Study Title: Benthic Ecology, Trophodynamics, and Ecosystem Connectivity

Task: US Geological Survey Deepwater Program: *Lophelia* II: Continuing Ecological Research on Deep-Sea Corals and Deep Reef Habitats in the Gulf of Mexico.

Study Start Date: 1 April 2008
Study End Date: 30 September 2011
Location: Gainesville, FL

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In cooperation with the Minerals Management Service

Overview and Objectives:

The project goal is to improve understanding of the benthic communities and their associated environment that reside in deep coral ecosystems and how they compare to other hard bottom and soft bottom ecosystems. Specific objectives include:

1. Examining the benthic community structure and function, community succession, and deep-coral environmental characteristics.
2. Describing food webs and trophodynamics, and developing models of energy flow.
3. Assessing the degree of habitat and ecosystem connectivity among deep sea environments including coral, other hard bottom, and soft sediment ecosystems.

Introduction and Background:

Deep-sea hard substrates (e.g., coral ecosystems) are complex, at the landscape level of biodiversity and at the local community level. While these systems may harbor substantial levels of biodiversity (e.g., Baco and Smith 2003), they remain inadequately investigated. Deep coral ecosystems are of interest because the corals are long-lived and are likely vulnerable to human activities including bottom trawling, anchoring, pollution, and offshore oil and gas development. Deep corals and associated fauna may enhance deep-sea habitats through the provision of structure and possibly food for mobile fish and invertebrates. The complex structure of deep corals may be an important factor in enhancing microhabitat diversity (Krieger and Wing 2002; Tissot et al. 2006), possibly promoting benthic diversity. However, the influence of habitat heterogeneity on the structure of the benthic communities, particularly the meio- and macrobenthos (< 1mm in size), is poorly understood. A better understanding of these animals is imperative, as they serve several important ecosystem functions. First, they are an important food source for higher trophic levels such as fish and larger invertebrates. Second, they are bioturbators and aerators of sediments, affecting the cycling of pore-water nutrients and chemicals. Third, they stimulate microbial activity. They may also provide a significant proportion of the faunal diversity in deep-coral habitats.

Very few studies have examined macro and meiobenthic communites associated with coral mounds and environments, and these were primarily confined to the NE Atlantic. Most of the work to date has involved shipboard sampling with minimal *in situ* collections of deep-coral communities. However, these remote collections have proven to be informative. Overall,

diversity of macrofauna (> 500 μm) is higher on coral mounds than in background sediments and several species are exclusive to coral mound environments (Henry and Roberts 2007; Raes and Vanreusel 2006). Several of these fauna were undescribed species (Henry and Roberts 2007; Raes and Vanreusel 2006). Preliminary studies indicate that certain meiofauna have an affinity for coral substrates, in particular on coral degradation zones (Raes and Vanreusel 2006). Dominant feeding groups associated with corals are carnivorous and suspension feeding forms (Henry and Roberts 2007). In addition, there appears to be distinct communities that reside within different microhabitats: the meio-epifaunal community found on live and dead coral fragments were distinct from the meio-infauna residing within the sediments below coral (Raes and Vanreusel 2006). These preliminary results suggest that biogenic structure found in coral and associate habitats promotes certain species and habitat affinities.

Understanding trophodynamics of deep coral ecosystems is in its infancy. Deep corals are suspension feeders, reliant on benthic-pelagic coupling for their food supply (Duineveld et al. 2004). Food supply and availability, swift currents enhancing turbidity, and water temperatures appear to control coral growth and development of deep-coral mounds (Mienis et al. 2007). Preliminary stable isotope data indicate that deep *Lophelia* reefs in the Gulf of Mexico contain complex food webs, encompassing multiple trophic levels (CSA International 2007). It is critical to understand the nature and periodicity of particle flux and the degree of trophic interactions of deep-sea coral communities in order to evaluate ecosystem controls on abundance, biodiversity, and community function.

Purpose and Scope of Study:

This study will focus on understanding benthic community structure, function, and trophodynamics within and among deep water hard bottom communities. In addition, we will examine species affinities for deep-coral environments and address questions of habitat residency. Specific questions of interest include: How do deep coral ecosystems compare to other deep-sea environs in terms of productivity, diversity, and community function? What are the faunal associates, and are they associated with corals because they provide structure, food, or both? By addressing these questions using the approaches outlined below, we will be able to develop an ecosystem model, generalizing the local and landscape connections and energy flow among deep corals and associated habitats.

This research will address these questions by examining connectivity over different spatial and temporal scales:

1. Among habitat types:

Benthic community structure and function, including densities, diversity, and functional groups will be examined in live and dead coral communities and adjacent off-coral mound environments, including soft sediments and other coral ecosystems in the Gulf of Mexico. Environmental characteristics such as organic carbon, particle size, and redox will be determined in each habitat type in order to develop hypotheses regarding which environmental variables control faunal distributions.

In addition, we are interested in understanding recruitment and successional dynamics in natural and disturbed environments. Specific questions include: Where do new recruits in a population originate? When habitat is disturbed, what are the patterns of succession? Specifically, who are

the initial colonists and subsequent recruits? To what degree do species within a coral community self populate and are retained within a system (e.g., Swearer et al. 2002). Understanding successional patterns of these ecosystems will provide insight into their resiliency to disturbance (e.g., increased sediment load, high temperatures, anthropogenic perturbations associated with oil drilling and petroleum extraction activities).

2. Among organisms: food webs and trophodynamics:

We are interested in defining species interactions (food webs) and understanding energy flow around deep coral and hard-bottom environments. What remains unclear is how dependent are coral and associates on seasonal particulate flux vs. *in situ* production. Who are the primary producers and primary and secondary consumers? This component will define trophic linkages and connectivity among deep coral benthos. Our study can be used to develop a conceptual model of energy flow through these systems using traditional diet analysis (see Ross and Nizinski study plan for more details) and natural abundance stable isotopes

3. Among habitats and ecosystems:

This research component will examine connectivity both at the population level and ecosystem level (e.g., with other environments, seeps), and will define ecosystem linkages. Questions include: Who are the habitat residents and transients? Are there organisms that use the deep corals at different life stages (i.e. ontogenetic shifts in habitat use)? This work will focus on understanding how life history strategies may dictate use of coral habitats at different life stages. It is important to understand how habitat use may change over different time scales (weeks, months, years) in order to effectively define and preserve critical habitats of concern.

We plan to examine connectivity over different time scales using natural chemical markers to track animal movements. Specifically, in order to understand habitat use (e.g., coral versus adjacent sediment flat) over short time scales (weeks to months) we will use stable isotopes and other chemical elements to define habitat associations. Three complimentary approaches will be used.

1. Stable isotope values reflect time-integrated diets, with the time interval depending on the metabolic rate of the tissue being measured (Tieszen et al. 1983). For example, animal muscle tissue turns over more slowly than blood, on the order of one month versus a few days (Fry et al. 2003). We will select dominant fish and mobile invertebrate species found in coral ecosystems and compare stable isotope values of tissues that have different turnover rates (digestive gland, muscle = fast, bone, carapace = slow). If the short and long turnover tissues have similar reproducible isotope values, then the animal is likely staying close to the food source over time (e.g., Fry 1981; Fry et al. 2003).
2. If animal movements are local, combinations of markers could distinguish among individuals collected in different areas. However, if the animals move more freely among different habitats (e.g., coral mounds and seeps or adjacent hard bottom), little chemical distinction would be expected. Measuring chemical elements of animal tissues and the environment (e.g., water, sediment) will enable us to understand if animal populations are resident over short time scales.
3. If the animals feed exclusively in coral ecosystems with little movement among adjacent habitats, they should have stable isotope compositions similar to those of

coral ecosystem "residents" those animals that spend their adult life stages in coral environments and restrict their movement over small spatial scales (e.g., Demopoulos et al. in press). Alternatively, if they are consuming a mixture of coral residents and adjacent ecosystem residents, then we would expect that their isotope compositions to fall between these two habitat endmembers.

In addition, we will examine habitat connectivity over longer time scales using isotopes and other chemical elements to track animal movement. Animals record the chemistry of their environment in their hard parts as they grow; in otoliths for fish and shells for molluscs (Thorrold et al. 2001). The microchemistry of these hardparts can be used to trace animal movement as they grow and develop. Stable carbon and nitrogen isotope analysis of these hard parts can be used to examine if animals change their food resources with age (i.e. ontogenetic shifts in diet). Stable oxygen isotope analysis ($\delta^{18}O$, proxy for water temperature changes) can be used to track movement between habitats or water masses with different water temperatures that may occur with age (Killingley and Rex 1985). In this case, $\delta^{18}O$ analysis could reveal if the habitat of pre-settled larval gastropods differed from adult habitat and if the water temperatures were distinct. This is particularly important for larval species that have a planktotrophic larval life history and a distribution dependent on ocean currents. Lastly, an emerging technique is to use chemical signatures of fish otolith and mollusc shells to examine changing habitat use with age (Becker et al. 2005; Thorrold et al. 2001; Zacherl et al. 2003). Because larval and adult life histories are imprinted in the organism, this technique eliminates the problem of collecting elusive larval forms. This method has yet to be tested in deep coral ecosystems but may prove to be useful in understanding larval movement and connectivity as well as defining critical habitat.

Lophelia I:

Lastly, there are sediment samples from Lophelia I that remain to be sorted and species identified. In addition, there are a few isotope samples left to be analyzed and these samples will provide a useful baseline dataset for our current research.

Products/Deliverables:

A brief annual update will be delivered on 1 December of each year, beginning 1 December 2008. This report will include study progress, accomplishments, notable findings, and a list of products delivered or in preparation. A final report will be delivered by 30 September 2011.

In addition, other products from this research will include peer-reviewed publications (e.g., *Deep-Sea Research*, *Limnology and Oceanography*), oral presentations (MMS Information Transfer Meeting, 4th International Deep-Sea Coral Symposium, December 2008, New Zealand, and other national and international conferences), and at least two factsheets that will be intended for scientists, managers, and the public. In addition, a website will be developed to house current information regarding the deep coral program, including the vision, overall goals and objectives for each principal investigator, cruise reports, and research results.

Specific planned products include at least two peer-reviewed publications for components one and two which will examine the benthic community structure, function, and trophodynamics of deep-coral ecosystems. If the pilot study from component 3 proves successful, several publications (~ 2-3) will result from continued elemental analysis and focus on this work as we expand our scope to include additional fish and invertebrate species and sites.

Methods:
We plan to use a total of 24 ship/ROV operations days in late summer or fall of 2008 (exact schedule pending) and at least 12-14 days of ship/ROV (or submersible) operations in summer or fall in each year of 2009 and 2010. Sampling areas in deep-sea coral habitats will range from the central GOM through the West Florida Slope (300-800 m). Two or three sites (e.g., VK 826, Gulf Penn, W. FL slope) will be selected for focused research. *In situ*, quantitative samples of *Lophelia* meio- and macrobenthic communities and their environment will be collected using specialized sampling gear enabling minimal sample loss. We will also use box cores and multicores deployed from surface ships to complement the *in situ* collections. These coring devices will enable larger scale collections at several locations, whereas the in situ collections will be focused on a few study sites. Larger invertebrates and fish will be collected using Otter and Tucker trawls. See Ross' study plan for more details regarding cruise operations. Specific details regarding the methods used for each study component are included below.

1. Benthic community structure and function:

We will collect samples from different habitat types (e.g., live coral stalks, coral rubble) to understand how habitat heterogeneity may structure benthic communities. Specifically, collections of live and dead coral fragments and sediment will be used to quantify epifaunal and infaunal densities, diversity, and biomass. These collections will target macrofauna (> 300 μm) and meiofauna (> 45 μm) communities. In addition, animals collected will be analyzed for stable carbon and nitrogen isotopes. Additional collections for benthic environmental characterization include sediment organic carbon, nitrogen, particle size, redox, pore-water sulfide, and sulfate.

Meio- and macrofaunal diversity will be examined with Biodiversity Pro and PRIMER Statistical Software (Clarke and Warwick 2001; McAleece et al. 1999) using number of species (S), normalized species richness per core ($d = S-1/\ln N$, where N= number of individuals), Shannon-Weiner Information index (H'; log base 2), evenness (J') per core, Fishers α and rarefaction. Similarities and differences in benthic communities will examined using non-metric multidimensional scaling (MDS), based on Bray-Curtis similarity indices. Pairwise comparisons will be made between live coral and other habitats using Analysis of Similarity (ANOSIM) and similarity percentages (SIMPER) will determine the percent dissimilarity/similarity and the taxa responsible for differences between groups.

This component also outlines the plan for years beyond FY08, including a colonization experiment to examine recruitment and succession over different time scales (months to years). We plan to place colonization substrates in representative habitats, including on benthic landers, and collect over time. Sampling will be conducted in *Lophelia* communities, other hard substrates, and soft sediment communities along same depth contour. Benthic landers will quantify various oceanographic parameters (temperature, salinity, currents, particle flux) enabling understanding of the environmental conditions in which various larval forms settle.

2. Trophodynamics in deep coral ecosystems:

Sample collection and preparation

Net tows and sediment traps will be used to collect primary producers for isotope analysis. In addition, various methods will be used to collect fish and larger invertebrates for this study component (see Ross and Nizinski for specific methods on collections). Surface sediments will also be analyzed for isotopes. Coral fragments and sediment collections will be sorted live, identified for meiofauna and macrofauna, and then analyzed for stable carbon, nitrogen, and

sulfur isotopes (Demopoulos et al. 2007). In addition, muscle and bone or carapace from certain fish and invertebrates will be analyzed for stable isotopes to address the question of habitat connectivity. We will characterize deep-coral benthic food webs, and, by collaborating with Ross and Nizinski, scale up to include higher trophic levels; primarily sessile and mobile benthic invertebrates and fish.

Isotope analysis.

The compositions of ^{13}C, ^{15}N, and ^{34}S in an organism are similar to its food sources, with 0-1 ‰ enrichment for $\delta^{13}C$ and $\delta^{34}S$, and 3-4 ‰ enrichment for $\delta^{15}N$ for each increase in trophic position (McCutchan et al. 2003; Peterson and Fry 1987; Post 2002). Samples will be analyzed for C, N, and S isotope compositions referenced to Vienna PeeDee Belemnite (VPDB), atmospheric N_2, and Vienna Canyon Dioblo Troilite (VCDT) respectively (Peterson and Fry 1987). Analyses will be performed using an elemental analyzer interfaced to a Finnegan MAT Delta-S or Delta-Plus stable isotope ratio mass spectrometer via a Finnigan MAT ConFlo II interface.

3. Habitat and Ecosystem connectivity:

(In collaboration with S. Ross and M. Nizinski).

This component gives details beyond FY08 because if the elemental fingerprinting pilot study is successful, then we will expand to more species and possibly use museum specimens to look at available historical records. We will select fish and molluscs that are well represented at several coral sites enabling sufficient replicate samples. We will extract the otolith and shells and process them for elemental analysis (Becker et al. 2005; Thorrold and Shuttleworth 2000). Recent technological advances enable direct analysis of specific regions of the otolith and shells using laser ablation-inductively coupled mass spectrometry. Briefly, a laser ablates small portions of the shell/otolith and creates a vapor that is transferred to a high resolution mass spectrometer for analysis.

We will analyze the center of the otolith, which represents deposition during the larval stage. The chemistry of this region of the otolith records the chemistry of the nursery habitat (i.e. where the fish spawned). We will also analyze the edge of the otolith, which represents the chemistry of where the fish was collected. By comparing the chemistry of these two regions, we will evaluate whether the nursery habitat and adult habitat are the same. During the pilot phase of this component, we will select isotopes that yield repeatable values and use those for our subsequent comparisons. For molluscs, we will use either the prodissoconch (larval shell of bivalves) and/or the statolith (gastropods) to determine if they can be used as potential tags of location. We will ablate different regions of the shell, which will correspond to different periods of the organism's development. The abundance of each trace element will be expressed as a ratio relative to the amount of calcium in order to control for differences in amount of material analyzed per sample. The resulting elemental ratios will then be analyzed using linear discriminant function analysis (DFA) in order to address our research questions.

Management Implications and Justification:

The USGS has a long-term commitment to assist the Minerals Management Service (MMS) meet their information needs in the Gulf of Mexico. MMS is concerned with preserving and protecting hard-bottom communities as the need for oil and gas exploration increased in the northern Gulf of Mexico. Our research will enhance understanding of the structure and function

of significant biological communities (deep coral ecosystems) and help MMS define and delineate critical habitats in the Gulf of Mexico.

Deliverables and Milestones:
Findings will be presented to resource managers at appropriate meetings and workshops.
FY 2008-Q1

- USGS Deep-Sea Studies Planning Meeting. Gary Brewer and project PIs, Steve Ross, Martha Nizinski, Cheryl Morrison, Chris Kellogg, and Amanda Demopoulos, met at UNCW to discuss Lophelia II science strategy and cruise logistics. UNCW, Wilmington, NC, 23-25 October, 2007.
- USGS and MMS Joint Planning Meeting for Lophelia II Project. Objectives of this meeting were to brief MMS on planned Lophelia II research and to discuss funding, research vessel and platform requirements, and cruise logistics. USGS, Leetown, WV, 31 October, 2007.
- Study plan development-Draft study plans are due December 2007.

FY 2008-Q2

- Lophelia I wrap up
    - Finish isotope samples
    - Sort and ID macrofauna, meiofauna from previous work
    - Resolve any megafauna IDs needed

FY 2008-Q3

- Lophelia I wrap up
    - Draft manuscript detailing the meio and macrofaunal communities residing in deep coral ecosystems: what is there, preliminary diversity study (Note prepared for *Marine Ecology Progress Series* or *Deep-Sea Research*).
- Lophelia II cruise planning and preparation

FY 2008-Q4

- Submit draft Lophelia benthic community manuscript to FSP and subsequently to journal
- Continued Cruise preparation for Lophelia II
- Lophelia II cruise in Gulf of Mexico

References:

Baco AR, Smith CR (2003) High species richness in deep-sea chemoautotrophic whale skeleton communities. Marine Ecology Progress Series 260:109-114

Becker BJ, Fodrie FJ, McMillan PA, Levin LA (2005) Spatial and temporal variation in trace elemental fingerprints of mytilid mussel shells: A precursor to invertebrate larval tracking. Limnology and Oceanography 50:48-61

Clarke KR, Warwick RM (2001) A further biodiversity index applicable to species lists: variation in taxonomic distinctness. Mar. Ecol. Prog. Ser 216:265-278

CSA International I (2007) Characterization of northern Gulf of Mexico deepwater hard bottom communities with emphasis on Lophelia coral. In. U.S. Department of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, p 169 + app.

Demopoulos AWJ, Cormier N, Ewel K, Fry B (in press) Use of multiple chemical tracers to define habitat use of Indo-Pacific mangrove crab, *Scylla serrata* (Decapoda: Portunidae). Estuaries and Coasts

Demopoulos AWJ, Fry B, Smith CR (2007) Food web structure in exotic and native mangroves: A Hawaii-Puerto Rico comparison. Oecologia 153:675-686

Duineveld GCA, Lavaleye MSS, Berghuis EM (2004) Particle flux and food supply to a seamount cold-water coral community (Galicia Bank, NW Spain). Marine Ecology Progress Series 277:13–23

Fry B (1981) Natural stable carbon isotope tag traces Texas shrimp migrations. Fishery Bulletin 79:337-345

Fry B et al. (2003) Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. Estuaries 26:82-97

Henry L, Roberts JM (2007) Biodiversity and ecological composition of macrobenthos on cold-water coral mounds and adjacent off-mound habitat in the bathyal Porcupine Seabight, NE Atlantic. Deep-Sea Research I 54:654-672

Killingley JS, Rex MA (1985) Mode of larval development in some deep-sea gastropods indicated by oxygen-18 values of their carbonate shells. Deep-Sea Research 32:809-819

Krieger KJ, Wing BL (2002) Megafaunal associations with deepwater corals (Primnoa sp.) in the Gulf of Alaska. Hydrobiologia 471:83-90

McAleece N, Lambshead PJD, Paterson GLJ, Gage JD (1999) Biodiversity Pro. A program for analyzing ecological data. Freewater at http://www.nrmc.demon.co.ud/bdpro/.

McCutchan JH, Jr., Lewis WM, Jr., Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378-390

Mienis F, Stigter HCd, White M, Duineveld G, Haas Hd, Weering TCEv (2007) Hydrodynamic controls on cold-water coral growth and carbonate-mound development at the SW and SE Rockall Trough Margin, NE Atlantic Ocean. Deep-Sea Research I 54:1655-1674

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18:293-320

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703-718

Raes M, Vanreusel A (2006) Microhabitat type determines the composition of nematode communities associated with sediment-clogged cold-water coral framework in the Porcupine Seabight (NE Atlantic). Deep-Sea Research I 53:1880-1894

Swearer SE et al. (2002) Evidence of self-recruitment in demersal marine populations. Bull. Mar. Sci. 70:S251-271

Thorrold SR, Latkoczy C, Swart PK, Jones CM (2001) Natal Homing in a Marine Fish Metapopulation. Science 291:297-299

Thorrold SR, Shuttleworth S (2000) In situ analysis of trace elements and isotope ratios in fish otoliths using laser ablation sector field inductively coupled plasma mass spectrometry. Can. J. Fish. Aquat. Sci 57:1232-1242

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32-37

Tissot BN, Yoklavich MM, Love MS, York K, Amend M (2006) Benthic invertebrates that form habitat on deep banks off southern California, with special reference to deep sea coral. Fishery Bulletin 104:167-181

Zacherl DC et al. (2003) Trace elemental fingerprinting of gastropod statoliths to study larval dispersal trajectories. Marine Ecology-Progress Series 248:297-303