, together with the acceptance angle of the lens, was used to calculate the area of the bottom and the size of the subjects in each photograph (Rosman et al. 1987).

Dives consisted of a series of short (100 to 200 m) transects, during which the pilot of the submersible attempted to maintain constant speed, altitude and heading. Start and end times were recorded for each transect. The submersible's range and bearing from the support ship were monitored with a Northstar Doppler sonar. The submersible's absolute position at the start and end points of each transect was fixed by maneuvering the ship to a position directly over the submersible (zenith ±5 m) and recording the ship's position with LORAN C.

The forward sphere of the submersible provided the pilot and scientist with an unobstructed 180° view of the bottom in the submersible's path. During each transect, the video camera was allowed to run continuously while the scientist recorded a narrative describing the objects in view, the time of observations and the submersible's bearing. The zoom on the video camera was kept at maximum wide angle while on transect. The camera was panned to aim the lasers at faunal clusters that the submersible passed. Contemporaneous notes and subsequent measurement of the video images indicated that the video camera was consistently able to scan a 6 m swath along the bottom. Small organisms (<5 cm) could be distinguished when they appeared in the center of the field of view where the lighting was best.

Collections of organisms, sediment cores and water samples were made while the submersible was stationary between transects. Organisms were collected with the scoop and claw devices of the mechanical arm and were placed in numbered buckets on the front basket. Cores of the upper 30 to 40 cm of surface sediments were collected with the punch cores and placed in numbered, water-tight quivers. Water samples were collected at distances that ranged from 0.1 to 3 m above the bottom. The intake tube was flushed with ambient water prior to the collection of each sample to prevent cross-contamination of samples. Each water sample consisted of a 50 ml syringe and a capped 300 ml bottle. Seawater samples were fixed when collected with zinc acetate and sodium azide for measurement of total sulfides (Cline 1969, Goldhaber et al. 1977) and low molecular weight hydrocarbons (Brooks et al. 1981), respectively.

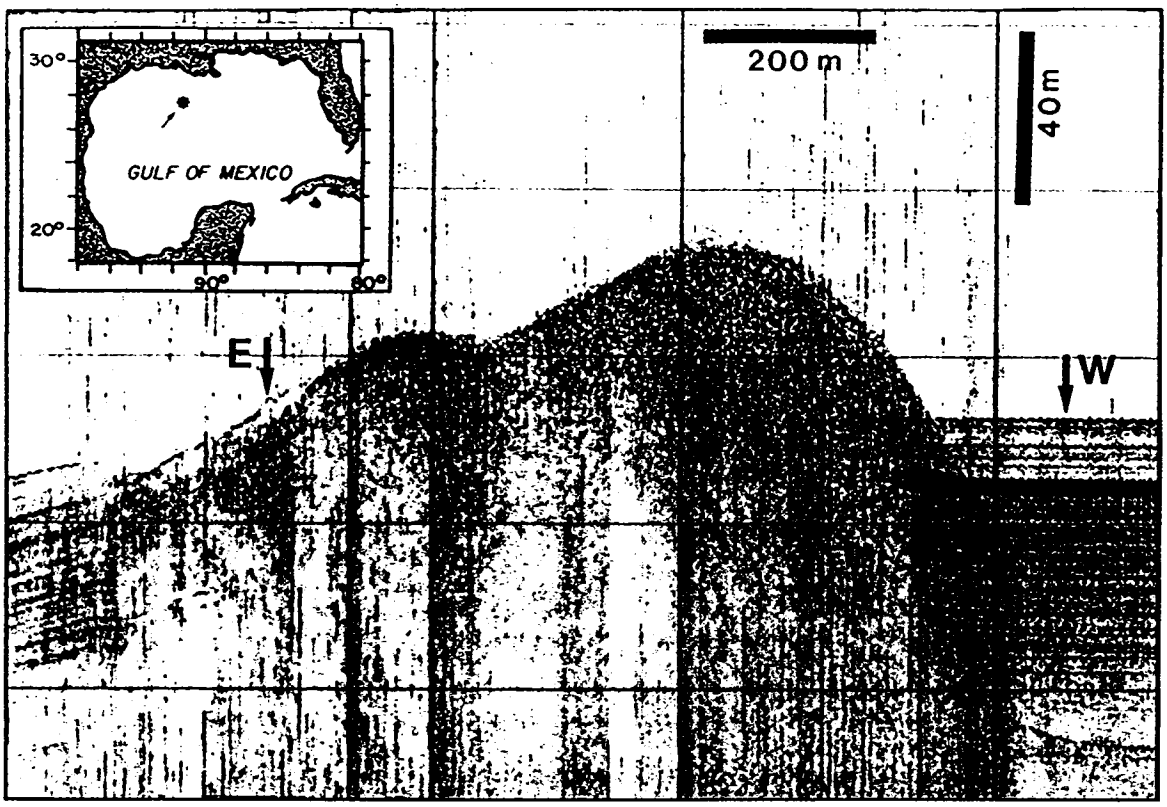

Fig. 1. Precision depth-recorder, PDR (3.5 kHz) trace of Bush Hill diapir. Inset shows location of study site in Gulf of Mexico. Arrows E and W mark eastern and western extent, respectively, of study site. Placement of PDR trace in study site is shown in Fig. 2B

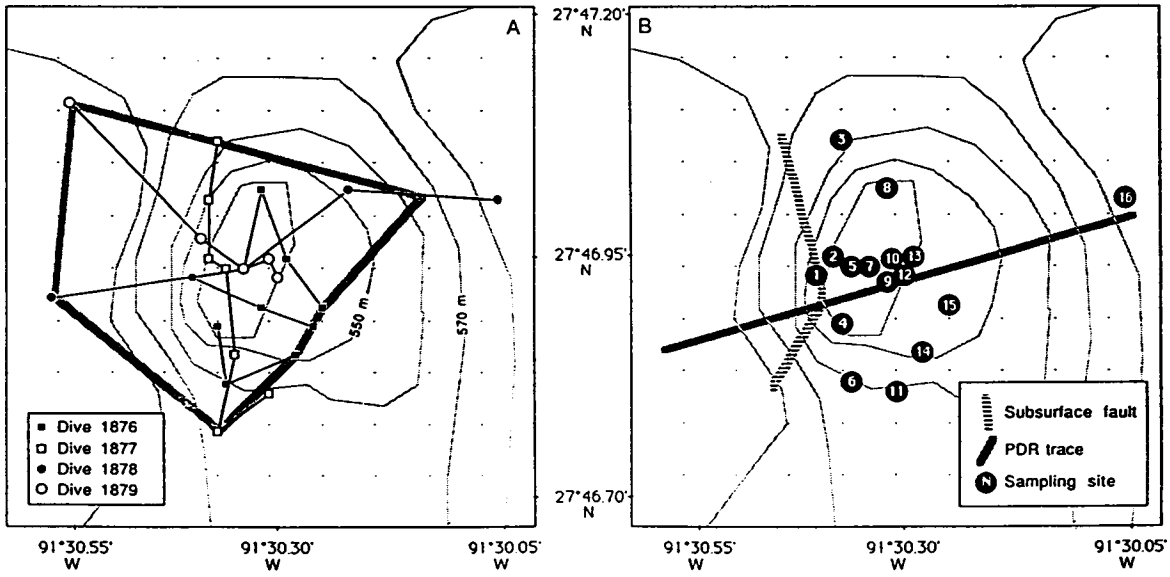

Fig. 2. Area surveyed by submersible "Johnson-Sea-Link I". Maps are 1 km², with grid points at 100 m intervals. (A) Continuous lines show submersible transects during each dive; stippled outline, outer extent of video coverage. (B) Locations of sediment and water sampling sites; stippled line shows placement of PDR trace (Fig. 1)

Rigorous sample control was maintained to ensure that the location of each sample site was accurately recorded.

Observations of the Bush Hill community were concentrated at the top of the carbonate cap, with dives originating at points on its periphery and proceeding inward (Fig. 2A). Usable video records were collected on 20 transects, which together surveyed 2716.2 linear meters and collected 112.7 min of data. The envelope surrounding the outermost ends of the video transects enclosed 20 ha of the bottom (Fig. 2A), and the swath of the transects covered approximately 7% of this area. Sediment cores and/or water samples were collected at 16 sites along the transects (Fig. 2B); cores were taken at 13 of these sites, water samples at 12. Video data were not recorded on the transect extending beyond the envelope to the east on Dive 1877 (Fig. 2A); however, the observers reported no sightings of tube worms or mussels on this transect. Video records made while transiting to Sample Site 11 (Fig. 2B) were unusable. A single, isolated cluster of tube worms was observed at this site. A sediment core was taken adjacent to the cluster and a collection of the organisms was made.

Chemical analysis

Hydrocarbons were recovered from sediments by extraction with CH_2Cl_2. Sediment samples were lyophilized and then extracted with CH_2Cl_2 for 12 h in a Soxhlet apparatus. All glassware was cleaned with solvents and combusted at 450 °C for 4 h before use. The collected extracts were concentrated by roto-evaporation and analyzed by capillary gas-chromatography with flame ionization detection (GC-FID). Extract components were separated on a fused-silica capillary-column with splitless injection (film thickness 0.2 μm; i.d. = 0.31 mm; length = 25 m). GC-FID conditions for analyses were an initial temperature of 60 °C with no hold time and a programmed increase of 12 C° min^{-1} to 300 °C (9 min hold time). The injection port and detector were held at 300 °C. Chromatography was performed with either a Hewlett Packard Model 5880 or 5790 gas chromatograph. The chemical analytical methods have been described in greater detail elsewhere (Brooks et al. 1986, Kennicutt et al. 1988).

Samples to be analyzed for stable carbon-isotope composition ($\delta^{13}C$) were processed by standard methods with both Craig-type and closed-vessel combustion techniques (Sackett et al. 1970, Schoell et al. 1983). Carbon dioxide was analyzed on a Finnigan MAT-251 isotope-ratio mass spectrometer. The carbon isotope composition is reported in the usual δ-notation (vs Pee Dee Belemnite standard). Low molecular weight hydrocarbons (C1–C4) were analyzed by the method of Brooks et al. (1981).

Video analysis

The video records of the transects were replayed on a recorder equipped with freeze-frame and single-frame-advance controls. Subjects sighted in the video record included the larger mobile epifauna (fish and crabs), clusters of sessile epifauna (seep mussels, Vestimentifera and Gorgonacea) and prominent benthic features (carbonate boulders and gas seeps). Each subject observed in the video record was identified to lowest practical taxon; and the time of observation, relative to the start or end of the transect, was recorded. The resolution of the video images was sufficient to distinguish objects of 2 to 3 cm size; however, identification of smaller objects was based on 35 mm photographs or on close-up video images obtained when the submersible was stationary. Video records obscured by disturbed sediments were deleted from the data. The diameters of clusters of seep fauna were measured directly on the monitor screen with the scale provided by the laser ranging device. If the diameter of a cluster exceeded the size of the field of view (usually less than 3 m), its diameter was estimated from the distance that it remained in view while it was traversed by the submersible. These latter estimates were confirmed by viewing the sequences one frame at a time and estimating the scale from the laser dots.

With the assumption that the submersible maintained constant speed and bearing during transects, the observation times could be used to determine the relative position of a video subject between navigation fixes. By comparing the time that elapsed between the beginning of a transect and an observation with the total time between the start and end of the transect, its relative position along the transect was estimated. All samples and observations, including the location of a subsurface fault evident in the sub-bottom profiles, were mapped onto a 1 km square (Fig. 2) with southeast corner coordinates 27°46.67′N; 91°30.6′W, and northwest corner coordinates 27°47.21′N; 91°30.01′W. The points of the navigation fixes at the ends of transects were converted to Cartesian coordinates within this square. The distance between any two points within the square was determined with elementary trigonometry. All organism, sediment and water samples and all video observations were then indexed by their respective coordinates.

Statistical methods

The individual sites where sediment or water samples were collected were ranked according to the abundance of both tube worms and mussels in the immediate vicinity of the sites. The vicinity of each site was arbitrarily divided into a series of concentric rings of equal width (i.e., a sequence of radial distances from the site such as 0.0 to 1.5 m, 1.5 to 3.0 m, etc.). Abundance was estimated from the measured diameters of the clusters of tube worms or mussels recorded on the video transects that led up to, away from, or tangentially past the sites (Fig. 2). Because all observations of faunal clusters were spatially indexed within the study area, the distance between clusters and sampling sites could be readily determined. A computer program was written to determine whether a given faunal cluster recorded in the video data occurred near a sampling site; if so, its diameter

was added to the proper ring surrounding the site. When the program had examined all clusters, the resulting data file provided a basis for ranking any set of areas equally distant from the sampling sites.

Sampling sites were also ranked according to the concentrations of the chemical parameters measured in the sediment or water samples. Correlations between site rankings produced by chemical concentrations and those obtained by comparing faunal abundances were examined by the non-parametric two-tailed Spearman's rho test (Conover 1980). The confidence level for the tests of hypotheses was set at $\alpha = 0.05$. Repeated searches for significant cross-correlation were carried out in this manner for all the chemical parameters and for different sequences of ring widths.

The distribution of tube worm clusters and mussel beds was examined by plotting their location within a map of the study area. Given a range in size among these clusters, however, a point distribution does not accurately represent the evident spatial distribution of abundance. With the assumption that all clusters were circular, their measured diameters were used to calculate the areal cover of tube worm clusters and mussel beds observed within the community. A regular surface of relative areal cover was then fitted to these data (Sammarco and Andrews 1988). The grid size for the surface was 10 m, and within each square the areal cover of faunal clusters in each square was the distance-weighted, moving average of all observations (Ripley 1981). The averaging function had the following form:

$$\Sigma w\,(d_i/h)\, z_i / \Sigma w\,(d_i/h);$$

where, for each grid square, the intensity z_i is the weighted average of the $i = 1, 2, \ldots, n$ estimates of areal coverage; and d_i is the distance from the location of the observation to the center of the grid square. A uniform band-width, h, of 60 m was applied to estimates of areal cover of tube worm clusters; a band width of 10 m was applied to estimates of the more sparsely distributed mussel beds. Selection of band-widths was subjective. Those selected produced smooth surfaces without obscuring the variability of the data. A short-tailed weighting function, w, treated the distance, x, of the observation from the grid square:

$$w(x) = 0.9375(1 - x^2)^2$$

for $-1 < x < 1$.

Results

Community description

The sediments of the biologically depauperate periphery of Bush Hill were pale ochre in color, with an easily-disturbed flocculent layer. Although the bottom in this region showed extensive ichno-traces, including burrows, shallow depressions and mounds, very few organisms were seen or photographed. Generally, the color of the sediment changed to a slate-grey toward the top of the carbonate cap, and the lebesspuren became less frequent. Carbonate outcroppings ranged in form from rubble to prominent boulders. Along the western side of the carbonate cap, the carbonate outcroppings formed an escarpment about 15 m at its steepest margin. The larger boulders were topped by gorgonians, which frequently supported large ophiuroids. Large colonies of the scleractinian oral *Lophelia* sp. were attached to the exposed portions of the boulders. White, filigreed patches of bacteria occurred on the sediments (Fig. 3A). Toward the center of the community, the bacterial patches increased in area and were interspersed with the slender (3.5 mm) black tubes of a pogonophoran, *Galathealinum* n. sp., family Polybranchiidae (E. Southward personal communication). The most prominent features of the community were bush-like clusters of tube worms, which occurred both among the carbonate outcroppings and on soft sediments away from surface rubble.

Two species of vestimentiferan tube worms were identified as *Lamellibrachia* sp., family Lamillibrachidae, and *Escarpia*-like species, family Escarpidae (M. L. Jones personal communication). The escarpiid, distinguished in the 35 mm photographs by its distinctive flaring of the tube opening (Fig. 3B), was the less common of the two and generally formed sparse clusters of recumbent individuals. The *Lamellibrachia* sp. formed bush-like clusters, in numbers ranging from a few tens to many thousands of individuals. Although *Lamellibrachia* sp. was clearly dominant, mixed clusters of *Lamellibrachia* sp. and the escarpiid did occur.

Numerous clusters of tube worms were observed in the video records (Table 1). Morphology, both of the individual tubes and of the clusters of tubes, changed as the number of tube worms in a cluster increased. Low tangles, 30 to 40 cm in diameter, consisted of individuals with twisted and spiral-shaped tubes (Fig. 3D). Larger clusters, up to 1 m in diameter, had a collapsed, basket-like center and consisted of longer, less convoluted individuals (Fig. 3C). The largest clusters were dome-shaped, 2 m or greater in diameter and 1.5 m in height; they consisted of long, relatively straight individuals (Fig. 3A). Obturacular plumes were clearly visible on the individuals that formed the outer surface of the

Table 1. Clusters of chemosynthetic fauna enumerated and measured in video records. Clusters of *Lamellibrachia* sp. (Vestimentifera) that contained the epifaunal bivalve *Acesta bullisi* are included in total for *Lamellibrachia* sp. Clusters of seep mussels (Mytilidae) were discrete beds of living individuals. Diameters of seven tube worm clusters and three mussel beds were estimated from transect length. All other clusters were measured directly with laser ranging device mounted on video camera

Taxon forming cluster	No. of clusters observed	Mean measured diam (cm)	Standard deviation
Total *Lamellibrachia* sp.	174	101.3	91.08
Lamellibrachia sp. with attached *Acesta bullisi*	11	114.6	39.78
Seep mussels	17	209.1	133.40

mussels reflect those of EOM and methane, respectively. The contrast between the distribution of tube worms and mussels suggests that these patterns can be appraised at two scales of variation. In the vicinity of chemical sampling points (15 to 30 m scale), sources of both methane and EOM are discrete and discontinuous. Within the surveyed area (100 to 500 m scale), both compounds are most abundant on the top of the carbonate cap near a major subsurface fault. Methane sources are relatively restricted; EOM sources are relatively dispersed.

Behrens (1988) attributes the seepage of gas and oil to activity within faults. Sibuet et al. (1988) observed correlation between aggregations of *Calyptogena* sp. and the locations of faults in a deep-sea subduction zone off Japan. The coarse-scale distribution of tube worms and seep mussels might therefore be explained by the present-day pattern of seepage through the fault. It is reasonable that liquid-phase oil would be more widely dispersed by diffusion through porous sediments than gaseous-phase methane would be. However, we know of no geological study that compares the horizontal distribution of seepage at the level of resolution that is evident in the faunal distributions.

Methane and oil are both seeping to the surface from a deep (2 000 to 3 000 m) reservoir (Kennicutt et al. 1988). The oil production platform being established in the Green Canyon 184 lease block will exploit this reservoir. Surface hydrocarbon seepage decreases or disappears after prolonged oil production in terrestrial oil fields (Horvitz 1972). Oil production from the Green Canyon 184 reservoir may have a similar result and have an impact on the nearby Bush Hill chemosynthetic community.

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Gulf of Mexico Hydrocarbon Seep Communities: VI. Patterns in Community Structure and Habitat

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Gulf of Mexico Hydrocarbon Seep Communities: VI. Patterns in Community Structure and Habitat

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Abstract

Communities of chemosynthetic fauna that depend on seeping oil and gas have been found in the Gulf of Mexico at approximately 45 sites between 88°W and 95°W and between the 350 and 2,200 m isobaths. Investigations suggest that the number of sites and the range of occurrence will increase with additional exploration. The dominant fauna consist of species within four groups: tube worms, seep mussels, epibenthic clams, and infaunal clams. These species co-occur to some degree, but tend to form assemblages dominated by a single group. Community development is closely coupled to the geological and geochemical processes of seepage.

Introduction

The initial discovery of hydrothermal venting at the Galápagos Rift sea floor spreading center (Lonsdale 1977) was occasioned in part by the observation of anomalous concentrations of clams (*Calyptogena* sp.) in an otherwise depauperate abyssal zone. Subsequent exploration of this first known chemosynthetic habitat (Corliss and others 1979) revealed other, remarkably dense, aggregations of species including the tube worm *Riftia pachyptila* and the mussel *Bathymodiolus thermophilis*. These species all host bacterial endosymbionts that fix primary carbon via chemical energy (Cavanaugh and others 1981, Felbeck 1981) from the sulfides produced by interaction of basalt and super-heated seawater (Edmond and others 1982). Chemical enrichment of the benthos necessary for chemosynthesis has subsequently been attributed to a number of "cold seep" processes, including continental-margin brine seepage (Paull and others 1984), hydrocarbon seepage (Kennicutt and others 1985), conate out-watering at subduction zones (Suess and others 1985, Ohta and Laubier 1987), decay of whale carcasses (Smith and others 1989), and sediment slumping (Mayer and others 1988). These attributions were all initially inferred, at least in part, from the presence of chemosynthetic fauna. Biological observations have clearly provided a rich source of fact and speculation regarding geochemical enrichment at the sea floor.

There have been many attempts to explain the distribution of benthic fauna of the deep sea in terms of variations in abiotic parameters at a zonal scale, that is, tens to hundreds of kilometers (Carney and others 1983), or at a fine scale, that is, centimeters to meters (Jumars and Eckman 1983). Use of deep-water camera sleds, remotely operated vehicles (ROV's), and manner submersibles, as well as improved methods for interpretation and analysis of photographic data, have produced a number of recent deep-water studies in which patterns in faunal distribution were resolved at an intermediate scale, that is, meters to hundreds of meters (Hecker 1990, Messing and others 1990, Schneider and others 1987). However, although both the physical structure of a habitat and the local distribution of its characteristic fauna can be accurately mapped at this scale, the basic trophic control of community development and

structure must be inferred empirically where primary production is absent. Ecologists typically invoke some combination of advective processes to explain observed patterns of faunal distribution.

Chemosynthetic fauna represent a genuine exception to this situation. Where reduced inorganic compounds are available at the sea floor, the bacterial symbionts and their metazoan hosts enjoy a relatively unlimited food supply and form aggregations that have much greater densities than the surrounding benthic community (Jannasch 1984). Because the inorganic compounds have a highly localized source at the sea floor (for example, hydrothermal vents), such communities are characterized by distinct clusters of animals (Grassle 1986). Development and spatial structure of a chemosynthetic community are therefore tightly coupled to specific geological processes and structures (Hessler and others 1985).

This line of argument suggests that a map of chemosynthetic fauna at a Gulf of Mexico oil or gas seep is also a map of the surface expression of seepage. In fact, it may be easier to examine chemosynthetic communities in deep water than to map seepage directly. Thus, a description of an oil seep community may yield information regarding the extent and history of seepage that is unavailable by other means. Here we describe varieties of chemosynthetic communities that have been found at hydrocarbon seeps on the continental slope of northern Gulf of Mexico and provide some details of their local geological settings.

Materials and Methods

The initial discovery and early exploration of slope hydrocarbon seep communities were conducted with otter trawls (Kennicutt and others 1985, Brooks and others 1987a) and camera sleds (Rosman and others 1987). Subsequent explorations have relied on submarines. The nuclear-powered submarine USS NR-1 was deployed on the Texas and Louisiana continental slope during the course of three field seasons (1987–1989) for a total of 90 days of continuous submerged operation. This ship continuously records its depth, its altitude above the bottom, and its position relative to an initial "mark on top" on magnetic tape. These data are normally recorded every 10 sec, though intervals of 1 sec can be used. Unique among naval submarines, the ship has three 7.6 cm view ports in the bow; instrumentation comprises forward-looking and side-looking sonar, 3.5, 7, and 25 kHz subbottom profilers, two emulsion cameras, and an array of monochrome video cameras. The vertically mounted video camera was used to estimate the areal cover of chemosynthetic fauna by scaling the recorded images to its altitude and the acceptance angles of its lens. Overlapping images from this camera were compiled to directly map large areas of the bottom.

The submarines Johnson SEA-LINK I and PISCES II were deployed on the Louisiana slope during four field seasons (1986–1989) for a total of 38 dives. These craft generally make two 3-hour dives per day. Their range is limited and their navigational capabilities imprecise; however, their maneuverability and wide range of sampling equipment (sediment corers, grabs, scoops and suction hoses for collecting organisms, maneuverable water intake ports, and Nisken bottles) have permitted collection of numerous physical samples and living specimens. Their cameras, video and emulsion, were superior and could be maneuvered to provide detailed *in situ* documentation of the communities and their physical settings.

Findings and Discussion

Zonal Distribution of Chemosynthetic Fauna on the Continental Slope

Chemosynthetic fauna have been collected or observed in at least 45 locations on the northern Gulf of Mexico slope, between 88°W and 95°W and between the 350 and 2,220 m isobaths (Fig. 1). This geographic and bathymetric range represents the limits of exploration, not a zoogeographic range. The most recent extensions of this range were S.J. McDonald's discovery (unpublished data 1990) of tube worms and mussels in Alaminos Canyon (26°21.3'N and 94°29.8'W, water depth 2,222 m) and vesicomyid shell beds with sparse clusters of tube worms in the Viosca Knoll region south of Mobile, Alabama (K. Graham, personal communication 1990). These findings extend the southern and eastern geographic limits of the slope communities and more than double their known bathymetric range. There is every indication that further exploration will increase the number of sites and may extend the range.

Broadly speaking, exploration for chemosynthetic fauna has been conducted where seismic "wipe-out" indicated oil or gas seepage in the near surface sediments (Behrens 1988, Kennicutt and others 1988a, b) and, although the abundance and species composition varied, the results were usually successful. Stable carbon isotope ratios of animal tissues collected at many of these sites distinguish heterotrophic ($\delta^{13}C = -14$ to $-20‰$) sulfur-based ($\delta^{13}C = -30$ to $-42‰$) and methane-based ($\delta^{13}C < -40‰$) energy sources (Brooks and others 1987b).

There is difficulty fixing the exact number of sites, partly because the trawl collections were inherently

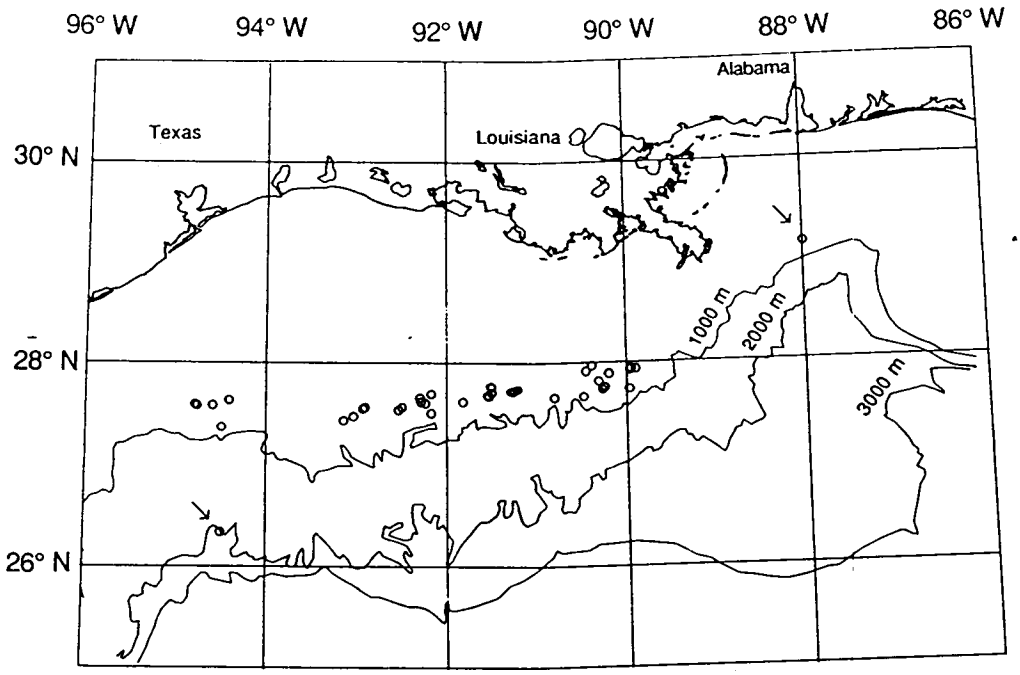

Figure 1. Continental slope south of Texas, Louisiana, and Alabama. Open circles mark the nominal locations of chemosynthetic communities that have been found to date; occurrence of these communities is frequently patchy; therefore the precise number of communities cannot be fixed with certainty. These findings represent the limits of present exploration, not the zoogeographic range of chemosynthetic communities on the slope. The most recent discoveries (arrows) considerably extend the geographic and bathymetric range of known communities.

imprecise, but primarily because no broadly acceptable definition exists of what constitutes a discrete community. Management concerns (Minerals Management Service 1988) refer to "high-density" communities that might be affected by oil exploration or production. This is not totally satisfactory because, as will be described below, aggregations of clams occur in low densities or beneath the sediments, often dispersed over areas of several hectare, whereas a "high-density" occurrence of tube worms may be confined to a single, 1-m wide cluster. Sites shown in Figure 1 designate the following: 1) mid-points of trawl tracks on which chemosynthetic fauna were collected (see Kennicutt and others 1988a), 2) a substantial number of high-density clusters within a radius of about 500 m, or 3) a substantial number of low-density aggregations within a radius of about 5,000 m.

Communities Dominated by Tube Worms

At least two undescribed species of tube worm, *Lamellibrachia* sp. and *Escarpia* sp., are commonly found at seep sites (MacDonald and others 1989). The species, assigned to the recently erected phylum Vestimentifera (Jones 1985), lack mouth, anus, and digestive system and live in a tube about 1 cm in diameter and up to 2 m in length, which consists of tough chitin (Gaill, personal communication 1989). The posterior end of the tube may be either buried in soft sediments or attached to a rocky substratum; a small, red-orange plume, the obturaculum, is extended from the anterior opening and serves as a gas exchange organ. Most of the body volume consists of trophosome, the organ that contains the bacterial symbionts (Jones 1985). *Lamellibrachia* sp. is generally the more abundant of these species and occurs in clusters that range from sparse tangles of recumbent individuals to dense, hemispherical "bushes" up to 2 m in height and 1 to 3 m in diameter (Fig. 2). The largest tube worm communities comprise tens of thousands of individuals that grow nearly vertically to a height of 2 m or more in unbroken stands up to 20 m in diameter.

Epifauna are common among the tube worms; sessile forms include sponges, gorgonians, and a scallop-like bivalve *Acesta bullisii*, which attaches to the anterior ends of the tubes and actually encloses the plume in its mantle cavity without apparent harm to the worms (Boland 1987). The soft corals are noteworthy because they are slow-growing forms; their presence indicates a slow growth rate for the tube worms as well. Mobile fauna include fishes and crustaceans that are commonly found on the continental

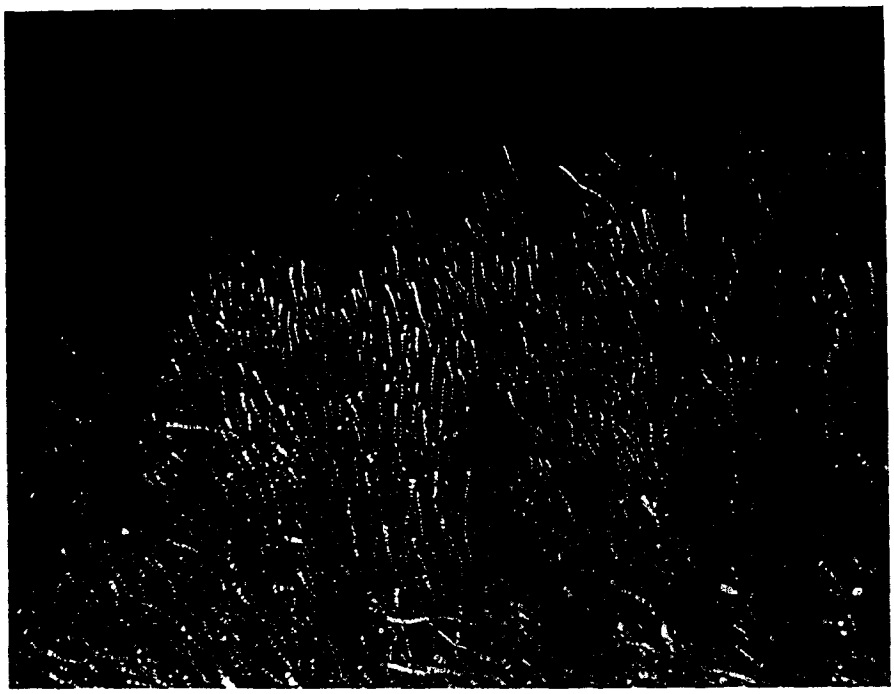

Figure 2. Clusters or "bushes" of the vesitmentiferan tube worm *Lamellibrachia* n. sp. near 27°44′50″N, 91°13′30″W at a depth of ~550 m. The scale of the photograph is approximately 2 m across the bottom edge of the frame.

slope, but are particularly abundant around the tube worm communities.

Tube worm communities have been found on grabens or half grabens at the crests of diapirs. A primary example is the "Bush Hill" site (27°47′N, 91°30′24″W) described by MacDonald and others (1989). More substantial communities were subsequently found in the northeastern corner of the Green Canyon 234 lease block (27°44′50″N, 91°13′30″W). The geology of this region has been described by Behrens (1988), who perceived a network of diapir crests that converge to the west of the site as an east-west trending diapir ridge. Seismic wipe-outs (Behrens' type II) are widespread, and substantial quantities of oil are dispersed in the sediments (Behrens 1988, J.M. Brooks, unpublished data 1987). The surface topography is uneven with sometimes massive deposits of authigenic carbonate either exposed or thinly draped with sediment. Tube worm communities appear to be restricted to terraces where the seismic reflections (25 kHz) are wiped out within ~3 m of subbottom (Fig. 3) and extensional faults approach the mud line.

Massive tube worm clusters, with their tough and densely intertwined tubes, present a formidable obstacle to collection, so the attachment substrata of such aggregations are not known. Most of the specimens collected to date have been relatively small individuals taken from the periphery of larger clusters. Generally, the posterior portions of these tube worms were buried beneath the sediments at depths of 10–20 cm and extended laterally for lengths up to 1 m. This burial poses a perplexing problem because the Vestimentifera are thought to grow from their attachment point (Southward 1975); if so, they must become buried after attachment. Given the sedimentation rates on the upper slope (10 to 60 cm per 1,000 yrs), burial at a depth of 10 cm implies a very slow growth rate. C.R. Fisher (personal communication 1989) has suggested that the clusters may increase local sedimentation in a manner analogous to formation of snow drifts around bushes; a more accurate analogy would be the accumulation of clay within mangrove thickets (A.H. Bouma, personal communication 1990).

Tube worm growth and burial rates are germane because of the uncertainty regarding the actual mechanism for sulfide uptake. At hydrothermal vents, significant concentrations of sulfides occur in the anoxic vent effluents and are absorbed by the vestimentiferans from the water column via the obturaculum (Arp and others 1987). At hydrocarbon seeps, sulfides are concentrated in the sediments but are absent from the overlying water column at the level of the obturacula of seep tube worms (MacDonald and others 1989). A probable, but untested, mechanism for uptake of sulfides by seep tube worms is direct absorption through the portions of the tubes that are on or beneath the sediments. This suggests that tube worm communities form on patches of oily sediment within wipe-out zones where sulfides either occur with the oil or via the anoxia caused by microbial

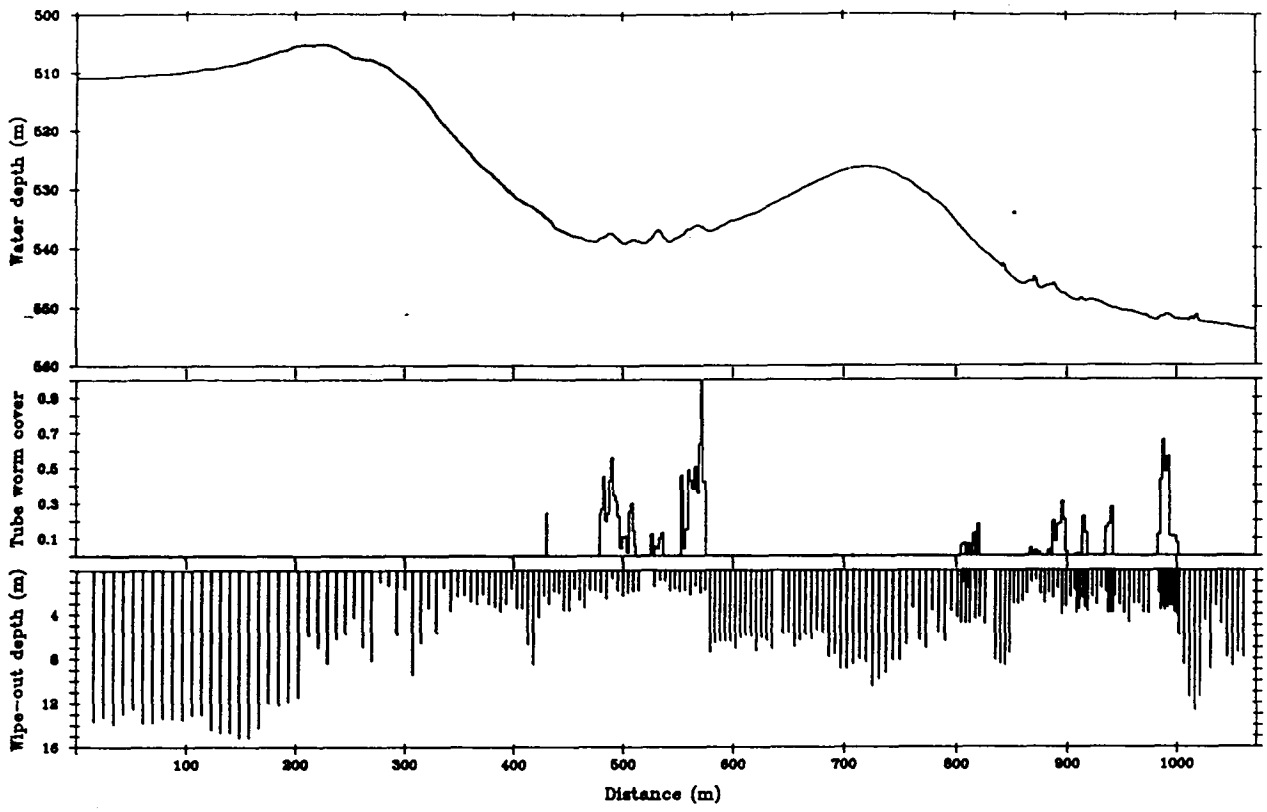

Figure 3. Observations of wipe-out depth, tube worm cover and topographic profile (water depth) along a 1,080 m east to west transect of tube worm community site near 27°44′50″N, 91°13′30″W with NR-1. Wipe-out depth indicates the maximum depth of seismic reflectors detected by 25 kHz subbottom profiler; wipe-out depth was measured at intervals ≤30 sec from a continuous record. Tube worm cover gives the proportion of the video frame filled by tube worms (*Lamellibrachia* sp.); video records were continuously recorded along the entire transect with a vertically mounted camera. Water depth was recorded at 1 sec intervals along transect.

oxidation of the hydrocarbons and where the combined effects of carbonate precipitation and increased sedimentation cause a gradual mounding of the substratum.

Communities Dominated by the Seep Mussel

The seep mussel (*Bathymodiolus* n. sp.: Mytilidae) has been shown to possess methanotrophic symbionts (Childress and others 1986), which are contained within the greatly enlarged gills of the species. Beds of seep mussels occur within tube worm dominated communities, often at sites where the escape of methane into the water column is visible as a stream of bubbles. Mussel abundance was found to be significantly correlated with elevated concentration of dissolved methane (up to ~60 μM) in seawater collected at 12 sampling locations on Bush Hill (MacDonald and others 1989). However, the largest assemblages of mussels yet discovered on the slope have occurred at sites where dissolved methane is available as a component of hypersaline brine.

MacDonald and others (1990a) investigated one such community, dubbed Mussel Beach (27°44′5″N, 91°32′16″W), that occupies a 10 ha area of relatively level (total change in depth <10 m) bottom at a mean depth of 630 m. This site lies at the head of a broad graben that extends to the southeast for at least 12 km (Behrens 1988) and is bounded to the northeast by a steeper slope. The subbottom signal (25 kHz) throughout this area is reduced in a thin (<1 m) layer immediately below the surface; however, surficial rocky strata are limited to small carbonate outcrops at the base of the slope. Thermogenic gas hydrates were collected from the surface sediments in this site (J.M. Brooks, unpublished data 1987), so it is likely that the signal attenuation is due to a diffuse layer of hydrates.

Mussels occurred in linear beds (Fig. 4) that comprised up to 50 m^2 in area, whereas tube worms were rare and occurred in much smaller (ca. 30 cm) clusters. Bubbling gas seeps were also rare and, although mussels were seen at these seeps, bubble streams were not seen around most of the mussel beds. The implication of this lack of bubbling gas seeps is that

Figure 4. Beds of the seep mussel (*Bathymodiolus* n. sp.) lining brine seep flow channels near 27°44′55″N, 91°32′16″W. Photograph taken with a vertically mounted camera; scale is ~1.25 m from left to right edges.

methane was becoming available to the mussels via a different process than at the Bush Hill-type communities.

A noteworthy characteristic of the Mussel Beach site was extensive patches of discolored sediments. Visually, these patches were blue-green, tended to occupy shallow (<20 cm deep) depressions, and often formed linear patterns that resembled miniature deltas or meanders. The cause of the discoloration is brine that escapes from the sediments and flows or pools at the sediment/seawater interface. Although attempts to collect brines in fluid samples from the sediment interface were inconclusive (maximum salinity 39‰), the pore fluids that were squeezed out of the punch cores contained salinities of up to 100‰ (MacDonald and others 1990a).

Methane entrained in seeping brine (e.g., by decomposition of gas hydrates) provides an alternative means for conveying methane to the mussels. MacDonald and others (1990a) mapped the fine-scale distribution of mussels and brine seeps at Mussel Beach and examined the length frequency histograms of collections of mussels taken near areas of brine-stained sediments. They proposed that larval mussels selectively settle at or near the brine seeps. Settlement may cease, however, if the discharge of methane-rich brine is reduced. The result is a mix of mussel beds, some with a significant proportion of juvenile settlement classes, while others consist only of adult classes. These "young" and "old" beds are spatially discrete and may occur in close proximity to each other because the brine is confined to distinct pools and channels on the sediment surface.

More recently, a small (190 m^2) pool of brine (salinity 121.35‰) was found near 27°43′24″N and 91°16′30″W at a water depth of 650 m (MacDonald and others 1990b). The brine filled the pool to a depth exceeding 3.5 m and contained saturating concentrations of microbial methane ($\delta^{13}C = -63.8$). It was ringed by a large (540 m^2) bed of seep mussels. Mussels settled on the "shoreline" of the pool included numerous juveniles, whereas the periphery of the bed comprised a single settlement class without juveniles. The difference in length frequency supports the preference of settling mussel spat for open brine. The pool, dubbed Brine Pool NR-1, was situated on a mound with an elevation of about 6 m and a basal diameter of about 130 m. Given the local elevation of the pool, it was apparent that brine filled the pool by welling in from below, rather than by the side-filling process which has been described at other brine pools (Rezak and Bright 1981) and basins (Shokes and others 1977). Furthermore, although brine is undoubtedly lost from the pool by diffusion into the water column and lateral percolation through the sediments, the continuous band of mussels surrounding the pool counter-indicates channelized runoff and may help to stabilize the rim.

Video records and field notes from the operations with NR-1 were reexamined in the light of these findings and it became clear that at least two other brine pools had been observed at 27°26′30″N,

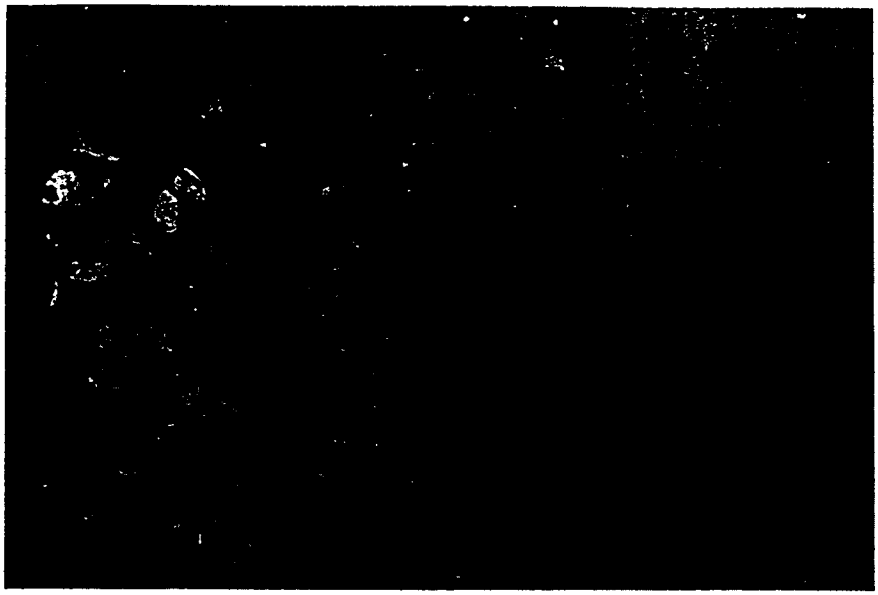

Figure 5. Epibenthic aggregation of the vesicomyid clam *Calyptogena ponderosa* near 27°45′30″N, 89°58′18″W, 940 m depth. Note the trails formed by living clams as they plow through the surface sediments. Living clams are frequently associated with pavements of dead shell. Scale of photograph (taken with vertically mounted camera) is ~2.25 m from left to right edges.

91°40′30″W, 662 m depth and 27°35′59″N, 92°55′58″W, 590 m depth, respectively. The former was evidently in an earlier stage of formation than Salt Well NR-1 because large plumes of sediment were being ejected with the escaping gas bubbles and the very steep (~80°) rim was still collapsing into the pool. Despite evident quantities of methane at both sites, neither had been colonized by mussels. The features may have formed too recently for colonization to have occurred, or the rapidly eroding rims may not yet provide a stable substrate for settlement.

Assemblages Dominated by Vesicomyid Clams

Vesicomyid assemblages comprise the species *Calyptogena ponderosa* and *Vesicomya cordata* (Boss 1968). Carbon isotope ratios in tissue samples from these species (Brooks and others 1987b) indicate chemoautolithothrophic utilization of sulfides. Both species are epibenthic and mobile; ventral margin is down in life position, with the anterior part of the umbo pointing toward the sediment at an angle of ~40° (Fig. 5). They move by plowing through the surface sediments, leaving distinctive vee-shaped trails (Rosman and others 1987). This life position and trail-making habit was now known to be widely characteristic of the Vesicomyidae when they occur on soft substrata, having been reported from abyssal habitats in the Japan Trench (Ohta and Laubier 1987) and the Laurentian Fan (Mayer and others 1988).

Rosman and others (1987) described two vesicomyid aggregations on the Louisiana Slope that had minimum widths of 95 and 145 m, respectively, and comprised a mixture of living individuals and dead, disarticulated shells. N.L. Guinasso (unpublished data 1989) has described aggregations several times larger based on surveys with NR-1. He reports numerous doughnut-shaped sonar reflectors from this site. One of these, which was examined in detail, was a small blow-out crater or pockmark approximately 10 m wide by 3 m deep, clearly formed after the establishment of the clam aggregation. Physical samples collected near this site (27°41′N and 91°32′W) revealed a thin (<10 cm thick) layer of poorly consolidated and liquid-rich sediments with an underlying layer of heavily tarred, relatively impenetrable sediments. Because clam aggregations typically included many articulated dead valves in life position on the surface, it is likely that photo surveys like that of Rosman and others (1987) would tend to overestimate the number of living individuals in the aggregation.

Assemblages Dominated by Lucinid and Thyasirid Clams

Lucinid assemblages comprise primarily *Lucinoma atlantis, Lucimoma* n. sp. (*Pseudomiltha* sp. of Brooks and others 1987b, Powell and others 1989), and *Thyasira olephila*. Although it is highly probable that both species of *Lucinoma* host sulfur-oxidizing endosymbionts (Brooks and others 1987b), no living *T. olephila* have been collected, so the chemosynthetic potential of this species can only be inferred from its habitat and its abundance. All three species are typically associated with subsurface lithification in the form of authigenic carbonates that range in size from small granules to fist-sized nuggets. Surface plates of authigenic carbonate are not uncommon. Lucinid assemblages are often characterized by dead

Figure 6. Carbonate chimney formed around a gas vent near 27°33'27"N, 92°32'22"W, 575 m depth. Note seep mussel attached to chimney (arrow). Infaunal aggregations of lucinid and thyasirid clams were very abundant at this site, as were gas seeps and hydrates; however, the attached specimen was the only seep mussel observed at this site.

and disarticulated valves, generally well preserved, dotted over the surface sediments. Living specimens are seldom seen on the surface, but are frequently collected in box cores.

The depth of occurrence appears to be controlled in part by the occurrence of tars. At sites where the underlying sediments were heavily tarred, lucinids were restricted to the upper 5 cm. Elsewhere, they had been collected with box cores at sediment depths of 65 cm. Beds of *T. olephila* that consist entirely of dead, articulated valves in life position are noteworthy for their very high densities (up to several thousand individuals m^{-2}). Box cores in these localities frequently recover gas hydrates. Venting of gas from the surface leads to the formation of numerous carbonate chimneys (Fig. 6). Because of their infaunal habitat, photographic surveys and trawl sampling would tend to underestimate the importance of lucinid assemblages (Callender and others 1990).

Summary and Conclusions

Exploration to date has revealed thriving biological communities on the continental slope south of Texas, Louisiana, and Alabama in a bathymetric range of 350 to 2,200 m at sites where seepage of oil and gas produces seismic wipe-out zones. Utilization of reduced compounds associated with seeping hydrocarbons has been observed directly (in the case of methane-based chemosynthesis) or has been inferred from the stable carbon isotope ratios of animal tissue. Four types of communities can be recognized based on the predominant species of chemosynthetic fauna: 1) tube worms clusters or "bushes," with isolated seep mussel beds, 2) linear mussel beds at brine seeps, 3) beds of two species of surface-living vesicomyid clams, and 4) extensive areas having a scatter of dead lucinid clam shells on the surface sediments and a living population beneath the surface. Community development appears to be determined by whether seepage provides methane or sulfide compounds to the benthos and by the geological characteristics of the surface sediments. Seep mussels occur in discrete beds defined by the flowage patterns of methane saturated brine at the sediment/seawater interface. Results from Brine Pool NR-1 suggest that a re-examination of high-resolution seismic data from the slope would reveal additional brine pools and that these may support seep mussel communities. Tube worm clusters are most commonly associated with hummocky topography in the crests of grabens or half grabens. Clam communities tend to be dispersed over larger areas than tube worm or mussel communities and may be either infaunal (Lucinidae and Thyasiridae) or epibenthic (Vesicomyidae).

Acknowledgments

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Chemosynthetic Mussels at a Brine-Filled Pockmark in the Northern Gulf of Mexico

I. ROSMAN MACDONALD, JAMES F. REILLY II, NORMAN L. GUINASSO, JR.,
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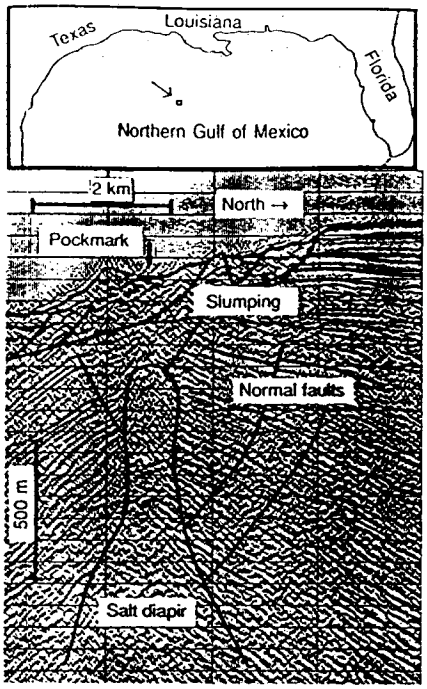

Fig. 1. Two dimensionally processed, common-depth-point (CDP) seismic data showing a north-south transect of the pockmark. Inset map shows its general location in the northern Gulf of Mexico. [Data provided courtesy of Halliburton Geophysical Services, Inc.]

Chemosynthetic Mussels at a Brine-Filled Pockmark in the Northern Gulf of Mexico

I. Rosman MacDonald, James F. Reilly II, Norman L. Guinasso, Jr., James M. Brooks, Robert S. Carney, William A. Bryant, Thomas J. Bright

A large (540 square meters) bed of *Bathymodiolus* n. sp. (Mytilidae: Bivalvia) rings a pool of hypersaline (121.35 practical salinity units) brine at a water depth of 650 meters on the continental slope south of Louisiana. The anoxic brine (dissolved oxygen ≤0.17 milliliters per liter) contains high concentrations of methane, which nourishes methanotrophic symbionts in the mussels. The brine, which originates from a salt-cored diapir that penetrates to within 500 meters of the sea floor, fills a depression that was evidently excavated by escaping gas. The spatial continuity of the mussel bed indicates that the brine level has remained fairly constant; however, demographic differences between the inner and outer parts of the bed record small fluctuations.

The violent escape of gas through surface sediment often forms sea floor pockmarks (*1*) or craters (*2*). During a submarine survey of the continental slope, northern Gulf of Mexico, we found pockmarks that were filled with hypersaline brine. These features are evidently a consequence of salt tectonism in a hydrocarbon province. One of the pockmarks, brine pool NR-1, was ringed by a large bed of *Bathymodiolus* n. sp., a mussel (*3*) that possesses methanotrophic symbionts (*4*). This discovery signals that the potential habitat for *Bathymodiolus* n. sp. on the slope may be greater than previously thought and demonstrates that chemosynthetic fauna, already known for their tolerance of toxic sulfides (*5*) and aromatic compounds (*6*), also tolerate hypersaline conditions.

Tectonic deformation of the Louann Salt, a Jurassic evaporite deposit, has created much structural complexity in the northern Gulf of Mexico (*7*); common features include salt diapirs and growth faults. The faults provide a conduit for natural hydrocarbon seepage (*8–10*), which is recognized as a widespread phenomenon on the Louisiana slope (*11*). Large chemosynthetic communities, which have been reported at oil and gas seeps in water depths of 500 to 900 m (*12*), are biological consequences of hydrocarbon seepage.

Because many salt diapirs penetrate recent sedimentary strata, sea floor brine seepage is also thought to be a common phenomenon in the Gulf of Mexico (*7*), but only a few actual seeps have been documented. Brine that originates from the Louann Salt has been found in a shallow (~25 cm deep) pool on the Texas shelf (*13*) and in a large (90 km^2) basin (*14*) on the lower Louisiana slope; both features are filled from the side by drainage from salt deposits located above. Brine that originates from saline aquifers in the Florida-Bahama platform saturates surface sediments at the abyssal base of the Florida escarpment (*15*). Reduced compounds associated with this brine nourish chemosynthetic communities that include a second species of mussel (*16, 17*).

Brine pool NR-1 was found (*18*) approximately 285 km southwest of the Mississippi Delta (27°43'24"N, 91°16'30"W) near the

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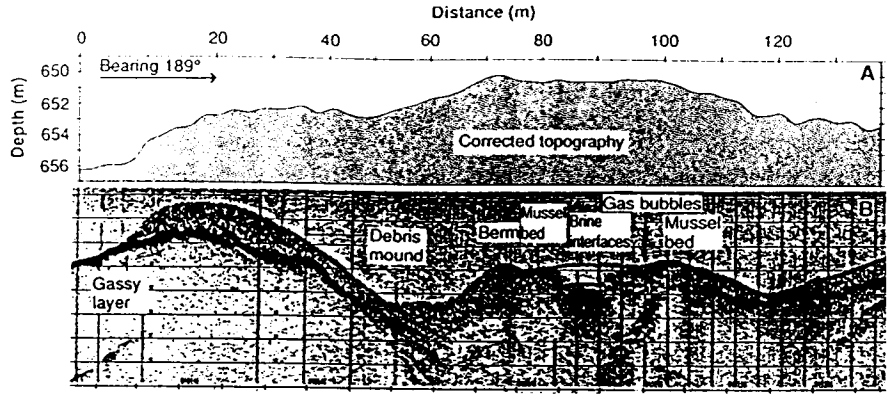

Fig. 2. A north-south transect of pockmark and brine pool recorded by Submarine *NR-1*. (**A**) Corrected topographic profile and (**B**) subbottom profile (25 kHz) at same horizontal and vertical scale. The apparent differences between the corrected topography and subbottom profile are an artifact of changes in the submarine's altitude along the transect. The horizontal axis is proportional to transit time so that distance on the figure is a function of the submarine's speed.

location where Anderson *et al.* collected sediment cores that contained crude oil (*8*). The pool lies at a water depth of 650 m among a series of recent slump terraces at the head of a broad, trough-like graben that deepens to the southeast (*10*). A salt diapir, the top of which is ~500 m below the sediment-seawater interface, is evident in seismic profiles across this site as a seismic anomaly and hyperbolic reflector (Fig. 1). Normal faults generated by salt uplift extend from the diapir and terminate against the base of the slump block. Shallow gas saturation in the near-surface sediments is indicated by a high-amplitude feature at the sediment surface and signal attenuation beneath the pockmark.

As shown in a 25-kHz profile, brine fills a 22-m-long, 11-m-wide, elliptical crater that is situated atop a mound with a height of about 6 m and a basal diameter of about 130 m (Fig. 2A). In nongassy sediments, such profiles typically show sedimentary strata to depths of ~20 m below the ocean bottom. On the periphery of the pool, the seismic signal was attenuated by highly reflective layers <2 m below the bottom. Judging from gas fractures and hydrates in piston cores we collected in nearby areas, these layers suggest that gas-rich sediments and possibly diffuse hydrates occur near the mudline at this site (Fig. 2B). Streams of fine bubbles were seen rising from the center of the pool and appear as a slight trace in the 25-kHz record. No bubbles were observed at the pool edges or in the mussel bed.

A debris lobe on the north side of the pool suggests that vigorous expulsion and down-slope slumping of sediment occurred when the pool formed. The subbottom profiles of the near-surface strata and direct observations gave no indication of a rocky substratum. We infer that the mound is composed of fine-grained and poorly consolidated sedimentary material that has been disrupted by upwardly migrating gas.

A berm, approximately 0.5 m high, encloses the mussel bed and the pool to the east, north, and west, but is reduced or absent to the south (Fig. 2A). Open brine in the pool can be readily distinguished in subbottom profiles by its sharp, flat density interface (Fig. 2B). A second horizontal reflector 1.5 m beneath the interface appears to indicate the maximum depth of the brine. However, when we attempted to plant a 3.5-m pole upright in the center of the pool, it sank straight down and disappeared. Therefore, the second reflector is probably also a density interface. The edges of the pool are well defined by the subbottom profiler, where layers of mussel shells provide an intense seismic reflector (Fig. 2B).

Brine was collected (*19*) from the upper density layer near the center of the pool. Although its salinity (121.35 practical salinity units, ~3.5 times seawater salinity) was less than saturation, it is likely that the density layer below contains more saline brine. The temperature of the upper layer (8.7°C) exceeded the ambient seawater temperature by 1.6°C. The brine was nearly anoxic (dissolved O_2 = 0.17 ml/liter); mea-

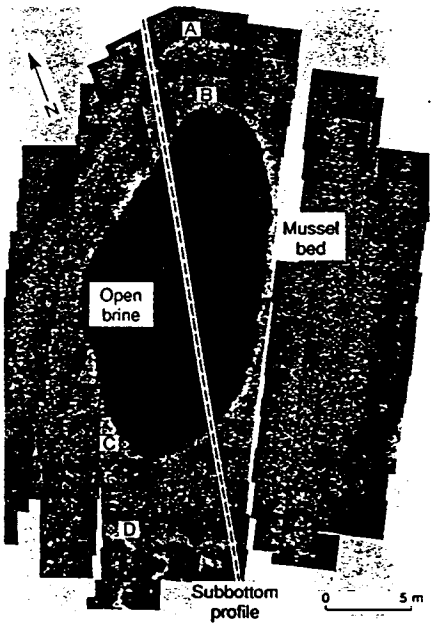

Fig. 3. A mosaic of 231 video photographs showing the brine-filled pockmark and surrounding mussel bed. The photographs were recorded on half-inch tape and digitally reduced to a constant scale. Inner "shoreline" of mussel bed was manually traced, and open brine was imaged with a constant gray tone. The irregular southern edge of the mussel bed is due to lateral seepage of brine from the low (southern) side of the pockmark. Approximate locations in mussel bed where mussels were collected for measurement are labeled as follows: (A) northern outer, (B) northern inner, (C) southern inner, and (D) southern outer.

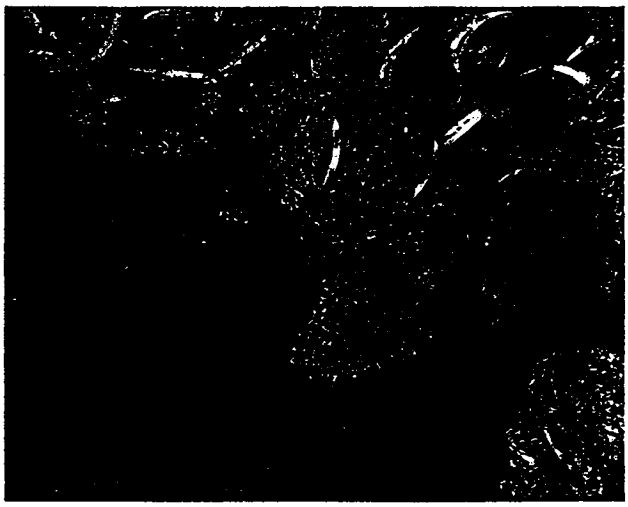

Fig. 4. Living *Bathymodiolus* n. sp. (Mytilidae: Bivalvia) partly submerged in anoxic brine at the edge of the brine-filled pockmark. Blackened shells of dead mussels can be seen completely submerged in brine at the lower edge of the frame. The anoxic-oxic interface at the surface of the brine is evident in the abrupt change in color from dark blue (reduced) to orange (oxidized) over the lengths of the mussels shells. The shaggy coatings visible on some mussels are silt-fouled byssus. The larger mussels are approximately 120 mm long.

ured dissolved oxygen may have resulted rom contamination during collection. Odor f H_2S was strong in the dive chamber when rine samples were collected. Vigorous ef- ervescence during collection indicated that ome dissolved gases were at saturation with espect to an ambient pressure of 1 atm.

Bubbles collected in a bell jar over the enter of the pool were predominantly CH_4 76.3%), with trace quantities of C_2H_6 (452 ppm) and C_3H_8 (145 ppm). The carbon isotope ratio of the CH_4 ($\delta^{13}C = -63.8$ per mil) and the low proportion of higher hydrocarbons indicate that the CH_4 in the bubbles was primarily microbial in origin (*20*). Biodegraded oil recovered in surface sediments on the periphery the pockmark indicated the presence of thermogenic hydrocarbons in the area.

Chemosynthetic mussels surrounded the open brine in a continuous band, approximately 3 m wide on the narrowest side, the northwest edge, and 7 m wide on the southeast edge (Fig. 3). The mussel bed was elevated only a matter of centimeters above the brine-seawater interface; its total area was ~540 m^2 whereas the area of the open brine was ~190 m^2. Transition from the mussel bed to bare sediment was abrupt on the north, west, and east, but more gradual to the south, where the berm was reduced or absent. To the south, gaps of stained sediments appeared as the continuous bed gave way to curvilinear stringers, intermingled with patches of disarticulated mussel shells (which were mostly broken and pitted) and filamentous colonies of white bacteria. Shells continued to dot the sediments 20 m south of the bed. There was no sign of a brine-outflow channel through the bed. We infer that the irregular southern edge of the mussel bed results from lateral percolation of brine through surface sediments.

A diverse and abundant biological community was associated with the mussel bed. Chemosynthetic tube worms, *Lamellibrachia* n. sp., which are abundant at other Louisiana slope oil seeps (*12*), were represented here by a few solitary individuals. Demersal fishes included *Chaunax pictus*, seen resting on the bottom outside the bed, the eel *Synaphobranchus* sp., and the hag fish *Eptatretus* sp. We collected a severely disoriented fish, *Nezumia* sp., as it swam in circles upside down just above the open brine, possibly affected by hydrogen sulfide toxicity. Our operations over the brine created internal waves, which lifted well-preserved dead fish from the bottom of the pool and into view around its edges. Epifaunal crustaceans observed among the mussels included a large magid crab, *Rochinia crassa*, as well as numerous shrimp, *Alvinocaris stactophila*, and galatheid crabs, *Munidopsis* sp., which browsed the interstices of the mussel bed. An unidentified paranoid polycheate formed a dense mat beneath the bed.

Several differences were evident between the outer edges of the mussel bed and the inner margin adjacent to the open brine. Mussels on the outer edge rested flat on the sediments or were loosely shingled one on another, whereas those on the inner edge stood vertically, with their anterior ends down, firmly bound by their byssus into a dense mat. Mussels on the inner edge adjacent to the open brine were partly submerged in brine; the interface between oxic seawater and anoxic brine was distinctly visible here because their upper (posterior) ends were rust-colored whereas their lower (anterior) ends were black (Fig. 4). The innermost bed was formed from completely submerged, dead individuals, whose valves, heavily blackened by mineral deposits, were still articulated and in many cases contained poorly preserved remains of the soft tissues.

Bathymodiolus n. sp. require adequate supplies of dissolved methane and free oxygen for growth (*4*). Because the brine supplies dissolved methane to the mussels at this site whereas oxygen is obtained from ambient seawater, the spatial distribution and demography of the mussels record the local extent of the oxic-anoxic interface and may indicate subtle fluctuations in brine level. Mussels collected from the outer edge (*21*) apparently all belonged to a single settlement class of intermediate length, whereas inner-edge mussels formed four apparent settlement classes (*22*) that ranged from recently settled spat to the largest specimens collected from the site (Fig. 5). Individuals of the intermediate-length settlement class that was prevalent on the outer edge were rare on the inner edge.

Spat of chemosynthetic mussels from the Gulf of Mexico evidently settle or survive preferentially on substrata where brine supplies dissolved methane (*15, 16, 23*); this preference is evident in the prevalence of spat and juveniles on the inner edge of the bed. The mussels tolerate partial submergence in the anoxic brine; however, complete submergence would undoubtedly be lethal. A rise in brine level of a few centimeters would therefore kill many of the mussels on the inner edge but would also broaden the distribution of methane-rich brine and promote settlement on the outer edge. Settlement would cease on the outer edges with subsequent lowering of the brine level and would resume on the inner edges.

Lack of recent settlement on the outer edges, the broad size range of mussels on the inner edges, and the accumulation of dead individuals in the pool all indicate that these mussels exist at an equilibrium between an adequate concentration of nutritive methane and an oversupply of anoxic brine. Despite evident fluctuations, the overall spatial continuity of the mussel bed is striking. Compared with the patchy fine-scale distribution of chemosynthetic fauna in communities reported from elsewhere (*5, 12, 16, 23, 24*), this mussel bed maintains a relatively uniform density over a significantly larger area. The density of the brine traps methane beneath a distinct interface with oxic seawater, and the morphology of the pockmark edges provides a stable substratum beneath a thin layer of brine. Such conditions are clearly favorable for methanotrophic symbiosis.

We have also found brine-filled pockmarks at two other locations on the Louisiana slope (*25*). They occurred on low mounds, had raised rims, and contained a dense, light-refractive fluid from which bubbles were escaping. One of these was evidently in active formation because its steep

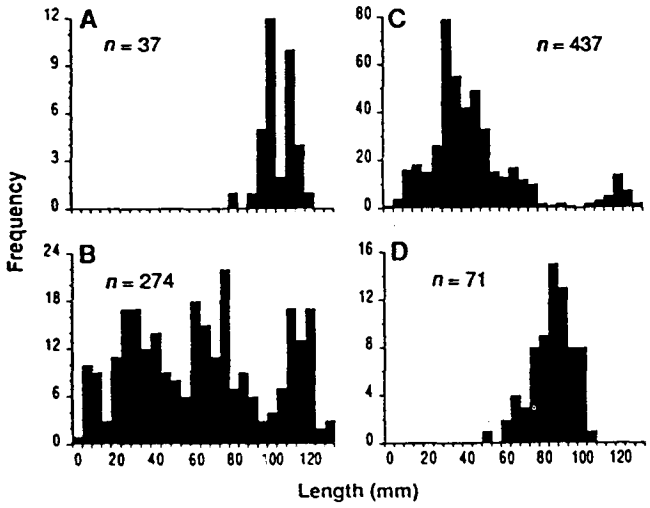

Fig. 5. Length frequency histograms of mussels collected from the mussel bed surrounding the brine-filled pockmark. Collection locations (**A–D**) refer to the description in Fig. 3. Mean lengths (±1 SD) for the samples are as follows: (A) 104 ± 8.7 mm, (B) 63 ± 35.3 mm, (C) 45 ± 26.6 mm, (D) 84 ± 11.5 mm.

pool while plumes of fine sediment were ejected by vigorous bubble discharges. Despite apparent concentrations of methane, neither feature had been colonized by mussels. Either the unstable substrata on the rims impeded settlement of mussels, or the features were so recent that colonization had not yet occurred.

Doming of the sea floor under pressure from upwardly migrating gas, followed by eruption, will excavate a pockmark with raised rims (*1, 2*). If hypersaline pore fluids are present, as they are in the vicinity of brine pool NR-1 (*10*), lateral seepage through the pockmark walls will fill the depression with brine. Sediments suspended at the bottom of the pool may continue to be excavated by bubble discharge after the initial eruption. Anoxic conditions may also inhibit microbial oxidation of methane and prevent the formation of authigenic carbonates, which are common at North Sea pockmarks (*5*) and Gulf of Mexico gas seeps (*10, 11*). Although these features may be relatively small, a careful examination of seismic records should reveal additional brine-filled pockmarks on the continental slope of the northern Gulf of Mexico. Their potential as habitats for chemosynthetic communities is evident.

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19. The mechanical arm of the Johnson *Sea Link I* was lowered about 1 m below the density interface. Brine was collected at 1 atm inside the dive chamber via a hose that ran from the end of the arm. Temperature was measured by a Sea Bird conductivity and temperature detector (CTD) held by the arm. Salinity was measured with a conductive salinometer after weight dilution to near seawater conductance. Dissolved oxygen was measured by Winkler titration. Gaseous hydrocarbons were determined by flame-ionized gas chromatography. Carbon isotope ratios are reported as per mil deviations from a PDB standard: $\delta^{13}C$ (per mil) = $10^3 \times ({}^{13}C/{}^{12}C_{sample})/({}^{13}C/{}^{12}C_{PDB} - 1)$.
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25. Near 27°26′30″N, 91°40′30″W at 662-m depth, and near 27°35′59″N, 92°55′58″W at 590-m depth.

26. We thank the captains and crews of the U.S. Navy Submarine *NR-1* and the Johnson *Sea-Link I* for enabling our submersible operations. R. A. Burke, Jr., supervised gaseous hydrocarbon analyses. Ship time was funded by Office of Naval Research and National Oceanic and Atmospheric Administration–National Undersea Research Program. Additional support was from the Texas A&M University and Louisiana State University Sea Grant Programs. We thank Exxon Corporation, U.S.A., and Ensearch Exploration, Inc., for support and suggesting dive sites for study.

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Gulf of Mexico Hydrocarbon Seep Communities: VIII. New Deep Water Petroleum Seep Communities in Alaminos Canyon

Gulf of Mexico hydrocarbon seep communities: VIII. New deep water petroleum seep communities in Alaminos Canyon

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ABSTRACT - Deep sea chemosynthetic communities were recently discovered in the western Gulf of Mexico (GOM) along a carbonate ridge in Alaminos Canyon at depths between 2010 and 2030 m. Two sites were discovered that had high densities of both mussels and vestimentiferan tube worms. Additionally, numerous isolated clumps of vestimentiferans were observed during transects of this area. A new species of mussel and a tube worm similar to those found at the Florida Escarpment chemosynthetic communities were collected at the first dive site. Two species of mussels and one vestimentiferan were recovered at the second dive site. Of the two species of mussels, one is identical to the mussel found at the first dive site, while the second mussel and the vestimentiferan are similar to species found at shallow petroleum seeps in the GOM. Enzyme data, ultrastructural information, and stable isotope values indicate that the vestimentiferans contain thiotrophic symbionts and that the mussel similar to those at shallow petroleum seeps is methanotrophic.

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Oil-stained sediment cores have been recovered near these sites and the extensive carbonate structures observed have $d^{13}C$ values consistent with bacterial utilization of gas and/or oil. Mussel and tube worm tissues contained concentrations of polynuclear aromatic hydrocarbons that are indicative of chronic exposure to oil. This data suggests that the Alaminos Canyon chemosynthetic communities are associated with seeping oil and gas.

INTRODUCTION

Chemosynthetic communities were first discovered in the Gulf of Mexico (GOM) at the base of the Florida Escarpment at >3000 m of depth (PAULL *et al.*, 1984). An unidentified methanotrophic mussel, a thiotrophic vestimentiferan tube worm, and a thiotrophic vesicomyid clam have been identified at the Florida site (CARY *et al.*, 1989). The seepage of brines from the interior of the Florida platform is the suggested source of methane and sulfide to the chemosynthetic fauna (PAULL *et al.*, 1984; COMMEAU *et al.*, 1987; CARY *et al.*, 1989). Chemosynthetic communities also occur at natural petroleum seeps on the Texas-Louisiana continental slope (KENNICUTT *et al.*, 1985). Petroleum seep fauna include vesicomyid and lucinid clams, and vestimentiferan tube worms that harbor thiotrophic bacterial endosymbionts. Also abundant at many of the petroleum seep sites are mussels that host methane-oxidizing bacterial endosymbionts (CHILDRESS *et al.*, 1986; FISHER *et al.*, 1987; BROOKS *et al.*, 1987). To date, approximately 40 sites at which chemosynthetic fauna are found have been documented on the Texas-Louisiana continental slope at depths between 400 and 1000 m (KENNICUTT *et al.*, 1988a; MACDONALD *et al.*, 1990a). The reduced chemical species (e.g., sulfides and methane) required to fuel

chemosynthesis are associated with seeping oil and brine (KENNICUTT *et al.*, 1985; BROOKS *et al.*, 1987; MACDONALD *et al.*, 1990b).

Natural oil and gas seepage is a widespread phenomenon on the northern GOM continental shelf and slope (SACKETT, 1977; BERNARD *et al.*, 1976; ANDERSON *et al.*, 1983; BROOKS *et al.*, 1984; KENNICUTT *et al.*, 1988b). The near surface seepage of hydrocarbons is controlled predominantly by fault systems developed during salt diapirism that serve as conduits to the sediment-water interface (GEYER and SWEET, 1973; MARTIN and CASE, 1975; BEHRENS, 1988). Features typically associated with hydrocarbon seeps include oil-stained sediments, gas hydrates, authigenic carbonates, brine seepage, mud volcanos with blow-out craters and acoustic "wipeout zones" (ANDERSON *et al.*, 1983; BROOKS *et al.*, 1984; 1986; 1987; KENNICUTT *et al.*, 1985; ROBERTS *et al.*, 1987; PRIOR *et al.*, 1989; KENNICUTT and BROOKS, 1990). The Alaminos Canyon area was considered a likely site for deep water chemosynthetic communities because many geological features associated with near sediment surface seepage are prevalent and oil-stained sediment cores were recovered during surface geochemical prospecting cruises in 1987 and 1988 (personal observation, JMB). The present study describes the chemosynthetic communities discovered in Alaminos Canyon and reports the results of enzyme, stable isotope, and tissue polynuclear aromatic hydrocarbon measurements of some of the fauna.

GEOLOGICAL SETTING

Alaminos Canyon is located approximately 194 km south of Galveston, Texas, between 26°-27°N and 94°-95°W (Fig. 1). It is a re-entrant in the Sigsbee Escarpment, which is a monocline separating the continental slope and rise in the northwestern GOM (BRYANT *et al.*, 1968). The canyon is

approximately 15 km wide, 40 km long and 800 m deep, and is oriented north-south. Unlike the classic submarine canyons described by SHEPARD and DILL (1966), Alaminos Canyon has sides with relatively low slopes and has no obvious channel connecting it with the shelf (BRYANT *et al.*, 1990). The morphology of Alaminos Canyon is thought to have resulted from salt tectonics. The canyon protrudes into the Sigsbee Escarpment, which is the leading edge of an allochthonous sheet of Jurassic salt that has been mobilized by the mass of overlying, younger clastic sediment. This sheet of salt has risen to near the seafloor and is now flowing downslope towards the deep GOM (BERGANTINO, 1971; HUMPHRIES, 1978). Because of its location at the boundary between the Texas-Louisiana and Rio Grande salt basins, where the Sigsbee Escarpment changes trend from east-west to north-south, Alaminos Canyon appears to have formed by the coalescence of salt sheets from the two different provinces (GARRISON and MARTIN, 1973). Alaminos Canyon is surrounded on the north and east by hummocky terrain that is also a result of deformation by salt flowage and tectonics (BERGANTINO, 1971).

MATERIALS AND METHODS

Sample collection

Areas containing geophysical and geochemical characteristics associated with seep communities were examined in the Gulf of Mexico in April 1990 using the DSRV *Alvin* (support ship the R/V *Atlantis II*). Two dives in Alaminos Canyon were completed on April 10 and 12. Animals and carbonate rocks were collected using *Alvin's* manipulator arm and placed in a temperature-insulated box for transport to the surface. Mussels and tube worms were transferred to chilled seawater (7°C) immediately upon recovery and stored chilled until dissection, usually within 2 h. Dissected

tissues were frozen at -70°C for subsequent laboratory analyses. Tissue samples from all mussels were provided to R. Vrijenhoek and C. Craddock (Rutgers University) for genetic analyses.

Enzyme assays

Enzyme assays were conducted on tissues that had been frozen in liquid nitrogen immediately upon dissection and stored at -70°C. All enzyme assays were conducted at 20°C. The tissues were homogenized with a ground glass tissue grinder in 0.2 mM Tris/HCl (pH=7.5, either 1:4 or 1:9 tissue:buffer). The homogenate was not centrifuged prior to use in assays, since a substantial portion of the enzymatic activity is often associated with the pellet. Homogenates were assayed for ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBP C/O; EC 4.1.1.39) activity using a ^{14}C incorporation method (WISHNICK and LANE, 1971, modified by FELBECK, 1981). Data were collected at three time points (5, 10, and 15 min) and corrected for non-RuBP dependent carboxylations with substrate-free controls. Adenosine-5'triphosphate sulfurylase (ATP sulfurylase; EC 2.7.7.4) and adenosine-5'-phosphosulfate reductase (APS reductase; EC 1.8.99.2) activities were assayed in additional homogenates by the methods of FELBECK (1981). Methanol dehydrogenase (MeDH) was assayed by the spectrophotometric method of ANTHONY and ZATMAN (1965).

Stable isotopes

Stable carbon and sulfur isotope compositions were determined on homogenized mussel body and gill tissues and tube worm soft tissues. Stable carbon isotope composition was also determined on carbonate samples. Tissue homogenates were freeze-dried and acidified to remove carbonates. Tissue samples were then combusted to CO_2 using standard closed-tube techniques. Carbonate samples were acidified to yield CO_2 (PRESLEY AND

KAPLAN, 1968) The $^{13}C/^{12}C$ of the resulting CO_2 was analyzed with a Finnigan MAT 251 isotope ratio mass spectrometer (IRMS) (SOFER, 1980; LEFEUVRE and JONES, 1988). Stable carbon isotope ratios are reported in the standard del (‰) notation relative to the PeeDee Belemnite (PDB) standard (CRAIG, 1953). Stable sulfur isotope ratios were determined on both elemental sulfur isolated from gill samples and freeze-dried tissue homogenates. Elemental sulfur was converted to SO_2 by the method of UEDA and KROUSE (1986) and the $d^{34}S$ of the purified SO_2 was determined with an IRMS. Organic sulfur in tissues was converted to $BaSO_4$ in a Parr Bomb under 30 atm of O_2 (FRY *et al.*, 1983). The $BaSO_4$ was converted to SO_2 (HOLT and ENGLELKEMEIR, 1970) and the purified SO_2 was analyzed by IRMS. $d^{34}S$ values are reported in the standard del (‰) notation relative to the Canyon Diablo meteorite standard.

PAH

The concentrations of PAH were determined for mussel and tube worm tissues. Surrogates were added to tissues homogenates (approximately 5 g) and digested with 6N KOH at 35°C for 18 h. The digested tissue was serially extracted with ethyl ether (WADE *et al.*, 1988). The eluate was dried with sodium-sulfate, concentrated, and purified by alumina column chromatography. The aromatic fraction eluted from the alumina column cleanup was further purified by high pressure liquid chromatography and then concentrated (KRAHN *et al.*, 1988). All hydrocarbon samples were analyzed quantitatively on a Hewlett-Packard Model (HP) 5890 gas chromatograph interfaced with a HP Model 5970 mass spectrometer, which was operated in the selected ion mode.

RESULTS AND DISCUSSION

Two dense communities of mussels and tube worms and several small isolated patches of vestimentiferans were discovered in Alaminos Canyon with the DSRV *Alvin* in April of 1990 (BROOKS *et al.*, 1990). The communities were found atop a small ridge, approximately 400 m above the seafloor, at the center of Alaminos Canyon (Fig. 2). Seismic reflection data show that the ridge is underlain by salt, but not connected to the salt sheets surrounding the canyon (J. MUELLER, Mobil Oil Company, personal communication). Thus, the ridge is probably a salt dome that arose from the deep Jurassic salt layer and fractured oil-bearing sedimentary layers causing seeps ascending to the seafloor. This discovery offshore of South Texas extends the range of known chemosynthetic communities from the Florida Platform to the western region of the Gulf of Mexico.

Community descriptions

Mussels and tube worms were collected at two sites. The first site was at 26°21'N and 94°30'W and was approximately 20 m^2 in size. Tube worm clumps and a few live mussels were observed and the site was strewn with dead mussel shells (Fig. 3). Due to the relatively rapid taphonomic loss of mussel shells from these types of environments, the substantial quantity of dead shells most likely indicates a recent population decline (CALLENDER *et al.*, 1990). Other fauna associated with this site included white shrimp and white galatheid crabs. The community was surrounded by authigenic carbonate rocks ranging in size from rubble to boulders. No venting gas or stained sediments were visually apparent near the community. The sediments associated with the fauna were a light tan color similar to the surrounding hemi-pelagic sediments. Although the presence of bacterial

mats on the surface of sediments is common at shallow hydrocarbon seeps (BROOKS *et al.*, 1989), no surficial bacterial mats were observed at this site.

The second site, about 1 nautical mile to the west of the first site, contained a dense community of both mussels and tube worms that covered an area of approximately 100 m^2. The community was surrounded by massive carbonate structures, covered by mussels. In general, tube worm clumps were located on the periphery of the mussel community. Dead shells were not as prominent at this site as they were at the first site and were evident only around the periphery of the site. White galatheid crabs, swarms of white shrimp, and a few fish were observed throughout the community. The surficial sediments were light tan in color with a few scattered patches of dark discoloration. No gas bubbles or seeping oil were observed.

Symbionts

Both symbiont-containing and symbiont-free tissues from mussels and tube worms were analyzed for APS reductase, ATP sulfurylase, RuBP C/O, and MeDH activities (Tables 1 and 2). APS reductase and RuBP C/O are indicative of chemoautotrophic sulfur bacteria (AMINUDDIN, 1980) and significant activities of ATP sulfurylase provide strong evidence for sulfur-based chemoautotrophic activity (FISHER, 1990). Methanol dehydrogenase catalyzes the oxidation of methanol to formaldehyde and is diagnostic for methanotrophic and methylotrophic organisms (ANTHONY, 1982). None of the enzymes characteristic of either methanotrophic or thiotrophic symbionts were detected in the symbiont-free tissues of any species tested.

Like all vestimentiferans tested to date, both species from Alaminos Canyon apparently harbor thiotrophic symbionts. Abundant symbionts were visible in electron micrographs of trophosome tissue from each species

(personal observation, CRF) and the levels of ATP sulfurylase and RuBP C/O present in all specimens indicate that these symbionts are thiotrophic. APS reductase activity was detected in only one individual; however, activity of this enzyme is often undetectable in thiotrophic symbiosis (FISHER, 1990). Preliminary taxonomic investigations indicate that the two species are closely related to other species found in the GOM (M. JONES, personal communication). The soft parts of *Lamellibrachia* sp. from Alaminos Canyon closely resemble those of *Lamellibrachia barhami* from shallow hydrocarbon seeps. However, the tubes of the Alaminos Canyon species are considerably more robust than those found at the shallow seeps, and most individuals were approximately twice as large. The *Escarpia* sp. from Alaminos Canyon is apparently similar to *Escarpia laminata* from the Florida Escarpment.

The presence of both methanotrophic and thiotrophic symbionts in the mussel (seep mytilid II) found at both sites in Alaminos Canyon has recently been reported (FISHER *et al.*, 1991). The other mussel (seep mytilid Ib), found only at the second dive site, is apparently very closely related to and may be the same species as a mussel (seep mytilid Ia) from a shallow hydrocarbon seep (R. VRIJENHOEK and C. CRADDOCK, Rutgers University, personal communication). The MeDH activities along with the absence of detectable RuBP C/O and ATP sulfurylase suggests that seep mytilid Ib, like its shallow water relative, harbors only methanotrophic symbionts. In fact the levels of MeDH activity found in gill samples from seep mytilid Ib are the highest reported for any petroleum seep mussel (BROOKS *et al.*, 1987; FISHER *et al.*, 1987).

Stable carbon and sulfur isotope analyses

The $d^{13}C$ values of mussels from Alaminos Canyon are listed in Tables 1 and 3. These values are within the range of values reported for

methanotrophic mussels collected at shallow petroleum seeps in the GOM and heavier than values reported for methanotrophic mussels from the Florida Escarpment, which are known to utilize biogenic methane (KENNICUTT *et al.*, 1985; 1991; PAULL *et al.*, 1985, BROOKS *et al.*, 1987). The mean $d^{13}C$ values of gill tissues from seep mytilid Ib and II, collected at the second dive site, are not statistically different. The mean gill $d^{13}C$ values of seep mytilid II collected at both dive sites are also not significantly different, thus indicating similar methane generating processes throughout the study site.

Methanotrophs and animals hosting methanotrophic symbionts are ^{13}C depleted because their cellular carbon $d^{13}C$ typically reflects the $d^{13}C$ of their methane source (BROOKS *et al.*, 1987). Methane can be produced either bacterially or thermogenically, and both processes result in methane that is depleted in ^{13}C. The primary source of methane to the Alaminos Canyon organisms is considered to be thermogenic since the $d^{13}C$ values of mussel tissues are more positive than -50 ‰ (BERNARD *et al.*, 1977).

Tissues from 10 Alaminos Canyon vestimentiferans were analyzed for $d^{13}C$ (Tables 2 and 3). These values are within the range of $d^{13}C$ values reported for tube worms found at shallow petroleum seep sites in the Gulf of Mexico (KENNICUTT *et al.*, 1985; 1991; BROOKS *et al.*, 1987). The $d^{13}C$ values of the two species of tube worms collected at the two sites are significantly different. Possible sources of inorganic carbon to thiotrophic petroleum seep vestimentiferans include both seawater and pore water containing dissolved inorganic carbon (DIC) (KENNICUTT *et al.*, 1991). The $d^{13}C$ of pore water DIC at shallow petroleum seep sites in the GOM ranges from -45‰ to +18 (BROOKS *et al.*, 1987; KENNICUTT *et al.*, 1989). A similar range of values in Alaminos Canyon could account for the differences

in $d^{13}C$ values observed in tube worms from the two sites Additionally, growth rate, size, symbiont complement, and gas uptake and transport capabilities could all affect the animal's tissue $d^{13}C$ values (FISHER, 1990; FISHER *et al.*, 1990; KENNICUTT *et al.*, 1991).

- The $d^{34}S$ values in Alaminos Canyon mussels range from -2.4 to +3.0‰ (Table 3). These values are among the lightest reported for mussels associated with chemosynthetic communities in the GOM (BROOKS *et al.*, 1987; CARY *et al.*, 1989; KENNICUTT *et al.*, 1991). The $d^{34}S$ values for tube worms from Alaminos Canyon are listed in Table 3. The $d^{34}S$ value of the tube worm tissue collected on dive 2211 is the lightest reported for the GOM (BROOKS *et al.*, 1987; CARY *et al.*, 1989; KENNICUTT *et al.*, 1991). The stable sulfur isotope ratio of an organism reflects its source of sulfur. Non-chemosynthetic marine organisms typically have $d^{34}S$ values that reflect seawater sulfate ratios (i.e., +13 to +20‰, FRY *et al.*, 1983). The sources of ^{34}S-depleted sulfur to petroleum seep chemosynthetic communities in the GOM are currently unknown. Possible sources of sulfur in the GOM include microbial sulfate reduction in pore waters and sulfides associated with seeping brines, oil and gas (CHANTON *et al.*, 1988; KENNICUTT *et al.*, 1989; VETTER *et al.*, 1991). Sulfides associated with seeping hydrocarbons may result from anaerobic sulfate reducers oxidizing simple organic hydrocarbons at depth as well as thermal decomposition of organic matter (VETTER *et al.*, 1991). The $d^{34}S$ of oils collected near Alaminos Canyon are near -4‰ (THOMPSON *et al.*, 1990). More negative tissue $d^{34}S$ values may reflect input from recent microbial sulfate reduction (VETTER *et al.*, 1991).

Elemental sulfur was observed in the gill tissues of mussels collected on dive 2209 (Fisher *et al.*, 1991). The $d^{34}S$ value of elemental sulfur

purified from gill tissues of seep mytilid II collected on dive 2209 is -2.8‰; whereas, the mean $d^{34}S$ value of total sulfur of the corresponding gill tissues is -1.8‰ (Table 3). The presence of elemental sulfur is an indicator of chemoautotrophic activity (FISHER, 1990). The $d^{34}S$ of elemental sulfur is considered to be the best indicator of the environmental source(s) of sulfide to symbionts because it is the result of a dissimilatory pathway that results in little isotopic fractionation (VETTER *et al.*, 1991). However, the $d^{34}S$ values of the purified elemental sulfur from mussel gill tissues is ambiguous and could be the result of microbial sulfate reduction and/or associated with seeping oil.

Carbonate $d^{13}C$ values

Two carbonate rock samples collected at site 2 had $d^{13}C$ values of -31.1 and -27.1‰. Authigenic carbonate is often formed at hydrocarbon seeps from pore waters containing excess CO_2 from the microbial degradation of oil and gas. The excess pore water CO_2 can combine with calcium and precipitate as authigenic calcium carbonate (BROOKS *et al.*, 1986; BEHRENS, 1988). The carbon isotopic composition of authigenic carbonate reflects the composition of the organic matter being degraded (BEHRENS, 1988, KENNICUTT and BROOKS, 1990). Carbonate resulting from the oxidation of oil and gas often exhibits anomalously negative $d^{13}C$ values (KENNICUTT *et al.*, 1989; BEHRENS, 1988). At shallow petroleum seeps carbonate $d^{13}C$ values have been reported to range from -47.5 to +2.0‰, reflecting various inputs from seawater ΣCO_2 and oil and gas oxidation (KENNICUTT *et al.*, 1989).

Polynuclear aromatic hydrocarbons

All tissues analyzed exhibited evidence of PAH exposure (Table 3). Naphthalenes (both parent and alkylated homologs) were the dominant PAH

detected. The concentration of total PAH in mussel tissues ranges from 439 to 1333 ng/g (dry weight). The single tube worm analyzed from site 1 contained 761 ng/g of PAH. These PAH concentrations are within the range of values reported for other petroleum seep fauna (36 to 7530 ng/g dry weight) in the GOM (WADE *et al.*, 1989; MCDONALD, 1990). Non-chemosynthetic fauna collected near petroleum seeps have significantly lower PAH concentrations (i.e., <10 - 66 ng/g dry weight, WADE *et al.*, 1989). The concentration and distribution of PAH in some seep fauna are similar to those measured in oysters from contaminated coastal sites in the GOM (WADE *et al.*, 1988).

PAH have been measured in waters and sediments associated with chemosynthetic communities at shallow petroleum seeps in the Gulf of Mexico (WADE *et al.*, 1989; MCDONALD, 1990). The concentration of total PAH in water samples collected at a shallow petroleum seep in the GOM was 28 ng/l, most likely resulting from the dissolution of seeping oil (WADE *et al.*, 1989). An indication of petroleum seepage in sediments is the amount of extractable organic matter (EOM). EOM can be composed of both biological (lipids) and petroleum hydrocarbons. Generally, sediments in the Gulf of Mexico contain less than 50 ppm of EOM that is of biological origin (KENNICUTT *et al.*, 1988c). Previous studies have reported EOM values that range from 21 to 5900 ppm in sediments associated with shallow petroleum seeps in the GOM (KENNICUTT *et al.*, 1988a). The EOM levels of sediments collected during geochemical surveys in Alaminos Canyon ranged from <10 to 2338 ppm (unpublished data). Additional evidence of hydrocarbon seepage in Alaminos Canyon was provided by total scanning fluorescence (TSF) and the analysis of head space gases. The intensity and spectral composition of sediment extracts analyzed by TSF were

characteristic of petrogenic PAH (BROOKS *et al.*, 1986; KENNICUTT *et al.*, 1988a). The concentrations of methane (>400 ppm) and ethane (>5 ppm) in head space gases of sediments were elevated, suggesting gas seepage (PHILP, 1987). Additionally, the high concentrations of C_2^+ gases in head space samples reflects an input from thermogenic sources (BROOKS *et al.*, 1984). The concentration and distribution of PAH in the tissues of Alaminos Canyon fauna indicate chronic exposure to sediment and/or water-associated PAH, most likely derived from the natural seepage of petroleum.

CONCLUSIONS

The chemosynthetic communities discovered in Alaminos Canyon fill the depth gap of previously known chemosynthetic communities at shallow petroleum seeps (400-1400 m) and at the Florida Escarpment (3000 m) in the Gulf of Mexico. No metazoan chemosynthetic communities have been documented below 400 m. Thus, the Alaminos Canyon discovery suggests that chemosynthetic communities can now be expected at depths below 400 m, wherever regional processes produce a sufficient flux of substrates suitable for chemosynthesis and/or methanotrophy at an oxygenated sediment-water interface. Although, oil and gas seepage were not observed during dives in Alaminos Canyon, previously collected oil-stained sediment cores, geochemical data, and data collected in this study support the conclusion that these communities are associated with oil and gas seepage.

The taxonomic similarity of a mussel and two species of tube worms in Alaminos Canyon to species found at chemosynthetic communities nearly 500 nautical miles apart suggests that these organisms may not be restricted to one geographic region. The discovery of two species of mussels, one of which hosts methanotrophic symbionts while the other hosts both methanotrophic and sulfide-oxidizers, at a single site raises questions as to

what environmental factors control microhabitat distribution of chemosynthetic and/or methanotrophic fauna.

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Table 1. Enzyme activity and $\delta^{13}C$ values in various tissues of two species of mussels collected in Alaminos Canyon

Dive #/Species Tissue	Sulfur (%GWW)	APS-R (IU/g^{-1})	ATP-S (IU/g^{-1})	RuBP C/O (IU/g^{-1})	MeDH (IU/g^{-1})	Tissue $\delta^{13}C$ ‰
2209/ seep mytilid II						
gill	0.585 ± 0.288*	nd*	2.56 ± 1.05*	0.022 ± 0.008*	2.27 ± 0.97*	-48.2 ± 0.4*
2211/ seep mytilid II						
gill	nd*	nd*	0.60 ± 0.39*	0.003 ± 0.001*	2.37 ± 1.19*	-48.8 ± 1.7*
2211/ seep mytilid Ib						
gill	nd	nd	nd	nd	6.96 ± 1.75	-43.9 ± 1.8

data is mean ± standard error, n=5 for all measurements
* Fisher *et al.*,1991
nd=not detected
IU=International Units, µmol substrate converted to product/min
sulfur is elemental sulfur in gills expressed as a percent of gill wet weight
APS-R = adenosine-5'-phosphosulfate reductase
ATP-S = adenosine-5'-triphosphate sulfurylase
RuBP C/O = ribulose-1,5-bisphosphate carboxylase-oxygenase
MeDH - methanol dehydrogenase

Table 2. Enzyme activity in trophosome tissue and $\delta^{13}C$ values of vestimentum and trophosome tissue from vestimentiferans collected in Alaminos Canyon

Dive #/species Tissue	APS-R (IU/g^{-1})	ATP-S (IU/g^{-1})	RuBP C/O (IU/g^{-1})	MeDH (IU/g^{-1})	Tissue $\delta^{13}C$ ‰
2209/Escarpia sp.					
trophosome	0.2* ± 0.2 n=4	19.2 ±11.0 n=4	1.2 ±0.48 n=4	nd n=4	-35.5 ± 1.0 n=5
vestimentum	-	-	-	-	-36.2 ± 0.5 n=5
2211/Lamellibrachia sp.					
trophosome	nd n=3	8.5 ± 2.0 n=3	0.4 ± 0.14 n=3	nd n=3	-20.7 ± 1.0 n=3
vestimentum	-	-	-	-	-21.4 n=1

data is mean ± standard error
nd=not detected
-=not analyzed
IU=International Units, µmol substrate converted to product/min
*APS reductase was detected in a single individual at 0.7 IU/g/min
APS-R = adenosine-5'-phosphosulphate reductase
ATP-S = adenosine-5'-triphosphate sulfurylase
RuBP C/O = ribulose-1,5-bisphosphate carboxylase-oxygenase
MeDH - methanol dehydrogenase

Table 3. $\delta^{13}C$ values and total PAH concentrations in various tissues of mussels and tube worms collected in Alaminos Canyon

Dive #/Species - Tissue	$\delta^{13}C$ ‰	$\delta^{34}S$ ‰	$\delta^{34}S$ of °S ‰	Total PAH (ng/g dry wt.)
2209/ seep mytilid II				
gill	-48.2 ± 0.4 n=5	-1.8 ± 0.2 n=5	-2.8 ± 2.1 n=2	-
body	-46.2 ± 0.3 n=5	-	-	946 ± 150 n = 5
2211/ seep mytilid II				
whole body	-49.1 ± 1.3 n=5	+1.6± 0.8 n=5		520 ± 38 n = 2
2209/ Escarpia sp.	-35.8 n=1	-8.4 n=1	-	761 n=1
2211/ Lamellibrachia sp.	-20.7 n=1	-23.2 n=1	-	-

data is mean ± standard error
-=not analyzed

Fig. 1. Location of Alaminos Canyon in the Gulf of Mexico.

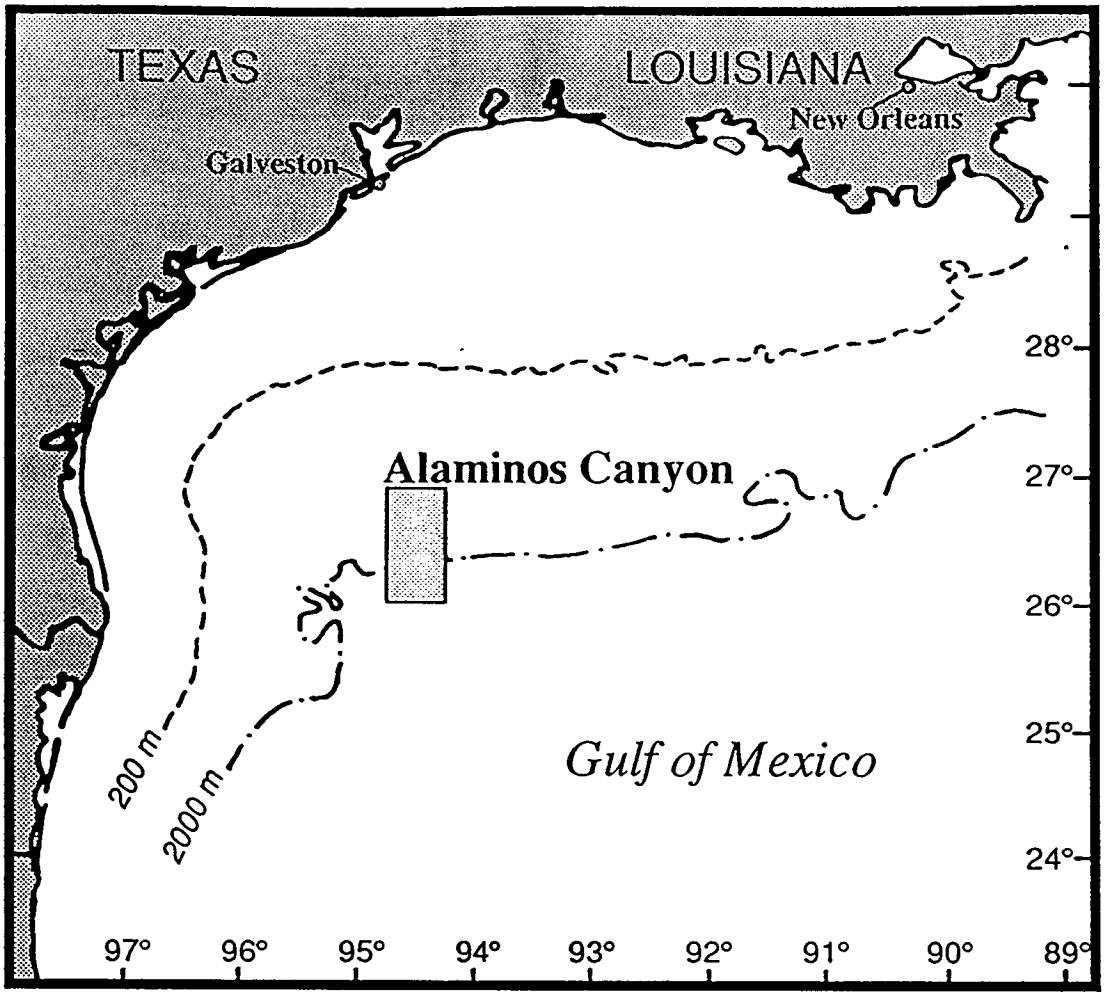

TEXAS
LOUISIANA
New Orleans
Galveston
28°
Alaminos Canyon
27°
26°
25°
Gulf of Mexico
200 m
2000 m
24°
97°
96°
95°
94°
93°
92°
91°
90°
89°

Fig. 2. Approximate location of chemosynthetic communities in Alaminos Canyon. Oblique perspective view of Alaminos Canyon derived from SEA BEAM multi-beam echo-sounder mapping. Depths were determined using an accoustic wave velocity of 1.5 km/sec. Vertical exaggeration 5:1; northwest is top.

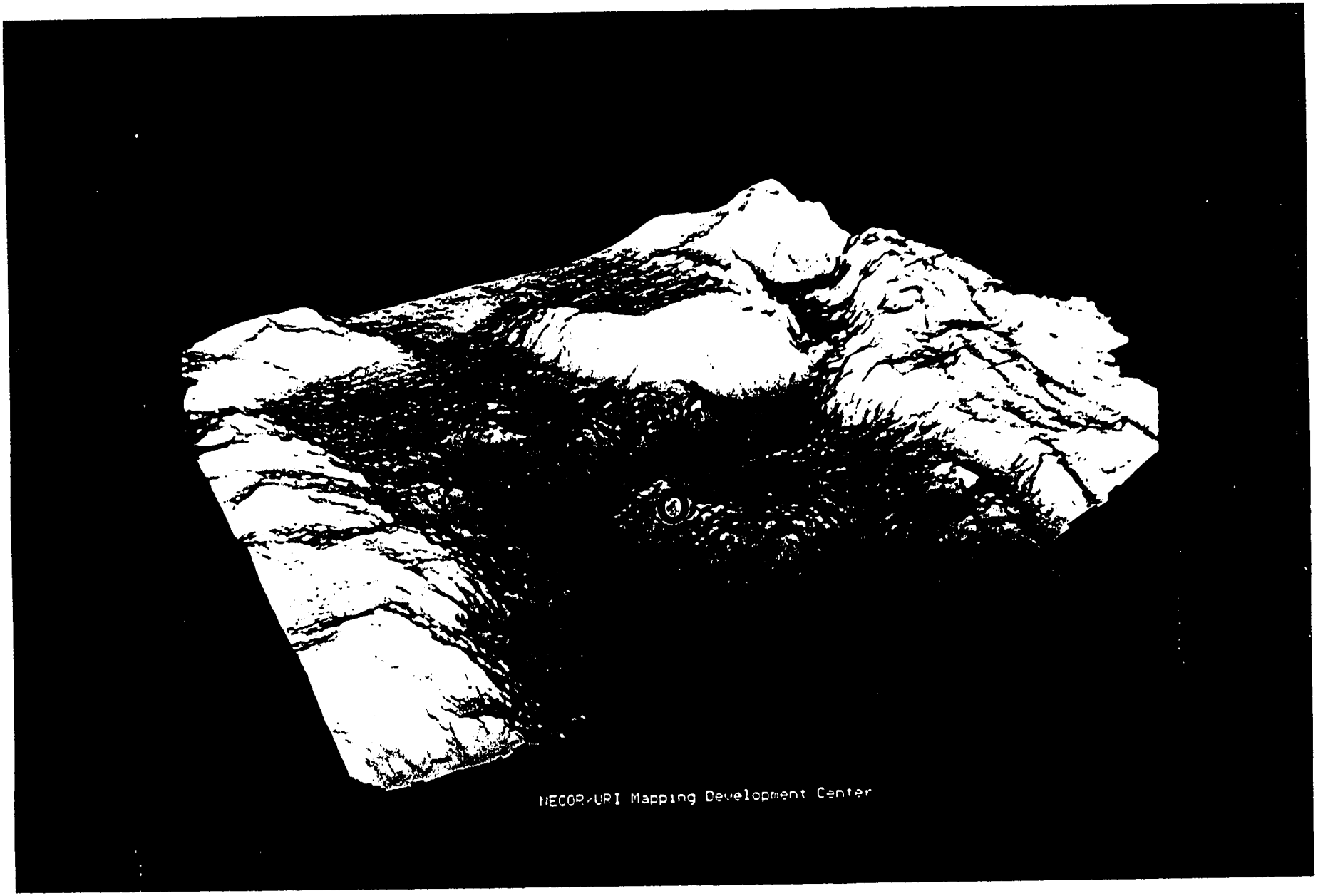
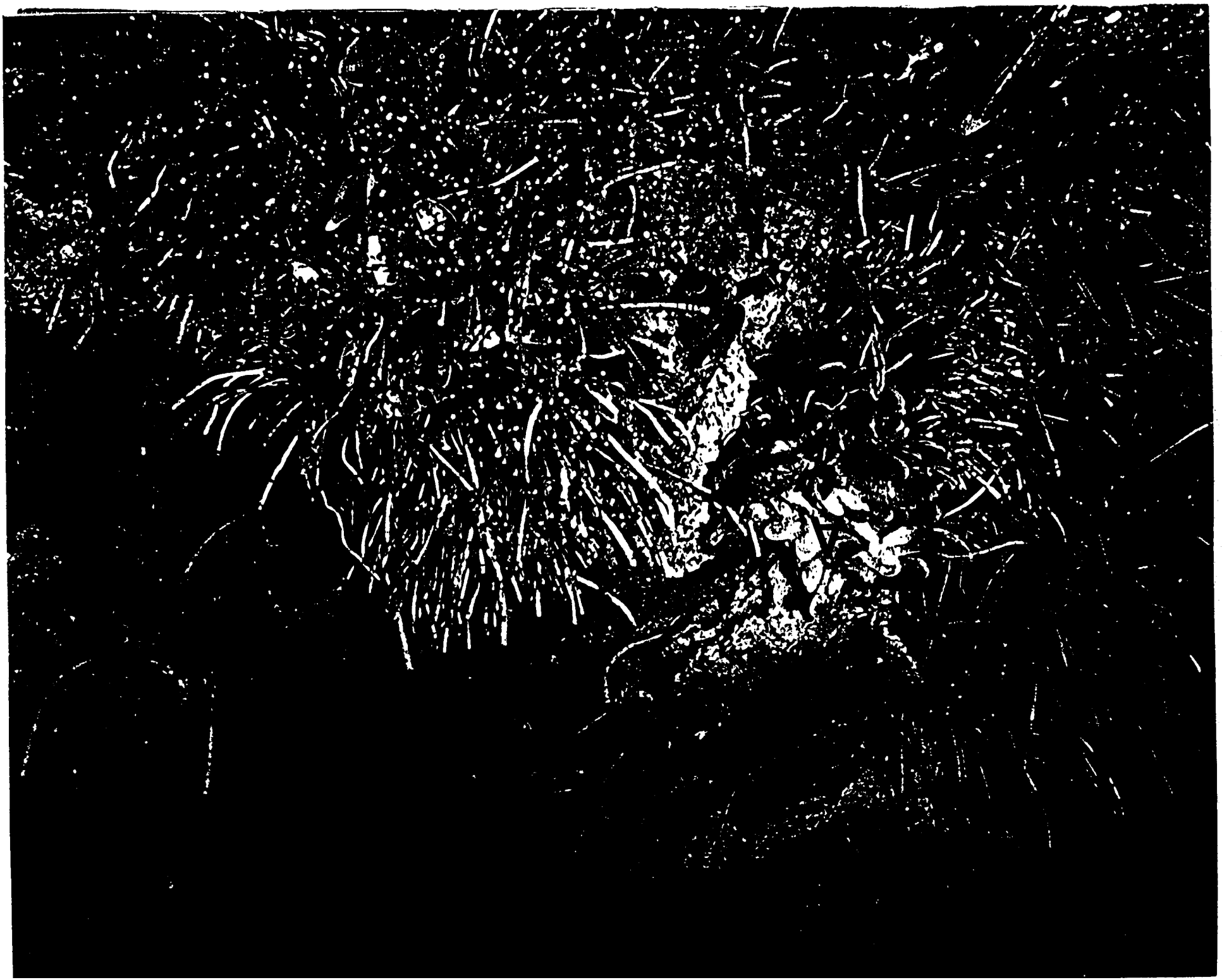

NECOR/URI Mapping Development Center

Fig. 3. Photograph of mussels (A) and tube worms (B) attached to a carbonate structure at site 1 (dive 2209) in Alaminos Canyon.



Notice to Lessees and Operators of Federal Oil and Gas Leases in the Outer Continental Shelf Gulf of Mexico OCS Region

Implementation of Measures to Detect and Protect Deepwater Chemosynthetic Communities

U.S. DEPARTMENT OF THE INTERIOR
MINERALS MANAGEMENT SERVICE
GULF OF MEXICO OCS REGION

No. 88- 11 Effective: February 1, 1989

NOTICE TO LESSEES AND OPERATORS OF FEDERAL OIL AND GAS LEASES IN THE OUTER CONTINENTAL SHELF GULF OF MEXICO OCS REGION

Implementation of Measures to Detect and Protect Deepwater Chemosynthetic Communities.

A. Introduction and Background

Deepwater (greater than 400 m) chemosynthetic organisms have recently been discovered in the Gulf of Mexico (GOM). Chemosynthetic communities are assemblages of tubeworms, clams, mussels, bacteria mats, and other associated organisms. Many of the species, while similar to those of other deepwater areas, including the vent communities of the Galapagos Ridge, are new to science. While generally widespread and in low densities, there are examples of very high densities of the organisms in apparently very small, isolated areas. These areas of high-density communities are apparently associated with hydrocarbon seeps, vents or gaseous sediments. Oil and gas activities, which disturb the seafloor where such dense communities are located, will cause damage to any of these communities with which they come into contact. Such activities would include (but not be limited to) anchoring, placement of seafloor templates, and installation of pipelines.

Regulatory authority to require avoidance or protection of chemosynthetic communities and avoidance of shallow hazards, such as vents or gaseous sediments, appear at several places in 30 CFR 250. General provisions concerning protection of fish and other aquatic life are at 30 CFR 250.5(a) and 30 CFR 250.20(a). Specific provisions concerning sea bottom hazards at particular sites are at 30 CFR 250.33(b)(1)(ix) for exploration plans, 30 CFR 250.34(b)(1)(vii) for development and production plans, and 30 CFR.250.139(b)(2) for foundations.

Detailed data regarding the extent, location, structure, and relationship of these communities to the local geophysical environment are at present unavailable. Currently, operators in water depths greater than 400 m are required to provide certain data in order for Minerals Management Service (MMS) to make determinations regarding the possibility of chemosynthetic communities being present and the potential of their being harmed by exploration and development activities. These requirements were previously imposed on a case-by-case basis, however these requirements are now included in a letter to lessees dated October 12, 1988, which specifies information required for Plans of Exploration (POEs) and Development Operations Coordination Documents (DOCDs) for oil and gas leases in the GOM OCS Region.

2. The requirements of this section may be revised as new information is received.

D. Intent

The provisions of this NTL are intended to provide clarification, description, or interpretation of requirements contained in OCS operating regulations and lease stipulations. This NTL does not impose additional requirements.

DEC - 9 1988

Regional Director
Gulf of Mexico OCS Region
Minerals Management Service

Date

Approved:

Associate Director for
Offshore Minerals Management
Minerals Management Service

12/14/88
Date

B. Purpose and Scope

It is the purpose of this Notice To Lessees and Operators (NTL) to inform all operators of leases in water depths greater than 400 m of the requirements currently being imposed by MMS and to provide a consistent and comprehensive approach which will avoid damage to high-density chemosynthetic communities. The requirement of Part C below shall apply to all operations of GOM leases in water depths greater than 400 m and shall remain in effect until superseded.

C. Implementation

1. Beginning February 1, 1989, owners and operators of leases on blocks in water depths of 400 m or deeper in the GOM shall comply with the following:

   a. Prior to approvals of Applications for Permit to Drill (APDs) and Pipeline Applications, the operators shall delineate all seafloor areas which would be disturbed by the proposed operations. Additionally, an analysis of geophysical information for these areas, as well as any other pertinent information available, shall be furnished which discusses the possibility of disturbing geological phenomena (such as hydrocarbon charged sediments, seismic wipe-out zones, anomalous mounds or knolls, gas vents, or oil seeps) that could support chemosynthetic organisms.

   b. If the subsequent review by MMS of the analysis required by 1.a. above results in a determination that high-density chemosynthetic communities may be present and could potentially be harmed by the proposed activities, the operator will be required to:

      (1) modify the application to relocate the proposed operations to avoid impacting possible chemosynthetic communities; or

      (2) modify the application to provide additional information (perhaps including a photo-survey, a video-survey, or already available information) which documents whether high density chemosynthetic communities exist in the areas of concern; or

      (3) adhere to certain conditions of plan or application approval, such as use of a remotely operated vehicle to precisely set anchors, or to ensure that the proposed anchor pattern does not impact chemosynthetic communities, to monitor impacts caused by the proposed work, or any other condition deemed necessary by the Regional Director.

Epifaunal Aggregations of Vesicomyidae on the Continental Slope off Louisiana

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Epifaunal aggregations of Vesicomyidae on the continental slope off Louisiana

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Abstract—Two species of the bivalve family Vesicomyidae living at a depth of 940 m in the central Gulf of Mexico were photographed, counted and measured. These species, which are related to bivalves in chemoautotrophic communities in the Gulf of Mexico and at hydrothermal vents in the Pacific Ocean, occurred here in two apparently distinct aggregations clearly visible on the sea floor surface. Living vesicomyids were generally found amid a scatter of dead shells and occurred in densities of 0.5–9.6 individuals per m^2. Distribution of living individuals within the aggregations was patchy. Living clams appear to plow actively through a substrate of silty clay, leaving behind distinctive, curving furrows up to 205 cm in length. Estimates of density, size distribution and spatial distribution provide a basis for detection of change in the aggregations over time and for comparison with similar aggregations.

INTRODUCTION

COMMUNITIES of deep-sea organisms resembling those reported from hydrothermal vents in the Pacific Ocean have been found in the Gulf of Mexico at a cold-sulfide seep at the base of the Florida Escarpment (PAULL *et al.*, 1984) and at a petroleum seep on the continental slope off Louisiana (KENNICUTT *et al.*, 1985). The Pacific Ocean hydrothermal vent communities are remarkable because they depend on a nonphotosynthetic carbon source obtained by metabolizing reduced inorganic compounds, particularly sulfides, present in the vent effluents (FELBECK, 1981; CAVANAUGH, 1985; JOHNSON *et al.*, 1986). Analysis of stable carbon-isotope ratios in the clams *Calyptogena ponerossa* and *Vesicomya cordata* (Vesicomyidae) from the Louisiana petroleum seep indicate that these organisms also depend at least in part on a nonphotosynthetic food source (KENNICUTT *et al.*, 1985). Ecological comparisons between seep communities in the Gulf of Mexico and Pacific Ocean hydrothermal vent communities have been limited. One would expect the physical and chemical differences between the two habitats to be reflected in the distribution patterns, densities, and population characteristics of seep organisms.

We analysed photographs of assemblages of two species of Vesicomyidae and were able to quantify their spatial distribution and to inventory their numbers. Our results describe characteristics of these assemblages that can be attributed to the animals' behavior and to properties of their environment.

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METHODS AND MATERIALS

Photographs of the sea floor were taken during an ecological survey of the northern Gulf of Mexico continental slope. The survey was conducted in a series of five cruises that occupied a total of 60 sampling stations (Fig. 1). Approximately 750 photographs were taken at each sampling station. The camera sled was suspended from the vessel so that the plane of focus was parallel to the bottom and was "flown" at altitudes of 0.5–4.5 m as the vessel slowly drifted along transects 1500–5000 m in length. The camera system included a clock and an altimeter (Benthos model 2210) that recorded the time and altitude above the bottom on each photograph. Camera altitude was maintained by adjustments to the winch in response to readings from the altimeter, which were transmitted to the vessel by a secondary pinger. The heading and drift speed, monitored on a LORAN C navigation system, were usually constant throughout the transect. The camera (Benthos model 372) had a 28 mm fixed focal-length lens and was loaded with an 800-exposure roll of 35 mm Ektachrome film (ASA 200). Photographs were exposed every 8 s. Illumination was provided by a high-intensity strobe (Benthos model 383) that was mounted at an angle of 45°.

The scale of the photographs was calculated from their exposure altitude and the acceptance angles of the camera lens. Knowing the scale of the photographs allowed us to interpret the area of the bottom shown in each photograph as a quadrat sample of the survey site (GRASSLE *et al.*, 1975; RIPLEY, 1981) and to measure the length of individual clams. Variations in exposure altitude caused the area of the quadrats to vary between 0.3 and 11.5 m^2 (mean = 2.2 m^2). Photographs exposed outside this range of altitudes were disregarded. The resulting sample consisted of a series of quadrats of various sizes,

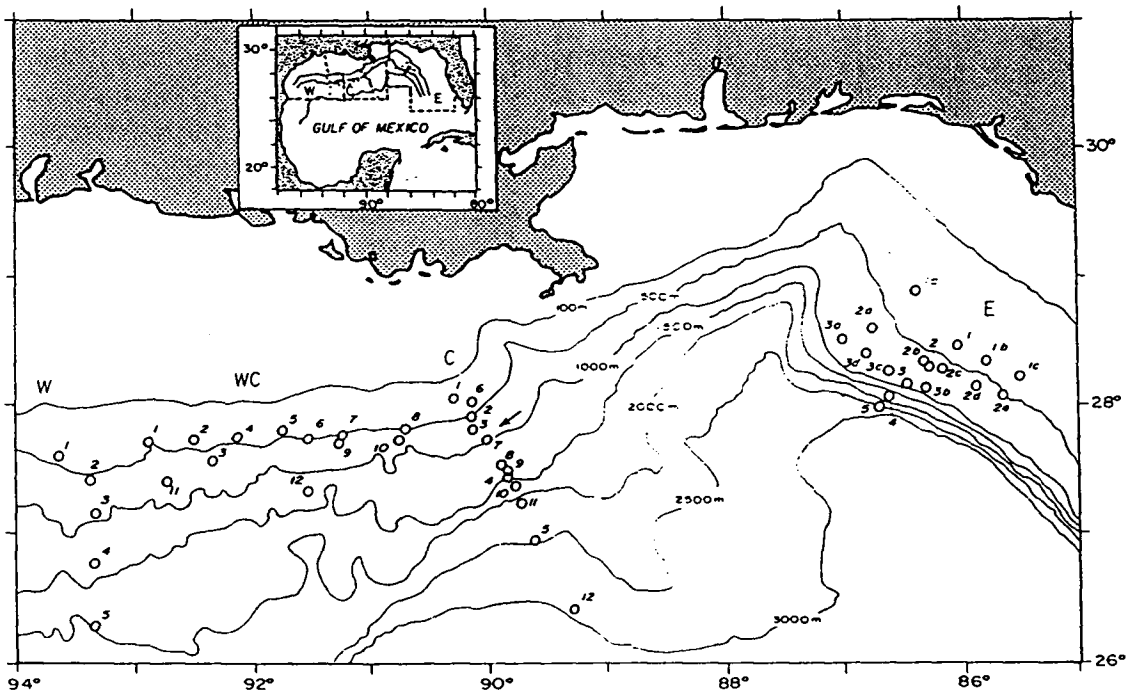

Fig. 1. Chart of study area showing sampling stations. The stations were grouped in four transects: west, west-central, central and east. Aggregations of vesicomyids were photographed at central transect station number 7 (arrow).

Table 1. Sample size of the photo-transect and abundances of three categories of vesicomyid specimens in the photographic quadrats

Transect segment	Sample size			Abundance		
	Transect length (m)	Number of quadrats	Sum of quadrat areas (m^2)	Dead valves	Living vesicomyids	Vesicomyid trials
Total transect	1520	493	1470	2460	305	420
Aggregation 1	140	46	117	913	90	155
Aggregation 2	95	38	159	1462	213	265

irregularly spaced along the transect (Table 1). The location of each photograph on the transect was calculated from its time of exposure and the total time of the transect.

All photographs that contained visible organisms were projected onto the platen of a digitizer, which was used to record the length and counts of the individuals in the photographs. The digitizer operator recorded the camera altitude and time of exposure for each photograph on a microcomputer connected to the digitizer, and then used the digitizer's cursor to count and measure the subjects in the photograph. Data records from the digitizer were transmitted from the microcomputor to a database. Custom software used the scale of the photographs to convert the digitized images and counts into estimates of lengths and densities of bivalves that were then available for analysis.

RESULTS

A total of 90,000 photographs was obtained from the study (Fig. 1). Organisms observed among the photo-transects included decapod crustaceans, echinoids and fishes; however, the overwhelming majority of individual photographs did not contain organisms. The most abundant organism was a small holothuroid that occurred in high densities (up to 86 per m^2) at several of the central transect stations. Bivalves and shell fragments were extremely rare. An exception to this occurred at Sta. C7 (Fig. 1), which is located at 27°45′30″N and 89°58′18″W at a depth of 940 m. Numerous bivalves were observed in the photographs from this station (Table 1). The overall abundance of clams in the photographs and their apparent density greatly surpassed that of bivalves at any other station and were second in abundance only to the holothuroids.

The bivalves in the photographs were identified by F. J. Rokop (personal communication) as two species of the genera *Calyptogena* and *Vesicomya* (Vesicomyidae). Vesicomyids collected at a similar depth from a site approximately 60 nmi from Sta. C7 were identified as *C. ponderosa* and *V. cordata* (Kennicutt *et al.*, 1985). Careful comparison of some of these specimens with a few well-photographed individuals showed that the species *C. ponderosa* and *V. cordata* were present. Most specimens in the photographs could only be identified as vesicomyids, and many could not be positively identified at the family level; however, general similarities in the shape and color of the shells and of trails left by living individuals suggested that the clams in the photographs were predominantly *C. ponderosa* and *V. cordata*.

Vesicomyids were by far the most abundant organism seen on the phototransect. The photographs showed living specimens, dead valves, and trails in the surficial sediment (Fig. 2). The living clams often appeared amid a dense scatter of dead and partly buried valves, both in groups (Fig. 2A) and as solitary individuals (Fig. 2B). Individual valves from dead clams were either widely gaped or disarticulated with the valve concavity

visible; each valve was counted (Fig. 2C). The specimens that could not be classified immediately were partly buried with the convex side uppermost (Fig. 2D); these specimens were added to the count of gaping or disarticulated valves. The total number of dead individual bivalves (Table 1) was calculated as half the number of gaping or disarticulated valves. Some of the doubtful specimens may have been alive, so classifying them as dead would over-estimate the number of dead specimens; however, dead valves clearly constituted the dominant category in almost all quadrats.

Living vesicomyids were usually oriented in a nearly horizontal position with the hinge and umbo visible above the sediment (Fig. 3a). The animals apparently plow through the surface layer of sediment, leaving behind distinctive trails. Vesicomyid trails were vee-shaped, curving furrows; both species left trails (Fig. 3a). A second, more delicate form of trail was sometimes seen among the clams (Fig. 3b). These trails, which were narrow, had distinct edges and disturbed only the uppermost layer of the sediment, were easily distinguished from the vesicomyid trails.

Counts of the specimens visible on the surface in each quadrat thus were divided into three categories: living vesicomyids, dead vesicomyids and vesicomyid trails. The results of these counts showed two regions of high abundance on the transect, which together constituted <19% of the total area photographed (Table 1). The distance between these regions suggests that we sampled two spatially distinct aggregations of vesicomyids (aggregations 1 and 2) that were at least 140 and 95 m in length, respectively, and separated by no more than 480 m. Figure 4 shows that although the proportions of living and dead varied, living clams were usually intermingled with shells from dead individuals. Dead clams (calculated as half the number of individual valves) outnumbered living clams by 5:1 and 3:1, respectively, in the two aggregations. Mean densities of living vesicomyids in aggregations 1 and 2 were 2.32 per m^2 (S.E. 0.67) and 2.71 per m^2 (S.E. 2.27), respectively. Variations in the densities of trails appeared to mirror the variations in the density of living vesicomyids, and both densities were reduced where the density of dead clams was highest (Fig. 4). The number of trails exceeded the number of living clams in photographs in which long trails were broken (i.e. those without a clam at one end) or the trail makers were out of the picture.

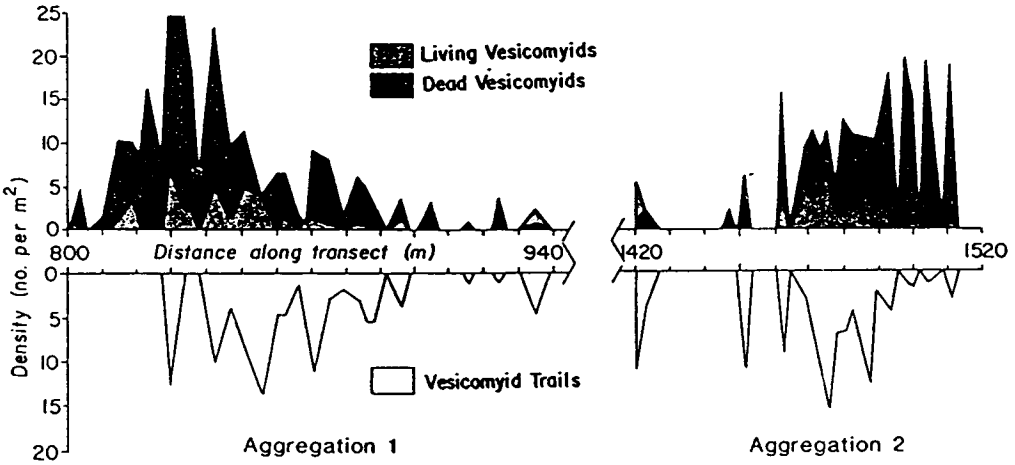

Fig. 4. Point estimates of density for living and dead vesicomyids and vesicomyid trails; obtained by dividing the count of each category in a quadrat by the quadrat area and plotted against their position along the transect. The number of dead individuals was calculated as half the number of individual dead valves.

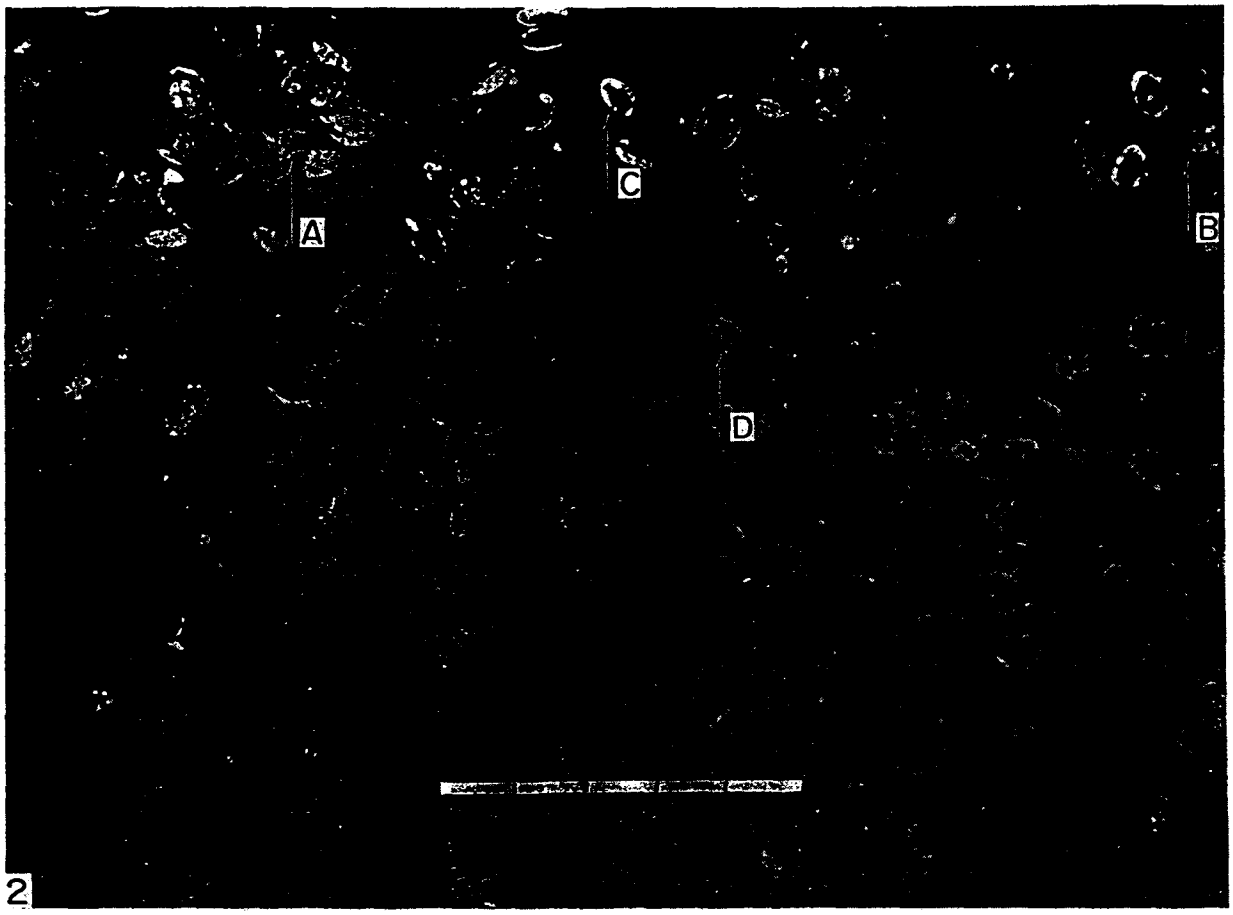

Fig. 2. This representative photograph from the photo-transect was exposed at an altitude of 2.1 m and is a quadrat sample of 2.3 m^2 of the bottom. Bar = 50 cm. Arrows indicate the following features: (A) a group of living vesicomyids, (B) a living vesicomyid in plowing position, (C) a disarticulated valve, and (D) and unclassified vesicomyid presumed to be dead.

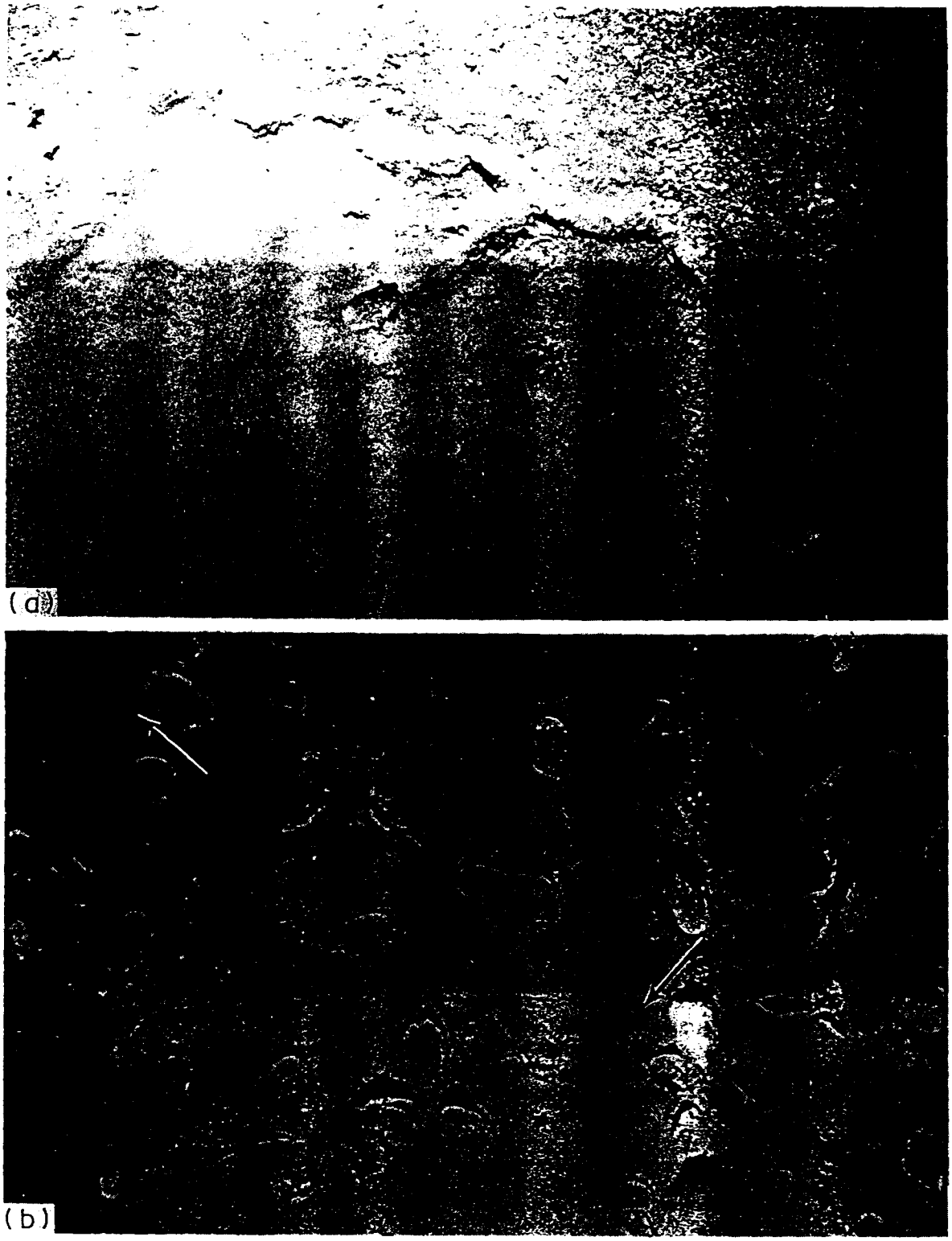

Fig. 3 Photographs of the two types of trails observed in the aggregations of vesicomyids. (a) A vesicomyid in characteristic plowing position and its vee-shaped, curving trail. (b) shows the faint surface trails formed by a second trail-making organism that occurred in the aggregations (arrows). Only trails similar to those in (a) were included in the analyses.

Valve lengths were recorded to the nearest millimeter whenever both ends were clearly visible. Partly buried individuals were not measured, so the measurement sample was a subset of the total number of clams in the photographs. The resulting frequency distributions are shown in Fig. 5. Because the living clams were often angled toward the camera, inaccurate measurements may have resulted from the foreshortening effect. Dead valves tended to be more visible, particularly when the concave side was uppermost. Trails were also measured, and they ranged in length from 4 to 205 cm (mean, 34 cm).

The sediment within the aggregations and throughout the photo-transect was uniformly fine-grained, with little surficial carbonate rubble apart from the clam shells, and the bottom contour of the surrounding area was regular. Despite the apparent homogeneity of the habitat, the density of clams varied considerably (Fig. 4), which suggested that their distribution was clustered or patchy within the aggregations. To determine the degree of patchiness, we computed three indices for the living vesicomyids within the aggregations (Grassle *et al.*, 1975; Ripley, 1981): the index of cluster size (ICS):

$$\mathrm{ICS} = (s^2/\bar{x}) - 1$$

the index of cluster frequency (ICF)

$$\mathrm{ICF} = \bar{x}/\mathrm{ICF}$$

and Morisita's I_δ

$$I_\delta = n\Sigma x_i(x_i - 1)/\{n\bar{x}(n\bar{x} - 1)\},$$

where x_i is the count in quadrat i and n is the number of quadrats. To calculate these indices, we pooled quadrats within selected intervals of quadrat areas. This allowed us to compare the effect of the scale of variation on patchiness (Table 2).

The values shown in Table 2 suggested that living vesicomyids had a patchy distribution within the aggregations. The Morisita's I_δ values were substantially greater than zero, and a Chi-square test for ICS values showed all to be significantly greater than zero ($P = 0.05$). There are two probable distributional forms that could result in the type of patchiness indicated by these indices: a point-cluster process and a random-rate Poisson process (Douglas, 1975). In a point-clustered distribution, the organisms might tend to occur in association with discrete environmental features (e.g. fish clustered around points of topographic relief; see Boland *et al.*, 1983). Diagnostically, this would result in

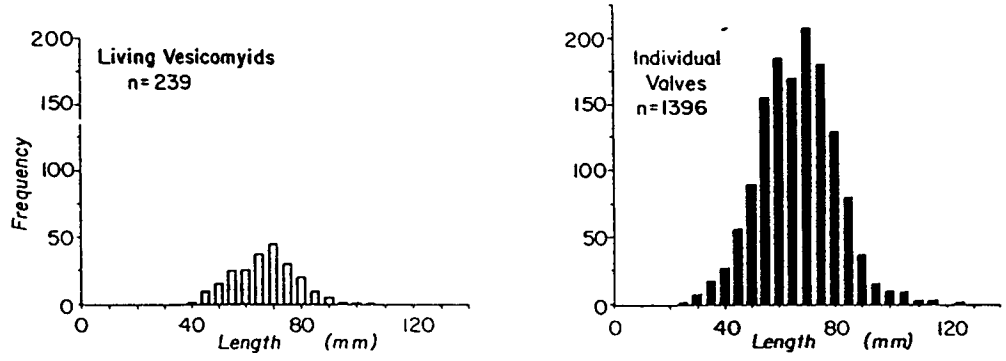

Fig. 5. Length distributions of living vesicomyids (left) and individual dead valves (right) measured the projected photographs with use of a digitizing platen. The measurement sample included all fully visible vesicomyid valves in the phototransect.

Table 2. Diagnostic indices for living Vesicomyidae photographed in two aggregations on the continental slope south of Louisiana. Indices shown are index of cluster size (ICS), index of cluster frequency (ICF) and Morisita's I_δ index. The altimeter on the camera system was accurate to tenths of meters; the photographic quadrat areas were pooled in 1 m intervals to increase sample size. Results obtained for actual quadrat areas were consistent with these intervals. ICS values greater than zero are an indication of patchiness. ICF values are diagnostic of the aggregating mechanisms. The I_δ values were calculated as a consistency check on the pooled results

Interval midpoint (m^2)	Number of quadrats	ICS $[(s^2/\bar{x}) - 1]$	ICF $(\bar{x}/\text{ICS})$	Morisita's I_δ $[n\Sigma x_i(x_i - 1)/\{n\bar{x}(n\bar{x} - 1)\}]$
1.5	8	2.619	0.573	2.996
2.5	24	4.867	0.728	2.403
3.5	18	14.192	0.348	3.914
4.5	8	4.591	1.552	1.674
5.5	8	12.000	0.500	3.064

ICF values that have a strong positive correlation with quadrat size (RIPLEY, 1981). This trend was not evident in the ICF values for vesicomyids in the two aggregations. It is likely therefore that the distribution of clams in the two aggregations is parsimoniously modeled with a random-rate Poisson process. This implies that within the aggregations the living clams occurred randomly, but with a varying rate of intensity.

DISCUSSION

At hydrothermal vents and other areas where inorganic compounds are issuing through surface or near-surface fissures, living *Calyptogena* align themselves in dense rows within the active fissures (HESSLER *et al.*, 1985; SUESS *et al.*, 1985). The utilization of reduced sulfides by *Calyptogena* suggests that an aggregation of these clams marks a discharge of sulfides at a site; their fine-scale distribution shows the localization of the points of discharge (CHILDRESS and MICKEL, 1985; CORLISS *et al.*, 1979). The age of such an aggregation of clams establishes the minimum period during which discharge has taken place. Aggregations of *Calyptogena* that consisted predominantly of living individuals of a uniform size were judged to be more recently established than aggregations of dead shells of the same size (HESSLER *et al.*, 1985; CORLISS *et al.*, 1979; CRANE and BALLARD, 1980). Aggregations consisting entirely of dead shells are presumed to have experienced a failure of nutrient supply (GRASSLE, 1985). The proportion of living and dead and the size and spatial distribution of individuals in aggregations of vesicomyids are all characteristics that can be used as a basis for comparison between central Gulf of Mexico seeps and other communities in chemically enriched environments.

The size range of clams in our photographs suggests that both living and dead individuals of a variety of ages occurred in the aggregations (Fig. 5). Growth curves for *C. ponderosa* and *V. cordata* are not available and, in any event, the mixture of species in the aggregations precludes the possibility of proposing an age distribution on pooled size distributions. The large sample size ($n = 1396$) suggests that the bimodal frequency distribution of the individual valves apparent in Fig. 5 was not an artifact of the measurement technique. This is consistent with the observation by Boss (1968) that *C. ponderosa* is more elongate than *V. cordata*. The symmetry of length distributions and the similarity between the length distributions of living and dead clams were evident. Growth cohorts did not appear in the size distribution curves. The clams in these photographs cannot be described as uniformly sized. Furthermore, careful examination

of sections of the transects in which dead shells predominated showed that there were always some living individuals amid the accumulated dead shells. The smooth shape of the size distributions and the presence of living individuals throughout the aggregations may be an indication of continuous recruitment and a stable population.

The siphons of both *C. ponderosa* and *V. cordata* are short, barely extending beyond the posterior margin of the shell (Boss, 1968). This would suggest activity at or near the surface sediments rather than in deep burrows. Adult *Calyptogena* (>90 mm) have been observed on the surface of soft sediments at depths of 900–1200 m in Sagami Bay, Japan; while younger forms buried themselves more completely (Ohta and Hashimoto, 1986). The adult clams in our photographs left a clearly etched record of their activity as they plowed through the surface sediments (Figs 2 and 3). The length of these meandering trails (up to 205 cm), and their presence throughout the aggregations, showed that the clams were capable of substantial movement. Accumulation of dead shells in areas still inhabited by living individuals indicated that the aggregations observed in the photographs remained in more or less discrete areas for a period greater than the life-span of individuals and the dissolution time of their shells.

These aggregations of vesicomyids appeared to extend across larger areas (95–140 m) than the thoroughly mapped aggregations of *Calypotogena* at Pacific Ocean hydrothermal vents. Similar transects across the aggregations would have been about 10–60 m in length (Hessler *et al.*, 1985; Crane and Ballard, 1980). Within the Gulf of Mexico aggregations, the vesicomyids did not occur in regular formations that could be detected by our photographs. In an area that lacked extensive hard substrate the only apparent constraint on the occurrence of bivalves was the absence of nutrients. Patchy distribution of clams within reasonably discrete regions of the transect indicates a nutrient source that was confined to the areas of the aggregations, but was discontinuous within these areas. These nutrients must have been sufficiently dispersed to require active searching rather than passive orientation. Random occurrence of living clams at varying density suggests gradients in the pore-water chemistry rather than point-sources of nutrients. This is consistent with the diffuse but variable concentrations of sulfides and hydrocarbons found in the sediments at the Louisiana seep sites (Brooks *et al.*, 1984; Kenninicutt *et al.*, 1985).

CONCLUSION

The Vesicomyidae have shown a variety of adaptations. Aggregations at central Gulf of Mexico petroleum seeps were found at depths of about 700 m (Kennicutt *et al.*, 1985); at other cold seeps they have been found from 1200 to 3000 m (Paull *et al.*, 1984; Suess *et al.*, 1985; Horikoshi and Ishii, 1985), and at eastern Pacific Ocean hydrothermal vents they occurred at about 3000 m (Hessler and Smithy, 1983). *Calyptogena* at hydrothermal vents were larger and had higher local densities (Crane and Ballard, 1980) than the *C. ponderosa* and *V. cordata* in our photographs, but faced the periodic loss of their nutrient supply (Grassle, 1985). Observations of *C. magnifica* show that members of this family can adapt to a relatively passive and epifaunal existence on the basalt substrate at hydrothermal vents (Turner, 1985). The clams in our photographs showed active epifaunal behavior on soft substrate. If they prove to be typical of petroleum seeps, their behavior demonstrates additional variety in the adaptation of Vesicomyidae to chemically enriched environments.

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Gulf of Mexico Hydrocabon Seep Communities: Part III. Aromatic Hydrocarbon Concentrations in Organisms, Sediments, and Water

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Gulf of Mexico Hydrocarbon Seep Communities: Part III. Aromatic Hydrocarbon Concentrations in Organisms, Sediments and Water

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ABSTRACT

Organism tissues from areas of natural oil seepage contain significant amounts of polynuclear aromatic hydrocarbons (PAH). Higher concentrations are found in sedentary organisms (i.e. mussels and tube worms) than in more mobile species (i.e. fish). The PAH distributions indicate that the seep organisms are exposed to sediment and/or water associated PAH. The concentration and composition of PAH in sedentary organisms are similar to that of an oyster from a coastal site indicating similar mechanisms of PAH uptake, depuration and accumulation. Tissue PAH concentrations indicate that these organisms are chronically exposed to high levels of petroleum in their environments and yet thriving communities are present at these locations. Microbial biomass in these seep areas is also substantially enhanced, and the carbon isotopic composition of tissues of organisms from higher trophic levels reflects the incorporation of bacterial biomass. The sedimentary sulfur cycle is heavily influenced by the process of natural oil and gas seepage and plays a key role in maintaining these communities. The primary source of isotopically light carbon to the system can be solely derived from chemosynthesis (H_2S or CH_4). The role of degraded oil as a partial source of light carbon to some organisms cannot be ruled out.

INTRODUCTION

Bivalve populations in coastal areas have been used extensively to determine the fate and effect of hydrocarbon contamination (Blumer *et al.*, 1970;

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Farrington & Quinn, 1973; Boehm & Quinn, 1976; Philips, 1980). Mussels (*Mytilus* sp.) and various oyster species (*Crassostrea* and *Ostrea*) are used as sentinel organisms to monitor hydrocarbon pollution in coastal waters (Bravo *et al.*, 1978; Cossa *et al.*, 1983; Farrington *et al.*, 1983; Martin & Castle, 1984; Lauenstein & O'Connor, 1988; Wade *et al.*, 1988). Bivalves exhibit low or undetectable enzyme activity for metabolizing xenobiotic compounds such as polycyclic aromatic hydrocarbons (PAH) when compared to fish and crustacea (Lee *et al.*, 1972), and, therefore, bivalves bioaccumulate xenobiotic contaminants without alteration.

Biological communities associated with petroleum seep areas in southern California have been extensively studied (Spies *et al.*, 1980; Davis & Spies, 1980; Montagna *et al.*, 1987). Deposit feeders from these seep areas have tissue carbon isotope ratios suggestive of a petroleum source (Spies & DesMarais, 1983). Microbial biomass is greatly enhanced at these seep sites, indicating that the microbes are responsible for the trophically enriched infaunal populations in these seep areas (Montagna *et al.*, 1987). The southern California seep areas are enriched in heterotrophic activity, have an unusual sedimentary sulfur cycle and are areas of enhanced colonization of invertebrates (Spies *et al.*, 1980). Similar communities have also been recently discovered at Gulf of Mexico seep sites.

Extensive communities of organisms (including mussels, clams and tube worms) are intimately linked to the natural seepage of gas and oil on the continental slope of the northern Gulf of Mexico in 500 to 600 m of water (Kennicutt *et al.*, 1985). Carbon isotope analyses, enzymatic studies, and electron microscopy confirmed that these communities are based on chemosynthetic utilization of reduced compounds (i.e. H_2S, CH_4) by bacteria (Brooks *et al.*, 1987*a*,*b*). Further studies have shown that mussels (family Mytilidae) contain endosymbiotic methane-oxidizing bacteria. Utilization of organic matter derived from bacterial oxidation of methane can potentially satisfy the mussels' entire carbon and energy needs (Childress *et al.*, 1986; Cary *et al.*, 1988).

The primary objective of the present study was to determine the PAH concentrations in tissues of organisms collected close to known oil seepage. The nutritional dependence of the symbionts, such as the mussel/bacterial system, on reduced compounds from seepages, requires them to be chronically in contact with high hydrocarbon concentrations. Though Spies *et al.* (1980) were unable to demonstrate adaptation to the high hydrocarbon environments, it was suggested that adaptation may account for mitigating effects. This obligate association of oil and organisms may provide information on the bioaccumulation of PAH by organisms in an ecosystem that has apparently evolved and thrives in the presence of high PAH concentrations. A secondary objective was to determine water column and

sediment PAH distributions to compare with the organism tissue PAH distributions.

MATERIALS AND METHODS

Sediment, organism, and water samples were collected with piston cores, benthic trawls, and clean 20-liter Niskin bottles, respectively, on R/V GYRE Cruises 85-G-5 and 86-G-2 (Table 1). Additional organism samples were collected with the submersible Johnson *Sea-Link* on Dive 1877, and sediments and organisms were also collected with the US Navy nuclear powered submersible *NR-1*. Upon retrieval, organism and sediment samples were frozen in precleaned aluminum foil or glass jars with teflon-lined screw caps. The sediment and organism samples were frozen and returned to the laboratory for further processing and analysis.

Internal standards were added to all samples before extraction. The aromatic internal standard contains d_4-1,4-dichlorobenzene, d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-chrysene, and d_{12}-perylene. Internal standards were added at a concentration level similar to that expected for sample analytes.

Water samples were extracted on the ship immediately after collection. The water was acidified to a pH of 2 or less with HCl and aromatic internal standards, containing d_4-1,4-dichlorobenzene, d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-chrysene, and d_{12}-perylene were added. The water samples (38 to 52 l) were extracted 3 times. The extraction was accomplished with a stirrer and motor in 3 to 5, 19 liter clean glass bottles. The contents of each bottle were extracted with a total of 900 ml of methylene chloride. The combined methylene chloride extracts were evaporated to near dryness in a rotary flash evaporator and analyzed directly by gas chromatography/mass spectrometry (GC/MS).

Approximately 15 g of freeze-dried sediment (dry weight) was extracted with methylene chloride for 16 h in a Soxhlet apparatus. Extracts were stored refrigerated if not immediately fractionated by column chromatography. A push core collected on the *NR-1* was visibly oil stained. After the core was extruded, the oil was removed with a spatula and dissolved in CH_2Cl_2. The CH_2Cl_2 extract was then filtered through a Gelman A/E glass fiber filter coated with $NaSO_4$ to remove particles and water. The sample was diluted and analyzed directly by GC/MS.

Soft tissue (bivalves, gastropods, tube worms) was removed from hard shells or tubes while still partially frozen, and the entire soft tissue and accompanying body fluids were homogenized with a Tekmar Tissuemizer. Fish samples were skinned and filleted frozen and the fillets homogenized

TABLE 1

Carbon Isotope Values ($\delta^{13}C$ in ‰ Relative to PDB) and Total PAH Concentrations (ng/g Dry Weight) in Organisms and Sediments from Seep Areas

	Description	*Location*	$\delta^{13}C$ (‰).	*Total PAH* (ng/g)*
Sediment core	4·6 m**	GC 287***		282
Sediment core	4·2 m**	GC 233/234		177
Sediment core	3·4 m**	GC 272		6 800
	Trawl Collections			
Clam	*Calytogena ponderosa*	GC 287	−37·0	129
Tube worm	*Lamellibrachia* sp.	GC 272	−36·4	1 120
Scallop		GC 272	−16·9	66
Shrimp	*Stereomastis sculpta*	GC 272	−17·6	32
Fish	*Halosaurus guentheri*	GC 272	−20·3	<10
Crab	*Geryon quinquedens*	GC 272	−26·2	58
Mussel	*Mytilidae* sp.	GC 233/234	−48·5	147
Snail	*Neogastropod*	GC 233/234	−27·3	46
Snail	*Gaza fisheri*	GC 233/234	−20·8	<10
Crab	Spider crabs	GC 233/234	−29·6	56
Shrimp	*Stereomastis sculpta*	GC 233/234	−17·1	50
Fish	*Halosaurus guentheri*	GC 233/234	−18·1	<10
	Sea-Link Collections			
Mussel	*Mytilidae* sp.	GC 185	−24·1	36
Tube worm	*Lamellibrachia* sp.	GC 185	−23·9	73
	NR-1 Collections			
Mussel	*Mytilidae* sp, push core 9	GC 185	−42·9	3 550
Snail	*Gaza fisheri*, Basket No. 2	GC 235	−38·7	319
Mussel	*Mytilidae* sp, Basket No. 2	GC 235	−50·8	121
Mussel	*Mytilidae* sp, body	GC 235	−31·1	243
Mussel	*Mytilidae* sp, gill	GC 235	−38·0	1 660
Tube worm	*Lamellibrachia* sp, Basket No. 2	GC 235	−20·3	87
Mussel	*Mytilidae* sp, Basket No. 1	GC 185	−39·3	712
Mussel	*Mytilidae* sp, Push core 8	GC 185	−39·5	7 530
	Status and Trends Mussel Watch Collection			
Oyster	*Crassostrea virginica*	Barataria Bay	−21·9	1 900

* Total PAH includes the sum of naphthalene, methylnaphthalenes, biphenyl, dimethylnaphthalenes, acenaphthene, fluorene, phenanthrene, anthracene, methylphenthrenes, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(e)pyrene, benzo(a)pyrene, perylene, dibenz(a,h)anthracene.

** Core section is 20 cm cut starting at this core depth (i.e. 4·6 m).

*** GC stands for Green Canyon, the number refers to the lease block number.

with a tissuemizer. A subsample of these homogenates was removed for dry weight determination by oven drying.

Approximately 15 g (wet weight) of tissue were required for analyses. Tissue analytical techniques were similar to those of MacLeod *et al.* (1985). The tissue was macerated in CH_2Cl_2 and Na_2SO_4 with a Tekmar Tissuemizer for 5 min. The mixture was centrifuged and the extract decanted. This process was repeated three times. Extracts were concentrated with a Kuderna-Danish (KD) Evaporative Concentrator to 0·5–1 ml (60°C). Again, concentrated extracts were stored refrigerated if not immediately fractionated.

Each set of samples (6–8) was accompanied by a complete system blank and a spiked blank which was carried through the entire analytical scheme in a manner identical to the samples. System blanks include only reagents and internal standards. Spiked blanks include quantitative amounts of all analytes as well as all internal standards.

Separation of aromatic hydrocarbons from other organic compounds was by alumina/silica gel chromatography. Alumina (10 g, 400°C/4 h, deactivated 1%) was slurry packed in CH_2Cl_2 over silica gel (20 g, 170°C/12 h, deactivated 5%). Copper powder was added to the column for sediment samples to remove sulfur. The CH_2Cl_2 was replaced with hexane, and the extract, in 1 ml of hexane, was transferred to the column. The column was then eluted with 50 ml of pentane (f_1) and 200 ml of 1:1 CH_2Cl_2:pentane (f_2). This recovered the aliphatic (f_1) and aromatic hydrocarbon (f_2) fractions. The f_2 fraction was concentrated with KD concentrators.

The aromatic fraction for organism samples was further purified by Sephadex LH-20 chromatography in order to remove interfering lipids. The alumina/silica gel aromatic hydrocarbon (f_2) fraction was dissolved in 2 ml of 6:4:3 hexane:CH_3OH:CH_2Cl_2 (Ramos & Prohaska, 1981). Sephadex columns were calibrated with azulene and perylene standards before samples were applied. The extract was transferred to the column and 150 ml of the 6:4:3 mixture was added. The first 40 ml were discarded. The next 60 ml of eluate were collected. After each sample, 50 ml of the 6:4:3 mixture was used to wash the column and was discarded. The collected fraction was then concentrated with a KD concentrator apparatus. Some of the tube worm samples were treated with copper powder to remove sulfur.

Quantitation of the aromatic (f_2) fraction was by selected ion monitoring (SIM mode) by gas chromatography/mass spectrometry (GC/MS). Mass fragmentation was accomplished in an electron impact mode with 70 eV electrons. Sample components were separated on 30 m (DB-5, 0·25 mm id) fused-silica capillary columns with carrier flow (He) of 2–3 ml/min. Sample injections were cold-trapped on the capillary column for 1 min at 40°C. The

GC oven was then heated to 300°C at 12°C/min. Each component of interest was quantified based on the calibration of the peak area of its molecular ion with an authentic standard. Deuterated internal standards were easily differentiated due to the differences in the molecular ions monitored with no interferences (i.e. naphthalene $m/z = 128$, d_8-naphthalene = 136). Two fully automated GC/MS systems were used: Hewlett–Packard (HP) 5996 GC/MS/DS and HP 5970 GC/MSD/DS. The HP Aquarius software uses a triple point calibration at three concentrations for each component for quantitative analysis. Blanks and spike blanks were run with each sample set. Analyte concentrations were corrected for blank levels. The limits of detection for individual aromatic compounds for tissues, sediments and water were 10 ng/g, 5 ng/g and 0·1 ng/liter, respectively. The PAH analyzed include naphthalene, methylnaphthalene, biphenyl, dimethylnaphthalenes, acenaphthene, fluorene, phenanthrene, anthracene, methylphenanthrenes, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(e)pyrene, benzo(a)pyrene, perylene and dibenz(a,h)anthracene.

Samples were prepared for stable carbon isotope analyses by the closed-tube combustion method (Brooks *et al.*, 1987*c*). The sample is combusted at 800°C for 24 h in the presence of cupric oxide. The carbon dioxide produced was analyzed on a Finnigan MAT 251 isotope ratio mass spectrometer with a triple collector. Abundance of carbon isotopes in the samples are reported relative to the Pee Dee Belemnite standard.

RESULTS AND DISCUSSION

Sample collection locations, descriptions, $\delta^{13}C$ values and concentrations of the sum of PAH measured are reported in Table 1. All of these samples were collected in areas of known oil seepage. However, the exact juxtaposition of oil seepage organisms collected with the benthic trawl is unknown.

The $\delta^{13}C$ values of organisms are useful in tracing the source of their carbon (Brooks *et al.*, 1987*b*). Seep organisms that derive a substantial proportion of their carbon directly or indirectly from methane have very negative $\delta^{13}C$ values (Kennicutt *et al.*, 1985; Brooks *et al.*, 1987*b*). Organisms that derive their carbon mainly from marine phytoplankton have carbon isotope values ranging from −19 to −21‰ (Gearing *et al.*, 1984). The carbon isotope values measured for organisms from these sites can be divided into three groups: (1) heterotrophic (−14 to −20‰); (2) sulfur-based (−30 to −42‰); and (3) methane-based (< −40‰). Immobile (tube worms, mussels, clams), moderately mobile (scallops, gastropods, crabs), and very mobile (fish and shrimp) species were analyzed.

The $\delta^{13}C$ values of organisms reflect their carbon source or sources.

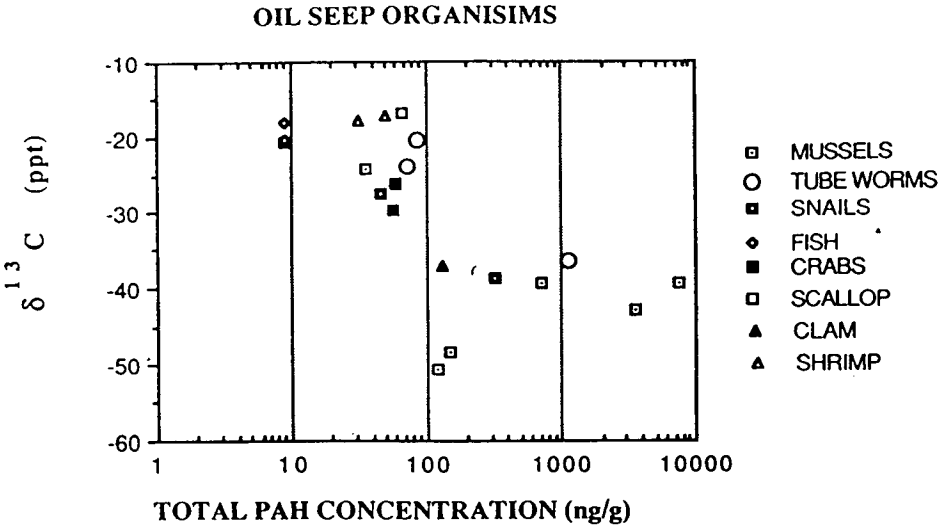

Fig. 1. Relationship between $\delta^{13}C$ values and total measured PAH concentrations (see Table 1 for explanation).

Because there is seepage of both methane ($\delta^{13}C = -40$ to -45‰) and oil ($\delta^{13}C = -26{\cdot}5$ to $-27{\cdot}5$‰) and the oil contains PAH in these areas, a covariance in tissue $\delta^{13}C$ and total PAH concentration of organisms collected at these sites was expected. A plot of tissue $\delta^{13}C$ values and total measured PAH concentrations is illustrated in Fig. 1. The fish and shrimp analyzed had heavier, ^{13}C-enriched, carbon isotope values ($-16{\cdot}9$ to $-20{\cdot}8$), reflecting a heterotrophic source of carbon for these mobile organisms. The range of tissue PAH concentrations for these organisms is <10 to 66 ng/g. In contrast, the tissues that are depleted in ^{13}C ($-36{\cdot}5$ to $-50{\cdot}8$), confirming the presence of chemosynthetic carbon, have limited mobility, with the exception of one snail sample ($-38{\cdot}7$‰). Tissue PAH concentrations for these organisms range from 121 to 7530 ng/g. The organism tissues with intermediate carbon isotope ratios ($-23{\cdot}9$ to $-29{\cdot}6$‰), possibly resulting from mixed carbon sources, have limited or moderate mobility. These tissues also have intermediate PAH concentrations ranging from 46 to 73 ng/g. Tissue $\delta^{13}C$ values and PAH concentrations may reflect: (1) proximity to oil and gas seeps for limited mobility organisms or (2) residence time in these areas for the more mobile organisms. Bivalves are known to have little or no enzymes for metabolizing PAH, as compared to fish and crustacea (Lee *et al.*, 1972). Therefore, an alternative explanation for the lower PAH content of the non-bivalve organisms may be their innate ability to metabolize PAH.

The variability of $\delta^{13}C$ values of tube worms ($-36{\cdot}4$ to $-23{\cdot}9$‰) and mussels ($-48{\cdot}5$ to $-24{\cdot}1$‰) indicates that these seep organisms may derive their carbon from methane, oil, and/or other sources. The $\delta^{13}C$ values of snail tissues ($-20{\cdot}8$ to $-38{\cdot}7$‰) reflect the carbon they most recently

ingested. Generally, organisms with isotopically lighter carbon values have higher PAH concentrations, possibly reflecting greater exposure to the seeping oil. The PAH concentrations were highest in the sediments collected near tube worms with the highest PAH body burden, though oil concentrations in the sediments are highly variable.

The gill and remaining body tissue of one mussel sample were analyzed separately (Table 1). The gills had a $\delta^{13}C$ value of −38·0‰ and PAH concentration of 1660 ng/g, while the corresponding values for the remaining body tissues were −31·1‰ and 243 ng/g. In contrast, previous studies found that mussel gill and body tissues had similar $\delta^{13}C$ values (Childress *et al.*, 1986). The resolution of this discrepancy based on the one sample analyzed as part of this study will require additional analyses. The gill represents about half of the total tissue weight of these mussels. Therefore, the gills analyzed in this study contain approximately 90% of the total PAH. The symbiotic bacteria that convert methane to organic material are located in the gills, which are also the probable entrance route of PAH into the mussel. Further study is required to confirm and understand the reasons for elevated PAH concentrations in the gill.

The concentration of naphthalene, total methylnaphthalenes, biphenyl, total dimethylnaphthalenes, acenaphthene, fluorene, and phenanthrene in water, sediments and organisms was divided by the sum of the concentrations of these compounds in a sample and multiplied by 100 to allow for comparison of sample compositions. The distribution of PAH in water, sediment, oil, and various organisms is illustrated in Fig. 2. The water

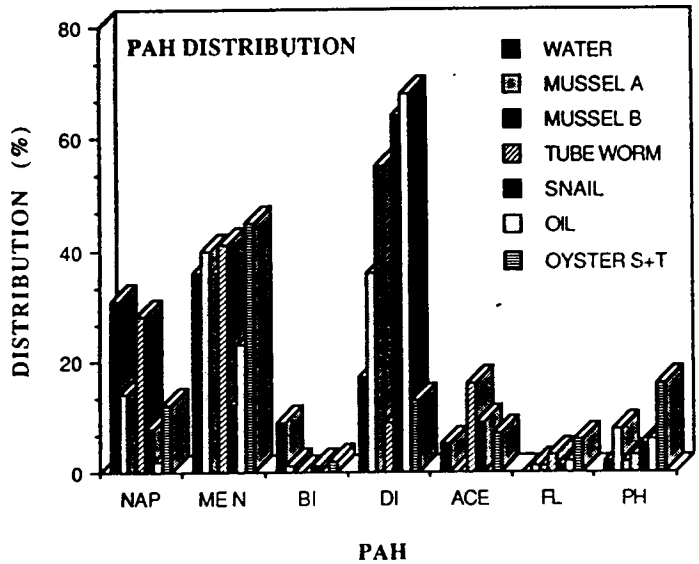

Fig. 2. Weight percentage composition of PAH in selected samples. Nap = naphthalene, MeN = methylnaphthalenes, Bi = biphenyl, Di = dimethylnaphthalenes, Ace = acenaphthene, Fl = fluorene, Ph = phenanthrene. For example, %Nap = (Nap ÷ Nap + MeN + Bi + Di + Ace + Fl + Pb) × 100.

contains a higher percentage of naphthalene and methylnaphthalenes than does the oil, while the oil contains a higher percentage of dimethylnaphthalenes, fluorene and phenanthrene. The oil from the sediment also contains PAH of molecular weights higher than phenanthrene while the water did not.

The concentration of total measured PAH in the water samples from 570 m depth at Green Canyon 233/234 was 28 ng/liter and most likely results from dissolution of seeping oil. Gas bubbles (probably methane) continuously emanate from the sediments in these seep areas and entrain traces of higher molecular weight hydrocarbons that can dissolve in the overlying water column. Gas bubbles have been observed breaking at the surface and producing an iridescent sheen of oil at the water surface at this site (Brooks *et al.*, 1987*a*). The relatively greater water solubility of lower molecular weight aromatic hydrocarbons (i.e. naphthalene) results in their enrichment relative to higher molecular weight PAH in the overlying water. The extractable PAH concentrations measured in this Gulf of Mexico deep water seep area are an order of magnitude lower than volatile PAH concentration (0·15–5·2 μg/liter) at a California seep area (Stuermer *et al.*, 1982).

The distribution of PAH in selected mussels, tube worms, and snails from the seep area, as well as an oyster sample from Barataria Bay, Middle Bank, LA (Brooks *et al.*, 1987*c*) is compared in Fig. 2. The organisms' PAH distributions are intermediate when compared to the water and oil compositions. The organisms generally contain relatively more lower-molecular-weight aromatic compounds than the sediments or separate phase oil. This may be due to the availability of naphthalenes dissolved in the water or preferential uptake of lower molecular weight PAH by the organisms. Similarities in PAH distributions of the near-shore Barataria Bay, as well as other Gulf of Mexico oysters (Wade *et al.*, 1988) and seep organisms indicate that similar mechanisms of uptake and depuration may be occurring at these sites.

Acenaphthene was detected only in the water, tube worms, snail and coastal oyster samples. Its source is unknown. Fluorene, a structurally similar compound, was detected in the oil and all organisms, but not in the water sample. These differences may reflect variations in water and oil composition with time whereas organism and sediment PAH represent a time averaged composition over months or years.

CONCLUSIONS

Tissues of organisms that are obligately associated with areas of massive oil seepage contain significant amounts of PAH. Higher concentrations are

found in the tissues of less mobile or sessile organisms. Organisms collected near sediments containing high concentrations of PAH have the highest concentration of tissue PAH. It is unclear whether the seep organisms accumulate PAH from the water or sediment or a combination of both, though certainly the largest repository of PAH is the sediments. The similarity of PAH distributions and concentrations in a coastal oyster and in tissues of organisms from the seep area indicates that similar mechanisms of uptake and depuration may be occurring at these sites. The seep organisms are able to survive and thrive in an environment of high PAH exposure. The apparent ability to cope with these elevated levels of PAH may involve specially adapted and/or evolved enzyme systems. There is ample physiological evidence that the primary source of isotopically light carbon to the system can be solely derived from chemosynthesis (H_2S or CH_4), though a contribution of light carbon from the degradation and/or assimilation of oil cannot be ruled out. The nature, interactions and mechanisms of the life strategies of the communities at these seep sites are yet to be fully understood.

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The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.