Environmental Studies Program: Ongoing Study

Study Area(s): Central GOM

Administered By: GOM OCS Region

Title: Genetic Affinities in Populations of the Invasive IndoPacific Coral *Tubastraea micranthus* on Northern Gulf of Mexico Platforms: Multiple Invasions? (GM-09-01-13)

BOEM Information Need(s) to be Addressed: This study will analyze the invasive species, *Tubastraea microcanthus*, found on oil and gas platforms in the GOM and determine how closely the population is related, if there were multiple introductions, and the direction of spread of this invasive species in the GOM. Platforms may be controversial "incubators" of this species, and an early identification of the point of origin may help to control or eradicate this species before it spreads.

Total BOEM Cost: $310,539 **Period of Performance:** FY 2012–2017

Conducting Organization(s): Louisiana Universities Marine Consortium (LUMCON)

Principal Investigator(s): Name and e-mail

BOEM Contact(s): Nicole Charpentier (nicole.charpentier@boem.gov)

Description:
Background: Before the 1940s, there was little hard substratum in shallow water in the Gulf of Mexico (GOM) between Texas and Florida (Curray, 1965a,b; Frost, 1977; Schroeder et al., 1995; Blum et al., 1998, 2001). Since the 1940s, the US has drilled at least 30,000 wells in the northern GOM. At this time, there are between 3,000 and 3,500 platforms remaining (Francois, 1993). These platforms represent islands of hard substratum not previously present in these waters since the Holocene, 14,000-20,000 years ago. The new substratum being available in the shallow euphotic zone created hard substratum for fauna and flora to settle on, grow, and establish new populations (Gallaway and Lewbel, 1981; Driessen, 1989; Bright et al., 1991; Adams, 1996; Boland, 2002; K. Deslarzes, pers. comm.). Among this new community of organisms were hermatypic (reef-building) Caribbean corals (Bright et al., 1991; Boland, 2002; K. Deslarzes, pers. comm.). Recent data has shown that as many as 12 coral species, both hermatypic (reef-building) and ahermatypic (non-reef-building), have colonized oil/gas platforms in the northern Gulf, extending W-E from the Matagorda Island (MI) to the Main Pass (MP) lease areas, and as far south as the Garden Banks (GB) (Sammarco et al., 2004).

Surveys performed on 48 platforms under the BOEM research program (Sammarco, in press) revealed that average densities of *Tubastraea coccinea* (Cairns and Zibrowius, 1997) reached 28/m2, with percent cover reaching an average of ~50%. This is

extraordinary, since *T. coccinea* is a species invasive to the western Atlantic from the Indo-Pacific. It was first recorded in 1943 in Puerto Rico, then in 1948 in Curacao, where they were collected on ship's hulls (Cairns, 2000). By the late 1990s/early 2000s, the species was reported off Belize and Mexico (Fenner, 1999); Venezuela, northern GOM, and the Florida Keys (Sammarco et al., 2004;); Brazil (Figueira de Paula and Creed, 2004); Colombia, Panama, the Bahamas, and throughout the Lesser and GreaterAntilles (Cairns, 2000; Humann and Deloach, 2002), and the Florida Keys (Shearer, 2008). It is one of the most successful species introductions into the western Atlantic, being the single most abundant scleractinian coral, hermatypic or ahermatypic, in the northern GOM on artificial substrata (Sammarco et al., 2004). It occurs in abundances of hundreds of thousands of colonies per platform (Sammarco et al., 2012, in press). It also occurs on deep banks in the northern GOM but in lower abundance (Schmahl and Hickerson, 2006). There are very few reports of successful invasions of corals in the Atlantic. Another is the accidental introduction of *Fungia scutaria* to Discovery Bay, Jamaica, W.I. (P.W. Sammarco, pers. obs., 1973; J. Lang, pers. comm.; Lajeunesse et al., 2005). There are also reports of other species of *Tubastraea* being introduced into Brazilian waters).

Previous Surveys. Since the late 1990s, PWS led a series of SCUBA and ROV surveys regarding the distribution and abundance of scleractinian corals on a total of 48 platforms throughout the northern GGOM – from Corpus Christi, Texas to Mobile, Alabama, including standing production platforms and some toppled "Rigs-to-Reefs" structures (Sammarco et al., 2004, 2012, in press). An additional 53 platforms were surveyed by S.A. Porter independently. Surveys were conducted in the High Island (HI), West Cameron (WC), and East Cameron (EC), Matagorda Island (MI), Brazos (BZ), Garden Banks (GB), South Timbalier (ST), Ship Shoals (SS), and Main Pass (MP) sectors. Surveys were also performed within the Grand Isle (GI) and ST lease areas (SAP). In addition, some platforms that had been transported to shore for salvage were examined onshore (SAP).

A New Invasive Coral Species for the GOM – Tubastraea micranthus. In his surveys, Porter found a new invasive species for the GOM, and for the Atlantic Ocean— the Indo-Pacific species *Tubastraea micranthus* (ID verified, Smithsonian Inst., Wash., D.C.; Sammarco et al., 2010). This species is a congener of *Tubastraea coccinea* and was observed at a depth of 18-22 m on GI-93 (28°32.96' N, 90°40.11' W) near two major safety fairways, SW of New Orleans, LA. This appeared to be the sole colonization point for this species. We estimate that colonization took place in ~2005. These corals are easily distinguishable from *Tubastraea coccinea* in the field because their color and their growth habit. Their tissue pigmentation is generally dark green or black vs. the bright orange-yellow pigmentation of *T. coccinea* (Humann and DeLoach, 2002). Population growth was evident from expansion of colonies covering ~3 m, to > 30 m covering up to 80% of the substratum within three- years (SAP, pers. obs.). This indicates that the species is viable in these waters and a good competitor for space.

Results from the CMI research program have indicated that *Tubastraea micranthus* colonized 9 out of the 14 platforms surveyed in the region of GI-93- C. The platforms possessing viable populations of this coral are (in order of abundance) GI- 93C, GI-116C,

MC-109A, ST-206A, SP-87D, MC-311A, GI-115A, SP-89B, and GI-90. Some exhibited populations with average densities as high as 15/m2. The average populations have a high growth rate and are spreading steadily. All platforms to the north, east, and south of GI-93-C possessed populations; none were found to the west of GI-93-C, implying that the spread is occurring down-current. The corals were found in waters as shallow as 3-6 m depth and, in some cases, near the seafloor at 132 m. This is a robust species and an effective disperser which may have the potential of spreading throughout the northern GOM and eventually the western Atlantic.

Platforms have been the subject of controversy as potential "incubators" of invasive species. One of the most recent examples of this was colonization by *Didemnum perlucidum*, a highly effective invasive ascidean from the Indo-Pacific (Culbertson and Harper, 2002).

The earlier study on *Tubastraea micranthus* is now confirming the presence and spread of this species at its point of origin in the western Atlantic (Sammarco et al., 2010). At this stage of development, such an introduction may be subject to eradication or at least controllable, if enough is understood about the species. What would make eradication impossible and control difficult is if these populations are not due to a single invasion, but repeated ones through time (Prober and Lunt, 2009). The only mechanism by which to tell if this is the case is to conduct a genetic study of these platform populations. Such would help reveal whether the current spread is derived from a single introduction, or whether it is the result of two or more introductions. Multiple introductions would increase the effectiveness of the original introduction. Historically, conducting population genetic analyses on corals has been very difficult, firstly because of the type of tissue they have, and secondly because they possess symbiotic zooxanthellae (microalgae) with potentially confounding DNA. D.A. Brazeau has developed a technique by which to purify and analyze the DNA, without zooxanthellar contamination. In addition, we have successfully applied population genetic analyses to both juvenile and adult coral populations in the northern GOM and the Bahamas (Brazeau et al., 2005; Atchison et al., 2008; Sammarco et al., 2012). The technique is Amplified Fragment Length Polymorphisms (AFLPs).

Amplified Fragment Length Polymorphisms - AFLP is a DNA-"fingerprinting" technique (Sunnucks, 2000) that detects polymorphisms based upon the selective PCR amplification of numerous restriction fragments. These fragments are generated by two different restriction enzymes (Vos et al., 1995; Mueller and Wolfenbarger, 1999). AFLPs are highly polymorphic but not co-dominantly expressed. AFLPs were originally used primarily in agricultural or commercially valuable species, and until recently, not commonly on animals (Bensch and Akesson, 2005). They have become more popular, however, in recent years and applied to many types of species. They have been used successfully to estimate migration rates (He et al., 2004), species boundaries (Lopez et al., 1999; Fukami et al., 2004), and degree of parental contributions to populations (Van Toai et al., 1997). AFLPs perform quite well for population assignment or allocation studies (Mueller and Wolfenbarger, 1999; Blears et al., 1998; Brazeau et al., 2005, 2008, in press), where the number of polymorphic loci surpasses allelic diversity in

importance (Duchesne and Bernatchez, 2002). In an earlier study (Sammarco et al., 2012), 117 polymorphic markers were generated and utilized.

The analyses used to analyze resultant data and differentiate variable populations are AMOVA (Analysis of Molecular Variance), AFLPOP, and STRUCTURE (see Atchison 2005; Atchison et al., 2006, 2008; Lopez et al., 1999; and others). These techniques can reveal whether these populations have been derived from a single introduction or several. They will also help reveal which population seeded other platforms, and, because corals disperse via planktonic larvae, the direction and extent of the spread from each seeding. To collect, analyze, and interpret such information at this early stage of a species introduction would be valuable to the field of marine ecology.

Objectives:

• Determine whether a single or multiple introductions of this species have occurred in this region;

• Determine how strongly differentiated the populations are on these platforms; that is, how closely or distantly related they are to each other; and

• Determine the geographic pattern of genetic relatedness in this region of the GOM, and the direction(s) of spread.

Methods:

Collection of Coral Tissue. Platforms possessing *Tubastraea micranthus* in the study area are already known, along with their depths of occurrence. Small tissue samples will be collected by hand from *Tubastraea micranthus* from colonies occurring at depths accessible by SCUBA divers - < 37 m. Samples up to 2.5 cm sq will be taken from each colony, using a hammer and small chisel. Supplementary samples will be collected by ROV equipped with lights and a manipulator from depths below this, extending to the bottom of the platform. Individual samples of the coral will be grabbed and traversed to a special basket designed and built at LUMCON. The basket is designed to accept and store samples in isolated acrylic tubes, insuring lack of DNA cross-contamination. The basket will be deployed at depths most appropriate for ROV collection, marked with a strobe for easy location. The tissue samples will be returned to the ship and once on-board, placed in a high-salt DNA preservation buffer for genetic analysis. Tissue will later be isolated, DNA will be pre-processed in Sammarco's laboratory, and samples will be forwarded to the University of New England for detailed molecular analysis.

Genetic Identification, Comparison of Adult Corals. AFLP DNA assays will be used to generate the large number of polymorphic markers necessary to develop population-specific markers for the corals (Atchison, 2005; Brazeau et al., 2005, 2008; Atchison et al., 2008; Sammarco et al., 2012). The AFLP technique has been chosen because: (1) it is based upon the polymerase chain reaction (PCR); only minute amounts of tissue (0.1 to 100 g) are required for analysis; (2) it yields potentially limitless numbers of polymorphic markers; (3) AFLPs employ two PCR amplifications under high stringency conditions, with adapter-specific primers, (4) much time will be saved by not having to

repeat each reaction for each sample; and (5) much of the analytical process will be automated. The DNA fingerprinting will be based upon similarity indices calculated from >100 markers.

Data Analysis. Genetic variation within and among sites will be assessed by one or more of the following analytical techniques: Analysis of Molecular Variance (AMOVA, L. Excoffier, LGB, Univ. Geneva); AFLPOP – an analysis written specifically to analyze AFLP data (Duchesne and Bernatchez, 2002); and/or STRUCTURE. Data will be considered in the geographic context of the GOM using SURFER 8.6, which creates three-dimensional graphics. Univariate data will also be analyzed by standard parametric and non-parametric statistical analyses, using BIOMStat 2.0 & 3.0 and SIMSTAT. Multivariate data will be analyzed via PATN and SAS. 2-D and some additional 3-D graphics will be constructed using SigmaPlot 10. Final graphics will be performed with the aid of MS PowerPoint 2007. Data will be presented in the form of graphs and tables, and data derived from earlier work will be utilized and cross-referenced wherever appropriate.

Data Management, Data Security, and Data Archiving Plan - Data collected in this study will be made available once they are reviewed for quality control and analyzed for patterns. Data will be held on the LUMCON server and made available to the public.

The data will consist of genetic affinity values for platform populations and genetic distances between populations. The data will be analyzed by the above techniques as well as other standard statistical commercially available packs. Data will carry internal codes for identification, with codes being defined in a key; e.g., BOEM-InvasSp-2-MolecGen-GI93C-TM-1 (BOEM Program, 2nd Invasive Species Project, Molecular Genetics Study, Platform #GI-93-C, *Tubastraea micranthus*, Sample #1). Data will be made available online, as well as the key. BOEM will make data available to other researchers and the public, but request the privilege to publish the analyzed data before others do so, as a matter of respect of intellectual property. That is, BOEM request viewers consult the PIs prior to utilizing the data for publication.

At LUMCON, all quantitative data will be logged in EXCEL files. Files will be stored on the main workstation. All data will be backed-up locally on a 1 TB Western Digital GBook external hard-drive, updated daily. The LUMCON computer network is protected by a 3Com Firewall as well as a Panda internet security suite (anti-virus, -malware, software firewall), updated continuously. All accounts are password-protected and secure. In addition, copies of all data files will be forwarded to UNE for archiving.

General. Copies of all images and data files will be archived at LUMCON and UNE. Each file will be annotated with respect to date, time, location, and depth (where appropriate). Data will be made available to general users upon request. Backups will be made prior to shipping of information**.**

Current Status: Data for this study have been collected and analyzed. The report for this study has been drafted by the PI and is currently in the final stages of review. Final Report will be available at the website provided below upon completion of technical review.

Final Report Due: April 2018

Publications Completed: N/A

Affiliated WWW Sites: https://marinecadastre.gov/espis/#/search/study/100026

Revised Date: February 15, 2018