TITLE: Phylogeography and population genetics structure of the deep-sea coral *Lophelia pertusa*

TASK: *Lophelia* II: Genetics studies of deep corals and associated communities

DESCRIPTION:

Background:
This four-year project builds upon our initial *Lophelia* population genetics and coral phylogeny studies (Study Plan 2100, Task 1.7). Additional sampling for both *Lophelia* population genetics and coral phylogeny will expand the scope of this work through inclusion of new sites and habitat types (such as shipwrecks and oil platforms), allowing refinement of estimates of gene flow and coral biodiversity. Spatial genetic connectivity estimates for Gulf of Mexico (GOM) *Lophelia* will be interpreted in conjunction with newly acquired physical oceanographic data. Connectivity of selected *Lophelia*-associated invertebrates and fishes will be surveyed in a phylogeographic approach using DNA sequences. Further background information on each of these topic areas follows.

The degree of demographic connection between populations has profound implications for the survival, regeneration, and evolution of marine species (Hellberg et al. 2002; Palumbi 2003; Cowen et al. 2007). Resilience to disturbance may be greater if exchange of recruits is common among populations (i.e., the gene pool is homogeneous), creating the chance for re-colonization. Population genetic analyses for GOM *Lophelia* conducted in the initial stage of this work (Task 1.7) did not support a homogeneous gene pool; instead, genetic structuring, or incomplete mixing, was apparent on a smaller scale than expected (e.g., less than two km between areas on VK 826). Gene flow was highly restricted between ocean basins (between the GOM and northwestern Atlantic Ocean (NAO)). Genetic estimates of gene flow will be improved and refined through the addition of samples from the West Florida Shelf and by supplementary sampling of Western Gulf sites such as Mississippi and Green Canyons. Targeting discrete *Lophelia* samples from different areas of the shipwreck Gulfpenn in Mississippi Canyon would help satisfy this goal with the added benefit of known maximum sample age from historical records of the date of the ship sinking. Fine-scale limits of connectivity will be investigated through the genetic analysis of evenly spaced coral samples within sites. The collection and genetic analysis of newly settled larvae on colonization substrates as well as inclusion of physical oceanographic data will enhance estimates of effective gene flow.

Obtaining a classification system that reflects evolutionary history is an important goal towards accurately describing biodiversity. High species diversity and a limited understanding of skeletal characters traditionally used for coral classification make estimation of evolutionary relationships among corals difficult (Romano and Cairns 2000). DNA sequence data provides additional characters for estimating evolutionary relationships. Molecular phylogenies have shown that species of the family Caryophylliidae, containing *Lophelia* and many other deep-sea species, do not fall into one grouping that matches their taxonomic placement in this family (Romano and Cairns 2000; LeGoff-Vitry et al. 2004), but instead are found in both the 'complex' and 'robust' clades. Phylogenetic analysis of DNA sequence data from our initial studies revealed that several caryophylliid species (including GOM species *Tethocyathus* and *Labyrinthocyathus*) comprise an additional, and likely ancient, lineage. Coral species collected during this study will be added to the phylogenetic framework we have developed for deep corals that includes *Lophelia* and other reef-building taxa (e.g., *Enallopsammia, Madrepora*) as well as solitary corals (e.g., *Caryophyllia, Javania*). This

molecular framework allows for rapid assessment of the evolutionary relationships of new species encountered, species identification of juveniles or larvae, and verification of morphological identifications.

Associations between certain macro-invertebrates and fishes have been documented by the USGS research team in both the GOM and NAO, suggesting close ecological ties with deep coral habitats. Samples of several *Lophelia*-associated macro-invertebrates have been collected from nine sites, including the squat lobster *Eumunida picta*, the sea urchin *Echinus*, a eunicid polychaete, and other deep-sea scleractinian coral species. Additional samples of these invertebrates, plus several fish species (hagfish *Eptatretus* n. sp., blackbelly rosefish, coral hake, and conger eel) will be used in a comparative phylogeographic assessment (community genetics). Genetic data (predominantly DNA sequences) will provide an overall estimation of biodiversity and may serve to clarify taxonomic issues, including identification of cryptic species. Genetic comparisons across latitudes, oceans, and evolutionary time frames will provide a unique ecological perspective on broad patterns of linkages (i.e., connectivity) in deep reef areas. Through the comparison of connectivity patterns across species with different life histories, a comprehensive understanding of population dynamics in the *Lophelia* deep reef communities will be obtained and the dominant scales of dispersal important for maintaining reef communities can be estimated. Comparative population genetic studies of other scleractinian coral or octocoral species that differ in larval type (i.e., dispersal potential) will broaden the ecological scope of this work. A good candidate for further population genetic investigation is the cosmopolitan scleractinian coral species *Madrepora oculata,* which differs from *Lophelia* in periodicity of spawning and in fecundity (Waller and Tyler 2005). *Madrepora oculata* samples have been obtained throughout a similar geographic area as our *Lophelia* samples. Additionally, this species has been observed at a deep site in the GOM, Green Canyon 852 (~1400 m). Samples of *M. oculata* were obtained from this site through the Chemo III project (Study Plan 2100, Task 1.2), allowing for genetic comparisons across depth gradients.

Objectives:

Specific objectives for this Task include:
1) Quantification of local and regional patterns of genetic variation in *Lophelia* through the application of microsatellite markers developed in Task 1.7., including assessments of genetic connectivity between natural reefs and manmade structures, further examination of the relative contributions of clonal (asexual) and sexual reproduction, and inferences regarding larval dispersal patterns,
2) Documentation of biodiversity of scleractinian corals using informative nuclear and mitochondrial DNA markers and appropriate phylogenetic analyses,
3) Characterization of levels of connectivity between *Lophelia*-associated mobile fauna (selected invertebrates and fishes),
4) Interpretation of genetics results in relation to environmental variables and patterns of microbial diversity being studied by members of the USGS Deep Coral Ecology Research Team.

Methods:
Four to five submersible/ROV cruises are planned during the first three years of the study with sampling areas ranging from the central GOM through the West Florida Slope (300-800 m). Two or three sites (e.g., VK 826, shipwreck Gulfpenn or similar, W. FL slope) will be selected for focused research. One of these sites, VK 826, has been sampled most extensively, but additional samples from certain areas are needed to provide even sampling. Most new *Lophelia* samples will be obtained from sites not visited previously and when possible, collections will be evenly spaced, allowing for resolution of the fine-scale limits of connectivity. Genetics samples

will be collected in separate containers and will be georeferenced. On deck, samples will be photographed and a sub-sample will be preserved in 95% ethanol or DMSO, with the remaining sample serving as a voucher. The USGS–Leetown Science Center is equipped to perform DNA isolation, amplification (PCR), genotyping using *Lophelia*-specific microsatellite markers, and DNA sequencing. DNA will be extracted at Leetown using the Puregene DNA extraction kit (Gentra Systems Inc., Minneapolis, MN). Amplified DNA fragments will be subjected to capillary electrophoresis on an ABI Prism™ 3100 Genetic Analyzer (Applied Biosystems). Software and expertise are available to assemble DNA sequences, perform phylogenetic analyses, to score microsatellite alleles, and to perform population genetic analyses.

Products/Deliverables:
A brief annual report indicating study progress will be delivered to USGS-BRD headquarters, MMS, and those associated with cruises on 1 December of each year, beginning with 1 December 2008. A final report following MMS Report Guidelines will be delivered by 30 September 2011. Oral presentations will be given upon request at MMS Information Transfer Meetings, and at national and international conferences, such as the 4th International Symposium on Deepsea Coral to be held in Wellington, New Zealand, in December 2008. Technical reports will be prepared for submission to peer-reviewed journals, such as *Molecular Ecology* or *Marine Biology*. Factsheets, presentations, and other educational materials will be prepared that target scientists, managers, and the public. All products will be prepared under the Bureau's guidelines for Fundamental Science Practices and in collaboration with other Lophelia II team members. MMS will be provided review copies of reports and Powerpoint presentations upon request. DNA sequences will be submitted to the public database GenBank.

JUSTIFICATION:
This is Part III of a U.S. Geological Survey (USGS) study in support of collaboration with the FY 2008-2010 competitive procurement titled "*Deepwater Program*: *Lophelia* II: Continuing Ecological Research on Deep-Sea Corals and Deep Reef Habitats in the Gulf of Mexico". The proposed genetics work will expand and complete our assessment of regional gene flow in the Gulf of Mexico (Study Plan 2100, Task 1.7) and add an assessment of gene flow between manmade structures, such as shipwrecks and oil drilling platforms, and natural reef areas. This study component will benefit from added perspective through comparisons with northwestern Atlantic Ocean *Lophelia* populations. *Lophelia* population genetic structure will set the stage for genetic comparisons of *Lophelia*-associated invertebrates, fishes, and microbes, which will provide an integrated view of community structure and the physical factors that may limit connectivity in these poorly studied communities. Taken together, this information can be translated into estimates of sensitivity of different reef areas, allowing refinement of mitigation measures and effective prioritization of areas for avoidance on the Gulf of Mexico continental slope.

References

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