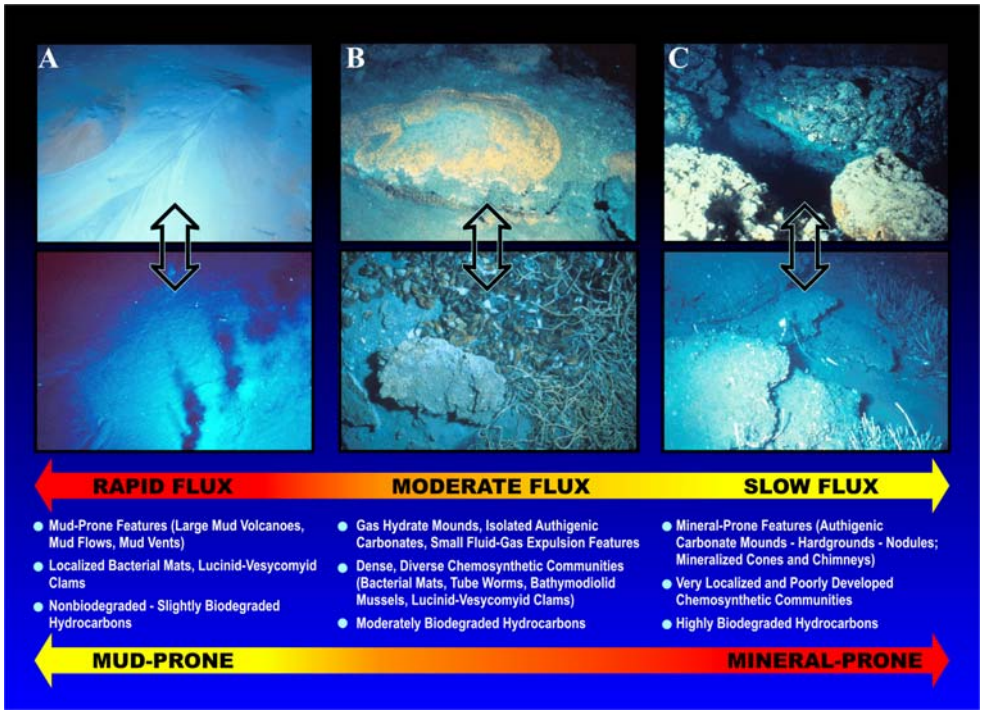
News Item

for

Investigations of Chemosynthetic Communities on the Lower Continental Slope of the Gulf of Mexico

TDI-Brooks has just been awarded a **$3.16 million contract** (#0105CT39187) with the Minerals Management Service (MMS) for a 4-year study of chemosynthetic communities on the lower continental slope of the Gulf of Mexico. As a bureau of the US Department of the Interior, the MMS has primary responsibility for the management of the mineral resources located on the nation's Outer Continental Shelf (OCS). The MMS administers the OCS competitive leasing program for oil and gas exploration, and oversees the safe and environmentally sound exploration and production of our nation's offshore natural gas, oil, and other mineral resources (www.gomr.mms.gov).

The award to TDI-Brooks includes a comprehensive study of existing 3-D seismic information for the purpose of locating the chemosynthetic communities, using a camera sled and other high-tech gear for the reconnaissance of identified areas, visiting known communities with the manned submersible ALVIN (http://oceanexplorer.noaa.gov/technology/subs/alvin/alvin.html), and documenting the communities with chemical sensors, microbiological examinations, and digital video using an autonomous underwater vehicle (AUV). This is an exciting, cutting-edge, multi-disciplinary scientific study for which TDI-Brooks is honored to be the prime contractor. In this capacity, TDI-Brooks will collaborate with a group of world-class and internationally-recognized scientists in the field of chemosynthetic communities.



(read more)

Scope and Objectives of This Project

A. To characterize known, or newly discovered chemosynthetic communities at depths below 1,000-meters in the central and western Gulf of Mexico.
B. To characterize all other hard bottom biological communities encountered regardless of association with active hydrocarbon seep activity or living chemosynthetic community species in the central and western Gulf of Mexico.
C. To determine the comparative degree of sensitivity of anthropogenic impacts for both A and B above through a variety of approaches such as rarity, unique taxonomy/biodiversity, or other environmental risk assessment methodologies. This objective includes understanding how these deep communities are similar or different from their shallower water counterparts.
D. To further develop successful assessment methodologies for correlation of remote sensing information such as bathymetry, seabed acoustic reflectivity, subbottom structure, and other geophysical signatures obtained by non-visual techniques with the "potential" presence of non-soft bottom biological communities at depths below 1,000 meters. The objective is specifically targeted to result in some level of predictive capability that can be used by MMS to avoid impacts to lower slope sensitive biological communities.
E. To contribute to assessing and explaining diversity distribution and abundance of marine species at depths below 1,000 m in the central and western Gulf, as well as improving the understanding of the functional role of marine species in areas of active hydrocarbon seep activity or living chemosynthetic communities.

These objectives will be accomplished through a combination of both exploratory work and more focused studies including process-based work on known communities.

Directed Missions of MMS

Acting under acts of Congress, MMS serves as a prudent manager of the nation's seafloor mineral resources. That management role requires development of critical energy resources without unacceptable impact on other ocean users, the natural environment, and the human environment. The primary strategy that MMS employs to eliminate or minimize environmental impact is to identify sensitive habitat and then restrict or otherwise mitigate exploration, development, and production activity. Typically, reefs, other live bottoms, critical fish habitats, etc. are classed as sensitive on the basis of accumulated prior knowledge and directed MMS studies. The vast areas of seemingly homogenous soft bottoms are classed as insensitive. Even in the case of such insensitive areas, MMS and EPA regulations seek to limit the area and degree of impact.

Increased oil and gas activity beyond the shelf break and US recognition of the 200 m EEZ greatly increased MMS's environmental coverage. Faced with a very poorly studied and remote environment, MMS supported a series of deep surveys (Carney, 2001) along the Atlantic and Gulf of Mexico continental margins in the 1980's. These studies confirmed the existence of vast soft sediment habitats, but also recognized previously known live bottoms, and found unexpected chemosynthetic communities. Both deep live bottoms and chemosynthetic communities can be classed as sensitive habitats. It can be noted that the core participants in the propose work participated in these previous surveys in many ways.

The results of MMS's first study of upper slope chemosynthetic communities, Chemosynthetic Ecosystems Study (MMS Report 95-2001) began the trend continuing to this day of submersible-based investigation in a mixed exploration and detailed-study mode. A second study, Stability and Change in Gulf of Mexico Chemosynthetic Communities (MMS Report 2002-036) greatly increased knowledge of the ecology of these systems. The scientific value of these studies was increased by the initiative of the core members who successfully sought competitive funds from NOAA, DOE, and NSF.

The work proposed herein is designed to meet MMS' information needs concerning the location and functioning of seep communities deeper than the artificially imposed limit of 1000 m. Preliminary studies have shown that seep communities at the slope base are different from those on the upper slope in much the same way that the normal background fauna differ. Therefore, MMS can not simply extrapolate upper-slope data down the entire margin.

Background for This Project

Over the last half century, offshore exploration for hydrocarbons in the northern Gulf of Mexico has advanced from the bays and inner shelf to the continental slope-to-continental rise transition. Geophysical and geotechnical data collected in support of both exploration and production has largely been responsible for the foundation of our present understanding of slope geology. This database emphasizes the extremely complex geological framework of the northern Gulf's continental slope and the surprisingly important role that the expulsion of subsurface fluids and gases has on shaping surficial geology and biology of the modern seafloor. Regional topography of the slope consists of basins, knolls, ridges, and mounds derived from the dynamic adjustments of salt to the introduction of large volumes of sediment over long time scales. Superimposed on this underlying topography is a smaller class of mounds, flows, and hard grounds that are the products of the transport of fluidized sediment, mineral-rich formation fluids, and hydrocarbons to the present sediment-water interface. The geologic response to the expulsion process is related both to the products being transported and the rate at which they arrive at the seafloor. Mud volcanoes and mudflows are typical of rapid flux settings where fluidized sediment is involved. Slow flux settings are mineral-prone. Authigenic carbonate mounds, hard grounds, crusts, and nodules are common to settings where hydrocarbons are involved. Barite in the form of small cones, chimneys, and crusts may also be found where expulsion of barium-rich water occurs. In settings between mud-prone rapid flux and mineral-prone slow flux environments, unique conditions occur to support and sustain densely populated communities of chemosynthetic communities. These areas correlate well with the occurrence of surficial exposures of gas hydrate. Direct observation and sampling of these unusual geologic and biologic environments by the key personnel of our contract started in the mid-1980s using manned submersibles. To date, most submersible-supported research has been concentrated on the upper slope (<1,000 m). However, fluid and gas expulsion features, chemosynthetic communities, brine seeps, and slope instabilities occur over the slope's full depth range as imaged on geophysical data and confirmed by limited numbers of deep submersible dives and remotely operated vehicle (ROV) bottom imaging transects and samples.

Project Team

TDI-Brooks International Inc. (TDI-Brooks) has assembled a team that combines the most knowledgeable and experienced researchers specific to the requirements of this project in the Gulf of Mexico and includes scientists that are internationally recognized for their contributions to the global understanding of cold seep ecology, microbiology, physiology and geology. All of the key US members of the team are also very experienced with, and have led, large multidisciplinary projects and cruises. Dr. James Brooks will be the Project Manager and will take the lead in administration of this project and assist in the geochemical studies. Dr. Charles Fisher (Pennsylvania State University) will coordinate the biological studies, Dr. Harry Roberts (Louisiana State University) will coordinate the geological/geophysical studies, and Ms. Liz Goehring (Penn State and NSF Ridge 2000 office) will coordinate the education and outreach activities.

Dr. Charles Fisher will oversee the biological aspects of the study and the interface with geochemical measurements and studies. He will work closely with Dr. Roberts and other PIs to

plan and conduct the submersible/ROV portions of the field work. His research group will take responsibility for in situ methane analyses, growth studies, quantitative collections, community composition and structure analyses, and trophic studies of the endemic and other closely associated seep and coral fauna. He will also be responsible for coordination with international collaborators. Dr. Erik Cordes will work with Fisher's team on studies of seep communities and take a leadership role on synthesis and publication of results for other hard bottom communities discovered. Dr. Stephane Schaeffer will oversee work in his laboratory, including molecular phylogenetic screening of foundation species and their symbionts (tubeworms, mussels and clams) and other potential new species (and symbioses) as needed. Dr. Robert Carney will lead the studies of interactions with background fauna and trophic exchange between seep/hard bottom communities and larger mobile fauna. Drs. Fisher, Carney, and Cordes will share responsibility for coordination with taxonomists and molecular phylogenists and proper curation of samples. Dr. Ian MacDonald will direct the use of digital imagery in all phases of the study, from the initial site survey and selection process to site descriptions and contributions to faunal inventory. Dr. Samantha Joye will be responsible for the microbial ecology and sulfide geochemistry studies.

In addition to this core team, we have assembled a team of collaborators that significantly expands our taxonomic expertise and brings in some of the top international seep research groups, at a very small additional cost to the project (transportation costs to cruises, minor supply costs, and shipping and curation costs for samples). Dr. Tim Shank (WHOI) has indicated his willingness to phylogentically characterize any potential new species of megafaunal crustaceans and to include at least the shrimp in his ongoing biogeographic analyses. Dr. Bob Vreijenhoek (MBARI) will do the same clams and their symbionts and other gastropods as needed. Limpets and snails will also be sent to Anders Waren (Swedish Museum of Natural History) and chitons to Julia Sigwart (University College Dublin) for morphological characterization. Dr. Stéphane Hourdez (Stacione Biologique de Roscoff, France) will take the lead on polychaete phylogenetic characterizations and descriptions of new species of polynoids and siboglinids (using both molecular and classical approaches). He will also assist with molecular characterization of foundation species during visits to PSU after the cruises (working in S. Schaeffer's laboratory at PSU). Dr. Stephane Cairns (Smithsonian) will oversee curation and identification of cnidarians, with assistance of Daphne Fautine (University of Kansas) and Dennis Opreska (Oak Ridge). Dr. Cheryl Morrison (USGS Leetown Science Center) has confirmed her willingness to include any samples of *Lophelia pertusa* collected in her ongoing studies of the phylogeography and population genetics of this foundation coral species, and also to collaborate with Dr. Cairns by contributing to the molecular systematics of other hard corals as needed. Dr. Sabine Stohr (Swedish Museum of Natural History) has agreed to examine all ophuiroids collected and is already working a brittle star that was one of the dominant species collected with mussels at some of the deeper sites in 2003. Dr. Monika Bright and her research team (Univ. Vienna) will sort and identify meiofauna collected with mussel and tubeworm communities and in sediment cores. Other faunal groups will be sent to appropriate experts as needed.

Additionally, two internationally recognized research groups from the Max Planck Institute of Marine Microbiology in Bremen will bring unique expertise and equipment to bear on the study. Nicole Dublier's group will use quantitative mRNA analyses to determine the relative activities of chemoautotrophic and methanotrophic symbiont populations in the dual symbiont-containing

mussels. Antje Botieus' group will bring their in-situ seep-chemistry analysis system and expertise on the ALVIN and ROV cruises. Letters of commitment from all collaborators are available upon request.

TDI-Brooks' management and analytical team will provide critical support for the project. Dr. Bernie Bernard, TDI-Brooks Director and Laboratory Manager, will coordinate the isotope, hydrocarbon and ancillary measurements that are conducted in our laboratory. Dr. Thomas McDonald will be the principle hydrocarbon chemist for the project. Dr. Gary Wolff will act as the projects Data Manager as he has for numerous previous large multi-disciplinary MMS projects. Mr. Kathy Allen will be the projects technical editor. She along with Ms. Suzanne Cardwell will provide financial and project administrative support.

As described above, each of these PIs will have their own areas of responsibility with respect to analyses, equipment, manpower and deliverables. However, the activities of the groups will be integrated from planning and site selection through implementation and interpretation of the results. All biological studies will be interpreted in the context of the geochemical and geophysical characterization of the region and the specific sites. Nested within these base maps will be studies of fine scale geochemistry, mobile fauna densities, and microhabitat distribution of specific types of chemosynthetic or coral communities. Dr. Carney's work will provide the context on the background fauna expected at different depths necessary to interpret Dr. MacDonald's time lapse optical sampling of mobile fauna to the sites. Dr. Carney's trap and targeted collection samples will allow analysis of trophic interactions between this component of the deep sea fauna and the communities that are the focus of this project. Fisher's quantitative collection-based analyses of community structure and function will be interpreted in the context of these studies and Dr. MacDonald's photo surveys of each site. Dr. Carney's detailed studies of bivalve/gastropod interactions will be interpreted in the context of Dr. Fisher's quantitative studies and those of collaborating European investigators. The animal distribution and activity data will be interpreted in the context of sediment microbial activity and geochemical data collected by Dr. Joye's group and in situ water column data obtained by Dr. Boetius' group. Outreach activities will include input from all teams and will be coordinated by Liz Goehring, who is currently the Education and Outreach coordinator for the NSF Ridge 2000 Program. In addition to integrating this project into programs she has designed, she will work closely with the GoM area COSEE center in Southern Mississippi and the Ocean Exploration Program.

Scientific and Technical Context

General Patterns of Deep-Sea Oceanography and Ecology

Since the inception of deep seafloor sampling in the later 1800's, three global-scale distribution patterns have been repeatedly found for the fauna inhabiting vast areas of soft bottom:

- The species composition of fauna changes progressively with depth
- The biomass of fauna decreases exponentially with depth
- Overall species richness remains high relative to shelf depths and may display a maximum on the middle to lower slope.

Since deep-sea sampling remains quite sparse, there is still much need to explore and map out the details of these patterns so that the causative drivers may become more obvious. The results of the most recent study of deep soft bottom fauna in the Gulf of Mexico conform to the general patterns found elsewhere in the Atlantic.

Attempts at preliminary identification of causality have produced conflicting and incomplete results. The uncontested biomass decline must ultimately be due to a similar exponential decline of labile carbon influx to the bottom, but correlations between benthic biomass and estimated surface productivity have proved unconvincing so far. The processes supplying food to deep heterotrophs may not be as simple as previously thought. The consistency of bathymetric faunal change worldwide seems strong evidence for piezo-thermal control, but community effects (species interactions) may also play an important role. Inconsistent methods, taxonomic focus, and severe design-induced biases, however, plague even simple generalizations of the nature and rate of depth changes. Most perplexing of the three is the high biodiversity in a homogenous, food poor environment; contemporary diversity theory predicts greatly reduced species richness in such systems.

The most important recent conceptual advancement in deep-sea ecology has arisen from the combined results of exploration and careful consideration of the origins of deep biodiversity. Exploration has lead to the discovery of a lengthening list of distinctive habitats on the continental margin (Tyler, 2003), and biodiversity theory suggests that even the soft-bottom areas have a high level of habitat heterogeneity (Snelgrove and Smith, 2002; Carney, 1997). The work proposed herein focuses on two distinctive habitats, chemosynthetic communities exploiting hydrocarbon seepage (***Seep Communities***) and sessile faunal communities associated with deep hardgrounds. The tasks proposed examine both seeps and hardgrounds in the larger context of the surrounding deep Gulf of Mexico ecology.

Hydrocarbon Seep Communities

The cold seep communities on the upper Louisiana slope (ULS) of the Gulf of Mexico were among the first deep-sea cold seep communities discovered and are arguably the most intensively studied of any such community in the world. Because these animals can live at ambient pressure we (and other groups like the Childress and Young laboratories) have been able to conduct numerous studies with live animals and have learned a lot about their biochemistry,

physiology, and reproductive biology. As a research community, we have reached the point where we understand the physiological ecology and life history of many of the dominant species and have documented patterns of biogeography and community composition, structure and succession. We have formulated both quantitative and qualitative models that explain the community patterns documented, and tested many of the hypotheses generated by these models. The core team assembled for this project includes the most active and experienced research groups for discovery and description of cold seeps and their associated communities in the country. All members of the group have extensive experience and knowledge gained from work with communities on the ULS as well as experience working with deep-sea coral communities and with seep communities at depths below 1,000 m in the Gulf of Mexico. In addition we have assembled a team of collaborators that significantly expands our taxonomic expertise and complements our geochemical expertise.

The approach we will use to characterize seep and other hard bottom sites on the lower slope is predicated on our knowledge of and experience with the upper slope seep and coral sites. Therefore a review of our working models of the upper slope seep and hard bottom communities is appropriate in this context.

The Upper Slope Communities

The most significant (high productivity and biomass) chemosynthetic communities on the ULS of the GoM are characterized by bacterial mats, mussels and tubeworms. Bacterial mats are present at all sites visited to date, and both mussels and tubeworms are present at most sites that have been well studied. Based on our current understanding of the geology, geochemistry and microbiology of these sites, along with our understanding of the physiology, life history, and ecology of the dominant megafauna, we have proposed and refined a qualitative model of seep community succession at hydrocarbon-rich sites (MacDonald et al., 2002; Bergquist et al., 2002, 2003 a; b; Cordes et al., 2003; 2005; in press).

The initiation of seepage through a particular point on the sea floor (whether it be caused by salt-tectonics, hydrate decomposition, or explosive release of gas) stimulates microbial activity, which ultimately generates many of the bulk features that characterize cold seeps. For example, microbial respiration increases dissolved inorganic carbon concentrations and alkalinity, generating authigenic carbonates, one of the macroscale geologic features of seeps. Microbial respiration generates other reduced metabolites, such as hydrogen sulfide (H_2S), which support the accumulation of significant biomass of macrofauna and their microbial symbionts. Though microorganisms are present and active throughout the sediment column (to at least meters beneath the seafloor), the only microbial assemblages visible to the naked eye are the dense, surface mats of giant sulfur oxidizing bacteria (Sassen et al., 1993; Sager et al., 1999; MacDonald et al., 2002; Nikolaus et al., 2003; Arvidson et al. 2004; Joye et al. 2004**).** Megafauna are occasionally seen on microbial mats but the mats remain relatively free of visible grazers, despite the widespread acceptance that free-living bacteria form the foundation of the seep food web as they do at hydrothermal vents (Fisher et al., 1994; Van Dover and Fry, 1994; Carney 1994; Fisher 1996; MacAvoy et al., 2002; 2005).

The next successional stage is characterized by settlement of *B. childressi*, a mussel with methanotrophic symbionts (Childress et al., 1986) that requires the presence of free methane above the sediment to grow (Cary et al., 1988; Nix et al., 1995). Unlike the bacterial mats, mussel beds offer an extensive three-dimensional habitat structure with hard surfaces on which to graze and interstices within which to seek refuge from predators. A high biomass but low diversity community, dominated by endemic macro- and megafauna characterize these productive mussel beds (Bergquist et al., 2005). Although some small predatory species are present, primary consumers, particularly deposit-feeding polychaetes and grazing gastropods and decapods dominate (Bergquist et al., 2005). When the substrate is sufficiently stabilized, settlement by tubeworms begins and persists for 20 – 60 years (Bergquist et al., 2002; Cordes et al., 2003). Newly metamorphosed tubeworms require the co-occurrence of sulfide and oxygen on their settlement substrate (Southward et al., 1988). As a result, the small but complex biogenic structures created by young vestimentiferans tend to be bathed in reduced chemicals (Freytag et al., 2001; Bergquist et al., 2003b; Cordes et al., in press), facilitating free-living bacterial production but also producing a habitat toxic to many species. During this settlement and early growth period, communities associated with tubeworm aggregations are dominated by endemic primary consumers, similar to those found in mussel beds, apparently grazing on primary bacterial production (Bergquist et al., 2003a, b; MacAvoy et al., 2005; Cordes et al., in press).

Settlement of vestimentiferans ultimately ceases when sufficient sulfide is no longer released above the sea floor (Bergquist et al., 2002). Lasting upwards of 100 years is a post-settlement period where tubeworm individuals, and therefore aggregations, increase in size (Bergquist et al., 2002, Cordes et al., 2003; 2005), and release of reduced chemicals from the sediment decreases (Bergquist et al., 2003a: Cordes et al., 2005, in press) while tubeworms increase their reliance on their roots to obtain sulfide (Cordes et al., 2005). During this period, the amount of habitat can be quite large (individual structures more than a meter in height and measuring several meters in diameter) and reduced chemicals may still be present in portions of the aggregation nearest the seafloor but are absent from portions further from the seafloor (Freytag et al., 2001; Bergquist et al., 2003a; Cordes et al., in press). The diversity of associated fauna during these intermediate stages can be quite high (up to 47 species in a single aggregation), and the food web complex, including many non-endemic and predatory species not typically seen in earlier successional stages (Bergquist et al., 2003b; Cordes et al., in press). As the aggregations continue to age, sulfide expression above the sediment surface largely ceases and the diversity of the associated fauna begins to drop off. The relative proportion of endemic animals (proportion of species and biomass), steadily declines, and there is an increase in the abundance of animals in higher trophic levels (Cordes et al., in press).

Eventually, perhaps as a result of carbonate precipitation, depletion of resources in the sediments, and/or very old age, the tubeworm aggregations begin to senesce and thin-out as a result of mortality (Bergquist et al., 2003a; Cordes et al., 2003; 2005). In this stage, the diversity of associated fauna, proportion of endemics and overall biomass supported are low, but the tubes are heavily colonized by sessile fauna (Bergquist et al., 2003b; Cordes et al., in press). These aggregations sometimes appear as clusters of recumbent individuals in heavily sedimented areas (with up to a meter of the tube above the point of initial attachment buried) or as the tops of sparse aggregations protruding from the surface of carbonate outcrops. Authigenic carbonates produced during periods of more active seepage persist and are often colonized by corals that can

co-occur with mature and/or apparently senescent tubeworm aggregations. The ULS coral associated fauna includes some of the same species found in senescent tubeworm aggregations, although there are also common coral-associated species not found in association with tubeworms (Fisher and Cordes, MMS project in prog.).

With respect to the communities associated with the foundation species, the most notable trends are shifts in community structure from an almost complete absence of macro- and megafauna on bacterial mats; to endemic primary consumers in productive and toxic small structures; to highly diverse and trophically complex communities on large chemically heterogeneous structures; to less diverse, predator-dominated communities in large, non-productive, chemically benign structures. The extent to which the observed changes in community structure are a result of changes in productivity, toxicity, physical habitat structure, or predatory activities is not well resolved at this time. However, studies of biogeographic and depth-related differences in community composition and structure must be interpreted in the context of these well-documented successional stages that can co-occur within a single site.

The dominance of larger and less-productive habitats by high-order consumers also suggests that individual structure-associated communities are not self-contained and self-sustaining. No predators have been identified that utilize tubeworm biomass directly for their bulk nutrition (MacAvoy et al., 2002, 2005; Cordes and Fisher, unpublished) and lower-level consumers are generally lacking as a nutritional source within the older aggregations (Bergquist et al., 2003b; Cordes et al., in press). Preliminary data from coral-associated fauna suggests a similar pattern in these communities, with a single species of snail the only known coralivorous species. The majority of the mobile fauna occupying coral structures are higher-level predatory crustaceans and fishes, with species of suspension-feeding solitary corals and polychaetes secondarily colonizing the chemically benign habitat. This suggests that at least some of the predators found in these structures are seeking refuge from larger predators while foraging in surrounding habitats (Bergquist et al., 2003b; Cordes et al., in press). In many marine and freshwater environments, intermediate predators seek refuge (from higher order predators) within and around physical structures and subsequently forage on fauna living on and within the surrounding soft-bottom (Weinstein and Heck, 1979; Parrish, 1989; Frazer et al., 1991). This often results in changes in the abundance of various infaunal taxa near the structure (Summerson and Peterson, 1984; Gilliam, 1989; Ambrose and Anderson, 1990; Posey and Ambrose, 1994). In general, cold seeps support abundant infauna that are typical of reducing habitats and their abundance often increases with increasing proximity to active seepage (for example: Sahling et al., 2002; Levin et al., 2003; Robinson et al., 2004). Infaunal communities associated with cold seeps on the ULS of the Gulf of Mexico are no exception. The sediments at these sites, whether influenced directly by active seepage (colonized by bacteria and containing crude oil and sulfide) or not, contain diverse communities of polychaetes, amphipods and copepods with some taxa increasing (polychaetes, ostracods) or decreasing (decapods) with increasing proximity to active seepage (Robinson et al., 2004; Bergquist pers. com.; Bright, pers. com.). Although, sediment infauna are not a focus of our proposed work, Dr. Monika Bright (University of Vienna) will send one person on each cruise to make collections for a preliminary description of the abundance and diversity of infauna at all sites visited over the course of this study. This information will also contribute to our understanding of the trophic dynamics of the macro and megafauna associated with seep and coral communities.

Lower Slope Communities

Compared to the ULS, our understanding of seep and other hard bottom communities below 1000 m in the GoM is quite limited. This is largely due to the vast difference in research effort at sites below JSL operating depth to date and the resultant lack of known sites and process oriented studies at the known sites. Nonetheless, there have been numerous deep-water submersible and ROV cruises to a few deep seep sites in the GoM over the past 2 decades, and we have sufficient information to design a study plan for the types of communities we are likely to encounter over the course of this study.

The foundation species at the known seeps of the lower slope and base of the continental shelf of the Gulf of Mexico include the tubeworms *Escarpia laminata* and at least one unidentified lamellibrachid and 3 species of bathymodioline mussels, *Bathymodiolus childressi*, *B. heckeri*, and *B. brooksi* (Paull et al., 1984; Craddock et al., 1995; McMullin et al., 2003; MacDonald et al., 2003). The three best-known sites are Atwater Valley (AT-425) at 1900 m (Milkov and Sassen, 2000; MacDonald et al., 2003), Alaminos Canyon (AC-645) at 2200 m (Brooks et al., 1990; Carney 1994) and Florida Escarpment (VN-945) at 3300 m (Paull et al., 1985; Carey, 1989; VanDover et al., 2002; Turnipseed et al., 2004). Atwater Valley contains aggregations of *E. laminata* and isolated individuals of the undescribed lamellibrachid. Alaminos Canyon mussel beds consist of *B. childressi* and *B. brooksi*, and *E. laminata* aggregations and lamellibrachid individuals have also been collected. At the Florida Escarpment sites, *E. laminata*, *B. heckerae* and *B. brooksi* are the predominant foundation species. Vesicomyid clams (*Calyptogena* sp.) have also been collected from the Florida Escarpment, and were seen but not collected at Alaminos Canyon (Fisher, pers obs).

During a recent expedition to these sites, a few quantitative samples of the communities associated with tubeworm aggregations and mussel beds were obtained, greatly expanding the list of known seep-associated species from these deep sites (Cordes, Fisher, Carney, unpublished data). A total of 48 species of associated macro- and megafauna were collected in 8 quantitative samples from the three deeper sites. There was very little overlap between this species pool and the 116 species we have collected to date from the upper slope sites. The only described species obtained in both sets of collections were the sipunculan *Phascolosoma turnerae*, and the symbiotic mussel *Bathymodiolus childressi*. Three polychaetes *Eunice* sp. nov., *Eurythoe* sp. nov., and *Nereis* sp. nov. resemble undescribed species from the upper slope collections and their bathymetric distribution awaits description of the species. There is a high degree of similarity at the family level in the communities associated with mussel beds and tubeworm aggregations at the deep sites. Lower trophic levels are dominated the bresiliid shrimp *Alvinocaris muricola*, trochid gastropods (*Fucaria* sp.), and neoleptopsid limpets (*Paraleptopsis* sp.). Polynoid polychaetes and galatheid crabs, common groups at ULS seeps, are the most abundant likely predators of the grazing species. The most significant difference at higher taxonomic levels between the communities of the ULS and the deeper sites is the dominance of a recently described genus and species of ophiuroid *Ophienigma spinilimbatum* (Stohr and Segonzac, 2005). Though only recently collected, this species is present at all three of these deep sites and is the numerical dominant in half of the collections. It appears to replace some of the common grazing species from the ULS, primarily provannid and neritoid gastropods. In addition, these primary consumer species (shrimp, gastropods, and ophiuroids), presumably relying on seep

productivity, dominate all of the communities collected to date, with the exception of one collection with roughly equal proportions of biomass in the primary and secondary consumer trophic level (Cordes et al., in prep.).

The range of foundation species at abyssal sites and the types of seep habitat generated by hydrocarbons were also extended by recent discoveries in the southern Gulf of Mexico (MacDonald et al., 2004). At two sites in the Campeche Knolls region in water depths between 2,000 and 3,300 m, so-called asphalt volcanism supported dense chemosynthetic communities aggregated around massive deposits of solidified asphalt. Chemosynthetic fauna were identified in the photographic material and recovered in grab samples. Large bivalve shells, many clearly identifiable as the chemosynthetic Family Vesicomyidae, were ubiquitous on the seafloor surrounding the asphalt flows and among the pillow boulders and cobble. The edges and fractures within and along the bitumen were colonized by vestimentiferan tubeworms (c.f. *Lamellibrachia* sp.) that had small obturacular plumes and robust, slightly segmented tubes with long, slender posterior ends extending into the sediment under asphalt deposits or down into fractures within flow fields. Tubeworm densities varied from mostly small clusters of one or two specimens to 23 individuals in one single bundle. Shells and living specimens of chemosynthetic mussels (c.f. *Bathymodiolus* sp. and *Solemya* sp) were recovered from highly oiled sediments. Heterotrophic fauna included galatheid crabs, shrimp resembling *Alvinocaris* sp. as well as non-endemic deep-sea invertebrates (*Benthodytes* sp., *Psychropotes* sp., *Pterasterias* sp.) and fish. The probability that asphalt based communities will be encountered in the northern Gulf of Mexico is not known, but these findings demonstrate the adaptive capacity of seep foundation species to previously unknown processes.

Another significant difference in the upper and lower slope tubeworm-communities is the presence of a potential tubeworm parasite. The majority of *E. laminata* in the collected aggregations contained a small group of phyllodocid polychaetes on the obturaculum. The coelomic cavities of all of the phyllodocid individuals were full of blood that appeared to be from the tubeworms they infested. Additional investigations of this relationship are underway, but the presence of this apparent parasite could provide a trophic link between tubeworm and associated fauna biomass that is absent on the ULS.

The abundance and activity of microorganisms at lower slope cold seeps has not been examined. Thus, we do not know how similar the lower slope microbial communities are to those present at ULS cold seeps (16S ribosomal RNA gene (16S rDNA) libraries are available from several ULS cold seep sites (Lanoil et al., 2001; Mills et al., 2003; 2005) nor do we know how similar they are to microbes at other cold seeps (Knittel et al., 2003, 2005). Documenting patterns of microbial abundance and activity are key because microbial activity generates the sulfide that provides the metabolic energy used by several of the metazoan foundation species and other productive microbes. Linking patterns of microbial activity with those of animal abundance is an integral part of this project and will significantly improve our ability to form a predictive understanding of cold seep dynamics.

Relation to Other Cold Seep Communities

The known cold seep communities of the Gulf of Mexico are not insular communities. It is becoming increasingly apparent that seep communities are widespread not only in the Gulf of Mexico, but throughout the world's oceans. Furthermore, some cold seep foundation species have ranges that extend for 1000's of miles (McMullin et al., 2003). The communities of the deep Gulf of Mexico bear some similarity to the chemosynthetic communities inhabiting seeps on the Barbados accretionary prism, Blake Ridge, and sites off the western coast of Africa. The known Barbados accretionary prism sites are located at depths of 1300, 1700, and 2080 m (Olu et al. 1997). The dominant tubeworm at the 1300 m site is reported by Sibuet and Olu (1998) to be the same *Lamellibrachia* sp. as the ULS species now described as *L. luymesi* (Gardiner and Hourdez, 2003) and the abundant ULS endemic species, *Bathynerita naticoidea*, *Cataegis meroglypta*, and *Alvinocaris* cf. *stactophila* are also present at this shallowest Barbados site (Olu et al., 1997). The communities at the Alaminos Canyon and Florida Escarpment sites are more similar to the 1700 m and 2080 m Barbados sites than they are to the ULS seeps in terms of the foundation species present (*E. laminata, B. heckerae*) and because they share seven species of associated fauna. There are even greater similarities between the communities at the deep Gulf of Mexico sites and the Blake Ridge seeps at 2150 m with to 10 species in common between these sites (Van Dover et al., 2003; Turnipseed et al., 2003). This includes the foundation species, *Bathymodiolus heckerae*, that has only 1.4% sequence divergence of mt ND4 sequence between the Blake Ridge and the Florida Escarpment (S. Carney, Schaeffer, and Fisher, unpublished). However these mussels are larger and resemble *B. boomerang* from the Barbados site, for which there is unfortunately no molecular data currently available (von Cosel and Olu, 1998; Van Dover et al., 2003).

In 2001 a French team discovered seep communities in the Gulf of Guinea at a depth of 3,150 m. Based on presentations made at the Second International Symposium on Hydrothermal Vent and Cold Seep Biology in Brest France in 2002, these communities are structurally similar to the GoM sites, dominated by large aggregations of tubeworms and with abundant mussel beds and similar associated fauna (Olu et al., Symposium Abstract Volume). The only formal description of the fauna from this site is of a new species of tubeworm, *Escarpia southwardae* (Andersen et al., 2004). These authors erected a new species for this Escarpid based on important morphological criteria, but the molecular (mt COI) data suggests it is very closely related to the *E. laminata* from the GoM (and *E. spicata* from the W. coast of the US, which is very closely related to *E. laminata* based on standard molecular markers). More recently, TDI Brooks collected tubeworms, 2 species of mussels, and alvinocarid shrimp in box cores from off the coast of Nigeria at 1,600 meters depth. Preliminary molecular (mt COI and 16S sequences) analyses of the mussels from the Nigerian seeps indicates that the short type is most closely related to *B. childressi* and the long type to *B. heckerae* (Brooks, S. Carney and Fisher). Preliminary microsatellite data (McMullin, Brooks, Schaeffer, and Fisher) indicates that the tubeworms from the two sites off Africa are from an interbreeding population (Fst = 0.009), but they are reproductively isolated from the GoM Escarpids (Fst=0.1). We will expand these analyses when more samples from the African seeps become available and with additional genetic markers as part of this project.

Deep Hardground Communities

The most common and well-known deep-sea coral (DSC) species is *Lophelia pertusa*. This is a cosmopolitan coral found in water depths from 80 m in Norwegian fjords to over 3000 m on the Mid-Atlantic Ridge and some seamounts (Bett, 1997; Rogers, 1999). In the Gulf of Mexico, *L. pertusa* (=*prolifera*) was first recovered in the late 1800s by the *U.S. Coast Survey Steamer Blake* (Cairns, 1978) and *L. pertusa* "reefs" were first reported from a deep water trawl taken by the *M/V Oregon* in 1955 (Moore and Bullis, 1960). More recently, extensive areas of *L. pertusa* mound-like formations have been observed on the upper De Soto Slope (in and near lease block VK-826) (Schroeder, 2002) and on the upper Louisiana Slope in lease blocks GC-234 and GC-354 (Cordes, Fisher and MMS Co-PIs, unpublished data) between 430 and 540 m depth. Because the majority of investigations of hard substrata in the deep Gulf of Mexico have been associated with studies of chemosynthetic habitats, continued exploration is likely to expand this range both geographically and bathymetrically.

L. pertusa prefers water temperatures from 4-10˚C (Frederiksen et al., 1992) and produces planula larvae which require hard substrata for settlement (Wilson, 1979). Other factors controlling its distribution are not well understood. *L. pertusa* seems to prefer accelerated current regimes associated with topographic highs (Freiwald et al., 1997; Masson et al., 2003). Whether this is related to concomitant increases in food availability, removal of waste products, or protection from burial by sediments is unclear (Reed, 2002). It has been suggested that there is a link between *L. pertusa* distribution and the presence of microseepage of light hydrocarbons (mostly methane) (Hovland et al., 1998). The frequent occurrence of *L. pertusa* at cold seep sites has been documented in the North Atlantic (Hovland and Thomsen, 1997) and on the upper Louisiana slope of the Gulf of Mexico (Schroeder, 2002; MMS PIs unpublished data). This may be a result of hard substrate availability from authigenic carbonate precipitation as a byproduct of anaerobic oxidation of methane (Aharon and Fu, 2000; Boetius et al., 2000). It may also be due to increased food availability as a result of locally enhanced chemosynthetic productivity. This possibility is currently under study by Fisher and Cordes as part of the ongoing MMS deep coral project on the ULS. Chemosynthetic production appears to constitute a small component of *L. pertusa* diet in relatively shallow Norwegian fiords, based on stable carbon isotope evidence (Mikkelsen et al., 1982). However, the importance of seep chemosynthetic production to corals may be significantly greater at greater water depth, where the input of good quality photosynthetically produced organic matter is greatly reduced.

While *L. pertusa* remains the best known DSC species in the Gulf of Mexico in particular and the world in general, many other species of DSC exist. The number of described deep-water (defined as inhabiting aphotic waters greater than 200m depth) scleractinian (hard) coral species now exceeds the number of known shallow water scleractinians (Cairns, 2001). In the Gulf of Mexico, 63 species of azoozanthellate scleractinians have been reported (Cairns et al., 1993). In addition to *L. pertusa*, 3 other species are known to form reef-like structures. *Madrepora oculata* is a cosmopolitan reef forming species which has been found down to 1500 m depth (Cairns, 1978). *Enallopsammia profunda* is found in waters down to 2165 m depth and is a common component of the deep-water reef-forming coral assemblage (Rogers, 1999). *Solenosmilia variabilis* has been documented in waters down to 3383 m (Cairns, 1978). This

species is also a contributor to coral frameworks in the Atlantic (Rogers, 1999) and is the most common deep water reef species on Southwest Pacific seamounts (Koslow et al., 2001).

In addition to scleractinian corals, a number of gorgonian and antipatharian corals are present in deep waters in the Gulf (Cairns, 1978; Cairns et al., 1993). The upper Louisiana slope hard-ground communities contain at least 10 species of gorgonians and antipatharians (Cordes, Fisher and MMS Co-PIs, unpublished data). These taxa can add significant habitat heterogeneity in the form of vertical relief on *Lophelia* reefs, and can also dominate carbonate outcrops in the periphery of active seep sites (Fisher and Cordes, pers. obs.). The structure provided by gorgonian corals is reported to be a significant component of rockfish (*Sebastes* spp.) habitat in the deep waters of the Gulf of Alaska (Heifetz, 2002). Gorgonians, like tubeworms, are relatively long-lived organisms, with single colonies of *Primnoa reseadiformis* estimated to be between 150 (Andrews et al., 2002) and 500-years old (Risk et al., 2002).

The complex physical structure of anastomosing branches created by large *L. pertusa* reefs and many other colonial cnidarians can host a diverse community of associated organisms, often with abundances orders of magnitude above the surrounding seafloor (Jensen and Frederiksen, 1992). In Northeast Atlantic alone, 886 species have been recorded living in and on *L. pertusa* habitats (Rogers, 1999). On two reef structures in Norway, 256 species were recorded from recovered coral blocks (Jensen and Frederiksen, 1992). The diversity of the community on these reefs, as measured by a Shannon-Weiner diversity index of 5.50 (Jensen and Frederiksen, 1992), rivals the diversity of many tropical zooxanthellate coral reefs (Rogers, 1999). Often, the most abundant and diverse communities are found among the dead branches of coral in the center of large thickets (Mortensen et al., 1995). On the upper Louisiana slope, a total of 40 species directly associated with *L. pertusa* thickets were collected and 11 additional species of mobile megafauna identified from photographic sampling of the thickets during the first year (2004) of the ongoing MMS coral study (Cordes and Fisher, unpublished data).

Deep Ocean Environmental Issues and Impacts

Resource exploitation of the deep-sea has experienced a dramatic increase in the past decade both in terms of petroleum activities and deep fisheries (Glover and Smith 2003). Prudent management of this poorly studied system is dependent on the best possible understanding of deep sea ecology. The core investigators are active in the discussions concerning deep-sea management policy in the US, Canada and Europe, as well as in international waters. The following material provides a brief overview of impact issues in the deep Gulf of Mexico as relevant to the work.

It has been suggested that normal deep-sea species will be more sensitive to impacting activities because of generally low levels of detrital food input, slow growth, low fecundities, and limited natural habitat variability (Carney, 1997; Thiel and Koslow, 2001; Glover and Smith, 2003). Risks to deep hardgrounds are now being actively assessed in Europe, but the same general concerns apply to these food-poor deep systems. Chemosynthetic communities are quite distinct in the sense of being largely independent of detrital influx and consisting of widely separated populations.

Impacts in the deep sea are most likely to be caused by the same activities that cause impacts in shallow water.

- Drilling discharges of cuttings and fluids will smother fauna, cause some mortality and alter both the geological and geochemical habitat. Such impacts may be greater in deep development due to increased use of multiple wells drilled through a single seafloor template and use of bio-active synthetic drilling fluids. The dependence of deep hardground fauna on very sparse suspended detritus may make these communities especially sensitive to the added particulate influx since this may clog filter apparatus and lead to stress responses. While seep foundation species do not filter feed, they must keep respiratory organs clean and functioning and may have heightened sensitivity. Initial assessments of deep drilling impacts are now underway (CSA/MMS in prep., CSA/API in prep.) for typical hard bottoms, and could be refined and applied to seeps and hardgrounds in future efforts.

- Production discharges of hydrocarbons and produced waters should largely mimic natural seepage and would not be expected to greatly impact seep communities. Indeed, such discharges might promote community development. Heterotrophic hardground communities should show responses similar to shallow reefs and other sessile communities. In shallow water corals, direct exposure to hydrocarbons can cause reductions in growth and fecundity, premature release of planulae, prevention of settlement, and even whole colony mortality (Loya and Rinkevich, 1980). The impact of production impacts has been successfully carried out in shallow water by the GOOMEX study (Kennicutt et al., 1996). A similar multi-site comparision should be carried out for seeps and deep hard grounds but is beyond the scope of the proposed work.

- Physical damage by anchors, pipelines, and seafloor templates is similar to that found in shallow reefs. The chains and wire ropes used to anchor floating deep-water oil platforms have been implicated as a cause of damage to *Lophelia* reefs in the Viosca knoll region of the GoM (Schroeder, 2002). The standoff and site approval requirements of NTL 2000-G20 will provide adequate protection once sites are identified.

- Reservoir depletion remains an unresolved issue and lies beyond the scope of the proposed work. Long-term monitoring of seepage rates and community condition in heavily developed fields should be the primary method of assessment. The site characterizations proposed herein are intended to serve as a benchmark for such monitoring.

Hypotheses

Our study has components that are exploratory in nature, in addition to components aimed directly at specific hypotheses. Although there have been numerous dives to deep sites in the Gulf of Mexico, most have been to only a few fairly well known places and most of the deep Gulf has never been imaged. We have sufficient knowledge to pose a number of cogent hypotheses addressing the composition, occurrence, and function of chemosynthetic and hard bottom communities in the deep GoM, but it should also be emphasized that there is a significant exploratory and descriptive component to the work outlined here. We will investigate a number of different types of sites on the seafloor, all of which show geochemical and/or geophysical indicators of seepage and/or interesting hard bottom topographies. As a result, we expect to discover and characterize new types of communities. Discovery of new types of communities will certainly lead to discovery of new (undescribed) species of animals. In addition, the relatively small sampling effort at hard bottom and seep communities in the deep Gulf of Mexico to date suggests that discovery of new species within even known types of communities is also almost certain to occur. Two discovery-based hypotheses can therefore be put forward:

Exploration Hypothesis 1. *Undescribed types of hard bottom and/or seep communities are present below 1000 m in the Gulf of Mexico and will be discovered over the course of this study.*

Exploration Hypothesis 2. *Undescribed species of hard bottom and/or seep fauna are present below 1000 m in the Gulf of Mexico and will be discovered over the course of this study.*

Improved Prediction of Occurrence

Central to this study is the collection of data that will allow increased confidence in the use of surface collected geochemical and geophysical data for prediction of occurrence of significant chemosynthetic and other hard-bottom communities. Based on our extensive work on the ULS over the past two decades we will test the following hypotheses addressing occurrence of significant chemosynthetic and hard-bottom coral communities. Our ideas concerning the relationship between seep and hardground biology with abiotic seepage features are based on the simple observations that: (1) seep communities occur below 450 m at some sites of seepage and seep-associated carbonate substrates; (2) live bottoms associated with carbonate hardground occur from the intertidal downward; (3) not all apparent seeps and not all deep hardbottoms have associated animal communities; (4) there is a community shift associated with a long-time transition from liquid to mineral phase seepage; (5) where seep and hardground communities occur there is a composition change with depth. Two general hypotheses can be put forward about the occurrence of these systems:

Occurrence Hypothesis 1: *Below 1000 m the general types and functional groups of biological communities present will show the same relationship to qualitative rates of fluid and gas expulsion as documented on the upper slope.*

Occurrence Hypothesis 2: *The geophysical signatures for seafloor geologic conditions that support significant seep and hardground communities above 1000 m will be the same for deep and ultradeep parts of the slope (1000 – 2800 m).*

Understanding General Changes in Community Composition

In addition to working towards improving the predictive power of surface surveys, and discovering and describing new sites and species, we will conduct this study in the context of what is known about seep and hard bottom communities in the GoM and worldwide, and test several hypotheses that are derived from these previous studies and our general knowledge of the bathymetric ranges and distributions of mobile and soft bottom fauna in the GoM. Preliminary data from the few well-studied deep seep sites in the GoM (described in the Introduction to this proposal), suggest: (1) a major transition in the bathymetric distribution of seep fauna is similar to the composition transition of non-seep fauna; and (2) there are a variety of phylogenetic relations among GoM, Caribbean, and pan-Atlantic faunae. We will determine the generality of these findings by testing the following three hypotheses for both seep and hard bottom communities:

Community Composition Hypothesis 1: *Chemosynthetic (and hardground) communities are sharply depth zoned such that composition below 1700 m in the Gulf of Mexico will have very few species in common with analogous communities on the ULS.*

Community Composition Hypothesis 1 (alternate): *Chemosynthetic communities undergo gradual species replacement with depth such that composition between about 1200 and 1700 m depth will have many species in common with both the deeper GoM communities and with communities on the ULS.*

Community Composition Hypothesis 2: *Chemosynthetic (and hardground) communities are depth zoned such that within depth ranges composition will be more similar to the communities found at similar depths in the Atlantic Ocean than to geographically closer communities on the ULS.*

Community Composition Hypothesis 3: *In terms of genetic differentiation, endemic fauna at seep (and hardground) sites deeper than 1700 m in the Gulf of Mexico will be more closely related to morphologically similar species at similar depths at other sites in the Atlantic than to similar species on the ULS.*

The hypotheses above focus upon endemic species and colonists of seep and hardground communities. The complete fauna found at seeps and hardgrounds worldwide consist of a combination of animals endemic to the sites, species from the 'background fauna" that have colonized the sites in elevated abundance relative to the surrounding seafloor, and vagrants from the background fauna that regularly visit the sites while foraging over larger areas. Quantifying the most mobile and larger members colonist and vagrant fauna is especially difficult for several reasons as follows: (1) they are seldom collected; (2) they may avoid the sites during times of submersible activities there; and (3) distinguishing between the vagrants and colonists requires knowledge of time spent in the seep environment and their abundance at seeps relative to the

surrounding area. To better understand these potentially important components of the seep communities, we will test the following hypothesis with deployments of 3 rotary time-lapse camera systems.

Community Composition Hypothesis 4: *The density of larger mobile species is greater in areas characterized by seep or hardbottom communities than it is in the adjacent background benthos.*

The composition of background fauna around the seeps has profound impacts on the structure and function of the seep communities. Two well-documented depth changes in the background fauna with depth are expected to significantly impact the interactions between this species pool and seep endemic fauna. The first is that the degradation of photosynthetically-derived detritus results in an exponential decrease in biomass of normal fauna with depth. The second is the marked change in functional groups of normal fauna with depth: abundance of large predatory fishes, crabs, and seastars declines below 1000 m while abundance of deposit feeding echinoderms increases. The overall consequence of these major changes will be determined by the proposed survey, sampling and characterizations. As a general prediction, we expect a decreasing role for background species and thus test two associated hypotheses.

Community Composition Hypothesis 5: *There will be fewer predatory vagrant species exploiting lower-slope than upper-slope seeps with possible replacement by endemic predators.*

Community Composition Hypothesis 6: *There will be fewer abundant background species colonizing lower-slope than upper-slope seeps with possible replacement of functional groups by endemic species.*

Tubeworm Component Hypotheses

Due to the long life span of tubeworms, their role as foundation species, and their role as ecosystem engineers including their ability to actively influence sulfide generation, this group comprises an especially important component of the seep fauna. Our intensive study of the upper slope communities and preliminary work on the lower slope has led to the following set of refined hypotheses.

Tubeworm Hypothesis 1: *Although the depth ranges of the shallow and deep species of tubeworms may overlap, they will not co-occur in significant numbers due to competition between the foundation species.*

Tubeworm Hypothesis 2: *Either the undescribed rare species of tubeworm from the ULS ("new escarpid sp."), the rare undescribed lamellibrachid from the lower slope, or another new species of tubeworm will dominate aggregations between 1200 and 1700 m depth.*

Tubeworm Hypothesis 3: *Lower slope tubeworms will in general have lower growth rates and the growth pattern of E. laminata on the lower slope will be more similar to S. jonesi than L. luymesi (the common ULS species)*

Tubeworm Hypothesis 4: *Changes in community structure will follow the same successional trends as documented for ULS tubeworm communities:*

- *Mussels will be present in young tubeworm aggregations but rare or absent in older aggregations.*
- *Proportion of endemic species of associated fauna decline with age of tubeworm aggregations.*
- *Associated fauna biomass declines in older aggregations.*
- *Proportion of primary consumers declines in older aggregations.*
- *Reliance on seep productivity will decline in older aggregations.*
- *Corals will co-occur with tubeworms at sites with abundant carbonate outcrops, but will not co-occur with mussels (at sites with very active seepage).*
- *Communities closely associated with deep water corals will share a few (but only a few) species with deep tubeworm communities.*
- *Some species of mobile fauna will show fidelity to mussel beds, tubeworm aggregations, or coral colonies.*

Trophic Interactions

Feeding relationships within communities are one of the most important aspects of community function, since they can help identify interactions that are strong, weak, or absent. Tissue stable isotope (SI) analysis allows assessment of overall trophic relations and can constrain specific trophic interactions from samples taken during survey and census stage for newly discovered sites and communities. Previous research has identified several simple relationships in upper seep systems: (1) the seep foundation species (symbiotic tubeworms and mussels) and free-living microbes produce organic matter that can be distinguished from photosynthetically produced organic matter when a combination of stable C, N and S are used together; (2) the tissue SI values indicate that most of the fauna closely associated with seep foundation species obtain the bulk of their nutrition from seep primary production, although not necessarily from the foundation species; (3) there is a high level of tissue SI variability in consumers associated with trophic shifts and diets; (4) a limited suite of vagrant predators export seep production; and (5) another suite of large mobile organisms are present at seeps but show little or no indication of consuming the chemosynthetic production. As noted above, the availability of phytodetritus decreases exponentially with depth, while seep productivity is independent of detritus supply. Thus, the relative importance of phytodetritus to seep consumers and vagrant fauna must decrease with depth as well. Our expectation is that seep productivity will show less dilution at depth and be more evident in background fauna. Using stable isotopes we will test the following hypotheses as part of the within-seep and seep-background trophic analyses:

Trophic Hypothesis 1: *Mobile predators collected within 1 km of lower-slope seeps will show a greater chemosynthetic signature than at upper slope seeps, with an inverse correlation to overall benthic biomass.*

Trophic Hypothesis 2: *Within-seep endemic and colonist consumers will show less mixing with phytodetrital sources than found on the upper slope, with an inverse correlation to overall benthic biomass.*

Trophic Hypothesis 3: *Suspension feeding, hard-ground communities have greater ties to seep production in deep water than similar communities on the ULS.*

Microbial Hypotheses: Understanding the Geochemical Engine

While hydrocarbon seepage ultimately drives seep processes, sulfide generated by microbial anaerobic oxidation of those compounds is critical to the persistence of most foundation species and may provide toxic barriers to exploitation by most background species. As such, place-to-place differences in fauna are at least in part due to different underlying microbial systems.

Increased availability of reduced metabolites will support higher microbial abundance and also increased rates of activity in the near vicinity of point sources of seepage. At shallow water seeps, microbes are abundant 10's of meters away from areas of active seepage and are capable of rapidly responding to increased substrate availability (Joye et al., 2004; Joye, unpublished data). We suspect that at deep-water seeps, the microbiological 'imprint' of seepage (i.e. increased cell numbers and high rates of potential activity) will be apparent significant distances (10's of meters) from the active seeps.

Persistent and numerous gas seeps are prominent features of the sites along the ULS, but may be less abundant at deeper sites, as a result of greater stability of the methane hydrate deposits due to lower temperatures and higher pressure. Sulfate reduction (SR) is coupled to the anaerobic oxidation of methane, so lower methane fluxes could result in lower sulfate reduction rates. Sulfate reduction can also coupled to the oxidation of higher hydrocarbons (e.g., oils) so SR at deeper sites may be more extensively coupled to oil oxidation.
The available data regarding the identity of sulfate reducing bacteria at GoM cold seeps is limited to two shallow sites along the ULS (Lanoil et al., 2001; Mills et al., 2003; 2005). Furthermore, most available data on the phylogenetic composition of SRBs is from gassy, not oily, cold seeps (e.g., Knittel et al., 2003). We expect the deep water sulfate reducers in the GoM to be distinct from those observed at ULS sites and to be more similar to those found at deep, oil-rich sites, such as the Guaymas Basin (Dhillon et al., 2003) or from oil field reservoirs (Voordouw et al., 1996, Orphan et al., 2000) than at gassy cold seeps (like Hydrate Ridge, Knittel et al., 2003).

Microbial Hypothesis 1: *At deep water sites, microbial abundance and activity (sulfate reduction rates) will be highest in areas of active seepage of gas and hydrocarbons and will occur over a greater surrounding spatial area than on the upper slope.*

Microbial Hypothesis 2: *Sulfate reduction rates in sediments will decrease progressively as depth increases and will be correlated to a decrease in gas or fluid seepage.*

Microbial Hypothesis 3: *The species composition of seep sulfate reducing bacteria (SRBs) will undergo a change with depth and will be distinct from SRBs found at other gassy cold seeps.*

Technical Approach

Selection of Scientific Review Group (SRG)

This task specifies the selection of a minimum of three individuals to be selected by TDI-Brooks to serve as a Scientific Review Group (SRG) for the Study. These individuals will be utilized to review progress and results of the Study and make recommendations to the COTR regarding the effectiveness and possible changes in the directions of the field work, analysis and/or interpretations. Following MMS precedent, we have selected two individuals from the academic community and one individual from industry. All three (3) individuals for consideration by MMS for the SRG have broad experience in chemosynthetic and hardbottom studies and Gulf of Mexico expertise. Each of these individuals is budgeted at five (5) days per year in the project. These individuals are as follows:

Dr. James P. Barry – *Associate Scientist, Monterey Bay Aquarium Research Institute*

Dr. Barry's research program focuses principally on the biology of deep-sea benthic communities. Research themes that have dominated his program over the past decade include, but are not limited to; 1) studies of chemosynthetic biological communities in the eastern Pacific and Japan, 2) benthic-pelagic coupling in polar and temperate continental shelf and slope habitats, and 3) the consequences of increased ocean carbon dioxide concentrations on marine biota. Much of his research has integrated the influence of physical processes on populations and communities. Community studies on the Antarctic continental shelf involved a characterization of biological patterns in relation to physical features and processes, as well as coupling with upper ocean productivity. Similar studies of pelagic-benthic coupling are ongoing of Central California, as well as measurements of the influence or role of Monterey Submarine Canyon on benthic community patterns and processes. Chemosynthetic community studies have been a major theme – these studies have focused on the influence of pore fluid chemistry on the biology of vesicomyid clams, the dominant megafaunal species inhabiting central California chemosynthetic communities. Studies of the physiology of these interesting animals and their symbiotic relationships with sulfide-oxidizing bacteria have also been an important element of this program. More recently, he has emphasized studies of the consequences of rising carbon dioxide levels in the ocean on marine ecosystems. His recent experimental work has evaluated the impacts of deep-sea carbon dioxide sequestration (a climate warming mitigation option presently under consideration) on the survival of deep-sea biota (from bacteria to fish). This work has involved experimental manipulations (releasing liquid CO2 into replicate pools on the seafloor) at 3600 m depth, in which the survival of various animals is evaluated during month-long exposure to CO2-rich dissolution plumes emanating from CO2 pools. He will soon expand the scope of our CO2 studies to include the effects of elevated CO2 on deep-sea corals. Changes in acidity, as well as a reduction in the calcite saturation state, could have important implications for all marine calcifiers, including deep-sea corals. Nearly his entire research program has been performed using remotely operated vehicles, and to a lesser extent, manned submersibles. He has also been involved in the development of advanced technology for deep-sea ecological studies (benthic respirometers, deep-sea camera systems, deep-sea fish trap / respirometers).

Dr. William W. Schroeder – *Professor, Biology and Marine Science, University of Alabama*

Dr. Schroeder has been involved in interdisciplinary oceanographic investigations for over 40 years and has conducted research along the coast, on the continental margins and in the deep water of the northern Gulf of Mexico for the past 36 years. In addition, he has participated in international research endeavors in the Australia, Azov Sea, Bahamas, Caribbean, Gulf of Papua and South Africa. He has authored and coauthored over 125 scientific publications. On going research activities include: coupled biological-geological-physical studies of deep-water corals in the Gulf of Mexico; an integrated study of physical and biological processes along the west coast of Australia; Late Quaternary sea level and paleoceanography investigations of hardbottom sites in the northern Gulf of Mexico; validation of distributed marine-environment forecast systems; and model validation of the coupled katabatic wind, coastal ocean and ice systems in Antarctica. He is currently serving on the following advisory and review boards: USDI-MMS-OCS-SAC 'Deepwater Development Issues' and 'Arctic' Subcommittees; Gulf States Marine Fisheries Commission 'Bottom Mapping Work Group'; Third International Symposium on Deep-Sea Corals 'Science Advisory Committee'; and USDI-MMS/Texas A&M University, Scientific Review Board, 'Deepwater Program: Northern Gulf of Mexico Continental Slope Habitats and Benthic Ecology'.

Dr. Daniel L. Orange – *VP, AOA Geophysics Inc, also Research Associate, Department of Earth Sciences, University of California, Santa Cruz.*

Dan Orange began his work on chemosynthetic "cold seeps" in 1986 as a graduate student of Casey Moore's at U.C. Santa Cruz. Moore was part of the 1985 ALVIN cruise program that identified the first seep communities as chemosynthetic (Kulm et al., 1985; Suess et al., 1985), and secured additional NSF funds to return to the area in 1986, with the goal of mapping the geology and distribution of seeps in order to determine what was controlling the plumbing. Dr. Orange participated in ALVIN dives on the Cascadia accretionary complex in 1986, 1988, 1989 and 1990. This collaborative work included geologic mapping (Moore, Orange, Tobin, Brown, Kulm), collection and analysis of authigenic carbonate (Carson, Ritger), collection and analysis of chemosynthetic fauna (Suess, Linke), geochemistry (Suess, Whiticar, Gieskes), and hydrology (Driess, Screaton).

Dr. Orange planned a dive in 1988 into a submarine canyon cutting into the first anticlinal ridge of the accretionary complex as a means of mapping the canyon headwall, and measuring the section. On this dive, he discovered a cold seep at the very base of the headscarp, where the canyon floor was relatively flat, and the headscarp formed a sharp inflection point with an amphitheater shape in map view. He interpreted this combination of observations to indicate that these canyons were forming by internal forcing as seepage-induced slope failures. Orange's work on submarine canyons, and his work mapping the structures controlling the seeps in this area, were significant portions of his Ph.D. research.

Because of his background as a structural geologist, and his experience mapping seeps, Dr. Orange was invited to participate on ALVIN dives to the Kane Fracture Zone on the Mid-Atlantic Ridge (J. Karson, chief scientist), and collaborated with GEOMAR on a series of Jason-2 ROV dives in the Aleutian Trench (which discovered seep-related pogonophoran tube worms and vesicomyid clams in 5000-m of water). Erwin Suess was Chief Scientist of this program.

He collaborated with Gerhard Bohrmann on the geology, and with Peter Linke and Rich Lutz on the seep biology.

Dr. Orange joined the Monterey Bay Aquarium Research Institute in 1992 as a staff researcher, and continued to investigate seeps. He also began work in the Eel River Basin as part of ONR's STRATAFORM program at this time. In Monterey Bay, he evaluated co-located side scan sonar and multibeam bathymetry to identify possible seep sites, and discovered a number of new seep locations associated with mud volcanism, with headless slumps, and with faults. In the Eel River Basin, he used co-registered multibeam bathymetry and backscatter, along with multi-channel seismic data, to identify possible seep sites. MBARI-sponsored ROV dives to the Eel River Basin demonstrated seeps associated with the surface expression of anticlines (authigenic carbonate, vesicomyid clams, chemoautotrophic bacteria), and at the surface expression of a mud volcano in shallow water (chemoautotrophic bacteria).

After leaving MBARI in 1997, he joined AOA Geophysics to pursue applications of his work in the private sector. He maintained, and still maintains, an academic soft money position at U.C. Santa Cruz. At AOA Geophysics, he has discovered and sampled cold seep fauna offshore West Africa and in the Makassar Straits. In both cases, he was prepared with a biological sampling kit, and sub-sampled and preserved the fauna at sea per standard protocols. In both cases, he convinced the client to release the samples to academia, and Bob Vrijenhoek (formerly of Rutgers, now of MBARI) analyzed the fauna and bacteria.

Selected bibliography of seep related publications:

Orange, D.L., Yun, J., Maher, N., Barry, J., and Greene, G., (2002) Tracking California Seafloor Seeps with Bathymetry, Backscatter and ROVs, *Continental Shelf Research,* v. 22, p. 2273-2290.

Hovland, M., and **Orange, D.** (2001) Gas hydrate and seeps – effects on slope stability: The "hydraulic model". ISOPE, International Symposium of Offshore and Polar Engineers, Stavanger, Norway. 11 pp.

Orange, D.L., Greene, H.G., Reed, D., Martin, J.B., McHugh, C.M., Ryan, W.B.F., Maher, N., Stakes, D., and Barry, J. (1999), Widespread Fluid Expulsion on a Translational Continental Margin: Mud Volcanoes, Fault Zones, Headless Canyons, and Aquifers in Monterey Bay, California, *Geological Society of America Bulletin*, v. 111, p. 992-1009.

Stakes, D.S., **Orange, D.,** Paduan, J.B., Salamy, K., and Maher, N., (1999), Origin of authigenic cold-seep carbonates from Monterey Bay. *Marine Geology*, v. 159, p. 93-109.

Greene, H.G., Maher, N., Naehr, T.H., and **Orange, D.L.,** (1999) Fluid flow in the offshore Monterey Bay region, in: Garrison, R.E. Aiello, I.W., and Moore, J.C., eds. Late Cenozoic Fluid Seeps and Tectonics along the San Gregorio Fault Zone in the Monterey Bay Region, California, Pacific Section American Association of Petroleum Geologists Volume and Guidebook GB-76, Bakersfield, California, p. 1-20.

Yun, J.W., **Orange, D.L.**, and Field, M.E., (1999) Gas distribution in the Eel River Basin and its link to submarine geomorphology. *Marine Geology.* v. 154, p. 357-368.

Lorenson, T.D., **Orange, D.L.,** Brewer, P.G., Davisson, M.L., McLaughlin, R.J., Kvenvolden, K.A., and Martin, J.B., 1998, Comparison of offshore and onshore gas occurrences, Eel River Basin, northern California: U.S. Geological Survey Open-File Report 98-781, 1 sheet

Kohn, M. J., Riciputi, L. R., Stakes, D., and **Orange, D. L.** (1998) Sulfur isotope variability in biogenic pyrite: Reflections of heterogeneous bacterial colonization? *American Mineralogist*, v. 83, p. 1454-1468.

Suess, E., Bohrmann, G., von Huene, R., Linke, P., Wallmann, K., Lammers, S., Sahlig, H., Winckler, G., Lutz, R.A., and **Orange, D.** (1998) Fluid venting in the Aleutian subduction zone, *Journal of Geophysical Research*, v. 103, p. 2597-2614.

Orange, D.L., McAdoo, B.G., Moore, C., Tobin, H., Screaton, E., Chezar, H., Lee, H. Reid, M., and Vail, R., (1997) Headless submarine canyons and fluid flow on the toe of the Cascadia accretionary complex, *Basin Research*, v. 9, p. 303-312.

Orange, D.L. and Campbell, K.A., (1997), Modern and ancient cold seeps on the Pacific Coast - Monterey Bay, California and offshore Oregon as modern-day analogs to the Hoh Accretionary Complex and Quinault Formation, Washington, *Washington Geology*, v. 25, p. 3-13.

Martin, J.B., **Orange, D.L.**, Lorenson, T.D., and Kvenvolden, K.A., (1997) Chemical and isotopic evidence of gas-influenced flow at a transform plate boundary: Monterey Bay, California, *Journal of Geophysical Research*, v. 102, p. 24903-24916.

Barry, J.P., Greene, H.G., **Orange, D.L.,** Baxter, C.H., Robison, B. H., Kochevar, R.E., Nybakken, J., Reed, D.L., and McHugh, C.M. (1996) Biologic and geologic characteristics of cold seeps in Monterey Bay, California. *Deep Sea Research*, v. 43, p. 1739-1762.

Kahn, L. M., Silver, E. A., **Orange, D.L.**, Kochevar, R., and McAdoo, B. G., (1996) Surficial evidence of fluid expulsion from the Costa Rica accretionary wedge, *Geophysical Research Letters*, v. 23, p. 887-890.

Orange, D.L., Anderson, R. S., and Breen, N. A. (1994), Regular canyon spacing in the submarine environment: the link between hydrology and geomorphology, *G.S.A. Today*, v. 4, p. 29-39.

Tobin, H.J., Moore, J.C., Mackay, M.E., **Orange, D.L.**, and Kulm, L.D. (1993) Fluid flow along a strike-slip fault at the toe of the Oregon accretionary prism: Implications for the geometry of frontal accretion, *Geological Society of America Bulletin*, v. 105, p. 569-582.

Breen, N.A. and **Orange, D.L.** (1992) The effects of fluid escape on accretionary wedges I: Variable porosity and wedge convexity. *Journal of Geophysical Research*, v. 97, 9265-9275.

Orange, D.L. and Breen, N.A. (1992) The effects of fluid escape on accretionary wedges II: Seepage force, slope failure, headless submarine canyons and vents. *Journal of Geophysical Research*, v. 97, 9277-9295.

Orange, D.L., (1991) The effects of fault zones and fluid flow on the structural, hydrologic and geomorphic evolution of accretionary complexes: Examples from Cascadia, *Ph. D. Thesis, University of California, Santa Cruz*, 140 p.

Moore, J.C., **Orange, D.L.,** and Kulm, L.D., (1990), Interrelationship of fluid venting and structural evolution: Alvin observations from the frontal accretionary prism, Oregon. *Journal of Geophysical Research*, v. 95, p. 8795-8808.

Refinement of Hypotheses, Field Methodologies and Logistics

This RFP identified five Study Objectives and eight Tasks that together largely define the study. The eight Tasks are addressed individually in **Sections A.3.1** to **A.3.8**. There is not a direct correlation between individual objectives and individual tasks, nor a one to one correlation between individual objectives and the hypotheses outlined in the previous section. Meeting the objectives with a series of related and carefully integrated field and laboratory studies, while testing key hypotheses and balancing exploration for new communities with intensive study of a few communities, were our primary goals in designing the study outlined in the following sections.

The five "Broad Study Objectives" identified are defined in **Section A.1.2**. In the following section an overview of the proposed work is presented in the context of these Study Objectives. The overview is presented in an order that logically proceeds from the proposed work, not the order of presentation of the objectives in the RFP. More detail on all aspects of the proposed work is presented in the following sections (particularly **Sections A.3.3, A.3.4,** and **A.3.5**).

Objective D: To meet Objective D, it is first necessary to compile and analyze all of the appropriate available data to predict the location of significant chemoautotrophic or other hard-bottom communities at depths >1000 m in the GoM. This will be a primary area of effort for the first six months of the project and will continue at varying levels of effort throughout the project. This effort will result initially in selection of 10 – 20 sites for visitation during the Site Confirmation Cruise. In addition to providing specific locations for at least most of the dives for the first submersible cruise, ground-truth data collected during the site confirmation cruise will allow evaluation of the predictive value of the various criteria used for site selection, and will also provide data on the types of communities present at the sites. Multivariate analysis of this data (geophysical and geochemical predictors, and presence/absence of various community types) will allow initial testing and refinement of the hypotheses relating to community occurrence, which can be further tested on the subsequent submersible/ROV cruises. These analyses will be further enriched when selected sites are more intensively imaged and sampled for macro and microbiology and chemistry. A third level of information directly related to this objective will come from mapping community occurrence type and density onto the high resolution maps of surficial geology and seafloor topography that will be made at of each of our three to four primary study sites. Our "sub-goal" here will be to enrich the predictive value of

these high resolution data sets to include the occurrence of different types of communities/ habitats on a spatial scale of meters.

Objectives A and B: The next focus of the team (largely beginning with the first submersible cruise) will be to meet Objectives A and B. In short, to characterize whatever types of significant hard bottom communities we encounter. First order community characterization will be identification of component taxa and descriptions of communities present at different sites. Second order characterizations will include distributions and abundances of taxa with respect to chemistry and surficial geology, and measures of community structure and function. Third order characterizations/analyses will include interactions with background fauna, taxonomic relations of species from key taxa to related species at other depths and in other areas, and community-level comparisons between sites and between related communities at other depths and in other areas.

Although the design of the first phase (site selection) is aimed at seep communities, experience suggests that this effort will also lead to the discovery of other hard bottom communities associated with carbonates. We will choose a variety of representative community types discovered during the site confirmation cruise (or already known communities) to characterize during the submersible and ROV cruises. All sites visited by submersible or ROV (including 6-7 in the first year) will be at least preliminarily characterized with respect to surficial geology, geochemistry of sediments and epibenthic bottom water, types of communities present, microbial activities, and mega- and macrofaunal species present. At four sites we will conduct more extensive survey and experimentation to better characterize and understand the communities, and test the hypotheses relating to community composition, tubeworms, trophic interactions, and microbiology. During the submersible and ROV cruises we will collect imagery that will provide data on endemic species occurrence, distribution and densities, and visitation by vagrant mobile megafauna. We will make quantitative collections of communities that will provide the material needed for taxonomic, biogeographic, and trophic studies, and analyze the collections in ways that provide a variety of data on community structure and function as well as composition. We will make in situ chemical measurements to describe the microhabitat chemistry of the major community types. We will map the faunal distributions with respect to surficial geology and chemistry. We will characterize the microbial communities in the sediments, and initiate temporal (time lapse camera, base line imaging, and growth) studies of the communities.

Objective E: During the course of fully addressing Objectives A and B, we will also address Objective E. Contributing to assessing the diversity, distributions and abundance of marine species below 1000 m in the GoM will result from our descriptions of the communities encountered (as described above) and from analyses of background fauna trapped, trawled and imaged over the course of the study. Contributing to the explanation of these patterns will be correlation analyses of faunal occurrence with geologic features and seep chemistry. Our trophic analyses, time-lapse camera data, community analyses, and growth studies will greatly improve our understanding of the functional role of many of the marine species we encounter. By working under the auspices of the Census of Marine Life ChEss program and providing all data collected for their database we will assure wide-spread international access to all biodiversity and biogeography data collected.

Objective C: While addressing Objectives A, B, and E, we will collect the data and perform most of the analyses to address Objective C. Direct determination of sensitivity of individual species to particular potential anthropogenic impacts is beyond the scope of this study, particularly as relevant physiological investigations would require high-pressure aquaria and a very significant effort. Instead we will address this objective indirectly, through assessment of rarity and unique taxonomy/biogeography of key species and communities, biodiversity of communities, and by interpretation made in the context of the degree of similarity to related communities on the upper Louisiana slope and what is known about those communities. The comparisons of community-level associations to similar communities elsewhere, and the proposed vestimentiferan growth studies will strengthen the power of these analyses.

Our team's collective familiarity with both GoM and other chemosynthetic and coral communities, including the associated fauna, will allow us to quickly recognize sites of rare or unique community composition. The Fisher lab at PSU maintains a voucher collection of 46 species collected with mussel beds or tubeworm aggregations at water depths of over 1000 m in the Gulf of Mexico, and 111 species collected with tubeworm aggregations and 40 species collected with *Lophelia pertusa* thickets on the upper slope. These collections will allow us to identify species with distributions extending to the upper slope, as well as previously unknown species. Existing collaborations with molecular and classical taxonomic experts familiar with the Gulf of Mexico seep and coral associated fauna, as well as other seep fauna world wide, will facilitate the identification of unknown species. Although we are not proposing a population genetic component *per se*, the proposed molecular analyses of foundation and other key species will provide information necessary to detect significant levels of genetic isolation at any site, analyze relations to taxa at other sites, and determine bathymetric ranges of the metapopulations.

Site Selection

The selection of sites for the study of chemosynthetic communities, hard substrates, and hard bottom faunas is of primary importance to the success of this proposed project. As **Figure 1** clearly shows, most sites on the northern Gulf of Mexico continental slope that have received significant scientific attention are concentrated above the 1000 m isobath. Only a few sites in water depths below 1000 m have been studied using a manned research submersible or ROV. Only four (4) of these deep-water sites, as designated in **Table 1**, support chemosynthetic communities. Three of the sites are in the Alaminos Canyon blocks (AC-601, 645, and 818) and one is in the Atwater Valley lease area (AT-425). Therefore, the vast deep-water area of the northern Gulf's slope below 1000 m water depth to the basin floor seaward of the Sigsbee Escarpment is virtually unknown territory regarding the existence of chemosynthetic communities, hard bottoms, and hard bottom faunas. This glaring void in our understanding of the deep Gulf presents some interesting and important geological and biological questions in the context of what we have learned from studies of the upper slope. These questions are:

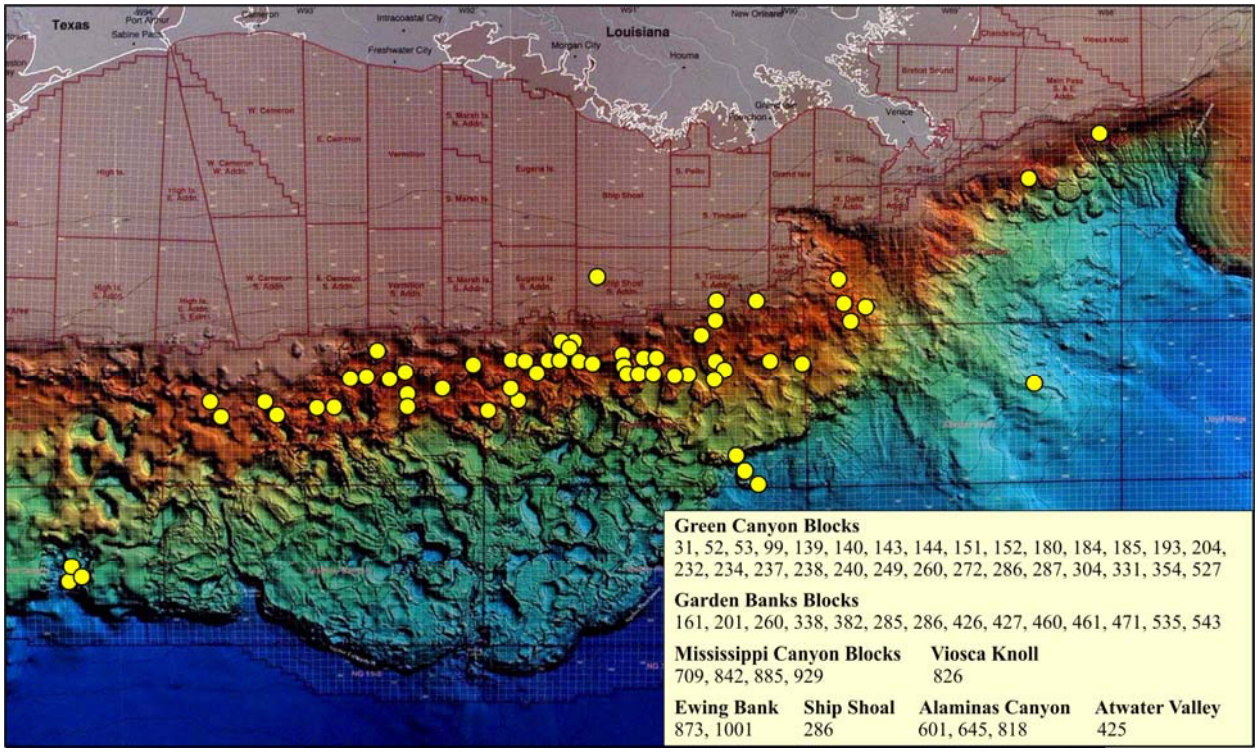


Figure 1. Superimposed on the shaded multibeam bathymetry map of the northern Gulf are the locations of upper slope sites fluid and gas expulsion that have been identified using 3-D seismic surface amplitude plus profile data. All sites have been field verified using manned submersible dives (Roberts, 2001; Roberts and Coleman, In Preparation). Deeper water sites (below 1000 m) represent both study sites (submersible and ROV) and reconnaissance sites identified using a variety of data bases (3-D seismic, deep-low high resolution acoustic data, and geochemistry). Note the glaring lack of coverage below sites clustered on the upper slope (< 1000 m WD).

1. Do sites of fluid and gas expulsion at the modern seafloor in water depths below 1000 m follow the same geological-biological relationships found by Roberts and Carney (1997) for sites in water depths shallower than 1000 m?
2. Are the methodologies for identifying and categorizing fluid and gas expulsion sites on the upper slope applicable to deep and ultra-deep water parts of the northern Gulf?

Table 1. Known Chemosynthetic Community Sites in Deep Water (> 1000 m)

Block	Depth
AC 601	2,255 m
AC 645	2,200 m
AC 818	2,750 m
AT 425	1,930 m

Because of the availability of shallow-diving (to 1000 m) manned submersibles to the Gulf of Mexico, the upper continental slope has been the focus of hydrocarbon seep research since the mid-1980s. The body of geologic data from well-studied sites (**Figure 1**) suggests that there is a

general relationship between the rate at which crude oil-laced fluids and gases arrive at the seafloor and overall geologic-biologic response. Roberts and Carney (1997) and Roberts (2001) have presented a spectrum of responses to be expected under a variety of flux rate conditions varying from very slow seepage to rapid venting. **Figure 2** schematically depicts these changes from mineral-prone slow seepage to mud-prone rapid delivery cases. In the case of very slow seepage conditions, consumption of hydrocarbons by microorganisms is intimately associated with carbonate precipitation and the production of hard substrates. Even though methane oxidation in an aerobic environment produces CO_2 and decreases pH, favoring dissolution of carbonates (Aloisi et al., 2002), anaerobic microbial sulfate reduction using hydrocarbon substrates causes sulfate depletion and simultaneous bicarbonate and hydrogen sulfide enrichments in sediments. The increase in carbonate alkalinity of pore fluids produces calcium-magnesium carbonate by-products leading to nodular masses in the sediments, hardgrounds, and mounds (Ritger and Seuss, 1987; Roberts et al., 1990). Very slow seepage sites do not support complex chemosynthetic communities, rather, they usually only support simple microbial mats (*Beggiatoa sp.*). In the upper slope environment, however, these hard substrates frequently are associated with communities of sessile cnidarians. At the rapid flux end of the spectrum fluidized sediment generally accompanies hydrocarbons and formation fluids arriving at the seafloor. Mud volcanoes and mud flows result. Localized microbial mats along with lucinid-vesycomyid clams are most common to these settings. Somewhere between these two end members exists the conditions that support densely populated and diverse communities of chemosynthetic organisms (microbial mats, siboglinid tube worms, bathymodioline mussels, lucinid and vesycomyid clams, and associated organisms). These areas are frequently associated with surface or near-surface gas hydrate deposits. They also have localized areas of lithified seafloor, generally authigenic carbonates but sometimes more exotic minerals such as barite are present. Although Rowan et al. (1999) clearly indicate that the across-slope geologic framework changes because of changing salt geometries, it is expected that the same general relationships between seafloor geology and biology will exist at sites below water depths of 1000 m. Therefore, a working hypothesis to be tested as part of the site selection process is as follows:

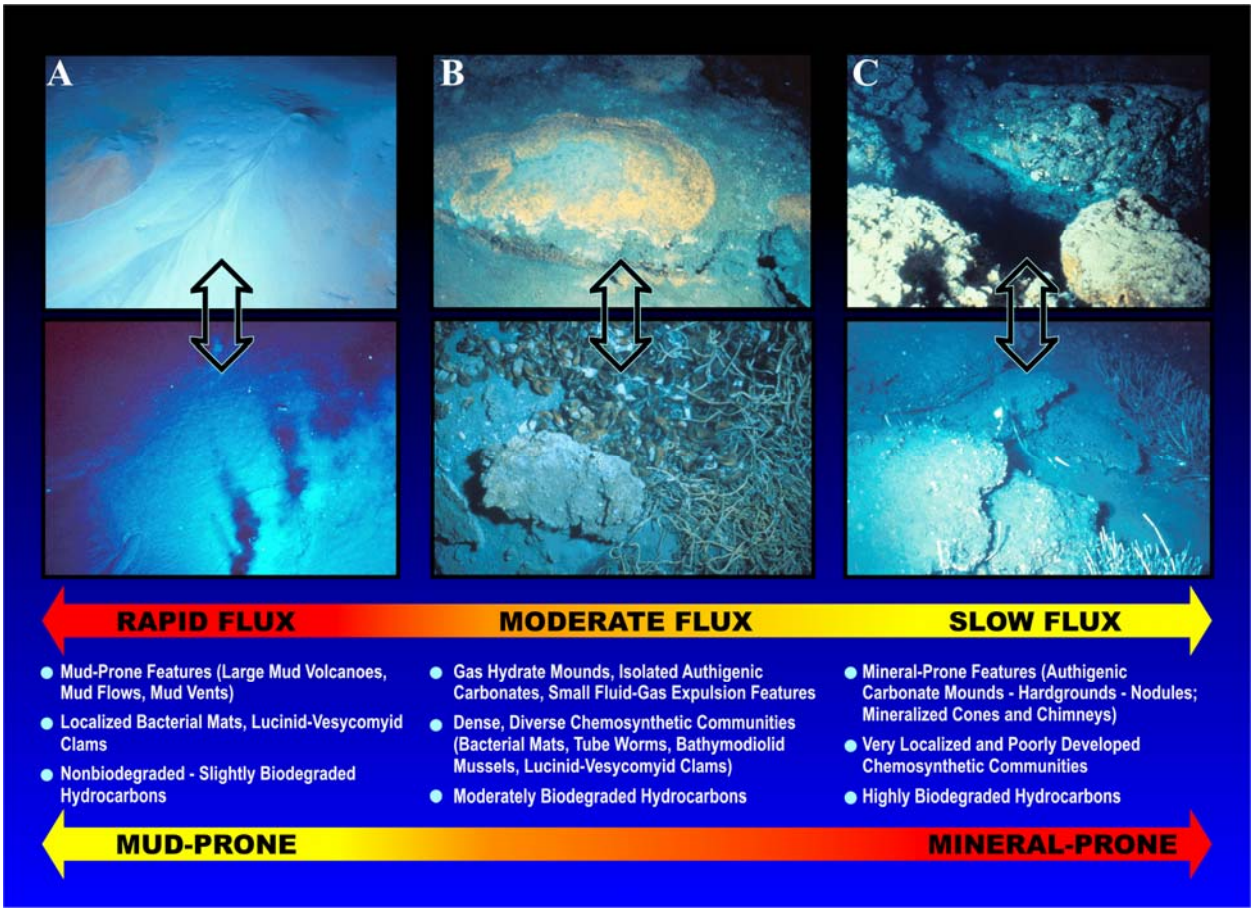


Figure 2. This figure summarizes the qualitative relationships between delivery rate of fluids and gases to the modern seafloor and general geologic-biologic response (modified from Roberts and Carney, 1997). The spectrum ranges from mud-prone rapid flux conditions to slow flux settings that are mineral-prone.

Hypothesis 1: *Across-slope similarity in surface geology-biology relationships – The general response of biological communities to a qualitative spectrum of fluid and gas delivery rates at the modern seafloor will be the same below 1000 m as above.*

Selecting new study sites in water depths below 1000 m will follow procedures well-documented by previous MMS-supported research (Roberts, 2001; Roberts and Coleman, In Preparation). New sites will be selected so that biological and geological questions involving along-slope and across-slope variability can be addressed. During the early part of Year 1, existing data sets (AUV data, geochemical data from cores, SAR data, published deep water studies, and site-specific seismic data held by industry and released for our use) will be analyzed simultaneously with review of the MMS 3-D seismic database. Work with the MMS seismic data will be carried out in conjunction with MMS geophysicists/geologists as in the aforementioned studies of the upper slope. From this exercise, candidate sites will be identified using 3-D seismic data surface amplitude mapping and seafloor wave form analysis plus geologic framework analysis from selected seismic profile data. The wave form analysis will indicate polarity of the seafloor reflector as well as polarity of subsurface reflectors. In conjunction with surface amplitude

mapping it is possible to identify gas-charged surface sediments and areas of hard bottom. This approach has worked successfully on the upper slope. **Figures 3** and **4** illustrate two sites identified using 3-D seismic data. Each site was ground-truthed by direct observation and sampling using the JSL on predetermined transects carefully planned to encounter various features and amplitude patterns observed in the seismic data. One site, VK826, is characterized with hard substrates inhabited by colonies of *Lophelia pertusa*, a deep-water scleractinian coral. No chemosynthetic communities were observed within the sampled area of these transects, although extensive areas of chemosynthetic communities have been observed nearby (Cordes et al. in prep.). The other site, MC-118, is characterized by localized outcrops of gas hydrate and chemosynthetic communities. The geophysical data predicted that chemosynthetic communities, though localized, would occur at this site.

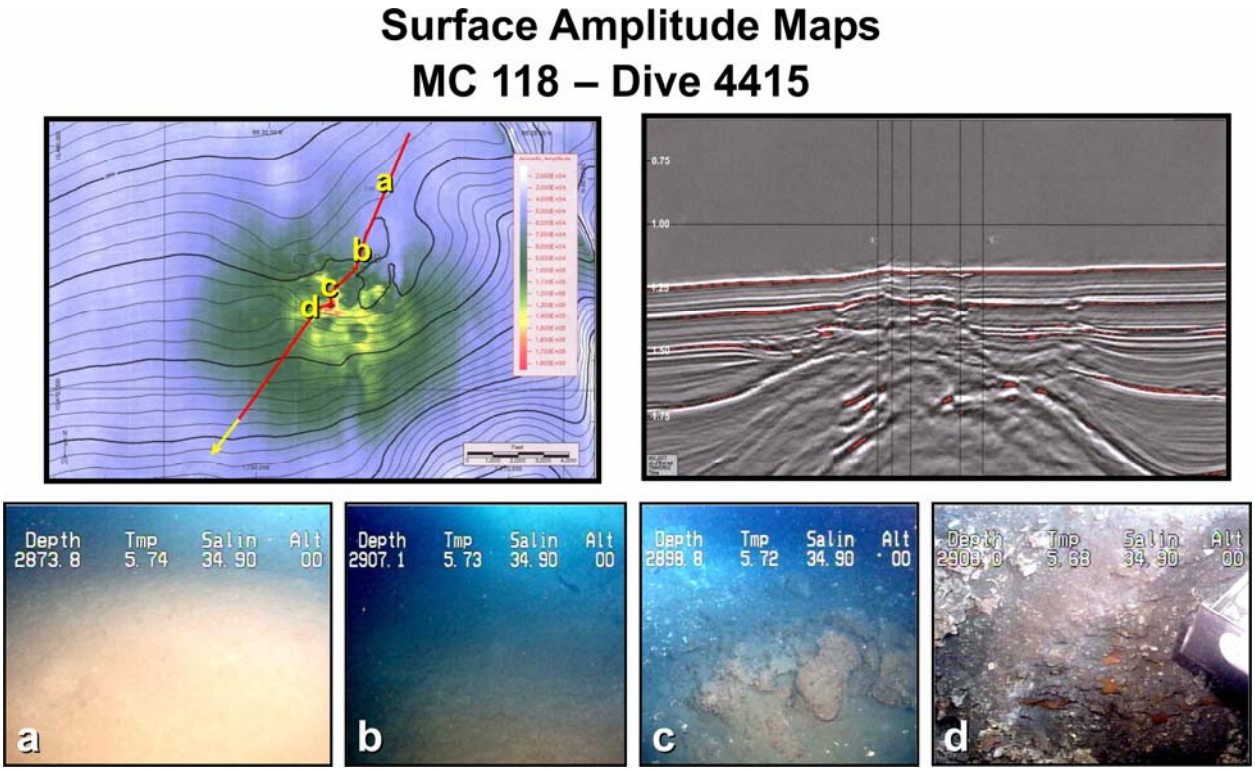


Figure 3. This figure illustrates the method of identifying and field verifying hard bottom areas of the continental slope. The 3-D seismic surface amplitude map (top left) is the template for making seafloor observations to "calibrate" the seismic data. The seismic profile (top right) depicts subsurface structure. A positive polarity is present for the surface return (no gas). The three bottom photos from a JSL transect show: (a) mud bottom outside the feature (low amplitude), (b) *Lophelia* on hard substrate (high amplitude), and (c) coral fragments and nodular masses in the sediment (moderate amplitude). No chemosynthetic organisms were found at this site.

In identifying sites that may be candidates for investigation we will rely heavily on the primary data sets: (1) 3-D seismic amplitude maps and profile data (Roberts, 1996); (2) the database of approximately 8,000 surface geochemical exploration cores acquired by Dr. Brooks and team at TDI-Brooks Int'l Inc. and GERG; and (3) SAR data for locating sea surface oil slicks

MacDonald et al. (1996). If AUV data have been acquired by industry in areas of interest identified from the above data, we will petition owner companies for use of the AUV data in support of this project. Our areas of interest will be confined to the central and western parts of the northern Gulf of Mexico slope. The southern limit will be defined by the boundary of the U.S. Exclusive Economic Zone (EEZ). We will focus on hard bottom areas that may be products of: (1) authigenic carbonate precipitation as observed on the upper slope (Roberts and Aharon, 1994), (2) gas hydrate exposures (MacDonald et al., 1994), and (3) salt which may be commonly exposed along the Sigsbee Escarpment (Orange et al., 2003).

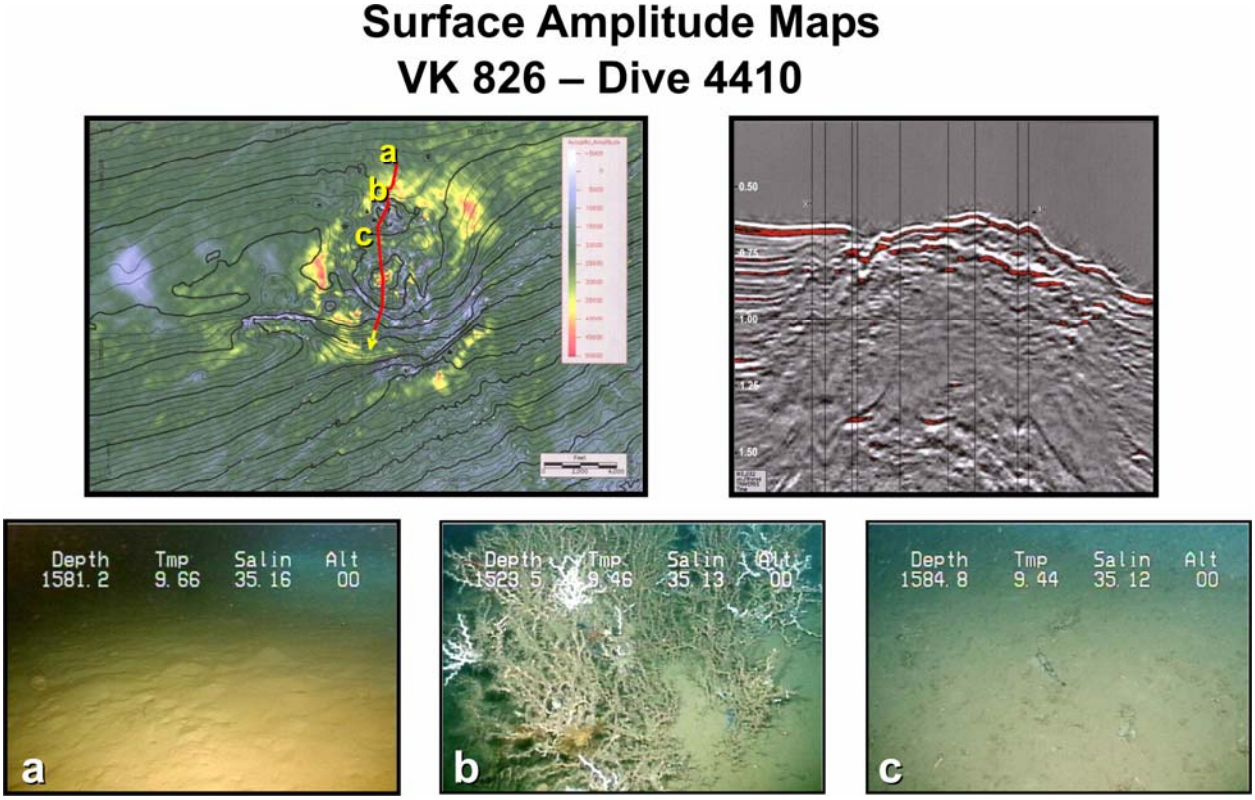


Figure 4. This figure illustrates the same method used to analyze the site at VK826, however, this site is quite different. The amplitude map (top left) shows a relatively small area of high amplitude response with localized low amplitude zones within. The seismic profile (top right) gives the geologic structure and even though it is not clear on this particular line, a phase reversal (negative polarity) occurs in local areas at the seafloor suggesting gas-charged sediments. The four bottom photos acquired by the JSL clearly correlate well with the surface amplitude map. Both mussel beds and tube worms were found in the vicinity of authigenic carbonates (c), and gas hydrate exposures (d). Free-flowing gas was also found at the hydrate exposure sites.

The database for chemosynthetic sites in deep and ultra-deep water is small but there is some indication that the methodologies used for site identification and characterization on the shallow slope may not apply as directly in deep water, especially the use of 3-D seismic surface amplitude and polarity analyses from seismic profiles. Therefore, an important working hypothesis to be tested during this deep and ultra-deep water study is as follows:

Hypothesis 2: *Similarity of detection methods for shallow and ultra-deep chemosynthetic communities – Geophysical signatures for seafloor geologic conditions that support chemosynthetic communities in the shallow slope setting (< 1000 m WD) will be the same for deep and ultra-deep parts of the slope (1000 – 2800 m WD).*

SAR Images: Persistent oil seepage, with quantities of oil rising from the seabed to the ocean surface, is a well-documented feature of Gulf of Mexico hydrocarbon seep communities from the upper slope. This process generates well-understood remote-sensing signatures that can be detected by a variety of satellite platforms, including RADARSAT (Espedal and Wahl 1999; Mitchell et al. 1999). Modeling exercises (MacDonald et al. 2002) and replicated observation with sea-truthing (De Beukelaer et al. 2003) have shown that oil reaches the ocean surface with a small and consistent lateral offset from the seafloor source. Episodic releases of large amounts of oil from mud volcanoes has been observed (MacDonald et al., 2000) and may be a feature of the lower slope. Commercial exploration has made routine use of satellite imagery for over a decade. In this program, we will use archived and previously acquired satellite imagery to supplement and enhance our interpretation of geophysical and geochemical data for predicting locations of significant seep and coral communities on the outer continental slope.

Data used in this task will come from several sources. Principally, Dr. MacDonald has an inventory of 12 RADARSAT synthetic aperture radar scenes that were acquired in 2001 and 2002. These data cover portions of the Green Canyon lease area out to the Sigsbee Escarpment. However, the coverage is attenuated on the outer slope. An additional 15 to 20 RADARSAT SAR scenes collected in the past two years will be purchased from RADARSAT International for use by the program. These data, as well as MacDonald's inventory, will be available to MMS for its subsequent use. Perusal of commercial seep-detection programs will also be available to confirm interpretations.

Coverage for the combined data-set will provide a minimum of two images over all potential areas on the outer continental slope. We will analyze the images to locate SAR signatures that indicate ongoing seepage from a seafloor source (MacDonald et al. 1993; De Beukelaer et al. 2003). Appearance of these signatures at the same location in two or more images will be taken as robust evidence for ongoing seepage. The seafloor source of this seepage will be localized by averaging the positions at which oil first reaches the surface in multiple images. For site selection, we will geo-rectify the satellite images to a common datum with available geophysical and geochemical data. Comparison of seafloor source predicted from satellite data to seabed features evident in geophysical data will constrain or support designation of a given locality as a probable seep community site.

Asset Utilization and Timing: The fall of 2005 and winter of 2006 will be consumed by analyzing data and compiling a list of candidate sites for further investigation. It is anticipated that between 20-40 sites can be identified by spring 2006 using the methods and databases outlined in the section above. At this point the site selection data base will be acted on in two ways: (1) reconnaissance–level data collection at sites that have no ground-truth verification data and (2) thorough sampling of known sites listed in **Table 1**.

The reconnaissance–level data collection will be conducted from either the R/V JW POWELL or P/V PELICAN in the early spring of 2006 using a Drift Camera system (see **Section A.3.4.1**). This activity is designed to determine whether sites identified by geophysical signatures, geochemical data from cores, and SAR data have chemosynthetic communities, hard bottoms, and/or hard bottom faunas (particularly corals). Drift Camera transects will be guided by the geologic character of the seafloor as determined primarily by the 3-D seismic surface amplitude expression of the area.

In late spring-early summer of 2006, DSV ALVIN time awarded to Drs. Roberts and Carney for a deep-water coral study will be added to dive days dedicated to this project by NOAA OE. The greatest part of the dive time in Year 1 will be dedicated to sites of known hard bottoms and chemosynthetic communities. Three of these sites are in the Alaminos Canyon lease area (see **Table 1**). A new site, AC-601, has been confirmed as having chemosynthetic communities in the spring of 2005 using a deep-diving commercial ROV. Another site outside of the Alaminos Canyon area is AT-425. However, this site is a considerable distance from Alaminos Canyon and therefore transit time may eliminate dive time. Therefore, it will be to our advantage to identify high quality intermediate sites through the reconnaissance-level photo sled drift camera work that will be conducted prior to the manned submersible dives. Sites such as GC-600 that have excellent indicators of macroseepage (repeat SAR data of oil slicks, favorable hydrocarbon geochemistry from cores, and expulsion features on seismic data) will receive special attention. Using intermediate sites between Alaminos Canyon and Atwater Valley is consistent with project goals. A total of 6-7 sites are envisioned as a realistic objective for ALVIN dives during Year 1.

Year 2 of the project will likely involve the ROV Jason-2. Proposals are being submitted by participants in this proposed research to a variety of funding agencies to provide additional dive days to this project (see **Section A.3.4.4**). Jason-2 will be used to both complete ground truth investigations of the potential sites identified in Year 1 and conduct detailed sampling at sites judged worthy of intensive study. As potential sites are identified through the site selection process, four (4) sites will be identified for AUV surveys. The results of these surveys will provide a template for biological, geochemical, and geological sampling (see **Section A.3.4.2**).

Field Sampling

Field sampling for this project will include at least four (4) cruises/components. Previous to any fieldwork, review of existing data (as described in **Section A.3.3**) will be used to select approximately 20 to 40 probable high-density seep and/or hard-bottom community sites.

Site Confirmation Cruise

In the spring of 2006 we will use a variety of cost-effective methods to conduct a preliminary survey of minimally ten (10) to twenty (20) sites from a surface ship, using over-the-side imaging equipment and shipboard acoustic methods. Survey sites will be selected from the 20 to 40 high probability sites based on MMS priorities, depth distribution, and transit time logistics. Either TDI-Brooks' research vessel, the R/V JW POWELL, or LSU's R/V PELICAN will be

used for this cruise. The primary purpose of this field effort will be to make a final determination on the sites to study using NOAA/MMS provided submersible assets in FY-2006 and 2007. An ancillary purpose of this cruise will be to conduct trawling and box coring off and near sites for isotopic characterization of the seep-background interactions in the vicinity of seep sites in the deep GoM (described in **Section A.3.5.6**, Interactions with Background Fauna).

During this field effort we will collect 37 kHz echo sounder profiles to image gas bubbles in the water column (**Figure 5**). Echo sounder profile transects will be surveyed across the target area as soon as the ship arrives in a study area. Detection of gas plumes will provide additional targeting information for camera surveys and subsequent work.

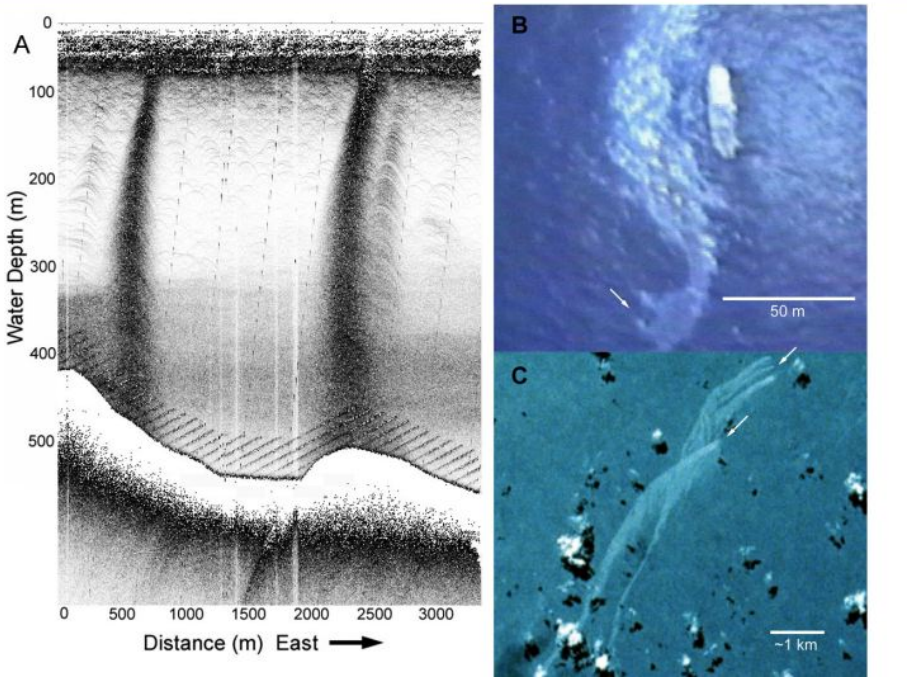


Figure 5. Transect across a deep-water seep site showing the location of gas plumes emanating from the seafloor.

The primary focus of this cruise will be to visually confirm the presence of a significant community of chemosynthetic or hard bottom fauna at some of the potential sites and to locate these communities precisely on the sea-floor. The primary tool for this portion of the project will be a Drift Camera system. This is an updated version of the drift camera system used for investigation of the LCS during the Continental Slope Study and includes a 3.2 mega-pixel Nikon digital camera with strobe illumination as well as a low-light video camera. The Drift Camera will be deployed on a frame lowered from the surface ship and held 2-5 m above the bottom based on feedback from a SeaBird CTD with altimeter. The low-light video camera will give a preliminary view of the seafloor, including compass heading, to assist in targeting and transecting. Precise navigation of the drift camera is obtained from an ultra-short baseline transponder calibrated with the ship's DGPS. The TDI-Brooks field group uses this system for taking piston cores at preset locations and is routinely able to do so within a 5 m offset from the

target. A rendered drawing of the complete system and examples of imagery collected from the system is shown in **Figure 6**.

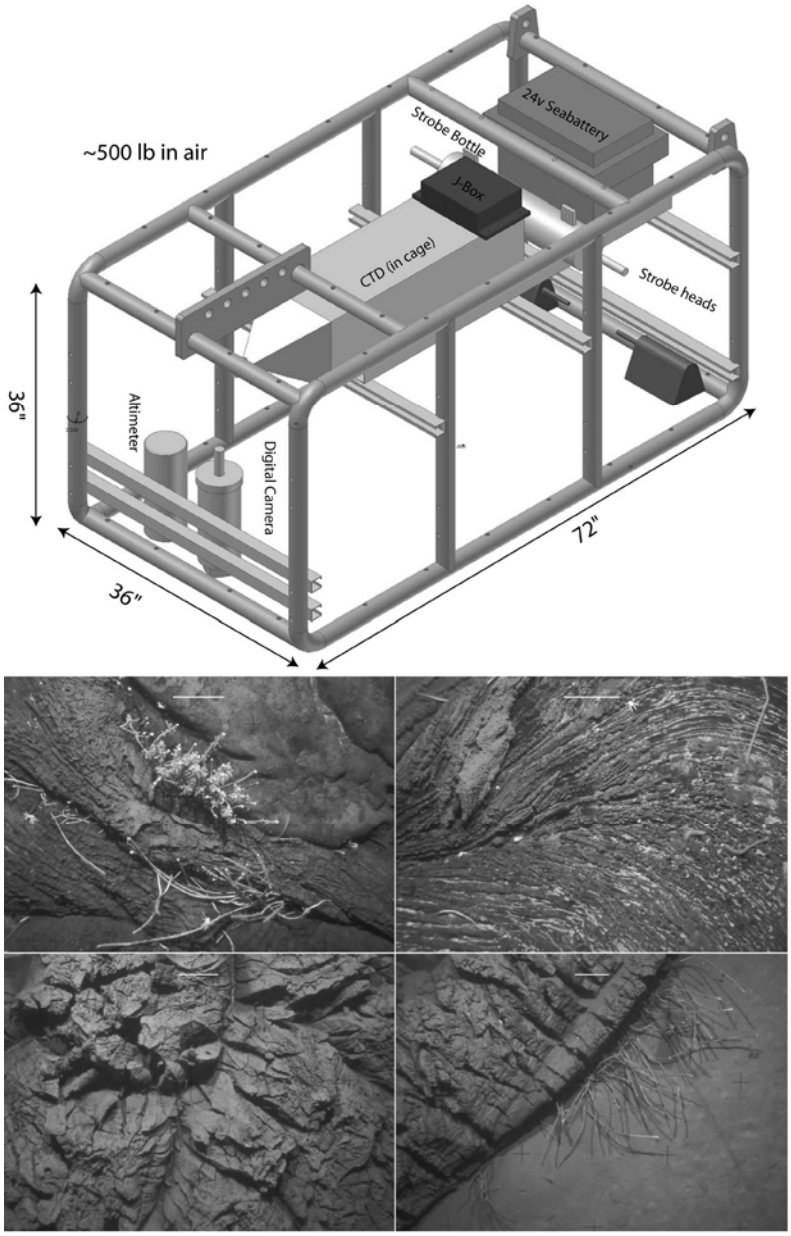


Figure 6. The upper panel shows a rendering of the drift camera frame and components. The drift camera is lowered with a 3-pt suspension and communicates to the surface through a conducting cable. The lower panel shows four black and white examples of high resolution color photographs taken with a similar system at depths of 3200 m in the Gulf of Mexico.

Collections of sea-surface oil-slick samples will be made to provide case-by-case information regarding the surveyed sites. Geochemical signatures of these oils can determine the rates of release and the degree to which oil is degraded by biological processes before being released into

the water column. Preliminary synthetic aperture radar (SAR) evidence indicates that some deep-water seeps are particularly active on the lower slope.

C&C Technology AUV Data Collection Cruises

After review of available data the team will chose four (4) sites that are likely to be targeted for more extensive study and contract C&C Technology to conduct Autonomous Underwater Vehicle (AUV) surveys of these areas (see **Section B.2**). These surveys could occur at any time after the sites to be surveyed have been chosen, as they will be conducted opportunistically when the equipment is in the region (thus saving mobilization and transit costs). The C&C Technologies, Inc. Hugin 3000 AUV will be used in both Years 1 and 2 of the project. This system collects high resolution acoustic data (multibeam bathymetry, side-scan sonar swaths, and chirp sonar subbottom profiles) that will allow us to map the surficial geology and seafloor topography of each of our four (4) primary study sites with great precision and accuracy. The AUV will travel at 20 m above the bottom while collecting data. Side-scan sonar and multibeam swaths will be acquired with a 25% overlap so that high quality mosaics with 100% coverage of bottom features and bathymetry can be prepared for each of the four (4) high priority study sites. These maps and the supporting data sets from which they will be derived will provide both an excellent template for biological/geochemical/sedimentological sampling and a database for interpreting details of the geologic framework. The locations and types of chemosynthetic organisms and communities will be related to this framework. For cruise planning purposes, side-scan sonar and multibeam mosaics will be distributed to all key scientific personnel once AUV data are collected and processed.

2006 DSRV ALVIN/ATLANTIS Cruise

In 2006, we anticipate having between 15 and 20 dive days available for this project. This is based on combining the "$500,000" of DSRV ALVIN time already awarded to Dr. Harry Roberts by NOAA NURP for fully integrated studies and the "15 days" that will be made available through the NOAA OE program specifically for this project. The exact number of days on station will depend mainly on exactly how the transit costs are split between NOAA and NSF, the number of transit days to the region, and port locations and days. To a minor extent, final site selections may affect the amount of time available for bottom work. However, with a cruising speed of 11 knots and 15 hours between 5 pm recoveries and 8 am launches most transits between potential sites can be accomplished overnight without loss of dive days. Site selection and cruise planning will be designed to avoid any loss of dives due to transit between sites.

During 2006 we will plan on visiting a total of six (6) to seven (7) sites. We will conduct a preliminary site and community characterization and sampling program at every site visited. At three (3) of the sites we will make more extensive collections and initiate some temporal, process-oriented studies. To accomplish this we plan on spending between three (3) and four (4) days at each of the three (3) sites for intensive study, and one or two days at each of three (3) to four (4) additional sites for preliminary site and animal community characterizations and collections.

At all sites visited we will make the following suites of measurements and collections:

- We will use a digital camera system in forward-looking mode to collect high quality images of communities of seep foundation species, and attached hard bottom fauna for a qualitative inventory of the dominant species present.
- We will characterize the geochemical habitat near the sediment-water interface using *in situ* chemical sensors to measure levels of methane, sulfide, oxygen, and determine pH, at appropriate points within whatever aggregations of animals are present at the sites. For example, for tubeworms we will make the measurements near their plumes and in the middle of the aggregations, for mussels we will make the measurements near their siphons and within the aggregations, for corals we will make the measurements near living polyps and within thickets.
- We will determine species composition by means of quantitative collections of whatever communities are present (seep as well as hardground sites), using the most appropriate devices for these collections. For example, we will use one of the bushmaster devices for tubeworms and corals and the mussel pots for mussel beds and clam aggregations. These collections will be used for all studies of these communities (including sclerochronolgy, genetics, ecology, size frequency, symbiont content, and trophic analyses).
- We will characterize the subsurface geochemical habitat and its inhabitants by taking replicate push core collections of sediments associated with each significant faunal feature (including microbial mats) for analyses that will include: grain size, carbonate nodule content, hydrocarbons, sulfide/oxygen profiles, sulfate reduction rates, microbial population surveys, and meiofaunal species identifications and descriptions.
- We will collect samples of hard substrates to represent the variability of hard bottom types associated with each study site. These samples will be analyzed for mineralogy, isotopic composition, character of cementing carbonates and other cementing minerals such as barite (SEM and microprobe), and age dating.
- We will make targeted collections of tubeworms, mussels, clams, and hard and soft corals for voucher specimens and genetic studies. This will supplement the quantitative collections and ensure the collection of representative samples and necessary levels of replication for population genetic studies of all key groups of chemosynthetic and hardground fauna.
- We will use baited traps and direct submersible collecting to make targeted collections of larger mobile fauna for voucher specimens and trophic studies.
- We will collect water samples for determination of microbial abundance, hydrocarbon analyses, and particulate organic C content.

At the sites chosen for more intensive study, we will nest these and additional replicate collections and chemical surveys within small scale mosaics (approximately 25 – 50 m^2) of each type of community, generated from digital photographs taken using a down-looking camera and parallel laser system. These mosaics will in turn be nested within high resolution side scan data collected by AUV and integrated with the collection and chemistry data into a comprehensive GIS map of the sites. Re-visitation and re-collection of the imagery will allow us to evaluate any changes to the communities over the period of the study (one year certainly, perhaps longer). We will also deploy traps at these sites for collection of mobile background fauna for trophic studies. Three baited traps will be deployed at each of the intensively-studied sites. One within

the area colonized by seep fauna, one ~50 m away, and one at ~1 km distance. The offsite traps will be rigged for free return for recovery without the use of submersible time.

In addition we will initiate two other temporal studies. We will deploy a total of four (4) time-lapse camera systems for year-long monitoring of activity of mobile fauna within and outside of seep and/or hard-bottom sites. We will also stain tubeworms within at least 3 aggregations at each of these sites. After collection of these aggregations in 2007 we can use our extant models of tubeworm growth on the ULS (Bergquist et al., 2000; 2002; Cordes et al., 2003; 2005) for a preliminary assessment of their growth rate and growth pattern in comparison to the ULS species.

We will also conduct trawls, boxcoring, and remote trapping on soft bottom ~5-km from seep sites to provide a wide range of feeding types in trophic studies. Six (6) trawls and 12 box cores will be taken across a wide depth range. These samples will be taken during night operations. Traps will be deployed 12 times and will be free return.

2007 ROV Cruise

At this point in time, details of the system and number of operation days available for this project in FY-2007 are uncertain. The PIs will be submitting proposals to both NOAA NURP and NOAA OE with the primary intent of increasing the number of operation days available for this project, and if possible to secure Jason-2 for the project. Considering this uncertainty, a very general plan based on priorities is all that is appropriate at this time:

- Completing the studies begun in 2006 will be our first general priority in 2007.
- Our first priority will be to collect the time-lapse cameras and the contained data from each of the intensive study sites.
- Our second priority will be to collect the stained tubeworm bushes from each of the most intensive study sites. Note that by collecting intact aggregations whenever possible (using a bushmaster), we will be replicating our community collections and significantly increasing our descriptive and comparative power for the community analyses.
- Our third priority will be to re-mosaic each of the communities surveyed in 2006, and re-survey chemical conditions within these aggregations. The amount of time spent on this task will depend in part on any changes apparent in the communities.
- Our fourth priority will be targeted collection of species which show a high likelihood of providing special ecological insight based on 2006 collections, or were missed in 2006. These animals may include species of particular interest for trophic studies, species with specific associations to particular features or other species, and mobile species that were abundant in photographs but not in the quantitative collections.
- Our fifth priority will be to collect any additional data necessary (not completed in 2006) for complete the GIS database for these sites.

After studies begun in 2006 are completed, we will change our focus to a combination of re-visitation of the other sites visited in 2006 and preliminary survey of any new sites discovered in the interim or not visited in 2006 because of time constraints. This decision would be based on evaluation of data collected up until this point in the program and made in consultation with

MMS. If preliminary evaluation of the data or collections made in 2006 indicated that re-visitation of a site would yield high returns relative to the goals of this study, then this would be our next priority. Alternatively, if new discoveries or information suggests that visitation of a new site is likely to yield high returns relative to the goals of this study, then that would be our next priority. In either case, we would have all the tools mentioned above (and described below) available for the study and would use the tools appropriate to the effort.

Sample, Data Collection and Analysis

Imagery

Imagery will be a key data component and analytical product of this program. Geophysical and remote-sensing images are discussed in other sections of this proposal. Optical images will be used to rigorously and quantifiably document the communities and substrates at hydrocarbon seeps and hard bottom areas that are the focus of the program. They will be used to estimate the abundance and diversity of fauna and to test hypotheses concerning the associations between fauna and geochemical gradients or geological features. Images will also be important for planning biological and geochemical sampling. All imagery will be obtained in-situ by submersibles or similar platforms using high-resolution, digital still-cameras or video-cameras. All in-situ images will be archived and indexed by (minimally) collection time, location (dive, site, station, etc.), photographer, and subject matter. Additional ship-board photography will document the personnel and procedures of the program. Depending on the analytical requirements, various levels of post-collection processing will be completed.

Faunal Characterizations

Documenting the fauna, including species, growth forms, habitat utilization, and behavioral adaptations will be accomplished by targeted and opportunistic photography. The non-living components of the study sites will also be documented. The first level of post-processing will be selecting photographs for a comprehensive archive of seep and hard-bottom imagery from the Gulf of Mexico. The photo-documentation process will be deliberate during the field program, with regular discussion of images that have been obtained or are needed. Taxonomic experts will be consulted as necessary to confirm identification of species. A variety of cameras will be available for this program via the submersible operators and the PIs. MacDonald will provide a digital macro-camera system with dedicated lighting that can be used to document fine scale features of the fauna and habitats. MacDonald's autonomous rotary time-lapse camera system can collect panoramic images that document the submersibles operating in the deep-sea environment. By the end of the project, the goal of this task is to have a digital archive of photographs and video clips with database descriptors; selected to be scientifically accurate and aesthetically pleasing. This archive will be available for use by the scientific community, managers, by the Census of Marine Life, and by educators.

Integration of Imagery with Sampling Plan and Ecological Analyses

More intensive levels of post-processing will produce imagery that can be used to guide sampling operations to and quantify abundance, diversity, and fine-scale distribution of

seep/hard-bottom fauna. Images from the drift-camera survey cruise will provide a preliminary overview of the fauna present at prospective study sites. The survey procedures are described elsewhere. Images from the survey will be geo-positioned using an ultra-short baseline transponder, oriented using a digital compass deployed on the drift camera platform and scaled according to their altitude above bottom. This information will provide rough geo-referenced information so that the images can be merged into a GIS layer for comparison to bathymetry, geophysical data, and other information regarding the sites. Presence or absence of fauna and other seep indicators will be a first-order test of predictions of active seepage and community formation based on the remote sensing data. These images will also be used to inform decisions about where to conduct detailed sampling and characterization efforts. Finally, the images, bathymetry and other information can be used to target the initial dives on the selected sites.

During the detailed sampling program, intensive image collection will be carried out to compile mosaics of small (10-20 m^2) sampling stations within the chosen study sites. Mosiacking images was a component of the CHEMO I and II and *Lophelia* studies, but now has potential for much greater precision and utility because of advances in submersible navigation and image processing software. The basic operation is to collect separate, overlapping images at a constant scale; then to merge these images into a larger continuous mosaic that covers the area of interest. Precision control of ROVs greatly reduces scale and orientation errors, while software recently developed by the WHOI deep submergence group makes it possible to rapidly compile the mosaics at sea (C. Fisher pers. obs). These mosaics will be utilized for several purposes. (1) Estimation of spatial coverage and abundance of foundation species, e.g. seep mussels or coral, which will be ground-truthed by physical collections. (2) Producing detailed maps for planning chemical surveys, collection of samples, or placement of instruments. (3) Providing context for analysis of relations between environmental chemistry, geological features, and animal distributions. (4) Providing information on density and distribution of the common mobile megafauna. (5) Documenting baseline data that can be used to test for habitat changes during the course of the program and in future years.

An additional use of imagery will be in the autonomous time-lapse camera systems. The methods and objectives of this task are described in section A.3.5.3.1. The images collected by the time-lapse system will be high-resolution digital pictures that can be archived with standard meta-data. The rotary camera system has the additional capability of producing 360° panoramas that can be used for public outreach and education (http://oceanexplorer.noaa.gov/explorations/05arctic/welcome.html).

Collection, Census and Analysis of Fauna and Communities

In addition to census data derived from imagery, we will use specialized quantitative collection devices to make collections that facilitate compositional and functional analyses of the wide variety of megafaunal community types that we expect to encounter during the course of this project. Choice of the specific device used for a particular community will depend on the type of community, its 3-dimensional shape, and the substrate it is on. All of the collectors have been used on hard substrates and on sediments. In some cases obtaining the highest quality quantitative collections from hard substrates is impossible and in these cases we will supplement data on these communities with imagery and qualitative collections using manipulators and nets

as appropriate. We will also collect mobile megafauna opportunistically and use push cores to supplement the collections of meiofauna.

The Bushmaster Collection Devices

The quantitative collections of vestimentiferan aggregations and some types of coral aggregations, along with the associated communities, will be accomplished using hydraulically actuated nets. Bushmaster Jr. and Sr. have been used successfully by the Johnson Sea Link Submersibles on vestimentiferan aggregations and for sub-sampling *Lophelia* thickets on the ULS of the Gulf of Mexico; by Alvin for vestimentiferan aggregations in the Gulf of Mexico, on the East Pacific Rise and on the Juan de Fuca Ridge; and by the ROV ROPOS on the Juan de Fuca Ridge (Bergquist et al., 2002; 2003a; 2003b; Cordes et al., 2003; 2005; in press; Urcuyo et al., 2003; in press; Govenar et al 2002; in press; Cordes and Fisher, unpublished results from ongoing MMS GoM coral project). The open diameters of the Bushmaster Jr. and Sr. collection devices are 0.7 and 1.5 m respectively. In short, the devices are nets that are suspended and held open by a framework of flexible ribs, with a "drawstring" stainless steel cable that can be hydraulically actuated to close the net completely. Each device is now lined with 63 µm nylon mesh and retains all fauna above that size. The smaller device can also be fitted with a plastic liner and used for staining entire small vestimentiferan aggregations.

The Mussel Pot Collection Device

The quantitative collections of mussel and clam communities, as well as any other appropriate communities encountered, will be accomplished using our new "mussel pot" collection devices. These new devices were modeled after mussel pots designed and used numerous times by Dr. Cindy Van Dover at vents and seeps around the world. We kept the same general dimensions and operation parameters (Van Dover et al. 2002) in order to allow comparison to her extensive data sets on seep and vent mussel community structure that were collected using her devices (Van Dover and Trask, 2000; Van Dover 2002a, 2002b; Van Dover et al., 2002; Doerries and Van Dover, 2003; Turnipseed et al., 2003, Van Dover, 2003, Van Dover et al., 2003). We added considerable strength to the devices, including much more robust outer shells and moving parts, Kevlar bags, and high tensile strength Vectran® draw strings. We also added the capability to deploy a ring that delineates the collection location and extent. These new collection devices consist of a 'pot' made of 1/8" thick rolled aluminum . The interior diameter is 26 cm and the height of the pot is 29 cm. The Kevlar bag is attached to the pot and is closed by rotating a handle on the top of the pot that cinches the bag closed using a draw string. This can be done with a single manipulator capable of 360° rotation by using a hydraulic ram on the manipulator and an anti-rotation bar on the pot. When the bag is cinched shut a 10-cm high aluminum ring is released that marks the collection location and allows photographic documentation of the collection scar (and therefore quantification of mega fauna missed on uneven hard substrates). We have 6 of these devices and all can be used during a single dive if there is room on the vehicle or elevators are used to transport them through the water column. These new devices were used by the Fisher lab for the first time on the East Lau Basin Spreading center, with the ROV Jason II in June 2005 to successfully collect quantitative sub-samples of mussel and snail (a foundation, symbiont-containing group at Western Pacific Back Arc Basin Spreading Centers) communities on hard substrates (Fisher et al., in prep).

The pot is approximately 29cm in height with a 26 cm internal diameter. In use a submersible's manipulator arm rotates the T handle in a counter clockwise direction spooling the draw string and closing the open end of a Kevlar fabric tube. The other end of the tube is attached to the pot with Stainless banding. Before closure the fabric is folded on the outside of the pot. The anti-rotation bar contacts a plate on the sub arm and prevents the whole unit from rotating as the T handle is turned. After one full rotation the shackles are triggered and the outer ring cords are detached allowing the ring to fall free. A ratchet mechanism prevents the drawstring from uncoiling once the bag is cinched. The outer ring is recovered after photo documentation of the collection.

Processing of the Quantitative Collections

On board ship, the Bushmaster or mussel pot collections are fully and painstakingly processed to maximize the potential yield of information and minimize the need for further collections. Each collection is separated into attached and unattached fauna in a cold room. Foundation species (tubeworms, mussels, clams, or corals) are counted, measured, and volumes or weights determined on board (as time allows). Loose small fauna, sediment and other material is sorted through a series of sieves (2 mm, 250 µm, 63 µm), and material passing through a 250 µm sieve but caught on the 63 µm sieve is preserved for meiofaunal analyses by the Bright group (Univ. Vienna). Associated macrofauna (>250 µm) are separated based on morphology and preliminary identifications are carried out. Prior to sub-sampling, most associated fauna will be enumerated and weighed (to the extent that time allows on board the ship) using a motion-compensated ship-board balance (Childress and Mickel, 1982). Sub-samples of foundation species and associated fauna are formalin-fixed and ethanol preserved (for morphological taxonomic studies and voucher collections), frozen (for SI studies and determination wet weight to ash-free dry weight conversion factors), preserved in ethanol (for molecular systematic and phylogeographic analyses), or preserved appropriately for other analyses requiring non-formalin fixed material (symbiont studies, etc.). Care is taken to freeze and fix paired samples of all species, particularly unknown (or unrecognized) species found at this point. Full sets of subsamples will only be possible for abundant associated fauna, therefore samples for SI, molecular phylogeographic and weight conversion will only be obtained for the more common species of associated fauna. Weight conversion for other species will be based on published conversion factors (Ricciardi and Bourget 1998; Bergquist et al., 2002). Live or fresh material required by other investigators is also removed and logged, and then the remainder of the collection is fixed in 7% buffered formalin, sealed in museum bags, and placed in a 55 gallon drum for shipment back to PSU. In the laboratory, the aggregation is transferred to alcohol and placed in a large aquarium with a tight fitting lid. Here the remaining sessile and all remaining associated fauna is removed, sorted (sieved and partitioned as described above), identified and quantified (numbers and biomass of each taxon).

The populations of foundation and other dominant species will be further characterized for analysis of size frequency (using taxon-appropriate measures of size and mass). The total surface areas of tubes (tubeworms), shells (bivalves), or skeleton (corals) of the foundation species will be determined and used to standardize the sample area and the density of associated fauna for comparisons between both like and unlike community types.

Taxonomic Identification and Phylogenetic Characterization

As described above, we will sort the fauna and identify all specimens possible using expertise within the core team of PIs and close collaborators. The Fisher lab at PSU maintains a formaldehyde-fixed, ethanol-preserved voucher collection of 46 species collected with mussel beds or tubeworm aggregations at water depths of over 1000 m in the Gulf of Mexico, and 111 species collected with tubeworm aggregations and 40 species collected with *Lophelia pertusa* thickets on the upper slope. In addition, the Fisher lab has a collection of DNA samples from the most common species, and either frozen or ethanol preserved voucher samples from many others. These collections will allow us to identify many of the species collected and help recognize unknown species. We will continue to use existing collaborations with molecular and classical taxonomic experts familiar with the Gulf of Mexico seep and coral associated fauna, as well as other seep fauna from around the world. These experts will facilitate the identification of unknown species (see **Section C.1**). Several of these collaborators have also confirmed their interest in pursuing biogeographic and population genetic analyses of particular groups both within the GoM and in the context of other seeps or hardbottom communities around the world (Cheryl Morrison for *Lophelia*, Timothy Shank for shrimp, and Robert Vrijenhoek for vesicomyid clams and some gastropods).

We will conduct the molecular characterization of species from key groups for which we already have expertise and the appropriate molecular tools and data. This will include siboglinids (tubeworms), polynoid polychaetes, and bathymodioline mussels and their close relatives. To phylogenetically place any new species discovered as well as have the power necessary to detect significant degrees of genetic isolation between populations, we will initially use mtCOI, mt16S, mtND4, 28SD9-10, and ITS2 markers that span a range of variability in the genome. Different taxonomic groups will require the use of different markers, depending on the level of comparison needed and on the rate of evolution of the considered gene within the group. In the case of the siboglinids and polynoids we will describe any new species collected (Stephane Hourdez will take the lead on these descriptions). These analyses will also allow us to define the depth ranges of metapopulations of these groups and to begin to constrain the relations of populations and related species from different sites in the GoM to each other, and to other populations of closely related species.

We will also sequence appropriate standard genes (most commonly mtCOI and 18S) for classical taxonomic collaborators upon request to assist in descriptions of new species as needed.

Within-Community Trophic Studies

We will conduct a preliminary study of trophic interactions in the communities using a combination of tissue stable C, N, and S determinations and the quantitative data for each species present. For this study 3-6 replicate individuals of all key species (high biomass or numbers present) and free-living attached bacteria (if present in sufficient amounts) in each community type will be analyzed to provide information that will allow us to constrain the possibilities of types of nutritional interactions among the fauna and microbes, and construct preliminary food webs for each community type. This data will be interpreted in the context of the planned trophic studies of the more mobile colonist and vagrant fauna (see section A.3.5.7). For all

isotope based trophic studies, consistent tissue-type samples, usually muscle free of all hard parts, will be taken from freshly captured organisms, logged for data management, and frozen at sea. Upon return to the laboratory, samples will be dried at 60˚C for three days and homogenized for shipment to an analytical laboratory (tentatively identified as the Macko lab at UVA). Approximately 5 to 6 mg of dry tissue are required for $\delta^{34}S$ measurements, and 0.6 to 1.0 mg are needed for $\delta^{13}C$ and $\delta^{15}N$ measurements. A Carlo Erba elemental analyzer coupled to a Micromass Optima isotope ratio mass spectrometer (Fisons/VG/Micromass, Manchester, UK) is used to obtain $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values on acidified samples (Giesemann et al. 1994). The $\delta^{13}C$ and $\delta^{15}N$ will be obtained concurrently and $\delta^{34}S$ will be determined during separate analysis runs.

The isotope compositions will be reported relative to standard material and follow the same procedure for all stable isotopic measurements, as follows:

$$\delta^{x}E = [({}^{x}E/{}^{y}E)_{sample}/({}^{x}E/{}^{y}E)_{standard}] - 1) * 1000$$

where E is the element analyzed (C, N or S) and x is the atomic weight of the lighter isotope, and y the heavier isotope (x=13, 15, 34 and y=12, 14, 32 for C, N and S respectively). The standard materials to which the samples are compared are PDB (Pee Dee Belemnite) for carbon, air N_2 for nitrogen and CDT (Canyon Diablo Triolite) for sulfur.

Other Community-Level Analyses

Due to the exploratory component of this study, one first-order goal is as comprehensive a description of what is present at each site visited as possible. Because we will be using very effective and efficient collection devices we can expect to develop an inventory of species present that is considerably more comprehensive that would be possible otherwise with the dive time available. For example, the first bushmaster collection of a vestimentiferan aggregation on the well studied and heavily collected Endeavour Segment of the Juan de Fuca Ridge yielded 39 macrofauna and 9 meiofauna taxa, while Tsurumi and Tunnicliffe (2003) reported a total of 37 macrofauna and 14 meiofauna taxa from 51 other collections of tubeworms from 4 segments of the Juan de Fuca Ridge (Bergquist et al., in prep).

Faunal inventories will be compared to what is known of similar communities in other parts of the GoM and around the World to further refine current ideas of seep and vent biogeographical patterns (Sibuet and Olu, 1998; Van Dover et al. 2002; Taylor et al., 2003). In the cases of foundation species (tubeworms, mussels, corals), and at least some crustaceans and gastropods, genetic relatedness will also be examined and contribute to this effort.

In addition, we will compare the communities to each other and to other appropriately analyzed related communities with respect to a variety of ecological measures of community structure and function. The amount of surface area per volume of sample will provide a measure of habitat complexity. Trophic structure (biomass per trophic level), species richness, diversity (Shannon-Weiner diversity index H') and evenness (Pielou's evenness index J) will be compared among quantitative community collections. Multidimensional scaling (MDS) analysis will be used to examine the similarity in the community structure of each site. Briefly, this involves the construction of a similarity matrix using the Bray-Curtis index of similarity (or dissimilarity) and

mapping the samples in two dimensions according to their rank similarity in community structure.

Potential explanatory variables for the differences in community type and structure determined will be explored using the biology-environment (BIO-ENV) procedure in the multivariate statistics package, PRIMER (Clarke and Warwick, 2001). This procedure compares the rank similarity in community structure to the rank similarity in subsets of environmental variables (depth, location, methane concentration, sulfide concentration, hydrocarbon concentration, substrate type, etc) measured using the Spearman's rank correlation coefficient. This will identify the subset of abiotic variables which vary most closely with the observed changes in community structure. This summary data analysis will provide additional predictive capability to our understanding of the relation between geological and geochemical characteristics of a site and the types of communities that colonize the site.

Time Series and Growth Studies

Deep water coral species and communities are generally quite long lived (Andrews et al., 2002; Risk et al., 2002). Seep sites, communities, and the foundation species on the ULS are also quite long lived (Bergquist et al., 2000, 2002; Cordes et al., 2003, 2005) and we expect that the same is generally true for the deeper GoM sites. Nonetheless, interannual variability has been documented in the seep mussel communities (Nix et al., 1995), and occasional dramatic changes in sites have been documented in association with hydrate activity (C. Fisher, Pers. Obs.). We will collect imagery and base line data that will allow time series monitoring of the sites both during and potentially after the end of this study. We will also use time-lapse camera systems to examine visits of mobile fauna that might not otherwise be documented because the visits are relatively rare or the animals avoid submersibles. Finally we will use a well established staining methodology to constrain and provide first order estimates of growth rates and ages of the deeper living species of tubeworms.

Time Lapse Camera Survey of Mobile Fauna

The project will utilize updated versions of digital time-lapse cameras used for long-term surveillance of chemosynthetic communities at Bush Hill (MacDonald et al., 2005). The rotary time-lapse camera (RTLC) is a commercial off-the-shelf product (SeaSnap 360, manufactured by AquaPix). A digital camera (Nikon Coolpix 5400, 5 megapixel) is housed in a thick-walled glass tube, mounted on a rotating turntable, and illuminated with a 120 watt-sec. strobe. The system is programmed to take a series of 10 pictures at preset intervals. After each picture, the camera turntable rotates 36°, so that 10 pictures complete a 360° panorama of the seafloor (**Figure 7**).

Analysis of the time-lapse records will address the objective of determining what mobile fauna, fishes and invertebrates, visit the sites and if their density is higher than that of the surrounding sea floor. These systems may also detect and document potential periodic or rare dynamic physical events such as gas venting, sediment resuspension, or changes in seafloor hydrate deposits, and provide preliminary information on lunar or seasonal patterns in the fauna. The mobile fauna is an understudied group because it is difficult to collect using submersibles. A

number of workers have advanced hypotheses concerning the relative abundance of "vagrant" fish or crustaceans at seep sites compared to the surrounding benthos (Carney, 1994; MacAvoy et al., 2002, Carney et al., 2002), however these predictions are difficult to test. By comparing data from deployments of the autonomous rotary time-lapse camera systems in the communities with control deployments in the non-seep/hard-bottom benthos, it will be possible to constrain and begin to quantify the degree to which mobile benthic fauna aggregate at seeps/hard-bottom communities in the deep GoM.

Three configurations of the RTLC will be used by the program: 1) short-term deployed and recovered by submersible; 2) long-term (1-year) deployed and recovered by submersible, and 3) long-term deployed and recovered autonomously. Configuration 1 is similar to the version as shown in **Figure 7**, except the plastic float is replaced with syntactic foam and an external battery is added. Battery life and data storage is sufficient for collection of ~2000 panoramic images, therefore, during a deployment of 4 days, an RTLC could collect an image every 3 minutes. The floatation makes the RTLC light in water and self-righting; so with a minimum of submersible time, the packages can be repositioned by the submersible to monitor different stations within the site.

The short-term RTLC deployments can be used to provide another view of mosaicked areas, obtain images of the research in action, and monitor collection sites for evidence of increased predator/scavenger activity in conjunction with submersible activities.

A total of three long-term RTLC systems will be used in this study. Two will be left in place at selected study sites found at similar depths when the submersible completes operations. The third will be deployed approximately 1 km from one of these sites at a similar depth. The long-term RTLC systems will utilize a larger battery package and will collect images at a much longer interval. Battery life is sufficient for a deployment of as long as one year, with collection of six images per day. Comparison of the images collected in the seep/hard bottom sites with those from the off-site location at a similar depth will provide a test of how the seep/hardbottom environment concentrates or attracts mobile fauna. For autonomous deployment and recovery, camera and tube are suspended beneath a Benthos TR6000 transponder in an 18-inch glass sphere, which essentially replaces the small buoy as shown in **Figure 7**. The TR6000 is a deep-sea transponder commonly used for long-baseline navigation of submersibles. Its position on the seafloor can be interrogated from a surface ship. For recovery, a command from the surface causes the TR6000 to release a disposable anchor. The camera frame will be lowered close to the bottom in an area of interest and the autonomous camera will be released when the video feed confirms location. For recovery, the release command will be sent from the surface and the camera recovered with a small boat.

Time Series Analysis of Photomosaics and Chemistry Surveys

We will re-mosaic the same areas imaged in 2006 when the intensive study sites are revisited in 2007, and conduct a chemical re-survey of the communities present. Because of the expected relative stability of the communities, and the expected slow growth rates of the fauna, we do not expect to see significant changes in the appearance or chemistry of communities between years. However, if any changes in the communities are apparent in a preliminary analysis of the new

mosaics, then we will conduct more detailed chemical surveys of the communities on the subsequent dives. Two years of photographic and chemical data will establish a rigorous base line for future work after more extended periods.

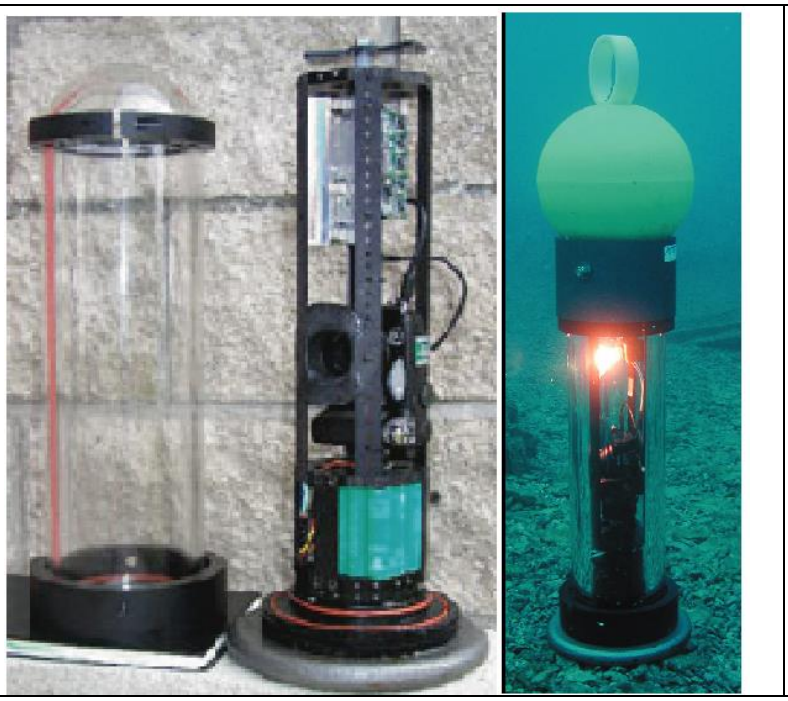


Figure 7. Autonomous time-lapse camera system. Far left shows camera with thick-walled glass and 120 watt second strobe. Left shows camera deployed in shallow water with lamp illumination. Lower panel shows panoramic image collected with the system.

Determination of Vestimentiferan Growth Rates and Ages

The primary tool for this aspect of the project is a staining device that uses a chitin stain (acid blue 158) to stain the growing ends of between 10 and 60 vestimentiferans simultaneously. The new growth is clearly apparent the next year as a band of white tube above the previously stained blue tube (**Figure 8**). It has been used numerous times by Alvin and the JSLs and can be used five or more times during a single dive (Bergquist et al., 2000; 2002; 2003; Urcuyo et al., 2003. in press; Cordes et al., 2003; 2005). We have also modified the smaller bushmaster device with a plastic inner net that allows this device to be used to stain small aggregations in their entirety (Cordes et al., 2005). This allows us to stain a few aggregations completely to study growth variation within the aggregation and stain species whose anterior ends are present deep in the aggregations (with the added benefits of assuring the stained aggregations fit in the collection devices and allowing staining and collection with the same equipment).

Ages of individual vestimentiferans are calculated using non-linear regression to fit a negative exponential model to the relationship between yearly growth rate and tube length (Bergquist et al., 2000). Repeated simulations of this growth model allow the determination of the average and confidence intervals for age-at-size for tubeworm species (Cordes et al., 2003, 2005). Mortality rates may be estimated by the frequency of empty vestimentiferan tubes in 10 cm size classes (Cordes et al., 2003, 2005), assuming tube degradation rates are comparable to other siboglinid species. Recruitment rate is estimated using a modified cohort analysis where size-specific growth and mortality rates are used to predict the size of the recruitment class forming

group of individuals in a given size class (Cordes et al., 2003). Aggregation-specific recruitment patterns are estimated using non-linear regression of the relationship between population size and annual recruitment rate (Cordes et al., 2003). Together, recruitment, growth, and mortality estimates are used to estimate age of each aggregation individually with greater accuracy than may be achieved by using the average length of tubeworms in the aggregation alone (Cordes et al., 2003, 2005).

Figure 8. In-situ photograph of L. luymesi from the ULS stained using the modified bushmaster as a whole-aggregation staining device. Above the blue-stained portions of the tubes, the white tips indicate 14 months of growth in a relatively young aggregation.

The level of effort for tubeworm staining and collection at the deep water sites will not allow the collection of data sets comparable to those obtained since 1997 from the shallow sites, that include thousands of individuals from dozens of aggregations at 3 central sites. However, the most significant comparisons will not require the same level of replication. Growth models will be developed for *E. laminata* and other species for which sufficient populations are discovered. We will compare the size specific growth parameters to those of the shallow species to obtain first order information on their growth rates in comparison to the shallow living species. We will compare the parameters of the non-linear growth functions at the different deep water sites to determine if growth is spatially variable and if it correlates to differences in site-specific geochemical conditions. We will compare the general form of the growth equation to that of *L. luymesi* and *S. jonesi* from the upper slope to reach qualitative conclusions regarding the form of growth in the deep-water species. For example, *L. luymesi* follow a clear pattern of decreasing growth with individual size. *S. jonesi* annual growth rate is not significantly related to its size; rather the probability of growth (growth rate > 0) significantly decreases with size. Similarity in

growth pattern will allow us to infer the relative importance of root (*L. luymesi*) vs. plume (*S. jonesi*) uptake for the deep-water tubeworm species.

Sclerochronology

Many deep-water sessile cnidarians possess annual growth bands useful for reconstructing the growth and environmental histories over their long life spans. In the case of corals, these historical records have provided abundant information about temperature, salinity, nutrient levels, water flow, turbidity and many other environmental factors important for growth and distribution (Dodge and Thompson, 1974; Hudson et al., 1976; Highsmith, 1979; Swart, 1983; Dodge and Brass, 1984; McConnaughey, 1989; Dunbar et al., 1994; Lough and Barnes; 2000; McCulloch et al., 2003; and many others). This information has advanced the understanding of the latitudinal limits, depth limits, and general distribution of most shallow water corals throughout the tropics. However, growth band studies have generally omitted in deeper cold-water sessile species, the exceptions being Andrews et al. (2002) and Risk et al. (2002). A known deep water coral in the Gulf of Mexico is *Lophelia pertusa*. Growth banding occurs in *L. pertusa*, but few studies have analyzed these potentially valuable records (**Figure 9**). In spite of several studies of *L. pertusa* (Wainwright, 1964; Wilson, 1979; Mikkelsen et al., 1982; Freiwald et al., 1997; Mortensen and Rapp, 1998), we currently lack a detailed understanding of the annual growth pattern of this interesting coral. Growth patterns and rates are measures of long-term, site-specific environmental conditions. The aim of this component of the proposal is to develop this understanding over annual and decadal time frames.

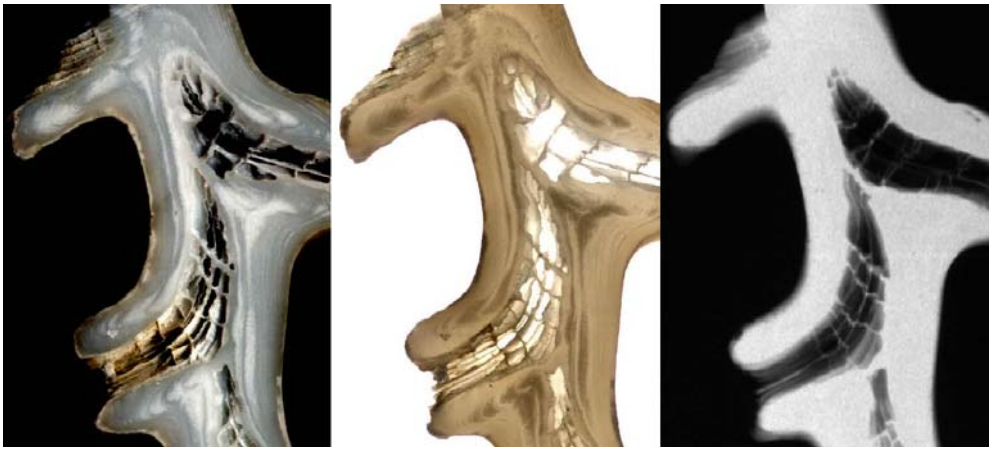


Figure 9. *Lophelia pertusa* sectioned longitudinally (1mm thick) and digitized as reflective (a), transmissive (b), and x-radiographic (c) images. Growth lines are present on reflective and transmissive images.

The annual nature of growth banding will be analyzed within individual sessile cnidarians, especially corals. A model will be developed for growth of an entire colony. The intent of this approach is to effectively describe the colony growth, and the growth model will be checked using radioactive decay for growth rates (e.g., ^{210}Pb dating, ^{14}C dating) and for seasonality within years using stable isotope analyses of carbon and oxygen in coral carbonates. The research should yield information on the general growth limits and environmental requirements necessary

for development. The cumulative goal of the growth-related studies is to develop a predictive understanding of hard bottom sessile cnidarian distributions in the deep Gulf of Mexico.

The major components of the growth studies are investigations of the density banding and stable isotope studies.

Density banding. The first step of this research is to confirm the annual nature of the growth bands in *Lophelia pertusa* by a combination of sclerochronological and geochemical analyses of the skeletons. The annual nature of these growth bands was recently reported by Mortensen and Rapp (1998) based on the timing of banding relative to a known collection date and a known time maintained in aquaria. Our approach is to use high resolution laser sampling of the skeleton (Sharp and Cerling, 1996) to reconstruct the seasonal variation within the isotope record and match this seasonal isotope pattern to the periodicity of growth banding. In previous studies (Ivany et al., 2000; 2003), high resolution sampling of biogenic carbonates (approximately 30 micrometer spot size) has been important to show annual growth patterns and infer seasonal temperature regimes. Growth lines will be measured using the Coral X-radiograph Densitometry System, Coral*XDS*, (**Figure 10**) (Helmle et al., 2002) to obtain linear extension data as well as bulk density along the growth transect.

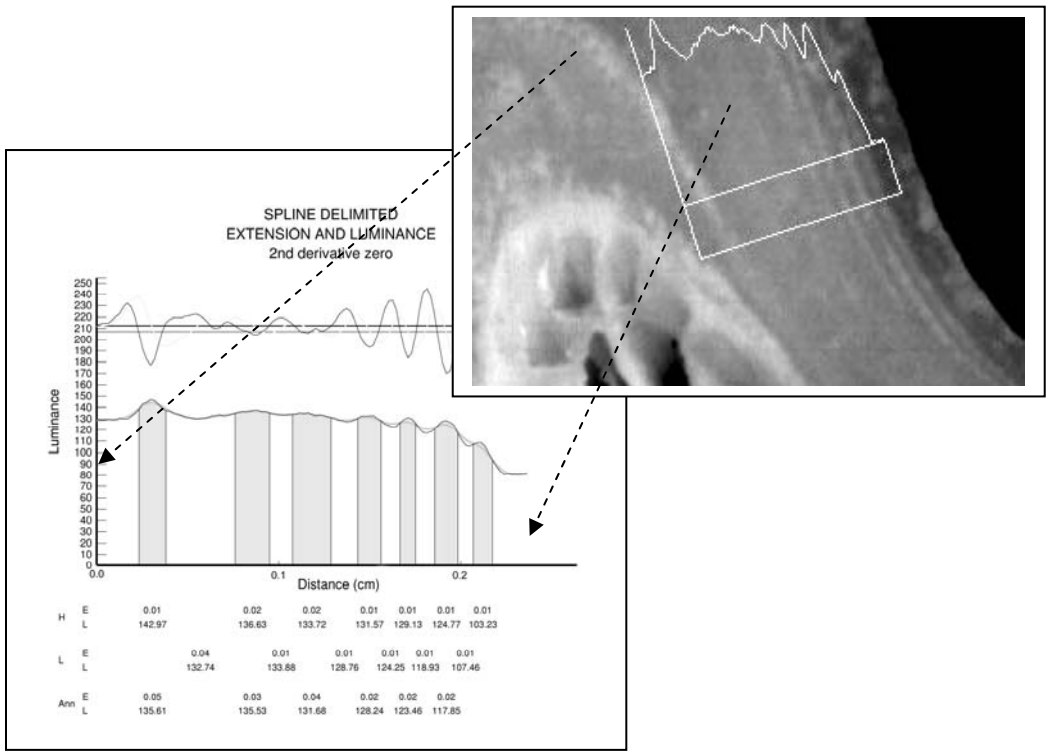


Figure 10. (Top)Transect box across annual bands and associated luminance profile showing annual bands as peaks and valleys. (Left) Delimiting of bands based on a threshold of the 2nd derivative of a cubic spline of the luminance data. Below, the extension data are broken down by High (light) bands, Low (dark) bands, and Annual (light and dark) bands for each year.

The colony age will also be corroborated by ^{210}Pb (Druffel et al., 1990) and ^{14}C dating at the base and growth tips. We will develop a relationship between growth rate, colony size, and colony age. The colony age/size relationship coupled with knowledge of the reef framework permit estimates of reef age and accretion rates. Beyond growth chronologies, isotopic and elemental analysis in the case of *Lophelia* should allow reconstruction of proxy records for temperature (Smith et al., 2000) as well as climate change (Adkins et al., 1998). Reconstruction of proxy records will allow refined definition of the environmental controls over growth and distribution.

Stable Isotopes. The stable isotope (δ^{13}C, δ^{18}O) values of skeletal carbonates generally reflect three components – growth rate, temperature and a small contribution of metabolic (dietary) carbon (e.g., Swart 1983; McConnaughey, 1989; Adkins, 1998; Heikoop et al., 2000; Spiro et al., 2000; Adkins et al., 2003; and many others). The growth rate information is expressed in terms of disequilibrium from expected isotopic compositions that should prevail if carbonate is precipitated slowly from seawater. Increases in growth rate are correlated with increases in disequilibrium for both δ^{13}C and δ^{18}O, with lower δ^{13}C and δ^{18}O isotope values pertaining at higher growth rates. The disequilibrium growth rate signal is generally the strongest component of the isotopic record (Mortensen and Rapp, 1998). However, it is also possible to estimate average growth temperatures from δ^{18}O values by accounting for disequilibrium effects (Smith et al., 2000), and similarly to estimate dietary C contributions from δ^{13}C values (Adkins et al., 2003). The dietary information may be especially useful if methane is an important ultimate source of nutrition for the corals, in which case the skeletal carbonate record could provide a historical view of how methane dependence has varied through time.

Geochemistry and Sediment Structure

Determining the biogeochemical signature of different cold seep habitats will help to characterize the geochemical input to the habitat, elucidate the controls on microbial distributions and activity and will help identify feedbacks between microbes, the geochemical environment and macrofaunal distributions. Replicate push cores will be collected at each site in a range of appropriate 'habitats" for the variety of geochemical and microbiological analyses described in this section. We envision these habitats will include; bacterial mats, near tubeworm aggregations, near mussel beds, among clam beds, and near coral-occupied hard substrates. A limited number of water samples will also be collected at appropriate locations among foundation species of megafauna for geochemical and microbial analyses.

Hydrocarbon Analyses in Push Cores

The concentrations of gaseous (C1-C5) and liquid (C15+) hydrocarbons in the sediments will be measured by standard GC-FID and GC/MS-SIM methods. These measurements will provide important information on the hydrocarbon thresholds controlling fauna distributions as determined from the photo-documentation and other studies at each of the study sites. The liquid aliphatic hydrocarbon analysis will provide important information on the degree of biodegradation of the hydrocarbon components fueling these chemosynthetic systems. Selected samples with elevated hydrocarbon concentrations will be analyzed for aliphatic and aromatic

biological markers in order to aid in understanding the origin of the hydrocarbon seepage. Key tricyclic terpane biomarkers are known which can distinguish between these source depositional types. Aromatic biomarkers will also be evaluated as well as various other molecular and bulk properties, including the following:

- Liquid chromatography for % saturate and % aromatic hydrocarbons and % NSOs
- Stable carbon isotope composition of both saturate and aromatic hydrocarbons
- Whole crude gas chromatography
- Molecular sieve separation of saturate hydrocarbons
- Quantitative GC/MS analysis for sterane and terpane biomarkers
- GC/MS analysis of aromatic hydrocarbons

Multivariate statistical techniques (cluster and principal component analyses) using isotopic and key biomarker ratios may aid in grouping seeps which share a common hydrocarbon source.

Other Geochemical Analyses in Push Cores

Ion specific microelectrodes and a UNISENSE picoammeter and computer-controlled micromanipulator will be used to quantify µm-scale depth profiles of dissolved O_2, H_2S, and pH in sediment cores. Standard pore-water and solid-phase biogeochemical variables will be determined in seep samples. For sediment samples, pore water will be obtained using a Reeburgh squeezer. The following dissolved species will be quantified: *Dissolved inorganic carbon* concentration will be determined using an infrared detector and *dissolved alkanes (C_1-C_5)* will be determined using gas chromatography. Dissolved H_2S and reduced iron (Fe^{2+}) concentrations will be determined using colorimetric methods. Dissolved organic carbon will be determined using a Shimadzu TOC 5000. Volatile fatty acids will be determined as 2-nitrophenyl derivatives using HPLC. Nutrient concentrations (N, P, Si) will be determined colorimetrically. Pore water *pH* and alkalinity will be determined with a high precision voltammeter (calibrated with TRIS standards) and by gran titration, respectively.

For solid phase samples, the following parameters will be determined. Total carbon, nitrogen and sulfur and inorganic (carbonate) carbon will be determined using a Carlo Erba elemental analyzer (total C/N/S) and a solid phase inorganic carbon analyzer (inorganic C). The concentration of elemental sulfur will be determined using the ferri-thiocyanate method. Sediment porosity will be estimated by drying a known amount of sediment to a constant weight.

Textural Analysis of Push Cores

Standard textural analysis will be performed on the sediment around designated study sites. This analysis is important because sediment size affects 3-D seismic surface amplitude response. Wet sieving will be used to separate the fine fraction from particles greater than 62 µm. A Gilson ultrasonic siever will be used on the coarse fraction. Graphic methods of Folk and Ward (1957) will be used on the coarse fraction where appropriate. Diagenetic inclusions will be picked from the coarse fraction for analyses listed above.

Geochemical Analysis of Water Samples

Two to four water samples will be taken at each site using the Alvin titanium "major" water samplers. The concentrations of gaseous (C1-C5) and liquid (C15+) hydrocarbons in the water will be measured by standard GC-FID and GC/MS-SIM methods. Aliquots from the same water samples will be filtered and analyzed for total bacterial and particulate organic carbon content.

In Situ Geochemical Analysis and Surveys

Measuring much of the biologically relevant chemistry in biologically relevant ways has been one of the longest standing challenges for hydrothermal vent and cold seep studies in deep water. This is largely due to two factors. The first is that sulfide and oxygen are very labile, reacting with each other in water samples that contain both (as is typical in seep and diffuse flow vent fluids). Thus, analyzing sulfide and oxygen in water samples after recovery of the submersible or ROV, often hours after collection, is of limited use. (No deep diving submersibles have the capability to take water samples into the sphere, as is possible with the JSL.) The second factor is that the most reliable water samplers available for use at this depth take a minimum of 500 ml samples, resulting in significant dilution of seep fluids with ambient bottom water entrained in the flow into the samplers.

The solution is to use in situ analysis tools. We will use a CAPSUM METS in situ methane sensor to survey methane levels at the sites and specifically among the communities of animals studied. We will partner with the Botieus lab at the Max Planck institute in Bremen and use their microsensors to assay oxygen, pH and sulfide levels in the same locations. In both cases the chemical survey strategy will be designed to provide site level general information on background levels in the bottom water as well as specific information on the pH and levels of methane, oxygen, and sulfide that different foundation species and faunal communities are exposed to.

A CAPSUM METS methane sensor in its 5 by 20 cm titanium housing is depth rated to 4,000 m with integral RS 232 sensor output. The sensor uses a semi-conductor type detector. Hydrocarbon molecules diffuse through a special silicone membrane into the detector where the adsorption of hydrocarbon on the active layer leads to electron exchange with oxygen and thus to modification of the resistance, which the electronics package transduces into a voltage. Voltage is proportional to methane concentration over a 200 fold range in concentration. The sensor will be deployed with the submersible's robotic arm, and data will be collected in real time inside the sphere of the submersible (or in the control van of a ROV). For the purposes of this project a sensor sensitive to 100 nM will allow detection of relatively low levels of methane and also discrimination between levels relevant to megafauna with methanotrophic symbionts that we are likely to encounter in some microhabitats (low µM: Nix et al., 1995; Smith et al., 2000)

Microsensors will be used to document substrate concentrations in the environment. With needle shaped microsensors, dynamics of substrates can be monitored with a spatial resolution of microns and a time resolution of seconds. The tip size of the microsensors is ca 5 microns, thus the measurements reflect the undisturbed environment. A microsensor array containing three types of sensors (O_2, pH and H_2S) will be deployed using the submersible's robot arm. The O_2

microsensors are Clark sensors, based on diffusion of oxygen across a silicone membrane to an O_2 reducing cathode that is polarized against an internal Ag/AgCl anode. The flow of electrons from the anode to the O_2 reducing cathode responds linearly to the oxygen partial pressure around the sensor tip and is in the pA range. The current is measured by a high quality pA meter that is housed inside the submersible. These O_2 sensors work extremely well even under low O_2 conditions. Microsensors respond linearly to changes in O_2 partial pressure. The sensors are pressure tolerant and appropriate for in situ use. The response time of the sensor is 1-3 seconds and the detection limit is 300 nM O_2; the accuracy is within ~100 nM O_2. pH microsensors are miniaturized versions of a conventional pH electrode, based on selective diffusion of protons though pH glass, and the determination of potentials between the internal electrolyte and a counter electrode. The measuring range is pH 4-10, the response time is 20 s, and the accuracy is within 0.1 pH unit. The microsensor for dissolved H_2S is also a Clark microsensor. H_2S diffusing through the silicone membrane at the sensor tip is deprotonated in the alkaline electrolyte. The response of the sensor relies on the oxidation of HS- to elemental sulfur on a platinum anode through a redox mediator $[Fe(CN)_6]^{3-}$. A platinum guard anode insures low and stable zero-current. The detection limit is 0.3 µM H_2S and the response time is 10 s; the accuracy is 100 nM H_2S.

Measurements in a particular spot can be made in seconds to minutes by the scientist in the submersible who will operate the microsensor array from a laptop computer.

Analyses of Hard Substrates

Analyses of hard substrates and sediments within fluid and gas expulsion sites will be conducted in order to determine the types and origins of hard bottoms and size plus composition of surrounding sediments. Hard substrates will be analyzed for mineralogy using X-ray diffraction (XRD). Whole rock samples and samples of lithified matrix drilled in order to avoid shell material will be investigated. Powders will be analyzed by XRD to determine the qualitative and quantitative mineralogical composition. A Phillip's 3720 APD or Siemens D5000 system available at LSU will be used for the analysis. These data will be generated using CuK radiation step scanning at 0.02° 2 increments with fixed counting times of 1.0 s. Nodular masses which are products of recent diagenesis will be sieve-separated from sediment samples and the same type of XRD analysis for mineralogy will be performed.

Scanning election microscopy using a JEOL-840 SEM unit will be used to determine cement morphologies and elemental compositions of lithfield materials. This SEM is equipped with an EDS X-ray and back-scattter electron detector unit for qualitative elemental analyses. Quantitative analyses will be made with an electron microprobe model JOEL-733. Both the SEM and microprobe are housed in the Department of Geology and Geophysics at LSU.

Carbon and oxygen isotope analysis, critical for establishing the link with hydrocarbons, will be conducted on hard substrate and nodular materials in the sediments. Analyses will be conducted by TDI-Brooks. Both AMS and standard ^{14}C dating of selected samples will be sent to BETA Analytic, Inc. of Miami, Florida. Previous studies by BETA Analytic, Inc. have been performed well and results have been delivered on time.

Microbial Studies

Microbial activity is responsible for many of the bulk features that characterize cold seeps. For example, microbial respiration increases dissolved inorganic carbon concentrations and alkalinity, which generates authigenic carbonates, one of the macroscale geologic features of seeps. Microbial respiration generates other reduced metabolites, such as H_2S, that support the accumulation of significant biomass of macrofauna and their microbial symbionts. We will apply a suite of microbiological, biogeochemical, and molecular techniques to document the activity and distribution of microorganisms at cold seeps in a variety of habitats within the deep habitats in the Gulf of Mexico. The overall goal is to identify and analyze the bacterial populations that inhabit seep sediments, with an emphasis on sulfate reducing bacteria. Microbial abundance and/or activity is likely related to seepage intensity (e.g., advection rates), thus, documenting patterns of microbial distributions and activity may help elucidate geophysical dynamics at our sites.

Quantifying Microbial Abundance and Associations. The goals of this work are to document patterns of microbial abundance, diversity and associations in different seep habitats and to isolate and characterize microbes from seep habitats. Samples for direct determination of bacterial abundance using epifluorescence microscopy will be collected and preserved with filter sterilized (0.2 um) buffered formalin (in 3.5% NaCl). Samples for molecular identification will be preserved in PBS/ethanol following formalin fixation. Microbes involved in key biogeochemical processes (e.g., sulfate reduction) will be enumerated using CARD-FISH (Amann et al. 1995, Pernthaler et al. 2002). CARD-FISH probes targeting sulfate reducing bacteria (e.g., *Desulfosarcina, Desulfococcus, Desulforhopalus, Desulfobulbus*; Boetius et al., 2000, Knittel et al., 2003) will provide data to monitor and quantify microbial abundance, associations (consortia), and diversity of functionally important cold seep microbes. 16S rDNA sequence information (see below) will be used to develop probes for other relevant groups of organisms (i.e., hydrocarbon-degrading microorganisms) to examine the correlation of these groups with observed biogeochemical signatures. DNA will be extracted from samples according to previously published methods. Samples will be amplified with domain-specific (*Bacteria* or *Archaea*) or group-specific (e.g., SRB) primers and cloned. Representative sequences will be obtained, edited and checked for chimera formation and similarity determined against databases using BLAST.

Microbial Activity. We will determine rates of sulfate reduction in cold seep samples using standard radiotracer techniques. All rates will be determined in duplicate or triplicate sub-cores to give a measure of spatial heterogeneity in activity. Shipboard experiments on sediment sub-cores will involve amending samples with high specific activity tracer, incubation (for 12-24 hours), termination and fixation, and recovery of product after acid digestion back at Joye's UGA lab.

All of the microbial data will provided for inclusion in the appropriate Census of Marine Life database.

Relation between Chemosynthetic and Other Hardbottom Communities to Normal Background Fauna

It is the intent of this component to characterize the extent of trophic linkage between seeps and hardgrounds, and the sparse fauna of the vast surrounding seafloor. It is well established that ULS seep communities interact with heterotrophic organisms of the surrounding seafloor in terms of species composition (Carney, 1994) and trophic links (MacAvoy et al., 2002, 2003; 2005). Similar hardground-background interactions are to be expected. Two general deep-sea phenomena are of special interest with respect to effects on seeps and hardgrounds: (1) the decline in benthic biomass with depth (Rex, in prep) and (2) the depth decline in large predators such as crabs, fish, octopods, etc. (Carney, 2005). Given the importance of keystone predators in shallow foundation systems (Naverrette and Menge, 1996), similar controls are to be expected in both deep seeps and hardgrounds. Large powerful predators are so prevalent on the shelf and shelf break that it has been speculated that predation limits the upper depth limit of seep communities (Carney, 1994; Sahling et al., 2003).

It is expected that seep and hardground communities at the slope base will have markedly different interactions with the surrounding fauna; the nature of the differences, however, is hard to predict. The more impoverished lower-slope background might produce less exploitation of seeps and hardgrounds, or make the high biomass even more heavily exploited. Similarly, the apparent paucity of predators down slope may correlate with less predation. Alternately, endemic large predators may be found (Voight, 2000), or the few background predators may be especially important.

Ultimately, the question of how seeps interact with the surrounding biota will be most effectively answered via well-designed experimental studies. Currently, however, research has progressed only to the level of refined survey and characterization. At this critically important level, three tissue isotopes, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, (see **Section A.3.5.2.6** for notation and analytical methods) provide good indication of trophic linkages, dietary mixing between chemosynthetic and photosynthetic sources, and apparent levels in a simplified trophic net. It is proposed to make these assessments and provide initial estimates of the standing stocks of carbon pools that can be related to initial estimates of the same for seep and hard ground communities. This requires adequate collection of targeted feeding types, assessment of population standing stocks, and isotopic assessment of trophic relations.

Since the normal slope fauna undergoes marked species turnover between the upper and lower slopes, it will seldom be possible to assess trophic relations for a single species across the full depth range. Based on a review of past work, 19 relatively abundant target species can be identified and grouped into four functional groups found at all depths (see **Table 2**). These are all megafaunal species for which an estimate of population density and size can be obtained from bottom images. Biomass can be correlated to size once ash-free dry weight relationships are determined. In an effort to extend this list to larger macroinfauna (reviewed by Levin, 2005), box coring will be conducted in addition to trawling and trapping as noted below.

The actual species analyzed will be determined by sampling success. As a planning estimate, isotopic analysis will be conducted on 100 samples following each of the field years

Sampling – Sampling will duplicate successful methods employed on the upper slope with the addition of box coring.

- Direct Collection – The ALVIN/ROV will collect megafauna species encountered around the seep area noting the position relative to seepage. Prior experience indicates that large holothurians and asteroids can be easily collected by this method and with increased effort crustaceans can occasionally be collected this way as well.
- Trapping – three styles/deployments will be used. First, a small 1/2"mesh baited trap with a smaller 1/8" inner trap will be deployed at a minimum of three detailed study sites/year on the 1st dive and recovered on the final dive in field Years 1 & 2. Second, a similar baited trap will be mounted on a free return elevator and deployed ~50m from seepage at the detailed-study sites. A minimum of two days between deployment and recovery is needed. Third, a larger baited trap rigged for surface deployment and free return will be deployed at least 500 m away and within 1km of known sites. Release will be via Oceanic Instruments acoustic release. A minimum of two days between deployment and recovery is required. Prior experience indicts that in-seep, near, and far will provide adequate numbers of fish and scavenging crustaceans.
- Trawling – a 40' semi-balloon trawl will be towed at least 5 km from detailed study sites to obtain a large number of background species. The trawling will be conducted during nigh operations on the R/V ATLANTIS. In addition, 6 background trawl samples will be taken during the 2006 survey cruise.
- Box coring – a Gulf of Mexico type box core (Boland and Rowe 1991) will be used to sample background benthic infauna at 1-km and 5-km from detailed study sites. Due to tissue requirements of isotope analysis, larger macrofauna will be sought and samples processed through a 0.5 mm screen. The box coring will be done as night operations on the R/V ATLANTIS. Off-site box coring will be initiated during the 2006 cruise.
- Specimen Processing – All samples will be sorted to separate morphospecies at sea. Taxonomic vouchers will be preserved as standard for each taxa. DNA vouchers will be preserved in ethanol. Consistent tissue types (usually muscle) will be frozen in liquid nitrogen for isotope analysis. Bulk samples for size x biomass determination will be frozen at –20ºC. Samples will be returned to LSU. Tissues will be submitted to Dr. Macko's lab at UVA for isotopic analyses, all other analyses will be carried out in Dr. Carney's lab at LSU. Ash-free dry weights will de determined via EPA standard methods of drying and combustion. Vouchers will be submitted to appropriate experts with instruction for final deposition at the USNMNH and/or the Field Museum of Chicago.

Table 2. Trophic target species by feeding type.

	Upper Slope (Past work)	Lower Slope (Proposed)
Microphagous Detritivore	Ophiuroidea	
	Ophiura spp. *Ophiomusium* spp.	*Ophiura* spp. *Ophiomusium* spp
Macrophagous Detritivore	Holothuroidea	
	Bathyplotes natans	*Mesothuria lactea* *Benthodytes typica* *Benthodytes lingua*
	Echinoidea	
	Echinus affinus	*Phormasoma* spp.
Chaelate Feeders	Galatheidae	
	Mundopsis spp.	*Munidopsis simplex*
	Lithodidae	
	Rochina crassa	*Lithodes agassizii*
	Brachyura	
	Chaceon quinquedens	*Chaceon quinquedens* *Ethusina abyssicola*
	Thalassinidae	
	Calaxius carneyi	*Axius* spp.
Predators/Scavengers	Gastropoda	
	Eosipho buccinae *Gymnobela sp.*	*Buccina spp.* *Gymnobela sp.*
	A-chelate Crustacea	
	Bathynomus giganteus *Lysianassia spp.*	*Bathynomus giganteus* *Lysianassia spp.*
	Asteroids	
	Sclerasterias tanneri *Dytaster insignis*	*Plinthaster dentatus* *Nymphaster arenatus*
	Pices	
	Synaphobranchus spp. *Chaunax pictus* *Eptatretus sp.* *Antimora rostrata*	*Synaphobranchus* spp. *Gadomus longifilis*

Two types of data will be generated: isotope triples and size x ash-free dry weight. Simple end-member diet mixing models will be applied to the first to characterize trophic relations to seeps as a function of feeding type and seep proximity. Once the trophic relations have been determined, size of the appropriate carbon pools will be estimated from abundance data (image derived) and size x biomass relationships.

Data Interpretation, Synthesis and Reporting

Data interpretation, synthesis and reporting is covered in the Project Management Plan, but the main reporting requirements of the project include:

- Two (2) narrative Interim Reports six (6) months after completion of each year's (FY-06 and FY-07) field work. The purpose of these reports is to disseminate results from the field sampling and initial results of analysis.
- Draft Final Report 45-months after contract award. The Final Report will include the assessment of all data collected during the Study, description of methods and analysis, interpretations of the analyzed information, and results, discussions, and synthesis of the findings. This will include synthesis of all the combined aspects of the entire data record including the discussion and synthesis of the recent worldwide research on chemosynthetic and hardbottom deep-sea communities.

TDI-Brooks acknowledges that no publication of these materials without the expressed permission of the MMS and the Contractor. However, we state that journal publication is the primary focus of our PI team and will be the most lasting product of this Study.

Development of Educational Outreach Material

The PI for the development of educational outreach material will be Ms. Liz Geohring. Ms. Goehring is the education and outreach coordinator for the Ridge 2000 program, an NSF-funded research initiative to study Earth's oceanic spreading centers as an integrated system. She is the founder and director of SEAS (Student Experiments At Sea), a web-based outreach program designed to bring authentic student inquiry about the deep-sea directly into the classroom through labs and experiment competitions. Ms. Goehring also coordinates and helps conduct deep-sea science workshops for teachers, with an emphasis on enhancing teachers' scientific inquiry skills through practice and interaction with scientists. She recently co-edited a special issue of Current, the Journal of Marine Education, titled *Deep Hydrothermal Vents*.

The education outreach component of this Study will build on the successes of both the NOAA OE educational materials and the Ridge 2000 SEAS (Student Experiments At Sea) educational program. Our objective is to develop one to two complementary secondary school "Classroom to Sea" laboratories along with teacher professional development, to be delivered in partnership with COSEE-CGOM.

The NOAA Ocean Exploration program has developed an extensive collection of hands-on lesson plans directly related to research cruises sponsored within the last 3-4 years. Several of these cruises have focused on research in the Gulf of Mexico (e.g., Bioluminescence, Deep-Sea Corals, Hydrocarbon Seeps) and curricular materials exist on the Ocean Explorer website for seep research in this geographic area (e.g., "Biochemistry Detectives" and "Chemosynthesis in the Classroom"). Additionally, NOAA offers excellent teacher professional development workshops where teachers explore available curricular materials, learn about current research, and interact with scientists.

The Ridge 2000 education outreach program has also developed teacher professional development and an exciting participatory educational program, SEAS, with the goal of helping facilitate authentic student inquiry related to deep-sea hydrothermal vent science. SEAS has several components including data-rich, inquiry-oriented curricula; a student-oriented website that features at-sea research; motivating experiment and report competitions; "Ask-a-Scientist" email forums for students to explore pre-proposal experiment ideas; and a "Classroom to Sea" Lab. All components of SEAS are geared towards modeling the scientific process and inviting student participation to engage the learner.

The focus of this education outreach program will be to develop one or two "Classroom to Sea" laboratories that feature the unique research on the chemosynthetic communities of the Gulf of Mexico. The philosophy behind the "Classroom to Sea" lab is to help students understand the remote environment of the deep sea by making direct comparisons with their own environment. The ocean floor and its communities can be challenging to understand, particularly since it is so different from our world on land and so difficult to visit. One way for students to begin to grasp the uniqueness of this environment is by making comparisons between it and things they are familiar with in their own environment, or can measure in their own classroom. The first "Classroom to Sea" lab, for example, focused on a comparison of shallow-water and deep-sea vent mussels, and guides students in exploring differences in anatomy based on different feeding strategies. Students collect data on shallow-water mussels and then compare this data to data collected at sea on deep-sea mussels. At-sea data, as well as log entries from sea are available to students through a website. Students are given guidelines for how to analyze the data and are invited to submit reports to a Report Fair, for scientist feedback.

This outreach program will have two phases. Phase I will involve developing the new Classroom to Sea Gulf of Mexico labs, and Phase II will involve disseminating the labs with teacher training workshops. In Phase I, we will work with three (3) expert teachers from the Gulf area to develop the labs. We'll work with the COSEE-CGOM to identify these teachers. We will test the labs on the first June cruise in 2006. In phase II, we will develop and conduct a 3-day teacher course, again in the Gulf area and offered through the COSEE-CGOM, to provide teachers with information on cutting-edge research, relevant OE lessons, inquiry skills related to the specific labs and the data analysis required, and practice working with the labs. The short course will be offered to coincide with the second June cruise so that teachers can access the cruise website, observe the at-sea lab, and download the data. The COSEE-CGOM will help to recruit teachers to the workshop, and to provide a venue for the course. We will also explore incorporating these labs and teacher professional development into their existing Summer Institute series for teachers.

Phase I: Explore & Develop

Objective: To develop 1-2 GOM "Classroom to Sea" labs, working with local teachers, and test on June 2006 cruise.
Identify and work with 1-3 expert teachers. Train teachers on current GOM chemosynthetic community science, and on inquiry process/data analysis if necessary (format: short workshop working with some of PIs and/or graduate students). Focus on lab techniques that might be

transferable to the classroom. Form teacher/scientist partners (1:1) to develop labs, with possible follow-on interaction.
Timeframe: First year of program
Phase I Tasks:

- Recruit 2-3 expert teachers, from biology, chemistry or earth science – COSEE-CGOM. Provide background training for expert teachers – 2 day workshop for teachers with 3 day follow-up in research labs (Teacher/Scientist Pairs). Focus on exploring research and lab techniques. Identify lab topics
- Test labs on summer cruise – Science party plus 1 teacher
- Analyze data - Pairs
- Revisions – Pairs
- Develop website – TBD

Phase II: Train and Disseminate

Objective: To provide workshop for ~20 middle/high school teachers on GOM Chemosynthetic Communities, on inquiry skills related to lab techniques and data analysis, on relevant OE curriculum, and on GOM "Classroom to Sea" Labs. Develop teacher workshop with focus on GOM chemosynthetic communities. Use PIs and/or grads as faculty, and existing OE and COSEE CGOM lessons. Work with COSEE – CGOM to recruit teachers for summer (2007) workshop to be held at Scott Aquarium. Hold workshop for teachers and tie into June 2007 cruise. Teachers will train in the summer, access lab data during summer cruise(s), practice working with data, and prepare to use "Classroom to Sea" lab with students during the academic year.
Timeframe: August 2006 – August 2007 (with evaluation and web presence continuing into Year 3)
Phase II Tasks:

- Develop 3-day short course, identify faculty - EG
- Advertise, recruit teachers, setup venue– COSEE-CGOM
- Conduct short course w/ unit plan assignment - EG
- Conduct lab at sea – Science party plus 1 teacher
- Post cruise follow-up, feedback on teacher reports – EG & PIs
- Program evaluation
-

Cooperation with International Marine Life Databases

The Census of Marine Life (CoML) effort includes a number of components, two of which are most relevant to this program. First, the ChEss program is dedicated to an understanding of the biogeography of deep-water chemosynthetic ecosystems. The ChEss program office is housed at the National Oceanography Center (NOC) in Southampton UK, and is co-directed by Chris German and Paul Tyler. Dr. Fisher is currently a member of the Steering Committee for the program and has been a scientific advisor to the program (and ex-officio member of the steering committee) since its inception. He is fully aware of the full spectrum of ChEss initiatives and has participated in their planning efforts for these initiatives. He has informed Dr. Daniel Desbruyeres (the lead scientist responsible for coordination of ChEss Atlantic Equatorial Belt studies) about this MMS project (and the MMS ULS Coral project and Fisher's NSF shallow

seep projects as well), and will provide a full report on the studies to the ChEss Steering Committee at their next meeting on September 11 in San Diego California. All data collected during this study that can be accommodated by the ChEss database (which is a component of the CoML OBIS data base) and will be provided to them, and all gene sequences will be submitted to the international genetic data base, GenBank. The work proposed here will contribute significantly to the goals of the Atlantic Equatorial Belt studies of the ChEss program, particularly the components that will allow interpretation of our findings in the context of seeps around the world.

The second component of the CoML program relevant to this project is the CoMargE component. Dr. Carney is the co-director of CoMargE along with Dr. Myriam Sibuet at IFREMER. Additionally, Dr. Carney is supported by the MMS Coastal Marine Program to transfer past MMS survey data into the CoML OBIS database system. Therefore, procedures and software are already in place to carry out data transfer to this International Marine Life database.

References

Adkins, J.F., H. Cheng, E.A. Boyle, E.R.M. Druffel, and R.L. Edwards. 1998. Deep-sea coral evidence for rapid climate change in ventilation of the deep North Atlantic 15,400 years ago. Science 280:725-728.

Adkins, J.F., E.A. Boyle, W.B. Curry, and A. Lutringer. 2003. Stable isotopes in deep-sea corals and a new mechanism for "vital effects". Geochim. Cosmochim. Acta 67: 1129-1143.

Aharon, P. and B.S. Fu. 2000. Microbial sulfate reduction rates and sulfur and oxygen isotope fractionations at oil and gas seeps in deepwater Gulf of Mexico. Geochim. Cosmochim. Acta 64:233-246.

Aloisi, G., I. Bouloubassi, S.K. Heijs, R.D. Pancost, C. Pierre, J.S.S. Damste, J.C. gottschal, L.J. Forney, J.M. Rouchy. 2002. CH4-consuming microorganisms and the formation of carbonate crusts at cold seeps. Earth and Planetary Science Letters 203:195-203.

Ambrose, R.F. and T.W. Anderson. 1990. Influence of an artificial reef on the surrounding infaunal community. Mar. Biol. 107:41-52.

Andersen, A.C., S. Hourdez, B. Marie, D. Jollivet, F.H. Lallier, and M. Sibuet. 2004. *Escarpia southwardae* sp. nov., a new species of vestimentiferan tubeworm (Annelida, Siboglinidae) from West African cold seeps. Can. J. Zool. 82: 980-999.

Andrews, A.H., E.E. Cordes, M.M. Mahoney, K. Munk, K.H. Coale, G.M. Cailliet, and J. Heifetz. 2002. Age, growth and radiometric age validation of a deep-sea, habitat-forming gorgonian (*Primnoa resedaeformis*) from the Gulf of Alaska. Hydrobiologia 471:101-110.

Arvidson, R.S., J.W. Morse, and S.B. Joye. 2004. The sulfur biogeochemistry of chemosynthetic cold seep communities, Gulf of Mexico, USA. Mar. Chem. 87: 97–119.

Bergquist, D.C., I.A. Urcuyo, and C.R. Fisher. 2002. Establishment and persistence of seep vestimentiferan aggregations from the upper Louisiana slope of the Gulf of Mexico. Mar. Ecol. Prog. Ser. 241: 89-98.

Bergquist, D.C., T. Ward, E.E. Cordes, T. McNelis, R. Kosoff, S. Hourdez, R. Carney, and C.R. Fisher. 2003a. Community structure of vestimentiferan-generated habitat islands from upper Louisiana slope cold seeps. J. Exp. Mar. Bio. Ecol. 289: 197-222.

Bergquist, D.C., J. Andras, T. McNelis, S. Howlett, M.J. van Horn, and C.R. Fisher. 2003b. Succession in upper Louisiana slope cold seep vestimentiferan aggregations: the importance of spatial variability. PSZN Mar. Ecol. 24:31-44.

Bergquist, D.C., C. Fleckenstein, J. Knisel, B. Begley, I. R. MacDonald, and C.R. Fisher. 2005. Variations in seep mussel bed communities along physical and chemical environmental gradients. Mar. Ecol. Prog. Ser 293:89-97.

Bett, B.J. 1997. Atlantic Margin Environmental Survey: Seabed Survey of the Shelf Edge and Slope west of Shetland. Published by Challenger Division for Seafloor Processes: Southampton Oceanography Centre, NERC/ University of Southampton. Pp.165.

Boetius, A., K. Ravenschlag, C. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann, B.B. Jørgensen, U. Witte, and O. Pfannkuche. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623-626.

Brooks, J.M., D.A. Wiesenburg, H. Roberts, R.S. Carney, I.R. MacDonald, C.R. Fisher, N.L. Guinasso Jr., W.W. Sager, S.J. McDonald, R.A. Burke Jr., P. Aharon, and T.J. Bright. 1990. Salt, seeps and symbiosis in the Gulf of Mexico. EOS 71(45):1772-1773

Cairns, S.D. 1978. A checklist of the ahermatypic scleractinia of the Gulf of Mexico, with the description of a new species. Gulf Res. Rep.6: 9-15.

Cairns, S.D. 2001. A brief history of taxonomic research on azooxanthellate Scleractinia (Cnidaria: Anthozoa). Bull. Biol. Soc. Wash. 10:191-203.

Cairns, S.D., D.M. Opresko, T.S. Hopkins, and W.W. Schroeder. 1993. New records of deep-water Cnidaria (Scleractinia & Antipatharia) from the Gulf of Mexico. Northeast Gulf Sci. 13:1-11.

Carney, R.S. 1994. Consideration of the oasis analogy for chemosynthetic communities at Gulf of Mexico hydrocarbon vents. Geo-Mar. Let. 14:149-159.

Carney, R.S. 1997. Basing conservation policies for the deep-sea floor on current diversity concepts: a consideration of rarity. Biodiv. Conserv. 6:1463-1485

Carney, R.S. 2005. Zonation of deep biota on continental margins. Oceanogr. Mar. Biol. Ann. Rev. 43:211-278

Cary, S.C. 1989. Multiple trophic resources for a chemoautotrophic community at a cold water brine seep at the base of the Florida escarpment. Mar. Biol. 100:411-418

Cary, S.C., C.R. Fisher, and H. Felbeck. 1988. Mussel growth supported by methane as sole carbon and energy source. Science 240:78-80.

Childress, J.J., C.R. Fisher, J.M. Brooks, M.C. Kennicutt II, R. Bidigare, and A.E. Anderson. 1986. A methanotrophic marine molluscan symbiosis: Mussels fueled by gas. Science 233: 1306-1308.

Clarke, K.R., and R.M. Warwick. 2001. Change in marine communities: An approach to statistical analysis. Primer-E, Plymouth, UK. 170 pp.

Cordes, E.E., D.C. Bergquist, K. Shea, and C.R. Fisher. 2003. High hydrogen sulfide demand of long-lived vestimentiferan tube worm aggregations modifies the chemical environment at deep-sea hydrocarbon seeps. Ecology Letters 6: 212-219.

Cordes, E.E., M.A. Arthur, K. Shea, and C.R. Fisher. 2005. Modeling the mutualistic interactions between tubeworms and microbial consortia. PLoS Biol. 3: e77.

Cordes, E.E., S. Hourdez, B.L. Predmore, M.L. Redding, and C.R. Fisher. In press. Succession of hydrocarbon seep communities associated with the long-lived foundation species *Lamellibrachia luymesi*. Mar. Ecol. Prog. Ser.

Craddock, C., W.R. Hoeh, R.G. Gustafson, R.A. Lutz, J. Hashimoto, and R.J. Vrijenhoek 1995. Evolutionary relationships among deep-sea mytilids (Bivalvia: Mytilidae) from hydrothermal vents and cold water methane sulfide seeps. Mar. Biol. 121(3):477-485

De Beukelaer, S.M., I.R. MacDonald, N.L. Guinnasso, and J. A. Murray. 2003. Distinct side-scan sonar, RADARSAT SAR, and acoustic profiler signatures of gas and oil seeps on the Gulf of Mexico slope. Geo-Marine Letters 23(3-4):177-186.

Dhillon, A., A. Teske, J. Dillon, D.A. Stahl, and M.L. Sogin. 2003. Molecular characterization of sulfate-reducing bacteria in the Guaymas Basin. App. Env. Microbiol. 69: 2765-2772.

Dodge, R.E. and J. Thomson. 1974. The natural radiochemical and growth records in contemporary hermatypic corals from the Atlantic to the Caribbean. Earth Planet Sci 36:339-356.

Dodge, R.E. and G.W. Brass. 1984. Skeletal extension, density, and calcification of the reef coral, *Montastrea annularis*: St. Criox, U.S. Virgin Islands. Bull Mar Sci 34:288-307.

Doerries, M.B. and C.L. Van Dover. 2003. Higher-taxon richness as a surrogate for species richness in chemosynthetic communities. Deep-Sea Res. I 50:749-755.

Druffel, E.R.M., L.L. King, R.A. Belastock, and K.O. Buesseler. 1990. Growth rates of a deep-sea coal using ^{210}Pb and other isotopes.

Dunbar, R.B., G.M. Wellington, M.W. Colgan, and P.W. Glynn. 1994. Eastern Pacific sea surface temperature since 1600 A.D.: The ^{18}O record of climate variability in Galapagos corals. Paleoceanography 9:291-315.

Espedal, H. and T. Wahl. 1999. Satellite SAR oil spill detection using wind history information. Int. J. Remote Sensing 20(1):49-65.

Fisher, C.R. 1996. Ecophysiology of primary production at deep-sea vents and seeps. In: Deep-sea and extreme shallow-water habitats: affinities and adaptations. R. Uiblein, J. Ott, and M. Stachowtish (eds.) Biosystematics and Ecology Series 11: 311-334.

Fisher, C.R., J.J. Childress, S.A. Macko, and J.M. Brooks. 1994. Nutritional interactions at Galapagos hydrothermal vents: Inferences from stable carbon and nitrogen isotopes. Mar. Ecol. Prog. Ser. 103: 45-55.

Frazer, T.K., W.J. Lindberg, and G.R. Stanton. 1991. Predation on sand dollars by gray triggerfish, *Ballistes capriscus*, in the northeastern Gulf of Mexico. Bull. Mar. Sci. 48:159-164.

Frederiksen, R., A. Jensen, and H. Westerberg. 1992. The distribution of the scleractinian coral *Lophelia pertusa* around the Faroe Islands and the relation to internal mixing. Sarsia 77:157-171.

Freidwald, A., R. Heinrich, and J. Patzold. 1997. Anatomy of a deep water coral reef mound from Sternsund, west Finnmark, northern Norway: James, N.P. and J.A.D. Clarke Eds. Cool-water carbonates. Society for Sedimentary Geology, Special Volume 56:144-146.

Freytag, J.K., P. Girguis, D.C. Bergquist, J.P. Andras, J.J. Childress, and C.R. Fisher. 2001. Sulfide acquisition by roots of seep tubeworms sustains net chemoautotrophy. Proc. Nat. Acad. Sci. 98; 13408-13413.

Gardiner, S.L. and S. Hourdez. 2003. On the occurrence of the vestimentiferan tube worm Lamellibrachia luymesi van der Land and Nørrevang, 1975 (Annelida: Pogonophora) in hydrocarbon seep communities in the Gulf of Mexico. Proc. Biol. Soc. Wash. 116(2):380-394.

Gilliam, J.F. 1989. Strong effects of a foraging minnow on a stream benthic community. Ecology 70:445-452.

Glover, A.G. and C.R. Smith. 2003. The deep-sea floor ecosystem: current status and prospects of anthropogenic change by the year 2025. Conserv. Biol. 30:219-141.

Heifetz, J. 2002. Coral in Alaska: distribution, abundance, and species associations. Hydrobiologia 471:19-28.

Heikoop, J.M., J.J. Dunn, M.J. Risk, H.P. Schwarcz, T.A. McConnaughey, and I.M. Sandeman. 2000. Separation of kinetic and metabolic isotope effects in carbon-13 records preserved in reef coral skeletons. Geochem Cosmochim Acta 64:975-987.

Helmle, K.P., K.E. Kohler, and R.E. Dodge. 2002. Relative Optical Densitometry and The Coral X-radiograph Densitometry System: Coral*XDS*. Presented Poster, Int. Soc. Reef Studies 2002 European Meeting. Cambridge, England. Sept. 4-7.

Highsmith R.C. 1979. Coral growth rates and environmental control of density banding. J. Exp. Mar. Bio. Ecol. 37:105-125.

Hovland, M. and E. Thomsen. 1997. Cold-water corals- are they hydrocarbon seep related? Mar. Geol. 137:159-164.

Hovland, M., P.B. Mortensen, T. Brattegard, P. Strass, and K. Rokoengen. 1998. Ahermatypic coral banks off mid-Norway: Evidence of a link with seepage of light hydrocarbons. Palaios 13:189-200.

Hudson J.H., E.A. Shinn, R.B. Halley, and B. Lidz. 1976. Sclerochronology: a tool for interpreting past environments. Geology 4:361-364.

Ivany, L.C., W.P. Patterson, and K.C. Lohmann. 2000. Increase in seasonality across the Eocene-Oligocene boundary inferred from fossil otoliths. Nature 407:887-890.Jensen, A., and R. Frederiksen. 1992. The fauna associated with the bank-forming deepwater coral *Lophelia pertusa* (Scleractinia) on the Faroe shelf. Sarsia 77:53-69.

Ivany, L.C., K.C. Lohmann, and W.P. Patterson. 2003. Paleogene temperature history of the U.S. Gulf Coastal Plain inferred from ^{18}O of fossil otoliths, pp. 232-251. In:D.R. Prothero, L.C. Ivany and E.A. Nesbitt Eds. From Greenhouse to Icehouse, The Marine Eocene-Oligocene Transition. Columbia Univ. Press, New York.

Joye, S.B., A. Boetius, B.N. Orcutt, J.P. Montoya, H.N. Schulz, M.J. Erickson, and S.K. Lugo. 2004. The anaerobic oxidation of methane and sulfate reduction in sediments from Gulf of Mexico cold seeps. Chem. Geol. 205: 219–238.

Kennicutt II, M.C., R.H. Green, P. Montagna, and P.F. Roscigno. 1996. Gulf of Mexico Offshore Operations Monitoring Experiment (GOOMEX), Phase I: Sublethal responses to contaminant exposure-introduction and overview. Can. J. Fish. Aquat. Sci. 53:2540-2553.

Knittel, K., A. Boetius, A. Lemke, H. Eilers, K. Lochte, O. Pfannkuche, P. Linke, and R. Amann. 2003. Activity, distribution, and diversity of sulfate reducers and other bacteria in sediments above gas hydrate (Cascadia Margin, OR). Geomicrobiol. J. 20:269-294.

Knittel, K., T. Lösekann, A. Boetius, R. Kort, and R. Amann. 2005. Diversity and distribution of methanotrophic Archaea at cold seeps. Applied and Environmental Microbiology, pp. 467–479.

Kornacki, A.S., J.W. Kendrick and J.L. Berry. 1994. The impact of oil and gas vents and slicks on petroleum exploration in the deepwater Gulf of Mexico. Geo-Marine Letters 14(2/3): 160-169.

Koslow, J.A., K. Gowlett-Jones, J.K. Lowry, T. O'Hara, G.C.B. Poore, and A. Williams. 2001. Seamount benthic macrofauna off southern Tasmania: community structure and impacts of trawling. Mar. Ecol. Prog. Ser. 213:111-125.

Kulm, L.D., E. Suess, J.C. Moore, B. Carson, B.T. Lewis, S.D. Ritger, D.C. Kadko, T.M. Thornburg, R.W. Embley, W.D. Rugh, G.J. Massoth, M.G. Langseth, G.R. Cochrane, and R.L. Scamman, 1986, Oregon subduction zone: Venting, fauna, and carbonates, Science, v. 231, p. 561-566.

Lanoil, B.D., R. Sassen, M.T. La Duc, S.T. Sweet, and K.H. Nealson. 2001. Bacteria and Archaea physically associated with Gulf of Mexico gas hydrates. Appl. Environ. Microbiol. 67(11):5143-5153.

Levin, L.A., W. Ziebis, G.F. Mendoza, V.A. Growney, M.D. Tryon, K.M. Brown, C. Mahn, J.M. Gieskes, and A.E. Rathburn. 2003. Spatial heterogeneity of macrofauna at northern California methane seeps: Influence of sulfide concentration and fluid flow. Mar. Ecol. Prog. Ser. 256: 123–139.

LGL Ecological Research. 1987. Northern Gulf of Mexico Continental Slope Study Technical Volume, Minerals Management Service, Gulf of Mexico OCS Regional Office**:** 632.

Lough, J.M. and D.J. Barnes. 2000. Environmental controls on growth of the massive coral *Porites*. J. Exp. Mar. Bio. Eco. 245:225-243.

Loya, Y. and B. Rinkevich. 1980. Effects of oil pollution on coral reef communities. Mar. Ecol. Prog. Ser. 3: 167-180.

MacAvoy, S.E., R.S. Carney, C.R. Fisher, and S.A. Macko. 2002. Use of chemosynthetic biomass by large, mobile, benthic predators in the Gulf of Mexico. Mar. Ecol. Prog.Ser. 225: 65-78.

MacAvoy, S.E., C.R. Fisher, R.S. Carney, and S.A. Macko. 2005. Nutritional associations among fauna at hydrocarbon seep communities in the Gulf of Mexico. Mar. Ecol. Prog. Ser. 225: 65-78.

MacDonald, I.R., N.L. Guinasso, Jr., S.G. Ackleson, J.F. Amos, R. Duckworth, R. Sassen, and J.M. Brooks. 1993. Natural oil slicks in the Gulf of Mexico visible from space. J. Geophys. Res. 98 C9:16351-16364.

MacDonald, I.R., N.L. Guinasso, Jr., R. Sassen, J.M. Brooks, L. Lee, and K.T. Scott. 1994. Gas hydrate that breaches the sea floor on the continental slope of the Gulf of Mexico. Geology 22:699-702.

MacDonald, I.R., J.F. Reilly Jr., S.E. Best, R. Venkataramaiah, R. Sassen, J. Amos, and N.L. Guinasso, Jr. 1996. A remote-sensing inventory of active oil seeps and chemosynthetic communities in the northern Gulf of Mexico. Hydrocarbon migration and its near-surface expression. D. Schumacher and M. A. Abrams, American Association of Petroleum Geologists. Memoir 66:27-37.

MacDonald, I.R., D. Buthman, W.W. Sager, M.B. Peccini, and N.L. Guinasso Jr. 2000. Pulsed oil discharge from a mud volcano. Geology 28(10):907-910.

MacDonald, I.R., R. Arvidson, R.S. Carney, C. F. Fisher, N.L. Guinasso Jr., S. Joye, P. Montagna, J.W. Morse, D.C. Nelson, E. Powell, W. Sager, R. Sassen, S. Schaeffer, and G.A. Wolff. 2002. Stability and Change in Gulf of Mexico Chemosynthetic Communities: Final Report. New Orleans, LA, U.S. Dept. Interior, Minerals Management Service, Gulf of Mexico OCS Region, Contract 14-35-001-31813

MacDonald, I.R., I. Leifer, R. Sassen, P. Stine, R. Mitchell, and N.L. Guinasso Jr. 2002. Transfer of hydrocarbons from natural seeps to the water column and atmosphere. Geofluids 5: 95-107.

MacDonald, I.R., W.W. Sager,and M.B. Peccin. 2003. Association of gas hydrate and chemosynthetic fauna in mounded bathymetry at mid-slope hydrocarbon seeps: northern Gulf of Mexico. Mar. Geol. 198:133-158.

MacDonald, I.R., G. Bohrmann , E. Escobar , F. Abegg , P. Blanchon , V. Blinova , W. Brückmann, M. Drews , A. Eisenhauer, X. Han , K. Heeschen , F. Meier , C. Mortera , T. Naehr , B. Orcutt , B. Bernard , J. Brooks, and M. de Faragó. 2004. Asphalt volcanism and chemosynthetic life, Campeche Knolls, Gulf of Mexico. Science 304: 999-1002.

MacDonald, I.R., L.C. Bender, M. Vardaro, B. Bernard, and J.R. Brooks. 2005. Thermal and visual time-series at a seafloor gas hydrate deposit on the Gulf of Mexico Slope. Earth and Planetary Science Letters 233:45-59.

Masson, D.G, B.J. Bett, D.S.M. Billett, C.L. Jacobs, A.J. Wheeler, and R.B. Wynn. 2003. The origin of deep-water, coral-topped mounds in the northern Rockall Trough, Northeast Atlantic. Mar. Geol. 194: 159-180.

McConnaughey, T. 1989. ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: I. Patterns. Geochim Cosmochim Acta 53:151-162.

McCulloch, M., S. Fallon, T. Wyndham, E. Hendy, J. Lough, and D. Barnes. 2003. Coral record of increased sediment flux to the inner Great Barrier Reef since European settlement. Nature 421:727-730.

McMullin, E.R., S. Hourdez, S.W. Schaeffer, and C.R. Fisher. 2003. Phylogenetics and biogeography of deep sea vestimentiferan tubeworms and their bacterial symbionts. Symbiosis. 34:1-41.

Mikkelsen, N., H. Erlenkauser, J.S. Killingley, and W.H. Berger. 1982. Norwegian corals: radiocarbon and stable isotopes in *Lophelia pertusa*. Boreas 5:163-171.

Milkov, A.V. and R. Sassen. 2000. Thickness of the gas hydrate stability zone, Gulf of Mexico continental slope. Marine and Petroleum Geology 17:981.

Mills, H. J., C. Hodges, K. Wilson, I.R. MacDonald, and P.A. Sobecky. 2003. Microbial diversity in sediments associated with surface-breaching gas hydrate mounds in the Gulf of Mexico. FEMS Microbiol. Ecol. 46: 39-52.

Mills, H. J., R.J. Martinez, S. Story, and P.A. Sobecky. 2005. Characterization of the microbial community structure in Gulf of Mexico gas hydrates: comparative analysis of DNA- and RNA- derived clone libraries. Applied and Environmental Microbiology 71: 3235-3247.

Mitchell, R., I.R. MacDonald, and K. Kvenvolden. 1999. Estimates of total hydrocarbon seepage into the Gulf of Mexico based on satellite remote sensing images. EOS Supplement 80(49): OS242.

Moore, D.R. and H.R. Bullis. 1960. A deep-water coral reef in the Gulf of Mexico. Bull. Mar. Sci. 10:125-128

Mortensen, P.B., M. Hovland, T. Brattegard, and R. Farestveit. 1995. Deep-water bioherms of the scleractinian coral *Lophelia pertusa* L. at 64^{o} on the Norwegian shelf: structure and associated megafauna. Sarsia. 80:145-158.

Mortensen, P.B. and H.T. Rapp. 1998. Oxygen and carbon isotope ratios related to growth line patterns in ckeletons of *Lophelia pertusa* (L) (Anthozoa, Scleractinia): Implications for determination of linear extension rates. Sarsia 83:433-446.

Nikolaus, R., J.W. Ammerman, and I.R. MacDonald. 2003. Distinct pigmentation and trophic modes in *Beggiatoa* from hydrocarbon seeps in the Gulf of Mexico. Aquat. Micr. Ecol. 32: 85-93.

Nix, E., C.R. Fisher, K.M. Scott, and J. Vodenichar. 1995. Physiological ecology of a mussel with methanotrophic symbionts at three hydrocarbon seep sites in the Gulf of Mexico. Mar. Biol. 122: 605-617.

Olu, K., S. Lance, M. Sibuet, P. Henry, A. Fiala-Medioni, and A. Dinet. 1997. Cold seep communities as indicators of fluid expulsion patterns through mud volcanoes seaward of the Barbados accretionary prism. Deep-Sea Res. 44(5):811-841.

Olu, K., V. Rigaud, A. Fifis, M.C. Fabri, P. Cochonat, H. Ondréas, and M. Sibuet. 2001. Spatial distribution of chemosynthetic fauna from video records and mosaic analysis at a new cold seep site in the Gulf of Guinea. Program and abstracts volume. Second Internatinal Symposium on Deep-sea Hydrothermal Vent Biology. October 8-12, Brest, France. Pp. 208.

Orange, D.L., M.M. Angell, J.R. Brand, J. Thompson, T. Buddin, M. Williams, W. Hart, and W. Berger. 2003. Geological and shallow salt tectonic setting of the Mad Dog and Atlantis fields: Relationship between salt, faults, and seafloor geomorphology: Proceedings of the Offshore Technology conference, Houston, Texas, May 5-8, 2003, OTC 15157, 16 p.

Orphan, V.J., L.T. Taylor, D. Hafenbradl, and E.F. Delong. 2000. Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum reservoirs. App. Env. Microbiol. 66: 700-711.

Parrish, J.D. 1989. Fish communities of interaction shallow water habitats in tropical oceanic regions. Mar. Ecol. Prog. Ser. 58:143-160.

Paull, C.K., B. Hecker, R. Commeau, R.P. Freeman-Lynde, C. Neumann, W.P. Corso, S. Golubic, J.E. Hook, E. Sikes, and J. Curray. 1984. Biological communities at the Florida escarpment resemble hydrothermal vent taxa. Science 226:965-967

Paull, C.K., A.J.T. Jull, L.J. Toolinand, and T. Linick. 1985. Stable isotope evidence for chemosynthesis in an abyssal seep community. Nature 317:709-711

Posey, M.H. and W.G. Ambrose Jr. 1994. Effects of proximity to an offshore hard bottom reef on infaunal abundances. Mar. Biol. 118:745-753.

Reed, J.K. 2002. Comparison of deep-water reefs and lithoherms off southeastern USA. Hydrobiologia 471:57-69.Ricciardi, A. and E. Bourget. 1998. Weight to weight conversion factors for marine benthic invertebrates. Mar. Ecol. Prog. Ser. 163:245-251.

Risk, M.J., J.M. Heikoop, M.G. Snow, and R. Beukens. 2002. Lifespans and growth patterns of two deep-sea corals: *Primnoa resedaeformis* and *Desmophyllum cristagalli*. Hydrobiologia 471:125-131.

Ritger, S., B. and Carson, E. Seuss. 1987. Methane-derived authigenic carbonates formed by subduction-induced pore-water expulsion along the Oregon-Washington margin: GSA Bulletin 98:147-156.

Roberts, H.H. 1996. Surface amplitude data: 3D-seismic for interpretation of sea floor geology (Louisiana slope): Transactions of the Gulf Coast Association of Geological Societies 46:353-362.

Roberts, H.H. 2001. Improved geohazards and benthic habitat evaluations: digital acoustic data with ground truth calibrations. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. OCS Study MMS 2001-050. 116 pp + appendices.

Roberts, H.H. 2001. Fluid and gas expulsion on the northern Gulf of Mexico continental slope: Mud-prone to mineral-prone responses: American Geophysical Union, Geophysical Monograph 124:145-161.

Roberts, H.H., P. Aharon, R. Carney, J. Larkin, and R. Sassen. 1990. Responses to hydrocarbon seeps, Louisiana continental slope: Geo-Marine Letters 10:232-243.

Roberts, H.H., and P Aharon. 1994. Hydrocarbon-derived carbonate buildups of the northern Gulf of Mexico continental slope: A review of submersible investigations, Geo-Marine Letters 14:135-148.

Roberts, H.H., and R. Carney. 1997. Evidence of episodic fluid, gas and sediment venting on the northern Gulf of Mexico continental slope, Economic Geology 92: 863-879.

Roberts, H.H. and J.M. Coleman. (In Preparation). Improving the predictive capability of 3-D seismic surface amplitude data for identifying chemosynthetic communities: Contract no. 143-01-99-CA-30951, task order 17801.

Robinson, C.A., J.M. Bernhard, L.A. Levin, G.F. Mendoza, and J.K. Blanks. 2004. Surficial hydrocarbon seep infauna from the Blake Ridge (Atlantic Ocean, 2150m) and Gulf of mexico (690-2240m). PSZNI Mar. Ecol. 25:313-336.

Rogers, A.D. 1999. The biology of *Lophelia pertusa* (Linnaeus 1758) and other deep-water reef forming corals and impacts from human activities. Int. Rev. Hydrobiol. 844:315-406.

Rowan, M.G., M.P.A. Jackson, and B.D. Trudgill. 1999. Salt-related fault families and fault welds in the northern Gulf of Mexico: AAPG Bulletin 83:1454-1484.

Sager, W.W., C.S. Lee, I.R. MacDonald, and W.W. Schroeder. 1999. High-frequency near-bottom acoustic reflection signatures of hydrocarbon seeps on the northern Gulf of Mexico continental slope. Geo-Mar. Lett. 18:267-276.

Sahling, H., D. Rickert, R.W. Lee, P. Linke, and E. Suess. 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin. Mar. Ecol. Prog. Ser. 231:121–138

Sassen, R., J.M. Brooks, I.R. MacDonald, M.C.Kennicutt, N.L. Guinasso, and A. G. Requejo. 1993. Association of oil seeps and chemosynthetic communities with oil discoveries, upper continental slope, Gulf of Mexico. Trans GCAGS 43:349-355.

Schroeder, W.W. 2002. Observations of Lophelia pertusa and the surficial geology at a deep-water site in the northeastern Gulf of Mexico. Hydrobiologia 471:29-33.

Sharp, Z.D. and T.E. Cerling. 1996. A laser GC-IRMS technique for *in situ* stable isotope analyses of carbonates and phosphates. Geochim. Cosmochim. Acta 60:2909-2916.

Sibuet, M. and K. Olu. 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep-Sea Res. 45:517-567.

Smith, J.E., H.P. Schwarcz, M.J. Risk, T.A. McConnaughey, and N. Keller. 2000. Paleotemperatures from deep-sea corals: overcoming 'vital effects'. Palaios. 15:25-32.

Snelgrove, R.V.R. and C.R. Smith. 2002. A riot of species in an environmental calm. Oceanogr. Mar. Biol. Ann. Rev. 40:311-342.

Southward, E.C. 1988. Development of the gut and segmentation of newly settled stages of *Ridgeia* (Vestimentifera): implications for relationship between Vestimentifera and Pogonophora. J. Mar. Biol. Ass. UK. 68: 465-487.

Stohr, S. and M. Segonzac. 2005. Deep- sea ophiuroids (Echinodermata) from reducing and non-reducing environments in the North Atlantic Ocean. J. Mar. Biol. Ass. U.K. 85:383-402

Suess, E., B. Carson, S. Ritger, J.C. Moore, M. Jones, L.D. Kulm and G. Cochrane, 1985, Biological communities at vent sites along the subduction zones off Oregon, Bulletin of the Biological Society of Washington, v. 6, p. 475-484.

Summerson, H.C. and C.H. Peterson. 1984. Role of predation in organizing benthic communities of a temperate zone seagrass bed. Mar. Ecol. Prog. Ser. 15:63-77.

Swart P.K. 1983. Carbon and oxygen isotope fractionation in scleractinian corals. Earth Sci Rev. 19:51-80.

Thiel, H. and J.A. Koslow, eds. 2001. Managing Risks to Biodiversity and the Environment on the High Sea, Including Tools such as Marine Protected Areas. Scientific Requirements and Legal Aspects, Proceedings of the Expert Workshop held at the International Academy for Nature Conservation, Isle of Vilm Germany, 27 February- 4 March 2001. www.bfn.de/09/090203.htm.

Turnipseed, M., K.E. Knick, R.N. Lipcius, J. Dreyer, and C.L. Van Dover. 2003. Diversity in mussel beds at deep-sea hydrothermal vents and cold seeps. Ecology Letters 6: 518-523.

Turnipseed, M., C.D. Jenkins, and C.L. Van Dover. 2004. Community structure in Florida Escarpment seep and Snake Pit vent mussel beds. Mar. Biol. 145(1):121-132

Tyler, P.A. (ed). 2003. Ecosystems of the deep oceans. Ecosystems of the World Vol. 28. Elsevier, Amsterdam, 569p.

Tyler, P.A., C.R. German, E. Ramirez-Llodra , and C.L. Van Dover. 2003. Understanding the biogeography of chemosynthetic ecosystems. Oceanol. Acta 25:227–241

Van Dover, C.L. 2002a. Community structure of mussel beds at deep-sea hydrothermal vents. Mar. Ecol. Prog. Ser. 230: 137-158.

Van Dover, C.L. 2002b. Trophic relationships among invertebrates at the Kairei hydrothermal vent field (Central Indian Ridge). Mar. Biol. 141:761-772.

Van Dover, CL. 2003. Variation in community structure within hydrothermal vent mussel beds of the East Pacific Rise. Mar. Ecol. Prog. Ser. 253:55-66.

Van Dover, C.L. and B. Fry. 1994. Microorganisms as food resources at deep-sea hydrothermal vents. Limnol. Ocean. 39:51-57.

Van Dover, C.L. and J.L. Trask. 2000. Diversity at deep-sea hydrothermal vent and intertidal mussel beds. Mar. Ecol. Prog. Ser. 195:169-178.

Van Dover, C.L., C.R. German, K.G. Speer, L.M. Parson, and R.C. Vrijenhoek . 2002. Evolution and biogeography of deep-sea vent and seep invertebrates. Science 295:1253-1257

Van Dover, C.L., P. Aharon, J.M. Bernhard, M. Doerries, W. Flickinger, W. Gilhooly, K. Knick, S. Macko, S. Rapoport, C. Ruppel, J. Salerno, R. Seitz, B.K. Sen Gupta, T.M. Shank, M. Turnipseed, R. Vrijenhoek, and E. Watkins. 2003. Blake Ridge methane seeps: characterization of a soft-sediment, chemosynthethically based ecosystem. Deep-Sea Res. 50:281-300

Vardaro, M., I.R. MacDonald, L.C. Bender, and N.L. Guinasso Jr. Accepted. Dynamic biological and physical processes observed at a gas hydrate outcropping on the continental slope of the Gulf of Mexico. Geo-Marine Letters.

von Cosel, R. and Olu K. 1998. Gigantism in Mytilidae. A new *Bathymodiolus* from cold seep areas on the Barbados Accretionary Prism. C. R. Acad. Sci (Ser. 3) 321:655-663.

Voordouw, G., S.M. Armstrong, M.F. Reimer, B. Fouts, A.J. Telang, Y. Shen, and D. Gevertz. 1996. Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate-reducing, fermentative, and sulfide-oxidizing bacteria. App. Env. Microbiol. 62:1623-1629.

Wainright, S.A. 1964. Studies of the mineral phase of coral skeleton. Exp Cell Research, 34:213-230.

Weinstein, M.P. and K.L. Heck. 1979. Ichthyofauna of seagrass meadows along the Caribbean coast of Panama and in the Gulf of Mexico-composition, structure, and community ecology. Mar. Biol. 50:97-107.

Wilson, J.B. 1979. 'Patch' development of the deep-water coral *Lophelia pertusa* (L.) on Rockall Bank. J. Mar. Biol. Ass. U.K. 59:165-177.