Photo courtesy of Zandy Hillis-Starr

Epidemiological Assessment of Reproductive Condition of ESA Priority Coral

CRCP Project 512 Interim Report

Reproduction is paramount for a populations' survival, however percent live coral cover and species abundance are predominant metrics to assess reef health and determine ESA listing status. Preliminary data from reproductive condition assessments of Acropora palmata indicate that a portion of colonies appearing sound, are in fact sterile or reproductively impaired; thus 'dead' from a population and evolutionary perspective--coral 'zombies'. This epidemiological assessment of reproductive condition of A. palmata will provide understanding of the prevalence of this condition in a geographic context, and in so doing also pinpoint reproductively successful (resilient) populations. This project directly relates to the Acropora Recovery Plan (draft) needs for recovery of the species.

December 2013

Interim Report CRCP Project 512

Epidemiological Assessment of Reproductive Condition of ESA Priority Coral

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Executive Summary

Reproductive failure in Caribbean Acropora has been identified as a critical factor preventing the species from recovery and delisting from their threatened status under the Endangered Species Act (ESA). Preliminary data from previous work in the Caribbean on *Acropora palmata* (elkhorn coral) reproductive conditions indicates that visually intact and 'apparently health' colonies can in fact carry mild to severe reproductive pathologies to the point of reproductive sterility. The fact that these observations are based on a small sample size suggests a much larger problem than previously recognized for this species.

This two year project seeks to provide a Caribbean-wide assessment of the prevalence and severity reproductive pathologies in elkhorn corals and in so doing identify locations with high levels of reproductive failure as well as those that are reproductively successful.

We have surveyed priority sites in the U.S. Caribbean, collecting tissue biopsies reefs across the three jurisdictions (Florida, Puerto Rico and USVI) for determining reproductive condition of *Acropora palmata* populations during July 30-August 5, 2013. This required coordination of multiple teams to simultaneously sample these locations within a 10 day window approximately 3 weeks prior to spawning. This time constraint was necessary in order to capture the gonads at similar stages of development for accurate comparisons during the histological evaluations. A total of 34 sites were sampled at reefs in the upper Florida Keys (4 sites), middle Florida Keys (4 sites), Puerto Rico (including Culebrita and Vieques) (9 sites), St. Thomas USVI (4 sites), St. John USVI (4 sites) and St. Croix USVI (Salt River Bay, 4 sites and Buck Island (5 sites). The target was to collect biopsies from 10 colonies per site. We achieved 95% of this goal with 327 biopsies. Noteworthy is that the 5% missing from our goal was due to three sites all within Puerto Rico that showed indications of degraded sites. Field observations described standing dead colonies, rubble fields of few standing dead and difficulty finding enough live colonies.

The culmination of this project is expected to identify populations that are reproductively viable and indicating a healthy 'resilient' population and those that are impacted and the degree of impact (i.e., from reduced capacity to sterile). This information will be spatially displayed and incorporated into other epidemiological efforts to understand drivers in reproductive success and/or failure. Further this information should serve as an indicator and model for other coral species proposed for ESA listing. This project has integrated resource managers and ESA specialists as part of the team who will be users of the information. The information derived from this work can be used not only to understand drivers of decline, but also populations warranting heightened protection (resilient populations).

This project directly relates to the Acropora Recovery Plan (draft) needs for the recovery of the species. Reproductive failure is a key listing factor for this species. This project addresses knowledge gaps by examining the reproductive potential of elkhorn coral populations, a keystone species providing essential habitat for numerous fish and invertebrates throughout the Caribbean. This knowledge can also help identify locations where further management actions are needed to promote ecosystem and species recovery.

INTRODUCTION

Caribbean coral reefs have undergone a downward spiral in their ability to thrive and reproduce naturally over the last 40 years. For example, Dustan & Halas (1987) reported that coral larval recruitment dropped over 73% (1975 to 1983) at Carysfort Reef in the Florida Keys. At the root of this collapse is an apparent relentless rate of coral mortality and a sustained decrease in juvenile coral recruitment (i.e., reproduction) across multiple coral species. For ESA listed elkhorn and staghorn coral, reproductive failure is recognized as the major impediment to recovery of these species. Data from *Acropora palmata* health assessment studies (2008-2010) (NCCOS- Woodley; Haereticus Environmental Lab-Downs) revealed a surprising prevalence of reproductive pathological conditions in *A. palmata* from the USVI, Guantanamo Naval Station in Cuba and Puerto Rico. The realization that though a reef may have an abundance of large coral colonies, it can be actually 'dead' from a demographic and evolutionary perspective, came from data showing that many corals in our study sites were sterile (no gametes) or their gonads (ova or spermaries) were deformed. These sites also exhibited little to no recruitment of juveniles. In essence, these corals are the 'living dead'—coral zombies. Sterility and the lack of coral recruitment are key indicators that populations with these symptoms are unsustainable and will eventually disappear. The extent of elkhorn sterility was not the focus of our original surveys, but this relatively small sampling suggests a much larger problem than has been previously considered. The goal of this project is to understand the prevalence of 'coral zombies' and related reproductive pathologies in elkhorn corals and identify key locations with high levels of reproductive failure (or success i.e. resilience). Our objectives are (1) to understand the magnitude and spatial distribution of reproductive pathologies of *A. palmata* populations, (2) identify *A. palmata* populations exhibiting reproductive success which is critical information for devising protective management actions to promote recovery of this species, (3) serve as an indicator for other corals under consideration for listing, and (4) identify environmental risk factors that may be causing or contributing to reproductive sterility. This assessment uses standard histopathological techniques to evaluate gonad (ova and spermaries) number and morphology from 1cm biopsies of coral tissue. The outcome of this study will assist recovery efforts of this species as well as identifying areas of reproductive success (resilience) for concerted management protection efforts.

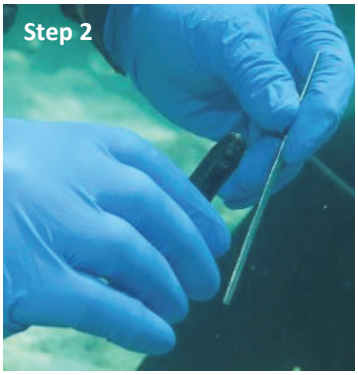
METHODS AND APPROACH

The goal of this project is to understand the prevalence of 'coral zombies' and related reproductive pathologies in *Acropora palmata* (elkhorn corals) and identify key U.S. Caribbean locations with high levels of reproductive failure (or success i.e., resilience). This assessment uses standard histopathological techniques to evaluate gonad (ova and spermaries) number and morphology from 1.4 cm biopsies of coral tissue.

Collection Sites. Samples were collected at 34 reefs within the U.S. Caribbean at each jurisdiction with *A. palmata* (Table 1). This includes the upper and middle Florida Keys, Puerto Rico, and each island of the U.S. Virgin Islands. Maps of each sampling site is shown in Figures 4-12.

Sample Collection. The sampling design required sampling at least 10 individual colonies per site all within a narrow window of time 3-4 weeks prior to the expected spawning date. This timing is critical to optimize gonadal development as well as to be able to make comparisons across all sampling sites. Biopsies were taken from the 'palm' of a branch where reproductive tissues are most abundant and the branch is most supported. An acetone cleaned 1.4 cm round stainless steel leather punch (Fig 1) was tapped into the skeleton using a hammer to a depth just below the tissue line. The divot of tissue was removed from the punch, using a modified dental probe to dislodge the tissue into a polypropylene vial (Fig 2). The divot was then backfilled with an artist clay (Roma plastilina #2) to discourage fouling while the tissue healed (Fig 3). At the surface, the biopsy was transferred into a histological fixative, Z-fix (zinc-formalin 3.7%) which had been diluted with artificial seawater. The samples were then stable at room temperature for transport.

Samples were transported back to the NOAA laboratory in Charleston, South Carolina where the samples currently are being processed for histological analysis. This involves decalcifying the tissue, embedding in paraffin, serial sectioning the tissue and staining with hematoxylin-eosin. Slides will be analyzed using image analysis to measure and quantify gonadal tissues (ova and spermaries) in addition to morphological assessments. These data will be used to develop an epidemiological model to assess reproductive condition of *Acropora palmata* in USVI waters. Data from each site will be synthesized and placed into a geographical representation as the initial steps in the spatial epidemiology of reproductive conditions of *A. palmata*. The outcome of this study will assist recovery efforts of this species as well as identifying areas of reproductive success (resilience) for concerted management protection efforts.



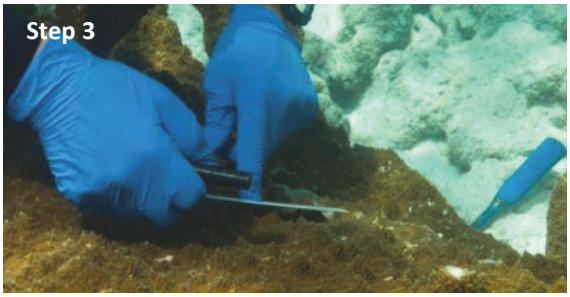


Figure 1 Preparing for coral tissue biopsy sampling. 1- Don gloves; 2- Use 1.5 cm steel leather punch and stainless steel probe; 3- Locate site on colony with supporting base to prevent breaking colony and mature areas suitable for reproduction (avoid growing tips).

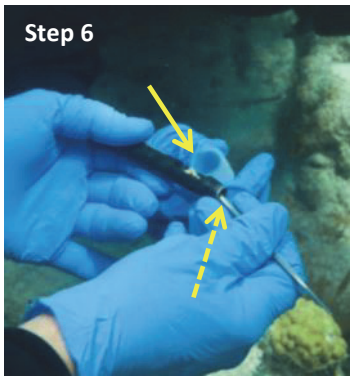


Figure 2 Creating the biopsy. 4- Using a hammer, tap leather punch to a depth approx. 1-2mm below polyp depth; 5-Biopsy should be intact within the punch; 6-Using the probe, gently push the biopsy from the base (skeleton side, dotted arrow) and into the notched opening at the side of the punch and into the collection vessel (solid arrow). This preserves tissue integrity for histology.

Figure 3 Filling biopsy divot to help protect from algal invasion. 7- The artist clay, Roma Plastalina No. 2 is used to backfill the divots that remain after the biopsy has been removed. This practice was recommended by the Florida Keys National Marine Sanctuary for use in their reefs. 8-Remove a small piece of clay from the block, kneading it slightly until pliable. 9-Press the clay into the biopsy divot. 10-Close-up of biopsy divot filled with clay.

RESULTS

Sample collection for this project occurred during July 30-Aug 5, 2013, simultaneously in six locations across the U.S. Caribbean requiring orchestration of multiple within a 10 day window approximately 3 weeks prior to spawning. This time constraint was necessary in order to capture the gonads at similar stages of development for accurate comparisons during the histological evaluations. A total of 34 sites were sampled at reefs in the upper Florida Keys (4 sites), middle Florida Keys (4 sites), Puerto Rico (including Culebrita and Vieques) (9 sites), St. Thomas USVI (4 sites), St. John USVI (4 sites) and St. Croix USVI (Salt River Bay, 4 sites and Buck Island (5 sites) (Table 1). The target was to collect biopsies from 10 colonies per site. We achieved 95% of this goal with 327 biopsies. Noteworthy is that the 5% missing from our goal were due to three sites, all within Puerto Rico that showed indications of degraded sites. Field observations described standing dead colonies, rubble fields of few standing dead and difficulty finding enough live colonies.

TABLE 1 SUMMARY OF U.S. CARIBBEAN *ACROPORA PALMATA* SAMPLED

LOCATION	SITE	# COLONIES SAMPLED	COLLABORATORS
UPPER FLORIDA KEYS	WATSON REEF	10	**MARGARET MILLER**
	HORSESHOE REEF	10	DANA WILLIAMS
	ELBOW REEF	10	ATHENA BURNETT
	MOLASSES REEF	10	
MIDDLE FLORIDA KEYS	SOMBRERO REEF	10	**KATE LUNZ**
	LOOE KEY	10	KEVIN MACAULAY
	ROCK KEY	10	ATHENA BURNETT
	SAND KEY	10	
PUERTO RICO	VEGA BAJA	10	**TOM MOORE**
	RINCON, TRES PALMAS	10	**SEAN GRIFFIN**
	GUANICA (WEST)	10	MICHAEL NEMETH
	GUANICA (EAST)	10	JOHN FAUTH
	VIEQUES	3	KATIE FLYNN
	FAJARDO	10	
	CAYO DIABLO	10	
	LUIS PEÑA	6	
	CULEBRITA	8	
ST. THOMAS, USVI	BOTANY BAY	10	**MARILYN BRANDT**
	INNER BRASS BAY	10	ROBERT BREWER
	HANS LOLICK	10	JEN KISABETH
	FLAT CAY	10	CHERYL WOODLEY
ST. JOHN, USVI	HAWKSNEST	10	**MARILYN BRANDT**
	REEF BAY	10	ROBERT BREWER
	YAWZE POINT	10	JEN KISABETH
	RAMS HEAD	10	CHERYL WOODLEY
ST. CROIX, USVI	BUCK ISLAND SITE 1	10	**ZANDY HILLIS-STARR**
	BUCK ISLAND SITE 2	10	ANNA TOLINE
	BUCK ISLAND SITE 3	10	
	BUCK ISLAND SITE 4	10	
	BUCK ISLAND SITE 5	10	
	SALT RIVER BAY SITE 1	10	
	SALT RIVER BAY SITE 2	10	
	SALT RIVER BAY SITE 3	10	
	SALT RIVER BAY SITE 4	10	
TOTAL SAMPLES		**327**	

FLORIDA SITES

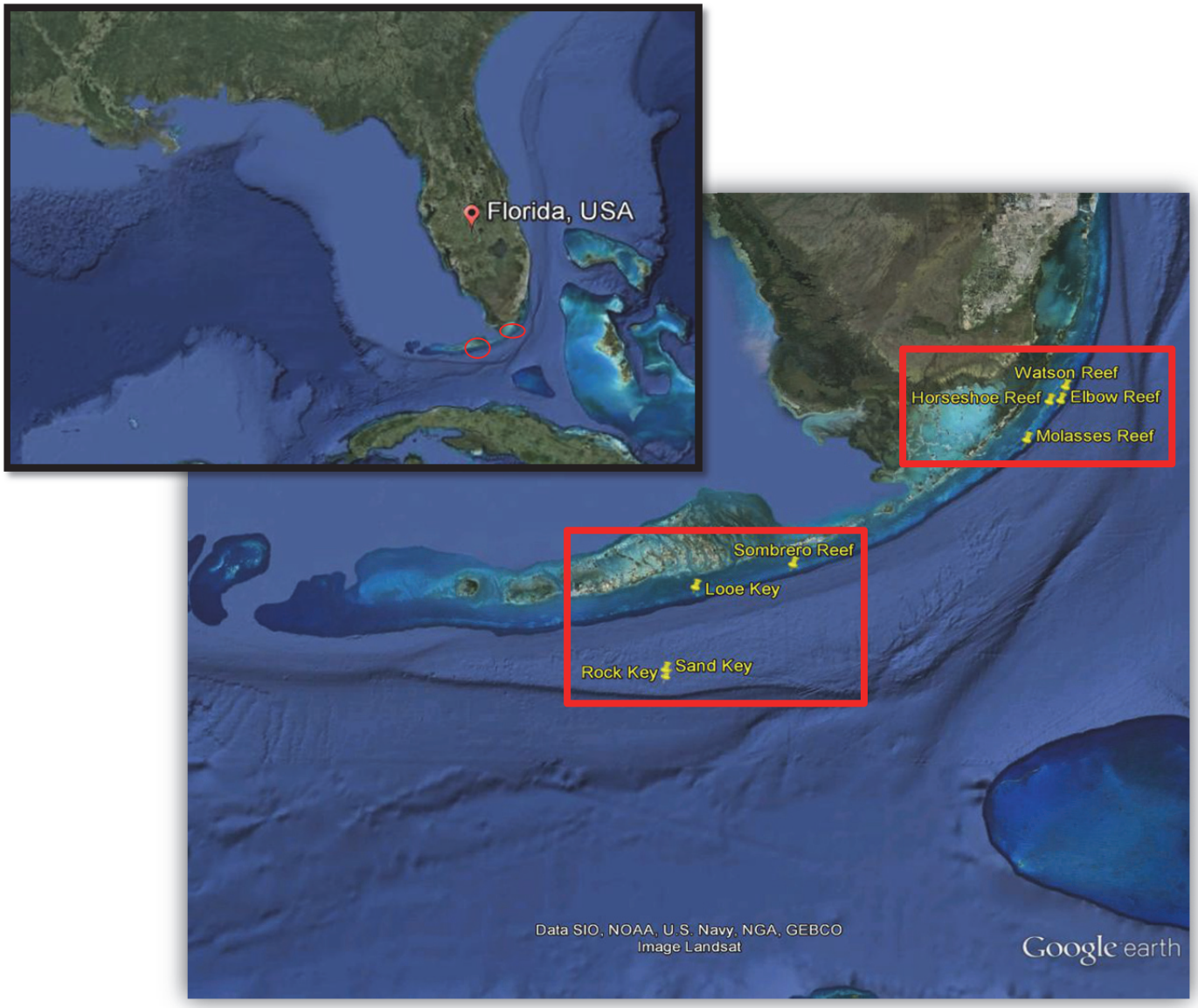


Figure 4 Overview of sampling sites within the Florida Keys.

SITES SAMPLED IN THE UPPER FLORIDA KEYS

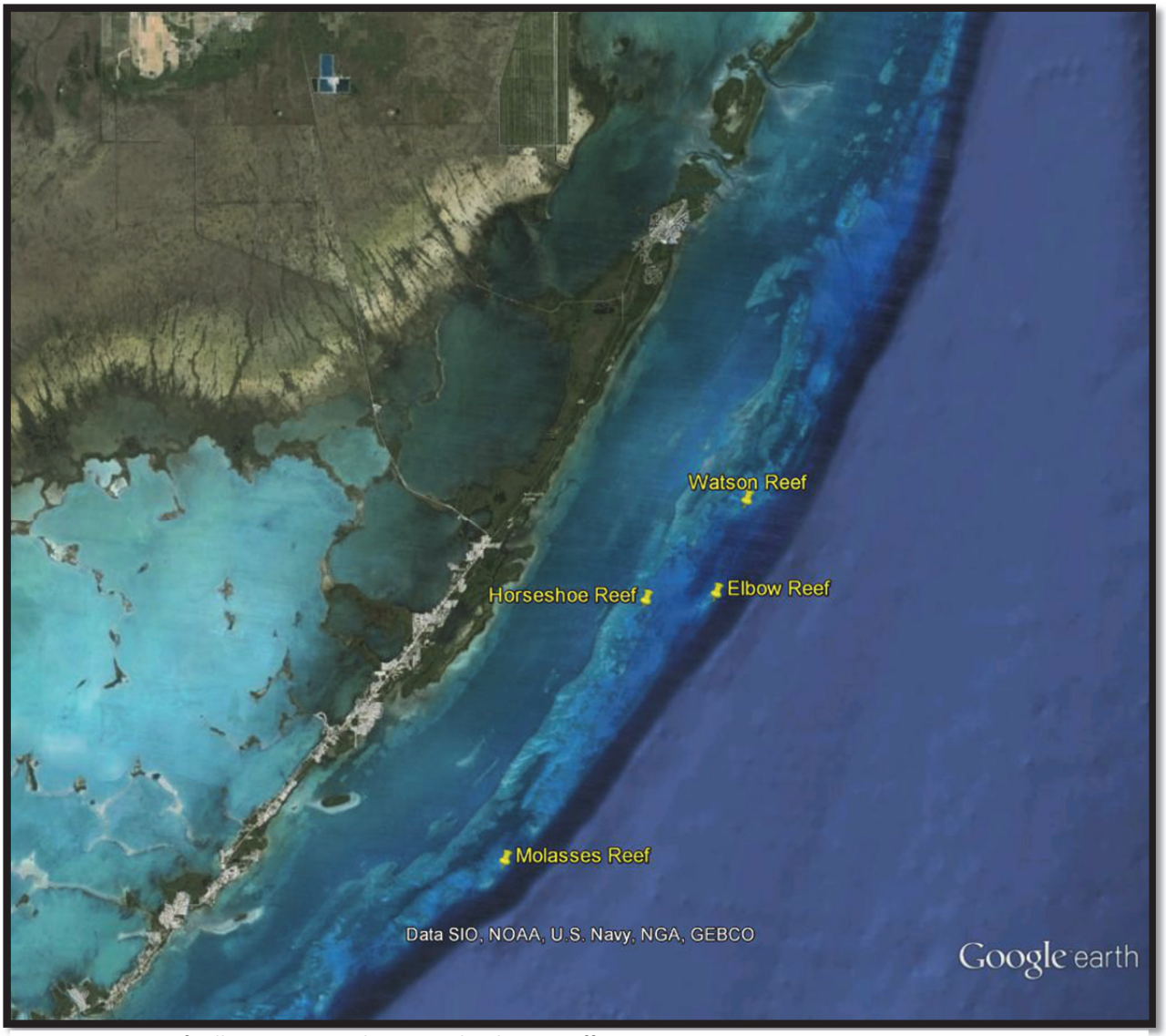


Figure 5 Location of collection sites in the upper Florida Keys, off Key Largo, FL.

SITES SAMPLED IN THE MIDDLE FLORIDA KEYS

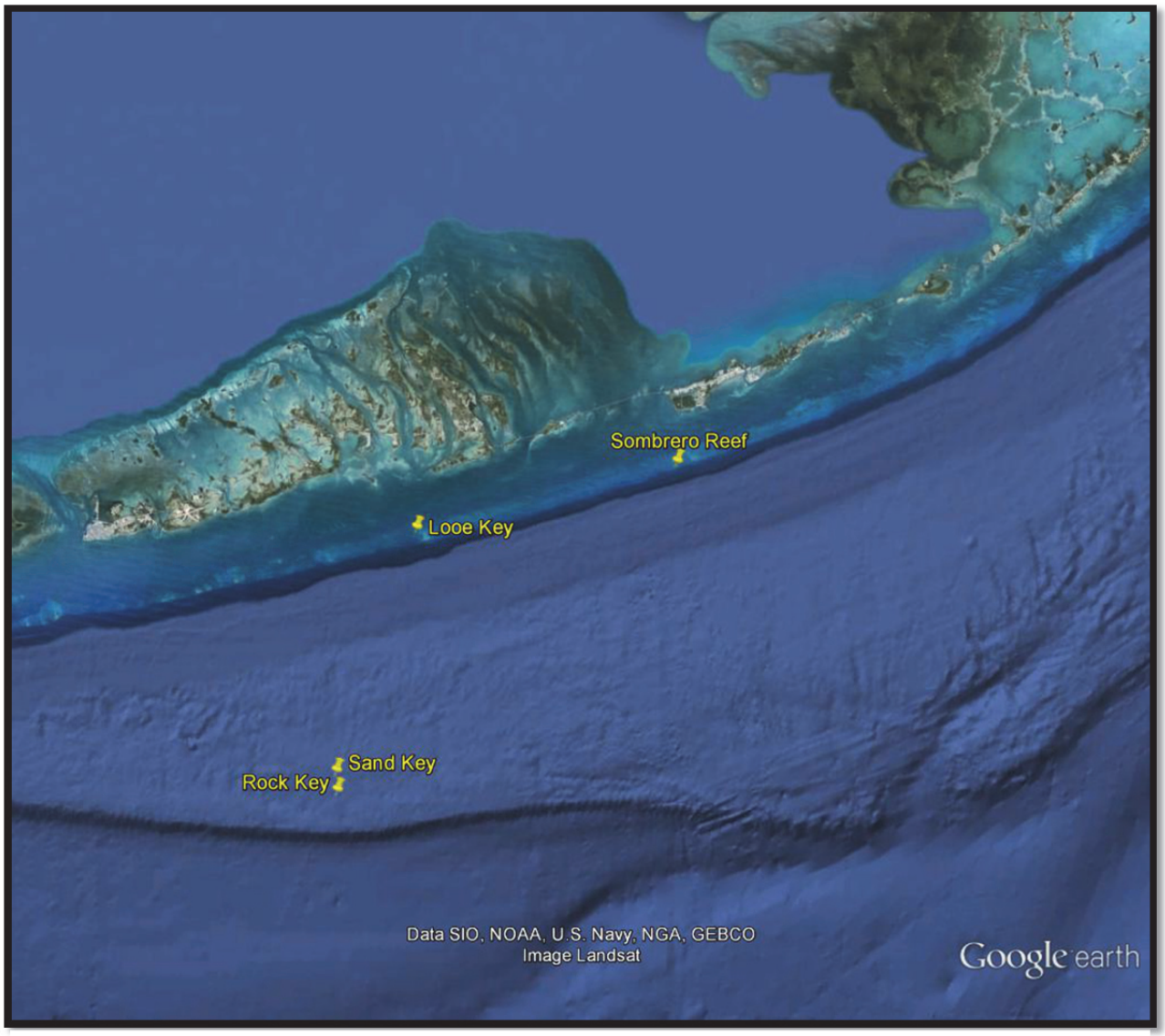


Figure 6 Location of collection sites in the middle Florida Keys, off Marathon, FL.

SITES SAMPLED IN PUERTO RICO

Figure 7 Location of collection sites in Puerto Rico

SITES SAMPLED IN ST. THOMAS USVI

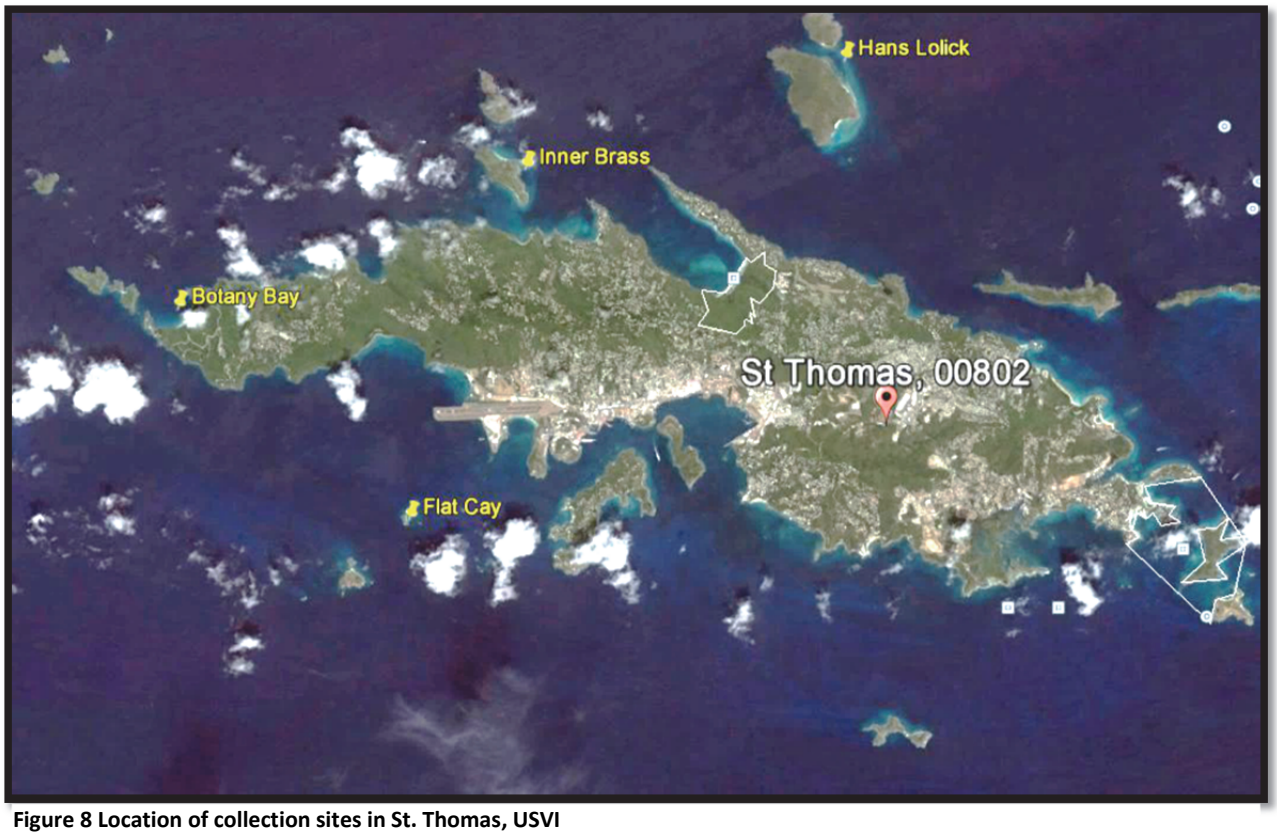


Figure 8 Location of collection sites in St. Thomas, USVI

REEFS OF ST. JOHN USVI SAMPLED

Figure 9 Location of collection sites in St. John, USVI.

REEFS OF ST. CROIX USVI SAMPLED

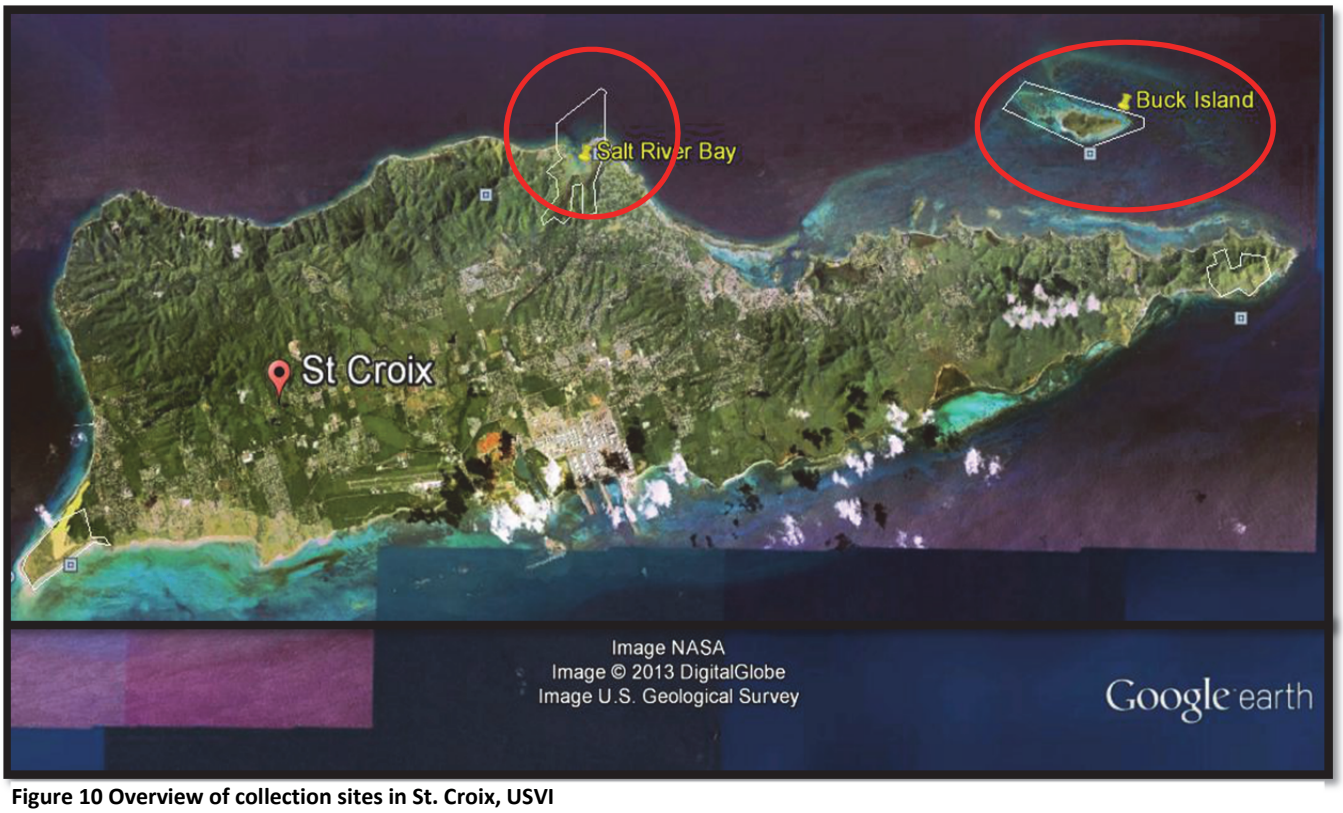


Figure 10 Overview of collection sites in St. Croix, USVI

REEFS OF SALT RIVER BAY NATIONAL HISTORICAL PARK, ST. CROIX USVI

Figure 11 Location of collection sites in Salt River Bay, St. Croix, USVI.

REEFS OF BUCK ISLAND REEF NATIONAL MONUMENT, ST. CROIX USVI

Figure 12 Location of collection sites at Buck Island Reef National Historical Monument, St. Croix, USVI.