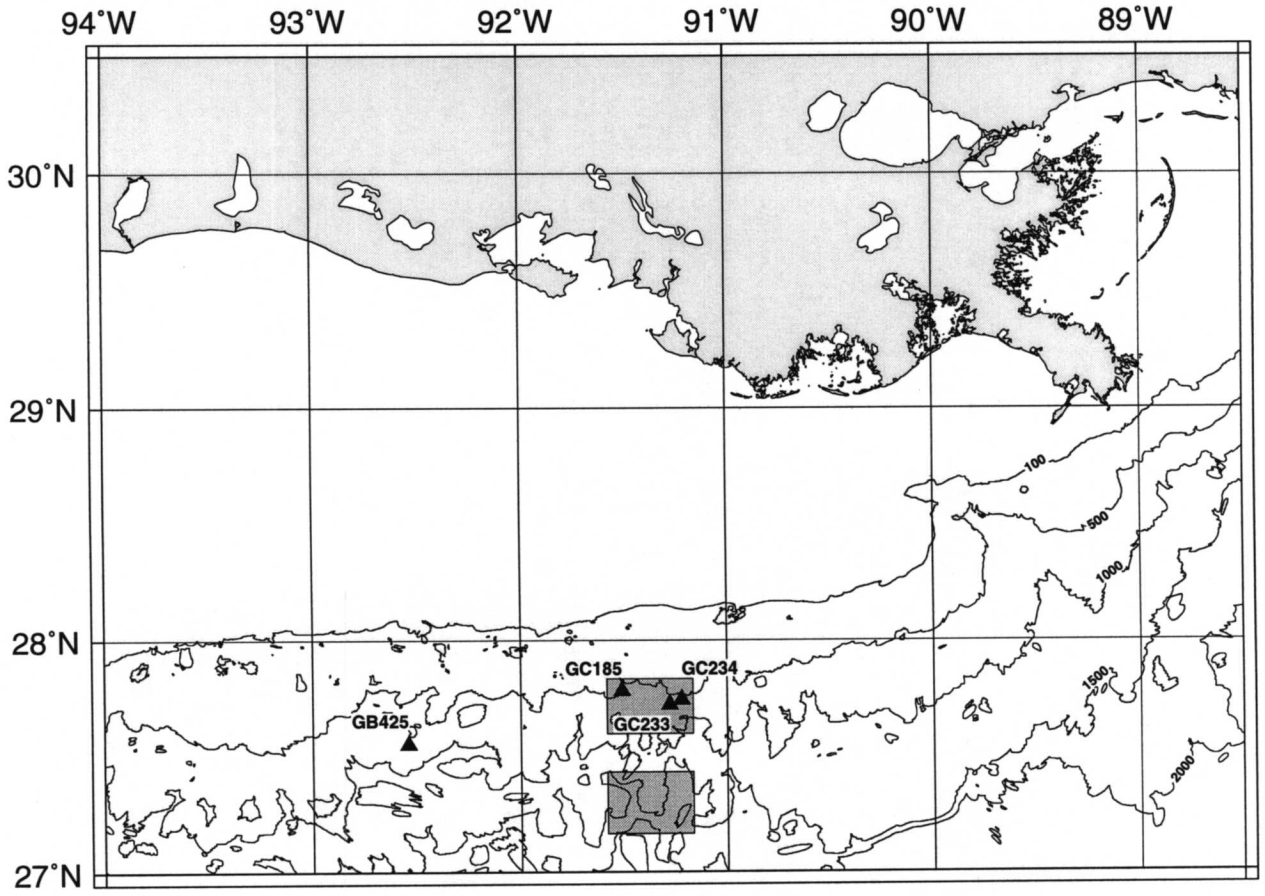
OCS Study
MMS 98-0034

Stability and Change in Gulf of Mexico Chemosynthetic Communities

Interim Report



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

OCS Study
MMS 98-0034

Stability and Change in Gulf of Mexico Chemosynthetic Communities

Interim Report

Editor

Ian R. MacDonald

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1.0 INTRODUCTION

This volume is the Interim Report for the research program entitled "Stability and Change in Gulf of Mexico Chemosynthetic Communities." The program is being conducted under the auspices of the Texas A&M Research Foundation under contract (Contract No. 1435-01-96-CT-30813) to the Department of the Interior, Minerals Management Service (MMS). The program was awarded on 31 October 1996 and is scheduled for 42 months of funding with the final report due 1 July 2000. Since award, the program team has completed one year of work, including two major field efforts and have made progress in analyzing the materials collected. This report details progress to date and presents preliminary findings for the program.

1.1 Objectives of the Program

The program was designed to aid the Minerals Management Service in the scientifically sound management of living resources found at hydrocarbon seeps on the northern Gulf of Mexico continental slope. An integrated, multi-disciplinary approach was proposed to address the complex issues associated with the protection of these unique living resources. As such, the program encompasses ecological studies at both regional and local spatial scales as well as an evaluation of temporal changes in these communities. Stability and change within these communities can only be understood in the context of their interactions within the geological, chemical, and oceanographic setting. An understanding of the processes that control the distribution, health, and succession of communities in these environments is necessary to forecast and forestall potential impacts derived from exploration and exploitation of fossil energy reserves on the northern Gulf of Mexico continental slope. Integrated studies were designed to collect ecological, geological, chemical, and oceanographic information related to the longevity, robustness, senescence, and recovery of chemosynthetic communities.

This program includes community ecological, regional geological, microbial, and site-specific chemical and oceanographic studies. At the community level, efforts focus on the abiotic factors that control the distribution, abundance, and health of the major chemosynthetic and associated fauna. Investigations of the life-history of these organisms are also included. At the regional level, efforts focus on the geological, chemical, and oceanographic processes that support communities including larval dispersion and circulation processes that maintain the genetic stability of these communities.

1.2 Key Personnel

The principal investigators, their area of expertise, and the members of the Scientific Review Board are summarized in Table 1.1.

1.3 Seep Fauna

The MMS requested that the proposed program be developed based on conceptual models that describe stability and change in chemosynthetic communities of the northern Gulf of Mexico. This section presents the current formulation of this model that was used to design the various

Table 1.1

Program Team and Roles

Texas A&M University Investigators

Department of Oceanography

Dr. John W. Morse Inorganic Chemistry	Dr. William Sager Geophysics

Geochemical and Environmental Research Group

Dr. Norman L. Guinasso, Jr. Physical Oceanography	Dr. Ian MacDonald Program Manager Imaging & GIS	Dr. Gary A. Wolff Data Management
Dr. Mahlon C. Kennicutt II Deputy Program Manager Environmental Chemistry	Dr. Roger Sassen Hydrocarbon Chemistry	

Principal Investigators Not at Texas A&M University

Dr. James M. Brooks TDI-Brooks Int'l. Inc. Industry Liaison for Geophysics and Geochemistry	Dr. Steven Macko University of Virginia Trophic Relationships	Dr. Douglas C. Nelson University of California, Davis Microbial Ecology
Dr. Robert Carney Louisiana State University Trophic Relationships	Dr. Paul Montagna The University of Texas at Austin Statistical Design	Dr. Eric Powell Rutgers University Histopathology and Community Health
Dr. Charles F. Fisher Pennsylvania State University Physiological Ecology	Dr. Kimberlyn Nelson Pennsylvania State University Molecular Ecology and Genetics	Dr. Steve Schaeffer Pennsylvania State University Molecular Ecology and Genetics
Dr. Samantha Joye University of Georgia Electrochemistry		

Scientific Review Board

Dr. James Barry Monterey Bay Aquarium Research Institute	Dr. Cindy Lee Van Dover University of Alaska	Dr. William W. Schroeder The University of Alabama

work elements. Two distinct processes, hydrothermal venting and cold seepage, can function as geologic mechanisms that generate habitats that can support chemosynthetic communities. The first section describes how cold seeps differ ecologically from hydrothermal vents. The second section gives the details of a hypothetical model for the formation of cold seep habitats in the Gulf of Mexico. References to the extensive published literature provide the framework for a model that proposes an explanation for stability and change in Gulf of Mexico chemosynthetic communities. The third section describes specific components of the proposed model with an emphasis on overarching concepts. Testing the validity of these concepts is the focus of the present program. Finally, the fourth section summarizes programmatic milestones.

1.3.1 Chemosynthetic Communities at Hot Vents and Cold Seeps

Since the original discovery in the late 1970s (Corliss et al. 1979), chemosynthetic communities dominated by tube worms (Vestimentifera), mussels (Bathymodilinae), and/or clams (Vesicomyidae) have been found in numerous locations in the deep ocean (Tunnicliffe 1992). The exceptional biological productivity that characterizes chemosynthetic communities depends on symbiosis between metazoan hosts and chemoautotrophic bacteria (Fisher 1990). The chemosynthetic habitat is a benthic environment in the ocean where geochemical enrichment produces high concentrations of electron donors--particularly sulfides or methane--and where oxygen-rich seawater is simultaneously available (Jannasch 1989). The original discoveries of chemosynthetic communities took place at mid-ocean ridge crests where active volcanism and seafloor spreading generates a dynamic geological and geochemical environment (Edmond et al. 1982; Fornari and Embley 1995). At hydrothermal vents the interface is turbulent which means reduced chemical compounds mix with seawater in the effluent plume that discharges from a vent orifice and subsequently bathes the vent organisms (Hessler and Kaharl 1995). At hydrothermal vents, chemosynthetic substrates are produced by high-temperature and high-pressure reactions between seawater and basalt (Hannington et al. 1995). There are two reasons formation and destruction of settlement surfaces available to hydrothermal vent chemosynthetic fauna are rapid and unstable: 1) the driving, sub-seafloor processes are dynamic and much larger in scale than active vent sites (Alt 1995) and 2) the precipitate structures (chimneys, etc.) are physically fragile (Tivey and McDuff 1990) and subject to rapid alteration by biological colonizers (Juniper et al. 1992; Juniper and Sarrazin 1995).

Subsequently, communities ecologically similar to those at hydrothermal vents were found under a diverse set of geological circumstances that are loosely termed "cold seeps." At these locations chemical enrichment results from more gradual processes (Paull et al. 1984; Kennicutt et al. 1985). The interface between oxidizing and reducing conditions at cold seeps tends to be laminar; that is, the physical setting maintains reduced compounds on one side of an interface and oxygenated seawater on the other. By physically bridging this interface, seep autotrophs are able to simultaneously access oxygen and reduced chemicals. The Gulf of Mexico hydrocarbon seeps are the archtypical example of cold seep habitats (Carney 1994; MacDonald et al. 1989). Remote sensing evidence has demonstrated that seepage is active over a wide geographic range of the northern Gulf of Mexico (Kornacki et al. 1994; MacDonald et al. 1993), so the potential magnitude of biological productivity due to these communities is high. Direct sampling of the seafloor by trawls, photo-sleds, and submersibles also indicates that chemosynthetic communities are common and widespread (Kennicutt et al. 1988; MacDonald et al. 1996;

Roberts and Aharon 1994). However, systematic surveys of the entire region have not been undertaken, so a comprehensive census of total biological production derived from seep communities is only gradually emerging.

1.3.2 Habitat Processes at Gulf of Mexico Hydrocarbon Seeps

At hydrocarbon seeps, reduced compounds are generated as by-products of microbial consumption of hydrocarbons in the upper few meters of the sedimentary column and are directly supplied by upward migration from deeply buried sources. Hydrogen sulfide is produced and consumed by biotic and abiotic processes at hydrocarbon seep communities at much higher rates than in normal sediments (Lin and Morse 1991). Biogenic reaction products are responsible for the formation of iron sulfide minerals and, in some cases, massive deposits of carbonate minerals at seep sites (Morse 1994). Seep sediments exhibit geochemical processes that differ from most models of normal marine sediment diagenesis in many important ways. Organic enrichment by hydrocarbon seepage is an important process in producing reduced compounds (Brooks et al. 1987). Hydrocarbon generation and migration occurs over timeframes of millions to tens of millions of years (Sassen 1987). The areal extent of chemosynthetic communities are dwarfed by the spatial scale of the subsurface hydrocarbon system, and are ephemeral features compared to the timeframe for hydrocarbon generation and migration. The volume of hydrocarbons withdrawn from reservoirs by humans is unlikely to impact the survival of chemosynthetic communities, since this volume is insignificant compared to total volume of the extant hydrocarbon system. Two styles of seepage have been described at Gulf of Mexico hydrocarbon seeps: sediment diffusion and brine pooling. Much of the community level diversity in cold seep communities can be explained by examining the environmental consequences of these two styles of seepage.

1.3.2.1 Sediment Diffusion

Sediment-diffusion hydrocarbon seeps have been discovered at many locations on the upper continental slope during energy prospecting activities. It has been widely recognized that upward migration of reservoired oils can result in high concentrations of liquid and gaseous hydrocarbons in seafloor sediments (Anderson et al. 1983). In these habitats; mussels, clams, and tube worms utilize reduced compounds--methane and H_2S--by extending body parts into the sediment or by bathing their brachia (gills or plumes) in the steep gradients immediately above the sediment/water interface (Figure 1.1). Gas seeps and gas hydrates--ice-like solids that form when methane and water combine at high pressure--are also recognized as important components of slope seep systems (Brooks et al. 1984; Shipley et al. 1979). The important characteristics of the sediment diffusion habitat are abundant, highly-altered hydrocarbons, including thermogenic gases, liquid petroleum, and tars which are distributed throughout the sedimentary section overlying a fault or fault nexus (Behrens 1988; Kennicutt et al. 1987; Reilly et al. 1996; Roberts and Aharons 1993). At the seafloor, a complex veneer tends to entrap and further contribute to the alteration of seeping fluids. Roughly, in order of greatest relative age, major components of this veneer are as follows (note that the citations provided refer to descriptions of the components, not their influence on habitat formation):

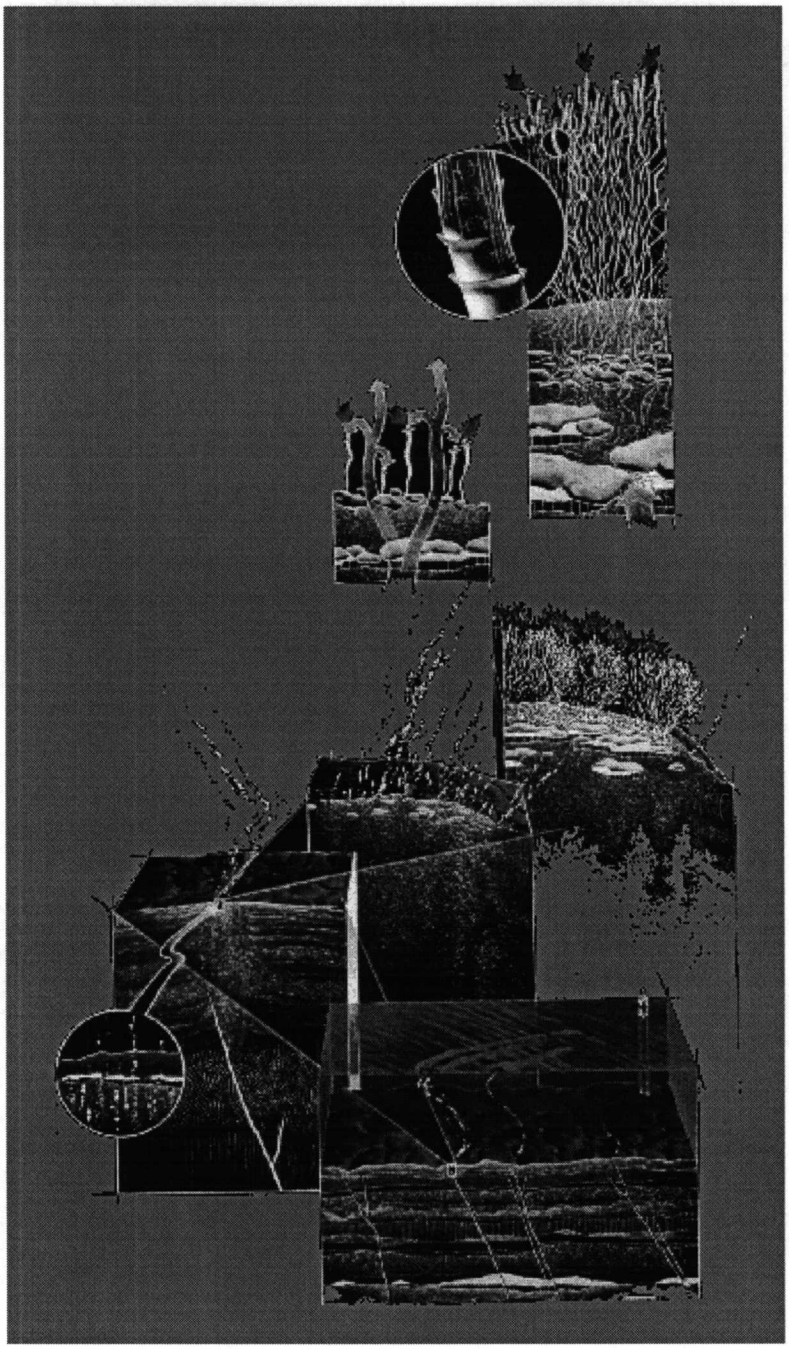

Figure 1.1. Illustration of the chemosynthetic community formation in a sediment diffusion style hydrocarbon seep. Gas and oil migrate along fault planes that penetrate reservoirs (lower), then diffuse through unconsolidated sediments approaching the seafloor (middle left). Formation of gas hydrate, layers of biota, and authigenic carbonate entrap hydrocarbons in the uppermost sediments (middle right), facilitating microbial degradation of hydrocarbons and subsequent reduction of seawater sulfate to sulfides needed for tube worm symbiosis (upper right). (Art by C. Bruce Morser.)

1) Authigenic carbonates in the form of rubble and consolidated pavements (Roberts and Aharon 1994; Sassen et al. 1994a).

2) Extensive aggregations of vestimentiferans, the "roots" of which extend into the upper sediment as extensive tangles (Fisher 1995).

3) Beds of bivalves, including living mussels and/or clams and layers of shell (Callender et al. 1990; MacDonald et al. 1990a).

4) Layers of gas hydrate, which can entrap the buoyant phases of hydrocarbons, but are subject to gradual decomposition and possible catastrophic failure due to temperature fluctuations in bottom waters (MacDonald et al. 1994).

5) Mats of the sulfide-oxidizing bacteria, *Beggiatoa* (Larkin et al. 1994), which occur as pigmented and non-pigmented forms (Sassen et al. 1993b).

Although there is good evidence for structural instability in rapidly forming seep habitats, particularly where mud volcanism produces massive fluid discharge (Neurauter and Roberts 1994; Roberts and Neurauter 1990), the formation of large chemosynthetic communities takes place in the context of increasing lithification and general seafloor stability (Roberts and Aharon 1994). The chemosynthetic fauna themselves will, with time, retard escape of hydrocarbons from the sediments (Sassen et al. 1994b).

1.3.2.2 Brine Pooling

Concentrated brines are often associated with petroleum migration and seepage (Behrens 1988). Where these discharge at the seafloor, their density allows for the formation of distinct pools on the seafloor. The discovery of brine-filled depressions in the Gulf of Mexico predates the discovery of chemosynthetic communities (Bright et al. 1980; Paull and Neumann 1987; Shokes et al. 1977). The discovery of chemosynthetic fauna on the Florida Escarpment demonstrated that brine could be a carrier of reduced compounds (Paull, et al. 1984; Cary et al. 1989). The significance of the pooling process became evident when a dense colony of Seep Mytilid Ia was discovered around the edges of a small, brine-filled crater (MacDonald et al. 1990c). Where brines accumulate on the seafloor, mussels can obtain methane through the uptake of water by placing their inhalant siphons just above the brine (MacDonald et al. 1990a). High concentrations of gaseous hydrocarbons are often dissolved in Gulf of Mexico brines. The defining characteristics of the brine pooling habitat are an extremely sharp (~1 cm) density driven interface between the brine and seawater and a surface which allows the development of a thin layer of brine that intercepts the sediment surface. Dense colonies of mussels become established at these seawater/sediment/brine interfaces. Where the brine interface is less sharp, for example in the large brine pool filling Orca Basin, which has a meters-thick separation between seawater and saturated brine (Shokes et al. 1977), the mussels are unable to bridge the distance between the anoxic brine and the oxygen-rich seawater, consequently, mussel communities appear to be absent (Brooks et al. 1990). The brine pooling habitat results from brine seeps on nearly level seabottoms or from brine-filled pockmarks or craters that exhibit a brine/seawater interface at the edges of the depression (Figure 1.2).

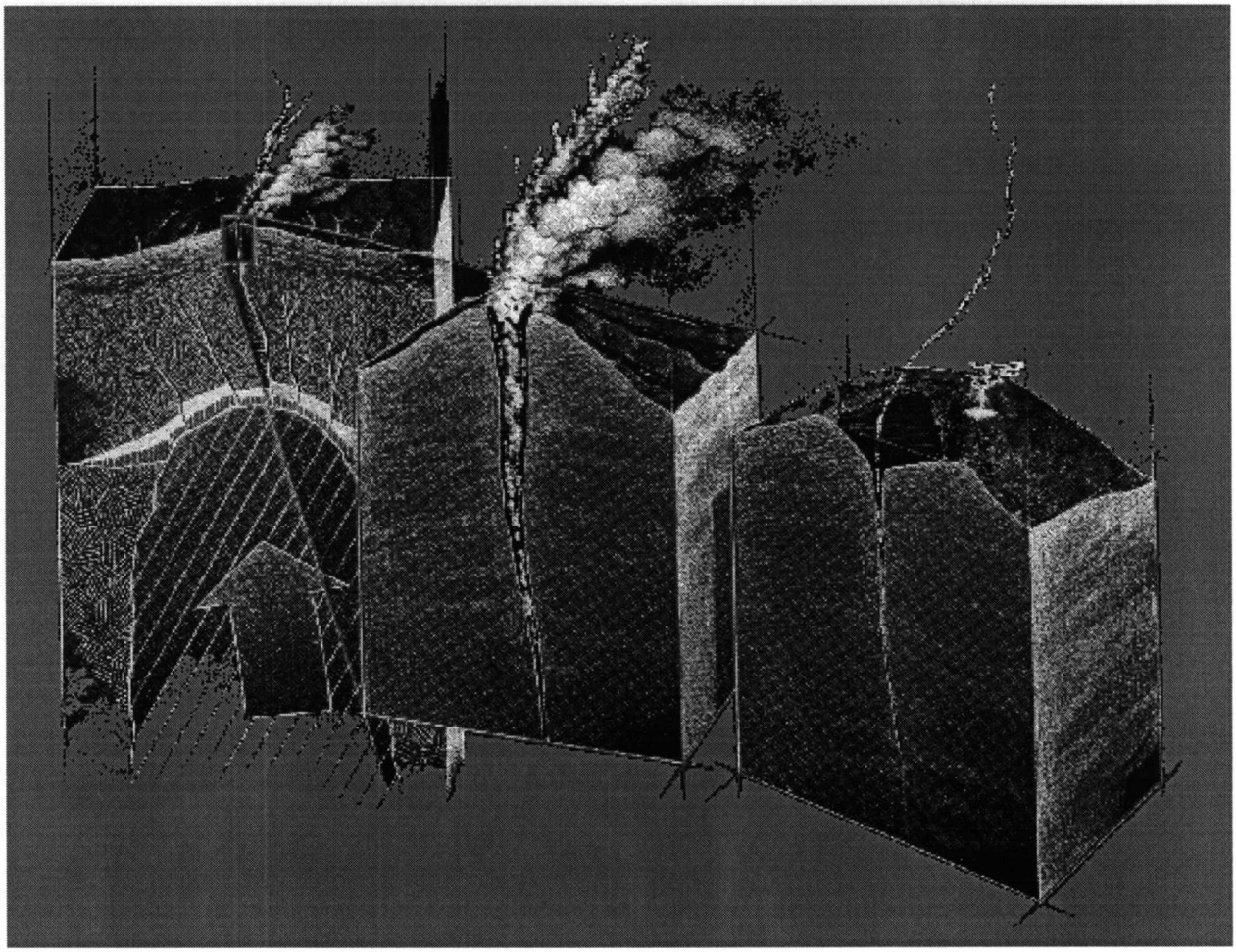

Figure 1.2 Illustration of the chemosynthetic community formation in a brine pool style hydrocarbon seep. Salt diapir pressurizes a shallow reservoir of methane--also known as a shallow gas hazard (left). Subsequent release of gas excavates a surface crater and diatreme (middle). High concentrations of methane are available to seep mytilids around the edges of the brine pool formed by dissolution of underlying salt (right). (Art by C. Bruce Morser.)

1.3.3 Model Components and Interactions

The following sections provide a brief description of the various biological components of an overall model of chemosynthetic communities including microbial communities, mussel colonies, and tube worm colonies. Interactions between the environmental setting and the biology are also considered including change at the community level, geological stability, regional characteristics, energy flow, and food webs.

1.3.3.1 Microbial Communities

Bacteria are a fundamental component of the seep community interacting with the chemical environments both as free-living bacteria and endosymbionts. The importance of bacterial occurrences and their role in mediating chemical transformations at seep sites is poorly understood. It is believed that autotrophic microbial (sulfide oxidizing) productivity is dependent on heterotrophic microbial (sulfate reducing) productivity. *Beggiatoa* filaments can extend 8-12 cm down into the anoxic sediment. *Beggiatoa* mats in the Gulf of Mexico sites have the potential for competing with higher organisms for sulfide over at least the top 10-12 cm of these anoxic sediments. *Beggiatoa* exhibits two growth forms: pigmented and non-pigmented. Enzyme activities in the pigmented and non-pigmented forms are consistent with heterotrophic and autotrophic metabolism, respectively. *Beggiatoa* may also be facultative consumers of hydrocarbons. *Beggiatoa* and the related genus *Thioploca* are the only bacteria known to contain a central vacuole. Similar filamentous, thio-autotrophic bacteria from Guaymas Basin hydrothermal vents (Gulf of California, 2000 m depth) and the Clam Field Seeps of Monterey Canyon (Monterey Bay, depth 900 m) contain intracellular nitrate at concentrations as high as 160 mM (4,000- to 8000-fold above ambient levels). Expanded investigations are needed to more fully understand the role microbes play in seep ecology.

1.3.3.2 Mussel Colonies

Seep Mytilid Ia (a provisional taxonomic designation), which is the major mussel species in these habitats, has evolved a symbiosis with a methanotrophic endo-bacteria (Childress et al. 1986; Fisher 1990). Mussel colonies are most abundant where gaseous hydrocarbon flux is high. Compared to tube worms, mussel colonies recruit more rapidly and are more mobile. Mussels, which inhabit the sediment diffusion habitat, experience greater variability in nutrient supply due to hydrate formation and decomposition and carbonate precipitation and are more ephemeral than mussel colonies at brine pools. Differences in the longevity, 'health,' and stability of mussel colonies depends on the following features of their life history and physiology. Mussels colonize a new site with motile larvae. Seep Mytilid Ia spawning is not seasonal and recruitment is episodic. The presence of settlement substrate enhances the chances of establishment of a mussel community. Mussels require high concentrations of methane around their siphons for growth and reproduction. The final size of individuals, as well as the density of the population, is primarily a function of ambient methane concentrations. The mussels can grow extremely fast as well as survive over long time periods.

Mussel communities in sediment diffusion habitats may begin to die out as carbonate precipitation blocks the seepage of methane into the community. In the absence of genetic isolation, a mussel community at a sediment diffusion habitat removed by physical disturbance can re-settle and mature within less than twenty years. Catastrophic mud burial is another cause for the demise of mussel communities. Mussel communities associated with brine seepage can be very long lived.

1.3.3.3 Tube Worm Colonies

Tube worms are the dominant biomass in sediment diffusion habitats. A significant seep community will typically contain many tens of bushes distributed across several hectare of seafloor. A mature bush indicates that petroleum flux and associated microbial activity has persisted at a location for more than 100 years. However, a bush forms and declines though a series of life-history stages. Vestimentiferan bushes display distinctive growth forms, species compositions, and levels of productivity at different stages of their development (newly recruited, mature, senescent). Vestimentiferan spawning is not seasonal, and recruitment is episodic. Vestimentiferans colonize a new site with motile larvae. A larval settlement substrate is required for the establishment of a vestimentiferan colony. This substrate of choice is authigenic carbonate which is formed in areas of active seepage. Both species of vestimentiferan recruit equally to new sites. Juveniles of both species grow relatively quickly to lengths of about 30 cm at sites where sulfide is found in the water around the plumes of the juveniles. Flow of seep fluids from the sediment column to the water column slows down as the bush matures. As a result of carbonate precipitation and sedimentation, the bases of vestimentiferan bushes become covered with an ever increasing amount of sediment. After 10 to 20 years, the transfer of sulfide to the water column is insufficient to carry sulfide to the plumes of the vestimentiferans and they begin to take up sulfide across the buried, terminal portion of their tube. Under these conditions, the lamellibrachid outcompetes the escarpid-like species and begins to dominate the assemblage. The lamellibrachid continues to grow very slowly. Individuals can reach lengths of three meters and live for hundreds of years (Fisher et al. 1997). Eventually, as a result of a combination of continued authigenic carbonate precipitation and sulfide depletion by animals on the edge of the aggregation, the animals in the center of the bush begin to die. Re-channeling of seepage due to carbonate precipitation eventually cuts off the supply of sulfide to the aggregations and the demise of the entire aggregation ensues.

1.3.3.4 Energy Flow and Food Web

Chemical energy is converted into biomass through chemoautotrophic fixation by free-living and symbiotic bacteria. Because of the advantages inherent in multi-cellular anatomy and physiology, both mussels and tube worms are very efficient at extracting chemicals from the environment and converting them into biomass. The endemic heterotrophic fauna rely almost entirely on seep chemosynthesis for their food. Free-living bacteria are the major source of the food for most of the endemic heterotrophic fauna. Primary production of seeps finds its way into the food chain of the deep Gulf of Mexico through crustaceans, fish, and other associated fauna that feed on the seep communities.

1.3.3.5 Change at the Community Level

Three processes contribute to change at the community level: recruitment, succession of aggregations, and episodic instability due to localized processes (e.g., gas hydrate formation/dissociation, mud expulsion). The current model suggests that changes in chemosynthetic community structures at seep communities occur over time scales of a few to tens of years. Substantial change is expected to be observed in the abundance and distribution of bacterial mats. Significant changes may occur in the abundance and distribution of mussels that are greatest for mussel communities that occur in the sediment diffusion habitats. Lesser changes will be evident in the tube worm colonies, particularly among mature bushes. Catastrophic events may cause localized effects in the community, but the frequency of these occurrences is unknown. The occurrence of these "rare" events can only be determined through long-term monitoring of the community including the *in-situ* measurement of various physical/chemical variables.

1.3.3.6 Regional Characteristics

The major regional feature of Gulf of Mexico communities is their prevalence across the northern continental slope. This suggests that these communities are routinely subjected to the effects of eddy-driven circulation events and variations in temperature, pressure, and sedimentation rates. Although physical oceanography is not a major focus of this program, the need for a minimum set of oceanographic observation is indicated by previous results.

Genetically isolated populations are inherently unstable, and thus susceptible to extinction as a result of disturbance. In contrast, populations characterized by extensive gene flow are insulated from catastrophic decline by the capacity for resettlement. Proximity of communities on the slope and circulation characteristics of the slope suggest that the gene pool of chemosynthetic species should be well-mixed, but existing taxonomic descriptions are not sufficient to determine if this is true. Modern molecular techniques can provide the information needed by delineating corridors of gene flow.

1.3.3.7 Geologic Stability

Geological evidence demonstrates that hydrocarbon and brine seepage has persisted on the northern Gulf of Mexico slope for thousands of years. Seeps are associated with migration conduits that connect the seafloor to deep sub-surface reservoirs containing gaseous and liquid hydrocarbons that were generated millions of years ago. Defining the processes that determine the geological cycle of seepage, i.e., reservoir dynamics, large-scale halokinesis, sedimentation rates, etc., is largely beyond the scope of this program. It is possible, however, to make qualitative evaluations of the relative age of the primary study sites and to identify additional sites of various ages. This will improve prediction of community presence/absence and increase the understanding of the long-term fate of communities.

1.4 Program Milestones

Program milestones for the first year are as follows and are shown in Figure 1.3:

1) Sample collection and electronic data entry in the field.
2) Sample transmittal to the laboratory.
3) Processing within the laboratory and completion of analyses.
4) Transmission of the analytical results to data management.
5) NODC data archiving agreement.
6) Completion of Cruises 1 and 2.
7) Cruise reports.
8) Interim Report Draft.

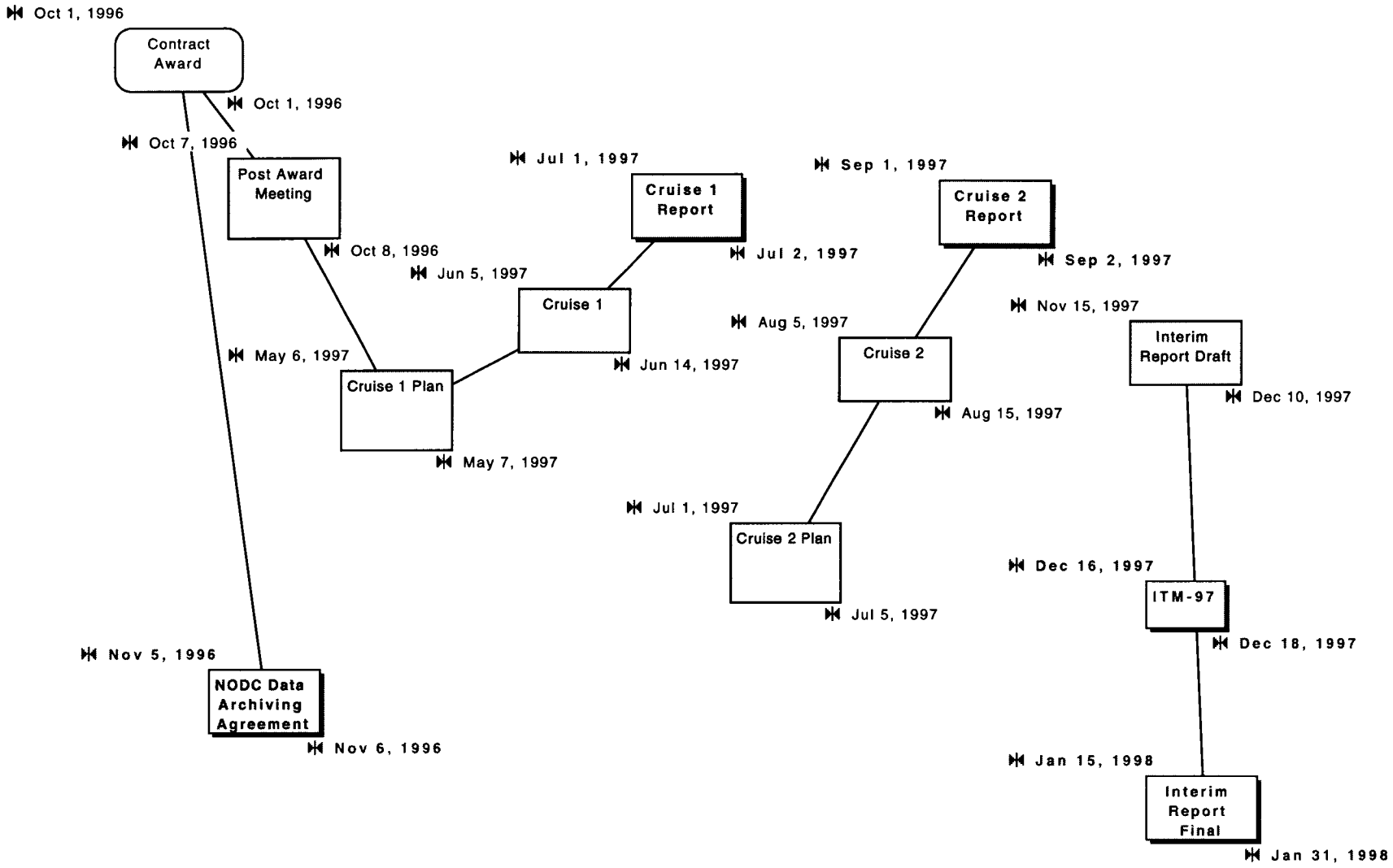

Figure 1.3. Program milestones for year one.

2.0 PROGRAM-WIDE ACTIVITIES

This section reports on activities that support all of the investigations being undertaken within the program. This includes the fieldwork to obtain materials for analysis and regional observations. The fieldwork during Year 1 included a geophysical survey with the TAMU^2 side scan and sub-bottom profiler and a submersible sampling cruise with the *Johnson Sea Link* submersible.

2.1 Study Sites

The program is focused in the northern Gulf of Mexico south of Louisiana. This region contains numerous natural seeps, several of which have been previously studied (Kennicutt et al. 1988; MacDonald et al. 1990b; Robigou et al. 1993; MacDonald et al. 1995; MacDonald et al. 1996). Pertinent historical information was synthesized during the selection of the study sites. One objective for the geophysical survey was to define large "Shallow" and "Deep" water sites. For the community-level studies, specific sites for biological and chemical samplings from submersibles were selected. The regional sites encompass a variety of seepage settings covering a wide bathymetric range. The community-level sites were selected based on previous information and represent the two distinct seepage settings described in the model. Locations of the mega-sites and community-level sites are shown in Figure 2.1. Details of the characteristics of the sites are given below.

2.1.1 Regional Studies

Two mega-sites are designated as survey sites for the development and testing of criteria to improve the prediction of the presence of chemosynthetic communities based on geophysical or other remote sensing data, an important objective of the program.

The size and location of mega-sites are designed to optimize survey operations and provide significant regional coverage. Both areas encompass several types of geological formations. Both contain more than ten (10) perennial sea surface slicks detected by remote sensing techniques. Both sites were surveyed with imaging swath bathymetry (TAMU^2 System). Additional survey data were collected at the Garden Banks (GB) 425 sampling site in support of the community-level studies (see Geophysical Survey section below) for:

1) Megasite 1 ("Shallow"; extended 4 nm south to include GC321) - area 1214 km^2

   a) Boundaries - 91°35'W-91°10'W; 27°36'N-27°50'N.
   b) Contains Green Canyon (GC) blocks 185, 233, and 234 sampling sites.
   c) Contains approximately 13 slicks.
   d) Water depths range from 400-900 m.

2) Megasite 2 ("Deep") - area 1214 km^2

   a) Boundaries - 91°35'W-91°10'W; 27°10'N-27°26'N.

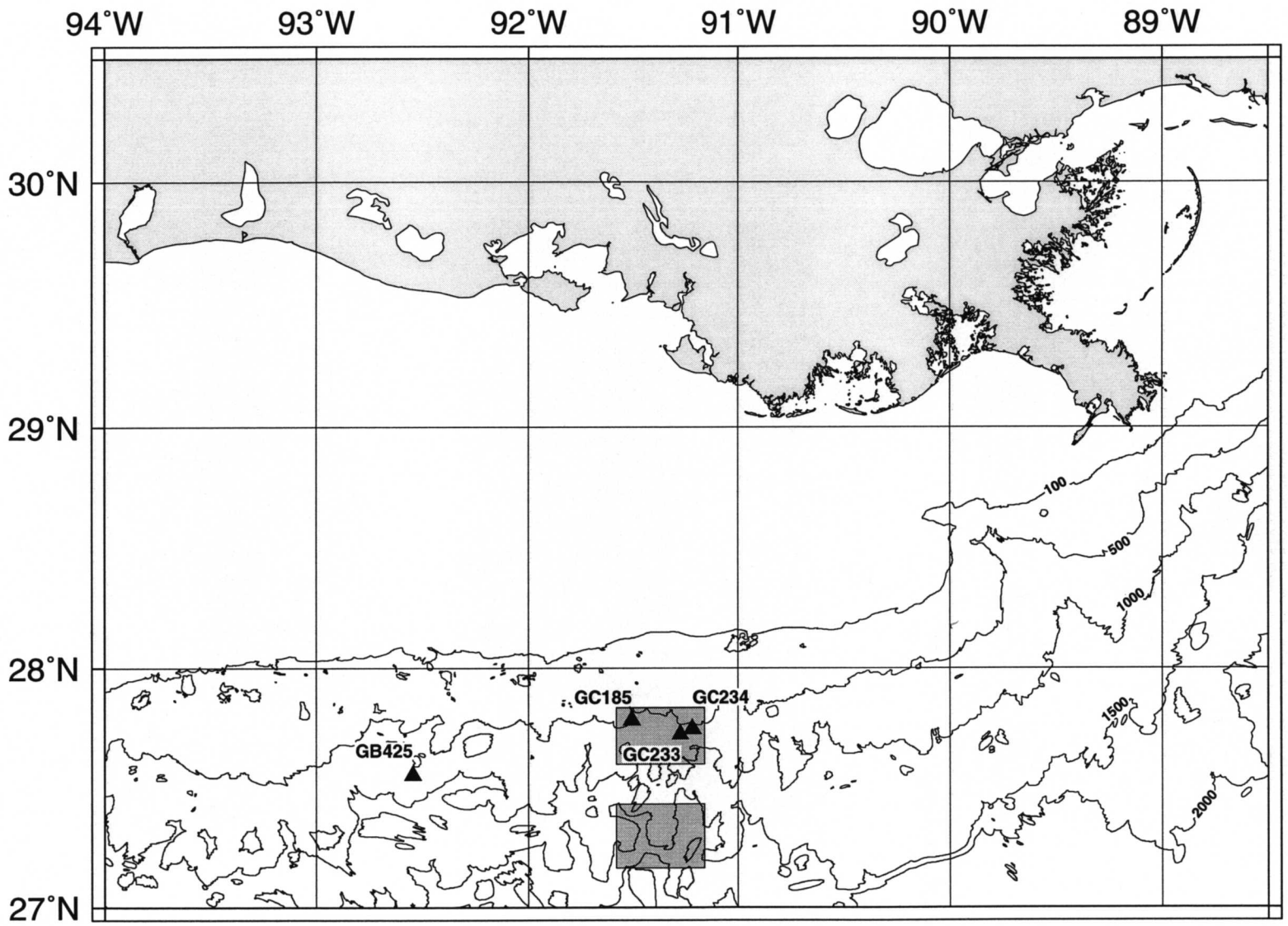

Figure 2.1. Northern Gulf of Mexico showing study site locations for submersible operations (triangles) and mega-site areas for geophysical survey (shaded rectangles). Depth contours in meters.

b) Contains northern Pygmy Basin; eastern Longhorn Basin; and most of Tiger Basin (intrasalt basins).
c) Contains 12 to 15 slicks.
d) Water depths range from 950 to 1250 m.

2.1.2 Community-Level Sampling Sites

Four principal community-level sites were designated for the intensive collection of samples and deployment of experiments and instrumentation. Sites are well-mapped and have been the subject of extensive historical studies. The sites contain the major faunal groups found in chemosynthetic communities (tube worms and mussels) and cover a range of environmental conditions so that hypotheses concerning abiotic controls on community ecology could be tested (Table 2.1).

2.2 Field Work

Field work during the first year consisted of two activities: a geophysical survey and a submersible sampling cruise.

2.2.1 Geophysical Survey

The geophysical survey cruise was conducted with the R/V *Gyre* (Cruise 97-G-4). The goal was to use the TAMU[2] digital side-scan sonar and a 3.5kHz echo-sounder to map the character and distribution of seafloor features in three areas of the Louisiana continental slope where hydrocarbon seeps are known to occur. The three areas are as follows:

1) A 36 sq. nmi (123 km^2) area centered on the mud mound at the border of lease blocks GB424/425 (henceforth "GB425" site).

2) A 315 sq. nmi (1050 km^2) area on the upper slope including the intensively-studied chemosynthetic community sites Bush Hill, Brine Pool *NR-1*, Mussel Beach, and GC234 (henceforth the "Shallow" site).

3) A 360 sq. nmi (1228 km^2) area on the lower middle slope due south of the Shallow site (henceforth the "Deep" site). After completing the geophysical surveys the strategy was to collect cores for 36 hours at the end of the cruise to "ground truth" the geophysical images.

The R/V *Gyre* departed Galveston on 3 June 1997. The GB425 site was surveyed from 4 to 5 June, collecting 45.5 nmi (84.2 km) of trackline data. Seven north-south tracks were surveyed and were spaced 1500 m apart with the sonar swath-width set at 3000 m. In addition, an east-west track was collected over the GB425 vent mound and a sub-circular feature to the west. The "Shallow" site was surveyed from 5 to 9 June, with data collected on 28 north-south tracks (387.0 nm/716.0 km), spaced 1500 m apart, and 7 north-south tracks (38.5 nm/71.2 km) situated between the main lines in the northwest corner (to fill in a shallower water area). As

Table 2.1

Principal Community-Level Sampling Sites and Pertinent Characteristics.

Sampling Site (abbreviation)	Latitude, Longitude	Depth (m)	Fauna present (by dominance)	Seepage properties
GC185	27°46.9' N	550-580	tube worms	high molecular weight hydrocarbons
[Bush Hill (BH)]	91°30.4' W		heterotrophs	free methane to pentane gases
			mussels	
GC234	27°44.1' N	525-560	tube worms	high molecular weight hydrocarbons
[Green Canyon (GC)]	91°15.3' W		heterotrophs	free methane to pentane gases
			mussels	
GC233	27°43.4' N	640	mussels	brine
[Brine Pool (BP)]	91°16.8' W		heterotrophs	free and dissolved methane gas
			tube worms	
GB425	27°33.2' N	600	mussels	brine
[Garden Banks (GB)]	92°32.4' W		heterotrophs (?)	free and dissolved methane gas
			tube worms	high molecular weight hydrocarbons

before, the sonar swath-width was set to 3000 m. Seventeen north-south lines (272.0 nm/503.2 km) were run at the Deep site on 9 to 11 June, but with a spacing of 2500 m and a sonar swath-width of 5000 m. All of the surveys produced high quality data showing faults, carbonate mounds, mud volcanoes, mud flows, sediment flows, and other features whose identity is uncertain.

During recovery of the side-scan sonar tow-fish on 11 June, the tow cable parted at the nose of the fish. After floating briefly, the tow-fish apparently sank. A search was conducted for about 24 hours, but the tow-fish was not found. On 13 June, *Gyre* met a crew boat to exchange technical personnel and obtain core liners. The ship returned to the "Shallow" site for coring. Thirty-one cores (16 piston cores and 15 gravity cores) were collected during a 30-hour period on 13 and 14 June. With the coring finished, the *Gyre* returned to Galveston, docking on 15 June 1977.

2.2.2 Submersible Sampling

A 24-day cruise on board the R/V EDWIN LINK, tender ship for the submersible Johnson SEA-LINK II, was completed from July 8-31, 1997. This cruise (JSL97) comprised a major portion of the field effort for Year 1 of the program. The goals for this cruise were to complete an extensive series of sample collections, to release marked animals for growth studies, to deploy in-situ monitoring instruments, and to map the study sites for future effort. Data produced by this effort was crucial for the success of the program. Despite a very ambitious cruise plan, all goals were achieved thanks to the dedicated efforts of the cruise participants. A total of 52 stations were occupied at four separate sites. A complete list of stations are provided in Table 2.2.2. All collections and observations were logged in a computerized database to facilitate data management. A detailed dive log has been issued as an Appendix to this volume.

Table 2.2

Complete Lists of 52 Stations Occupied at Four Separate Sites. (See Table 2.3 for station abbreviations.)

Site	Station	Depth (m)	Latitude	Longitude	Mosaic Latitude	Mosaic Longtidue	Description
CTDXCT	CTD1	1396	27° 29.56'	91° 16.68'			Site of CTD cast
CTDXCT	CTD12	941	27° 29.90'	91° 29.979'			Site of CTD cast
CTDXCT	CTD13	692	27° 29.90'	91° 29.916'			Site of CTD cast
CTDXCT	CTD2	792	27° 35.00'	91° 16.70'			Site of CTD cast
CTDXCT	CTD3	732	27° 40.00'	91° 16.70'			Site of CTD cast
CTDXCT	CTD4	518	27° 45.00'	91° 16.90'			Site of CTD cast
CTDXCT	CTD5	101	28° 8.48'	91° 16.68'			Site of CTD cast
CTDXCT	CTD6	168	27° 59.98'	91° 30.06'			Site of CTD cast
CTDXCT	CTD7	328	27° 50.06'	91° 30.01'			Site of CTD cast
CTDXCT	CTD8	690	27° 40.05'	91° 29.98'			Site of CTD cast
CTDXCT	CTD9	926	27° 30.01'	91° 30.00'			Site of CTD cast
GB425	CTD2882	567	27° 33.19'	92° 32.44'			CTD on Sub winch
GB425	CTD2883	567	27° 33.19'	92° 32.44'			CTD on Sub winch
GB425	CTD2884	567	27° 33.19'	92° 32.44'			CTD on sub winch
GB425	GBM1	567	27° 33.19'	92° 32.44'	27° 33.1887'	92° 32.4449'	On S side of pool, W side of mussel bed; visible on LLS
GB425	GBM2	568	27° 33.25'	92° 32.3618'			Not shown on LLS
GC185	BHAT1	543	27° 46.99'	91° 30.45'	27° 46.96'	91° 30.46'	Marked with T1 foam float, orange edge, 0.5 m N of small to medium-sized bush. Red Beggiatoa mats, mussel beds 2 and 3 m to south.
GC185	BHAT2	539			27° 46.93'	91° 30.50	MMS
GC185	BHAT3	0					
GC185	BHAT4	0					
GC185	BHAT5	552					
GC185	BHB1	550					Red Beggiatoa mat
GC185	BHB2	550					White Beggiatoa mat
GC185	BHD1	539	27° 46.95'	91° 30.28'			East of Bush Hill. Deployed current meter here.
GC185	BHF	538			27° 46.94'	91° 30.50'	
GC185	BHHYD1	539	27° 46.94'	91° 30.48'	27° 46.9417'	91° 30.4839'	Near ST2 marker and next to LSU Bubblometer.
GC185	BHJT1	541			27° 46.95'	91° 30.48'	

Table 2.2. (Continued).

Site	Station	Depth (m)	Latitude	Longitude	Mosaic Latitude	Mosaic Longtidue	Description
GC185	BHJT2	544			27° 46.97'	91° 30.46'	
GC185	BHJT4	0					
GC185	BHJT5	551					
GC185	BHJT6	0					
GC185	BHM1	552	27° 47.01'	91° 30.49'	27° 46.97'	91° 30.45'	Northern end of Bush Hill. Bearing 030T 50' from large rock with numerous sea sefans. Marked with orange&white foam float and black&white flat square.
GC185	BHM2	546			27° 46.97'	91° 30.47'	Ten ft.SE from large rock (marked with pinger in JSL97 cruise). Large rope, orange&white M2 float, black&white square float
GC185	BHM3	539			27° 46.93'	91° 30.50'	
GC185	BHM4	540	27° 46.945'	91° 30.48'	27° 46.96'	91° 30.46'	On S side of mussel bed.
GC185	BHM5	543	27° 46.00'	91° 30.46'	27° 46.96'	91° 30.47	Between M4 and AT1 when facing E.
GC185	BHR1	533	27° 47.19'	91° 31.05'	27° 47.19'	91° 31.05'	Trap deployment site approx 1 km from GC185 Site for Carney. Surface fix.
GC185	BHST1	541			27° 46.93'	91° 30.50'	
GC185	BHST2	539	27° 46.95'	91° 30.48'	27° 46.94'	91° 30.49'	
GC185	BHTT6	541					
GC185	BHTW2	539					
GC185	BHUN1	540					~315 degrees from LSU Bubblometer.
GC185	BHX1	544					Trap deployment site, near bucket 2, death's head float.
GC233	BPAT1	649					
GC233	BPBRN	650	27° 43.44'	91° 16.78'			This is the brine in the pool--incl sample of brine, gas, dead animals.
GC233	BPM1	649	27° 43.42'	91° 16.76'	27° 43.4245'	91° 16.7616'	Marked with orange-foam float labeled M1. On N side of pool, inner edge of mussels, directly in from milk crates.
GC233	BPM2	648	27° 43.44'	91° 16.76'	27° 43.52'	91° 16.77'	Toward N end of brine pool. Along outer edge of brine pool.
GC233	BPM3	649			27° 43.52'	91° 16.77'	Toward N end of brine pool. Between M2 and M4 as you move radially out from brine along mussel bed.
GC233	BPM4	649	27° 43.44'	91° 16.75'	27° 43.52'	91° 16.77'	Toward N end of brine pool. On inner rdge of brine pool. In line radially with M2 and M3.
GC233	BPM5	649			27° 43.53'	91° 16.77'	Near marker Q on outer edge of mussel bed.
GC233	BPM7	649			27° 43.51'	91° 16.77'	Old MI site (see dive log for dive #2860).

Table 2.2. (Continued).

Site	Station	Depth (m)	Latitude	Longitude	Mosaic Latitude	Mosaic Longtidue	Description
GC233	BPMI	0					
GC233	BPQ	0			27° 43.52'	91° 16.77'	
GC233	BPR1	651	27° 43.45'	91° 16.77'			Cages out North of Pool approx. 50 ft.
GC233	BPR2	652					Tube worm in small colonies 50 - 100 ft from pool at 210T heading.
GC233	BPRT1	0					
GC234	GCAT1	537	27° 44.76'	91° 13.30'			
GC234	GCAT2	536	27° 44.75'	91° 13.34'			
GC234	GCAT3	0					
GC234	GCAT4	0					
GC234	GCB	540					
GC234	GCHYD1	535	27° 44.74'	91° 13.31'			Protruding hydrate mound (first ice worms identified).
GC234	GCHYD2	540	27° 44.75'	91° 13.3'			Second hydrate mound. Deeper and on steep slope (camera deployment).
GC234	GCJ	538	27° 44.77'	91° 13.30'			J float; next to PVC box.
GC234	GCJT1	540	27° 44.75'	91° 13.33'			
GC234	GCJT2	538	27° 44.90'	91° 13.36'			
GC234	GCJT4	0					
GC234	GCJT5	0					
GC234	GCM1	538					
GC234	GCM2	535	27° 44.7'	91° 13.29'			
GC234	GCNM	558					
GC234	GCNM1	558					
GC234	GCNM2	558					
GC234	GCR1	549	27° 44.8'	91° 14.6'			Trap deployment location 1 km W of dive site.
GC234	GCST1	538	27° 44.76'	91° 13.31'			
GC234	GCST2	540	27° 44.76'	91° 13.31'			Flat marker and float. Float points to bush. Bush flanked by square floats Y and RI. Y is bnded and RI stained youngsters.
GC234	GCTx	539					To Carney float ~40-50 degrees.

2.3 Data Management

Data management plays a key role in complex environmental programs such as this that involve sample collection, monitoring, archiving, and chemical/physical/biological analyses. Data management is the central node for the flow of data to program participants and the government. As the central node, data management participates in and oversees the data flow among the various work elements of the program. Data management provides data to project management concerning the status and progress of sample/data collection and analysis to aid in identifying potential conflicts and problems during performance of the program.

Data management monitors, controls, and facilitates data flow, ensuring the integrity of the data through each phase of the program. To accomplish this objective, four interrelated elements are incorporated into the data management plan: 1) data management, 2) data control, 3) data utilization, and 4) archiving with the National Oceanographic Data Center (NODC).

2.3.1 The Data Management Plan

Data management and data analysis is based on four key elements that provide for a systematic approach: 1) comprehension of the program; 2) incorporating data management and analysis during the planning phases of a program; 3) execution of the data management plan during all phases of the program; and 4) analysis and interpretation.

Data management has prepared a detailed summary of the planned data and sample collections for the initial phase of the program. Using this summary, a unique sample designation was assigned to each planned sample collection element. These sample designators were then incorporated into the sample tracking system. This sample designation scheme is the central component for the sample tracking system and forms the basis for documenting the collection and labeling of samples in the field, monitoring their progress to and through the laboratories, and final submission of analytical results.

Appropriate forms for sample collection and data transmittal were developed prior to collection. The uniformity gained by preparation of standardized forms facilitates documentation and ensures the completeness of data entered into the program database. As each field effort was completed, collection information was entered into the database.

As the project investigators complete their sample analysis, they submit their data on task specific electronic data submission forms. Before an investigator's data file is incorporated into the program database, it is subjected to a data screening analysis that provides a quality control procedure that minimizes propagation of errors.

2.3.2 The Project Database

A computerized relational database system is invaluable for the effective search and retrieval of data to support project management and to provide data products and analyses to report authors. Relational systems insulate the end-user from the physical links in the database and allow the

relationships between data to be easily restructured, thus responding quickly to changing needs. The program database is an integrated repository for all information relevant to the study.

To accomplish these objectives, a database structure was implemented with the data management software Microsoft Access. The design of this database made it possible to record all the pertinent information for every sample collection and to associate this information with each sample record in a robust manner. These records were entered at sea during the submersible dive series to minimize transcription errors and to ensure compatibility among the sample logs that each individual investigator maintained. All samples collected from a station, i.e., fixed locations distinguished by certain attributes such as fauna present, could be uniquely identified. By naming these stations with appropriate abbreviations, much of the pertinent station information would be implicit in the station name. A combination of the site code, e.g., BH for GC185, the habitat designation, e.g., AT for adult tube worm, and a number to distinguish among multiple stations of the same type comprised a complete station name such as BHAT2. The list in Table 2.3 shows the complete abbreviation system.

To ask the database questions, the query mode is entered. Under a Query By Example (QBE) system, a display of the field names from the database is displayed. Columns to be included in the query are selected and the query is executed. In this way the database can provide summary information (e.g. the number of samples for trace metals analysis collected at a station).

The program database is divided into two modules. The first module is sample/data status inventory. It consists of the files necessary to track the status and custody of all data and samples collected during the program. Table 2.4 presents this module. The second module of the database contains the analytical results of the samples represented by the "X" in Module 1. The merging of these two modules results in the project database, as represented in Table 2.5.

2.4 Experimental Design and Statistical Analyses

The sampling plan reflects an interdisciplinary approach to meeting the program's objectives. The primary objective of the fieldwork is to determine the environmental factors that regulate stability and change in chemosynthetic communities. In this context, there are six different work elements covering several disciplines. A major goal of the project is to provide a cross work element synthesis of all data collected. This section outlines the approach and methods that will be used to complete the integrated synthesis.

There are two key variables in the sampling design: time and space. Temporal change is analyzed in two ways: measuring change in chemosynthetic communities based on a contrast of archived data with new data and measurement of new variables over a new two year sampling effort. Assessment of temporal change will allow us to determine those factors related to the stability of chemosynthetic communities. Assessing spatial change is more complex, because there are several levels of spatial variability that must be examined. There are four hierarchical levels of spatial variability: the total chemosynthetic community of the Gulf of Mexico (10-100 kms), sites with specific populations of chemosynthetic communities (kms), habitats within sites (which include tube worm colonies, mussel beds, and bacterial mats), and subhabitat gradients

Table 2.3

Site and Habitat Abbreviations

Site Abbreviation	
BH	GC185 (Bush Hill)
GC	GC234 (Green Canyon)
BP	GC233 (Brine Pool *NR-1*)
GB	GB425 (Garden Banks)
Habitat Abbreviations	
AT	Adult Tube Worms
ST	Senescent Tube Worms
JT	Juvenile Tube Worms
M	Mussel Bed
HYD	Outcropping Hydrate
R	Radial Station
UN	Unoccupied Seep Sediments
BRN	Open Brine in a Brine Pool

Table 2.4

Sample/Data Status Inventory.

Module 1	Cruise	Site	Station	Matrix	Habitat	Sub Matrix	Analysis 1	Analysis 2	Analysis n
Obs 1	Jul_97	GC (GC234)	GCAT1	Tissue	Tubewm	Tubewm	X		X
Obs 2	Jul_97	GC (GC234)	GCM1	Tissue	Muss	Muss		X	
Obs 3	Jul_97	GC (GC234)	GCM1	Sed	Muss	Sed	X	X	
Obs 4	Jul_97	BH (GC185)	BHB1	Tissue	Bact	Bact		X	

Table 2.5

Analytical Results of the Samples Represented by the "X" in Module 1.

	Cruise	Site	Station	Matrix	Habitat	Sub Matrix	Sample ID	Result 1	Result 2	Result n
Obs 1	Jul_97	GC (GC234)	GCAT1	Tissue	Tubewm	Tubewm	GCA1	X	X	X
Obs 2	Jul_97	GC (GC234)	GCM1	Tissue	Muss	Muss	GCM6	X	X	X
Obs 3	Jul_97	GC (GC234)	GCM1	Sed	Muss	Sed	GCM9	X	X	X
Obs 4	Jul_97	BH (GC185)	BHB1	Tissue	Bact	Bact	BHB19	X	X	X

(which vary according to habitat type). At the smallest spatial scales (meters to centimeters), heterogeneity in the physical, geological, and geochemical properties is a common occurrence. Characterizing spatial heterogeneity allows for determination of the factors that influence the distribution, abundance and growth of chemosynthetic communities on small spatial scales.

The components of the subhabitat level are different among the three main habitat types. For tube worm habitats, there are at least three identifiable kinds of colonies: young colonies, mature colonies, and senescent colonies. For the mussel beds, there is a gradient of mussel abundance with proximity to brine pools. Mussel beds are different on the inner and outer edges of brine pools. Bacterial mats are inhomogeneous but mainly fall into two groups, pigmented and non-pigmented. Measuring environmental factors and comparing with biological responses allows for the determination of the abiotic features that control stability and change in chemosynthetic communities thus meeting programmatic objectives.

2.4.1 Program Wide Hypotheses

A series of null hypotheses guided the design of a common synoptic sampling program for most of the program work elements. Therefore, the collection of samples among work groups have common elements. Data collected by these elements can be synthesized and interpreted. An overarching program also allows for planning an efficient sampling program that utilizes field logistical resources in a cost-effective manner.

H_o-1: There are no changes in chemosynthetic communities over time.
H_o-2: There are no differences among chemosynthetic communities within the Gulf of Mexico.
H_o-3: There are no differences among habitats of chemosynthetic communities.
H_o-4: There are no differences within subhabitats of chemosynthetic communities.

2.4.2 Elements of the Sampling Plan

Two cruises were planned to determine the stability of chemosynthetic communities. The cruises are to be one year apart. Therefore, time (with two levels: Year 1 and Year 2) is a main effect in the sampling design. The first sampling effort was completed from 8-31 July 1997.

There are three major chemosynthetic habitats being studied: tube worm dominated habitats, mussel dominated habitats, and microbial mat habitats. Four sites will be used to determine the abiotic factors that control the spatial distribution of chemosynthetic communities. The four sites (GC185, GC234, GC233, and GB425) differ principally in that two sites contain significant amounts of liquid hydrocarbons in the sediments and are dominated by tube worms (GC185 and GC234) and two sites are brine seeps that are dominated by mussels (GC233, and GB425). The principal abiotic differences among the two types of sites are that tube worm dominated sites have seepage of liquid hydrocarbons, free and dissolved methane to pentane hydrocarbon gases, and hydrates, whereas the mussel dominated sites have seepage of brine and free and dissolved methane gas. The only variance from this scheme is that the mussel/brine seep habitat at GB425 may contain small amounts of liquid hydrocarbons. An important aspect of this experimental design is that pseudoreplication is avoided by replicating treatment effects (i.e., habitat differences among the two types of sites). Microbial mats exist at all four sites, but are only common and easy to sample at the mussel sites. Therefore, microbial mats are studied at GC233 and GB425. Finally, non-seep or reference sediment is sampled and contrasted with the sediments from the chemosynthetic community sites.

There are also within habitat differences. Another level of spatial variability is being examined to determine the abiotic factors that control growth and development of chemosynthetic communities within a habitat. This level of spatial resolution is called the "subhabitat". In the tube worm habitat, subhabitats are related to colony size and age. There are (small) young assemblages, (large) mature assemblages, and senescent (old) assemblages. In the mussel habitat, there are mussel beds on the inner and outer edges of brine pools. There are two types of heterotrophic bacterial mats: pigmented and non-pigmented. Therefore, we have identified seven subhabitats: three for tube worm, two for mussel, and two for microbial. In addition, for each habitat there are two reference subhabitats, seep and non-seep.

The key elements of the design for the tube worm, mussel, and microbial mat studies are similar. There are three main effects: time, subhabitat, and replicate site. The study designs differ only in that there are a different number of subhabitats for tube worms (3) and mussels and mats (2). With the addition of the two reference stations at each site, this increases the total number of subhabitats to five for tube worms, and three for mussels and mats. However, because many of the habitats occur in the same sites, the reference stations are sampled only once and the data can be used for all three statistical analyses. In addition, the mussel and mat sites have site as a design difference, because two sites contain liquid hydrocarbons and two sites contain brine pools.

2.4.2.1 The Relationship Between Sites and Habitats

All three habitats occur only at the sites containing liquid hydrocarbons. Therefore, most of the study can be accomplished at just two sites (Table 2.6). The tube worm study is to be carried out only at liquid hydrocarbon containing sites. In this case, the site is a form of replication. The mussel study is being carried out at all four sites, so site is a major design element for the mussel study. The bacterial mat study could be carried out at all four sites or at just the liquid hydrocarbon containing sites, as in the tube worm study. Reference habitats (i.e., sediment unoccupied by mussels, mats, or worms) are used to determine the effect of biological communities on background sediment geochemistry. Unoccupied sites are available at all study sites.

2.4.2.2 Samples Taken During the July 1997 Cruise

All four sites and all three habitats were sampled during the first expedition (Table 2.7). The tube worm study was completed with a balanced design. Difficulties in sediment sampling, finding suitable substrate, and time constraints caused the sampling to be unbalanced for other habitats. For example, no push-cores could be obtained at GC233 due to substrate texture. Stations were established where replicate samples were taken. Within each site, most of the experimental design was accomplished (Table 2.8).

2.4.3 Univariate Statistical Model for the Sampling Plan

A statistical model is a mathematical representation of the relationships amongst the variables in the experimental design of the study. The statistical model is used to test null hypotheses. Model development is based on the elements of the design. The following 3-way analysis of variance (ANOVA) model represents the experimental design for the common sampling plan for time, habitats and sites that can be used to test the first three null hypotheses (H_{o1} - H_{o3});

$$Y_{ijkl} = \mu + \alpha_I + \beta_k + \alpha\beta_{jk} + \gamma_l + \alpha\gamma_{jl} + \beta\gamma_{kl} + \alpha\beta\gamma_{jkl} + \varepsilon_{i(jkl)},$$

where Y_{ijkl} is the ith observation, μ is the overall sample mean, α_I is the main treatment effect for time where there are 2 levels of j, β_k is the main treatment effect for subhabitat where there are 4 or 5 levels of k, $\alpha\beta_{jk}$ is the time-habitat interaction, γ_l is the main treatment effect for site where there are 2 or 4 levels of l, $\alpha\gamma_{jl}$ is the time-site interaction, $\beta\gamma_{kl}$ is the habitat-site interaction, $\alpha\beta\gamma_{jkl}$ is the second order interaction for all main effects, and $\varepsilon_{i(jkl)}$ is the random error associated with each of the three replicates. Time and subhabitat are fixed effects, but site is a random effect, because the two sites sampled are only chosen to represent the generality of a larger number of actual sites available. Because this is a mixed model with both random and fixed effects, the expected mean squares for each term must be calculated and the appropriate error term used for each null hypothesis test. The expected mean squares and F tests are the same for all habitats, but differ in the number of degrees of freedom because there is a different number of sites and subhabitats sampled (compare Tables 2.9-2.11).

Table 2.6

Habitats Found Within Sites. There are Two Types of Sites Based on Whether There is Oil (Liquid Hydrocarbon) or Brine Seepage and the Source of the Methane, Through Diffusion or Dissolution. The Letter X Indicates Habitat Present at Site.

	Habitat			
Site	Mussel	Bacterial Mat	Tube Worm	Reference
GC185 Oily, diffusion	X	X	X	X
GC234 Oily, diffusion	X	X	X	X
GC233 Brine, dissolution	X	X		X
GB425 Brine, dissolution	X	X		X

Table 2.7

Number of Stations Occupied During the July 1997 Expedition.

	Habitat			
Site	Mussel	Bacterial Mat	Tube Worm	Reference
GC185 Oily, diffusion	2	2	6	1
GC234 Oily, diffusion	2	2	6	
GC233 Brine, dissolution	6		1	
GB425 Brine, dissolution	2	2		

Table 2.8

Stations Sampled According to the Experimental Design Criteria.

Site	Habitat	Level within Habitat	Stations
GC185	Tube Worm	Adult	AT1, AT2, AT3, AT4, AT5
Oily diffusion		Senescent	ST1, ST2
Station prefix=BH		Juvenile	JT1, JT2, JT4, JT5, JT6
	Mussel		M1, M2, M3, M4, M5
	Bacterial Mat	White Mat	B1
		Red Mat	B2
	Reference	Unoccupied	boxcore from ship
GC234	Tube Worm	Adult	AT1, AT2, AT3, AT4
Oily diffusion		Senescent	ST1, ST2
Station prefix=GC		Juvenile	JT1, JT2, JT4, JT5
	Mussel		M1, M2
	Bacterial Mat		B
GC233	Tube Worm	Adult	AT1
Brine dissolution	Mussel	Inner edge	M1, M4, M7
Station prefix=BP		Outer edge	M2, M3, M5
GB425	Mussel		M1, M2
Brine dissolution			
Station prefix=GC			

Table 2.9

Experimental Design for the Tube worm Sampling Program. Sources are Time (T), Subhabitat (H), Replicate Site (S), Degrees of Freedom (DF), Variance Components That Make the Expected Mean Squares (EMS) for the Mixed Model, and Appropriate Mean Square (MS) Term Used as the Denominator in the F Test for Each Source. There are Four Subhabitats: Young, Mature, Senescent, and Reference. There are Just Two Sites.

Source	DF	EMS	Error Term
T	2-1 = 1	δ(Error)+ δ(T*H*S)+ δ(T*S)+δ(T)	MS(T*S)
H	4-1 = 3	δ(Error)+ δ(T*H*S)+ δ(H)	MS(T*H*S)
S	2-1 = 1	δ(Error)+ δ(T*H*S)+ δ(T*S)+δ(S)	MS(T*S)
T*H	(2-1)(4-1) = 3	δ(Error)+ δ(T*H*S)+ δ(T*H)	MS(T*H*S)
T*S	(2-1)(2-1) = 1	δ(Error)+ δ(T*H*S)+ δ(T*S)	MS(T*H*S)
H*S	(4-1)(2-1) = 3	δ(Error)+ δ(T*H*S)+ δ(H*S)	MS(T*H*S)
T*H*S	(2-1)(4-1)(2-1) = 3	δ(Error)+ δ(T*H*S)	MS(Error)
Error	(2)(4)(2)(2-1) = 16	δ(Error)	
TOTAL	(2)(4)(2)(2)-1 = 31		

Table 2.10

Experimental Design for the Mussel Program. Sources are Time (T), Subhabitat (H), replicate Site (S), Degrees of Freedom (DF), and Variance Components That Make the Expected Mean Squares (EMS) for the Mixed Model, and Appropriate Mean Square (MS) Term Used as the Denominator in the F Test for Each Source. There are Two Subhabitats: In the Center and at the Edge of the Brine Pool. There are Four Sites: Two Oily (Liquid Hydrocarbons) and Two Brine Seeps.

Source	DF	EMS	Error Term
T	2-1 = 1	δ(Error)+δ(T*H*S)+δ(T*S)+δ(T)	MS(T*S)
H	2-1 = 1	δ(Error)+δ(T*H*S)+δ(H)	MS(T*H*S)
S	4-1 = 3	δ(Error)+δ(T*H*S)+δ(T*S)+δ(S)	MS(T*S)
T*H	(2-1)(2-1) = 1	δ(Error)+δ(T*H*S)+δ(T*H)	MS(T*H*S)
T*S	(2-1)(4-1) = 3	δ(Error)+δ(T*H*S)+δ(T*S)	MS(T*H*S)
H*S	(2-1)(4-1) = 3	δ(Error)+δ(T*H*S)+δ(H*S)	MS(T*H*S)
T*H*S	(2-1)(2-1)(4-1) = 3	δ(Error)+δ(T*H*S)	MS(Error)
Error	(2)(2)(4)(2-1) = 16	δ(Error)	
TOTAL	(2)(2)(4)(2)-1 = 31		

Table 2.11

Experimental Design for the Microbial Mat Sampling Program. Sources are Time (T), Subhabitat (H), Replicate Site (S), Degrees of Freedom (DF), and Variance Components That Make the Expected Mean Squares (EMS) for the Mixed Model, and Appropriate Mean Square (MS) Term Used as the Denominator in the F Test for Each Source. There are Two Subhabitats: Pigmented and Non-Pigmented. There are Three Sites.

Source	DF	EMS	Error Term
T	2-1 = 1	δ(Error)+δ(T*H*S)+δ(T*S)+δ(T)	MS(T*S)
H	2-1 = 1	δ(Error)+δ(T*H*S)+δ(H)	MS(T*H*S)
S	3-1 = 2	δ(Error)+δ(T*H*S)+δ(T*S)+δ(S)	MS(T*S)
T*H	(2-1)(2-1) = 1	δ(Error)+δ(T*H*S)+δ(T*H)	MS(T*H*S)
T*S	(2-1)(3-1) = 2	δ(Error)+δ(T*H*S)+δ(T*S)	MS(T*H*S)
H*S	(2-1)(3-1) = 2	δ(Error)+δ(T*H*S)+δ(H*S)	MS(T*H*S)
T*H*S	(2-1)(2-1)(3-1) = 2	δ(Error)+δ(T*H*S)	MS(Error)
Error	(2)(2)(3)(2-1) = 12	δ(Error)	
TOTAL	(2)(2)(3)(2)-1 = 23		

Post hoc multiple comparison tests, such as the Tukey Test, are used to detect differences among sample means for main effects where there are more than two levels. Subhabitat is the only main effect that falls into this category. A priori linear contrasts will also be used to test if the reference station is different from the chemosynthetic subhabitat stations. A priori linear contrasts will be used to determine if there are site differences when both the liquid hydrocarbon and brine seep sites are sampled.

2.4.4 Multivariate Analyses for the Sampling Plan

The models in the above section are univariate, and can be used to test for differences among main effects for the variables measured. The models are also used to test the first three null hypotheses about differences due to temporal change and spatial variability among and within specific chemosynthetic habitats. However, to test the fourth null hypothesis, about differences among the types of chemosynthetic communities, a multivariate analysis is necessary. In this case, all data collected during the study can be synthesized in one general analysis where the experiment wide error rate is controlled. The purpose of the analysis is to identify the abiotic factors that control biotic factors among the three different chemosynthetic habitats.

In the end, the multivariate data set is a matrix consisting of rows of observations for each sample (identified with time, site, habitat, and subhabitat), and columns of values for each measurement taken at each station. Many of the columns of data in this matrix are autocorrelated, or co-vary, therefore, a simple bivariate test, such as calculation of correlation coefficients for all possible combinations of variables is inappropriate and introduces multiple testing error. Multivariate analysis is used so that important relationships are not ignored among the variables and to control the experiment wide error rate.

Principal Components Analysis (PCA) is the multivariate technique of choice. PCA is a transformation of the data set to create another data set with two desirable attributes: the principal components are mutually orthogonal (which means that the columns are now uncorrelated), and the components are extracted in order of decreasing variance. This gives us a data set that has a reduced number of variables, but still contains most of the information in the original data set. The reduced number of variables is used to test hypotheses or make predictions about how abiotic factors are related to biotic factors. Hypotheses are tested by visualizing plots of PCA scores for each row (i.e., observation) where the various elements of the experimental design are plotted as the symbols for the observation. This technique has been used successfully in many environmental studies.

3.0 INDIVIDUAL WORK ELEMENTS

Upon completion of the field work activities for Year 1, the remaining work efforts were carried out in the laboratories of the team members. The following sections provide summaries of the progress to date on individual work elements. Each section highlights sample collections, significant problems (if any), and the general approach to the analyses. Although different work elements have different timelines, the general objective was to have the preliminary results completed for a planning meeting held in early March 1998. This meeting provided an opportunity for plenary discussion of the overall program results, meetings between investigators with shared interests, and planning of field activities for Year 2.

3.1 Imaging and GIS

Principal Investigator: Ian MacDonald.
Institution: Geochemical and Environmental Research Group; Texas A&M University

Research at hydrothermal vents has shown that detecting stability and change in chemosynthetic communities requires precise mapping of sessile fauna in areas that are 100 to 1000 sq m in extent (Hessler et al. 1985; Fustec et al. 1987; Hessler et al. 1988). Mapping diverse data sets into a common coordinate space also makes it possible to test hypotheses regarding the physical/chemical dependencies of chemosynthetic organisms (MacDonald et al. 1989). One of the major challenges is accurate navigation of submersibles that operate at substantial depths. This is difficult because the field of view is extremely limited and the habitat is disorienting. It is often difficult to achieve repeatable sample collections during multi-year programs. Navigation of submersibles was formerly only accurate to within 100 m (LORAN) or 40 m (GPS with selective availability); however, many of the sampling stations are located much less than 40 m apart. Consequently, much of the navigation information recorded during previous visits to the program study sites is unreliable for determining the position of sample collections at a precision that would distinguish unique faunal clusters. The most reliable position information is believed to be the recorded descriptions that locate sample collection in proximity to durable features of the landscape (rocks, large faunal aggregations, etc.) or to prominent markers. Locations of markers deployed at sampling stations during the program's first submersible cruise were determined by combining precise differential GPS navigation with comprehensive seafloor imagery that covers the entire extent of the study sites. Additional effort is underway to determine the position of "old" features and markers so that results from previous studies can be compared with newly obtained results.

Developing and maintaining a common context for the program is crucial to developing an integrated model of community ecology. To accomplish this, all sampling efforts have been focused in study sites that are characterized by distinct seepage styles and at sampling stations within the sites that have definable ecological characteristics. The data management and experimental design sections of this report have previously described how study sites and sampling stations were defined and chosen. This section describes the geographic analyses that provide spatial control for the sampling stations and visual surveys of the study sites. Such mapping provides the context for ecological modeling and also yields standing stock estimates of the various critical components of the communities. The products of this effort will be used at

the completion of the program to determine what degree of change is detectable over the timeframe of the available observations. It is hoped that these products will also become reference materials for future studies that examine seep ecology over longer time scales.

3.1.1 Navigation

During the 1997 sampling cruise with *Johnson Sea Link II*, the tender ship (R/V *Edwin Link*) was navigated with differentially corrected GPS (accurate to approximately 5 m). The position of the submarine on the bottom was determined by use of a short base line acoustic system. An estimate of the submarine's absolute position was obtained from the offset between the two vessels. Although this method is adequate for reoccupying stations, it was sensitive to factors such as how the submarine was oriented and changes in the water column that affect acoustic transmission. Consequently, the navigation system might indicate that the submersible was occupying a particular station, while the estimated position of the station was only accurate to about 10 m. In many cases, errors of as much as 30 m apparently occurred. Efficient submarine sampling at multiple stations requires that the pilot and scientists know the relative bearings between stations. Putting this in perspective, the study sites encompass areas on the order of 100 by 100 m and include from 5 to 15 sampling stations. If station positions are only known with an error of 10 m, then bearings between stations will not be accurate enough for reliable navigation. Consequently, valuable bottom-time is expended and replication of samples is difficult to achieve.

3.1.2 Markers and Sketch Maps

To maximize spatial control, pragmatic operational procedures during the dives and image processing and geographic information system (GIS) methods were combined and applied to the best available information on the study sites. The pragmatic methods included standard markers for the stations and a set of simple sketch maps designed to be used by the submersible scientist and pilot during the dives.

Each station was marked with a combination float and visual scale (Figure 3.1). The float, which was painted with reflective paint, provides high-visibility marking from the vantage point of the submersible. The marker, a 30 by 30 cm square, painted with a black and white checker pattern, provides a marking for an over-head vantage point and a convenient scale for future visual survey efforts. Each marker was labeled with an abbreviated form of the station name, e.g., AT1 for BHAT1. (See Table 2.2 for a complete list of station names.) Sketch maps were hand-drawn maps that included locations of individual stations and relative bearings between stations (Figure 3.2). These sketches were continually updated and modified as stations were marked and occupied, or as better information became available about station locations, adjacent landmarks, etc.

3.1.3 Laser Line Scan Mosaics

Laser line scan system (LLSS) technology uses a solid-state laser and a rotating mirror to illuminate individual scan-lines. By passing the device across the bottom in a track perpendicular to the scan-lines, a LLSS can image a swath of the seafloor up to 30 m wide with

Figure 3.1. Combination float and bottom marker used to mark sampling stations.

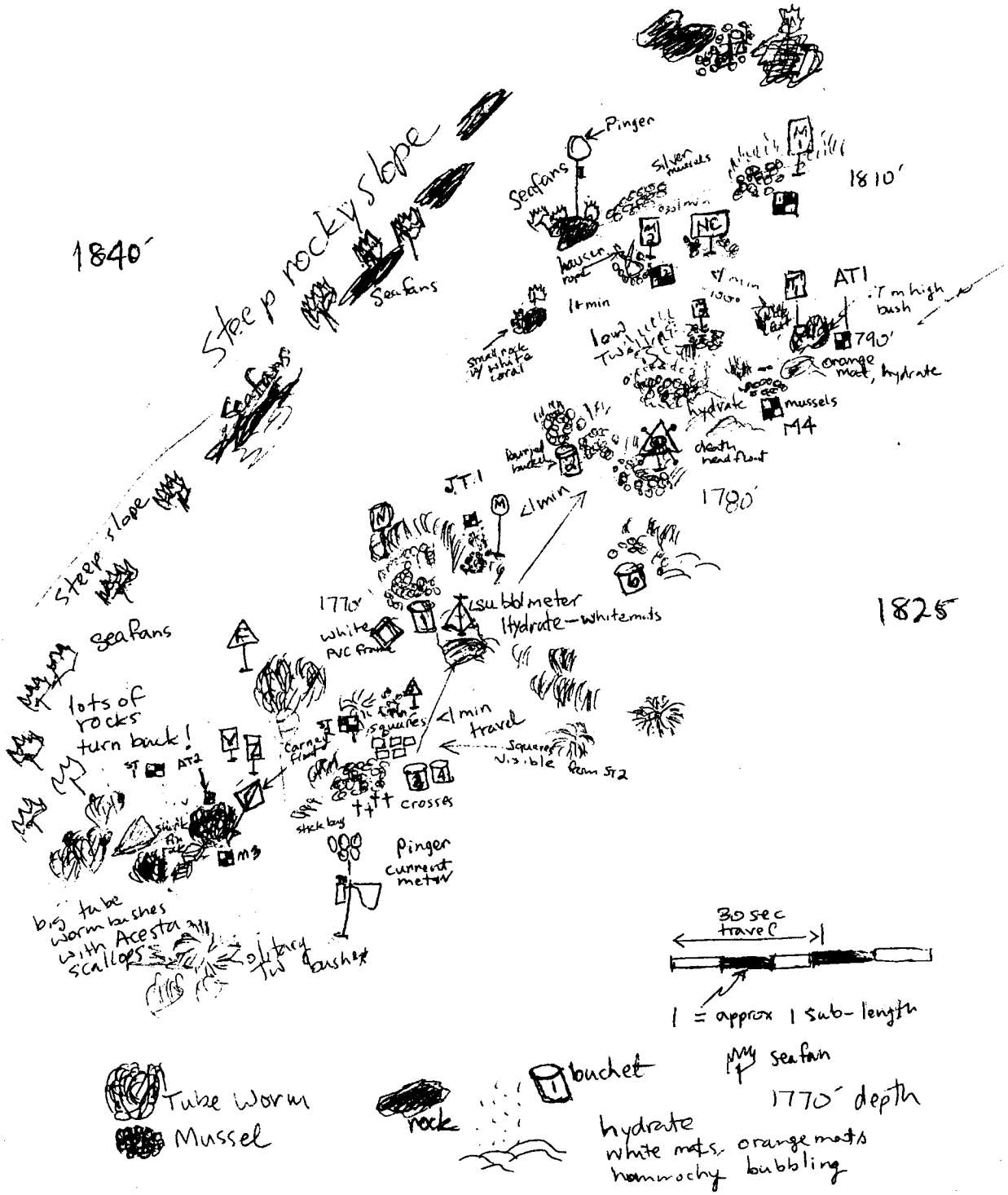

Figure 3.2. Hand-drawn sketch maps of locations for individual stations and the relative bearings between stations.

centimeter resolution. During a 1994 cruise, the Navy Submarine *NR-1* was used for collection of contiguous LLSS images that could be assembled into a mosaic of the seafloor. Construction of mosaics requires ER-MAPPER and PV-WAVE computational programs on UNIX workstations, and additional image-processing routines on PC workstations. The methods have been described in detail in MacDonald et al. (1997).

During the 1994 cruise, data were collected for large-area mosaics of the four sampling sites. The mosaics for three of these sites (GC185, GC233, and GB425) were completed prior to the 1997 submersible cruise. At three of the four study sites, the sketch maps were based on laser line scan mosaics covering all or most of the site. These mosaics provided detailed information about the location of prominent landmarks and faunal clusters. Mosaics also provided an accurate distance and bearing scale for the sketch maps. An example of a laser-line-scan mosaic (at the GC233 study site) with sampling stations overlaid is shown in Figure 3.3.

To combine the information in the laser-line-scan mosaics with the selection of sampling stations accomplished during the submersible cruise, the following procedure was adopted. First, the mosaics were geo-rectified in the ER-MAPPER software environment. No warping of the mosaics was performed; each image was simply pegged to the best-available coordinates of some prominent feature within the site, preserving the scale and rotation information calculated when the mosaic was constructed. The software would then return a latitude and longitude for any pixel in the mosaic. Then the video information and notes from the scientists in the submersible's sphere were used to locate each sampling station within the mosaic. Details of the mosaic are adequate for this in most cases, although some questions remain. Finally, the geo-rectified images were queried in ER-Mapper to determine the interpolated coordinates of each sampling station. Comparison of the interpolated coordinates, with coordinates obtained by the JSL acoustic navigation system, confirmed that there was general agreement between the two methods. The station coordinates determined by interpolation of mosaics and by acoustic navigation are given in Table 2.2.

Work during the first quarter of 1998 involved producing a series of standard maps for each of the study sites based on the combined laser line scan mosaics and observations from *Johnson Sea Link* during the 1997 cruise. Subsequent analysis will estimate total bottom area--and biomass where possible--occupied by seep fauna at each of the study sites. A repeat cruise with Submarine *NR-1* is planned for May, 1998 and will repeat the collection of laser line scan mosaics. Analysis of the new data will provide a basis for testing whether changes in faunal distribution have occurred.

3.2 Geophysical Detection and Characterization of Seep Community Sites

Principal Investigator: Dr. William Sager
Institution: Department of Oceanography, Texas A&M University

Petroleum seep chemosynthetic communities cover a fraction of the seafloor. Therefore, to manage and protect these resources, evaluation of techniques for using remote sensing or geophysical techniques to detect seep sites containing significant seep communities is an

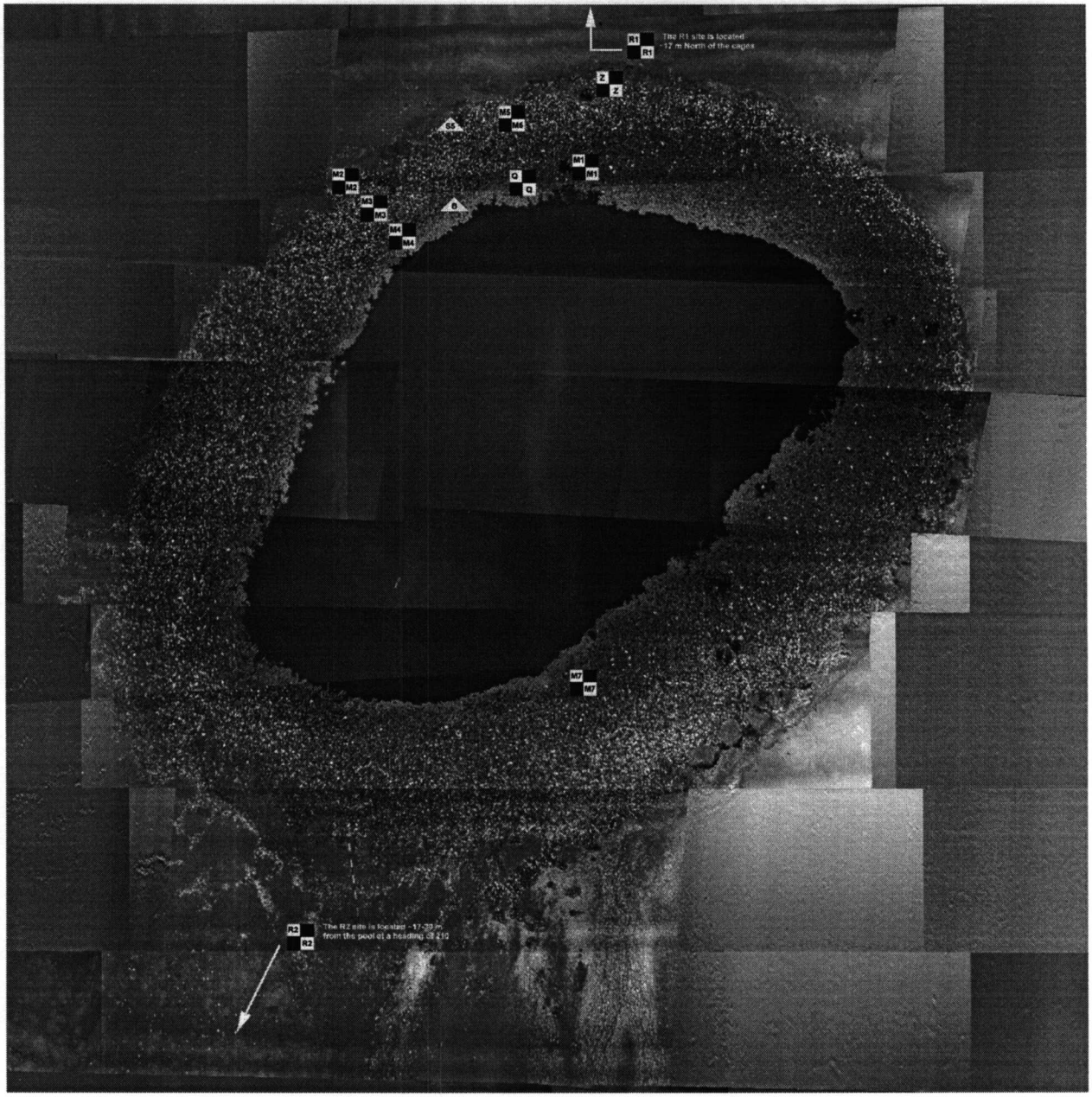

Figure 3.3 Laser line scan mosaic of Brine Pool *NR-1* at GC233 Study Site. Image covers an area 38 m wide by 40 m high. Stations markers not to scale.

important program objective. Although some research has occurred along these lines, it suffers from being done piecemeal -- typically small site surveys with little regional context. Furthermore, the number of sites investigated has been small, so the statistical significance of prior results is uncertain. It was proposed to collect geophysical data that would allow the detection of all sites that could harbor significant seep communities within an area known to contain a large number of active seeps.

Significant seep communities can only rarely be detected directly using geophysical techniques; they are typically too small. Ultimately, some form of near-bottom optical detection (e.g., submersible, ROV, or Laser Line-scanner) is needed. However, the geological conditions that allow significant seep communities to exist, i.e., hydrocarbon seeps, occur in a distinct geological setting and modify their surroundings. These settings and modifications can be remotely detected. One goal, therefore, is to define the geophysical techniques and characteristics that allow for rapid focusing of ground-truthing efforts to a small amount of seafloor.

Historical studies indicate that seeps occur along faults that cut through the sediment cover and intersect petroleum reservoirs (Hovland and Sommerville 1985; Kennicutt and Brooks 1990; Reilly 1995). What is more, not all faults have significant seeps, and even on those faults that do, the seepage is localized. The focused seepage gives rise to modifications of near seafloor physical properties, which can be detected by acoustic profiling techniques (Behrens 1988; Lee 1995; Reilly 1995; Roberts and Neurauter 1990). Briefly, known sediment modifications are: 1) precipitation of authigenic carbonate in the form of micronodules, nodules, or rock masses; 2) formation of gas hydrate; 3) concentration of hard chemosynthetic organism remains (such as shell fragments and layers); 4) formation of interstitial gas bubbles or hydrocarbons; and 5) formation of pockmarks by gas expulsion. These give rise to acoustic effects, such as "wipeout" (no echoes), "hardbottoms" (strongly reflective echoes), bright spots (reflection enhanced layers), or reverberant layers (Behrens 1988; Lee 1995; Roberts and Neurauter 1990). The missing link is a solid correlation of these signatures with chemosynthetic communities. Much is known about a few significant seep community sites, but not enough data exists to determine the certainty of finding a significant seep community at a given seep site.

One approach is to use large-area side-scan sonar surveys to delineate faults and seep-related sediment perturbations in example areas where there are known seeps and significant seep communities and then to ground-truth those sites with deep geophysical or optical data. Where available, industry geophysical data will be incorporated. Three sites were chosen for study surveys. One represents "Shallow" seep sites and another is typical of "Deep" seep sites. Both of these areas are located in the Green Canyon lease block area and are roughly 14 x 22 nm N-S (Figures 3.4 and 3.5). Both contain about 10 or more seeps productive enough to create long-lived slicks on the sea surface (MacDonald et al. 1993). The third survey is a 5 x 6 nm area centered over the active mud mound in Garden Banks blocks 424 and 425 (Figure 3.6). This area was chosen because of its known recent seep activity. The shallow Green Canyon area includes the well-known sites GC184/185 and GC234/235 and is in 400-900 m depths. In this area, an attempt to assess as many of the geophysically determined targets as possible using existing and more easily gathered ground-truth data will be made. The preferred method is to

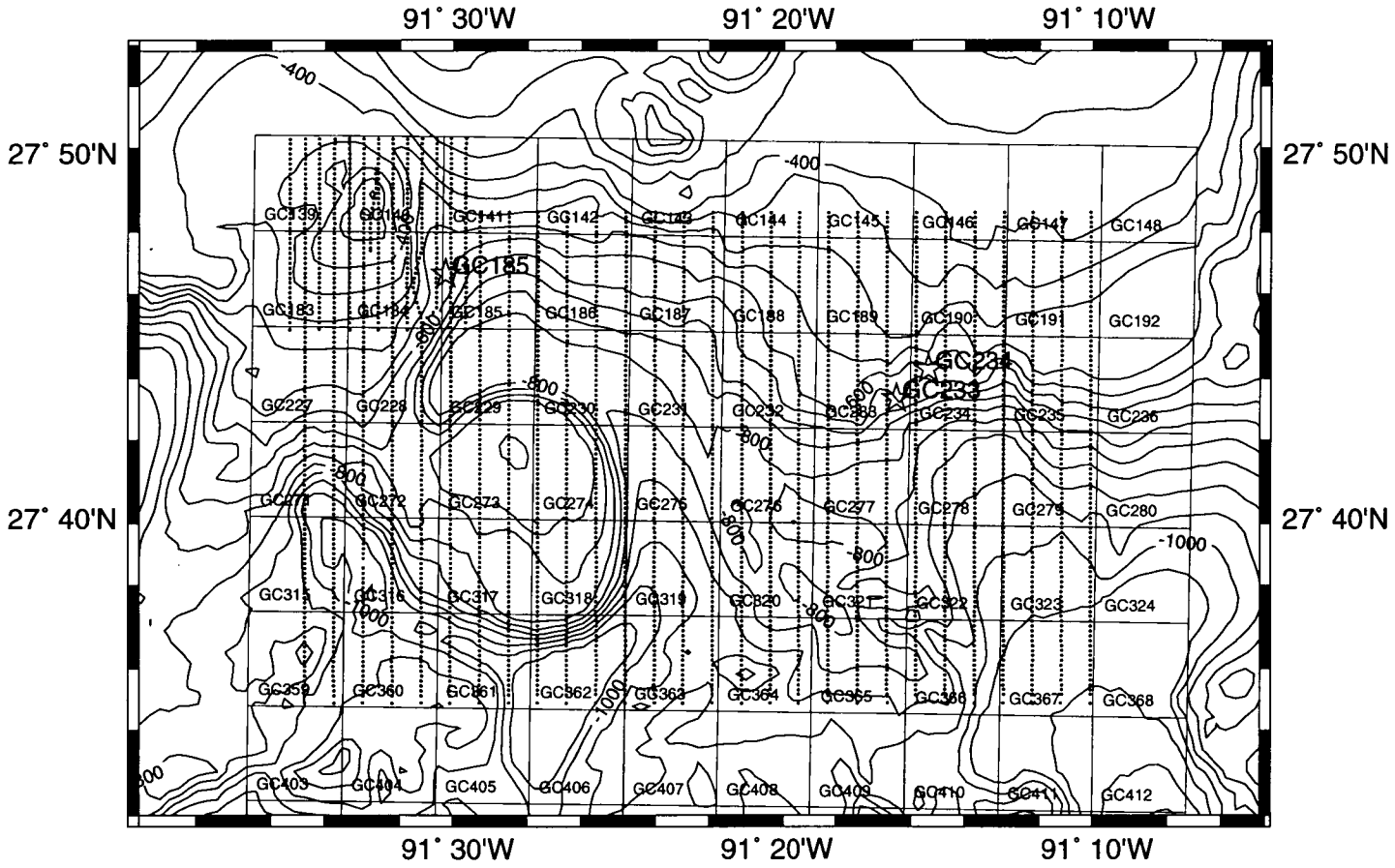

Figure 3.4. Shallow Green Canyon geophysical survey tracks (dotted lines). Stars indicate study site locations.

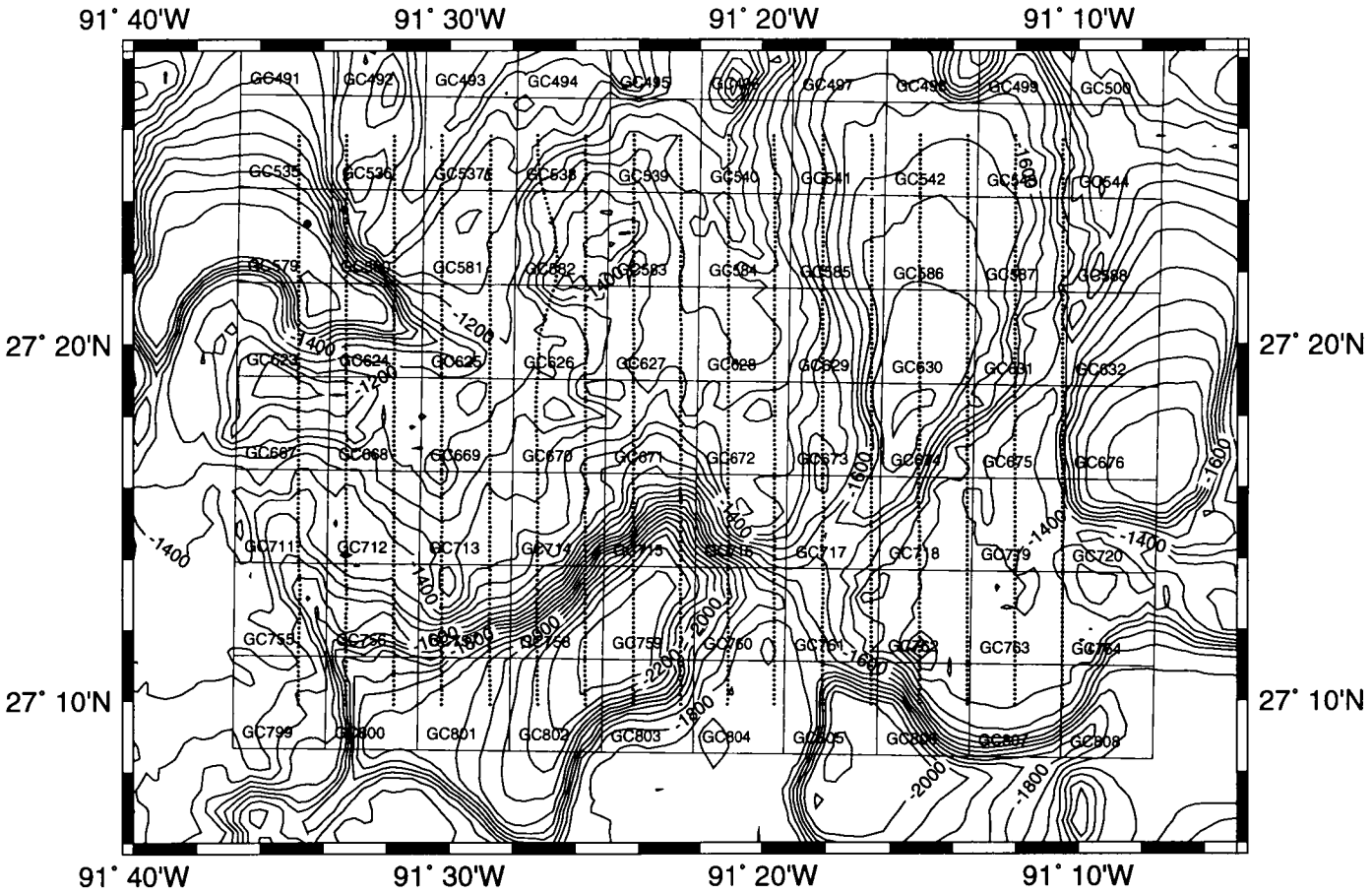

Figure 3.5. Deep Green Canyon geophysical survey tracks. Symbols as in Figure 3.4.

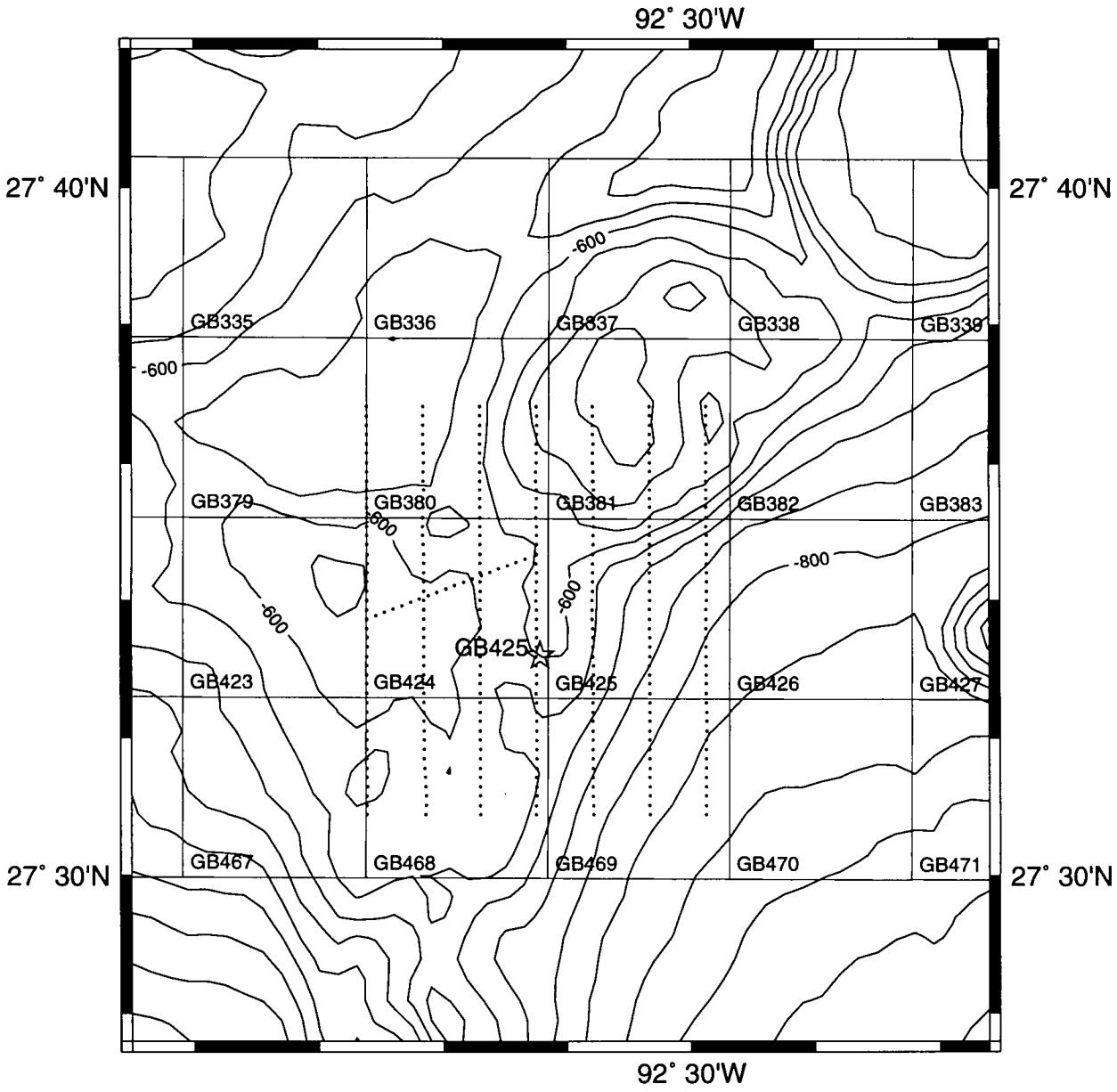

Figure 3.6. Garden Banks geophysical surey tracks. Symbols as in Figure 3.4.

gather Laser Line-scan, subbottom profiles, and side-scan sonar data from the submarine *NR-1* as well as piston cores from surface ships and push cores from submersibles, mainly the Johnson Sea-Link. The GB424/425 survey is in similar depths and will be investigated in the same manner. The deep Green Canyon area has water depths of 1200-2000 m, which is too deep for collection of ground-truth data by *NR-1* and most submersibles. It is valuable, however, because it allows comparisons with the shallow data, even if it is impossible within the scope of this project to ground-truth all sites.

3.2.1 Objectives

The protocol outlined by Reilly (1995) is the starting conceptual model. It proposes significant seep communities will be found at sites that produce long-lived slicks at the sea surface that can be detected by satellite or aerial photography. Having reduced the search area to these sites, they are examined with high-frequency echosounder records for shallow attenuation (wipeout). The idea here is that these zones are caused by shallow hydrocarbons that are necessary to support significant seep communities. Reilly then makes several generalizations designed to further reduce the number of prospective sites: 1) only regional faults (as opposed to faults over salt domes) have the steady, robust seeps needed to support significant seep communities; 2) only seeps with negative seafloor reflection coefficients (those with shallow hydrocarbons but no significant hardbottom) support significant seep communities; and 3) significant seep communities occur only at sites where cores show shallow hydrocarbons and in particular methane and higher hydrocarbons.

Although some of the details of Reilly's protocol may be questioned, it is a useful starting point and sets forth a number of testable hypotheses:

H_O-*1: No significant seep communities are located at a seep that does not produce a long-lasting sea surface slick, visible from the air or space.*

H_O-*2: Significant seep communities are always located on faults.*

H_O-*2a: Significant seep communities are always located on regional faults.*

H_O-*2b: Significant seep communities are never located on faults directly on salt diapirs.*

H_O-*3: Significant seep communities are always located in zones with shallow acoustic signal attenuation (wipeout).*

H_O-*3a: Significant seep communities always occur where the low-frequency seafloor reflection coefficient is negative.*

H_O-*3b: Significant seep communities never occur where the low-frequency seafloor reflection coefficient is positive.*

H_O-4: Significant seep communities always occur where hydrocarbons are present in piston cores.

H_O-4a: Significant seep communities always occur where sediment cores show significant concentrations of pentane and higher molecular weight hydrocarbons.

The main objectives of this work element are: 1) to use a geophysical data set to locate all of the potential sites of hydrocarbon seepage within an area broad enough to contain many seeps; 2) to use submersible observations and other ground-truth data to determine which sites support significant seep communities and what are their geophysical signatures; 3) and to use this comparison to evaluate geophysical methods of seep location. In particular, the objective is to test hypotheses set out by the geophysical detection protocol (Reilly 1995) and to modify the protocol after the results are completed.

More specific objectives, addressing different data sets, are as follows. High-resolution side-scan sonar data, collected from the sea surface will be used to map faults and areas of enhanced (or modified) acoustic backscatter caused by seep-induced carbonate or hydrate formation. These areas should be where significant seep communities occur. Subbottom profiler data, collected during the same cruise and subsequently with submarine *NR-1*, will be used to map the occurrence of surface faults and zones where the sediment properties have been modified by seepage (wipeout, reverberant layers, bright layers, etc). Existing NOAA multibeam bathymetry data will be compiled and compared with these two data sets to show seep-related bathymetric modifications (such as mud or carbonate mounds). Industry multichannel data will be examined for wipeout and reflection coefficient characteristics when available. The geologic and acoustic characteristics of piston cores and push cores collected from seep targets seeking evidence of sediment alteration by seepage and significant seep community remains will be analyzed. Finally, in order to test for significant seep communities at the target sites, Laser Line-scan, high-frequency side-scan sonar, and high-frequency subbottom profiler data collected for ground-truth by ROV, submersible, or the submarine *NR-1* (depending on availability) will be analyzed.

3.2.2 Methodologies

The survey design is a compromise using as large an area as feasible and the least expensive geophysical technique to adequately and rapidly cover the study area because geophysical data are expensive to collect and process. Much of the knowledge of the geophysical characteristics of seeps comes from seismic reflection data of either high or low frequency. Moreover, the petroleum industry typically gathers high-frequency reflection data for hazard surveys and low-frequency data for reservoir exploration. To be effective, such data must be collected on closely spaced ship tracks, making these types of data expensive to collect and interpret, especially the low-frequency multichannel lines. Therefore, a high-resolution, low-frequency side-scan sonar, the TAMU[2] system, was chosen to collect sonar images of the seafloor. These data will show fault traces that disturb the seafloor (i.e., active faults), as well as places where the seafloor sediment properties have been altered by seepage. This might take the form, for example, of highly reflective areas caused by authigenic carbonate precipitation as well as mounds or pockmarks owing to fluid expulsion.

The TAMU[2] sonar images will be mosaicked and interpreted by tracing seafloor features (a preliminary interpretation map of a portion of the shallow Green Canyon site is shown in Figure 3.7). This will be done in an interactive process that includes using subbottom profiler data (collected simultaneously with the sonar data), NOAA multibeam echosounder bathymetry, available industry seismic profiles, and other data to make maps of the fault traces and zones where sediments have been modified by seepage. Using these data, the following maps of each survey area will be made: 1) bathymetry, 2) side-scan sonar mosaic, and 3) seep-related reflection disturbance (from the subbottom profiler records). If industry multichannel seismic data is obtained that delineates the underlying salt bodies, origins of the faults will be determined. A preliminary interpretation of salt dynamics has already been done for the "Shallow" survey area using widely-spaced tracks (Mann et al. 1987).

From the maps, a number of targets will be identified that appear to be potential sites for significant seep communities. Industry seismic data will be requested as needed during the course of the program defining the geologic context and for testing the utility of low-frequency multichannel data to distinguish significant seep community sites. The hydrocarbon content of cores from these sites will be examined by taking a limited number of cores, by having them analyzed for hydrocarbons, and by using existing core analyses from data archives. Magnetic susceptibility profiles will be made for the new cores because studies indicate that the magnetic minerals are destroyed in organic-rich anoxic sediments (Machel and Burton 1991). This measurement quickly and inexpensively has the potential to show the presence of hydrocarbons in the sediments. Finally, near-bottom optical images and possibly geophysical data will be collected over significant seep community candidate sites using the submarine *NR-1*, an ROV, or the *Johnson Sea Link* submersible (whichever is available). If geophysical data can be obtained from the *NR-1*, it will analyzed in a manner similar to the previous study (Lee 1995). The reflection characteristics of the high-frequency subbottom profiler records will be analyzed, plotted in map form, and related to significant seep community occurrence, bathymetry, and seafloor character.

Finally, the augmented data set of significant seep community characteristics will be used to test the hypotheses implied by Reilly's (1995) protocol. Subsequently, the protocol will be modified according to the new findings.

3.2.3 Preliminary Results

Cruise 97-G-4 of the R/V *Gyre* collected side-scan sonar and subbottom profiler data along with gravity and piston cores from 3 to 15 June 1997. Side-scan sonar data were collected with the 11/12 kHz digital, long-range TAMU[2] system. Subbottom profiles were collected simultaneously with a 3.5 kHz echosounder. Subsequently, 31 cores (15 gravity and 16 piston cores) were obtained for ground-truth from the "Shallow" site (Table 3.1; Figure 3.8). The GB424/425 site was surveyed from 4 to 5 June, collecting 45.5 nm (84.2 km) of trackline data. Seven north-south tracks were run (Figure 3.6), spaced 1500 m apart with the sonar swath-width at 3000 m (1500 m on either side of the ship) to achieve 100% overlap. In addition, an east-west track was collected over the GB425 vent mound and a subcircular feature to the west. The

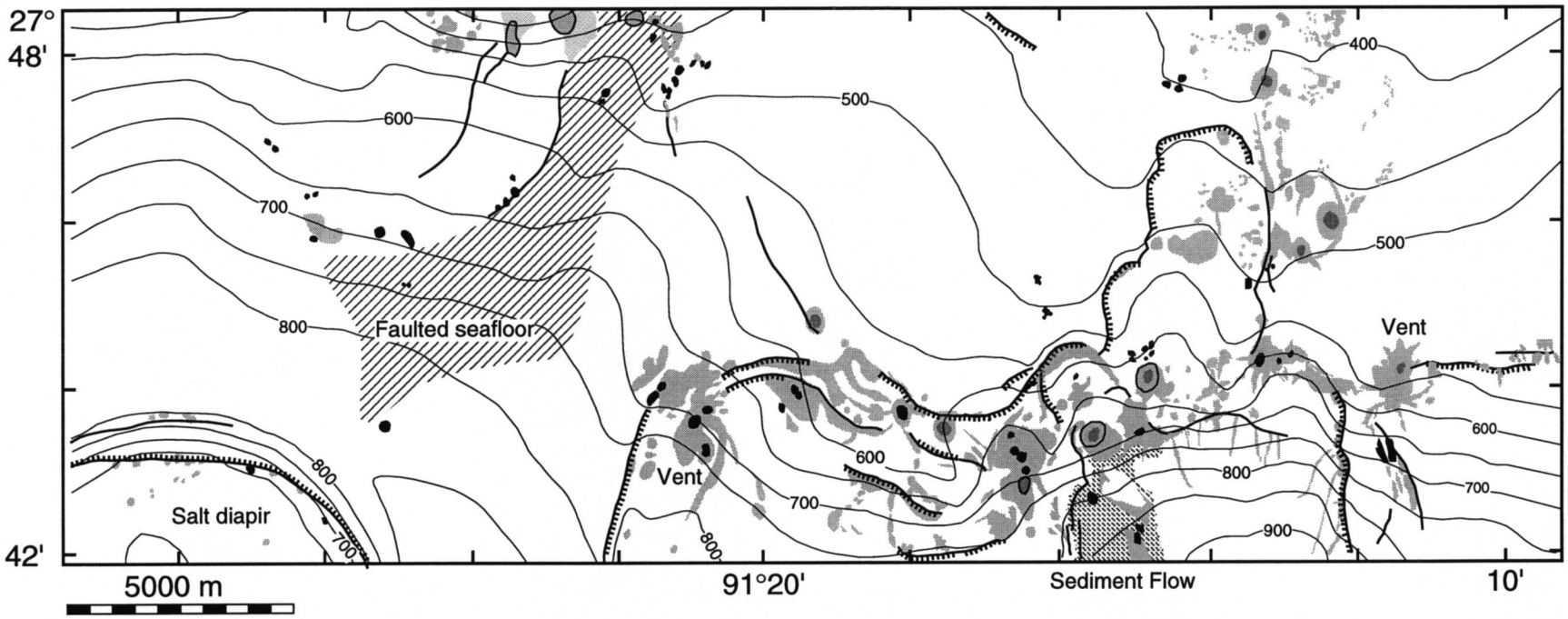

Figure 3.7. Interpretation map of a portion of the shallow Green Canyon TAMU[2] side-scan sonar survey. Bathymetry contours shown at 50-m intervals, labeled every 100 m. Shaded areas denote seafloor with high acoustic backscatter, perhaps caused by hard bottoms, carbonate precipitation, or gas-charged sediments. Dark spots show features that appear to be mounds from acoustic shadows. Heavy lines represent faults; teeth show down thrown sides where shown by acoustic shadows. Cross-hatched areas show zones of pervasively faulted seafloor (northwest part of figure) and a large sediment flow (southeast part of figure). Several "bulls-eye" features are noted and these may be mud mounds or brine pools. Some areas of high acoustic backscatter have radial arms suggesting sediment flow outward from vent sources.

Table 3.1
Cruise 97-G-4 Core Locations.

Core	On Bottom Date	Time (UT)	Depth (m)	Latitude (d)	Latitude (m)	Longitude (d)	Longitude (m)	Lease Block	Core Length (m)	Core Type*
GC1-1	13-Jun-97	13:32	420	27	48.097	-91	13.025	GC146	4.61	P
GC1-2	13-Jun-97	14:12	420	27	47.832	-91	13.370	GC146	1.42	G
GC2-1	13-Jun-97	14:52	405	27	46.351	-91	12.471	GC191	5.20	P
GC2-2	13-Jun-97	15:33	407	27	46.363	-91	12.475	GC191	0.04	G
GC3-1	13-Jun-97	16:18	519	27	44.277	-91	11.337	GC235	5.51	P
GC3-2	13-Jun-97	16:51	519	27	44.228	-91	11.513	GC235	1.47	G
GC4-1	13-Jun-97	17:55	641	27	43.475	-91	15.560	GC234	5.43	P
GC4-2	13-Jun-97	18:20	641	27	43.469	-91	15.644	GC234	1.03	G
GC5-1	13-Jun-97	19:04	809	27	42.401	-91	15.661	GC278	4.27	P
GC6-1	13-Jun-97	19:53	832	27	42.425	-91	15.193	GC278	4.16	P
GC7-1	13-Jun-97	20:50	559	27	43.762	-91	18.052	GC233	2.18	P
GC7-2	13-Jun-97	21:10	575	27	43.729	-91	17.946	GC233	1.34	G
GC8-1	13-Jun-97	21:42	639	27	43.436	-91	17.050	GC233	4.57	P
GC8-2	13-Jun-97	22:16	592	27	43.371	-91	17.417	GC233	1.30	G
GC9-1	13-Jun-97	22:55	688	27	43.457	-91	20.950	GC232	4.20	P
GC9-2	13-Jun-97	23:38	688	27	43.542	-91	21.004	GC232	1.48	G
GC10-1	14-Jun-97	0:36	766	27	38.859	-91	20.906	GC320	4.25	P
GC10-2	14-Jun-97	0:57	766	27	38.906	-91	20.684	GC320	1.36	G
GC11-1	14-Jun-97	1:46	766	27	38.910	-91	20.473	GC320	4.00	P
GC12-1	14-Jun-97	2:39	795	27	38.320	-91	20.340	GC320	4.08	P
GC13-1	14-Jun-97	3:34	890	27	35.779	-91	23.133	GC363	4.08	P
GC14-1	14-Jun-97	5:29	985	27	39.972	-91	33.805	GC315	3.78	P
GC15-1	14-Jun-97	6:44	726	27	40.363	-91	32.215	GC272	3.93	P
GC16-1	14-Jun-97	15:36	676	27	41.574	-91	32.398	GC272	0.74	G
GC17-1	14-Jun-97	16:17	630	27	43.054	-91	32.108	GC228	1.41	G
GC18-1	14-Jun-97	17:26	279	27	49.369	-91	33.055	GC140	1.28	G
GC19-1	14-Jun-97	17:52	327	27	49.234	-91	33.256	GC140	1.92	G
GC19-2	14-Jun-97	18:46	263	27	48.396	-91	33.129	GC140	0.25	G
GC20-1	14-Jun-97	19:21	401	27	47.947	-91	35.434	GC139	1.33	G
GC21-1	14-Jun-97	10:37	1152	27	23.764	-91	33.345	GC580	1.50	G
GC22-1	14-Jun-97	11:43	1542	27	23.365	-91	34.530	GC579	1.67	G

*Type: P = piston; G= gravity.

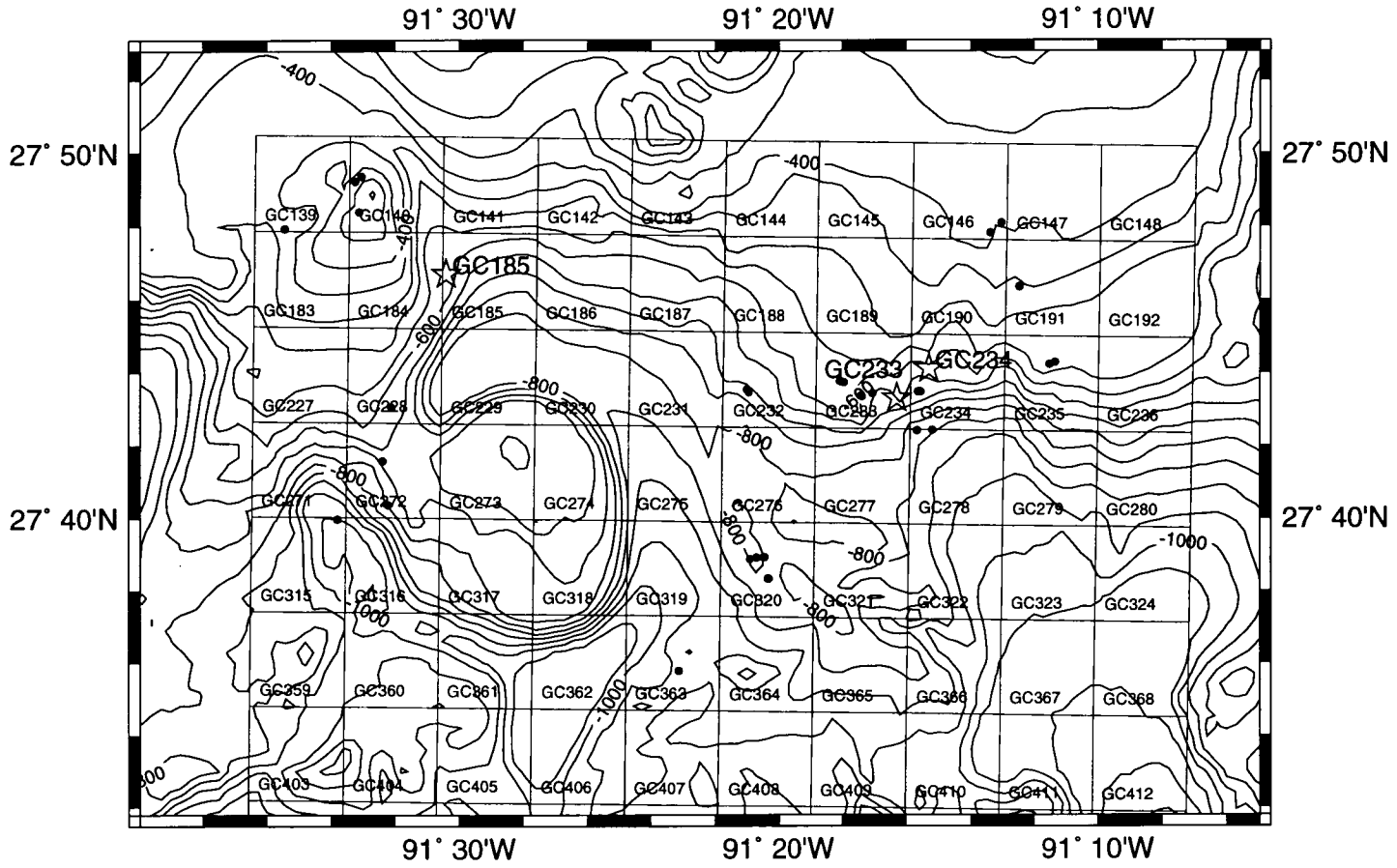

Figure 3.8. Locations of gravity and piston cores in the shallow Green Canyon survey area. Bathymetry and lease blocks as in Figures 3.4-3.6. Filled circles show core locations.

shallow Green Canyon site was surveyed from 5 to 9 June, with data collected on 28 north-south tracks (387.0 nm/716.0 km), spaced 1500 m apart, and 7 north-south tracks (38.5 nm/71.2 km) situated between the main lines in the northwest corner (to fill in a shallower water area over a salt dome). As before, the sonar swath-width was set to 3000 m. Seventeen north-south lines (272.0 nm/503.2 km) were run at the deep Green Canyon site on 9 to 11 June, but with a spacing of 2500 m and a sonar swath-width of 5000 m. All of the surveys produced excellent quality data showing faults, carbonate mounds, mud volcanoes, mud flows, sediment flows, and other features whose identity is not yet certain. These geophysical data will form the basis of our interpretations of significant seep community locations.

During 1998, the submarine *NR-1* will be used to collect Laser Line-scan, high-frequency subbottom profiler, and visual observations on potential seep targets picked from the TAMU[2] sonar images. These data will provide ground-truth information to help interpret the sonar images and to determine where significant seep community sites occur. Initial interpretation of the side-scan images shows there are many targets that are potential seep sites. Several of the blocks within the survey areas have industry-collected geophysical data sets. Some of these data will be used for comparison with new survey and ground-truth data.

3.3 Ecology of Seep Fauna

Principal Investigators: Drs. Charles Fisher, Kimberlyn Nelson, and Stephen W. Schaeffer
Institution: Pennsylvania State University

A primary objective of this program is to develop an understanding of the Gulf of Mexico seep communities that will allow determination of the relative robustness and ephemerality of these communities and allow development of conceptual models of seep community ecology. To achieve these goals it is necessary to: (1) determine the variables (physical, chemical, and biological) that control the occurrence, distribution, growth and health of the primary ecosystem structuring fauna and quantify the impact of these variables on the growth and health of the fauna; (2) understand the life history of the chemosynthetic fauna, including larval dispersal capabilities, recruitment periodicity and life spans; (3) identify the pools of reduced substrates utilized by the chemosynthetic fauna and their sources; (4) identify the significant biological interactions which effect community structure, succession, and senescence; and (5) determine if the populations at different sites are genetically isolated and if differences between communities at different sites are due to heredity or environment. The work proposed here will be integrated with that of the other investigators involved in this project to meet the five goals listed above. The specific hypotheses to be addressed and the methods are detailed below.

3.3.1 Objectives

The following hypotheses will be tested:

H_o-1 *Growth rate and final size of seep mussel 1a is a direct function of ambient methane concentration in the mussel bed.*

H_o-2 *In reciprocal transplant studies, growth of the transplants will mimic their new neighbors (no genetic influence).*

H_o-3 *Density and productivity of mussel beds is a direct function of ambient methane concentration in the bed.*

H_o-4 *The populations of seep mussel 1a in the Gulf of Mexico are not genetically isolated.*

H_o-5 *There is directional gene flow between populations of mussels in the Gulf of Mexico as a result of the prevailing currents used by the larvae.*

H_o-6 *Recruitment of seep mussel to the Brine Pool is seasonal, reflecting yearly spawning events.*

H_o-7 *Young vestimentiferans grow relatively quickly.*

H_o-8 *Sulfide is present around the plumes of young vestimentiferans.*

H_o-9 *Sulfide is not present around the plumes of individuals in mature bushes but is present near the point of original attachment of the tubes (buried under a variable depth of sediment).*

H_o-10 *In very mature (senescent) bushes, the individuals in the center of the bushes will be less healthy and grow slower than those at the periphery of the bushes.*

H_o-11 *Growth rate and final size of each species of vestimentiferan is a direct function of ambient sulfide concentration.*

H_o-12 *Vestimentiferans can live in excess of 200 years.*

H_o-13 *Size frequency of vestimentiferans in single bushes reflect recruitment of all individuals to that site within a period of 5 years.*

H_o-14 *Size frequency of vestimentiferans in very young aggregations reflect yearly recruitment events.*

H_o-15 *The population of vestimentiferans within a site are genetically homogeneous.*

H_o-16 *The populations of vestimentiferans at different sites are genetically isolated from each other.*

H_o-17 *There is directional gene flow between populations of vestimentiferans in the Gulf of Mexico as a result of the prevailing currents used by the larvae.*

H_o-18 *Mussel and vestimentiferan populations will not show the same patterns of genetic isolation due to differences in their larval biology (time as larvae, height in the water column of larval transport, etc.).*

H_o-19 The extreme age of the individuals in mature bushes will be reflected in genetic differences between these individuals and young individuals from the same site.

3.3.2 Methodologies

The following methodologies will be utilized:

- Small volume (1 mL) water samples will be collected from biologically relevant points among and beneath the tubeworms and mussels using the four samplers designed and built for use with the JSL submersibles. Water from above the sediment, at preset distances within the sediment (2.5, 5, and 10 cm depths), and from any depth up to 75 cm in the sediment can be sampled with this equipment.

- Water analyses: salinity by refractometry; dissolved gases (H_2S, CH_4, O_2, N_2, CO_2) by gas chromatography of water or blood samples injected into a custom in-line gas stripper.

- Mussel growth by mark and recapture within a site and with reciprocal transplants.

- Mussel condition by condition index (CI) and tissue water content.

- Factors influencing distribution, growth, and condition of the mussels by analysis of small volume water samples taken from 2.5 cm below the tops of their shells.

- Tube worm growth by videoscopic analysis of tagged individuals and single and double staining of tubes followed by videoscopic analysis and analysis of collected animals.

- Tube worm condition by modified CI and tissue water content.

- Factors influencing distribution, growth, and condition of the tubeworms by analysis of small volume water samples taken from plume height and under sediments, adjacent to the tube attachment site.

- Quantitative collection methods for mussels include collection of all fauna from a space delineated by a 0.5 m stainless steel ring using suction sampler and manual collection. For tubeworms, collection of intact bushes and associated fauna will be made using new equipment to be built under this contract.

- DNA fingerprints will be determined for each individual with up to 5 different genetic markers that will be developed from microsatellites, M13 fingerprinting, intron-targeted PCR, or RAPDs. The technology available with the ABI 373A Automated Sequencer to multiplex genetic markers and simultaneously analyze multiple individuals, will enable the generation of fingerprints on the large number of individuals necessary for this study. The results of the fingerprinting will be analyzed

using recent population genetic models developed for these types of polymorphic markers.

3.3.3 Preliminary Results

The following preliminary results have been obtained to date.

3.3.3.1 Mussel Community Ecology

The first field cruise was more than 100% successful with respect to the mussel community ecology studies. All proposed collections were made and all proposed experiments were initiated:

1) It was proposed to mark and measure 800 to 1200 mussels in Year 1 as part of establishment of the growth sites and transplant studies: 1,300 mussels were marked, measured, and released to initiate all proposed growth experiments.

2) It was proposed to subsample 80 mussels in Year 1 and analyze an initial 48 for Condition Index (CI) and tissue water content. 236 mussels were subsampled for CI and tissue water content analyses. These analyses are underway, about 1/2 of the shell volume determinations are complete, and processing of animals for water content and ash-free dry-weight determination has begun. The additional animals represent samples from an additional time course condition experiment designed to aid in the interpretation of these data (56 animals), collections of previously marked mussels (20 animals), and all ring collections (an extra 50 animals which will considerably enrich interpretation of the associated community data). Subsampling of the mussel collections for histopathology and tissue hydrocarbon burden analyses was conducted.

3) It was proposed to make and analyze eight (8) ring collections of mussels and associated fauna in Year 1. A total of 12 ring collections were made. All mussels in each collection were counted and measured. All fauna from each ring has been sorted, and the videos of each collection to add-in the "escapees" and "left-overs" documented by video are being reviewed. Biomass determinations will be conducted in the next quarter. All rings were subsampled for stable isotope analysis.

4) The results of the simulation studies for estimation of growth parameters from the marked recapture data are in press in the *Canadian Journal of Fisheries and Aquatic Science*, and the simulation studies are currently being used to investigate a nonlinear regression method for comparison of growth parameters between populations. Because bias in the estimation of growth parameters for a population was a function of the variability of the parameters within the population, methods of estimating the individual variability within a population are currently being examined (which will allow for reliable estimation of the error in growth parameter estimation from real populations). The results of these studies are essential to the interpretation of the growth data we are currently collecting. Simulation studies to examine the potential pitfalls in interpretation of size frequency data are being used.

5) A paper on the Brine Pool community (zones, biomass, size-frequency, microhabitat chemistry, growth rates, and associated fauna biomass) is being prepared. This paper will combine data from the past three years, with data from a few ring collections made this year, and the results of some of the above simulation studies. This manuscript should be ready for review by early next year.

3.3.3.2 Vestimentiferan Community Ecology

The objectives of establishing growth stations for the vestimentifera community studies was successfully accomplished. The monitoring of previously banded worms and restaining of previously stained individuals was not completely successful. Collections of stained individuals exceeded expectations and the new quantitative collection devices (dubbed Bush Masters) were used successfully several times (a year ahead of schedule). A "Bush Master" is a large, inverted basket of nylon netting that is open at the bottom and maintains its shape by means of several flexible rods. To collect a sample, the device is placed over a tube worm cluster. Hydraulic rams attached to the rods then tighten a steel cable that encircles the net opening. When the net is firmly cinched around the base of the cluster, the tube worms are pulled loose from the sediments by the submarine arm, which holds the sampler until the sample is removed on deck.

About thirty (30) of the previously banded individuals will be monitored during this program. Due to time constraints, only 11 animals were videoscopically documented in sufficient detail for growth measurements during Cruise 1. This can be rectified next year with minimal loss of interpretive value to the data.

The re-staining of previously stained animals (with a different color stain) was not accomplished during the cruise due to a combination of time constraints and technical problems with the new stain (which have since been rectified). However all twelve proposed aggregations (2 each, juvenile, adult, and senescent, at each of two sites) were stained in at least two positions. Additionally, 180 stained individuals were collected from five different aggregations, significantly exceeding expectations for Year 1. Additional collections of previous years' experiments demonstrated that the young worms can extend their tube below the point of original attachment. All stained animals have been measured and about 40 have been processed for condition index analysis.

The "Bush Masters" were used successfully three times. Some problems with the design were apparent, and both devices are being outfitted with stronger and more flexible "ribs" for next year's field effort. These collections were successful in providing both their quantitative collection of tube worms and recovering associated fauna for other work elements (e.g., food webs). Additionally, the habitat for the Seep Mytilid Species III (a rare mussel) was discovered at the base of young tube worm aggregations. All tube worms in each collection were counted and a subsample of each was processed for biomass determinations. Associated fauna have been sorted and subsampled for other work elements.

3.3.3.3 Mussel and Vestimentiferan Population Ecology

Significant portions of the collections necessary for the population genetics portion of this study were completed. As originally envisioned, some additional collections will be made during the second year field activities. In addition to the collections proposed (and described below), Dr. Harry Roberts, of Louisiana State University, provided additional collections of tube worms and/or mussels from three sites, a significant enhancement of the collections, at no additional cost. Additional subsamples from every individual were collected and archived for a parallel study of the population genetics of the symbionts of the mussels and tube worms (gill samples from the mussels and trophosome from the tube worms).

For the mussels (SM1a), ten (10) animals each were subsampled from collections at the Brine Pool, the Garden Banks site, two different mussel beds at GC234, and five different mussel beds at Bush Hill. A single collection from another mussel bed at Bush Hill will be needed to complete the collections as proposed.

For the tube worms, between six (6) and ten (10) animals of each species were sampled from a Brine Pool collection, a GC234 collection, and from six different bushes at Bush Hill [three (3) mature bushes and three (3) juvenile bushes]. Subsamples from three additional mature bushes at Bush Hill will be collected when stained worms are collected at the same location. Another collection from a fourth geographic area next year is needed to complete the collections proposed.

An additional, potentially valuable, set of unplanned collections was also made. Another species of mussel, Seep Mytilid III, were routinely collected along with the "Bush Master" vestimentiferan collections. Although aware of the existence of this "rare" species, its preferred habitat was only discovered this year (partially buried amongst the base of tube worm bushes). The three collections of this species of mussel (two from Bush Hill and one from the Brine Pool area) were subsampled. The genetic analysis of these samples will provide strong evidence for the generality of any findings with respect to the Seep Mytilid Ia mussels. However, this effort is not within the scope of the current program.

3.3.3.4 Microsatellite Development

The isolation of microsatellites from the two species of tube worms and the mussels is well underway. Microsatellites are highly repetitive sequences in the genomes of organisms. Their utility for DNA fingerprinting, forensics, paternity identification and questions pertaining to molecular ecology is undisputed, and a wealth of primary literature and a large body of court cases illustrates their practical uses for identifying related individuals and samples. A technique for the high quality purification of genomic DNA from these organisms has been refined and standardized. The routine purification of DNA from the population samples has begun. These DNAs will be available for screening with the microsatellites as they are isolated.

DNA for microsatellite isolation has been size-selected, and linkers for cloning have been placed on the restriction enzyme digested DNA. Microsatellites will be used to determine the relationships within and between populations of tube worms and mussels and to address issues of

gene flow between distant sites. The DNA is ready for the first round of cloning in *E. coli* and screening with repeat sequence oligonucleotides.

3.3.3.5 Characterization of Seep Microhabitats

The water sampling and analysis efforts were compromised because the gas chromatographic quantification of sulfide was undependable due to contaminated carrier gas. However, this did not effect the analysis of methane or oxygen from the mussel microhabitats. It was proposed to collect and analyze 32 water samples for mussel habitat characterization. Forty (40) water samples from mussel habitats were taken and analyzed on board ship and another three (3) were taken and analyzed in conjunction with the time-course condition index experiment. An additional sampling tool and analytical method was developed (in collaboration with D. Julian of San Francisco State University) which allowed for the detection of sub-micromolar levels of hydrogen sulfide in ambient bottom water (from around the animals plumes). Over 20 water samples were taken from among and above vestimentiferan bushes and analyzed for sulfide. Numerous additional samples taken with a water sampling apparatus were utilized by the other investigators.

3.4 Spatial and Temporal Comparison of Reproductive State, Histopathology, and Health of Seep Mussels and Tube Worms

Principal Investigator: Eric Powell; Co-Principal Investigator: Mahlon C. Kennicutt II
Institution: Haskin Shellfish Research Laboratory, Rutgers University; Geochemical and Environmental Research Group, Texas A&M University

One important programmatic concern is the spatial and temporal status (health) of communities at petroleum seeps, particularly the large sessile species harboring chemoautotrophic symbionts, such as mussels and tube worms. The assessment of community health can be approached in several ways. In one approach, community health can be examined at the population level by monitoring species composition, abundance, and size frequency. In a second approach, health can be evaluated at the level of the individuals within the population. Health, in this approach, is generally evaluated using a suite of physiological indices such as disease incidence, size and condition, and reproductive state. Both the recent MMS-sponsored GOOMEX program and the NOAA National Status and Trends (NS&T) program have demonstrated the usefulness of this approach. In particular, it was found that in the NS&T program, size, condition, reproductive state, and disease/parasite prevalence and intensity were important indicators of health in oysters and mussels (Powell et al. 1992b; Wilson et al. 1992). These variables responded to significant changes in the natural environment (Powell et al. 1992b; Wilson et al. 1992) and could be correlated with human activities as measured by land use (Craig et al. 1989; Wilson et al. 1990). In GOOMEX, size and parasite-infection intensity, in particular, were significantly impacted by nearness to oil and gas production activities in several species of shrimp, crabs, and starfish (Ellis et al. 1996).

Evidence indicates that site chemistry varies on time scales as short as weekly and at least as long as decadal (Callender and Powell 1997). Long-term changes eventually produce significant changes in the biota, including a complete demise of the seep community. Stratigraphic studies

show that seep bivalve communities can disappear (and appear) relatively rapidly on a geological time scale, perhaps over periods of about 10 years. However, these changes are relatively slow on a human time scale. Stratigraphic studies also indicate that time periods of hundreds of years are required to reestablish a seep community once it has disappeared, although it may reappear relatively quickly once the process begins. Finally, stratigraphic studies indicate that populations wax and wane in abundance and health (as measured by adult size) during a period of relative stability of the seep community. The waxing and waning of populations is likely tied to the availability of sulfide and methane. Discriminating populations that are in decline from those that are healthy and correlating the state of these populations with the availability of reduced molecules is, therefore, a key component to understanding the processes controlling the structure and persistence of the seep community.

Seep communities cannot be sampled intensively (e.g., monthly) over the year; the best achievable sampling frequency is yearly sampling. Thus, an early warning of changes in community health, which can be obtained from a single analysis or a time series of infrequent analyses, would be most useful. This approach has been used successfully by the NS&T program, now in its thirteenth year. The NS&T program uses a once-per-year sampling and basic health indices, including histopathology, condition, and size frequency, to document changes in population health. Powell et al. (1992a) and Wilson et al. (1992) review the success of this approach.

Classically, loss of condition and reduced fecundity have been used in bivalves to evaluate a decline in health. The NS&T program has once again shown the usefulness of these relatively coarse measures. However, both the NS&T and GOOMEX programs, as well as other studies and a number of recent theoretical treatments (Hofmann et al. 1992; Hofmann et al. 1994), have identified ambiguities in the interpretation of these measures when sampling intensity is low, and particularly when sampling can occur no more frequently than once per year. What has become clear over the last two decades, however, and what has been clearly demonstrated by the GOOMEX and NS&T programs, is the sensitivity of pathology, disease, and parasite intensity to changes in the environment. Small reductions in health result in significant increases in susceptibility to diseases and parasites, while prevalence and infection intensity increases. Similarly, pathologies such as digestive gland atrophy, tumors, and the like increase in frequency and intensity as overall health declines.

The concept that reduced health, brought on by pollutants, limitations in food resource availability, etc., results in increased susceptibility to a range of disease conditions was initially proposed by Laird (1961) and has received support from a wide array of subsequent studies. Recent modeling efforts (Hofmann et al. 1995; Powell et al. 1996) provide a theoretical underpinning for how small changes in the environment can produce large changes in the prevalence and intensity of parasitism and disease. Both GOOMEX and the NS&T programs have demonstrated the sensitivity of these measures of health to spatial and temporal changes in the environment. Accordingly, individual health, as measured by the prevalence and intensity of parasites, disease, and tissue pathology, should provide a valuable tool at petroleum seeps for comparing the health of populations, and may provide a useful early warning signal of long-term changes in the health of seep communities that may eventually result in local extinction.

3.4.1 Objectives

This component addresses the primary study objective: to characterize the age, growth rate, turnover rates, reproduction and recruitment, and patterns of senescence and death in the dominant chemosynthetic animals. This study will directly identify the stage of reproductive development in key chemosynthetic species and relate that stage to variations in community health and the physical environment. The study will also directly determine the health of key populations of chemosynthetic species and establish the usefulness of histopathology as a monitoring tool to evaluate long-term trends in community health.

The objectives of this work element are as follows:

1) Identify the prevalent pathologies and parasites present in mussels and tube worms taken from selected sites.

2) Evaluate the intensity of the occurrence of these parasites and pathologies from at least ten mussels or six tube worms from one or more populations at each selected site.

3) If sampling permits, also:

   a) evaluate the effect of changes in environmental conditions on histopathology in mussels using a reciprocal transplant experimental approach;

   b) evaluate the variation in health of individual tube worms within bushes to test models of bush senescence; and

   c) correlate these data with other measurements made at the same sites, including sediment chemistry, population attributes of the species (e.g. size and condition), and, where known, the time history of the population obtained from a time series of samplings or camera transects.

4) Evaluate the usefulness of histopathology in determining population health and in supplying an early warning signal of significant changes in population health.

For mussels, comparisons will also be drawn with a large set of data for various mussel species (*Mytilus edulis*, *Mytilus californianus*, and other *Mytilus* species) sampled by the NS&T program. Although these other species do not have chemoautotrophic symbionts, they fall victim to a number of pathologies, diseases, and parasites that might be expected to have analogues at petroleum seeps. The NS&T program has identified a number of locales where populations are not healthy (as defined by a high prevalence and intensity of selected pathologies and diseases). These populations will be used as reference populations in comparing relative health amongst the various seep populations.

Histopathological analyses will also permit an evaluation of gonadal state. Data on gonadal state for all analyzed specimens. As for histopathology, these data will be correlated with other measurements made at the same sites, including sediment chemistry, population attributes of the species (e.g., size and condition) and, where known, the time history of the population obtained

from a time series of samplings or camera transects. From these correlations, the usefulness of gonadal index in determining population health and in supplying an early warning signal of significant changes in population health will be evaluated.

One of the most common features of chemosynthetic community sites in the northern Gulf of Mexico is petroleum seeps. The original discovery of these communities was during trawling in areas of known seepage, testing the hypothesis that high levels of natural hydrocarbon seepage would suppress the local ecology. To date, it has not been demonstrated that seep animals are adapted to highly toxic environments and thus the question still remains whether toxins (i.e., polycyclic aromatic hydrocarbons) at the sites exert control over the occurrence, distribution, structure, and longevity of chemosynthetic communities. It is well documented that petroleum in the environment can cause a range of acute and sublethal effects in biological organisms. It has also been demonstrated that hydrocarbons can differentially effect species causing shifts or changes in community structure.

The use of bivalves as indicators of contaminant exposure is well established as the "mussel watch" concept. Bivalves are used to monitor the exposure of communities to toxins in many marine environments. Bivalves have been chosen because they are: 1) sentinel (attached); 2) relatively resistant to toxic effects; 3) unable to detoxify contaminants; 4) relatively long-lived; 5) dependent on filter feeding for nourishment providing a time integrated assessment of contamination; and 6) abundant. At seep sites, mussels are important members of the communities and therefore should provide insight into contaminant exposure histories. While seep mussels are less dependent on filter feeding than their shallow water variants, their lipophilic tissues will serve as accumulators of hydrophilic contaminants. It has also been demonstrated that there are large differences in the concentrations of hydrocarbons at seep sites. Therefore it was proposed to conduct a "mini-mussel watch program" using individual mussels at seep sites as assessors of hydrocarbon exposure history. Individuals at different sites and within sites in discrete mussel beds were collected to compare polycyclic aromatic hydrocarbon body burden with animal health. The primary measures of organismal health are provided by the histopathology portion of this program which was concomitantly collected.

3.4.2 Methodologies

Gonadal state and histopathological condition will be assessed on 222 specimens in each year. If sampling permits, these specimens will be distributed as described in Table 3.2.

Sampling is contingent on submersible operations. Submersible logistics may modify the above sampling plan as follows:

1) Transplant and senescence experiments may be replaced by additional population samples.

2) Tube worm samples may be replaced by mussel samples, because tube worms require more time for collection.

Table 3.2

Assessment of Gonadal State and Histopathological Condition.

Assessment	Year 1	Year 2
Primary site sampling[1]		
Mussel (5 sites, 1-2 replicates @ 10 per replicate)	110	100
Tube worms (6 sites, 1-2 replicates @ 6 per replicate)	72	52
Reciprocal transplants[2]		
Mussel (4 experiments @ 10 each)	10	40
Tube worm senescence[2]		
(4 bushes @ 15 per bush)	30	30
Total analyses	222	222

[1]Sample site designations are given by Fisher elsewhere in this report.
[2]Descriptions of these experiments are provided by Fisher elsewhere in this report.

3) Samples may be obtained in differing proportions in Years 1 and 2, depending upon submersible schedule.

In addition, if preliminary comparisons of hydrocarbon body burden and histopathology are promising, additional comparison sites may be included in Year 2.

The following indices of health will be measured on bivalves (mussels) and tube worms chosen from selected sites: condition index (for mussels), reproductive stage, and histopathology (prevalence and infection intensity of parasites and pathologies). Each of these indices has been used extensively by other "sentinel" monitoring programs to examine population health.

Reproductive stage and histopathology are measured using standard histological techniques. Upon recovery, the animals are preserved in Davidson's fixative. Davidson's-fixed animals is used for analysis of histopathology and reproductive stage following the methods used in the NS&T program (Powell et al. 1993). All assessments are based on quantitative measures or semiquantitative scales so that reproductive stage and parasite/pathology infection intensity can be rigorously evaluated statistically. Direct appraisal of this approach during the GOOMEX program showed the power of using quantitative scales of infection intensity, rather than just prevalence. Nearly all statistically-significant relationships were observed using quantified measures. Earlier work in the NS&T program had indicated that this approach would be advantageous (Wilson et al. 1990; Wilson et al. 1992). Reproductive stage and certain pathologies, like digestive gland atrophy, are assigned semiquantitative scales describing stage of development (for the former) or severity of the effect (for the latter). Most parasites and certain pathologies are subjected to direct counts using consistently-obtained tissue cross-sections (Powell et al. 1993; Sericano et al. 1993).

For mussels, the proposed analyses are standard histological analyses with well-defined semiquantitative and quantitative scales available by adaptation from their NS&T program equivalents for gonadal state and most anticipated pathologies, diseases, and parasites. For tube

worms, most semiquantitative and quantitative scales will have to be developed de novo. Basic protocols have been established for scale development during GOOMEX, when new taxa were included in the analysis (several species of crabs, shrimp, and in particular starfish, in that case). In that successful approach a standard is set for the use of quantitative counts for all discrete parasites and local pathologies (e.g., focal inflammation). For wide-spread, ramifying, or invasive diseases/pathologies (e.g., tumors, digestive gland atrophy, pathologies of gonadal developmental, or certain trematode infections), a semiquantitative scale ranging from zero for unaffected or healthy tissue, to five for the most affected or widespread state was set. The scale was then divided into as many as ten divisions (0.5 increments), carefully defined, and documented pictorially. Blind comparisons between technicians were used to confirm standardization of these semiquantitative protocols.

The mussel tissues were analyzed by standard NOAA National Status and Trends methods. These methods have been widely adopted and intercalibrated for marine pollution studies. Extensive QA/QC activities accompany all analyses assuring highly precise and accurate measures of contaminant levels in biological tissues. In brief, the tissues are homogenized, chemically dried, and extracted with methylene chloride. The extract is purified by gravity flow alumina/silica gel chromatography and size exclusion high pressure liquid chromatography. Hydrocarbons are quantified by gas chromatography/mass spectrometry in the selected ion monitoring mode. All analyses are verified by the analysis of standard reference materials and all data produced are comparable to most national monitoring programs.

3.4.3 Preliminary Results

Initial inspection of collected mussel samples indicates that fixation was satisfactory (Table 3.3). Coarse inspection revealed high prevalences of a number of important parasite types, including rickettsial bodies and the trematode Bucephalus which causes loss of fecundity or castration in heavy infections. If quantitative inspection of these initial samples confirms initial reports, seep mussels will be found to be highly parasitized in comparison to most oyster and mussel sites from the NS&T program. Such high prevalences will warrant modification of the sampling program to stress sampling of different populations and sites to determine how widespread high parasite prevalences are, with additional hydrocarbon analyses to determine possible causation.

Analyses of 62 individuals for tissue PAH burden have been completed. Ten (10) mussel beds were sampled at four sites - five (5) at Bush Hill (GC185), two (2) at the Brine Pool (GC233), one (1) at Garden Banks-425, and two (2) at Green Canyon-234. Total tissue PAH concentrations vary from 200 to 2500 ppb. There is some variability within a site, but, in general, replicate individuals exhibit consistent body burden so that a site can be characterized as to relative uptake or exposure to PAH. In general, the values are not high compared to chronic or acute coastal pollutant scenarios, but do show significant and varying exposure histories between locations. A ranking of the study sites from least to most oiled is as follows: BP M3 (214 ppb), BH M2 (283 ppb), BP M1 (565 ppb), GC M1 (614 ppb), BH M3 (635 ppb), BH M4 (697 ppb), GC M2 (1065 ppb), GB M1 (1160 ppb), BH M5 (1185 ppb), BH M1 (1346 ppb). Note that individual mussel beds within a site can be differentiated illustrating the heterogeneity of the distribution of seeps within a study site. Small scale heterogeneity may be important in

Table 3.3

Samples Collected in Summer 1997 Cruises.

Species	Dive	Station	Site	Number
Mussel	2852	M1	GC185	10
Mussel	2857	M2	GC185	8
Mussel	2868	M4	GC185	10
Mussel	2873	M5	GC185	10
Mussel	2850	M1	GC233	10
Mussel	2870	M2	GC233	3
Mussel	2870	M3	GC233	8
Mussel	2877	M1	GC234	10
Mussel	2883	M1	GB425	7
Tube Worm	2887		GC234	10

determining biological distributions. Toxicity may play a dual role at sites controlling community distributions as well as determining whether the site is habitable in the early stages of settlement. An aging (i.e., detoxification) of the sedimentary environment may be a requirement for community development. This aging may include not only hard substrate formation but also detoxification of the seep oil through microbial oxidation. Body burden data will be correlated with histopathological assessment of assemblage health at the study sites. Other studies have shown that variance within replicates as well as absolute concentration may indicate disturbance at a location.

3.5 Dissolved Gases and Free Gases: Their Sedimentary Biogeochemistry

Principal Investigator: John W. Morse
Institution: Department of Oceanography, Texas A&M University

Gases play an important role in the ecology of benthic seep communities and may act as major organic (e.g., methane) and inorganic (e.g., hydrogen sulfide) energy sources. They can also be produced as metabolic products that interact with other sedimentary components to exert a strong, and often dominant influence, on the master chemical environmental variables of solution pH and redox state (Eh). The interaction of dissolved inorganic gases with other sedimentary components can result in the production of new solid phases, such as sulfide minerals and calcium carbonate. Formation of these authigenic minerals can also exert a significant influence on the bioavailability of toxic metals that may be associated with seep brines. As part of this study, it is therefore essential that a careful investigation be made of the biogeochemistry of gases and related components of sediments. The two inorganic gases primarily considered are hydrogen sulfide and carbon dioxide. Both gases are part of complex chemical systems which will only be briefly described here and are often interrelated through biogeochemical processes (for further reading see, for example, Morse and Mackenzie 1990; Morse et al. 1987; Rickard et al. 1995). Both gases may be delivered to seep sites by migrating subsurface fluids at relatively high concentrations, or produced within a benthic community.

The major chemical reactions of hydrogen sulfide with aqueous solutions can be represented by the series of reactions:

$$H_2S_{(g)} \leftrightarrow H_2S_{(aq)} \leftrightarrow HS^- + H^+ \leftrightarrow S^{2-} + 2H^+$$

The speciation of hydrogen sulfide in solution is therefore governed by gas solubility and the dissociation reactions (note the second dissociation can often be neglected as its K is very small, $<10^{-15}$). Total dissolved hydrogen sulfide is generally analyzed and its speciation calculated. The sedimentary sulfide system is further complicated by redox reactions in which sulfide can be oxidized into other chemical forms and is often the dominant control on sediment Eh. Two of the most important oxidation products are solid elemental sulfur and dissolved sulfate. These reactions can occur both biotically (producing energy for organisms) or abiotically. Sulfide also interacts extensively with sedimentary iron to form a variety of iron sulfide minerals of which pyrite (FeS_2) is generally the most abundant and acid volatile sulfides the most reactive (e.g., Morse and Cornwell 1987). Many studies have demonstrated that formation of sulfide minerals is also important for sequestering toxic metals and limiting their bioavailability (e.g., Morse 1994).

H_2S may be produced either biotically, by sulfate reducing bacteria in anoxic environments, or during burial diagenesis, by thermogenic sulfate reduction. In both cases a reaction occurs between organic matter and sulfate. It can be represented as:

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$$

Therefore, both organic matter and dissolved sulfate are necessary for the production of hydrogen sulfide. Consequently, either reactive organic matter or dissolved sulfate will limit hydrogen sulfide production. In most sediments reactive (readily metabolizable) organic matter is limiting, but at seep sites the introduction of petroleum and gases such as methane may cause the transport of sulfate into sediments to be rate limiting for hydrogen sulfide production. A related major question for the seep community sites is whether the hydrogen sulfide present is being dominantly produced near the sediment-water interface by benthic bacteria or is it arriving from depth in association with the migrating fluids that are seeping to the sediment surface. If the latter is true, then organisms may (as has been observed for some hydrothermal vent communities) be using the hydrogen sulfide as an energy source according to the reaction:

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ + \text{energy}$$

The carbon dioxide system has many remarkable similarities to the hydrogen sulfide system and, as can be seen from the above reactions, the two systems are often tightly intertwined. In analogy to the basic reactions for hydrogen sulfide, the major reactions for the carbon dioxide system are:

$$CO_{2(g)} \leftrightarrow CO_{2(aq)} + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

Four analytical parameters for this system are total dissolved CO_2, P_{CO2}, pH, and alkalinity. If any two of these four parameters are known, everything else can be calculated. These reactions

often exert a dominant influence on sediment pH. The carbon dioxide system is also influenced by redox reactions, of which formation and remineralization of organic matter are usually most important. In some environments methanogenesis and methanotrophy can also be of considerable significance.

Interactions between the carbonate system and other sediment components can lead to extensive formation or dissolution of sedimentary carbonate minerals. If carbon dioxide is generated from oxic remineralization of organic matter, dissolution of calcium carbonate can occur via the general reaction:

$$CH_2O + O_2 + CaCO_3 \rightarrow Ca^{2+} + 2HCO_3^-$$

Dissolution of calcium carbonate can also be driven by the oxidation of hydrogen sulfide via the reaction:

$$H_2S + 2O_2 \ ^+ CaCO_3 \rightarrow SO_4^{2-} + Ca^{2+} + H_2O + CO_2$$

However, if organic matter is remineralized via sulfate reduction in the presence of reactive iron oxides, both calcium carbonate and iron sulfide minerals can be formed (ratios of reaction products depend on related complex factors).

$$9CH_2O + 4SO_4^{2-} + 4FeOOH + 4Ca^{2+} \rightarrow 4CaCO_3 + 4FeS + 5CO_2 + 11H_2O$$

This type of reaction is clearly of major importance in the sediments at seep sites and is probably largely responsible for the major formation of carbonate and sulfide minerals occurring in these areas. It should be kept in mind that all of these reactions are likely to be occurring leading to a very complex and dynamic system that may be variable on a microscale.

3.5.1 Objectives

The primary objective of this work element is to test the following hypotheses:

H_o-1 *The dominant source of hydrogen sulfide and carbon dioxide in benthic seep communities is local biogenic reactions rather than brines.*

H_o-2 *Unlike most siliciclastic marine sediments, seep site sediments have sulfate rather than metabolizable organic mater as the major limiting factor for hydrogen sulfide production.*

H_o-3 *Subsurface brines are a significant component of pore fluids in sediments underlying benthic seep communities that may play an important role in carbonate and sulfide mineral formation.*

H_o-4 The diagenetic "fate" of sulfides and carbonates at seep sites is unique and may also play a role in the "fate" of toxic metals which may often be associated with brines and the general physical properties of seep site sediments.

3.5.2 Methodologies

At each location, three push cores about 20 cm in length were obtained. Cores were generally utilized as follows for inorganic gas-related studies: 1) N_2 gas atmosphere squeezing for redox sensitive pore water components with squeeze cakes retained for solid phase analyses; 2) microelectrode measurements, followed by centrifugation for non-redox sensitive pore water components, with solids subsampled for non-redox sensitive components; and 3) integrated sulfate reduction rate measurements.

Depth profiles were made by sub-sampling cores as appropriate to their size and ability to yield analytically reasonable amounts of material, generally at about 2 cm intervals for many parameters. A summary of analytical parameters to be determined as part of the inorganic gas-related biogeochemistry study and analytical techniques are presented in Table 3.4.

Recent developments in solid state microelectrode technology (Brendel and Luther 1995) permit sediment geochemists to obtain contemporaneous, high resolution (< mm scale) vertical profiles of four important redox species (i.e., O_2, H_2S, Fe^{2+}, Mn^{2+}) in sediment pore water (Brendel 1995). To date, this technology has been applied in only a few sedimentary settings (sediments of the Scotian shelf and Delaware salt marshes, (Brendel 1995); sediments of the Mid-Atlantic Bight sediments, (Joye et al. in preparation). Microelectrode techniques provide an excellent complement to classic geochemical approaches (e.g., colorimetric analysis of analytes in water samples collected by sediment squeezing or centrifugation). The fine, mm-scale, spatial resolution, obtained using microelectrodes, may improve the ability of geochemical models to predict reaction schemes occurring in sedimentary environments. As part of this study, Au-Hg thin film microelectrodes were used to examine the pore water profiles of O_2, H_2S, Fe^{2+} and Mn^{2+}. These data will be used to corroborate and fine-tune data obtained using standard geochemical methods. Generally measurements were made using the microelectrodes over about 2 mm intervals of the top 5cm of sediment where fine scale variability was likely to be most significant.

H_2S and total CO_2 were determined because they are the primary inorganic gases being studied. Reduced Fe and Mn will soon be measured because most anoxic sediments tend to be iron or sulfide dominated, and the Fe and Mn concentrations are needed to help interpret the sediment geochemistry. Major ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, $SO4^{2-}$), alkalinity, and pH were (or are being) measured to help identify the source of pore fluids and make possible the characterization of the carbonic acid system. Nutrients (ammonium and nitrate) and dissolved organic carbon are being processed and will be used as a measure of diagenesis of sedimentary organic matter.

Total reduced sulfur and dissolved sulfate sulfur stable isotope ratios (when sufficient sample is available) will be measured as possible indicators of sulfide and sulfate sources. Porosity was determined in order to model fluxes, make mass balances, and calculate sulfate reduction rates. The silt and clay fraction of sediment was determined because many solid phase parameters tend to correlate well with this fraction. Organic carbon was determined because of its central importance to sedimentary biogeochemical processes, and (calcium) carbonate concentration was

Table 3.4

Gas-Related Analyses for Each Type of Component by Priority.

Type of Component	Component	Analytical Method
Pore water (microelectrodes)	O_2, H_2S, Fe^{2+}, Mn^{2+}	Solid state electrodes
Pore water	H_2S	Colorimetric (Cline method)
(N_2 atm. Squeezer)	Total CO_2	Infrared analyzer (on DOC analyzer)
	Reduced Fe and Mn	Colorimetric
Pore Water	Major ions	Ion chromatography
(by centrifugation)	Alkalinity	Gran titration
	pH	pH electrode/meter
	Nutrients	Auto analyzer
	Dissolved organic C	DOC analyzer
	Sulfate S stable isotopes	Mass spectrometer
Sediment Solids	S and C stable isotopes	Extract/mass spectrometer
	Porosity	Wt. change on drying
	Silt and clay fraction	Sieving
	Organic carbon	Leco C analyzer
	Carbonate carbon	Leco C analyzer
	Reactive Fe and Mn	Citrate dithionite. Extraction Atomic absorption spec.
	Acid volatile sulfides	Extract/colorimetric
	Total reduced sulfides	Extract/colorimetric
	Elemental sulfur	Extract/colorimetric
Sulfate Reduction Rate	$^{35}SO_4^{2-}$	Incubation and radiometric assay

measured because of the potential of carbonate minerals to cause major variations in bulk sediment chemistry and properties. Reactive iron and manganese (citrate dithionite extractable) will be determined in order to estimate the extent to which reactive iron has been sulfidized and the contributions of Fe and Mn as terminal electron acceptors. Acid volatile sulfides, total reduced inorganic sulfur, and elemental sulfur will be measured to determine probable products of hydrogen sulfide reactions in the sediments. Past experience has shown that seep areas may be characterized by much higher concentrations of total reduced inorganic sulfur and higher acid volatile sulfides/total reduced inorganic sulfur ratios than "normal" sediments in this area.

Sulfate reduction rates in sediments are being measured using the $^{35}SO_4^{2-}$ technique in a manner similar to that described by Lin and Morse (1991). Details of the method were adjusted to comply with the methodology being used to measure sulfate reduction rates in bacterial mats. Rate measurements were made at about 2cm intervals in each core to yield the "integrated sulfate reduction rate" as well as producing a profile of how these rates vary with depth in a core. Sulfate reduction rates will provide vital information on the production of hydrogen sulfide and thus contribute to the interpretation of sources of sulfide in benthic seep communities. This information can also be combined with dissolved profiles to obtain an estimate of dissolved component transport rates which can be used to calculate fluxes of other pore water components.

Other analyses that may shed further light on the biogeochemistry of seep community sediments will be performed based on the results of the previously described work and available resources. These analyses may include trace metal solid phase speciation, SEM/EDAX microscopy, and fine-scale elemental and carbon and oxygen stable isotope analyses of authigenic carbonates.

3.5.3 Preliminary Results

Activities during the first year can be divided into three major areas: 1) precruise planning and preparation, 2) participation in the two sampling cruises in mid-summer, and 3) post-cruise analytical work. Additionally, the development of an advanced computer model for sediment diagenesis that will play a major role in the interpretation of the data was initiated. More detailed summaries and commentaries on these activities are provided below. A good deal of careful planning of sediment sampling, onboard analytical activities, and how this phase of the project could be well integrated with other activities was necessary. This was accomplished via frequent phone and e-mail contacts, participation in the general planning meeting, and other meetings with the sediment subgroup. Cruise preparation included acquisition of supplies and materials, cutting of sampling cores, washing and labeling of well over a thousand sample bottles, and preparation of chemical reagents. Additionally a new coring device for hard sediments was developed for use on the submarine (made from titanium tubing).

Post-cruise analytical work will probably continue to occupy most of efforts of this work element until May, 1998. The major analytical parameters being determined and their current status are listed in Table 3.5. The currently "active" sample set is 140 samples, which does not include first leg samples, "mud sucker" samples, and brines. The only parameters for which are foreseen as difficulties, because of a lack of sufficient sample volume for analyses, are alkalinity (which can be fairly well calculated from other parameters), dissolved sulfate, and DIC for sulfur and carbon stable isotope determinations. Collecting extra samples for these dissolved components at sites of particular interest on the second cruise will be important. To make up for the short-coming, urea, phosphate, and silica will be determined to see if they provide further insight into the nutrient chemistry of these sediments.

A brief and very preliminary summary for the primary coring sites of some of the most interesting parameters determined so far are presented in Table 3.6. The descriptive adjectives are based on PIs familiarity with sediment geochemistry in the region. High pH refers to close (within a few tenths) the pH of normal seawater. Blank boxes are where analyses have not yet been completed. Clearly this is going to be an area of highly variable and dynamic sediment geochemistry. As previously mentioned, much of Year 2 will be involved with finishing the analytical work on samples obtained in Year 1 and interpreting the results. A second cruise will take place next summer. It and the associated analytical work will fill the rest of Year 2 activities. It is important to stress that research on sediment biogeochemistry was not a major part of the former program and it is, therefore, necessarily much more of an exploratory nature than most other elements of the present program. It is quite likely that the plans for the second cruise sediment biogeochemistry studies may be substantially modified based on the results of the first cruise. Any major changes will be made based on discussions with other participants in this study and the appropriate sponsor personnel.

Table 3.5

Major Analytical Parameters Being Determined and Their Current Status.

Analytic Parameter	Current (11/1/97) Status
Microelectrode measurements (dissolved O_2, H_2S, Fe^{2+}, Mn^{2+})	Measurements made, but considerable work left to turn them into values
Squeezer pore water extractions	Done
Centrifuge pore water extractions	Done
Components of pore waters	
Na^+	Not done
K^+	Not done
Mg^{2+}	Not done
Ca^{2+}	Not done
Fe^{2+}	Not done
Mn^{2+}	Not done
Cl^-	~60% done
SO_4^{2-}	Not done
Alkalinity	Not done (In many cases not enough sample)
Total CO_2 (DIC)	~70% done
pH	Done
H_2S	Done
NH_4^+	Not done
NO_3^-	Not done
Solid phase components	
Porosity	Done
Fraction <62 μm	Done
Wt. % organic carbon	Done
Wt. % carbonate	Done
Reactive Fe	Not done
Reactive Mn	Not done
Acid volatile sulfides	Not done
Total reduced inorganic sulfur	Not done
Elemental sulfur (S^o)	Not done
Sulfur stable isotopes	Not done
Pore water sulfate	Not done (In most cases not enough sample)
Carbon stable isotopes Authigenic carbonate minerals	Not done
DIC	Not done- Insufficient sample
Integrated sulfate reduction rates	Incubated-not analyzed
Special (as funds permit)	
Sulfide assoc. trace metals	To be determined on select samples
SEM/EDAX	To be determined on select samples

Table 3.6

Preliminary Summary for Primary Coring Sites.

Site	Station	Dive #	pH	DIC	H_2S	Cl^-	Org-C	$CaCO_3$
GC234*	GCST1	2871	High	Mod	V. Low	SW	High	Mod
GC235	BHm4	2873	High		V. High		V. High	High
GC235	BHAT1	2873	High		Low		V. High	Mod
GC235*	BHST1	2874	High	Mod	High		V. High	Mod
GC235	BHUN1	2875	High		V. Low	SW	High	Mod
GC235*	BHST2	2875	High	Mod	V. Low		High	Mod
GC233	BPR2	2876	Mod	Mod	Low		High	Mod
GC234	GCB1	2880	Mod	V. High	High	Brine	Mod	Mod
GC235	BHBC2	box	Mod		V. Low	SW	V. High	Mod
GB425	GBB1	2882	Low		High to	SW	Mod	Mod
GB425	GBM1	2883	Mod		V. Low		High	Mod
GB425	GBM2	2885	Mod/Low		Mod	Brine	Mod	Mod
GC234*	GCAT2	2886	Mod		V. High	SW	High	High
GC234	GCAT1	2888	Mod		Low to	SW	V. High	Mod
GC235	BHAT2	2891	Mod/High	Mod	Low		High	Mod

3.6 Origins of Hydrocarbons and Community Stability

Principal Investigator: Roger Sassen
Institution: Geochemical and Environmental Research Group, Texas A&M University

The Gulf of Mexico slope includes a stratigraphic section more than 10 km thick and has been profoundly influenced by salt movement. The largest volume of hydrocarbons at depth in the Gulf slope are thermogenic in origin. Oil in most Gulf slope fields is generated by Mesozoic source rocks from Upper Jurassic to Upper Cretaceous in age as a consequence of deep burial of organic-rich source rocks at high temperatures (Sassen et al. 1993a). Gas and oil migrate vertically to reservoirs of mainly Miocene, Pliocene, and Pleistocene age (Sassen et al. 1993a). The vertical scale of migration from deep Mesozoic source rocks to shallow Upper Tertiary reservoirs is therefore on the order of probably 6-8 km.

Most oils in subsurface reservoirs display compositions that are not greatly altered after emplacement in reservoirs. Jolliet Field in Green Canyon Block 185 serves as an example (Sassen et al. 1993b). Only small amounts of O_2 are available at the 3 km depth of Jolliet reservoir rocks to allow microbial oxidation. Only subtle evidence of microbial oxidation is shown in even the shallowest reservoirs at Jolliet Field (Sassen et al. 1993b).

Jolliet oils are from Upper Jurassic marine source rocks, and have a relatively low gas/oil ratio. Sulfur contents of Jolliet oils are between 1 and 1.7% by weight of the C_{15+} fraction (Sassen et al. 1993b). The sulfur of oils is mainly organically-bound in the aromatic and asphaltene fractions of oils; thus sulfur is not readily bio-available. CO_2 is a minor component of reservoir gas. The CO_2 carbon isotopic composition of the reservoir gas approximates that of normal

marine carbonates [±0‰ Pee Dee Belemnite (PDB)]. H_2S is in extremely low concentration of only a few ppm, if present, because it is readily fixed into by iron minerals in the reservoirs. Elemental sulfur is absent or below detection limits for the same reason.

There is a clear relationship between known hydrocarbon discoveries at great depth in the Gulf slope, and chemosynthetic communities, hydrocarbon seepage, and authigenic minerals including carbonates at the sea floor (Sassen et al. 1993a). Differences in scale are immense. In comparison to the subsurface hydrocarbon system which represents a broad area of the Gulf slope, 6-8 km thick, chemosynthetic communities are isolated point occurrences associated with active gaseous seeps in thin veneers of sediment a few meters or tens of meters thick. Migration conduits provide a pathway for material to reach the near-surface sediments where it is rapidly altered beneath seals of carbonate, hydrate, bacteria, etc. The rate of alteration is rapid on geological timescales.

Bush Hill on Green Canyon Block 185 is clearly related to the hydrocarbon system that gave rise to the Jolliet Field at GC 184 (Sassen et al. 1993a), but an unambiguous link to specific reservoirs has not been made. Unlike reservoired oils of Jolliet Field, seep oils at Bush Hill are heavily altered by microbial oxidation, reflecting the loss of saturated hydrocarbons, a major component of reservoired oils (Sassen et al. 1994b; Sassen et al. 1993b). The first oil components oxidized by bacteria are saturated hydrocarbons. The saturated hydrocarbons are converted to CO_2 by aerobic hydrocarbon-oxidizing bacteria. Relative to deep reservoirs, O_2 is relatively abundant in permeable shallow sediments, until it is depleted by hydrocarbon oxidizing bacteria or other processes.

In contrast to subsurface reservoirs at Jolliet Field where CO_2 is a minor non-hydrocarbon gas, CO_2 is very abundant at chemosynthetic communities. Large differences in the carbon isotopic composition of CO_2 in shallow sediments have been observed. The carbon isotopic composition of CO_2 in chemosynthetic communities is generally thought to be depleted in ^{13}C (-30‰ PDB or lighter). CO_2 is formed *in situ* at seeps and authigenic carbonate rock from CO_2 precipitation is found at seeps with a similar carbon isotopic composition. Carbon isotopic compositions ($\delta^{13}C$) of petroleum produced from the Jolliet Field is similar to that of carbonate on the sea floor, approximately -26.5‰ PDB (Sassen et al. 1993b).

In strong contrast to subsurface reservoirs, various inorganic sulfur species (particularly H_2S and elemental sulfur) are extremely abundant in sediments from chemosynthetic community sites at Bush Hill. Mass balance constraints argue against inorganic sulfur species originating from oil-related organic sulfur compounds. Moreover, seeps of pure biogenic methane (CH_4) are also associated with high concentrations of elemental sulfur (Sassen et al. 1993b). H_2S is formed in discrete, shallow anoxic zones by sulfate-reducing bacteria at seeps. The H_2S is rapidly oxidized by *Beggiatoa*, creating elemental sulfur concentrations as high as 19% by weight (Sassen et al. 1993b).

3.6.1 Objectives

The analytical approach, described below, is designed to address several hypotheses in conjunction with data produced by other work elements..

H_o-1 It is hypothesized that chemosynthetic community stability is mainly controlled by an optimal rate of hydrocarbon migration, with both lower and upper limits beyond which instability occurs. Hydrocarbons play a major role in driving organic-inorganic reactions at chemosynthetic communities, resulting in the accumulation of carbonate and the cycling of inorganic sulfur species (Sassen et al. 1993a; Sassen et al. 1994b).

H_o-2 Hydrocarbon production will not usually affect the long-term stability of chemosynthetic communities in the Gulf slope, since most hydrocarbons in a leaky basin (perhaps 90%) are not trapped and instead are lost to the sea floor. To effectively address this hypothesis requires an overview of petroleum geochemistry and geological context of the study areas.

H_o-3 The accumulation of authigenic carbonate is a contributing factor in the origin and stability of chemosynthetic communities. It has been suggested that there is a perturbation in carbon cycling at seep sites because of net accumulation of authigenic carbonate minerals (Sassen et al. 1993b).

H_o-4 Hydrocarbon migration rates could influence the sulfur cycle at chemosynthetic communities. The sulfur cycle appears to operate efficiently in active chemosynthetic communities (Sassen et al. 1993b), and H_2S is clearly a factor in the origin, stability, and ultimate destruction of chemosynthetic communities.

3.6.2 Methodologies

A number of samples were collected at the Bush Hill, GC 234, and the Brine Pool localities. One oil seep sample was analyzed for saturated and aromatic biomarkers by gas chromatography-mass spectrometry to confirm the source of the oil and gas. Since more samples were collected than originally envisioned, selected samples were selected for detailed molecular and isotopic characterization of methane to pentane hydrocarbon gases. Specifically, the molecular compositions were measured by gas chromatography [hydrocarbons include methane (C_1), ethane (C_2), propane (C_3), iso-butane (i-C_4), normal butane (n-C_4), iso-pentane (i-C_5), and normal pentane (n-C_5)]. In addition, CO_2 was quantified and gas chromatography/isotope ratio mass spectrometry was used to accurately measure the carbon and hydrogen isotopic composition ($\delta^{13}C$) of gaseous hydrocarbons and CO_2.

3.6.3 Preliminary Results

An oily sediment sample from Bush Hill was analyzed for biomarker and whole-oil $\delta^{13}C$ to confirm that the source of the oil is deeply buried source rocks of the Upper Jurassic. Thus, the

hydrocarbons at Bush Hill are part of a larger hydrocarbon system that extends east to west from Mississippi Canyon/Atwater Valley to East Breaks/Alaminos Canyon. The time-scale for oil and gas migration from this source system is on the scale of millions of years and involved 6 to 10 km of vertical migration to the present reservoirs.

Oil seeps differ from the reservoirs of Jolliet Field in that seep oil has been altered by bacterial oxidation. Little evidence is found for oil in the study areas that has not been impacted by microbial oxidation. Bacterial oxidation of oil has contributed to the precipitation of authigenic carbonate. Bacterial sulfate reduction is concomitant with the bacterial oxidation of oil.

Methane to pentane molecular distributions and $\delta^{13}C$ of the hydrocarbons from samples of vent gas entering the water column provide evidence of origin. The gas at the Bush Hill and GC 234 localities is related to an Upper Jurassic source system. The similarities observed at the Bush Hill and GC 234 localities with oil-related gas from the subsurface are consistent. In contrast, the molecular and carbon isotopic compositions of gas from the Brine Pool site indicate that the gas is of biogenic origin, formed by bacterial oxidation of sediment organic matter at low temperatures and shallow depths.

The gas of vents characteristically shows a lack of alteration by bacterial oxidation. In other words, the gas emanating from the subsurface at Bush Hill and GC234 can be more effectively utilized than oil by bacteria. The biogenic methane at the Brine Pool is being utilized by bacteria, possibly in the brine itself, and certainly in nearby sediments. Once the oil-associated with vent gases enters unconsolidated sediments at Bush Hill and GC234, it is rapidly altered by bacterial oxidation. This is shown by characteristic changes in molecular composition that result in preferential destruction of the ethane to pentane hydrocarbon gases. At the same time, some biogenic gas is being generated at these localities, adding methane as ethane to pentane gases are oxidized. This is confirmed by $\delta^{13}C$ measurements of the hydrocarbon gas molecules. Abundant CO_2 is observed in sediments that is enriched in ^{12}C, demonstrating bacterial oxidation. At the Brine Pool, high concentrations of CO_2 enriched in ^{12}C in free gas were measured suggesting that bacterial methane oxidation occurs in the brine pool itself, as well as in the surrounding sediments.

Although oil has been considered an important factor driving complex bacterial processes occurring at chemosynthetic communities, new data suggests that hydrocarbon gases are perhaps more important. Large volumes of unaltered gas arriving from the subsurface is readily metabolized by bacteria. The oil, however, is already highly altered and the most easily metabolized molecules have already been consumed. Gas flow thus plays a role in stability of chemosynthetic communities by supporting the complex bacterial consortia that produces CO_2 and H_2S. Gas flow also plays a role in recent and on-going carbonate precipitation.

The methane to pentane molecular and isotopic data suggest hydrates play an important role at chemosynthetic communities. Gas hydrates outcrop on the sea-floor at Bush Hill and GC 234. These hydrates have molecular signatures, confirmed by NMR, of structure II gas hydrate. There is also geochemical evidence of the rare structure H hydrate. Smaller volumes of gas hydrate are widely dispersed across chemosynthetic communities, based on the ability to detect the geochemical "fingerprint" of dispersed hydrate in sediments. The hydrates "freeze" large

volumes of hydrocarbon gases, retarding the rate at which bacterial oxidation occurs. Hydrocarbons in gas hydrate can be affected also by bacterial activity. CO_2 in hydrates is enriched in ^{12}C. Moreover, possible evidence of biogenically produced unsaturated hydrocarbons as cage-filling hydrate components has been produced. The occurrence of complex, unsaturated, biogenic hydrocarbon gases in hydrates and associated sediments has not been previously reported. In addition, the formation and decomposition of gas hydrate is a repetitive process commencing with rapid crystal growth, decomposition within a semi-closed system and recrystallization, providing for both chemical composition and probably crystal structure changes. The dynamic alteration of gas hydrate is hypothesized to play a role as a "buffer," slowly releasing hydrocarbon gases to fuel biogeochemical processes at chemosynthetic communities. Hydrates thus could play a significant role in community stability.

Preliminary reconnaissance data, in the context of previous data, suggests a new generalization about the importance of hydrocarbon gases to the stability of chemosynthetic communities. Oil seems less important than previously thought, at least within the more complex communities such as at Bush Hill and GC234. Methane is a key carbon source at the Brine Pool.

3.7 Electrochemistry of Sediments from Chemosynthetic Communities

Principal Investigator: Samantha Joye
Institution: Dept. of Oceanography, Texas A&M University

Recent developments in solid state microelectrode technology (Brendel and Luther 1995) permit sediment geochemists to obtain contemporaneous, high resolution (< mm scale) vertical profiles of four important redox species (i.e., S^{2-}, O_2, Mn^{2+}, and Fe^{2+}) in sediment pore water (Brendel and Luther 1995). To date, this technology has been applied in only a few sedimentary settings [sediments of the Scotian shelf and Delaware salt marshes, (Brendel 1995); sediments of Galveston Bay, TX (Escorcia and Joye 1997; Joye and Escorcia 1998); and sediments of the Mid-Atlantic Bight, (Joye et al. in preparation)].

3.7.1 Objectives

Microelectrode techniques provide an excellent complement to classical geochemical approaches (e.g., colorimetric analysis of analytes in water samples collected by sediment squeezing or centrifugation). The fine, mm-scale, spatial resolution, obtained using microelectrodes, improves the ability of geochemical models to predict reaction schemes occurring in sedimentary environments. As part of this study, Au-Hg thin film microelectrodes were used to examine the pore water profiles of S^{2-}, O_2, Mn^{2+}, and Fe^{2+}. These data will make it possible to corroborate and fine-tune results obtained using standard geochemical methods. In addition, application of microelectrodes to material collected with a novel sampling device enable measurement of redox species from micro-environments within seep habitats (e.g., the center of tube worm clusters) that have been largely inaccessible until now.

3.7.2 Methodologies

The microelectrodes fabricated for the this work element were fairly similar to those described in Brendel and Luther 1995. Several minor modifications were made to ensure good performance in these types of sediments. Soft soda lime glass was used for all microelectrodes. Instead of fabricating ultra-fine tip electrodes (tip diameter ~ 200 μm^2), a wider tip (tip diameter ~ 0.5 - 1 mm; sensing surface ~ 100 μm^2) or blunt tip (tip diameter ~ 5 mm; sensing surface ~ 100 μm^2) was made. These electrodes were robust and not prone to breakage. The tapered tip of the "wide" electrodes are capable of performing 100 to 200 scans before recalibration is necessary. Because of the organic-rich nature of the sediment, the scanning range was adjusted so that organic material was continually cleansed from the sensing surface. The square wave scan was inserted into the sodium window (-1.99 V for a few seconds) thereby creating H_2 and "burning off" any organic carbon on the sensing surface. This procedure was necessary for maintaining and assuring quality control. The selection of the proper electrode conditioning voltage (pre-scan) was also important (e.g., we used -0.4 instead of -0.6 V).

3.7.3 Preliminary Results

Preparation for the JSL-97 field effort included fabrication of electrodes, electrode calibration, software maintenance, packing, and shipping. All electrochemistry was accomplished at sea during the JSL-97B cruise using electrodes and other materials prepared in advance. Dr. Joye was assisted by Ms. Susie Escorcia. Measurements were obtained from selected push cores using the microelectrodes to scan at 2 mm intervals for the first centimeter, 3 mm intervals for the next three centimeters, and 5 mm intervals for the last two centimeters. The top six centimeters of sediment were chosen to scan as it is where fine scale variability will likely be most significant. Cores were selected to provide data from each of the major station types. Additional measurements were taken from the mudsucker samples immediately after they were brought on deck. These samples did not provide as detailed resolution as the push cores, but measurements (six holes) were provided at 10 cm intervals from all locations in which a push core was not recoverable because of hard substrate.

In total, pore water profiles were obtained using microelectrodes in 16 separate push cores. In each core, two six centimeter profiles were performed. The duplicate profiles totaled 37 individual depths (21 individual depths for the first profile and 16 individual depths for the second profile), and each scan at a particular depth was (at least) duplicated. A minimum of 1200 individual scans were obtained (an individual scan represents a concentration value for S^{2-}, O_2, Mn^{2+}, and Fe^{2+}) for the cores collected. Twenty (20) mudsucker collections were analyzed scanning each hole 2 to 3 times. This represents a total of ~250 scans. Finally, standardization and quality control samples accounted for 450 to 550 scans. Thus, approximately 1,900 scans were available for analysis. Details on sample distribution among sites and stations are presented in Table 3.7.

Thus far, a few of the scans have been analyzed, primarily "mudsucker" samples. Results to date illustrate: 1) steep gradients in pore water sulfide concentration; 2) very low, to undetectable concentrations of reduced Fe and Mn; and 3) no measurable oxygen gradients. The lack of

Table 3.7

Sample Distribution Among Sites and Stations.

Dive Number	Site	Station	Core Samples	Mudsucker Samples
Boxcore from surface	GC185	BHBC2	BHBC2-4	
2873	GC185	BHAT1	BHAT1-4	BHAT1-D1
2873	GC185	BHAT1		BHAT1-D2
2873	GC185	BHAT1		BHAT1-D3
2873	GC185	BHAT1		BHAT1-D4
2873	GC185	BHAT1		BHAT1-D5
2873	GC185	BHAT1		BHAT1-D6
2891	GC185	BHAT2	BHAT2-2	
2892	GC185	BHM2		BHM2-D1
2892	GC185	BHM2		BHM2-D2
2892	GC185	BHM2		BHM2-D3
2892	GC185	BHM2		BHM2-D4
2892	GC185	BHM2		BHM2-D5
2892	GC185	BHM2		BHM2-D6
2891	GC185	BHM3		BHM3-D1
2891	GC185	BHM3		BHM3-D2
2891	GC185	BHM3		BHM3-D3
2891	GC185	BHM3		BHM3-D4
2891	GC185	BHM3		BHM3-D5
2891	GC185	BHM3		BHM3-D6
2873	GC185	BHM4	BHM4-3	
2874	GC185	BHST1	BHST1-5	
2875	GC185	BHST2	BHST2-7	BHST2-D1
2875	GC185	BHST2		BHST2-D2
2875	GC185	BHST2		BHST2-D3
2875	GC185	BHST2		BHST2-D4
2875	GC185	BHST2		BHST2-D5
2875	GC185	BHST2		BHST2-D6
2875	GC185	BHUN1	BHUN1-2	
2869	GC233	BPM2		BPM2-D1
2869	GC233	BPM2		BPM2-D2
2869	GC233	BPM2		BPM2-D3
2869	GC233	BPM2		BPM2-D4
2869	GC233	BPM2		BPM2-D5
2869	GC233	BPM2		BPM2-D6
2870	GC233	BPM3		BPM3-D6
2870	GC233	BPM3		BPM3-D1
2870	GC233	BPM3		BPM3-D2
2870	GC233	BPM3		BPM3-D3
2870	GC233	BPM3		BPM3-D4
2870	GC233	BPM3		BPM3-D5
2879	GC233	BPM4		BPM4-D1
2879	GC233	BPM4		BPM4-D2
2879	GC233	BPM4		BPM4-D3
2879	GC233	BPM4		BPM4-D4
2879	GC233	BPM4		BPM4-D5

Table 3.7. (Continued)

Dive Number	Site	Station	Core Samples	Mudsucker Samples
2879	GC233	BPM4		BPM4-D6
2876	GC233	BPR2		BPR2-D1
2876	GC233	BPR2		BPR2-D2
2876	GC233	BPR2		BPR2-D3
2876	GC233	BPR2		BPR2-D4
2876	GC233	BPR2		BPR2-D5
2876	GC233	BPR2		BPR2-D6
2885	GB425	GBM1	GBM1-1	
2883	GB425	GBM1	GBM1-6	GBM1-D1-1
2883	GB425	GBM1		GBM1-D2-1
2883	GB425	GBM1		GBM1-D3-1
2883	GB425	GBM1		GBM1-D4-1
2883	GB425	GBM1		GBM1-D5-1
2883	GB425	GBM1		GBM1-D6-1
2884	GB425	GBM1		GBM1-D1-2
2884	GB425	GBM1		GBM1-D2-2
2884	GB425	GBM1		GBM1-D3-2
2884	GB425	GBM1		GBM1-D4-2
2884	GB425	GBM1		GBM1-D5-2
2884	GB425	GBM1		GBM1-D6-2
2882	GB425	GBM2	GBM2-6	GBM2-D2-1
2882	GB425	GBM2		GBM2-D1-1
2882	GB425	GBM2		GBM2-D3-1
2882	GB425	GBM2		GBM2-D4-1
2882	GB425	GBM2		GBM2-D5-1
2882	GB425	GBM2		GBM2-D6-1
2885	GB425	GBM2		GBM2-D1-2
2885	GB425	GBM2		GBM2-D2-2
2885	GB425	GBM2		GBM2-D3-2
2885	GB425	GBM2		GBM2-D4-2
2885	GB425	GBM2		GBM2-D5-2
2885	GB425	GBM2		GBM2-D6-2
2888	GC234	GCAT1	GCAT1-8	
2880	GC234	GCAT1		GCAT1-D1
2880	GC234	GCAT1		GCAT1-D2
2880	GC234	GCAT1		GCAT1-D3
2880	GC234	GCAT1		GCAT1-D4
2880	GC234	GCAT1		GCAT1-D5
2880	GC234	GCAT1		GCAT1-D6
2872	GC234	GCAT2	GCAT2-6	
2886	GC234	GCAT2	GCAT2-3	GCAT2-D1
2886	GC234	GCAT2		GCAT2-D2
2886	GC234	GCAT2		GCAT2-D3
2886	GC234	GCAT2		GCAT2-D4
2886	GC234	GCAT2		GCAT2-D5
2886	GC234	GCAT2		GCAT2-D6
2880	GC234	GCB	GCB-1	
2880	GC234	GCB	GCB-6	

Table 3.7. (Continued)

Dive Number	Site	Station	Core Samples	Mudsucker Samples
2881	GC234	GCHYD1		GCHYD1-D1
2881	GC234	GCHYD1		GCHYD1-D2
2881	GC234	GCHYD1		GCHYD1-D3
2881	GC234	GCHYD1		GCHYD1-D4
2881	GC234	GCHYD1		GCHYD1-D5
2881	GC234	GCHYD1		GCHYD1-D6
2872	GC234	GCJT1		GCJT1-D1
2872	GC234	GCJT1		GCJT1-D2
2872	GC234	GCJT1		GCJT1-D3
2872	GC234	GCJT1		GCJT1-D4
2872	GC234	GCJT1		GCJT1-D5
2872	GC234	GCJT1		GCJT1-D6
2888	GC234	GCJT2		GCJT2-D3
2888	GC234	GCJT2		GCJT2-D1
2888	GC234	GCJT2		GCJT2-D2
2888	GC234	GCJT2		GCJT2-D4
2888	GC234	GCJT2		GCJT2-D5
2888	GC234	GCJT2		GCJT2-D6
2877	GC234	GCM1		GCM1-D1
2877	GC234	GCM1		GCM1-D2
2877	GC234	GCM1		GCM1-D3
2877	GC234	GCM1		GCM1-D4
2877	GC234	GCM1		GCM1-D5
2877	GC234	GCM1		GCM1-D6
2890	GC234	GCM2		GCM2-D1
2890	GC234	GCM2		GCM2-D2
2890	GC234	GCM2		GCM2-D3
2890	GC234	GCM2		GCM2-D4
2890	GC234	GCM2		GCM2-D5
2890	GC234	GCM2		GCM2-D6
2871	GC234	GCST1	GCST1-5	GCST1-D1
2871	GC234	GCST1		GCST1-D2
2871	GC234	GCST1		GCST1-D3
2871	GC234	GCST1		GCST1-D4
2871	GC234	GCST1		GCST1-D5
2871	GC234	GCST1		GCST1-D6

measurable oxygen is probably due the high concentrations of organic matter (high oxidation rates), sulfide fluxes, and the loss of oxygen during the recovery process. For example, when cores were returned to the surface, the height of the water was adjusted in order to insert the microelectrodes, and the cores were permitted to recover from the disturbance associated with adjusting the height for one to two hours. During this period, cores were kept in a cold room (approximately 10°C) and the overlying water was not aerated. Aeration on the overlying water was not performed for fear of resuspending the flocculent surface layer. Therefore, during the time between collection and analysis (3 to 7 hours), O_2 was rapidly consumed. With high concentrations of hydrocarbons, frequently high concentrations of S^{2-}, and the on-going activity

of bacteria (respiration and oxidation reactions consuming O_2), it is no surprise that O_2 was rapidly depleted. In the future, aeration of the headspace of cores using aquarium pumps attached to air stones will alleviate this problem.

Despite the problems outlined above, excellent profiles of S^{2-} concentration were obtained. Sharp gradients exist in S^{2-} concentrations. The few complete profiles suggest a region of S^{2-} uptake exists within the upper 10 cm beneath adult tube worm bushes

3.8 The Effects of Physical Environmental Variables on GOM Chemosynthetic Ecosystems

Principal Investigator: Norman Guinasso
Institution: Geochemical & Environmental Research Group, Texas A&M University

Water temperature and currents play a role in determining the structure and spatial distribution of chemosynthetic communities. Two fundamental processes are important. One is hydrate formation and the other is the effect of the bottom currents on the distribution of larvae. The flux of hydrocarbons along faults appears to be modulated by the formation of hydrates. Hydrates can fill the interstitial pores of shallow sediments and produce mounds or diapirs at locations where the gas fluxes are large enough. Observations indicate that chemosynthetic communities may have a difficult time establishing themselves if the substrate is mostly hydrates or hydrate-rich sediments.

3.8.1 Objectives

It has been hypothesized that the decomposition of hydrates, caused by fluctuating bottom water temperatures, can play a role in controlling the morphology of chemosynthetic sites. Temperature and gas flow measurements were made at one site that appear to show that gas fluxes increase when water temperature increases. Time series measurements of bottom temperature measurements at Green Canyon 234 and 185 in the study area (500 m depth) indicate that the bottom water temperature in this region can undergo excursions of 4-5 degrees. The cause of these excursions is not well understood, but the presence of mesoscale features in the overlying water or Rossby waves propagating along the continental slope may play a role.

Pure methane hydrate is stable at 50 Atm (~500 m depth) at a temperature of 6.7°C. At 100 Atm (~1000 m water depth) pure methane hydrate is stable at 13°C. Addition of higher molecular weight hydrocarbon gases raises the stability temperature of hydrates significantly. At Bush Hill, the bottom water temperature is approximately 7°C. MacDonald et al (1994) propose that small increases in temperature can lead to the disassociation of hydrates, hence increasing gas fluxes. In regions where the bottom water temperature is at or near the stability temperature for hydrates, the geothermal gradient allows hydrates to form near the surface thus affecting the surface morphology.

The situation is different at deeper depths. At 1000 m for example the bottom water temperature is about 5°C and can be expected to be rather constant. Here at the sea floor, methane hydrate is colder than its stability temperature. Under these conditions the geothermal gradient might permit hydrates to form deeper in the sediments. Thus, hydrates may not form diapirs as seen in

shallower waters. At these deeper depths, hydrates possibly suppress the flux of light hydrocarbon gases to the surface and probably play a smaller role in affecting the surface morphology of chemosynthetic community sites.

3.8.2 Methodologies

In-situ instruments were deployed at all four study sites. A mooring with two current meters was deployed at a depth of 1798 m in GC185 at 27°46.95' N and 91°30.28' W. This site is about 100 m due east of the top of Bush Hill. The mooring has one current meter above the bottom Ekman layer, and one meter about 200 m from the surface (Figure 3.9). The lower current meter assesses the current and temperature variability in the nearbottom region of the chemosynthetic community. The upper meter serves to identify mesoscale features that may affect the bottom circulation. The mooring was deployed from the surface. It will be released by an acoustic release. As a backup, the mooring has a weak link beneath the acoustic release that can be cut using the manipulator arm on the *Johnson Sea Link.* Although the mooring was deployed at GC185, the information will also be relevant to conditions at the nearby GC233 and GC234 sites.

A bubblometer with two thermistors and a time-lapse video camera were deployed at the GC234 site. The bubblometer was deployed in a mussel bed (station number GCM2) where gas hydrate was semi-exposed below the mussels. This device, based on a design described by MacDonald et al. (1994), will record episodic release of gas as well as water and sediment temperature. The camera is set up to monitor an exposed hydrate mound and to record video images at hourly intervals for the entire year. The camera also has three thermistors that are monitoring the temperature of the water column and seafloor. These instruments will be deployed initially for one year. Additional deployments for the second year will depend on the state of the instrumentation at first recovery and recovery logistics.

Thermistor arrays were also deployed at the two brine pool sites, GC233 and GB425. The configuration of the arrays was designed to provide information about the stability of the brine-seawater interface and changes in brine temperature that would indicate fluid discharge events.

In addition to the bottom measurement, CTD stations in the water column above the chemosynthetic communities were taken during the submarine cruise. These observations were taken at night with the use of a Seabird CTD along north-south transect through the sites. A total of eleven CTD stations were occupied from the surface ship (Table 2.2.2). CTD records were also obtained from an instrument mounted on the submarine during each dive.

The time series data will be subjected to standard analysis techniques such as power spectra. The data will be interpreted in the light of satellite AVHRR and altimetry measurements in the area, CTD measurements, meteorological measurements from buoys and platforms in the region, and any other collateral data available to the investigators. The goal of this task will be to have a detailed data set of temperature, currents, and gas flow rates that can be used to help interpret any changes noted in the biological communities.

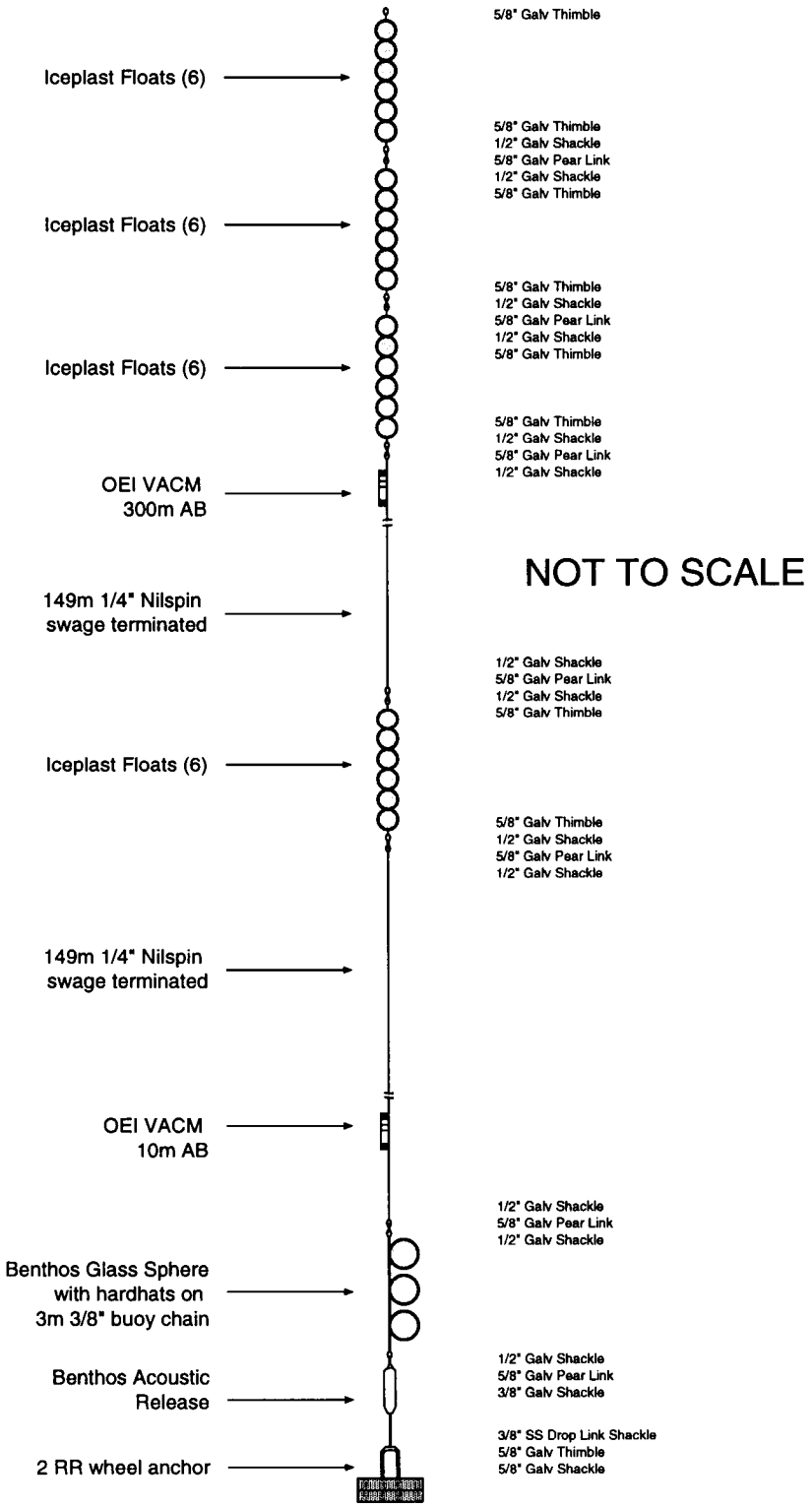

Figure 3.9. Engineering drawing of the current meter mooring deployed at GC185.

3.8.3 Preliminary Results

Apart from the CTD profiles mentioned above, none of the data is available until the instruments are recovered in 1998.

3.9 Bacterial Mats: Metabolic Diversity, Productivity, and Competition with Symbiont-Containing Animals

Principal Investigator: Douglas Nelson
Institution: Biological Sciences, University of California - Davis

Chemosynthesis is fundamentally a microbial process, whether it is carried out by bacteria living in symbiosis with higher animals or by free-living bacteria. Previous efforts have not adequately addressed the role of free-living bacteria. This work element focuses on the free-living bacteria at various sites in the Gulf of Mexico where numerous, sediment-associated mats of *Beggiatoa* sp. have been reported. As detailed below, these bacteria may compete with major fauna for sulfide and methane and may contribute significantly to overall production at these sites. Additionally, thioautotrophic and methanotrophic bacteria associated with worm tubes and bivalve shells are expected to contribute significantly to bacterial production. Based on the activity of the enzyme ribulose-1,5-bisphosphate carboxylase (RUBISC/O), the "non-pigmented" *Beggiatoa* mats are almost certainly chemolithoautotrophic sulfur bacteria (thioautotrophs), and the "orange" mats possess an undefined but non-autotrophic metabolism (Nikolaus 1995). All of the Gulf *Beggiatoa* filaments exceed 25 μm in cell diameter (Nikolaus 1995), a property always associated with filamentous sulfur bacteria with possession of a unique bacterial structure, a large central vacuole (Maier et al. 1990; Nelson et al. 1989). Furthermore, in sulfide-rich sediments these vacuolate bacteria were shown to accumulate intracellular nitrate at concentrations as high as 160 to 500 mM, 4,000- to 20,000-fold above ambient levels (Fossing et al. 1995; McHatton et al. 1996). This is presumably in the central vacuole, which comprises roughly 80% of the cellular biovolume. High membrane-associated activities of nitrate reductase indicate that these *Beggiatoa* employ nitrate as an electron acceptor (McHatton et al. 1996) in the microoxic or anoxic niches where they dominate, reaching population densities of up to 0.5 kg/m^2 of sediment (wet wt., excluding vacuole). Very recently (McHatton and Nelson, unpublished) it was observed that the vacuolate *Beggiatoa* filaments at sulfide-seeps in Monterey Canyon extend 8-12 cm down into completely anoxic sediment and appear to consume virtually all free sulfide in these regions. This suggests a strategy for anoxic oxidation of the sulfide based on the use of internally stored nitrate as the electron acceptor. Because neither oxygen nor free-nitrate will penetrate more than a few millimeters into such sediments, *Beggiatoa* filaments with this adaptation avoid competition with free-living thioautotrophs that respire exogenous nitrate or oxygen. Steep sulfide fluxes measured below the Monterey *Beggiatoa* sp. indicate a high rate of sulfide oxidation by these mats, and a massive ammonia maximum centered at 6 cm sediment depth suggests that this, rather than N_2, is the final product of concomitant dissimilatory nitrate reduction.

It seems likely that the mats of white *Beggiatoa* sp. found in the Gulf of Mexico can also store and use nitrate as a terminal electron acceptor. Therefore, it is possible that the non-pigmented filaments have the potential for avidly consuming sulfide, at least over the top 10-12 cm of these

anoxic sediments, which in turn suggests the potential for intense competition for sulfide between *Beggiatoa* sp. and symbionts of bivalve or tube worms. It also has implications for a sediment-localized nitrogen cycle driven by the sulfur and carbon cycles. If the oxidizable substrate of the orange *Beggiatoa* sp. proves to be, for example, methane, this suggests potential for competition between these bacteria and symbiont-containing methanotrophic mussels.

It has been recently demonstrated (Hagen and Nelson 1996; McHatton et al. 1996) that combined determinations of the activities of the enzymes 2-oxoglutarate dehydrogenase (OGDH) and ribulose-1,5-bisphosphate carboxylase (RUBPC/O) can tell us much about the autotrophic or heterotrophic growth status of obligate and facultative thioautotrophs. With no known exceptions, the Calvin cycle is the carbon fixation pathway employed by aerobic thioautotrophic bacteria, and RUBPC/O is the carboxylating enzyme of this cycle. Because of the selective pressure exerted for regulation of this inefficient enzyme, it is generally not produced in excess of the biosynthetic requirements of a particular bacterium. The enzyme MDH is a universal component of the energy generating pathways in C_1 bacteria that oxidize methanol or methane as their means of energy conservation and synthesize new bacterial biomass with these C_1 compounds as sole precursors. Because the biosynthetic pathways employed by these C_1 bacteria diverge after the common intermediate formaldehyde (the product of methanol dehydrogenase), MDH provides the best single estimate of their collective activity.

In regions of *Beggiatoa* mats, it was proposed to examine sediment profiles of nitrate, ammonia, and soluble sulfide at 2 cm resolution. Flux calculations should allow estimations of rates of bacteria consumption and production. Since considerable sulfide may also be produced via sulfate reduction in the *Beggiatoa* zone, but also rapidly consumed there, this process will be estimated using radiotracer techniques. Expertise within the program team will be needed to provide methane profiles from these sediments. Within these same sediments, vertical profiles (2 cm resolution) of *Beggiatoa* abundance (biovolume, protein) will be examined. Assay purified *Beggiatoa* filaments "diagnostic enzymes" (RUBPC/O, OGDH, MDH), intracellular concentrations of nitrate and ammonia, and the degree of contamination by unicellular bacteria will be examined. This data set will span on the reasons for different colors of *Beggiatoa* mats. It will also include two sets of control sediment core data for non-mat sediment regions immediately adjacent to *Beggiatoa*-dominated regions. To estimate the contributions of bacterial processes on surfaces, known areas of worm tubes and bivalve shells will be scraped to yield mixed bacterial populations which will be assayed for diagnostic enzymes (see above) and total protein.

3.9.1 Objectives

The objective of this work element will be to test the following hypotheses:

H_o-1 *Beggiatoa filaments from both orange and non-pigmented mats in the Gulf of Mexico contain massive central vacuoles with intracellular nitrate concentrations in the range of 150-500 mM.*

H_o-2 *For these Beggiatoa populations, their vertical distribution in sediments, their change in internal content of nitrogen ions with depth, and their*

impact on sediment sulfide and/or methane profiles reveal their dominant energy motifs with respect to electron donors and acceptors.

H_o-3 *Based on (1) and (2), Beggiatoa mats are capable of consuming energy sources (e.g. methane or sulfide) at sediment depths of up to 10cm. This is hypothesized to have placed strong adaptive constraints on the types of symbiotic associations that can flourish in this environment, i.e., the associations need to be capable of competing at considerable sediment depth for energy sources.*

H_o-4 *Assays of bacterial mats from sediment and animal surfaces for "diagnostic enzymes" (RUBPC/O, OGDH, and MDH) will identify the most productive mats. Furthermore, it will allow cataloguing of mats as predominantly chemoautotrophic, heterotrophic, or methanotrophic.*

3.9.2 Methodologies

Beggiatoa biovolume and percent contamination of samples by unicellular bacteria will be measured by epifluorescence microscopy (Nelson et al. 1989). Total *Beggiatoa* protein, a second measure of biomass, will be made by the Coomassie brilliant blue dye-binding technique (Nelson et al. 1989). The intracellular nitrate and ammonia concentrations of highly purified filaments will be measured by ion chromatography and normalized to intracellular volume as previously done (McHatton et al. 1996). *Beggiatoa* purification will be achieved by overlaying 1-2 cm vertical sections of sediment on agar containing 2-6 mM sulfide to induce filaments to glide out of sediments. Alternatively, purification will be by density gradient centrifugation, exploiting their high specific gravity due to internal globules of elemental sulfur. Sediment porewaters will be obtained by anaerobic centrifugation, and their concentrations of sulfide, ammonia, and nitrate will be determined by ion chromatography or standard wet chemical techniques. Enzyme activities (RUBPC/O, OGDH, and MDH) will be determined by previously employed (Hagen and Nelson 1996) or standard techniques. Sulfate reduction will be measured by injecting 35^S-sulfate at 1-2 cm intervals into intact sediment cores for 24 h shipboard incubations at *in situ* temperatures. Termination of the reaction, processing of cores, and rate calculations will be as detailed by Fossing and Jorgensen (1989).

3.9.3 Preliminary Results

Activities during the first year fell into three categories: 1) pre-cruise planning and preparation, 2) participation in the second leg of the 1997 cruise, and 3) post-cruise laboratory analyses. The general planning meeting was pivotal for integrating this work element plans with the other work elements. Correspondence was essential to agree upon common methodologies for sulfate reduction assays. Along with the normal cruise preparation tasks of assembling and shipping equipment and sampling vials. Sixty (60) plexiglas sediment cores (30 to 40 cm each) were cut to length and drilled at 1cm intervals. The holes were filled with silicone sealant, thereby forming injection ports for sulfate reduction assays. Due to space limitations, only one PI was able to participate for microbiology in the second cruise leg. The distribution of 64 large sediment cores (7.5 cm diameter, 20 to 40 cm long) between canning for hydrocarbon analysis,

microelectrode profile research, inorganic chemistry porewater processing, and microbiological studies was decided. The latter comprised of the following activities: 1) generating triplicate sub-cores of appropriate large cores; 2) freezing duplicates of these for porosity and porewater profile determinations; 3) injecting and incubating the third replicate for sulfate reduction rate determinations; and 4) harvesting appropriate surface populations of orange or white *Beggiatoa* mats for future analytical and biochemical studies (Table 3.8). Quantitative subsamples of the Beggiatoa mats were preserved for electron microscopy and biovolume determinations. The suction sampler of the *Johnson Sea-Link* (JSL) also provided several *Beggiatoa* mat collections. These were quantitatively subsampled and replicates were preserved in glutaraldehyde or by freezing.

Diagnostic enzyme analyses are approximately one-third complete on the various *Beggiatoa* collections. Considerable time has been spent training two undergraduate researchers in these assay methodologies. The methods are sometimes operating near the limit of detection because of the relatively small quantities of material that could be collected by pipette off the top of sediment cores. The ion chromatograph has just become available, and analyses of intracellular nitrate and ammonia have been initiated. Supporting information for sulfate reduction analyses is available from other groups.

Based on Year 1 sampling, *Beggiatoa* filaments only occupy the surface 2 centimeters of Gulf of Mexico seep sediments. Because this is in contrast to our expectations based on our findings elsewhere, density gradient centrifugation on preserved materials will be used to confirm this. More effort will be expended in the second year to obtain bacterial scrapings from the surfaces of tube worms and mussels for assay of epibiotic bacterial abundances. If sufficient quantities can be gathered, this material will be subjected to analysis of diagnostic enzyme activities. For the second cruise, the approach will be much the same as the first. However, more effort will be placed on obtaining larger *Beggiatoa* populations via the JSL suction sampler or one more suited to bacterial collections. This will assure that all diagnostic enzyme assays can be performed on a single population of bacteria.

3.10 Food Chain Dynamics

Principal Investigators: Robert Carney; Robert Feller; and Stephen Macko
Institution: Louisiana State University; University of Southern California; and University of Virginia

The design of this work element is based directly upon the results of previous studies. The following observations about food chain dynamics have been established by previous studies:

1) There appears to be some seep carbon in slope species. Background megafauna from the northern Gulf of Mexico. During the northern Gulf of Mexico Continental Shelf Study isotopic analyses showed a well-defined $\delta^{13}C$ signal consistent with a phyto-detritus food web with the very notable exception of a few specimens of the large red crab (*Chaceon quinquedens*) and an eel (*Synaphobranchus* sp.) which seemed to contain carbon from either terrestrial vascular plants or chemosynthetic carbon. Since

Table 3.8

Variables to be Measured - Proposed and Actual (Year 1)

Variable	Year 1 Proposed	Year 1 Actual
Sediment core profiles - nitrate, ammonia, and sulfide (2cm resolution)	n=6	n=9
Sediment core profiles - Beggiatoa protein, biovolume, intracellular nitrate, and % contamination (2cm steps)	n=4	n=9
Surface and deepest Beggiatoa populations - intracellular ammonia and diagnostic enzymes*	n=4	n=9
Sediment core profiles - sulfate reduction rates (2cm resolution)	n=6	n=14
Diagnostic enzymes* - bacterial scrapings from worm tubes and mussel shells	n=6	n=0

*These are: ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBPC/O); methanol dehydrogenase (MDH); 2-oxoglutarate dehydrogenase (OGDH).

terrestrial carbon is ubiquitous in the sediments but otherwise is not incorporated into deep benthic food webs, the interpretation of chemosynthetic carbon is preferred. This interpretation is consistent with the observation that both organisms are known vagrants (background fauna that exploit seeps) at seep sites.

2) Non-chemosynthetic seep organisms definitely exploit chemosynthesis, but analysis of these links proved more ambiguous than expected. During the initial studies $\delta^{13}C$ seemed to be the ideal tracer of chemosynthetic carbon. The underlying methane reservoirs have distinctive values which are transferred to methanotrophs directly, and indirectly to thiotrophs via carbon dioxide. Unfortunately, $\delta^{13}C$ values showed puzzling results; the range of values of many consumers was too great to be explained simply by feeding upon chemosynthetic sources. This might have been expected for vagrants like the crab Chaceon which may move in and out of seeps, but similar confusing ranges were found for partial predators like Munidopsis and the obvious mussel predator Sclerasterias.

These two important, but inconclusive, observations have been made. There may be slope populations at some distance from seeps that benefit from them and might be impacted by their demise. And, some of the consumers at seep communities may not be fully or even partially dependent upon chemosynthesis.

The work being carried out will resolve the ambiguities of the previous observations. There are two reasons for such ambiguity. First, the food web is actually complex with both normal phytoplankton-derived carbon somehow contributing a substantial portion of the diet of seep associates. Second, the pools of chemosynthetic carbon are complex and mimic phyto-detritus input as consumers feed on different patches of mussels, tube worms, and bacteria. To address this question, the more definitive isotope ^{15}N will be measured in conjunction with ^{13}C to confirm chemosynthetic origins. Immunoassay of stomach contents will be used to identify the primary chemosynthetic tissue in the diet. Immunoassay is very useful in benthic systems where complete mastication by consumers makes traditional stomach content analysis impossible.

Many of the small seep consumers are non-lethal predators consuming tiny snips of tissue, thus this is the best method of confirming feeding upon either mussels or tube worms.

This work-element is intended to provide a trophic study of interdependencies and interrelationships between chemosynthetic and non-chemosynthetic organisms and their abiotic environment. This work is necessary in that trophic classification of fauna provides:

1) determination of which species actually exploit chemosynthetic production rather than some other habitat value of seep-related topographic features.

2) identification of possible agents of predatory control on seep populations, and

3) identification of feeding dependencies of background species.

3.10.1 Objectives

This work element will provide a trophic answer about the interdependencies and interrelationships between chemosynthetic and non-chemosynthetic organisms and their abiotic environment. Previous investigations provided interesting but ambiguous results due largely to limitations of the methods employed. Through improved identification of food pools by adding ^{15}N and ^{13}C and improved stomach content analysis using immuno-assay the following will be achieved:

1) Determination of which seep and slope species actually exploit chemosynthetic production rather than some other habitat value of seep-related topographic features.

2) Identification of specific prey-predator pairs identifying food-web links important in trophic processes and as possible agents of predatory control of seep populations.

Specific hypotheses in the null form are:

H_o-1 *There is no distinguishable difference in the $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of consumer organisms collected at known and progressively decreasing proximity to seep clumps and seep areas.*

H_o-2 *The masticated stomach contents of seep consumers cannot be identified as to source, mussel, tube worm, or bacteria.*

H_o-3 *The seep endemic galatheid crabs, alvinocarid shrimp, orbinid polychaetes, nerite and buccinid snails obtain the bulk of their nutrition from mussels and tubeworms.*

H_o-4 *Selected species of non-endemic fauna (assorted crustaceans and fish) obtain the bulk of their nutrition from mussels and tubeworms.*

H_o-5 The heterotrophic faunal communities associated with mussel beds at different sites (and in different geological settings) are significantly different.

Approximately 300 specimens will be analyzed for isotopes and 40 for immune response to prey species antibodies. All sampling will be coordinated with general ecological sampling.

3.10.2 Methodologies

Immunoassay analyses will be conducted in the laboratory of Dr. Robert Feller. Dr. Feller pioneered the use of immunoassay in benthic ecology. Polyclonal antibodies to menthanotrophic mussels, thiotrophic tube worms, and chemosynthetic bacteria will be developed and used to test

advised. Five kilometer deployments were not made due to a lack of time. Specimens obtained this way number 186.

Surface-deployed trapping proved simple, but weather and operational concerns limited deployments to approximately 1 km from the three proposed sites. Use of two traps, luck with the weather, and better non-dive time management should allow completion of work element objectives in 1998. Submersible-deployed traps proved simple, but imposed space restrictions on the submersible. The proposed deployment within site at three locations was completed. A large number of samples were obtained for isotopic analysis, but representation of the targeted species was somewhat low. Tissue was submitted to initiate antibody development.

There was concern that a two day soak bait would cause nitrogen to enter the tissues of smaller invertebrates and confuse results. Thus very small baits were used, and these well wrapped in screening to avoid actual consumption. As a consequence, catches were meager, and none of the targeted crab Chaceon were taken. By adding small amphipods to the trapped fauna, the proposed number of 144 samples was exceeded.

The materials listed in Table 3.9 have been submitted for antibody production to determine gut contents.

Table 3.9

Material Submitted to Dr. Robert Feller.

Sample Type	Proposed	Actual
Mussel mantle	Adequate	20 individuals collected JSL dive 2852
Escarpia plumes	Adequate	10 individuals JSL 2857 10 individuals JSL 2850
Beggiatoa mat	Adequate	None
Total		40

Initial assessments are that enough mussel and tube worm tissue was obtained to have successful antibody production. Obtaining sufficient bacterial material will be attempted in 1998. The low biomass in cores and the amount of sediment contamination when suction is used pose technical problems that will be resolved.

In 1998, two traps will be rapidly deployed to complete the proposed design. They will be bulk baited and only megafauna will be included as originally intended. These larger organisms are less likely to show bait assimilation. Trap deployment is simple and makes no impact on submarine operations. Therefore, scheduling a total of six deployments and recoveries should be feasible. In 1998, three deployments will be made with bulk baiting.

4.0 SUMMARY

This report has documented the first year of work of a three and one-half year program. Work accomplished during this period sets the stage for completion of the remainder of the program over the next two and one-half years. At the outset of the program, the investigators agreed upon a comprehensive sampling and analytical design to examine processes that affect stability and change within chemosynthetic ecosystems of the northern Gulf of Mexico continental slope (Section 1.3). The field elements include three sampling cruises with submarines and a geophysical survey that utilizes a state-of-the-art side-scan and swath bathymetry instrument. Two of the four field efforts were completed during Year 1 with accomplishment of nearly 100% of the planned work (Section 2.2). An additional benefit included the discovery of a previously undescribed polychaete worm living in direct contact with gas hydrates.

A regional geophysical survey was completed and combined with submarine reconnaissance data from previous years. In Year 2, methods for remote detection of seeps and seep communities will be further evaluated (Sections 3.1 and 3.2). This effort is expected to significantly increase the number of known seep community sites. The survey extends investigations into the deeper waters of the northern Gulf, however, there is currently no plan for ground-truthing survey results with direct seafloor observation. Additional intense seafloor sampling builds on previous results at known seep communities to better delineate the biological and chemical processes that contribute to seep community ecology. By categorizing the types of seep communities on the basis of distinct seepage style (sediment diffusion and brine pooling), the program selected four well-documented study sites where the major faunal groups are found in association with seeps and where sampling can be accomplished with maximum efficiency. Within each site, sampling stations were chosen on the basis of robust biological characteristics (Section 2.1). Experience with the use of the *Johnson Sea Link II* submarine allowed investigators to design an ambitious, but doable sampling program that encompassed sediment, water, and tissue collections, as well and deployment of a variety of *in-situ* experiments to study animal growth and fluctuations in the physical/chemical environment. All of these samplings were conducted within the context of a rigorous meticulous data management and statistical design (Sections 2.3 and 2.4), respectively).

The overall program brings together a wide range of scientific disciplines to address a complex series of interrelated objectives (Table 4.1). The present report outlines the preliminary results of what, so far, has been primarily a sampling effort. Reports from individual work elements in Section 3.0 emphasize methods and approaches since this report is being prepared before most analyses are complete. The work elements include components that were part of previous research sponsored by MMS that are now being approached in an expanded and more comprehensive format and design. Research on the ecology of seep fauna (Section 3.3.) extends previous efforts related to the physiology and growth of seep mussels and tube worms and introduces techniques of molecular genetics to examine population stability within and among seep communities. Techniques of histopathology and assay of tissue hydrocarbon burdens (Section 3.4) address the "health" of seep mussels and tube worms. The hydrocarbon geochemistry of seep sediment has focused in particular on the influence of hydrate formation and is combined with an extensive inorganic chemistry program (Section 3.5, 3.6, and 3.7). New efforts include a series of experiments to study the currents and water temperature variations at

Table 4.1. Summary of Program Objectives, Content of the Final Report, and the Associated Work Elements.

Objective	Final Report		Work Element/PI
(a) Conceptual Model -Abiotic -Biotic	a1 a2	Discuss initial conceptual model and its refinement Estimate of the overall abundance and distribution of communities in the Gulf of Mexico	a1 Conceptual Model Group/IRM, JB, CF, PM a2 Detection of Communities/IM, WS
(b) Physical/Chemical Factors	b1	Physicochemical interdependicies	b1 Physical Oceanography and *in situ* instrumentation/NG, IRM Inorganic and Organic Chemistry/JM, RS
(c) Sources of Dissolved and Free Gases	c1	Seawater and substrate findings	c1 Inorganics/JM
	c2	Source of seep hydrocarbons (effects of reservoir depletion)	c2 Organics/RS c1/c2 Microbiology/DN
(d) Stability and Change or Ecology -robust or fragile	d1	Composition, abundance, distribution, variability, spatial relationships	d1 Photo Mosaics/IM
-permanent or ephemorol	d2	Growth, physiological and biological findings	d2 Ecology/CF, DN
-senescence and death	d3	Interdependencies and interrelationships between chemo-synthetic and non-chemosynthetic organisms and the abiotic environment	d3 Trophic Studies/RC, SM, DN
-recovery rates	d4	Possible effects of petroleum industry	d4 Molecular Biology (recovery?)/KN Ecology/CF Laser Mosaics/IRM
(e) Detection of Communities -remote acoustics -geophysical devices -imaging instrumentation -hydrocarbon measurements -other technologies	e1 e2	Geophysical and geological findings Reliability of various available methodologies	e1 Geophysics/WS e2 Geophysics/WS Industry/JB Remote Sensing and GIS/IRM
(f) Recomendations for future studies			f1 All PIs
(g) Review			g1 Scientific Review Board

seep sites (Section 3.8) and use of micro-electrodes to determine pore water sulfide levels (Section 3.7). Study of bacterial mats (Section 3.9) and seep trophic relationships by use of immunoassay and stable isotope techniques (Section 3.10) complement the other biological components of the program.

In conclusion, a team of internationally recognized scientists has been assembled to marshall the best possible field and analytical methods in pursuit of program objectives and goals. At the conclusion of the first year, the program is firmly on track and holds forth the promise of exceptional results that will be of management utility to the MMS.

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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 **Royalty Management Program** 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.