Rasmussen Summer Research Proposal: Delimiting species within deep-sea corals impacted by the 2010 Deepwater Horizon Oil Spill

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Abstract
The Deepwater Horizon (DWH) oil spill was one of the worst environmental disasters in U.S. history. Approximately 800 million liters of crude oil were released into the deep Gulf of Mexico over a 3-month period. This oil combined with 7 million liters of chemical dispersants deployed in surface waters resulted in a cascade of negative impacts to coastal, pelagic, and benthic communities. Among the communities impacted included deepwater coral communities. In the vicinity of the wellhead, numerous deepwater coral colonies were found to be either deceased or were exhibiting signs of stress. Species within the octocoral genus *Paramuricea* were among the most abundant deepwater corals impacted. Similar to corals in shallow waters, *Paramuricea* spp. provide habitat for a diversity of other animals. In fact, these corals are some of the most important foundation species inhabiting deep waters throughout the North Atlantic Ocean. Yet, we cannot clearly distinguish among *Paramuricea* spp.; thus, we presently do not know how many species were impacted by the oil spill. Because of our lack of knowledge of species boundaries, we also do not know the degree of endemism or whether populations expand outside of the Gulf of Mexico. These are critical data for ongoing management and restoration efforts to better understand the recovery potential of species impacted by the spill. In this project, we will use restriction-site associated DNA sequencing (RADSeq) to delimit species of *Paramuricea* collected from the Gulf of Mexico and throughout the broader North Atlantic Ocean. RADSeq is a relatively new method that uses next-generation Illumina Sequencing and bioinformatics to find single nucleotide polymorphisms among samples. A next-generation sequencing approach is necessary to resolve species boundaries within the genus *Paramuricea*. During this project, the summer research student will use a multidisciplinary approach to address research objectives. The student will learn a variety of techniques, including taxonomy, molecular methods, and bioinformatics, while contributing to better informed management decisions of vulnerable, deep-sea species.

Start Date and Location
This project will start June 27, 2016 and will continue for 10 weeks. Research will take place at Harvey Mudd College, in the Olin Science Center.

Proposed Research

Significance and Rationale

On April 20 2010, the Macondo wellhead and Deepwater Horizon (DWH) oil rig exploded in the Gulf of Mexico (GoM), causing a substantial amount of oil to be released in the deep sea. Since that catastrophic event, numerous research efforts have amassed to understand the oil spill's effects on benthic, midwater, surface, and coastal communities. Through these efforts, numerous questions have arisen as to the long-term effects of the DWH oil spill on deep benthic communities. These effects, however, are difficult to quantify, as it remains a particular challenge to study organisms at depth. Because the deep sea remains poorly explored worldwide, we still know very little about deep-sea communities in general. In fact, basic biological and ecological data (e.g., reproductive mode, distribution) are unknown for the majority of species. Although the area in the vicinity of the DWH well-head is now one of the best surveyed areas of the deep seafloor in the world, there has yet to be a complete account of how many (and what) species were negatively impacted because currently available data are insufficient to accurately delimit species in some taxonomic groups. To better understand the recovery potential of species impacted by the DWH oil spill, it is essential to determine what species were impacted. **In this project we will use a genomic approach to delimit species within an enigmatic genus of deep-sea coral that was negatively affected by the DWH incident.**

Deepwater octocorals were among the benthic species most negatively impacted by the DWH oil spill. The DWH oil spill caused substantial damage to deepwater corals at a site in 1370 m depth, 11 km southwest of the spill site (White et al. 2012). Many coral colonies, primarily within the octocoral genus *Paramuricea*, were found to be either dead or partially deceased. The majority (86%) of colonies exhibited signs of stress that included excessive mucus production, retracted polyps, and sloughing tissue. Since the initial discovery of damaged corals in 2010, negative impacts to additional deepwater coral communities dominated by *Paramuricea* were found at distances up to 30 km away from the wellhead (Fisher et al. 2014). Similar to other corals in the deep sea, *Paramuricea* spp. are foundation species, supporting a diversity of fishes and other invertebrates. Population dynamic studies of *Paramuricea* spp. within the northern GoM have revealed that individuals can have extreme longevities (500 years old) and communities are recruitment limited (Doughy et al. 2014, Prouty et al. 2015). Thus, loss of these foundation species would not only cause a collapse in ecosystem functioning, it could take decades to recover due to extreme longevities and slow growth rates. Understanding whether populations in the GoM can recover from the DWH oil spill requires documenting the degree and direction of gene flow among populations. Additionally, it is critical to determine whether species are endemic to the GoM or whether they have distributions that extend into other regions. The level of genetic connectivity among *Paramuricea* populations cannot be determined accurately without a better estimate of species boundaries.

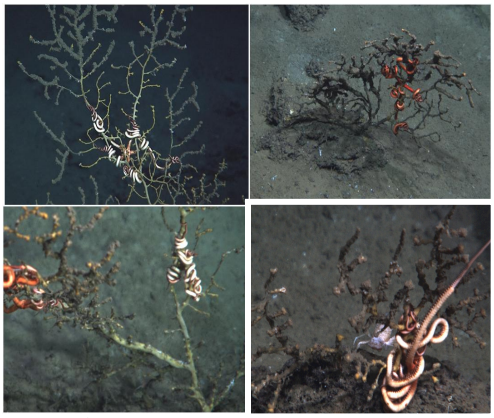

Figure 1. *Paramuricea* colonies covered in brown floc fingerprinted with Macondo Oil. Bare skeleton without living tissue demonstrates recent impacts.

Species boundaries within the genus *Paramuricea* have particularly been difficult to delimit because species definitions have been traditionally based on subtle differences in

morphology (Grasshoff et al. 1977) that are not always obvious. In addition, low genetic divergences (< 0.5% p-distance) at mitochondrial and nuclear rDNA genes exist among these putative species (Doughty et al. 2014), making it difficult to define species using these common genetic markers. The lack of clear species boundaries in *Paramuricea* and other numerous octocoral genera may be a consequence of insufficient molecular data due to the small number of currently available molecular markers and/or lack of clear, diagnostic morphological characters in species taxonomy (McFadden et al. 2010, 2011). Delimiting species boundaries using conventional DNA barcodes (e.g., mtCOI) has been challenging due to slow mitochondrial evolutionary rates (Shearer et al. 2002) and morphological plasticity (Todd 2008, Paz-Garcia et al. 2015). Recent experiments have even shown how environmental conditions can cause an individual to change its morphology to that of another "species" (Paz-Garcia et al. 2015), demonstrating that coral identification based on morphology alone can be misleading.

Phylogenomic methods can help overcome these methodological limitations. One such phylogenomic approach is restriction site associated DNA sequencing (RADSeq). RADSeq entails enzymatic fragmentation of genomic DNA followed by Illumina sequencing of tagged fragments, enabling 100s to 1000s of molecular markers to be generated (Baird et al. 2008). RADSeq has proven particularly useful at resolving recently diverged species across the tree of life, including deep-sea octocorals (Pante et al. 2015, Herrera & Shank in review). In this project, we will use RADSeq coupled with computational approaches to clarify species boundaries in the genus *Paramuricea.* Specifically, we will address whether the current morphologically defined species of *Paramuricea* represent distinct, separately evolving lineages. *Paramuricea* samples previously collected in the GoM as well as other areas of the North Atlantic will be incorporated into analyses. Expanding the focus to include putative species inhabiting other regions is essential to understand species' distributions. Our results can be used by managers to help establish protection measures of deepwater corals in the GoM. Results will also be of interest to numerous coral biologists, as our approach can be applied to other taxonomic groups; thus our efforts will help to unravel the perplexities of octocoral taxonomy.

Methods

The summer research student will extract genomic DNA from species (~16 putative species, 48 total individuals) previously collected in the GoM, off Canada, and off Ireland. S/he will conduct gel electrophoresis to determine quality of DNA, and measure quantity and quality of DNA using a NanoDrop© spectrophotometer and a Qubit© fluorometer. S/he will also use standard DNA barcodes (mtMutS, mtCOI, n28S rDNA) to amplify specimens at three gene regions (following protocols) in order to compare these results to RADSeq data. DNA will be sent to Floragenex, Inc (Portland, OR) for library preparation, where it will be sheared using the *Pst*I enzyme and ligated with adaptors and barcodes. The libraries will be sent to the University of Oregon for sequencing on an Illumina Hi-Seq 3000. After reads are delivered (approx. 3 weeks), the summer student will learn how to de-multiplex the reads, cluster the reads, and find single nucleotide polymorphisms among individuals using the programs STACKS (Catchen et al. 2013) and PyRad (Eaton 2014). Loci containing SNPs will be analyzed using RaxML (Stamatakis 2006) to construct a phylogenetic tree, and Bayesian methods (Yang and Rannala 2010) will be employed to delimit putative species of *Paramuricea.* The student will work closely with A. Quattrini to learn all steps of the bioinformatic pipeline.

Significance of the Project for Environmental Quality

Deep-ocean resources are under increasing threats from anthropogenic stressors. The negative impacts of the DWH oil spill on deepwater coral communities in the Gulf of Mexico is only one example of how human activities can negatively impact organisms in the largest environment on earth. With the additional potential for fishing and mining activities expanding into deep waters, ocean acidification affecting deep-sea corals, and global warming altering food supply to the deep ocean (Ramirez-Llodra et al. 2011), it is clear that human impacts could have detrimental effects on ecosystem function in the deep sea. *Paramuricea* spp. have also been severely affected by additional anthropogenic stressors that extend beyond the DWH; for example, destructive fishing and high temperatures have caused mass mortality events in the Mediterranean Sea (Linares et al. 2005). It is therefore a critical time to understand how and where changing ocean conditions will impact deep-sea biodiversity and how deep-sea organisms respond to and potentially recover from environmental changes. Resiliency from stress depends on several factors, including the amount of genetic diversity within a population and the level of migration (or connectivity) among populations. Yet, these attributes are unknown for the majority of deep-sea species. Defining species and species distributions are critical steps for estimating biodiversity and assessing connectivity. Thus, this project will aid conservation and mitigation efforts in the GoM and in other regions throughout the North Atlantic Ocean.

Educational Value

The student researcher will gain valuable experience in a variety of ecological and evolutionary methods, providing a foundation upon which to cultivate her/his interests in particular topics. The student will use a multi-disciplinary approach to address research objectives in this proposal and will be trained in molecular methods (i.e., DNA extraction, PCR amplification, Gel Electrophoresis), taxonomy, phylogenetic analyses, and bioinformatics. Because the student will learn both relatively new methods and conventional practices, this summer research experience will provide the student a solid background to increase his/her skillset, which can be transferred to other fields beyond ecology and evolution (i.e., cellular and molecular biology, computational biology). Although the library preparation for RadSeq will be outsourced (see budget), the student will prepare the DNA for sequencing and will computationally process the millions of reads generated from the Illumina sequencer. As the fields of bioinformatics and computational biology are in high-demand, gaining these skills while at HMC will increase the competitiveness of the student when applying to graduate programs or other positions after graduation. Finally, the student will have opportunities to improve upon his/her communication skills through journal club meetings in the McFadden lab and through presentation of research at the end of the 10 weeks. Although no student has yet been chosen for this project, we have several HMC students who have expressed interest.

Feasibility

The project start date is set for June 27 2016, as both advisors will be conducting field research until then. Both co-advisors will be available to the student throughout the rest of the summer and have extensive experience in all above-mentioned methods. Two to three weeks will be allotted to extract and prepare the DNA for sequencing. When the DNA is sent to Floragenex for library preparation and sequencing, the student will focus on PCR amplification of additional molecular barcodes (3-4 weeks). After the sequencing reads have been received, s/he will

conduct bioinformatic analyses in the remaining 3-4 weeks of the project. During this time, the summer student will also generate a poster of his/her results.

The McFadden lab has all necessary equipment to conduct DNA extraction and PCR amplification. The HMC server has sufficient computing power and currently contains all of the necessary software programs to run the bioinformatic analyses. We have successfully completed the bioinformatic pipeline and generated a RADSeq dataset for a related group of octocorals.

Budget and Justification

We request a total of **$4,880** to be used for RADseq library preparation and sequencing. The estimated total for RADSeq analysis is $7,880 [48 samples @ $110 per sample = $5,280; Library QC and management = $400; 1 lane Illumina sequencing = $2,200]. We have already received funds from HMC's HHMI SURP to support the student's summer stipend ($5,000 + benefits) and to cover a portion of the cost of RADSeq ($3,000). Fixed costs for library preparation and sequencing mean that 48 samples is the minimum number that is economically feasible to process and sequence at one time. Reducing the number of samples therefore will not reduce total cost, so in the absence of additional funding we will be unable to undertake this project.

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