Genetic connectivity of the gorgonian soft coral *Plexaura flexuosa* (Octocorallia) across the Florida reef tract.

Proposal to Biscayne National Park
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ABSTRACT

Gorgonian octocorals (soft corals) are ecologically important reef invertebrates. They provide shelter and refuge for many reef inhabitants (Jaap 1984) and contribute calcium carbonate to the reef structure (Bayer 1961). Despite their importance, however, little is known about the genetic connectivity of gorgonians across the Florida reef tract, and how this information may benefit conservation efforts. This proposal requests the non-destructive sampling (2cm branch tips) of the soft coral, *Plexaura flexuosa*, at four reef sites (25-60 individuals per site) within the Biscayne National Park (BNP) along its offshore eastern border - Fowey Rocks, Triumph reef, Ajax reef, and Pacific or Elkhorn reefs. The samples will contribute to a population genetic analysis of *P. flexuosa* across the Florida reef tract that will include reefs from the upper and lower Keys and Broward County. Results of this study will produce information about the dispersal potential and population structure of *P. flexuosa*, and will provide important information about connectivity that could be used to supplement conservation and management plans designed to protect the natural resources of BNP's reefs.

OVERVIEW

Recent evolutionary relationships within an individual species across its geographic range are not only valuable to understanding the genetic structuring of marine organisms (Grosberg & Cunningham 2001), but can serve as central information for conservation practices among marine and terrestrial organisms (*e.g.,* Avise *et al.* 1989, Ayre & Hughes 2004). Microsatellites, regions of nuclear DNA consisting of short (2-4bp) repeated nucleotide motifs, have been demonstrated to be a valuable genetic marker for quantifying the population structure and genetic connectivity of reef corals and octocorals (soft corals) (e.g. Gutierrez-Rodriguez & Lasker 2004 and Baums *et al.* 2005). Specifically in octocorals, microsatellites have been useful for a range of species/population-level applications (Chen *et al*. 2002, Liu *et al*. 2005, Costantini *et al*. 2007). For example, Gutierrez-Rodriguez & Lasker (2004) investigated the population dynamics of the shallow-water reef gorgonian octocoral (*Pseudopterogorgia elisabethae)* from the Caribbean using microsatellites. High genetic structuring was found among 18 populations, separated by 12.54–443.65 km, within the Bahamas island chain. Similarly, Baco *et al*. (2006) isolated six highly variable microsatellite loci in the deep-sea octocoral, *Corallium lauuense*, which are currently being utilized to study the genetic connectivity and dispersal *C. lauuense* along the islands and seamounts of the Hawaiian archipelago. No study to date, however, has attempted to examine the genetic connectivity of a Caribbean gorgonian octocoral across the Florida reef tract.

This study will investigate the population dynamics and genetic connectivity of *Plexaura flexuosa*, an ecologically important shallow-water octocoral, from geographic locations that cover the extent of the Florida reef tract. Gorgonians are important to the ecology of reef environments. They provide shelter and refuge to numerous commensal and epibiotic species, ranging from bacteria to fish that are central in the trophic structuring of reef communities (Jaap, 1984). Gorgonians are also important in reef formation by releasing an estimated 1 ton of calcium carbonate per acre as calcitic sclerites that are incorporated into the reef mass (Bayer, 1961). Yet, given the importance and pervasiveness of octocorals on Florida's reefs, little is known about their reproductive dispersal potential and how well reefs are connected genetically. It is, therefore, important to not only protect reefs from degradation, but to understand the fundamental processes governing the structuring of reef populations in order to make informed

decisions about their conservation.

Biscayne National Park (BNP) covers the upper extent of the Florida reef tract that includes the reefs offshore of Elliot Key and reef communities at the northeastern end of the Park at Fowey Rocks. *P. flexuosa* is a common gorgonian species found ubiquitously among Florida's reefs and hard bottom environments, and represents a good model species for studying the population dynamics of gorgonians on Florida's reefs. This proposal requests the non-destructive collection of small, 2cm branch tip clippings of *P. flexuosa* colonies (a benign sampling technique of *in situ* colonies from which the colony will recover within days to weeks, per. obs.). The sampling within BNP will be part of a more extensive sampling of the Florida reef tract that will include sites within the Florida Keys Marine Sanctuary (FKMS) and sites to the north of BNP within the Broward County reef communities. BNP is, thus, a strategically important location in order to establish a continuous and robust sampling design along the Florida reef tract.

Results from this study will provide valuable information regarding the connectivity of *P. flexuosa* among Florida's reefs. Since *P. flexuosa* is a broadcast spawner (egg and sperm fertilized in the water column, and fertilized larvae have a pelagic stage), larvae have the potential to move great distances. The extent to which how well Florida's reefs are genetically connected, however, is not known and recent studies into larval connectivity among reefs suggests that reefs may not be as "open" and genetically connected as traditionally assumed (Cowen *et al.* 2000, 2006). This study will provide genetic data that will help elucidate the degree to which *P. flexuosa* populations are genetically connected, and provide data to help identify reefs that may be acting as sources of larvae to other reefs, and reefs that may be sinks (i.e. depend on other reefs for new recruits into the population). Additionally, assignment tests can identify from which reefs individuals are likely to have migrated.

A better understanding of the genetic connectivity and population dynamics of this species within the reefs of BNP will provide important information regarding the genetic population structuring of its reefs and how they relate to other reefs along the Florida reef tract. Ultimately, this study will provide basic knowledge of recent evolutionary events of *P. flexuosa* regarding intraspecific radiations and dispersal potential, and provide valuable information that can be used to supplement conservation and management designs in order to better safeguard the valuable resources of BNP's reefs.

OBJECTIVES

1) Use microsatellite genetic markers and population genetic theory (e.g. Hardy-Weinberg analyses) to determine the population structure of *P. flexuosa* across the Florida reef tract including four reefs sites within the Biscayne National Park.

2) Utilize assignment tests in order to determine directionality of migrant individuals and to elucidate reefs that may act as source or sink populations.

3) Incorporate population genetic information about *P. flexuosa* into conservation and management plans concerning Florida's reefs, including BNP.

METHODS

Description of study area

The study will involve the collection small (2cm) *P. flexuosa* branch clippings from four reef sites along the eastern boundary of BNP (Figure 1). The reefs were picked in order to encompass a latitudnal gradient along the Park's offshore reef tract system. The four sites include Fowey Rocks, Triumph Reef, Ajax Reef and Pacific or Elkhorn reefs.

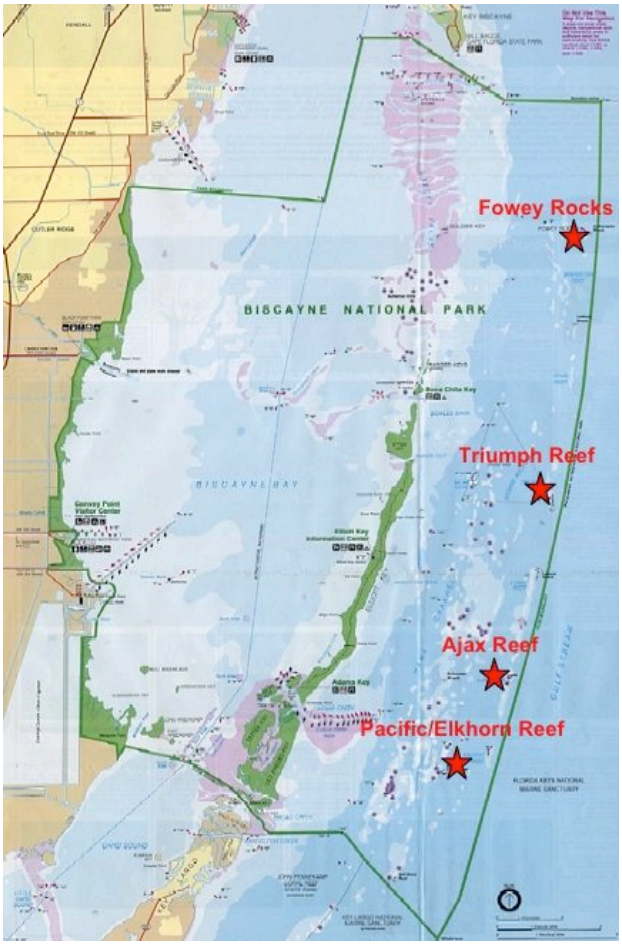

Figure 1. Map of Biscayne National Park (BNP) showing proposed collection sites along the Park's offshore eastern reef tract along a latitudnal gradient. The sites include Fowey Rocks, Triumph reef, Ajax reef and Pacific or Elkhorn reefs.

Procedures/Collections

All *P. flexuosa* samples will be collected using SCUBA. All boats, diving gear, and field supplies will be provided by the PI, Co-PIs at RSMAS (Rosenstiel School of Marine and Atmospheric Science, University of Miami). Boats will be launched at Matheson Hammock Park or Black Point Park. A dive knife or clippers will be used to clip a small 2cm long branch tip from 25-60 colonies at each site depending on their abundance. Twenty-five samples are necessary in order to satisfy a minimum number of individuals for population genetic statistical analyses. The preferred numbers of 50-60 individuals reduce variation and uncertainty, and provide more statistically robust results. Branch clippings will be stored in small (5-10ml) vials filled with 95% ethanol. The samples will be taken to the Coral Reef Conservation Research Laboratory of co-investigator Dr. Andrew Baker at RSMAS where the samples will be processed for genetic analyses. The genomic DNA of each individual will be used for downstream analyses using *P. flexuosa* specific microsatellite markers designed previously by the PI.

It is imperative to emphasize that all of the sampling is non-destructive and will not involve the removal or permanent damage to the soft coral colonies. The 2cm branch clipping is solely for genetic analyses, and the clipped area of the colony will recover and begin to re-grow within days, and within a few weeks the site of the removed clipping will be unnoticeable (pers. obs.). Also, collecting will involve one boat with a team of three personnel – two divers and a boat captain. Each collection trip to each site will only take a few hours, and includes travel time to the collection site by boat, dive time and the return trip. Impact to the park and its visitors will be negligible.

Analysis

All *P. flexuosa* samples collected will be extracted for total genomic DNA and amplified with Polymerase Chain Reaction (PCR) with at least 6 species specific microsatellite markers. Alleles will be scored as PCR products with an automated sequencer (ABI 3730) using ABI dye primer sets and an internal LIZ-500 size standard. Electropherograms will be analyzed using GeneMapper Software 4.0 (Applied Biosystems). Samples will be tested for deviation from Hardy-Weinberg Equilibrium (HWE) and for heterozygote deficiencies for each locus within each population using Genepop (http://wbomed.curtin.edu.au/genepop), and directionality of gene flow will be assessed using genetic exclusion/assignment tests (*e.g.*, GeneClass2).

Schedule

This study is part of the PI's Ph.D. dissertation and use of BNP will only involve the collection of samples. Sampling dates will be in the summer (June – September) of 2009. Exact dates will be dependent on the availability of the boat at RSMAS, weather, and personnel logistics. If all of the sampling is not completed during this preferred time, it will be completed by the end of the summer 2010 (i.e. one year will be sufficient to complete all required sampling with 4-5 individual boat trips). Analysis of the data will occur throughout 2010 and results are planned for completion by the end of 2010 to early 2011. The PI will publish the results of the study in a peer-reviewed scientific journal.

Budget

The collection of samples for this project is funded by the RSMAS Small Boat Fund. This award is for graduate students at RSMAS and supports 7 full boat days to field sites. The cost of processing and storing the samples will be supported by funds from the PI's National Science Foundation (NSF) Science Made Sensible Fellowship.

PRODUCTS

Publications and reports

The PI expects to publish the results of this study in a peer-reviewed scientific journal and is part of his Ph.D. dissertation.

Collections

After analyses are completed, collected samples will be permanently stored as total genomic DNA in a –80C freezer at RSMAS. Any left over tissue will be stored in small (5-10ml) vials of 95% ethanol.

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QUALIFICATIONS

The PI is a Ph.D. candidate in the department of Marine Biology and Fisheries at the Rosenstiel School of Marine and Atmospheric Science, University of Miami and has an M.S. in marine biology from Nova Southeastern University. For more information please see included CV.

SUPPORTING DOCUMENTATION AND SPECIAL CONCERNS

Safety

The only potential hazardous activity that will occur during this study is SCUBA and standard small boat operations.

Access to study sites

Access to study sites will be by boat and will require no more time and activity at the sample sites than a standard dive operation.

Use of mechanized and other equipment

N/A

Chemical use

Small vials containing 5-10ml of 95% ethanol will be brought on board the boat for the storage of samples immediately after their collection.

Ground disturbance

N/A

Animal welfare

N/A

NPS assistance

N/A

Wilderness "minimum requirement" protocols

N/A