Mississippi Canyon 252 Incident

Shallow Coral Tier 1 Plan

Approval of this work plan is for the purposes of obtaining data for the Natural Resource Damage Assessment. Each party reserves its right to produce its own independent interpretation and analysis of any data collected pursuant to this work plan.

This work plan describes the protocols and methodologies that will be employed to obtain baseline, pre-assessment data. A Tier 2 plan will be developed in preparation for a post-exposure assessment of Shallow Water Corals (stony corals that create reef structures in waters 30m or less in depth) of the Gulf region and South Florida, should the need arise. All portions of the overarching Tier 1 plan were developed in concert with each other, resulting in a comprehensive, integrated final plan.

APPROVED:

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Mississippi Canyon 252 Incident Shallow Coral Work Plan and SOPs

Tier 1

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This work plan has three major objectives for documenting baseline conditions for shallow water corals. Each of these objectives essentially has a geographic focus. Partly because of the differences in the ecosystem within each of these geographic regions, each objective relies on its own set of methods to accomplish the objective. In turn, each method focuses on different aspects of the ecosystem and generates unique measurement endpoints. Use of any method in this Tier 1 plan does not constitute a guarantee or imply that the method will be replicated under a Tier 2 work plan.

This work plan makes provision for the future addition of additional objectives and/or methods for characterizing baseline conditions. Potential expansion of Tier 1 efforts will be handled in the form of separate work plans, submitted to the Shallow Water Coral Technical Work Group, which will then become addendums to these overall Tier 1, baseline efforts.

All field teams performing work under this plan will be trained in, and adhere to, NRDA trustee sample handling and chain of custody protocols. All sample handling will be performed under the direction of a trustee agency employee, or their designee. Likewise, all sample containers will be maintained at all times under the chain of custody by a trustee agency employee.

All diving activities involved in this plan will fit the criteria for scientific dives (i.e. "...solely as a necessary part of a scientific...activity by employees whose sole purpose for diving is to perform

scientific research tasks..." 29 CFR 1910.402). All teams involved in diving under this Shallow-water Coral Tier 1 Plan will operate in accordance with institutional diving protocols and under each institution's diving emergency plans (NOAA, NPS, or AAUS {www.aaus.org}). For example, under NOAA operations, each team and operation has qualified and authorized divers involved, has an approved dive plan, has a Dive Accident Management Plan for the specific area of operations, conducts explicit daily dive briefing, and includes on site availability of emergency O2 (NOAA policies and regulations described in detail at http://www.ndc.noaa.gov/pdfs/Dive_regulations_and_policies.pdf). AAUS and National Park Service teams will follow analogous protocols to ensure dive safety as prescribed by their home agency/institutions.

Data Sharing

Each laboratory shall simultaneously deliver raw data, including all necessary metadata, generated as part of this work plan as a Laboratory Analytical Data Package (LADP) to the trustee Data Management Team (DMT), the Louisiana Oil Spill Coordinator's Office (LOSCO) on behalf of the State of Louisiana and to ENTRIX (on behalf of BP). The electronic data deliverable (EDD) spreadsheet with pre-validated analytical results, which is a component of the complete LADP, will also be delivered to the secure FTP drop box maintained by the trustees' Data Management Team (DMT). Any preliminary data distributed to the DMT shall also be distributed to LOSCO and to ENTRIX. Thereafter, the DMT will validate and perform quality assurance/quality control (QA/QC) procedures on the LADP consistent with the authorized Quality Assurance Project Plan, after which time the validated/QA/QC'd data shall be made available to all trustees and ENTRIX. Any questions raised on the validated/QA/QC results shall be handled per the procedures in the Quality Assurance Project Plan and the issue and results shall be distributed to all parties. In the interest of maintaining one consistent data set for use by all parties, only the validated/QA/QC'd data set released by the DMT shall be considered the consensus data set. The LADP shall not be released by the DMT, LOSCO, BP or ENTRIX prior to validation/QA/QC absent a showing of critical operational need. Should any party show a critical operational need for data prior to validation/QA/QC, any released data will be clearly marked "preliminary/unvalidated" and will be made available equally to all trustees and ENTRIX."

Laboratory Analysis

Unless otherwise specified or agreed upon by the Trustees and BP, all samples will be sent to TDI Brooks Lab.

Objective 1: Document baseline/pre-impact condition of shallow-water corals along the Florida Reef Tract.

Background

The Florida Reef Tract is the most extensive living coral reef system in North American waters and the third largest system in the world, extending approximately 530 km from Martin County, on the Atlantic coast, to the Dry Tortugas, west of Key West, in the Gulf of Mexico. Throughout this region shallow–water corals and associated organisms occupy the benthos to a depth of approximately 70 m, although the most significant accumulations of hermatypic reef corals occur within a water depth range of less than a meter to approximately 30 m. Within this region there are a number of state and federally managed marine protected areas that contain corals and coral reef communities within their borders, including the Florida Keys National Marine Sanctuary, Biscayne National Park, Everglades National Park, Dry Tortugas National Park, and numerous National Wildlife Refuges, State Parks, and Aquatic Preserves.

Two species of acroporid corals, Elkhorn Coral (*Acropora palmata*) and Staghorn Coral (*Acropora cervicornis*), are listed as threatened under the Endangered Species Act. Elkhorn coral generally occurs in the shallowest reef habitats from approximately 1 to 5 m depth, although it may occasionally be found in water two or three times this depth. Staghorn Coral may be present from a few meters depth, down to the deepest reaches of the fore reef zone of the offshore bank/barrier.

To appropriately characterize the baseline/pre-impact condition of the corals, it is essential to build upon existing monitoring programs. Several coral reef monitoring programs exist that collect data on the state of coral reefs throughout the Florida Reef Tract. The Coral Reef Evaluation and Monitoring Project (CREMP, including Southeast Florida CREMP [SECREMP]) has monitored the condition of coral reef and hardbottom habitats annually throughout the Florida Keys since 1996, southeast Florida since 2003, and the Dry Tortugas since 2004. It is one of the longest running coral reef monitoring projects in South Florida and has provided important documentation of the temporal changes that have occurred in recent years.

The National Park Service Coral Reef Monitoring Protocol guides the monitoring of coral reefs within four national parks of the South Florida/Caribbean Network (SFCN): Biscayne National Park (BISC), Dry Tortugas National Park (DRTO), Virgin Islands National Park (VIIS) and Buck Island Reef National Monument (BUIS). The Florida Reef Resilience Program (FRRP) is a collaborative effort among managers, scientists, conservation organizations and reef users implementing a disturbance response monitoring program of approximately 52 unique bio-zones throughout the Florida Reef Tract. The purpose of the NOAA Elkhorn Monitoring Program is to document demographic status and trends in remnant populations in the Florida

Keys and determine the relative importance of each potential 'threat' (disease, predation, etc). These programs provide a backdrop on which to overlay additional investigations to document the impact of the Deepwater Horizon oil spill (AKA Mississippi Canyon [MC] 252), should the oil reach these resources.

The intent of Objective 1 of this plan is to undertake two complementary sampling activities: 1) broad scale rapid assessment to document density, size structure, condition and species composition of corals throughout the Florida Reef Tract via the established CREMP, SFCN, and FRRP protocols, and 2) Biopsy sampling of tissues of strategic coral species and sediment collections at sites regularly distributed along the region.

Methods

Method 1: CREMP/SECREMP Monitoring Protocol

Data from CREMP has been instrumental in assessing how different disturbances have changed stony coral cover and altered benthic community composition in the FKNMS during the last 15 years. This includes severe declines after the 1997/1998 El Nino bleaching event, the 2004/2005 hurricanes seasons, and more recently the mortality associated with the winter of 2010. The design of established CREMP sampling protocols have been effective at detecting both short-term (year to year) changes as well as determining long-term trends in benthic community composition. The core field methods for CREMP continue to be the underwater videography of three transects at each sampling station within a sampling site, accompanied by a timed station species inventory which provides data on stony coral species richness. Image analysis from video provides relative estimates of benthic community composition and traditional station species inventories provides information on coral species richness, presence/absence of disease and bleaching, and *Diadema* abundance. In addition to the core field methods, the monitoring of selected benthic species such as *Montastraea annularis* species complex and *Xestospongia muta* has been incorporated to assist management efforts or to improve the project's ability to assess reef condition. The sampling effort proposed for 2010 includes 109 stations at 41 sites in the Florida Keys (17 patch, 12 shallow and 13 deep forereef sites) and 39 stations at 12 sites in the Dry Tortugas.

CREMP sampling site locations were chosen in 1994 using a stratified random sampling procedure according to the EPA Environmental Monitoring and Assessment Program (EMAP). Spatial stratification included four habitat types: nearshore hardbottom, patch, offshore shallow forereef, and offshore deep forereef; in three regions: Upper, Middle, and Lower Keys. Four permanent station markers were installed at 40 sites in 1995. Annual sampling began in 1996, and has continued through 2010. Three sites were installed in the Dry Tortugas and sampling began in 1999.

Prior to sampling in 2001, an analysis of CREMP data indicated that sampling effort could be reduced at the station level without compromising the overall power to detect change in coral community composition. Thus, stations that exhibited high levels of similarity within a site were excluded from subsequent monitoring. The current sampling effort includes 50 sites within the Upper, Middle, Lower Keys, and Dry Tortugas (Table 1). In the Keys, these sites represent 12 shallow and 11 deep spur and groove reef locations, and 15 patch reef locations and contain two to four stations, based upon station reduction and reef size (not all sites are large enough to support four stations). In addition to the three original locations selected in the Dry Tortugas, CREMP, in cooperation with the National Park Service, has added another 9 sites in the Dry Tortugas to monitor specific resource targets. All hardbottom sites were removed from monitoring due to consistently low stony coral cover.

In 2003, CREMP was further expanded to include 10 sites offshore southeast Florida in Miami-Dade, Broward, and Palm Beach counties. The project was expanded again in 2006 with the establishment of three sites in Martin County. In 2010, an additional four sites were added (see Table 1). This CREMP expansion, named the Southeast Florida Coral Reef Evaluation and Monitoring Project (SECREMP), is filling gaps in coverage of knowledge and monitoring of coral reef ecosystems in Florida and nationwide. (Unless specifically noted, CREMP herein refers to both CREMP and SECREMP). In 2010, the CREMP sites in the Middle Keys were sampled May 16 – 27. The CREMP sites in the Dry Tortugas are planned for June 6 -16, 2010; Lower Keys sites are planned for June 21 – 31, 2010; the Upper Keys sites are planned for July 6 – 16, 2010. If the sites are not visited prior to the arrival of oil, data from 2009 will be used as the baseline.

Table 1. CREMP and SECREMP sites by subregion.

SUBREGION	COUNT
Martin County	3
Palm Beach County	5
Broward County	4
Miami-Dade County	5
Upper Keys	12
Middle Keys	9
Lower Keys	16
Dry Tortugas	12

TOTAL	**66**

Each CREMP site consists of two to four monitoring stations delineated by permanent stainless steel markers. Stations are approximately 2 x 22 m and are generally perpendicular to the reef crest. The SECREMP stations have a north-south orientation, which is generally parallel to the reef tracts of Southeast Florida. Within each station, field sampling consists of a station species inventory (SSI), video transects (three transects per station) and targeted benthic surveys (Figure 1).

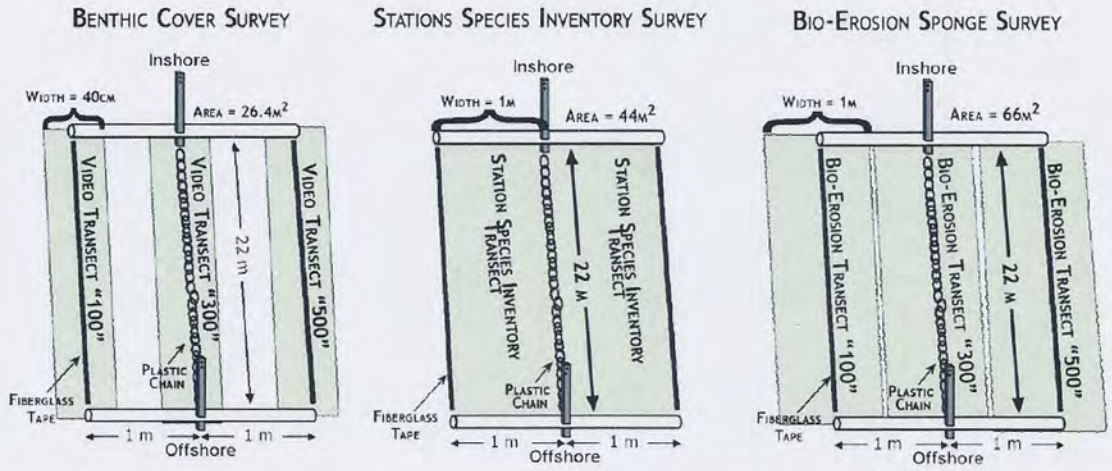


Figure 1. Illustration of survey area and station layout for CREMP video transects, station species inventory, and *Xestospongia muta* sponge survey.

Station Species Inventory (SSI), Coral Density and *Diadema* Abundance

SSI consists of counts of stony coral species (*Milleporina* and *Scleractinia*) present in each station to provide total stony coral species richness and the total number of colonies for each species present within the transect. Two observers conduct simultaneous, timed (one hour max) inventories within the SSI station area and enter the data on underwater data sheets. Each observer records all stony colonies ≥4 cm (hydrocorals are only marked as present) and enumerates long-spined urchin (*Diadema antillarum*) abundance within the station boundaries. Upon completion of the survey, observers compare data (5 minutes) underwater to confirm the species recorded by each observer. After surfacing, data sheets are verified again by both divers and mean density for each coral species is calculated by taking the average from both

observers. Data sheets are permanently stored at the Fish & Wildlife Research Institute after data entry and processing.

Coral Disease and Bleaching Assessment

During the station species inventory, any species within a station that exhibits specific signs of bleaching, disease, or clionid colonization is documented on the data sheet. Each colony with disease or condition is enumerated separately. The diseases identified include black band, white plague, white band, white pox/patch, dark spot, yellow band, and idiopathic diseases. Disease and bleaching prevalence is calculated by dividing the incidence of disease (total number colonies affected for each species) by the total number of species recorded in the station (22x2 m transect). The severity of each disease or bleaching incidence is also recorded by assigning a percentage to the portion of the colony affected. The prevalence of stony coral disease, bleaching, and condition is reported as the percentage of surveyed colonies affected for each coral species. A mean disease, bleaching, and severity is calculated by each SSI observer based upon the severity of all the colonies afflicted and the mean average is calculated for the station from the results of both observers. This yields a mean severity estimate for each species that is categorized according to a severity index of 1 through 4 (1 = 0-25% mean severity; 2 = 25-50%; 3 = 50-75%; 4 = 75-100%). CREMP survey methods have been adapted from Santavy et al. 2001.

Video Transects to Estimate Benthic Cover

Video is captured using a SONY TRV 900 to film three 40 cm x 22 m transects at each station (Fig. 1). All transects are filmed at a distance of 40 cm above the reef to yield a ~40 cm wide image. A convergent laser light system aids in maintaining the camera at the appropriate 40 cm distance above the reef surface. Prior to filming each transect, the videographer films a clapperboard that provides information on the date and location of each transect. Filming is conducted perpendicular to the substrate and follows a chain which marks the center of each transect. The videographer maintains a steady swim speed which roughly equals 4 meters per minute (~4 min to complete one 22 m transect).

In the lab, the video is captured digitally and each filmed transect is separated into about 9,000 digital frames. Digital image programs are used to rejoin a small subset of the 9,000 images to create a representative mosaic of the transect. Overlap between the rejoined images is set to 10% or less which results in 60-75 images for each transect. The images are then formatted for PointCount image analysis software. PointCount places 15 random points on each image and under each point, selected benthic taxa (stony coral species, octocoral, zoanthid, sponge, seagrass and macroalgae) and substrate are identified. The software uses a "point and click"

feature that enters the identification data into a spreadsheet. After all images are analyzed, the data are checked for quality assurance and entered into the Microsoft Access database.

Xestospongia muta Fate Tracking Study

Since 2007 a survey to track the fate of individual *Xestospongia muta* colonies has been included at all deep forereef CREMP sites excluding the Dry Tortugas and SECREMP sites. Giant barrel sponges are major contributors to reef systems but like stony corals, these sponges are susceptible to stressors such as bleaching and disease that can cause mortality. CREMP began monitoring the status of these sponges to provide another indicator for evaluating overall reef condition. Sampling methodology is based on existing CREMP station layout (Fig. 1). All *X. muta* colonies are enumerated by conducting three 1 m x 22 m belt transects at each station. A total area ~66m2 is surveyed at each station. Information on location, measurements to calculate volume, and any incidence of bleaching and disease are recorded for each *X. muta* colony. Due to the morphological plasticity of this sponge, volume is measured by approximation to various geometric shapes. The most common geometric shape for calculating the volume of *X. muta* is a frustum (truncated cone). The measurements used for calculating the volume of the frustum are the diameter of exterior sponge apex and base, and height. The sponge atrial (oscula) diameter and height are also measured and subtracted from the total volume to calculate the total sponge tissue volume. Methods for calculating the volume of the sponge are in McMurray and Pawlik 2008.

Site MC3 Stony Coral Colony Condition (Martin County)

Limited appropriate reef area within the Martin County sampling area did not permit the establishment of three standard SECREMP sites. Stony coral cover and density is low in this area, which limits the ability of the standard SECREMP sampling protocol to track changes in the stony coral assemblage. After discussions with project colleagues from the Florida Department of Environmental Protection (FDEP) and FWRI, it was decided that a third site (MC3) would be established; but this site will be used to fate track a representative sample of stony coral colonies. To delineate the site, five stakes were deployed in a reef area between sites MC1 and MC2. These stakes mark the center point from which stony coral colonies were identified and recorded. The distance and bearing from these center stakes to the colonies was also recorded. These measurements permit the same colony to be located and sampled each year. During the first monitoring year (2006), colonies within approximately 10 m of the stake were targeted. As colonies mapped and tagged in 2006 die or become missing, new colonies are added to the project by mapping and tagging colonies greater than 10 m from the stake or by adding colonies within 10 m of the stake that were not included in 2006.

Total colony size (length and width) and colony condition (presence of bleaching, disease, etc.) were recorded *in situ*. In addition to the *in situ* measurements, a digital image was taken of each colony. The images were taken with a digital camera attached to a PVC framer (0.38m2). Date and colony tag numbers were included within each image.

The framer allows all images from each monitoring event to be a consistent planar view of the colony. These consistent planar view images permit changes in tissue area between monitoring events to be measured. National Coral Reef Institute (NCRI) developed software (Coral Point Count with Excel Extensions, CPCe, http://www.nova.edu/ocean/cpce/index.html) (Kohler and Gill, 2006) is used to trace the tissue area (cm2) in each colony planar image. The software automatically calculates the area (cm2) encompassed by the traced portion of the image. If dead areas are present within the living area of a colony, these dead areas are also traced. The dead area(s) subtracted from the previously-traced living tissue area provides a more accurate measure of the living tissue area.

Method 2: Protocol for Combined Sediment/SPMD sampling at CREMP sites

Semi-permeable Membrane Devices (SPMDs) are temporally integrative, abiotic devices which sample some aqueous, nonionic organic compounds. As such, they can provide qualitative indications of exposure to these types of compounds. Similarly, some sediments (more accurately, fractions of benthic material) are capable of adsorbing certain hydrophobic compounds. Sediment concentrations of these compounds, given the proper time and conditions, reach an equilibrium status with overlying water and surrounding pore water. Chemical analysis of sediments or SPMDs are one approach that may provide indications of exposure to certain hydrocarbons under the proper conditions. Each approach has its own strengths and weaknesses and neither are to be taken individually or solely as absolute indicators.

Sample Collection of Sediment for Organic Contaminant Analysis – CREMP/SECREMP

Sediment sampling is an additional method that can help detect the presence of oil at the sampling sites, and will be included in the protocol. The purpose for sediment sampling (and SPMD deployments) is for exposure documentation via presence/absence, and not for toxicity or concentration calculations.

The protocols to be used for collecting sediment at CREMP stations will essentially be similar to those used at stations where coral tissues are also collected; a different number of samples will be collected however.

Materials:

- Sediment Jars, 250-500 ml – Glass, EPA certified w/ Teflon lined caps

- Teflon bags with clip closures (alternate protocol)
- Teflon syringe or metal coring device with Teflon caps (alternate protocol)
- Nitrile gloves

Collection Procedures

The particle composition and size of sediment may have an effect on the inferences that can be made from the contaminant chemistry data resulting from a sample site. The types of sediment at the sampling sites may vary from site to site. Oftentimes, inexperienced or uneducated sampling teams will sample sediments containing large sized stones (e.g., 1 centimeter or larger). Particulate size within a sediment sample should be, on average, smaller than two millimeters. Stones larger than 1 cm should be excluded from the sample. Exclusion of large stones requires that the sediment be resampled in the lab; do not manually remove the stones from the sediment jar.

It is also suggested that the divers make note in the field book a description of the texture, color and scent of the matrix of each sediment sample.

1. Pre-label EPA approved pre-cleaned amber collection jars with the bar-coded labels provided in each case, however these labels require securing with clear packing tape because they will come off the jar when wet. As a precaution we have also place a secondary label on the jars and lids with cryo-labels that adhere with wetting as well as freezing. Securing labels with cellophane tape wrapped completely around the jars is also acceptable.
2. At each sampling site with sufficient appropriate sediment, place up to 10 EPA approved jars that have been filled with distilled water into mesh bag. Clean nitrile gloves should be used when filling the jars with distilled water. If you do not dive deeper than 15 m, the pressure on the jar should not break the jar. Do loosen the lid on the jar so that it can be opened easily underwater.

   An acceptable alternate collection device can be a modified Teflon syringe or a metal tube with Teflon caps (rinsed with acetone) to core approx 250 ml of the sediment. This is then placed into a Teflon bag and sealed with the bag clips. The Teflon bag is then placed into a labeled zip-lock or evidence bag.
3. Diver should wear nitrile gloves and change gloves between samples.
4. At depth, open jar above sediment, using regulator (if necessary) remove distilled water and core into the sediment approximately 2-5 centimeters and facilitate getting the

sediment into the jar by scooping the sediment into the jar using the lid of the jar, taking care not to abrade the Teflon liner. Only collect the top 2-5 centimeters of sediment.

5. Try to ensure that the jar is completely filled with sediment, and contains little residual seawater.

6. At depth, cap jar with lid.

7. Prior to sediment samples arriving on the deck, ensure a cooler is in place at the work station that is large enough to hold the sediment samples being collected and that it is filled half-way with ice. If ice is not available use blue-ice packs to chill the samples.

8. The snorkeler (or diver) brings the sediment samples to the boat and transfers custody to the sample handler.

9. The sample handler next puts on a clean pair of nitrile gloves and removes sediment jars from the mesh dive bag.

10. The samples are then inspected to ensure the pre-label of the jar corresponds with the sample identification sheet and chain of custody sheet and the sample is then chilled. Place jar in a cooler with normal ice – not DRY ICE. If Teflon bags are used, place sealed Teflon bag into evidence bags. Tamper-proof evidence bags are provided to be used to as a secondary container to cushion the sample during transit. These bags have unique numbers which can also be used to uniquely identify the sample. If these are used then the chain of custody or data records should reflect this additional labeling.

11. When the sediment has settled, open the jar and decant the excess seawater carefully so as not to disturb the sediment and replace the lid. Place jar in the pre-labeled evidence bag and sign the label and place the sediment into the ice filled cooler.

12. Repeat this process for each sediment jar. Remembering to have separate chain of custody paperwork for different collection teams.

13. REMOVE YOUR NITRILE GLOVES.

14. When collection is completed, seal cooler with evidence tape and chain of custody tags. The evidence custodian should sign across the evidence tape and secure the samples in a way that they will be able to testify that the samples were not tampered with while in their custody. Once approval is given, ship the cooler to the lab designated for these samples (e.g., TDI Brooks). If the samples are not frozen, the cooler must arrive at lab in less than 1 week.

15. If the samples require holding prior to shipping, place the sediment jars in a -20°C freezer. BE SURE TO LEAVE SUFFICIENT SPACE IN THE JAR FOR EXPANSION OF THE SAMPLE TO AVOID BREAKING THE JAR AS THE SAMPLE FREEZES.

Using NRDA trustee Sample Forms, the GPS coordinates will be recorded, along with water depth at which the samples were collected. Complete all other necessary NRDA sampling forms, both chain of custody and description of sample type.

Samples should be taken as close to one of the stakes as feasible. In the case of spur and groove reefs where transects are located on top of spurs, it is preferable to find sediment at the same depth as the coral transect. Therefore, look for sediment pockets on the spur. If this is unavailable, then swim laterally to the nearest sand at the same depth as the coral, rather than dive down to the nearest sand. It is better to sacrifice lateral proximity to the stake, than vertical proximity.

Deployment of SPMD Passive Sampling Devices

Background

Diver-deployed semipermeable lipid bags (SPMDs) are used to detect hydrocarbons in seawater. SPMDs from EST labs will be utilized, in two sizes described below: Small (2.1) and Large (2.2).

SPMDs will be used to detect the presence or absence of MC252 products, not to measure concentration or toxicity.

The larger size may be more sensitive due to its greater surface area; however it is impossible to completely control for contamination at the sea surface, and in the water column above the deployment location because the "blank" does not follow the SPMD through the surface layer of the water or down to the bottom. Therefore, contamination from nearby boats or other sources cannot be ruled out.

The small SPDMs eliminate the risk of contamination prior to deployment, because they remain in clean, argon filled, sealed cans until the diver reaches the bottom where they are to be attached.

All remaining CREMP sites in the Upper and Lower Keys yet to be sampled in 2010 and a subset of sites in the Middle Keys will be targeted for SPMD deployment. Annual monitoring in the Middle Keys and Dry Tortugas has been completed for 2010 and thus a subset of sites in the Middle Keys will be revisited. Sites selected in the Middle Keys will be chosen to optimize the

spatial coverage to detect the presence of hydrocarbons within the region. These sites include two SPA locations monitored by CREMP (Tennessee and Sombrero Reefs) and two patch reefs (TBD). Dry Tortugas sites will not be revisited. At each site, 3 small SPMDs and 1 large SPMD will be attached to individual permanent stainless steel stakes. The merit in deploying both types of SPMDs at each site is to maximize the detection of hydrocarbons, mitigate against surface contamination on the large SPMDs, and account for potential tampering by recreational divers at heavily visited CREMP sites.

Deployment of Small SPMDs

Each small SPMD consists of one lipid bag contained inside its own individual aluminum box. Three of the small SPMD's (9.5 cm x 4.5 cm) will be deployed concurrently at each CREMP site. Each box has a wire-attachment loop securely tied to one end. It should be noted that loss of one or two of the devices does not invalidate SPMD utility but only reduces detection power within a site.

The small SPMDs are supplied in quart-size paint cans, with three devices in each can. The can is filled with argon gas. Argon gas is a neutral substance and requires no precautions for safe handing.

Deployment

At each site, divers will take a single can to the bottom. Deployment starts by punching a small hole in the bottom of the can using a standard can-opener. After the can floods with seawater, the can is opened with a paint can opener. The aluminum boxes will be handled underwater using a fresh pair of latex gloves for each dive. Three zip ties will be used to attach each SPMD to a stainless steel stake. There is maximum of 4 to 8 stakes per CREMP site. Stakes will be chosen at random. Each SPMD will be deployed for a maximum of 30 days.

Retrieval

SPMDs will be collected after 30 days. Zip ties will be cut and all devices will be placed into a quart-sized paint can. The can will be closed underwater and returned to the surface. The cans will be stored on ice aboard the vessel and then transferred to a -20 °C freezer. Sampled will be shipped frozen to the analytical facility. At the time of collection, 3 more small SPMDs will be deployed for collection. This process will continue until confirmation that oil from the Deep Water Horizon will not impact the Keys.

Deployment of Large SPMDs

At each CREMP site, one (1) large (30cm high x 16cm wide) SPMD will be deployed. The methods described above for attachment for small SPMDs to CREMP stainless steel stakes will

be followed. Two D rings on the outside of the large SPMD containers are provided to secure the device with zip ties to the CREMP stake. The time of deployment will be concurrent the small SPMDs and length of deployment will be the same (30 days).

Collected SPMD's will be preserved and shipped according to requirements (http://wwwaux.crc.cr.usgs.gov/SPMD/; Huckins et al., 2002) to designated laboratory for analysis.

CREMP will follow the guidelines issued by the manufacturer to properly deploy and retrieve the large SPMDs. CREMP will be inserting 5 standard length membranes "spiders" (91.4cm) into the large SPMD container for hydrocarbon collection.

Items required for SPMD deployment or recovery:

- Gallon cans containing SPMDs
- Small cans that contains single field blank SPMDs.
- Pint-sized can with metal spacers (not always included)
- Deployment canisters
- Soap for washing hands
- Data sheets
- Church key to open cans
- Tie wraps for deployment
- Wire clippers for recovery

Important to know:

- Keep SPMDs in freezer or on ice until time of deployment preparation.
- Conduct the deployment steps with **clean hands**.
- Select a location where air contamination is least likely (e.g. open air upwind of any ship exhaust, inside the cabin – turn ship/boat engines off, if possible) for deployment prep and deployment. SPMDs can adsorb contaminants from the air as well as water.
- Handle SPMDs quickly and carefully, minimizing air exposure.
- Expose field blank SPMD during all deployment and retrieval procedures.

Procedures for deployment:

1. At deployment site, unscrew nut securing lid of deployment canister. (Notice the rod in the center of the canister. The "spiders", which carry the SPMDs, will slide onto this rod.)

2. Using a church key, work open the gallon can containing the SPMDs on "spiders." (Save this can to return the SPMDs in. Avoid using a screwdriver etc. in place of a church key as it may damage or deform the lid.)
3. Open the smaller can containing one loose SPMD. This is your field blank. It will remain in its can and should be exposed to the same surface conditions as the SPMDs to be deployed. Once the SPMDs enter the water, this can should be immediately resealed and placed in the freezer. It will be opened again during the retrieval process.
4. With clean hands, grasp the spiders by either the metal plate or center post and lift it slightly. Do not touch the SPMD (the membrane)—it could ruin the project. Notice that there is a metal plate that has flaps that are bent upward. These flaps allow the carrier to slide out of the can diagonally. Gently turn and pull the carrier out of the can, taking care not to touch, damage, or abrade the membrane.
5. While holding the spider, slide it into the deployment canister. Make sure the threaded rod in the canister runs up through the spider's center post. The spider may be placed in the canister plate up or down.
6. Place a spacer onto the canister rod then repeat steps 3 and 4 to add the four additional spiders/SPMDs, placing a spacer between them and after the last one (if available).
7. Place lid on deployment canister. Be sure that the mounting ring on the side of the canister matches up with the mounting ring on the lid. These rings must be fastened together to fully secure the spiders/SPMDs inside.
8. Deploy canister, closing the lid on the field blank once the deployment device enters the water. (Deployment is best if SPMD canister can be attached to something upright, up to a meter above the bottom. This reduces sediment abrasion and movement. Attachment can be with large tie wraps or other secure means.)

For retrieval, the above procedures can essentially be followed in reverse. A rubber mallet works well for resealing the can lids. Once retrieved, the SPMDs must again be kept in a freezer or on ice.

Procedures for retrieval:

1. The diver clips the tie wraps holding the SPMD canister. The canister is simply brought to the surface and passed to surface support for disassembly. Remember, to expose the field blank whenever the SPMDs are exposed to air.
2. When the canister surfaces and is exposed to air, the top is removed from the can containing the blank. Note time on the data sheet. As quickly, but carefully as possible, the field canister is opened (cut tie wrap, remove butterfly nut, and canister top). There are five SPMD/spider assemblies and four spacers (if available) inside.

SPMD/spiders should be gently lifted out, quickly inspected for tears, and placed one on top of the other inside the gallon can. They may need to be tilted to insert into the can. **The membranes should not be touched**. Place spacers in pint-sized can for reuse.

3. As soon as all five spiders are placed in the gallon can, the top of that can and the top of the can containing the field blank should be replaced and securely sealed (be careful not to bend lids, as the seal may be lost). Again, note the time on the data sheet.
4. All samples (blanks and SPMD/spiders) should be placed on ice until they are returned to shore. They should then be placed in a freezer until shipment.
5. Complete the data sheet and make two copies.
6. All samples will be shipped to EST (SPMD manufacturer) for initial processing prior to being transported to TDI Brook for analysis.

Place the gallon can (spiders) and the small can (blank) on ice (dry ice, if available), and along with a copy of the data sheet, send overnight.

Method 3: South Florida/Caribbean Network Coral Reef Monitoring Protocol

The South Florida/Caribbean Network (SFCN) coral reef monitoring protocol guides the monitoring of coral reefs within four national parks of the SFCN: Biscayne National Park (BISC), Dry Tortugas National Park (DRTO), Virgin Islands National Park (VIIS) and Buck Island Reef National Monument (BUIS

The objectives for this protocol include:

- Determine whether percent cover of major taxonomic groups (e.g., coral, algae [turf, calcareous, macroalgae], gorgonians, sponge, substrate), coral species diversity, coral community structure, and rugosity are changing through time within selected coral reef sites.
- Track trends and severity in reef-associated covariates such as coral bleaching, coral disease and presence of the herbivorous sea urchin, *Diadema antillarum*.
- Maintain and archive a video record of the transects to allow for quantification and future analyses of benthic components not identified in this protocol.

To achieve these objectives, coral reef monitoring involves the following components:

1) Selection of the reef study site, 2) selection of twenty, 10-m transects within the mapped perimeter of that study site, 3) filming the benthic community with a digital video camera in an underwater housing, and 4) quantification of benthic organisms found under random "dots"

applied to the video images. This reef monitoring is supplemented by the simultaneous collection of data on presence and size of disease lesions, presence and severity of coral bleaching, presence of *Diadema antillarum*, and rugosity along the transects. Monitoring is conducted annually and episodically, as necessary. The SFCN analyzes and archives data and videotapes. The network will also prepare an annual report detailing the status and trends of the coral cover at the selected sites, termed index sites. The full protocol can be accessed at http://science.nature.nps.gov/im/units/sfcn/phase3/SFCN_Coral_index_site_protocol.

Method 4: FRRP Disturbance Response Monitoring Protocol 2010

Site Selection

All sites will be predetermined. GPS coordinates will be assigned to each dive team in advance. The FRRP approach is a probabilistic survey design using randomized two-stage stratification. Although the design was not created for natural resource damages sampling, it is beneficial for population census and prevalence. The primary sampling unit is a 'site' defined as a 200 m x 200 m cell and the secondary sampling unit is a 'transect' defined as 1 m x10 m belt transect. At a minimum, the survey design calls for two sites per reef 'stratum' and two replicate transects per site. More sites are allocated to higher variability strata such as patch reefs. The strata have been developed and refined over the past five years and are based on sub-regional divisions, cross shelf divisions, habitat type, and bathymetry. This stratification framework continues to be revisited each year and is being iteratively improved to more accurately describe how reef community types are organized.

The sites selected for the baseline characterization are represented in Map 1 and summarized in Table 2. The locations of the Coral Reef Evaluation and Monitoring Program (CREMP) monitoring sites were included as strategic sites. The following table represents the distribution of the sites across the stratification framework.

Table 2. FRRP sites by subregion and reef zone.

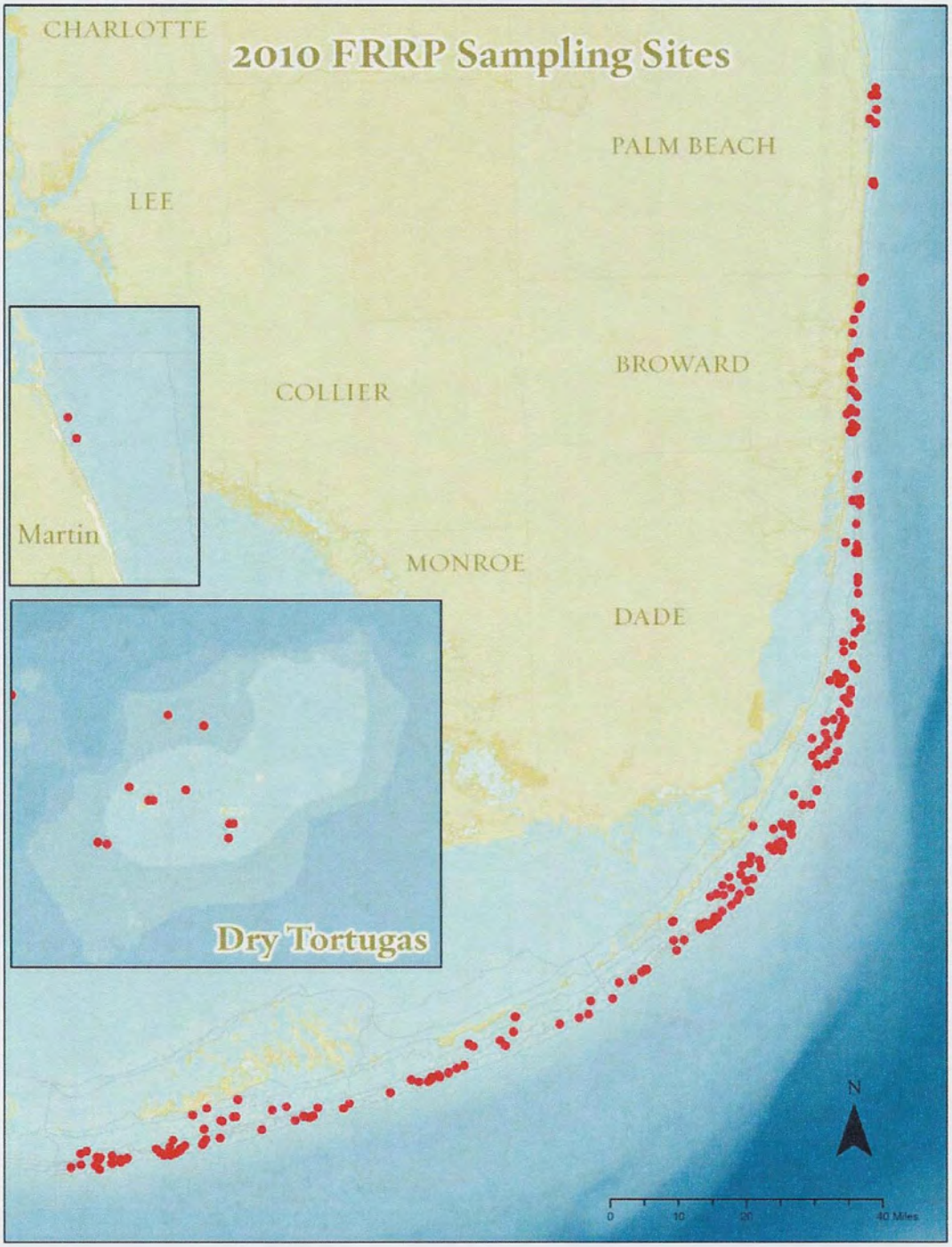
SUBREGION	ZONE NAME	COUNT
Martin County	Undetermined	6
Palm Beach	Inshore	2
Palm Beach	Reef Ridge Complex	7
Broward	Inner Reef	9
Broward	Inshore	17
Broward	Outer Reef	9
Biscayne	Forereef	24
Biscayne	Inshore	3
Biscayne	Mid Channel	15
Biscayne	Offshore Patch Reef	2

Upper Keys	Forereef	42
Upper Keys	Inshore	3
Upper Keys	Mid Channel	9
Upper Keys	Offshore Patch Reef	12
Middle Keys	Forereef	15
Middle Keys	Inshore	3
Middle Keys	Mid Channel	2
Middle Keys	Offshore Patch Reef	3
Lower Keys	Forereef	31
Lower Keys	Inshore	3
Lower Keys	Mid Channel	10
Lower Keys	Offshore Patch Reef	7
Tortugas		12
TOTAL		**246**

Each survey team will be assigned sites that they are responsible for surveying. The sub-regional leads, Mote Marine Lab, University of Miami and Nova Southeastern University, will allocate the sites to each team and coordinate the data entry.

Each survey site will have a primary and secondary set of GPS coordinates (first attempt to locate coral habitat at the primary coordinate - search an area of 100 m radius around the GPS point). If any suitable (non-soft bottom) habitat is found, drop a float and anchor the boat. If no suitable area is found within the 200x200 m cell, then go to the secondary location (within the same zone) and repeat the procedure. Based upon the expertise of the trained observer, if the secondary site is either too far away or also unsuitable, go to an area known to have hardbottom/reef with THE SAME FRRP ZONE and survey that location. If the site is not within 200 m of the primary or secondary coordinate, upon logging into www.FRRP.org to record the data, it will assign a site number which denotes a strategic location instead of a pre-determined, selected site.

Once at the proper site, it is critical that the exact location of the actual survey (transect locations) be recorded using a GPS. Drop a float at the site based on the assigned location and then try to anchor or attach to a mooring ball as close as possible. Teams should use a small weighted float to mark transect locations - lower or throw two weighted line when near the suitable reef survey area. Before doing this, adjust the length of the buoy line to be similar to the water depth. Swim down to the weight end of the line and start transect from that location by laying out the line. When done with the benthic survey (two transects per site), the GPS location of the transect buoy should be recorded.



Map 1. Location of 2010 FRRP sample sites.

Benthic Survey Protocols

1. At each site, record the following information on the underwater datasheet before each dive. (Each team member should fill in every category.)

- Name of recorder: Use four letter codes- first two initials of first name, first two initials of last name. Example: John Smith= JOSM
- Date: As day with two digits/abbreviation of month/year with two digits;
- Latitude: As determined by dGPS.
- Longitude: As determined by dGPS.
- FRRP site code ID number
- Reef Zone/Habitat: e.g., reef crest, reef front, spur and groove, etc. (Note- if the reef zone/habitat surveyed appears different than predicted, please describe the actual reef zone/habitat following the survey)

2. In Time Start: Record the time at which the first transect is started.

Haphazardly (ie, disregarding the distribution and orientation of the corals and reef structures) lay the 10 m transect line just above the reef surface. Make sure the line is taut.

Note: Be sure to avoid and don't cross the other transect that is being set by a second surveyor. Lines should be at least 5 m apart so data from each transect are less likely to be pseudoreplicated, which can happen if too close (within 5 meters) and features of one transect impact the other (big corals for example). Stay away from the edges of the reef. Also try to avoid areas with abrupt changes in slope, deep grooves, large patches of sand or unconsolidated coral rubble. Swim without looking down at the bottom as the line is unreeled.

Each transect can be surveyed in two "passes" of the transect line as follows:

First pass:

3. Swim a belt transect along the 10 m line. Tie the first end of the transect line off to a dead piece of coral, fire coral, gorgonian, or other feature that is not living coral. Once at the end of the transect (past the 10 m mark), pull tightly and secure the line. Note the depth at the start and the end of the transect line (0 m and 10 m).

Second pass:

4. Swimming from one end of the transect line to the other, assess the cover, size and condition of each stony coral that is 4 cm in length or greater, and for which any live or dead part of its skeleton is within 1 m of the transect line. Lay down the 1 m measuring pole perpendicular to the transect for scale. Try to work the same side of a transect line.

a. Identify stony corals to species and record using four letter species codes.

b. For all scleractinian corals measure the x, z dimensions of the colony with the 0.5 m measuring bar (or tape): *i.e.,* the maximum diameter (x) of the outward-facing colony surface (perpendicular to the axis of growth) as seen from above in planar view, and the maximum height (z) (parallel to the axis of growth) as seen from the side of the colony. Record these measurements to the nearest cm.

Note: Colony boundaries can be difficult to recognize when parts of the coral have died and are overgrown by other organisms–particularly other colonies of the same species. Look for connected live tissues, connected skeletal deposits above a common base, and at the size and color of separated polyps.

Colonies derived from new recruits:

1) Live tissue, generally concentric with clear edge boundaries. Often have a raised "lip" at edges approximately 1-2 mm above underlying substrate/old dead coral.

2) Upward growth, branching evident.

3) Underlying substrate is very old dead.

Colonies derived from resheeting:

1) Live, often with preferred growth in one direction, edges on at least one side often "merge" with underlying substrate/dead coral.

2) Live tissue rarely displays upwards growth (branching) except at tips.

c. Estimate the partial mortality (old and recent) of the whole colony surface. Try to round the percentage to the nearest 5% unless it is very small or very large, in which case try to round to the nearest whole number (e.g., 1%, 97%).

"Old dead" is defined as any non-living parts of the coral in which the corallite structures are either gone or covered over by organisms that are not easily removed (certain algae and invertebrates). If it is entirely "old dead", indicate this on the data sheet as 100% "old death", as long as the colony can be identify it to either the species (e.g., *Acropora palmata* by gross

morphology; *Montastraea cavernosa* by polyp size and shape) or to the genus (e.g., *Diploria* by size of meandering ridges and valleys).

Note: In some cases, a coral may be partially or completely overgrown by one of the species of brown, zooxanthellate clionid sponges. If you look closely, you will observe the in/ex-current holes of the sponge and sponge tissue instead of live coral polyps. If you can see the coral skeleton beneath the sponge, and are able to identify it to genus or even species, include the affected area in your estimate of "old death" and note "Cliona overgrowth" in the corresponding Comments box.

"Recently dead" is defined as any non-living parts of the coral in which the corallite structures are either white and still intact, or slightly eroded but identifiable to species. Recently dead skeletons may be covered by sediment or a thin layer of turf algae, generally within two to four weeks.

Note: How to assess corals that are detached from the substratum:

i. If it has recently fallen, the length, height and % mortality should be measured as if it were still upright; write "fallen" in comments box.

ii. A detached but wedged coral should be marked as "wedged" in the comments section (as it is likely to remain in this position for an extended period). If it has been fallen for long enough to have reoriented to grow upward in its new position, the "new" maximum length and maximum width should be measured, and the new outward-facing surface used for calculating % mortality.

d. Scan over the surviving portions of the ENTIRE coral colony for any DISEASES and/or BLEACHED tissues present.

e. Characterize any DISEASES by the following color categories:

BB = Black band

WB = White band (*Acropora* only)

WS = White patches/white pox/patchy necrosis (*Acropora* only)

WP = White plague

YB = Yellow-band/yellow-blotch

RB = Red band

UK = Unknown

For more information about coral diseases, see the disease cards (Bruckner & Bruckner 1998) or one of the following web sites:

http://www.unep-wcmc.org/marine/coraldis/cd/index.htm

http://www.coral.noaa.gov/coral_disease/

Characterize any BLEACHED tissues as approximate severity of discoloration:

blank = No bleaching

P = Pale (discoloration of coral tissue)

PB = Partly Bleached (patches of fully bleached or white tissue)

BL = Bleached (tissue is totally white, no zooxanthallae visible)

Many severely bleached corals are translucent, but the polyp tissues can still be seen above the skeleton. Bleached tissues should not be included with the "recently dead" estimates.

Note: It is important to be able to differentiate between tissues that are alive (but look white because they are bleached) and white, recently dead skeletons.

f. For Milleporid species (*Millepora alcicornis* and *Millepora complanata)* that are 4 cm or greater within the belt transect, record only the presence of each colony and the degree of bleaching.

g. Complete two transects per site. After surveying, either transcribe slates to paper and then enter data to spreadsheet, or enter data into spreadsheet and print out a copy. Enter the data into ww.frrp.org according to the prompts in the website. Please check the data to verify its accuracy then close the site.

h. This protocol may be revised based on input from survey teams and approval from the NRDA Shallow Water Corals Technical Working Group.

Data Management:

All survey data will be entered into the online database located at www.frrp.org. Each surveyor will be assigned a login and password for the database and initial QA/QC will be conducted by the sub-regional leads and further QA/QC of the data will be conducted by the database manager.

Method 5: Protocol for Monitoring *Acropora palmata*

The targeted *Acropora palmata* demographic monitoring sites are listed below (Table 3). Sampling for the regular spring monitoring interval is underway to document current colony density and status (See Williams et al. 2006 – NOAA Tech Memo NMFS-SEFSC-543 available at http://sero.nmfs.noaa.gov/pr/pdf/Williams2006.pdf for protocol implemented at the following 24 sites):

Table 3. Acropora palmata demographic monitoring sites.

Site	Plot	Latitude	Longitude	Mean Depth (m)	# Ap colonies
Turtle Rocks	TR1	25.280670	-80.208880	3	81
Turtle Rocks	TR2	25.280720	-80.209070	3	37
Turtle Rocks	TR3	25.280780	-80.208820	3	39
Carysfort	CF1	25.221940	-80.210550	3	6
Carysfort	CF2	25.221780	-80.210600	3	40
Carysfort	CF3	25.222900	-80.209560	4	9
Elbow	EL1	25.142590	-80.258350	4	78
Elbow	EL2	25.142900	-80.258220	4	31
Elbow	EL3	25.143940	-80.257800	5	16
Elbow	EL4	25.145080	-80.257340	6	13
Elbow	EL5	25.145180	-80.257400	6	8
French	FR1	25.033930	-80.349410	7	54
French	FR2	25.033690	-80.349590	6	22
Key Largo Dry Rocks	KL1	25.123600	-80.297360	2	10
Key Largo Dry Rocks	KL3	25.122550	-80.298260	3	14
Molasses	ML1	25.009580	-80.374810	4	7
Molasses	ML2	25.009120	-80.374730	6	24
Molasses	ML3	25.010150	-80.373280	4	26
Sand Island	SI1	25.018270	-80.368880	4	26
Sand Island	SI2	25.018250	-80.368450	3	42
Sand Island	SI3	25.018130	-80.368350	4	18
Looe Key	LK1	24.545850	-81.405070	3	35
Looe Key	LK2	24.545000	-81.409180	4	45
Looe Key	LK3	24.546930	-81.407680	4	31

Method 6: Protocol for Collection of Coral Tissue and Sediments

Adapted from Downs 2005. Sampling, Biomarker, and Contaminant Chemical Target Analyte Protocols

Sampling Protocol Strategy

All sampling teams will be approved by Dr. Cheryl Woodley (NOAA/NOS) and Dr. Margaret Miller (NOAA/NMFS) for their competency in completing the protocol. All biological samples will be taken and stored in sealed Teflon bags, tubes or jars. Chain of Custody protocols and labeling will be used. Coral tissues and sediments will be collected at a subset of sites monitored under the other schemes described here (FRRP, CREMP, SFCN, and Elkhorn Demographic), targeting sites distributed along the outer fore-reef in the Keys where remnant elkhorn (*Acropora palmata*) populations are concentrated, analogous habitats in the northern areas of the reef tract will be targeted. Four sites within each subregion (e.g., Dry Tortugas, Lower Keys, Middle Keys, Upper Keys, Broward, etc) are proposed. Elkhorn coral (*A. palmata*) will be targeted in the tissue biopsy sites (ESA threatened status and relatively shallow depth limitation making it vulnerable), as well as *Porites astreoides* (ubiquitous, common in very shallow reef crest habitats). At northern sites, where elkhorn coral is not present, the ESA-listed staghorn coral (*A. cervicornis*) will be sampled. Because of the lack of *A. palmata* and *A. cervicornis* on the reefs of the Florida Middle Grounds, East and West Flower Garden, Stetson, and Sonnier Banks, tissue biopsies will not be taken as part of the sampling protocols within these areas.

Sample Collection & Preparation for Coral

Materials Needed:

- Al Stohlman Brand Drive Punch 13/64" or similar stainless steel hole-punch 1-1.5 cm
- Stainless steel dissection probe
- Heavy-duty Aluminum Foil
- 5 mm Teflon Layflat bags
- Ziploc bags
- Evidence bags that are tolerant to liquid nitrogen vapor temperatures
- Evidence tape
- Cable ties

- Dry Shipper
- Liqui-nox detergent
- Pencil, permanent markers, fine tip, rubber bands
- Clear packing tape to secure labels (wrapped around label on jars)
- CryoTags to label jar lids and bottom – use permanent markers to label
- Underwater paper – datasheets
- Clipboard
- Acetone
- Double Distilled water or MilliQ purified water
- Squirt bottle – Teflon or Teflon lined
- Nitrile Gloves of sizes fitting all samplers and sample handlers
- Hammer
- Forceps
- Sharp knife
- Tags for colonies (e.g., cattle tags) and stainless steel nails or other material to secure the tags
- Digital camera with new or empty digital cards that is equipped with underwater housing and standard metric for photographing lesions.
- Cooler with ice or blue ice for sediment
- Amber 250ml glass jars pre-cleaned to EPA's highest cleaning standards (this designation can vary with supplier's specific coding (e.g., Grade A or Grade 1) with seal and certification
- Padlock with key. Key will be in the custody of only one custodian at a time and transfer will be reflected in the chain of custody
- Sediment samples will be kept on ice or frozen at -20°C prior to shipping to approved lab but should be kept in a lockable container with access limited to the designated

evidence custodian at any one time (i.e., transfer of access to the samples is conveyed along with the evidence via the chain of custody.

Preparation for Cleaning

Arrange equipment and materials so they can be easily accessed once the cleaning process is started. Once cleaning has begun, do not touch any other equipment or surfaces with the gloves you are wearing. If you must touch something else, remove the gloves and put on a new pair of clean gloves. Any stray contamination introduced during the cleaning process has the potential to affect the subsequent analysis. Equipment and gear should be cleaned and stowed in a clean area the day before the intended sampling dive to provide sufficient time for the underwater collections.

A. Cleaning of biopsy equipment and packaging-

Processing and packaging of the biopsy equipment and kit assembly should be conducted in a laboratory setting by technicians knowledgeable in organic contaminant chemistry, and ideally by one or a small number of laboratories to assure consistency in the cleaning and assembly process.

B. Preparing packaging materials and work surfaces

1. Fill a cleaned Teflon-coated squirt bottle with acetone (100%). Identify the bottle with "acetone".
2. Fill a cleaned Teflon-coated squirt bottle with the distilled water. Identify the bottle with "water".
3. Place the roll of aluminum foil on a counter or table for cutting.
4. Cut a 30 cm x 30 cm section. Either mark the top left-hand corner use a #2 pencil to inscribe an "A". The letter will let you know which side you have cleaned later or you may choose to use the shiny or the dull side of the foil to be the cleaned side, and then remain consistent and document this protocol with how you assure you are working with a clean surface.
5. Cut a piece of foil into approximately 10 cm x 20 cm pieces.
6. Put on a clean pair of nitrile gloves.

7. Wash the side of the 30 cm x 30 cm piece of aluminum foil that was pre-determined to be the clean side (see # 6 above) with acetone using the squirt bottle. Use acetone-cleaned forceps to manipulate the foil piece; handling of the aluminum piece with a gloved hand should be kept at a minimum.

8. Wash one side of each piece of 10 cm x 20 cm aluminum foil with acetone using the squirt bottle. Set the unwashed side face-down on a clean counter. Allow the foil to dry (15 minutes).

9. Rinse the forceps with acetone. Use the forceps to pick up each piece of foil for cleaning. Wash both sides of the foil square with acetone using the acetone squirt bottle. Allow to air dry on a 10 cm x 20 cm piece of acetone-rinsed aluminum foil.

10. Allow all pieces of foil to air dry, about 15 minutes.

11. Collect a portion of the final aluminum foil rinse into Teflon or EPA glass jar with Teflon liner and store for analysis.

C. Clean Instruments & Package for Field Use

1. Prepare a solution of 10% Liqui-nox detergent and place in a wash pan.

2. Wearing the nitrile gloves, wash the Drive Punches (leather punches) and dissection (dental) probes (or other steel probes for use in dislodging biopsies from the punch) in the 10% Liqui-nox detergent.

3. Remove all residual detergent on the instruments by rinsing the instruments with distilled water or MilliQ ultrapure water using the squirt bottle.

4. Rinse instruments with acetone using the squirt bottle.

5. Place instruments on acetone-cleaned aluminum foil and allow to completely dry.

6. Place one Drive Punch and one probe onto a single 10 cm x 20 cm piece of aluminum foil (on the side of the foil that was washed with acetone).

7. Wrap the instruments with the acetone-cleaned aluminum foil.

8. Place one aluminum-covered instrument set into labeled Ziploc bag, add one layflat Teflon bag and one seal-n-clip.

9. Seal the Ziploc bag.

10. These kits are then ready to be labeled with the unique sample identifier. Each kit is good for only 1 colony.

IT IS RECOMMENDED TO PRE-LABEL ALL SAMPLING KITS AND SAMPLE CONTAINERS PRIOR TO DEPARTING THE DOCK OR STARTING THE DIVE. THIS AVOIDS HAVING TO LABEL ANYTHING ON THE BOAT AND WILL STREAMLINE THE PROCESS AND REDUCE LABELING ERRORS.

Cleaning Diver and Dive Gear before Collection

Cleaning divers and dive gear is intended to remove gross contamination of existing organics that may be introduced into the samples being collected. These can come from numerous sources such as lotions, personal care products, and a variety of other hydrocarbon sources that may accumulate on gear or suits. Equipment and gear should be cleaned and stowed in an area cleaned with 10% Liqui-nox the day before the intended sampling dive to reduce the preparation time the day of the underwater collections. A large cleaned holding area on the boat or a large cooler or container would assist in this effort.

1. Dive gear and tanks should be cleaned with 10% Liqui-nox lab detergent. The detergent needs to be removed by rinsing with clean water. Ideally the rinse is distilled water. This may not always be feasible. In this case a sample of the water (i.e., tap water) used to rinse the equipment and divers should be archived in case questions arise concerning contamination.

2. At the dock or immediately prior to the dive (i.e., on a live-aboard), divers should wash with 10% Liqui-nox in preparation for the collection, rinsing to remove all soap with distilled water. If distilled water is not feasible, then tap water may need to be substituted. If this occurs then an aliquot of the water is archived.

3. 200-250ml of the last rinse water should be collected and archived to serve as a control for any residual contaminants.

Collection of Coral Biopsies

A. General Considerations:

- Each site needs to be pre-mapped and colonies to be biopsied identified and the availability of appropriate sediment identified and those collection sites identified and a unique identifier determined. This should be done prior to the day of the collection

dive. This serves to expedite the collection underwater because the colonies and the order of collection has been pre-determined AND this allows the sample identifications to be known ahead of the dive so all collection equipment and sampling vessels can be pre-labeled.

- Operations should always move from "clean to dirty".
- Collection of samples is assumed to be conducted by SCUBA diving off a boat; however some sites may be amenable to shore-entry.
- There should be a minimum of three people for the collection, the two collection divers and the 'sample handler' to process the samples for stabilization and documentation. If there is more than one collection team in the water then a second sample handler is needed on the boat.
- The boat should be down-current from the collection site 50-100 m. The divers may use various means of moving to the site, e.g., swimming, dive kayak, scooter, flotation device equipped with small electric motor. Snorkelers may also be used to ferry samples from the collection site back to the boat for processing and stabilization.
- Divers need to re-clean themselves and gear between sites. This may be accomplished by coming back to shore or bringing duplicate equipment to change before diving at the second location.
- Based on the protocol evaluation, sediment samples should be taken first at the site and the jars returned to the boat for processing before the biopsy collections begin.

Sample Collection of Sediment for Organic Contaminant Analysis

The purpose for sediment sampling is for exposure documentation not concentration calculations. A minimum of 3 sediment samples will be collected from the coral tissue sampling sites in order to characterize the area occupied by the corals. For larger areas a maximum of 10 samples per site will be taken in as close proximity to sampled corals as possible, with approximate distances noted on the collector's datasheet.

Materials:

- Sediment Jars, 250-500 ml – Glass, EPA certified w/ Teflon lined caps
- Teflon bags with clip closures (alternate protocol)
- Teflon syringe or metal coring device with Teflon caps (alternate protocol)

- Nitrile gloves

Collection Procedures

The particle composition and size of sediment may have an effect on the inferences that can be made from the contaminant chemistry data resulting from a sample site. The types of sediment at the sampling sites may vary from site to site. Oftentimes, inexperienced or uneducated sampling teams will sample sediments containing large sized stones (e.g., 1 centimeter or larger). Particulate size within a sediment sample should be, on average, smaller than two millimeters. Stones larger than 1 cm should be excluded from the sample. Exclusion of large stones requires that the sediment be resampled in the lab; do not manually remove the stones from the sediment jar.

It is also suggested that the divers make note in the field book a description of the texture, color and scent of the matrix of each sediment sample.

16. Pre-label EPA approved pre-cleaned amber collection jars with the bar-coded labels provided in each case, however these labels require securing with clear packing tape because they will come off the jar when wet. As a precaution we have also place a secondary label on the jars and lids with cryo-labels that adhere with wetting as well as freezing. Securing labels with cellophane tape wrapped completely around the jars is also acceptable.

17. At each sampling site with sufficient appropriate sediment, place up to 10 EPA approved jars that have been filled with distilled water into mesh bag. Clean nitrile gloves should be used when filling the jars with distilled water. If you do not dive deeper than 15 m, the pressure on the jar should not break the jar. Do loosen the lid on the jar so that it can be opened easily underwater.

    An acceptable alternate collection device can be a modified Teflon syringe or a metal tube with Teflon caps (rinsed with acetone) to core approx 250 ml of the sediment. This is then placed into a Teflon bag and sealed with the bag clips. The Teflon bag is then placed into a labeled zip-lock or evidence bag.

18. Diver should wear nitrile gloves and change gloves between samples.

19. At depth, open jar above sediment, using regulator (if necessary) remove distilled water and core into the sediment approximately 2-5 centimeters and facilitate getting the sediment into the jar by scooping the sediment into the jar using the lid of the jar, taking care not to abrade the Teflon liner. Only collect the top 2-5 centimeters of sediment.

20. Try to ensure that the jar is completely filled with sediment, and contains little residual seawater.

21. At depth, cap jar with lid.

22. Prior to sediment samples arriving on the deck, ensure a cooler is in place at the work station that is large enough to hold the sediment samples being collected and that it is filled half-way with ice. If ice is not available use blue-ice packs to chill the samples.

23. The snorkeler (or diver) brings the sediment samples to the boat and transfers custody to the sample handler.

24. The sample handler next puts on a clean pair of nitrile gloves and removes sediment jars from the mesh dive bag.

25. The samples are then inspected to ensure the pre-label of the jar corresponds with the sample identification sheet and chain of custody sheet and the sample is then chilled. Place jar in a cooler with normal ice – not DRY ICE. If Teflon bags are used, place sealed Teflon bag into evidence bags. Tamper-proof evidence bags are provided to be used to as a secondary container to cushion the sample during transit. These bags have unique numbers which can also be used to uniquely identify the sample. If these are used then the chain of custody or data records should reflect this additional labeling.

26. When the sediment has settled, open the jar and decant the excess seawater carefully so as not to disturb the sediment and replace the lid. Place jar in the pre-labeled evidence bag and sign the label and place the sediment into the ice filled cooler.

27. Repeat this process for each sediment jar. Remembering to have separate chain of custody paperwork for different collection teams.

28. REMOVE YOUR NITRILE GLOVES.

29. When collection is completed, seal cooler with evidence tape and chain of custody tags. The evidence custodian should sign across the evidence tape and secure the samples in a way that they will be able to testify that the samples were not tampered with while in their custody. Once approval is given, ship the cooler to the lab designated for these samples (e.g., TDI Brooks). If the samples are not frozen, the cooler must arrive at lab in less than 1 week.

30. If the samples require holding prior to shipping, place the sediment jars in a -20°C freezer. BE SURE TO LEAVE SUFFICIENT SPACE IN THE JAR FOR EXPANSION OF THE SAMPLE TO AVOID BREAKING THE JAR AS THE SAMPLE FREEZES.

31. If desired, grain size distribution for each sediment sample may be determined so that contaminants analysis results can be normalized against this grain size distribution. Grain size should be determined either by sieve fractionation or by gravimetric pipetting. The major fractions of particle distribution (based on the Wentworth scale) include the following classes:

    a. cobble (-6 phi and above)

    b. gravel (-2 phi to -5 phi)

    c. sand (+4 phi to -1 phi)

    d. combination of both silt and clay (+5 phi and below)

32. Chain of Custody

The chain of custody should be pre-printed with all the samples to be collected listed on the sheet with the associated collection team and snorkeler. Once the collection team completes their collections, the team lead will sign the appropriate chain of custody associated with their team. The team lead also documents in their log the underwater collection team and snorkeler that handled the samples

To make this run smoothly, sites, sample designations and collection teams need to be determined ahead of time. If for some legitimate reason sample identifications cannot be predetermined, then extreme care must be given to labeling collection kits and sample bags or jars on the boat. Detailed records and quality control by an observer needs to be implemented to avoid mislabeling of samples. At no time should a non-Trustee representative handle any samples.

Each sample collected by this team of the same individuals can be grouped onto one chain of custody form by listing each individual piece of evidence as a batch. These will then move as a batch under one chain of custody. If the complement of the collection team changes, this dictates a new chain of custody for those samples. In other words, there is one chain of custody per team of divers, snorkelers and sample handlers.

Once the group of samples has been placed into custody, they will be transported only by approved carriers that ensure they are stored in a secure manner to maintain and ensure the integrity of the chain of custody. The instructions for shipping should be provided by the SWC working group leader.

Collection of tissue biopsies

1. Once collection divers have completed sediment sampling, they are given a biopsy kit from the sample handler. This bag contains two pair of nitrile gloves (An alternate strategy is for the divers to place multiple gloves on just prior to entry and remove a pair after each sampling) and a Ziploc bag that has been pre-labeled with the colony designation to be sampled. Ideally these will be in the order predetermined in the mapping phase.

2. When the divers reach the colony, they should open the first sampling bag put on nitrile gloves and verify the label on the Ziploc corresponds to the colony tag number or other designation being sampled.

3. Remove the drive punch/dissecting probe from its protective covering; the drive punch and the hammer are taken by the lead diver.

4. The wing diver will handle the Teflon bag, the sealing clip and camera to assist the primary collector as necessary.

5. Once at the target site on the colony, the lead diver will place the drive punch on the apex of the coral colony, if it is a mounding coral. The apex of the coral is the area of the coral that is perpendicular to the surface of the water, and should receive the greatest amount of incident light. Do not collect the biopsy from the coral edge, as you may collect noncoral tissue, thereby introducing significant artifact into the study. For *Acropora palmata*, the goal is to sample at the base of the 'palm' branch where there is support from the colony's base, thus reducing the risk of breaking a branch. Each of the replicate samples should be taken from an adjacent or similar location on the colony.

6. With several taps with the hammer, the drive punch should be driven into the colony about 1 cm or the depth of the coral's polyp. The diver must go deep enough into the coral colony so that the biopsy contains all visible tissue. For most boulder corals, the tissue can extend into the skeleton from 1 mm to as much as 10 mm. If boring sponge is present, try not to collect the sponge with your biopsy – resample from the same coral colony, or sample from a different coral colony. The coral biopsy is likely to remain in the drive punch. The exact procedure to remove and place the biopsy into the Teflon sample bag depends on the dive team and their preference.

7. The lead diver may hand the drive punch that contains the biopsy to the wing diver. The wing diver, using the dissecting probe, pushes out the coral biopsy into a Teflon bag. If more comfortable, the lead diver may use the probe themselves to push the biopsy into the Teflon bag being held by the wing diver. A minimum of three biopsies are collected per colony. The three biopsies can all be placed in one Teflon bag. The Teflon bag is sealed with the clip and then placed into a Ziploc bag that has been labeled. THIS IS

CRITICAL because you cannot write on Teflon, so it is imperative that the tissue bag is returned to the correctly labeled Ziploc. Therefore it is advised that each kit is in one mesh bag, however this is not mandatory.

8. Wing diver will then return the drive punch to the lead diver.

9. A newly cleaned drive punch/probe will be used for each colony sampled.

10. Biopsy lesions are photographed in a front-on plane using a fixed distance metric that is included in the photograph and colony identifier. This will serve as the initial basis for the lesion regeneration assay, which will require follow-up photo-documentation to calculate healing/regeneration rates. These are characteristic for a given species.

11. The lesions will need to be re-photographed at an interval that is appropriate to the species. For example *A. palmata* in healthy habitats have been reported to heal within 2-4 weeks, while *Porites astreoides* is slower growing and is more likely to need monthly – quarterly photos taken to calculate lesions regeneration rates.

Field Sample Processing

1. During the dive interval, the sample handler should organize dive bags with biopsy kits to hand off to the snorkeler to deliver to the sampling team.

2. When coral tissue samples arrive at the surface, the sample handler should attempt to quickly work in a shaded area to conduct the processing to stabilization steps.

3. The sample handler puts on a clean pair of nitrile gloves prior to opening the sample bag. Only one bag is processed at a time. The time between sampling and freezing tissues should be less than 20 min.

4. The Ziploc bag is first opened and the Teflon bag containing tissue biopsies is retrieved. The Teflon bag is opened by removing the clip closure and the seawater poured from the bag with any remaining water removed by pressing the Teflon bag to force the water out (i.e. squeezing smoothing the bag forcing the water out at the open end). There are two methods to accomplish removal of water, 1) pour samples out into your hand and carefully shake the samples to gently flick off residual water or 2) leave the biopsies in the bag, if the seawater can be removed by pressing out of the bag. Removing seawater is an important step for down-stream analyses, so care should be taken to remove as much water as possible while working quickly to move to the next step. If samples are intact, use method 1; if broken then method 2 is suggested. Once the seawater is removed the samples are returned to the Teflon sampling bag, re-seal with the closure clips. This is then placed into a tamper-proof evidence bag and

documented on the sampling sheet and chain of custody form. If official evidence bags are used, the bag should be labeled with the colony designation, signed and the unique number of the bag notated on the sampling sheet. If these are unavailable, an alternate container that is resistant to breakage in liquid nitrogen will need to be used as a secondary container carrying the sample identification number. Use muslin mail bags that have write-on labels inside the bags, or wrap the Teflon bags in aluminum foil (pre-washed with acetone) with a label written in pencil on water proof paper inside the wrapping. There may be other materials that can withstand liquid nitrogen freezing that can be used. This, however, is not ideal and measures need to be taken to maintain the integrity of the sample identification labeling.

5. The configuration of each size and type of dry shipper is different. Be familiar with the dry shipper you will be using, its proper operation, its charge duration, and precautions about mishandling that can cause loss of the charge and warming of the samples, which will destroy them. You will likely need to roll the evidence bag to its smallest size to place it into the dry shipper canister to optimize the number of samples that can be placed into the dry shipper.

6. Establish and maintain the chain of custody as described for the sediment samples above.

7. Remove gloves and dispose of them. Repeat the process from step one with the arrival of each sample bag.

SUMMARY and QUICK REFERENCE

- Document each sample fully.
- DO NOT cross contaminate samples by working on more than one colony's samples at a time. Put on clean nitrile gloves with each biopsy set
- Remove the Ziploc bag from the coral sample mesh dive bag
- Open Ziploc bag & remove Teflon bag containing the coral tissue biopsies
- Remove clip closure from the Teflon bag and empty bag of seawater and excess seawater from tissue biopsies
- Return tissue to the Teflon bag and seal
- Place Teflon tissue bag into a secondary labeled container that is resistant to liquid nitrogen freezing (i.e. doesn't shatter)

- Seal the evidence bag and fill out required information as noted on the bag and sign the evidence bag.
- Roll evidence bag with contents to minimal size and place into dry shipper.
- Remove nitrile gloves and dispose of them.
- Repeat with each new tissue sample.

Documentation

Ideally, the sample handler will be accompanied by an assistant that can document the process as well and help the sample handler maintain continuity of the procedures. The documentation or field notes should be entered into a bound notebook with pages numbered and dated. It is recommended to note the time of different activities. The documentation should include notes related to any deviations from the written and accepted protocol, descriptions of samples and activities conducted during a particular noted time period and any additional descriptions of conditions or observations related to the samples or the sampling process. For example: at the beginning of an operational cycle, the day and start time should be noted along with the name of the person making the log entry. A brief description of the activity: This may include a description of a test, such as testing the integrity of the evidence bag when placed in liquid nitrogen, documenting that the sample handler performed each step in the protocol to which they were assigned, or description of samples sets entered into a chain of custody.

Laboratory Analysis of Coral Biopsy Samples

Although the determination will be made at a later date regarding what, if any, analyses of coral biopsy samples will be undertaken, the Trustees and BP agree that the coral biopsy samples will be transported by the coral biopsy lead, Cheryl Woodley, to the NOAA Forensic Lab in Charleston, SC, rather than to TDI Brooks. This exception is necessary due to the unique expertise of Dr. Woodley's laboratory, also in Charleston, and the relative instability of coral biopsy samples once they have been stored cryogenically, causing later long-distance transport to put the samples at risk.

Objective 2: Document baseline/pre-impact condition of shallow-water corals in the Florida Middle Grounds.

Hydrocarbon analysis is a part of the protocol for Objective 2; however, only the costs of data collection are included in the budget. Costs associated with laboratory analysis for hydrocarbons are not included in the budget.

Background

The Florida Middle Grounds (FMG) is series of reefs in the form of banks, pinnacles, and knolls rising from a carbonate platform in the NW Florida shelf. The platform lies at the depth of about 45 to 50 m, and the reefs rise to the depth of 24 to 30m (Picture 1). According to previous studies, scleractinian coral fauna is depleted in comparison to normal Caribbean reefs; coral cover is patchy, and reefs are dominated by sponges, octocorals, and the hydrocoral *Millepora alcycornis*. There have been 16 to18 scleractinian coral species reported on the FMG (Grimm and Hopkins, 1977; NOAA CRCG, 2002). During a survey of the FMG in 2002, maximum cover by *Millepora alcycornis* was about 32% at Site FMG 491, and maximum cover of scleractinian corals was13% at Site FMG 251 (NOAA CRCG, 2002).

Patches of monospecific dense coral growth of few square meters are present in some locations at the break of reef tops; patches can be formed by *Madracis* species or *Porites porites* (Shinn; Ruzicka; personal communications). Neither of the major reef building species, *Acropora palmata* and *Montastraea* spp, are present in the FMG. The only common reef corals recorded within stations were *Dichocoenia stokesii*, *Porites porites, Stephanocoenia intersepta*, and *Siderastrea siderea* was recorded outside of the stations (NOAA CRCG, 2002).

Scleractinian corals of the FMG mostly include those typically found in semicryptic, deeper reef, and temperate/subtropical hardbottom environments. Species includes *Madracis* spp, *Agaricia fragilis*; *Scolymia spp.*, *Oculina* spp., *Phyllangia americana*, and *Cladocora arbuscula*.

The fauna of octocorals at the FMG is common for the Caribbean and is relatively diverse (about 15 species) and abundant in contrast to shallow-water hardbottom communities of southwest Florida. Typical shallow-water hardbottom in southwest Florida supports only a few octocoral species, and octocorals are absent on the Flower Garden Banks, TX. In general, reef communities of the FMG banks are dominated by algae, sponges, octocorals, and hydrocoral *Millepora alcycornis* (NOAA CRCG, 2002).

According to Bullock and Smith (1979), the conversion of water masses from the NW Florida shelf and tropical waters of the Loop Current creates a situation of high water column productivity in the area of the FMG. These observations of influence by water bodies outside the FMG, with the Loop Current influence, indicate the possibility that oil from the Deep Water

Horizon event may reach the FMG reefs with the Loop Current. The intensive mixing processes of the upper layer of water in the Gulf of Mexico in the area of the FMG increase the potential risk of impact to the reefs. We do not have information about the current status of these reef communities. Therefore, it is important to conduct a baseline survey in the FMG as soon as possible.

Methods

The proposed sampling protocol is subject to modification after the initial field reconnaissance and during the field work if the modifications would result in higher quality or more extensive information.

Setting of sites and stations

The sampling will be conducted by SCUBA divers at water depths of 25m to 35 m, using SCUBA (NITROX and/or air). Up to seven sites will be sampled which approximately correspond to the seven locations surveyed during the NOAA CRCG 2002 Habitat Characterization of Pulley Ridge and the Florida Middle Grounds (NOAA CRCG, 2002). These sites are being revisited for this sampling effort because the living coral cover on the Middle Grounds is rather scattered and patchy and these sites were selected for the previous habitat characterization study on the basis of their relatively high concentrations of stony corals for this region.

Each sampling unit will be a site consisting of two stations. Each station will consist of two 25m-long transects spaced 1m from each other, and 25 m^2 rectangle formed by these two transects. Permanent stations will be established in order to achieve high repeatability of monitoring surveys. Biased allocation will be used for establishment of the monitoring station. Each station within a site will be set in such a way that it will include the areas with highest cover of scleractinian corals. Most of the coral growth is concentrated in the areas at or near the break from the relatively flat tops of the banks to the steeper outer slopes. These are the areas which will be sampled during the survey. Coordinates of the seven sampling sites are given below in Table 4; however, the exact location of sites and stations will be established based on the initial reconnaissance at each location.

Video survey

Standard CREMP/SECREMP protocol will be used for the video surveys, data processing, and QA/QC procedures (general information: http://research.myfwc.com/features/view_article.asp?id=30988; data processing: http://spinner.cofc.edu/~coral/pc99/pc99.htm?referrer=webcluster&; or later development of this method in NOVA Southeastern University: http://www.nova.edu/ocean/cpce/ ; Kohler and Gill, 2006). This protocol provides quantitative characteristics of coral reef communities through an estimation of cover by major benthic groups (macroalgae, turf algae, scleractinian corals, octocorals, and sponges) and open substrate (hardbottom, sand).

A variety of additional information can be obtained from the analysis of recorded video and frame-grabbed images if needed (e.g. coral abundance). The set up of the station will be different than CREMP due to the deeper water depth and the character of the task (described below).

Video surveys will be conducted using a digital video camera set to fully automatic operation, maximum wide angle, and "progressive scan" mode. The recording will be taken from a permanently-maintained distance of 40 cm above the bottom using convergent laser guidance or other similar devise used to assist the maintenance of a permanent distance from the bottom. The video operator shall swim along each transect with a speed of no more than 5m/min. The width of the recorded belt will be about 40 cm and thus the entire recorded area for the station would be 20 m^2.

Fate tracking of representative scleractinian coral colonies

Larger individual colonies of scleractinian corals and hydrocoral *Millepora alcycornis* within or outside of the monitoring station will be designated for photo monitoring (up to 5 colonies per station). Colony size (length, width, height) and colony condition (presence of bleaching, disease, etc.) will be recorded *in situ*. Colonies will be tagged, and photos of the colonies will be taken from a standard distance using a digital camera attached to a PVC framer (0.5 m^2). Date and colony tag numbers will be included within each image using special display.

The framer allows all images from each monitoring event to be a consistent planar view of the colony. These consistent planar view images permit changes in tissue area between monitoring events to be measured. NCRI developed software (Coral Point Count with Excel Extensions, CPCe, http://www.nova.edu/ocean/cpce/index.html) (Kohler and Gill, 2006) is used to trace the tissue area (cm^2) in each colony planar image. The software automatically calculates the area (cm^2) encompassed by the traced portion of the image. Such calculations will be used for the analysis of the status of corals, including a ratio of life tissue versus dead; diseases, lesions, physical damage, and bleaching.

In situ sampling of benthic communities

Quantitative *in situ* data on scleractinian corals and octocorals [species diversity, abundance, class sizes of scleractinian corals (recruits <3cm; 3-5cm; 5-10cm; 10-25 cm; >25 cm) and octocorals (<5cm; 5-10cm; 10-25 cm; 25cm-50cm; >50cm)], description of colony status/health (diseases, signs of stress, bleaching) will be recorded using a 1-m wide belt transect for a distance of up to 25 m (25 m^2) at each sampling location. Scleractinian coral recruits (less than 2 cm in diameter) will be identified and counted within the first 10 m of each the 25-m belt transects.

Fish sampling

A third diver of the surveying group, working with buddies doing benthic assessments, video, and photo surveys, is responsible for collecting data on the fish using a belt-transect visual census technique, assessing over an area of 100 m^2 (25 m length x 4 m width), swimming along one of transect stations, and using the transect line as a centerline for the survey. Horizontal visibility at each station must be at least 2 m to allow for identification of fish in the 4 m wide belt.

To identify, enumerate or locate new individuals of fish, the surveyor may move off the centerline of the transect as long as he/she stays within the 4 m transect width and do not look back along area already surveyed. The surveyor is allowed to look forward toward the end of the transect for the distance remaining (i.e., if the surveyor is at meter 15, he/she can look 10 m distant, but if he/she is at meter 23, he/she can only look 2 m ahead).
On-site, no attempt to avoid structural features within a habitat such as a sand patch or an anchor should be made as these features affect fish communities and are "real" features of the habitats. The transect should take 15 minutes regardless of habitat type or number of animals present. This allows more mobile animals the opportunity to swim through the transect, thus standardizing the samples collected to allow for comparisons.

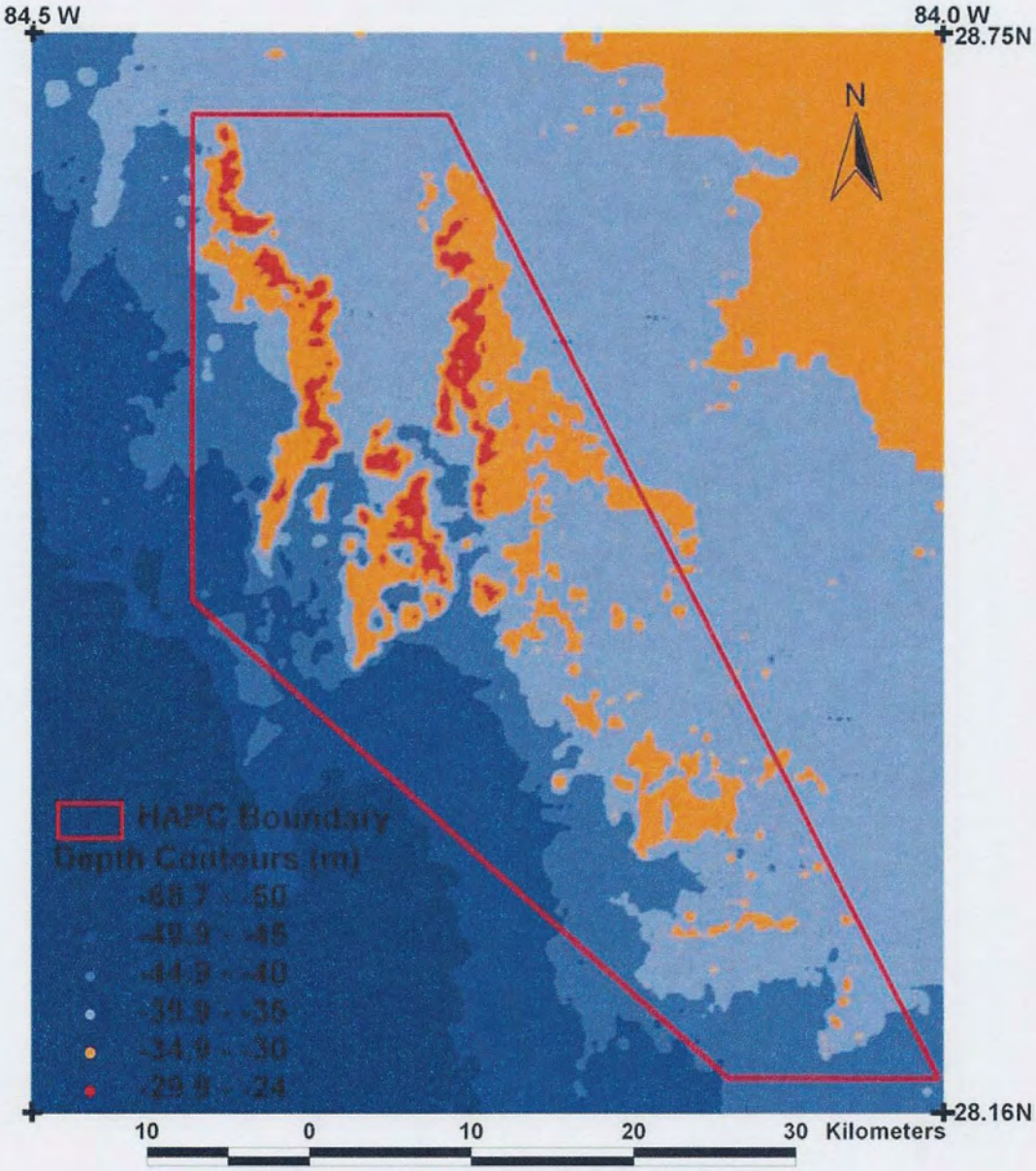
Data are collected on the following:

1. Identification - as the surveyor swims at a relatively constant speed, he/she records all fish species to the lowest taxonomic level possible that come within 2 m of either side of the transect and towards the end of the transect. To decrease the total time spent writing, four letter codes are used that consist of the first two letters of the genus name followed by the first two letters of the species name. In the rare case that two species have the same four-letter code, letters are added to the species name until a difference occurs. If the fish can only be identified to the family or genus level then this is all that is recorded. If the fish cannot be identified to the family level then no entry is necessary. Individuals too difficult to identify or unique in some manner may be photographed for later clarification.
2. Abundance and size - the number of individuals per species is tallied in 5 cm size class increments up to 35 cm using visual estimation of fork length. If an individual is greater than 35 cm, then an estimate of the actual fork length is recorded.
3. Logistic information - diver name, dive buddy, date, time of survey, site code, transect bearing.

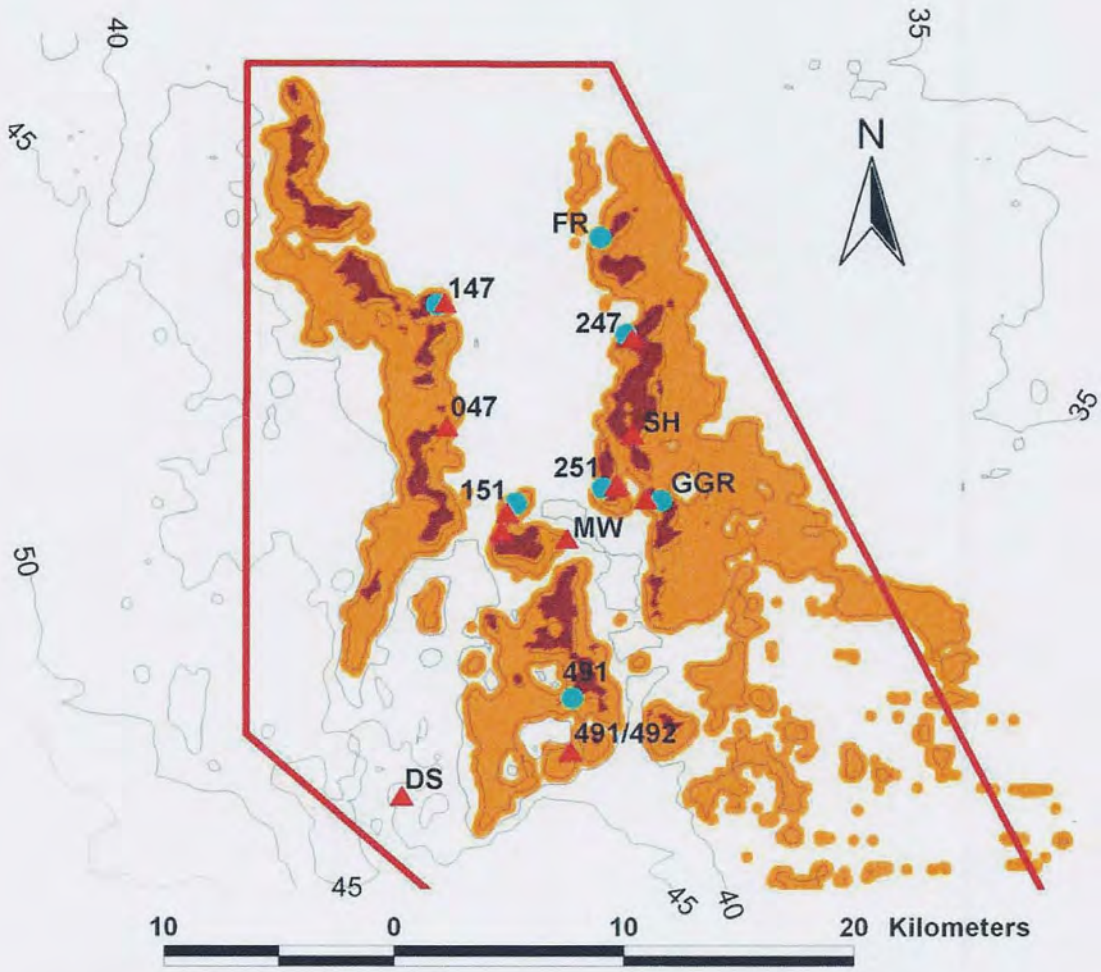
Table 4. Florida Middle Ground SCUBA sampling sites, May 2003. Sites with the prefix'Hopkins' were sampled in the 1970s by Hopkins et al. (1981). Summary of information on SCUBA dives by location including date, location, and survey time. (Table 3 from Final..., 2004)

Dive#	Location	Date	Latitude	Longitude	Video Time (min)
1	Hopkins 147	5/21/2003	28.61293	-84.33972	25.8
2	Hopkins 247	5/25/2003	28.60113	-84.26570	13.8
3	Hopkins 251	5/25/2010	28.54047	-84.27473	13.8
4	Hopkins 151	5/26/2010	28.53460	-84.30868	25.3
5	Hopkins 491	5/26/2010	28.45772	-84.28653	12.0
6	GGR	5/27/2010	28.53572	-84.25195	13.6
7	FR	5/27/2010	28.63960	-84.27593	28.7

GGR = Goliath Grouper Rock; FR = Fish Rock



Map 2. Relief and location of Florida Middle Grounds (Figure 3 from NOAA CRCG, 2002).



Map 3. Sampling locations at the Florida Middle Grounds during the 2003 cruise. Red triangles indicate ROV sites and light blue circles SCUBA sites. (Figure 4 from NOAA CRCG, 2002)

Water quality monitoring sampling with Semi-Permeable Membrane Devices (SPMD's)

Water quality monitoring utilizing the SPMD system will be used for documenting possible presence of certain hydrocarbons in the water passing the FMG. SPMD is designed to sample lipid or fat–soluble (nonpolar or hydrophobic) semivolatile organic chemicals from water and air. The SPMD is an integrative sampler which provides a time-weighted average concentration of sampled chemicals over a deployment period ranging from days to months. SPMDs sample chemicals from dissolved phase, mimicking only the bioconcentrations of organic contaminants coming from sea water into fatty tissues of organisms. SPMDs have been accepted for use by several federal agencies (e.g. USGS; US EPA; National Park Service and the US FWS).

Special containers with three standard SPMDs will be deployed at each station. The container will be secured 0.5 m above the bottom on the stainless steel rod (marker for the monitoring station). The recovery of SPMDs is targeted for 28 days of exposure.

Collected SPMD's will be preserved and shipped according to requirements (http://wwwaux.crc.cr.usgs.gov/SPMD/; Huckins et al., 2002) for extraction and then to a designated laboratory for analysis. Costs of analysis are not included in the budget of this Tier 1 project.

Items required for SPMD deployment or recovery:

- Gallon cans containing SPMDs
- Small cans that contains single field blank SPMDs.
- Pint-sized can with pre-cleaned metal spacers (not always included)
- Deployment canisters
- Alconox or Liquinox for washing hands
- Data sheets
- Church key to open cans
- Tie wraps for deployment
- Wire clippers for recovery

Important to know:

- Keep SPMDs in freezer or on ice until time of deployment preparation.
- Conduct the deployment steps with **clean hands**.
- Select a location where air contamination is least likely (e.g. open air upwind of any ship exhaust, inside the cabin – turn ship/boat engines off, if possible) for deployment prep and deployment. SPMDs can adsorb contaminants from the air as well as water.
- Handle SPMDs quickly and carefully, minimizing air exposure.
- Expose field blank SPMD during all deployment and retrieval procedures.

Procedures for deployment:

9. At deployment site, unscrew nut securing lid of deployment canister. (Notice the rod in the center of the canister. The "spiders", which carry the SPMDs, will slide onto this rod.)
10. Using a church key, work open the gallon can containing the SPMDs on "spiders." (Save this can to return the SPMDs in. Avoid using a screwdriver etc. in place of a church key as it may damage or deform the lid.) Cans must be labeled according to the site and re-sealed tightly for re-use.

11. Open the smaller can containing one loose SPMD. This is your field blank. It will remain in its can and should be exposed to the same surface conditions as the SPMDs to be deployed. Once the SPMDs enter the water, this can should be immediately resealed and placed in the freezer. It will be opened again during the retrieval process.
12. With clean hands, grasp the spiders by either the metal plate or center post and lift it slightly. Do not touch the SPMD (the membrane)—it could ruin the project. Notice that there is a metal plate that has flaps that are bent upward. These flaps allow the carrier to slide out of the can diagonally. Gently turn and pull the carrier out of the can, taking care not to touch, damage, or abrade the membrane.
13. While holding the spider, slide it into the deployment canister. Make sure the threaded rod in the canister runs up through the spider's center post. The spider may be placed in the canister plate up or down.
14. Place a spacer onto the canister rod then repeat steps 3 and 4 to add the four
15. additional spiders/SPMDs, placing a spacer between them and after the last one (if available).
16. Place lid on deployment canister. Be sure that the mounting ring on the side of the canister matches up with the mounting ring on the lid. These rings must be fastened together to fully secure the spiders/SPMDs inside.
17. Deploy canister, closing the lid on the field blank once the deployment device enters the water. (Deployment is best if SPMD canister can be attached to something upright, up to a half meter above the bottom. This reduces sediment abrasion and movement. Attachment can be with large tie wraps or other secure means.)

For retrieval, the above procedures can essentially be followed in reverse. A rubber mallet works well for resealing the can lids. Once retrieved, the SPMDs must again be kept in a freezer or on ice.

Procedures for retrieval:

7. The diver clips the tie wraps holding the SPMD canister. The canister is simply brought to the surface and passed to surface support for disassembly. Remember, to expose the field blank whenever the SPMDs are exposed to air.
8. When the canister surfaces and is exposed to air, the top is removed from the can containing the blank. Note time on the data sheet. As quickly, but carefully as possible, the field canister is opened (cut tie wrap, remove butterfly nut, and canister top). There are five SPMD/spider assemblies and four spacers (if available) inside. SPMD/spiders should be gently lifted out, quickly inspected for tears, and placed one on top of the other inside the gallon can. They may need to be tilted to insert into the can. **The membranes should not be touched**. Place spacers in pint-sized can for reuse.

9. As soon as all five spiders are placed in the gallon can, the top of that can and the top of the can containing the field blank should be replaced and securely sealed (be careful not to bend lids, as the seal may be lost). Again, note the time on the data sheet.
10. All samples (blanks and SPMD/spiders) should be placed on ice until they are returned to shore. They should then be placed in a freezer until shipment.
11. Complete the data sheet and make two copies.
12. All samples will be shipped to EST (SPMD manufacturer) for initial processing prior to being transported to TDI Brook for analysis.

Place the gallon can (spiders) and the small can (blank) on ice (dry ice, if available), and along with a copy of the NRDA Trustee Sampling Form data sheet and Chain of Custody, send overnight.

Sample Collection of Sediment for Organic Contaminant Analysis at the Florida Middle Grounds

Sediment sampling is an additional method that can help detect the presence of oil at the sampling sites, and will be included in the protocol. The purpose for sediment sampling is for exposure documentation and not for toxicity or concentration calculations.

At all sites, sediment samples will be collected using glass, EPA certified jars (250-500 mL) with Teflon lids. Pre-labeled jars filled with distilled water will be carried by divers to the bottom and opened just above the sediment sampling location. Two samples will be collected from each study station (four samples per site). Samples will be collected in the upper 2-5 cm layer of the sediments. Sediments should be as finely grained as is available from the location selected, and should not contain any material coarser than sand. Sediments will be collected only at the survey station and only in case there are sediments of appropriate grain size are available.

Upon return to the surface, sample jars will be stored in a cooler on ice until they are transferred on shore to the lab. Chain of custody documentation protocols will be maintained throughout.

Sampling procedures

At each site, a buoy will be dropped at the coordinates provided in Table 1 above. Initial reconnaissance using a towed camera system will be conducted in the vicinity of buoy. Selection of the site will be adjusted by these observations and the buoy will be re-established.

A reconnaissance group of two divers will identify the position of the two stations (i.e. 25-m transects) within the site. As the position of each station is identified, the reconnaissance

divers will anchor buoys at the beginning and end of Transect 1 at both stations, and will stretch the measuring tape in between the buoys of the transect. The next group of four divers will be establishing permanent markers at the beginning and the end of the transects in both stations (cinderblocks with steel rods cemented into them, or by drilling hole and cementing a stainless steel rod into it). Next team of four divers will set up the station by placing a weighted line or chain along Transects 1 and 2. GPS coordinates of the beginning and the end of Transects 1 will be recorded. Transect 2 will be established one meter E of Transect 1 if the direction of transect is NS, or one meter N if the direction is EW.

After the establishment of the monitoring stations within the site, two trios of divers will conduct video, photo, benthic, and fish surveys within the two stations. Standard CREMP protocol will be used for video surveys.

While one diver is videorecording, the second diver will conduct the estimate of the diversity and abundance of scleractinian coral and octocoral species within the rectangle of $25m^2$ formed by two transects of a station. Larger individual colonies of scleractinian corals and hydrocoral *Millepora alcicornis* within or outside of the monitoring station will be designated for photo monitoring (up to 5 per station). Colonies will be tagged, and photos of the colonies will be taken from a standard distance using a framer ($0.5\ m^2$). The position of each colony relative to one of transects will be recorded and each colony will be tagged.

Survey Team and Equipment

The survey team will consist of fourteen to sixteen divers. The boat will be equipped with an air/NITROX compressor; a towed camera system; two or more digital video cameras with lights; three or more photo cameras with strobes; weighted line or light chain (four sets); meter sticks; cinderblocks with 0.5m steel rods cemented into them (30-35), and buoys in order to establish permanent stations. It is suggested to use crew of scientific divers with experience in similar coral reef surveys, and data processing/evaluation in Florida (Coastal Eco-Group and PBS&J). Crew comes with dive gear, video and photo equipment, station set up and survey gear.

All diving activities involved in this plan will fit the criteria for scientific dives (i.e. "...solely as a necessary part of a scientific...activity by employees whose sole purpose for diving is to perform scientific research tasks..." 29 CFR 1910.402). The team involved in diving under this Florida Middle Grounds Coral Tier 1 Plan will operate in accordance with institutional/agency diving protocols and under each institution's diving emergency plans (FDEP, NOAA, or AAUS {www.aaus.org}). For example, under NOAA operations, each team and operation has qualified and authorized divers involved, has an approved dive plan, has a Dive Accident Management Plan for the specific area of operations, conducts explicit daily dive briefing, and includes on site availability of emergency O2 (NOAA policies and regulations described in detail at http://www.ndc.noaa.gov/pdfs/Dive_regulations_and_policies.pdf). AAUS and contracted

teams will follow analogous protocols to ensure dive safety as prescribed by their home agency/institutions.

Objective 3: Document baseline/pre-impact condition of shallow-water corals in the East and West Flower Garden, Stetson, and Sonnier Banks.

Earliest Potential Cruise Dates: mid-June

Cruise duration: 10-12 days

Objectives

- **Baseline Demographics:** Determine baseline conditions for benthic and fish populations and key service indicators (e.g. colony condition, disease incidence).
- **Exposure:** Collect samples that may help determine whether benthic and near-bottom habitats of the banks have been exposed to hydrocarbons.
- Note: Fish data are proposed for two reasons. First, we are not aware of other groups planning to collect fish data from these banks, and will need pre-exposure data. Second, fish data collection protocols are already a component of the data gathering protocol for these banks, and divers who conduct the work are very experienced with the techniques. All data will be shared with the fish NRDA technical working group.

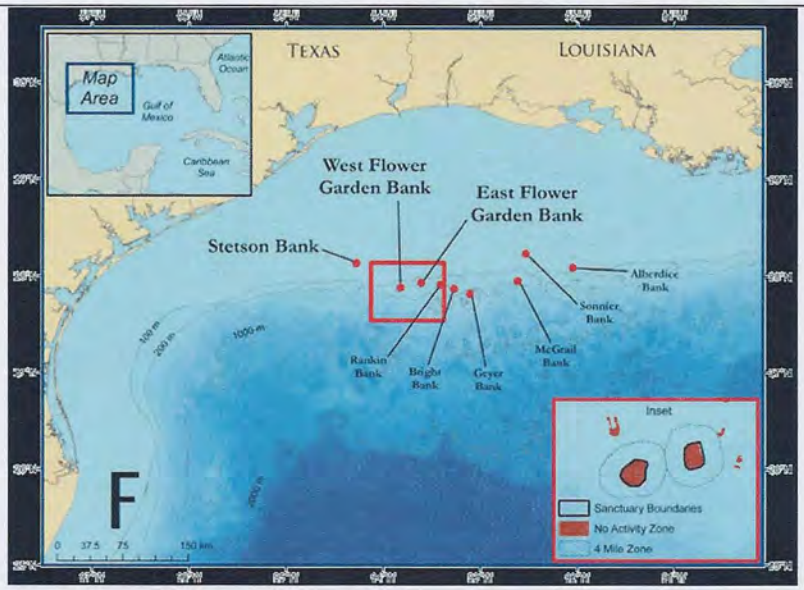
Approach

- Compile existing monitoring data from East and West Flower Garden Banks, through 2008, to establish baseline conditions.
- Use protocols of the long-term monitoring program at Stetson Bank to quantify populations and condition on both Stetson and Sonnier Banks.
- Collect sediment and semipermeable membrane device (SPMD) samples on all four banks and coordinate with other working groups to determine exposure regime of oil and oil/dispersant mixtures on the banks.

Introduction

Numerous topographic features in the northwestern Gulf of Mexico contain sensitive hard bottom or coral assemblages of tropical and subtropical origin (see Map 4). Several of these banks have summits above 30 m and contain reef corals and associated coral communities. The well known coral reefs of the Flower Garden Banks are widely considered to be among the healthiest reefs remaining in the western Atlantic. In 1992 they were designated the Flower Garden Banks National Marine Sanctuary. The coral reef zones of the banks have been

monitored for over 30 years, primarily to evaluate the effects of nearby oil and gas development facilities. Studies to date suggest that the lease stipulations designed to protect biological or other resources of concern in that region have been effective for that purpose. Coral cover has remained around 50%. Growth rates have not changed, and any significant changes in reef community structure have been traced to natural or human events unrelated to those of the oil industry.



Map 4. A subset of topographic features in the northwestern Gulf of Mexico. Those shown are being considered for addition to the Flower Garden Banks National Marine Sanctuary, and include the four proposed for investigations related to spill impacts.

Monitoring on Stetson Bank has occurred intermittently since the early 1990s. Conditions on that bank have changed considerably over the years, primarily attributable to the soft substrate of the bank, and the consequent effects of storms and fishing activities.

Sonnier Bank is the least studied of the banks, but is under consideration for addition to the Flower Garden Banks National Marine Sanctuary, which already contains the other three banks. Sonnier also appears to be subject to the forces of change that cause Stetson populations to vary so much. Both are much more dynamic than the Flower Garden Banks (Robbart et al., 2009).

There is no documentation that direct oiling has ever occurred on any of these banks. This may be partly due to the depth of the banks, the shallowest point on any of them being 18 m below the surface. Generally, floating oil is not considered a significant threat to the benthic communities at such depths. However, the addition of large amounts of dispersants, and dispersant use at depth, may result in conditions which do not meet these risk assumptions.

Assessment Approach

The demographic assessment plan described here uses protocols that are, for the most part, elements of past MMS and NOAA sponsored monitoring. For the Flower Garden Banks, data collected over many years, the latest being from 2008, will be used to determine baseline conditions for benthic populations, condition, and growth rates. Because of the historically stable population data on these banks, it is not considered necessary to collect another set of baseline data prior to the regularly scheduled monitoring later this summer. For Stetson and Sonnier Banks, data will need to be collected to determine current benthic conditions. For all four banks, sediment and SPMD samples will be collected to help determine background hydrocarbon presence.

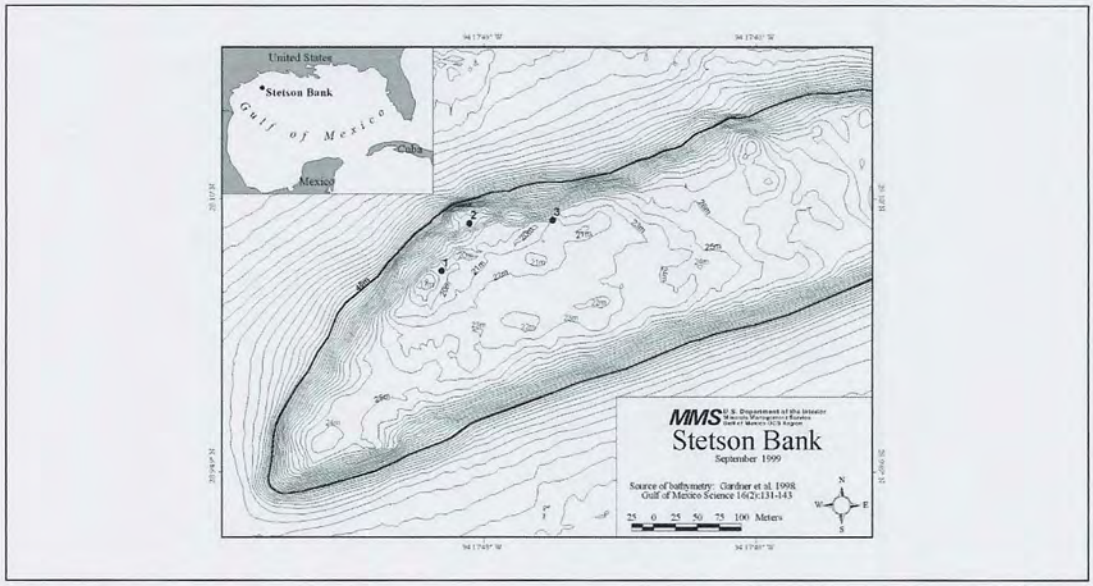
The initial field work will be performed during early summer of 2010 to establish baseline data for benthic conditions and contaminants. Exposure data will be collected in consultation with NRDA teams conducting water column and sediment sampling plans. A Tier 2 sampling plan will be developed to track changes in benthic conditions within and outside potentially exposed areas.

All sampling and survey work will be carried out with appropriate collecting permits in compliance with NOAA's National Marine Sanctuary Regulations and the FGBNMS Office. Dive operations will be conducted under the auspices of NOAA.

Scope of Work

Study Area

Study sites will include those used in the previous monitoring studies at the Flower Gardens and Stetson Bank (Map 5) and the shallowest habitat area on one of the pinnacles of Sonnier Bank. On the Flower Garden Banks, those are in areas of the higher-diversity, coral reef communities. These historical study sites are 100-meter by 100-meter squares located in less than 30 meters of water on the reef crests of each bank. Permanent corner pins drilled and cemented into the reef substrate mark the four (4) corners of each study site.



Map 5. Stetson Bank, showing the location of three mooring buoys at the study site.

The study site at Stetson Bank is between 20 and 30 m, between and including an area of claystone pinnacles that mark the shallowest part of the bank. At Sonnier Bank, the shallowest of the three pinnacles that characterize that bank will be sampled (called "Primary Peak" by Kraus et al., 2006 and 2007; see Figure 2). Sampling on this bank was conducted in 2004 and 2005, then after Hurricane Rita in 2006, an event that caused substantial change to benthic assemblages (dominated by algae, two sponge and one hydrocoral species; Kraus et al., 2007).

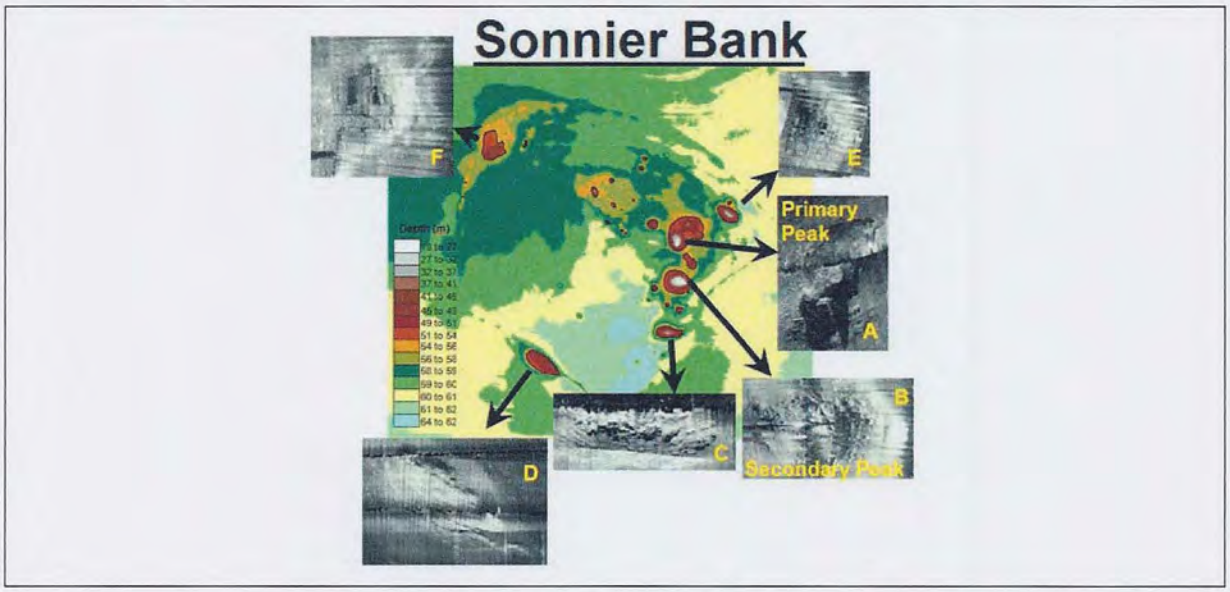


Figure 2. The peaks of Sonnier Bank (in red). The location marked "Primary Peak" is the proposed study site.

General Survey Overview

Water and sediment samples were recently (May 2010) collected from the Flower Gardens and Stetson Bank and are being analyzed by Anacon, Inc. in Houston, Texas. Water was taken from the surface, mid-water, and reef crest (two per depth using 5L Niskin bottles). Two sediment samples were taken from each bank. This collection was made as part of the ongoing long term monitoring project. They are not officially part of the NRDA action, but are part of a project funded through a discharge settlement (Rowan).

Sediment sampling is an additional method that can help detect the presence of oil at the sampling sites, and will be included in the protocol. The purpose for sediment sampling is for exposure documentation and not for toxicity or concentration calculations.

Initial sediment and SPMD samples from the Flower Gardens, Stetson, and Sonnier Banks, along with other baseline surveys will be performed as soon as possible following approval of this plan (presumably July 2010). Exposure assessments would continue as soon as modeling or observational data indicate that oiling is possible, or monthly, whichever is less. Monthly sampling may allow detection of exposure from subsurface plumes that modeling or surface observations may miss.

Sediment Sampling Protocol

For all banks, sediment samples will be collected using glass jars with Teflon lids. Pre-labeled jars filled with distilled water will be carried by divers to the bottom and opened just above the sediment sampling location. Three samples will be collected from each study site, each containing sediments from the upper 2-5 cm of the bottom. Sediments should be as finely grained as is available from the location selected.

Upon return to the surface, sample jars will be stored in a cooler on ice until they are transferred on shore to the lab. Chain of custody documentation protocols will be maintained throughout. More specific instructions are provided in Objective 1 of this plan.

Semi-Permeable Membrane Device (SPMD) Protocol

Protocols for SPMDs at the banks will be similar to that for the Keys. At one location on each of the four banks, SPMDs will be deployed for approximately four weeks (28 days) to provide integrated exposure data from the water column. SPMDs will secured to existing mooring locations approximately 50 cm above the bottom.

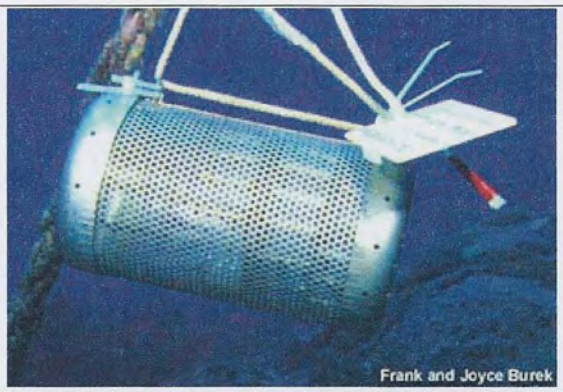


Figure 3. Semipermeable Membrane Devices (SPMD's) are housed in cages such as that shown here. An SPMD is a passive sampling device used to monitor for organic contaminants.

Items required for SPMD deployment or recovery:

- Gallon cans containing SPMDs
- Small cans that contains single field blank SPMDs.
- Pint-sized can with pre-cleaned metal spacers (not always included)
- Deployment canisters
- Alconox or Liquinox for washing hands
- Data sheets
- Church key to open cans
- Tie wraps for deployment
- Wire clippers for recovery

Important to know:

- Keep SPMDs in freezer or on ice until time of deployment preparation.
- Conduct the deployment steps with **clean hands**.
- Select a location where air contamination is least likely (e.g. open air upwind of any ship exhaust, inside the cabin – turn ship/boat engines off, if possible) for deployment prep and deployment. SPMDs can adsorb contaminants from the air as well as water.

- Handle SPMDs quickly and carefully, minimizing air exposure.
- Expose field blank SPMD during all deployment and retrieval procedures.

Procedures for deployment:

1. At deployment site, unscrew nut securing lid of deployment canister. (Notice the rod in the center of the canister. The "spiders", which carry the SPMDs, will slide onto this rod.)
2. Using a church key, work open the gallon can containing the SPMDs on "spiders." (Save this can to return the SPMDs in. Avoid using a screwdriver etc. in place of a church key as it may damage or deform the lid.) Cans must be labeled according to the site and re-sealed tightly for re-use.
3. Open the smaller can containing one loose SPMD. This is your field blank. It will remain in its can and should be exposed to the same surface conditions as the SPMDs to be deployed. Once the SPMDs enter the water, this can should be immediately resealed and placed in the freezer. It will be opened again during the retrieval process.
4. With clean hands, grasp the spiders by either the metal plate or center post and lift it slightly. Do not touch the SPMD (the membrane)—it could ruin the project. Notice that there is a metal plate that has flaps that are bent upward. These flaps allow the carrier to slide out of the can diagonally. Gently turn and pull the carrier out of the can, taking care not to touch, damage, or abrade the membrane.
5. While holding the spider, slide it into the deployment canister. Make sure the threaded rod in the canister runs up through the spider's center post. The spider may be placed in the canister plate up or down.
6. Place a spacer onto the canister rod then repeat steps 3 and 4 to add the ur additional spider/SPMDs, placing a spacer between them and after the last one (if available).
7. Place lid on deployment canister. Be sure that the mounting ring on the side of the canister matches up with the mounting ring on the lid. These rings must be fastened together to fully secure the spiders/SPMDs inside.
8. Deploy canister, closing the lid on the field blank once the deployment device enters the water. (Deployment is best if SPMD canister can be attached to something upright, up to a half meter above the bottom. This reduces sediment abrasion and movement. Attachment can be with large tie wraps or other secure means.)

For retrieval, the above procedures can essentially be followed in reverse. A rubber mallet works well for resealing the can lids. Once retrieved, the SPMDs must again be kept in a freezer or on ice.

Procedures for retrieval:

1. The diver clips the tie wraps holding the SPMD canister. The canister is simply brought to the surface and passed to surface support for disassembly. Remember, to expose the field blank whenever the SPMDs are exposed to air.

2. When the canister surfaces and is exposed to air, the top is removed from the can containing the blank. Note time on the data sheet. As quickly, but carefully as possible, the field canister is opened (cut tie wrap, remove butterfly nut, and canister top). There are five SPMD/spider assemblies and four spacers (if available) inside. SPMD/spiders should be gently lifted out, quickly inspected for tears, and placed one on top of the other inside the gallon can. They may need to be tilted to insert into the can. **The membranes should not be touched**. Place spacers in pint-sized can for reuse.

3. As soon as all five spiders are placed in the gallon can, the top of that can and the top of the can containing the field blank should be replaced and securely sealed (be careful not to bend lids, as the seal may be lost). Again, note the time on the data sheet.

4. All samples (blanks and SPMD/spiders) should be placed on ice until they are returned to shore. They should then be placed in a freezer until shipment.

5. Complete the data sheet and make two copies.

6. All samples will be shipped to EST (SPMD manufacturer) for initial processing prior to being transported to TDI Brook for analysis.

Place the gallon can (spiders) and the small can (blank) on ice (dry ice, if available), and along with a copy of the data sheet, send overnight.

Demographic Sampling at the East and West Flower Garden Banks

Baseline population data for the Flower Garden Banks already exist. Annual sampling for many years has resulted in a robust data set on coral populations, diversity, relative abundance, cover, growth rates, disease and bleaching incidence, sea urchin and lobster abundance, fish abundance and diversity, and water quality. Variability for coral parameters has been low over the years, so this information will be relied on and considered a baseline against which to compare post spill data.

Demographic Sampling at Stetson Bank

Monitoring on Stetson Bank has focused on assessments of community condition and cover. Repetitive photostations are established along the pinnacles of the bank, where the majority of scleractinian and hydrozoan corals occur. The following methods are used to measure community condition on the bank:

- Repetitive Quadrats: Currently there are approximately 45 pins in use. At each location a single photograph is taken using a frame that ensures exact positioning of the camera directly above the pin at a specified height above the bottom. Each photograph covers 1.6 square meters. Benthic cover data will be collected using the NCRI-developed software package Coral Point Count with Excel Extensions (CPCe) statistics (see http://www.nova.edu/ocean/cpce/index.html) (Kohler and Gill, 2006). The software uses random point overlays on photos or captured video images to guide an analyst, who quantifies taxa and benthic information under each point. The program then produces mean and variance estimates for abundance, cover, and species diversity, which can be compared over time using standard statistical comparison techniques.

- Video and Invertebrate Transects: Transects between mooring buoys are surveyed visually and with video at each study site. Visual surveys are done for sea urchins and lobsters. Video surveys are for characterization of the general condition of the benthic community, for incidence of disease, and for evidence of human disturbance.

- Stationary and roving fish surveys are performed to estimate community composition, density, and size. Roving surveys (Pattengill, 1998) have been conducted on Stetson Bank for over ten years and extensive data are available for comparison (see www.REEF.org). Stationary surveys have been collected in recent years using protocols described below.

Surficial sediment will be collected from the bank at three locations (three mooring buoys locations) and analyzed for hydrocarbons using protocols approved in the NRDA sediment collection protocol. Water column contaminants will be measured using an SPMD deployed at one of the mooring locations.

Sonnier Bank

At Sonnier Bank, there are currently no monitoring programs in place. We will use a protocol that has proven effective at the Flower Gardens for benthic and fish assemblages. It was developed by NOAA's National Centers for Coastal Ocean Science and is presented with detailed analysis in the report, *A Biogeographic Characterization of Fish Communities and Associated Benthic Habitats within the Flower Garden Banks National Marine Sanctuary*. It is based on a randomized design for site selection, then transect-based assessments of benthic

communities and associated fish assemblages. The individual methods are outlined below (modified from Caldow et al., 2009).

Site Selection and Benthic Sampling

The boat captain navigates to previously selected random locations using a handheld GPS unit. On-site, divers are deployed and maintain visual contact with each other throughout the entire census. One diver is responsible for collecting data on benthic composition. This diver follows the belt-transect (fish) diver and records data on small-scale benthic habitat composition and structure within a 1 m^2 quadrat divided into 100 (10 x 10 cm squares) at four separate positions along the transect. Each position is randomly chosen before entering the water such that there is one random point within every 6 m interval along the transect. Percent cover is obtained as if looking at the quadrat in a two dimensional plane (i.e., a photograph) versus three dimensions where percent cover could add up to greater than 100%. To estimate percent cover, the diver first positions the quadrat at the chosen meter mark along a randomly chosen side of the transect tape. The remaining quadrats are placed on alternating sides of the transect at the subsequent three locations.

Data are collected on the following:

- Logistics information - diver name, dive buddy, date, time of survey, site code and meter numbers at which the quadrat is placed.

- Habitat structure - to characterize the benthic habitats of the dive site, the habitat diver first categorizes the habitat structure of the site (high or low relief). This is done by quantification to the nearest 5% of the dominant coral and sponge forms within a 25 m radius of the transect starting point. High relief habitat is characterized by the dominance of pinnacle features or large coral colonies, while the low relief habitat is characterized by flat substrates or sometimes the dominance of low growing corals like *Madracis aurentenra*.

- Transect depth profile - the depth at each quadrat position. Depth is measured with a digital depth gauge to the nearest 0.3 m (1 ft).

- Abiotic footprint - defined as the percent cover of hard bottom, sand, rubble and fine sediments within a 1 m^2 quadrat. Rubble refers to rocks and coral fragments that are moveable; immovable rocks are considered hard bottom. The percent cover given as a part of the abiotic footprint should total 100%.

- Biotic footprint - defined as the percent cover (to the nearest 0.1%) of live corals, algae, sponges, gorgonians and other biota (tunicates, anemones, zooanthids and hydroids) within a 1 m^2 quadrat. The remaining cover is recorded as bare substrate to bring the total to 100%. The diver should use a planar view to estimate percent cover of the biota. Species covering less than 0.1% of the area are not recorded. Taxa are identified to the following levels: stony coral to species, algae to morphological group (macro, turf, crustose), two dominant sponges to species (*Neofibularia nolitangere* and *Ircina* sp.) and other sponges to morphological group

(barrel/tube/vase or encrusting). Macroalgae is defined as algae equal to or greater than 1 cm in height whereas turf is identified as a mix of short algae less than 1 cm high. For stony corals, the approximate area covered by living coral tissue is recorded. Coral skeleton (without living tissue) is usually categorized as turf algae or uncolonized substrate. Data on the condition of coral colonies are also recorded. When coral is noticeably bleached, the entire colony is considered affected and is recorded as bleached to the nearest 0.1%. Diseased/dead coral refers to coral skeleton that has recently lost living tissue because of disease or damage, and has not yet been colonized by turf algae.

- Maximum canopy height - for each soft biota type (e.g., gorgonians, sponges-except encrusting form, algae) the maximum height is recorded to the nearest 1 cm.
- Abundance and maturity of queen conchs (*Strombus gigas*) - conch encountered within the 25 x 4 m belt transect are enumerated. The maturity of each conch is determined by the presence or absence of a flared lip and labeled mature or immature respectively.
- Abundance of spiny lobsters (*Panulirus argus*) - a count of the total number of lobsters encountered within the 25 x 4 m belt transect.
- Abundance of long-spined urchin (*Diadema antillarum*) - a count of the total number of urchins encountered within the 25 x 4 m belt transect.
- Photos – Two photos are taken in opposite directions at each location to document the surrounding habitat. Additional photos may be taken to document disease, bleaching or other events of note.

Fish Sampling

Fish data will be collected due to efficiency of the divers being present at these remote locations. The data will be supplied to the Fish NRDA Technical Working Group as their process develops.

A second diver, working with his/her buddy doing benthic assessments, is responsible for collecting data on the fish communities using a belt-transect visual census technique over an area of 100 m^2 (25 m length x 4 m width). The belt-transect diver obtains a random compass heading for the transect prior to entering the water and records the compass bearing (0-360°) on the data sheet. Visibility at each site must be sufficient to allow for identification of fish at a minimum of 2 m away. Once reasonable visibility is ascertained, the diver attaches a tape measure to the substrate and allows it to roll out for 25 m while they are collecting data.

Although the habitat should not be altered in any manner by lifting or moving structure, the observer should record fish seen in holes, under ledges and in the water column. To identify, enumerate or locate new individuals, divers may move off the centerline of the transect as long as they stay within the 4 m transect width and do not look back along area already covered. The diver is allowed to look

forward toward the end of the transect for the distance remaining (i.e., if the diver is at meter 15, he can look 10 m distant, but if he is at meter 23, he can only look 2 m ahead).

On-site, no attempt to avoid structural features within a habitat such as a sand patch or an anchor should be made as these features affect fish communities and are "real" features of the habitats. The only instance where the transect should deviate from the designated path is to stay above 33.5 m (110 ft). The transect should take 15 minutes regardless of habitat type or number of animals present. This allows more mobile animals the opportunity to swim through the transect, thus standardizing the samples collected to allow for comparisons.

Data are collected on the following:

- Identification - as the tape roles out at a relatively constant speed, the diver records all fish species to the lowest taxonomic level possible that come within 2 m of either side of the transect and towards the end of the transect. To decrease the total time spent writing, four letter codes are used that consist of the first two letters of the genus name followed by the first two letters of the species name. In the rare case that two species have the same four-letter code, letters are added to the species name until a difference occurs. If the fish can only be identified to the family or genus level then this is all that is recorded. If the fish cannot be identified to the family level then no entry is necessary. Individuals too difficult to identify or unique in some manner may be photographed for later clarification.

- Abundance and size - the number of individuals per species is tallied in 5 cm size class increments up to 35 cm using visual estimation of fork length. If an individual is greater than 35 cm, then an estimate of the actual fork length is recorded.

- Logistic information - diver name, dive buddy, date, time of survey, site code, transect bearing.

Surficial sediment will be collected from the bank at three locations and analyzed for hydrocarbons using protocols approved in the NRDA sediment collection protocol. Water column contaminants will be measured using an SPMD deployed at a mooring location.

Tasking Leads

Flower Garden Banks

- Benthos - Data compilation – Flower Garden Banks National Marine Sanctuary

- Fish - Data compilation – Flower Garden Banks National Marine Sanctuary

- Sediment - Flower Garden Banks National Marine Sanctuary, samples to TDI-Brooks

- SPMDs - Flower Garden Banks National Marine Sanctuary, samples to Environmental Sampling Technologies (SPMD technology, covering both assembly and dialytic recovery,

is the subject of two US Government patents and one Canadian patent that are exclusively licensed to Environmental Sampling Technologies, Inc.)

Stetson Bank

- Benthos – Flower Garden Banks National Marine Sanctuary
- Fish - National Centers for Coastal Ocean Science, Biogeography Group
- Sediment - Flower Garden Banks National Marine Sanctuary, samples to TDI-Brooks
- SPMDs - Flower Garden Banks National Marine Sanctuary, samples to Environmental Sampling Technologies

Sonnier Bank

- Benthos - National Centers for Coastal Ocean Science, Biogeography Group
- Fish - National Centers for Coastal Ocean Science, Biogeography Group
- Sediment - Flower Garden Banks National Marine Sanctuary, samples to TDI-Brooks
- SPMDs - Flower Garden Banks National Marine Sanctuary, samples to Environmental Sampling Technologies

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Appendix – Budgets

This budget represents only those costs that are specific to the implementation of this sampling plan. This budget excludes Trustee labor and contract employee labor costs, which will be documented and accounted for at a later time as part of the Trustees' assessment costs.

This Tier 1 Plan does not include cost of analyses for tissues or sediments. These costs will be determined by the approved laboratories.

Budget for FRRP surveys:

Personnel:

The FRRP DRM will be managed and coordinated by The Nature Conservancy under the direction of the NRDA Shallow Water Corals Technical Working Group. Mote Marine Lab, University of Miami and Nova Southeastern University provide the sub-regional coordination. Survey teams consist of professional scientists from NOAA/Florida Keys National Marine Sanctuary, Florida Fish and Wildlife Conservation Commission, Florida Fish and Wildlife Research Institute, Florida Department of Environmental Protection, Miami-Dade Department of Environmental Resource Management, National Park Service, and Broward County Environmental Protection and Growth Management Department.

TNC Expenses:

BUDGET CATEGORIES	
a. Personnel	7,889
b. Fringe Benefits	3,313
c. Travel	1,200
d. Equipment	-
e. Supplies	3,000
f. Contractual	62,850
g. Construction	-
h. Other	-
i. Total Direct Charges (sum of a-h)	78,252

j. Indirect Charges (@ 23.13%)	18,100
k. Totals (sum of i and j)	96,352

TNC subcontracts from above:

Sub-Contractor	Amount	Includes
Mote Marine Lab	$14,850	Science Staff, Boat time, fuel, travel, dive tanks/gear, Sub-regional coordination, data entry and QA/QC
University of Miami	$20,000	Science Staff, Boat time, fuel, travel, dive tanks/gear, Sub-regional coordination, data entry and QA/QC
Nova Southeastern University	$20,000	Science Staff, Boat time, fuel, travel, dive tanks/gear, Sub-regional coordination, data entry and QA/QC
Ken Marks	$8,000	Management, Data QA/QC

Budget for CREMP Sediment Collection/SPMD Deployment

Travel

Pre-Assessment Sampling:

Travel for fieldwork for CREMP to and from coral research group's headquarters at FWRI, St. Petersburg, Florida, to Upper and Lower Keys regions. Two 14 day stays (July 6th-20th and August 1st- August 15th) are planned. Seven FWRI researchers and 2 subcontracted support staff (9 Total) are required for all field activities. Lodging is estimated at 14 days for each region (total 28 days). Per Diem is for 1 day per person at end of trip.

meals	$36/day/person	$9,072
lodging	$100/day/person	$25,200
per diem	$80/day/person	$1,440

SPMD Deployment & Reovery:

Travel for 2 CREMP team members to deploy and recover SPMDs throughout Florida Keys. Travel is from group's headquarters at FWRI, St. Petersburg, FL to Florida Keys. A total of 30 days spanning July thru September are budgeted to deploy and retrieve SPMDs from all 3 regions (Upper, Middle, Lower Keys). Three days are budgeted for each region and a minimum of 2 deployments will be performed providing continuous monitoring thru September. Per Diem is for 1 day per person at end of trip and 3 trips are planned (Middle Keys deployment, Retrieval (1st Deployment) and 2nd Deployment and Retrieval (2nd Deployment)

meals	$36/day/person	$1,512
lodging	$100/day/person	$4,200
per diem	$80/day/person	$480

TOTAL TRAVEL EXPENSES **$41,904**

Supplies

Fuel (vehicles, SCUBA compressor)	$1,000
Field & Dive Supplies	$3,500
Office Supplies	$500
Computer supplies/software	$2,500

Underwater video & camera supplies		$1,500
	TOTAL SUPPLIES	**$9,000**

Transportation

Vessel Cost: Pre-assesment monitoring — $14,000

38' FWRI Research Vessel (RV Tortugas) will be provided to support field operations at anticipated cost of $500/day for 28 days. This includes cost for fuel but not other maintenance to prepare vessel.

Vessel Cost: SPMD Deployment & Recovery — $5,250

FWRI Research Vessels (23' Parker or 26" Whaler) will be provided to support deployment of SPMDs in the Middle Keys and retrieval of SPMDs from all locations at a cost of $250/day for 21 days. Cost includes 2nd round of deployment (concurrent with 1st retrieval) and retrieval to continuously monitor through September. This includes cost for fuel. Three days are estimated for deployment and retrieval in each region (Initial deployment in Upper and Lower Keys will occur concurrently with pre-assessment monitoring)

TOTAL TRANSPORTATION	**$19,250**

Other

Vessel (RV Tortugas Maintenance & Prep)		$10,000
Reports and Publication costs		$1,000
	TOTAL OTHER	**$11,000**
	TOTAL DIRECT COSTS	**$81,154**

INDIRECT COSTS — 5% of total direct costs

FWC's current federally negotiated indirect cost agreement is attached but does not apply to this project. FWC accepts instead a rate of 5% on total direct costs for this project.

TOTAL INDIRECT	**$4,058**
TOTAL REQUEST 09/10	**$85,212**

Budget for Florida Middle Grounds sampling

Estimate of Labor and Expense Costs

Task I Field Work		
	Field/Equipment Preparation	$4,505.52
	Mobilization/Demobilization	$24,508.80
	Monitoring	$153,180.00
	Subtotal	**$182,194.32**
Task II Data Analysis and Reduction		
	CPCE	$22,015.20
	In-situ belt transects and fish counts	$16,800.00
	still photo quads	$2,907.60
	QC	$6,514.56
	Subtotal	**$48,237.36**
Task III Reporting		
	Report Production	$13,946.40
	QA/QC of Report	$1,292.40
	Subtotal	**$15,238.80**
Task IV SPMD Retrieval		

	Mobilization/Demobilization	$9,765.72
	Monitoring	$12,655.44
	Subtotal	**$22,421.16**
	TOTAL LABOR COST	**$268,091.64**
Expenses		
FMG Expidition Expenses		$12,660.00
	Field Expenses Subtotal	**$12,660.00**
FMG Expidition Boat Rental Expenses		
	Boat Rental (boat, fuel, lodging)	$91,000.00
	Tanks	$4,000.00
	Boat Rental Subtotal	**$95,000.00**
SPMD Retrieval Expenses		
	Boat Rental (boat, travel, fuel)	$7,347.50
	Tanks	$240.00
	SPMD Retrieval Expenses Subtotal	**$7,587.50**
	TOTAL EXPENSES	**$115,247.50**

	TOTAL LABOR AND EXPENSES	$383,339.14

Total for Tier 1 baseline/pre-impact condition of shallow-water corals in the Florida Middle Grounds survey: **$383,339.14**

Costs of staff from FDEP are not included in the budget

Budget for Flower Gardens/Stetson/Sonnier Surveys:

Travel	
2 cruise participants @ $1,500 per person	$3,000
Contracts (data collection, data analysis)	
Contract labor:	$15,000
Supplies	
Sampling gear parts, underwater paper, miscellaneous supplies	$2,500
Vessel Costs	
16 days x $5K/day (fuel, food and crew)	$80,000
	Total $100,500

GRAND TOTAL COST OF TIER 1	**$665,403.14**