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STUDY TITLE: Deepwater Program: Characterization of Northern Gulf of Mexico Deepwater Hard Bottom Communities with Emphasis on *Lophelia* Coral

REPORT TITLE: *Lophelia* Reef Megafaunal Community Structure, Biotopes, Genetics, Microbial Ecology, and Geology (2004-2006)

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KEY WORDS: Gulf of Mexico, *Lophelia pertusa*, deep coral fishes, megafaunal invertebrates, biotopes, submersible, Viosca Knoll, video image analysis, species richness, multivariate analyses, clustering, ordination, deep-sea coral, scleractinian biodiversity, mitochondrial 16S, phylogeny, microsatellites, population structure, dispersal, gene flow, gene expression, cDNA, Hox, ParaHox, DM, calcium L-channel, biology, geology, nitrogen, carbon, stable isotopes, ^{210}Pb dating.

BACKGROUND: There is a rapidly growing social, political, and scientific awareness of deep coral reefs, and the potential loss of unexplored biodiversity by human activities including bottom trawling, anchoring, cable-laying, ocean dumping, pollution, and offshore oil and gas development. To be responsible ecological stewards for deep reefs, research into the ecology of deep reefs is necessary.

OBJECTIVES: The U.S. Geological Survey (USGS) community structure team investigation, together with companion investigations by USGS microbiology and genetics research teams, has addressed gaps in knowledge of *Lophelia pertusa*

(Linnaeus, 1758) deep reef ecosystems in the Gulf of Mexico, with particular emphasis on the fish and mobile invertebrate megafaunas. Basic objectives included:

- Define species composition, diversity, and numerical dominance of fishes and mobile megafaunal invertebrates.

- Define and compare mobile megafaunal, infaunal macrofaunal, and meiofaunal biotope affinities, population densities, biomass, diversity and population dispersion for the *Lophelia* reef biotope, other reef-associated biotopes, and comparative non-reef biotopes.

- Obtain tissue samples for analysis of stable carbon, nitrogen, and sulfur isotopes from taxa forming the *Lophelia* community, as well as comparative taxa forming the overall oceanic food web, surface to substrate.

- Develop variable microsatellite DNA markers for *Lophelia,* and document biodiversity of *Lophelia* and other scleractinian corals using nuclear and mitochondrial DNA markers and appropriate phylogenetic analyses.
- Investigate the physiological state of *Lophelia* by determining the expression of genes related to basic life functions such as growth, differentiation, and reproduction.

- Characterize the microbial community associated with *L. pertusa* and investigate whether the coral-associated microorganisms are acting as symbionts.

DESCRIPTION: Two geological features identified by reference to MMS Lease Blocks Viosca Knoll-826 (VK-826), and Viosca Knoll 906 & 862 (VK-906/862) were extensively surveyed during three research cruises in 2004 and 2005. Twenty submersible dives were conducted, and surface deployed traps and samplers were set and retrieved. The overall scientific team consisted of 28 personnel, with a wide range of specialties.

Eighty-nine sampling stations were conducted over the course of the study, yielding over 100 hours of submersible video imagery, 493 still images, 634 fish specimens from 83 different species, and thousands of invertebrate collections of approximately 90 different species. Sediment, coral, and rock collections were made, and 691 stable isotope samples were taken, as well as multiple fixes of samples for microbial and genetic analysis.

STUDY RESULTS: The demersal fish fauna of *Lophelia pertusa* coral reefs and associated hard-bottom biotopes was investigated at two depth horizons in the northern Gulf of Mexico using a manned submersible and remote sampling. The Viosca Knoll fauna consisted of at least 54 demersal fish species, 38 of which were documented by submersible video. On the 325 m horizon, dominant taxa determined from frame-by-frame video analysis included Stromateidae, Serranidae, Trachichthyidae, Congridae, Scorpaenidae and Gadiformes. On the 500 m horizon, large mobile visual macrocarnivores of families Stromateidae and Serranidae were absent, while a zeiform

microcarnivore assumed importance on reef 'Thicket' biotope, and the open-slope taxa Macrouridae and Squalidae gained in importance. The most consistent faunal groups at both depths included sit-and-wait and hover-and-wait strategists (Scorpaenidae, Congridae, Trachichthyidae), along with generalized mesocarnivores (Gadiformes). The specialized microcarnivore, *Grammicolepis brachiusculus,* appears to be highly associated with *Lophelia* reefs.

Megafaunal invertebrates were quantified by occurrence from high-quality digital video frame grabs using Coral Point Count software. Megafaunal invertebrate assemblages indentified by Primer v6 multivariate analyses of the occurrence data were used to characterize and differentiate key biotopes used by demersal fishes associated with *Lophelia* coral and comparative biotopes. Multivariate analyses fundamentally supported the a priori empirical classification of biotopes on Viosca Knoll, including *Lophelia* coral 'Thicket', 'Rock', 'Plate', 'Plate/Chemo' and 'Open'. In contrast to *Lophelia* reefs from the eastern North Atlantic, coral 'Rubble' biotope was essentially absent. *Lophelia* coral 'Thicket' biotope was extensively developed on the 500 m site. *Lophelia* occurred only sporadically and as individual colonies on the 325 m site. Mixed species oases comprised of *Lophelia*, black corals, sponges and other taxa occurred primarily on the shallower site. In places clusters of individuals of a single species inhabited broad expanses of 'Plate' and 'Rock' biotope. Among hard-substrate and structured biotopes, species richness was highest for 'Rock' biotope, and lowest on *Lophelia* 'Thicket'. Thus, contrary to expectations, *Lophelia* biotope in the northern Gulf of Mexico does not support a richer invertebrate megafaunal assemblage than that found on comparative hard-substrate or soft-substrate biotopes. The height and slope of the rarefaction curve for 'Open' biotope suggested that this inadequately sampled biotope probably supports the highest megafaunal invertebrate species richness, also contrary to expectations.

Geographic patterns of genetic diversity in *Lophelia pertusa* were examined by quantifying genetic diversity present in populations, and assessing levels of genetic differentiation within the Gulf of Mexico (5 sampling locations, <1-290 km apart). Patterns of differentiation observed within Gulf *Lophelia* were compared to *Lophelia* populations from the Southeastern U.S. continental slope (6 sampling locations, 18-990 km apart) and with Europe (5400-7900 km away from sampled U.S. populations). A suite of nine microsatellite markers for Gulf of Mexico *Lophelia* were developed; 190 individuals have been genotyped. The microsatellite markers were highly variable, ranging from 11-53 alleles per locus with an average of 27.4 alleles per locus. Eighteen (9%) individuals with identical multi-locus genotypes were identified as clones. Populations of *Lophelia* harbored substantial genetic diversity. The majority of populations had unique alleles indicative of little gene flow. Pairwise chord distances were high among all populations (0.42 – 0.62), and regional groupings of populations resulted from a neighbor-joining clustering analysis. North versus south areas of Viosca Knoll 826, the most intensively sampled area, had fixation index estimates significantly greater than zero, suggesting little larval mixing. Comparisons of all Gulf *Lophelia* populations with the shallowest site, VK862, produced significant fixation indices. Quantitative estimates of hierarchical gene diversity (AMOVA) indicated significant population structure at every level: between the three regions examined; between Gulf

and southeastern U.S. regions; and within the Gulf and southeastern U.S. regions. Mantel tests identified significant correlations between geographic and genetic distance (an isolation-by-distance pattern) at larger spatial scales, but not within regions. Thus, dispersal of *Lophelia* larvae is generally localized, with occasional long distance dispersal occurring such that some genetic cohesion is retained regionally within the Gulf and Southeastern U.S. Genetic differentiation observed between these regions suggests more restricted gene flow than expected, suggesting that the most effective management plan for *Lophelia* may be regional reserve networks.

Gulf of Mexico deep-sea scleractinian coral biodiversity was put into a phylogenetic framework by comparison of 16S mitochondrial DNA sequences. Four basal lineages were revealed, including the 'complex' and 'robust' corals, the genus *Anthemiphyllia*, plus several species belonging to the family Caryophylliidae. The latter basal coral lineage appears diverse since three Gulf species grouped within this clade. Members of the family Caryophylliidae were not monophyletic, but appeared in six clades; the majority of which were in the 'robust' coral group. The high estimate of genetic distance reported previously between *Lophelia* in different oceanic regions was not supported.

Utilizing polymerase chain reaction, the expression of several gene families was investigated to study the molecular mechanisms functioning in *Lophelia* that are known from studies of other metazoan species to be associated with vegetative growth, division, gamete development, and skeletal biomineralization. Four previously unknown expressed genes were discovered. Expression of these genes were compared in budding (presumably immature) and unitary (presumably mature) polyps. Two members of the *Hox/paraHox* gene family, thought to be associated with segmentation and neuronal development were found to have elevated expression in budding polyps. An L-type calcium channel gene associated with the importation of Ca^{2+} into calcioblastic cells was also more highly expressed in budding as opposed to unitary polyps. In contrast, a *DM*-containing gene, a member of a family of genes notably associated with sexual development and gamete differentiation, was strongly expressed in both budding and unitary polyps. Interestingly, the samples analyzed were taken at a time thought to be the approximate spawning period for *Lophelia pertusa* in the Gulf of Mexico.

The microbial associates of *Lophelia pertusa* in the Gulf of Mexico were characterized. This study included both culture-based and molecular data. We collected the coral samples in individual insulated containers and preserved coral samples at depth in an effort to maintain *in situ* microbial diversity by minimizing contamination and thermal shock. There are a few links between *Lophelia*-associated bacteria and bacteria from shallow-water corals and deep-sea octocorals, but both cultured isolates and clone libraries revealed many novel bacteria associated with *Lophelia*. There are many bacteria and clone sequences that are similar to symbionts of fish, squid, and methane seep clams. In particular, there is a sequence, VKLP1, present in all *Lophelia* colonies analyzed to date (n=6), which is related to a sulfide-oxidizing gill symbiont of a seep clam. This microbe may be a *Lophelia*-specific bacterium and links the coral to cold seep communities. Molecular analysis of bacterial diversity showed a marked

difference between the two sites, Visoca Knoll 906/862 and Visoca Knoll 826. The 16S rRNA bacterial clone libraries from VK826 were dominated by a variety of unknown *Firmicutes*. The dissimilarity between the dominant members of the bacterial communities at these two sites may be evidence of diseased *Lophelia* or thermal stress at one site, or may indicate biogeographical differences. There was no overlap between the bacteria identified in this study and those from a recent study of *Lophelia* in the Mediterranean. This may indicate biogeographical differences, however, it is more likely due to the significant methodological differences in collection, extraction, and analysis of the *Lophelia* samples. No archaea have been detected to date, however, a fungus similar to marine species of *Paecilomyces* and *Acremonium* was found.

Deep-water (307–697 m) antipatharian (black coral) specimens were collected from the southeastern continental slope of the United States and the north-central Gulf of Mexico. The sclerochronology of the specimens indicates that skeletal growth takes place by formation of concentric coeval layers. We used 210Pb to estimate radial growth rate of two specimens, and to establish that they were several centuries old. Bands were delaminated in KOH and analyzed for carbon and nitrogen stable isotopes. Carbon values ranged from -16.4‰ to -15.7‰; the oldest specimen displayed the largest range in values. Nitrogen values ranged from 7.7‰ to 8.6‰. Two specimens from the same location and depth had similar ^{15}N signatures, indicating good reproducibility between specimens.

Incidental collections of live *Lophelia pertusa* fronds, coral rubble, rocks and reef sands enabled an opportunistic group of primarily geological analyses. Radiometric ages of living coral and dead sub-fossil coral were obtained. One substrate rock was analyzed for mineralogy via x-ray diffraction and for stable ^{13}C and ^{18}O isotopic signatures. Gravimetric analyses of specific gravity were undertaken for fresh coral, coral rubble, and rocks. Reef sand collected was analyzed to identify major biotic contributors. Results suggest an age of <400 yrs for contemporary Viosca Knoll *Lophelia* reefs, and of 25.0-26.0 ky for the overall *Lophelia* ecosystem in the northern Gulf of Mexico. This indicates that reefs flourished during the low sea-level stand of the Pleistocene Wisconsonian Glaciation. From the young age of contemporary reefs, relative to the much greater age of sub-fossil *Lophelia*, it may be hypothesized that reef-building has occurred episodically over geological time, a concept raised by Paull et al. (2000), but not further elaborated. Results of analysis of one black substrate rock revealed unexpected goethite mineralogy, whereas methanogenic carbonates had been anticipated in the area of methane seeps. The atypical rock substrate mineralogy, and the exclusive occurrence of well-developed *Lophelia* reefs on Viosca Knoll suggest a uniquely favorable environmental context for reef development on this feature, relative to other similar slope-depth features further to the west. The absence of coral mounds and of extensive rubble fields indicates a distinct difference in the development of *Lophelia* reefs and associated biotopes in the northern Gulf of Mexico, relative to reefs off the southeastern U.S. East Coast, and in the northeastern Atlantic. Soft substrates found on Viosca Knoll may be characterized as biogenic reef sands, comprised predominantly of eroded calcium carbonate shells, spines, and skeletons. Thus,

Lophelia reefs do create a unique sedimentary regime very different from that of the surrounding abiogenic fine sediment of the open slope.

SIGNIFICANT CONCLUSIONS: Fish fauna of *Lophelia pertusa* coral reefs and associated hard-bottom biotopes changes with depth, becoming more depauperate as depth increases. While *Lophelia* has a higher biomass, there is higher species richness of invertebrates on more open substrates than within *Lophelia*. Populations of *Lophelia* harbored substantial genetic diversity, while indicationg little gene flow between populations. In RNA gene expression studies, four previously unknown expressed genes were discovered. Many bacteria similar to symbionts of fish, squid, and methane seep clams were found, one that may be *Lophelia* specific. Individual antipatharian specimens were shown to be several hundred years old, and to incorporate isotopic signatures that varied over time. Radiometric aging of *Lophelia* suggested an age of <400 yrs for contemporary Viosca Knoll *Lophelia* reefs, and of 25.0-26.0 ky for the overall *Lophelia* ecosystem in the northern Gulf of Mexico.

STUDY PRODUCTS: MANUSCRIPTS

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