

NOAA CIOERT Cruise Report
Report Title: Characterization of the Mesophotic Benthic Habitat and Fish Assemblages
from ROV Dives on Pulley Ridge and Tortugas during 2012 and 2013 R/V *Walton Smith*
Cruises

R/V *Walton Smith* – Cruise Nos. WS1213 & WS1312
UNCW *Super Phantom S2* ROV
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Project Title: Connectivity of the Pulley Ridge - South Florida Coral Reef Ecosystem:
Processes to Decision-Support Tools

John Reed, Stephanie Farrington
Cooperative Institute of Ocean Exploration and Technology
Harbor Branch Oceanographic Institute, Florida Atlantic University

Lt. Heather Moe, Stacey Harter
NMFS/Southeast Fisheries Science Center (SEFSC)

Dennis Hanisak
Cooperative Institute of Ocean Exploration and Technology
Harbor Branch Oceanographic Institute, Florida Atlantic University

Andrew David
NMFS/Southeast Fisheries Science Center (SEFSC)



HARBOR BRANCH

FLORIDA ATLANTIC UNIVERSITY

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EXECUTIVE SUMMARY

Two research cruises were conducted in 2012 and 2013 to the region of Pulley Ridge and Dry Tortugas to study and survey the mesophotic reef communities and fish populations. The University of Miami's R/V *Walton Smith* Cruise No. WS1213 was conducted from August 14 to 25, 2012 and Cruise No. WS1312 was conducted from August 12 to 27, 2013. Pulley Ridge is the deepest known photosynthetic coral reef in continental U.S. waters and is located in the Gulf of Mexico, 100 miles west of the Dry Tortugas at the far end of the Florida Keys. This research and cruises were funded by the NOAA-NOS-NCCOS grant titled 'Connectivity of the Pulley Ridge - South Florida Coral Reef Ecosystem: Processes to Decision-Support Tools'. Ship and ROV time was funded in part by the Cooperative Institute for Ocean Exploration, Research, and Technology (CIOERT) at Harbor Branch Oceanographic Institute-Florida Atlantic University (HBOI-FAU). This cruise was conducted in collaboration with the University of Miami, HBOI-CIOERT, NOAA Fisheries, and the University of North Carolina at Wilmington (UNCW) which provided the UNCW *Super Phantom S2* ROV.

This Cruise Report provides a detailed characterization of the benthic habitat, benthic macrobiota, and fish populations for each ROV dive site. Appendix 1 provides the complete species list and percent cover of benthic biota and substrate for each site at Pulley Ridge (PR) and Tortugas (T). Appendix 2 lists the fish species and their densities for each site. Prior to the cruises, we pursued a statistically rigorous sampling protocol for the ROV surveys. In ArcGIS a fishnet grid of 1 km x 1 km blocks were overlaid on the available bathymetric maps at both regions. A total of 42 random blocks were selected for the quantitative ROV surveys in 2012 and 2013 (28 for PR and 14 blocks for T). Within each sampling block ("site"), we conducted five random 100-m transects with the ROV to characterize that block.

A total of 44 ROV dives were conducted, covering 45.45 km (PR = 32.6 km; T = 12.83 km), at depths from 94.5 m to 23.1 m (PR = 94.5 m - 60.3 m; T = 58 m - 23.1 m). A total of 99.75 hours (PR = 74.5; T = 24.5) of ROV video were recorded and 7,563 *in situ* digital images were taken which included quantitative transect images (7,336), general habitat images, and species documentation images.

All fish were identified for each ROV dive to species level and counted. The total distance (km) of each dive was used to calculate the linear density (# individuals/km) of each fish species. A total of 111 fish taxa were identified from both Pulley Ridge and Tortugas dives in 2012 and 2013. The most common species included sharpnose puffer, cherubfish, reef butterflyfish, yellowtail reeffish, sunshinefish, purple reeffish, squirrelfish, wrasse bass, orangeback bass, chalk bass, greenblotch parrotfish, and bicolor damselfish. Unfortunately, lionfish were also abundant, mostly associated with red grouper burrows, and present in 72% of the blocks. A total of 703 lionfish were counted over both years. Fifteen species of commercially and recreationally important grouper and snapper species were counted (total 681 individuals). The dominant species were vermilion snapper (428), black grouper (13), graysby (19), mutton snapper (41), red grouper (88), and scamp (56).

Quantitative photo transects were conducted during each ROV dive using a digital still camera pointing straight down (or perpendicular to the substrate as possible) with parallel lasers (10 cm)

for scale. In general, digital images were taken every 30 seconds within the 100-m transects throughout the dive. Percent cover of substrate type and benthic macrobiota was determined by analyzing the quantitative transect images with Coral Point Count with Excel extensions (CPCe 4.1©) (Kohler and Gill 2006). Fifty random points overlaid on each image were identified as substrate type and benthic taxa. Substrate categories included: soft bottom (unconsolidated sand, mud) and hard bottom, which was subdivided into rock (pavement, boulder, ledge), rock rubble/cobble (generally, 5-20 cm diameter), and bare dead coral plate. All benthic macrobiota (usually >3 cm) were identified to the lowest taxa level possible.

A total of 216 benthic biota taxa were identified from Pulley Ridge and 196 taxa from Tortugas transects. These included 102/60 taxa, respectively, of Porifera, and 47/54 Cnidaria which included 32 Scleractinia, 27 Alcyonacea (gorgonacea), and 4 Antipatharia. The density and diameter of plate corals (*Agaricia* sp., *A. fragilis*, *A. lamarcki/grahamae*, *Leptoseris cucullata* and *Montastraea cavernosa*) were calculated for all the Pulley Ridge transect photos for both 2012 and 2013 cruises. Mean percent cover of plate corals was 0.55% and mean density was 0.54 colonies/m². The southern part of the Main Pulley Ridge had the greatest coral cover (1.69%) and density (3.66 colonies/m²).

What is chilling is the apparent loss of coral cover over the past 10 years. In surveys conducted by USGS in 2003, they documented a mean coral cover on Pulley Ridge of 11.90%, with a maximum of 23.23% in the central region of the ridge. By 2013, we report an average coral cover of 0.85%, and a maximum of 5.62% (Block 18). The overall mean coral cover went from 11.90% to 0.85% which is a 92.8% loss of coral cover in 10 years! At this time we cannot say with certainty the cause of this loss. On a possible positive note, a large number of the corals are relatively new recruits: 47.7% were <5 cm in diameter, and 35.4% were 5-9 cm.

Ultimately these data from the various cruises will be used to characterize and document the habitat, benthic communities, and fish populations inside and outside the Pulley Ridge HAPC and between the North and South Tortugas Ecological Reserves. These data may then be compared to future research cruises to better understand the long-term health and status of these important mesophotic ecosystems. These data will be of value to the regional Fishery Management Councils, NOAA Fisheries, NOAA Mesophotic Reef Ecosystem Program, NOAA Deepsea Coral Research and Technology Program (DSCRTP), NOAA Coral Reef Conservation Program (CRCP), and NOAA Marine Sanctuaries for management decisions on these habitats and managed key species.

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Connectivity in the Gulf of Mexico – Pulley Ridge to the Florida Keys”). Ship and ROV time was funded in part by CIOERT at HBOI-FAU. This cruise was conducted in collaboration with the University of Miami, HBOI-CIOERT, NOAA Fisheries, and the University of North Carolina at Wilmington (UNCW) which provided the UNCW *Super Phantom S2* ROV. This is Harbor Branch Oceanographic Institute Technical Report Number 147.

PROJECT OVERVIEW

The Gulf of Mexico Fishery Management Council (GMFMC) and Department of Commerce through the Magnuson-Stevens Fishery Management Act established the Pulley Ridge Habitat area of Particular Concern (HAPC) in 2005. This project proposes to document and characterize the mesophotic benthic habitat, benthic macrobiota, and fish populations within and adjacent to Pulley Ridge and within mesophotic sites adjacent to the North and South Tortugas Ecological Reserve and the Florida Keys National Marine Sanctuary (FKNMS).

Pulley Ridge is the deepest known photosynthetic coral reef in continental U.S. waters (USGS 2005; Hine et al. 2008; Halley et al. 2013; NOAA 2013). It lies in the Gulf of Mexico, 100 miles west of the Dry Tortugas at the far end of the Florida Keys (Figure 1). Pulley Ridge is a submerged 100 km x 5 km barrier island that was originally discovered in 1950. It has less than 10 m of relief across the 5 km wide ridge at depths of 65 to 75 m. According to USGS (2005), the coral on Pulley Ridge was “considerably healthier than coral from shallow water reefs nearly worldwide”. This is of particular interest because research shows that shallow water reefs worldwide are stressed due to climate change, habitat loss, human impact, and coral diseases. It was for this reason that Pulley Ridge HAPC was designated in order to receive protection from targeted fishing activity and specifically bottom longlines. The second area of study was near the Dry Tortugas, west of the western boundary of the FKNMS and adjacent to the North and South Tortugas Ecological Reserves, but outside of both protected areas.

Ultimately these data from the various cruises will be used to characterize and document the mesophotic habitat, benthic communities, and fish populations within the Pulley Ridge HAPC and between the North and South Tortugas Ecological Reserves (TER). These data may then be compared to future research cruises to better understand the long-term health and status of these important ecosystems. These data will be of value to the regional Fishery Management Councils, NOAA Fisheries, NOAA Mesophotic Reef Ecosystem Program, NOAA Deepsea Coral Research and Technology Program (DSCRTP), NOAA Coral Reef Conservation Program (CRCP), and NOAA Marine Sanctuaries for management decisions on these habitats and managed key species. In addition these data will be of interest to the various agencies by documenting deepwater coral/sponge habitat and Essential Fish Habitat (EFH) that are currently unprotected and may be potential additions to the protected areas.

Pulley Ridge Benthic and Fish Assemblages- Background

Southern Pulley Ridge has an atypical array of photosynthetic hard corals, macroalgae, sponges, and large variety of tropical fishes (Halley et al. 2013). These reefs are termed mesophotic reefs which are relatively deep compared to shallow water reefs. At depths of 50 to about 100 m,

mesophotic reefs still receive enough sunlight to support photosynthetic algae and corals with zooxanthellae (algal symbionts). Based on photographs collected by the USGS's SeaBOSS camera system in 2003, Hine et al. (2008) reported that Pulley Ridge is dominated by coralline algae which covers 45-65% of limestone bottom. Pulley Ridge is also home to a wide variety of fleshy macroalgae including *Halimeda tuna*, *Dictyota* sp., *Kallymenia* sp., and the endemic species *Anadyomene menziesii*, which look like large heads of lettuce and can be as dense as tens of plants per square meter. Halley et al. (2013) also reported *Agaricia* spp. and *Leptoseris cucullata* as the two most abundant species of scleractinian coral which form flat plates as large as 50 cm in diameter and make up almost 60% of the live coral cover in some locations. These species are typically found only on the deeper slopes of shallow water reefs in the Caribbean and Florida. *Montastraea cavernosa* (the giant star coral) is another species found on Pulley Ridge but is also common on shallow water reefs. Pulley Ridge is home to more than 60 species of fish including both shallow water and deep reef species. These include the commercially caught species *Epinephelus morio* – the red grouper. Red grouper form large 6-10 m wide pits in the sand and rubble bottom that provide an oasis-like shelter for numerous smaller reef fish. Unfortunately lionfish are also showing up here recently in virtually every red grouper burrow. Since HBOI-CIOERT discovered the first lionfish on Pulley Ridge in 2010, our research cruises in 2011 and 2012 have shown the population to have exploded (Reed et al. 2012).

At the Tortugas sites which are outside of the TERs and FKNMS, no deepwater benthic surveys, fish surveys or multibeam mapping have been conducted previously. However, areas within the TERs such as Miller's Ledge, Riley's Hump, and Sherwood Forest have been mapped and relatively well studied (Lee et al. 1999, Schmidt et al. 1999, Cowie-Haskel and Delaney 2003, Weaver et al. 2006, Ault et al. 2013).

OBJECTIVES

Objectives for this NOAA-NOS-NCCOS grant "Connectivity of the Pulley Ridge - South Florida Coral Reef Ecosystem: Processes to Decision-Support Tools" are to:

1. Population Dynamics:
To estimate the spatial distribution, abundance, and size structure of key economically and ecologically important reef-fish species in Pulley Ridge compared to those regionally located on the west Florida shelf and the Dry Tortugas-Florida Keys areas.
2. Community Structure:
To quantify preliminarily two key reef processes: benthic primary productivity and settlement/recruitment potential of corals.
3. Decision Support Toolkit:
To create software specific for the Pulley Ridge dataset that will organize and integrate the data, models and other information products produced by the project

This 5 year grant hopes to supply timely information useful to natural resource managers. These different sub-themes within the grant will produce outputs that are vital to providing managers with knowledge to make informed decisions about the spatial scales of connectivity and

functioning of the overall South Florida coral reef ecosystem, and whether specific actions are warranted for the Mesophotic Coral Ecosystem within the area.

PURPOSE

The most important data that can be acquired from Pulley Ridge is on shallow water species which are able to live at such great depths – almost out of the zone of impact from climate change and human contact. The interaction and genetics of the mesophotic and shallow water reef species may provide a baseline denoting impacts of coral bleaching and other effects of climate change (NOAA 2013). This is exactly the research that was conducted by the science team aboard the University of Miami's *R/V Walton Smith* on 2012 and 2013 Coral Ecosystem Connectivity cruises to Pulley Ridge and the Tortugas regions. This project is a unique collaboration of more than thirty scientists pooling the established expertise from within two NOAA CI's: the Cooperative Institute for Marine and Atmospheric Studies (CIMAS) at the University of Miami, and the Cooperative Institute for Ocean Exploration Research and Technology (CIOERT) at Harbor Branch Oceanographic Institute/Florida Atlantic University, as well as the wider Gulf of Mexico scientific and management communities.

METHODS

ROV video and photographic surveys were made at each site to ground-truth multibeam sonar maps, quantify and characterize the benthic habitats, benthic macrobiota, fish populations, and coral/sponge cover. Prior to each ROV dive, georeferenced sonar maps with overlaid random 1 km² blocks were uploaded to the ROV navigation software and 100-m circles were added for the quantitative transects. Typically one 4-hour ROV dive would complete five 100-m transects per random block. Two ROV dives were made each day during night time and daylight hours in 2012 and daylight hours only in 2013; CTD operations were conducted at night.

ROV Operations

Surveys were conducted with the UNCW *Super Phantom S2* ROV which was equipped with standard definition digital video and digital still cameras mounted on tilt bar, and parallel lasers for scale.

ROV Navigation:

The ROV uses an integrated navigation system consisting of Hypack® Max software on a Dell 1.6 GHz computer, ORE Offshore 4410C Trackpoint II Underwater Acoustic Tracking System with an ORE Offshore 4377A transponder with depth telemetry, Northstar 951XD differential GPS, and Azimuth 1000 digital compass. This system provides real time tracking of the ROV and ship to the ROV operator and the support vessel's bridge for navigation. Ship and ROV positions are logged every 1-2 seconds and processed after each dive and provided to the scientist in an Excel file. Geo-referenced TIFF files obtained with multibeam sonar can be entered into Hypack as background files to display target sites and features of interest to aid in ROV and support vessel

navigation. All data documentation (digital images, video, dive annotations) are geo-referenced to ROV position by matching the date and time to the ROV navigation files.

ROV Survey Protocol:

During each dive the primary objectives were to document benthic habitat, benthic macrobiota, and fish populations, and to conduct photo/video transects which were used for quantitative analyses of the habitat and biota. The general protocol included:

1. During the photo/video transects, the ROV was kept <1 m off bottom with a speed over ground of $\sim\frac{1}{4}$ knot. During quantitative still images, the ROV ascended to ~ 1.3 m to provide a 1-2 m² image. Each ROV dive was $\sim 1-2$ km in length for a duration of 3-4 hours and was documented with continuously recording digital video and digital photographs. The direction of each transect was based on flip of coin, and ship's maneuverability due to wind/current.
2. Video transects were used for analysis of fish populations and general habitat characterization. The video camera was angled $\sim 20-30^\circ$ down with 10 cm parallel lasers for scale. Underwater video was viewed in real time on the support vessel by PIs familiar with the local deep-water fauna; audio annotations describing habitat, benthic biota, and fish were recorded onto the video and transcribed into a Microsoft Access 2010, CIOERT At-Sea Access database. All fish were identified and counted from the video transects and densities determined, emphasizing commercially and recreationally important species.
3. Digital still images were used for quantitative analysis of habitat and benthic macrobiota. The digital still camera was pointed down 90° with 10 cm parallel lasers for quantitative photo transects. Still images were captured with the digital still camera every 30 seconds throughout the dive at a height of 1.3 m to provide relatively consistent area for each image. Still images captured from the photo transects were analyzed using CPCe[®] 4.1 software to determine relative percent cover of benthic biota and habitat types as well as coral colony diameter and count.
4. All data documentation (digital images, video, and dive annotations) were geo-referenced to ROV position after the cruise by matching the date and time to the ROV navigation files in our CIOERT At-Sea Access database.

Selection of Random Blocks for ROV Surveys

A statistically rigorous sampling protocol was used for the ROV surveys at Pulley Ridge and Tortugas. In ArcGIS 10.0 a fishnet of 1 km x 1 km blocks were overlaid on the available bathymetry maps at both regions. A total of 42 random blocks were selected for the quantitative ROV surveys in 2012 and 2013 (28 for Pulley Ridge and 14 blocks for Tortugas) targeting the main ridge and west ridge of Pulley Ridge and areas adjacent to but outside the Florida Keys National Marine Sanctuary (FKNMS) and the Tortugas Ecological Reserves (TER) (Figs.1-2). Within each sampling block ("site"), we conducted five random 100-m transects with the ROV to characterize that block. The direction of each transect was based on flip of coin, and ship's maneuverability due to wind/current. Then a 100-m radius circle was placed on the ROV navigation screen with the ROV in the center. Each 100 m transect was conducted at ~ 0.25 kn for 15-20 minutes or until the ROV passed through 100 m radius. Off transects between the

photo transects were 10-15 minutes, also with the heading determined by flip of coin. The five transects generally covered the length and breadth of the 1 km block (Figs. 3-6).

Fish Surveys

A Sony standard resolution, single-chip color video camera (410x380 pixels; 79° diagonal angle in water) with 12:1 zoom, and auto/manual focus provided video documentation during ROV operations. An On-Screen Display (OSD) video overlay recorded time, date, ROV heading, and ROV depth. The video footage was recorded continuously throughout each dive from surface to surface and recorded to 2 TB hard drives and copies to DVDs. The camera was typically angled down ~30° to view both near and far to the horizon for fish aggregations and habitat. A microphone was used for continuous audio annotations by the PIs describing events, habitat, and fauna which were recorded onto the video recordings and transcribed into the Microsoft Access CIOERT At-Sea Access database. Along with being used as the main “pilot” view, the video was the primary data source for the quantitative analysis of the fish populations. All fish were identified for each ROV dive to species level and counted. The total distance (km) of each dive was used to calculate the linear density (# individuals/km) of each fish species. The video camera angle precludes an accurate calculation of areal density of the fish (i.e., # km⁻²); however, we estimate that the field of view width was generally about 10 m, and most fish were identified within a 5 m distance. So the densities listed in Appendix 2 could be multiplied by 0.1 to get an estimate of the number of fish km⁻² (based on an average 10-m width field of view).

Benthic Surveys

Geo-referenced digital still images were acquired with an Insite Pacific Inc. Scorpio Plus digital still color camera and strobe. This camera features a 4X zoom lens; internal electronics and imaging device are a Nikon Coolpix 995. In fine resolution setting, the 1 GB, compact flash card can store 664 images in JPG format (approximately 1.0 Mb per image), which were copied to DVD media. Quantitative photo transects were conducted during each ROV dive using the digital still camera pointing straight down (or perpendicular to the substrate as possible) with parallel lasers (10 cm) for scale. In general, digital images were taken every 30 seconds within the 100-m transects throughout the dive. Each photo filename was coded with corresponding EDST time and date code (using Stamp 2.8 by Tempest Solutions[©]) which was imported into MS Access and linked to the ROV navigation data for site specific data of coordinates and depth and then imported into ArcGIStm 10.0. Non-transect photos, such as purposeful images to document a specific species, were not included in the quantitative analyses. Poor and unusable photos (blurred, black, off bottom) were also removed from the quantitative analyses.

Benthic Analyses

Percent cover of substrate type and benthic macrobiota was determined by analyzing the quantitative transect images with Coral Point Count with Excel extensions (CPCe 4.1[©] (Kohler and Gill 2006), and following protocols established in part by Vinick et al. (2012) for offshore, deepwater surveys in this region. For each random block, a total of 120 images were randomly selected and each was overlaid in CPCe with 50 stratified random dots to identify the substrate

and biota. For Pulley Ridge, 28 blocks were surveyed resulting in 3,360 images and 168,000 points scored; Tortugas had 16 blocks and 96,000 points scored.

To determine how many images and points were needed for CPCe point count analysis, we first tested Dive 14 from 2012 which had the most images as well as *Agaricia* coral colonies. Using PRIMER statistical software we plotted the species curves using four different tests: 180 images/50 points, 120/50, 120/25 and 60/50. The data were tested for percentage cover of sessile species only. The two statistical models, analyzed in PRIMER 6 ® v 6.1.13, CHAO2 and Michaelis Menton (MM) both approached asymptotic values. Although, the results showed no difference in the PRIMER 6 test between 50 and 25 points, and both were asymptotic below 120 images, we decided to use the larger number of images, since we have used that number for several previous deepwater and mesophotic surveys. Also although we attempted to take ~ 30 images per transect, overall nearly all blocks had at least 120 images. So in order to keep samples size similar, we selected 120 random images from each block for the point count analysis.

Random points overlaid on each image were identified as substrate type and benthic taxa. All benthic macrobiota (usually >3 cm) were identified to the lowest taxa level possible. For this report we used the following terminology: hard bottom is sometimes referred to as live bottom due to the amount of living organisms attached to these substrates (SAFMC 1998). Hard bottom provides anchorage for sessile or semi-sessile organisms (e.g., corals, octocorals, anemones, hydroids). Coral is defined by NOAA (Lumsden et al. 2007) as hard corals (stony corals-Scleractinia) and other taxa with solid calcareous skeletons (e.g., Stylasteridae), as well as non-accreting taxa such as octocorals (Alcyonacea- “gorgonacea”) and black corals (Antipatharia).

Prior to point count analysis, all images were reviewed and a species list was made in a Taxonomic Photo Album using Microsoft Access. We tried to identify to the lowest possible level of taxa (in some cases to species, but some only to family, order, or higher taxa). We included all benthic algae; and sessile macroinvertebrates including Porifera, Scleractinia, Alcyonacea- gorgonians, Antipatharia, Corallimorpharia, Alcyoniina soft corals, other non-coral cnidaria (hydroids), and ascidians; and all mobile benthic macroinvertebrates including: echinoderms, mollusks, arthropods, and annelids. Species images were sent to the following taxonomists for identification verification:

Sponges- Pomponi, C. Diaz, P. Cardenas
Cnidaria- S. Cairns, P. Etnoyer, C. Messing, J. Voss
Algae- S. Hanisak, S. Reed, M. and D. Littler.
Echinoderms- D. Pawson, C. Messing

Some common taxa could be identified to genus or species level but many could only be identified to a higher level such as family, class, order or even phylum. Sponges, gorgonians, and black coral are especially difficult to identify without a specimen in hand. In these cases a general descriptive taxa was used, e.g., “brown lobate sponge” or “unidentified Demospongiae”, which could consist of numerous species. These designations should not be considered equivalent to species level and should not be used for diversity (H’) indices calculations. Many deepwater species in this region look nearly identical, such as fan sponges which are

polyphyletic and may actually include different orders or classes. Once the Microsoft Access Taxonomic Album was completed these were coded for CPCe analysis.

Coral Analysis

In CPCe every point that landed on a scleractinian coral was identified to species level if possible and percent cover calculated for each transect. The agariciids were identified as *Agaricia* sp., *A. fragilis* or the combination of *A. lamarcki* and *A. grahamae*. Example images were sent to various coral experts who all agreed that these species are nearly impossible to tell apart without a specimen in hand. Therefore our analyses simply grouped all *Agaricia* as *A. spp.* *Leptoseris cucullata* could usually be distinguished from *Agaricia* by the raised corallites.

Density Analysis of Plate Corals

Density and maximum diameter of all plate corals (i.e., *Agaricia* spp., *Leptoseris cucullata* and *Montastraea cavernosa*) were calculated for all the Pulley Ridge transect photos for both 2012 and 2013 cruises. 2013 Tortugas sites were not analyzed. Only 3 blocks at Tortugas encountered reef habitat and all were typical shallow reef habitat and species. Since these Tortugas sites were primarily shallow water patch reefs (25-35 m), they did not have the same species of *Agaricia* or *Leptoseris*, and therefore were not included in the analysis.

Density was calculated by the following protocol. All transect images were used, and not filtered by the 120 random filter; however, any purposeful images or duplicate overlapping images were removed. All visible corals were counted and diameter measured using CPCe (total images with coral = 839; total corals counted = 2,272; total measured = 2,075; minimum size measured was 0.47 cm diameter). To calculate density, any transect that had even one coral was analyzed for density. Of these transects, the area of every transect image with lasers was calculated with CPCe ARA (3,528 images had lasers and area determined). Then the total number of corals for a transect was divided by the total photo area of the transect to get density (# coral/m²). Density (ρ) by Block = Sum of Coral Count per Block \div Sum of Image Area by Block:

$$\rho = \frac{\sum \text{Count of Corals by Block}}{\sum \text{Image Area by Block}}$$

To calculate coral size, the CPCe Area Analysis tool was used calculate the maximum diameter of each plate coral. Coral colonies that were only partially visible in a photograph were measured if it appeared that >50% of the colony was visible.

Protocol for Benthic Habitat Characterization

This following defines the habitat categories that were used to define and characterize the benthic habitats of Pulley Ridge and Tortugas. These data are result of the ROV video observations and multibeam sonar maps where available. These habitat categories were then entered into the HBOI Microsoft Access at-Sea Database for each ROV dive. These data are used along with the CPCe Point Count data from the photo transects to characterize the benthic habitat and distribution of benthic biota, and also used with the video data for the fish population analyses.

1. [*Habitat_Zone= Geomorphology*]: This describes the geological feature; e.g., Main Ridge- East Slope, Main Ridge- West Slope; Main Ridge- Top, Off Ridge- East Base, Off Ridge- West Base, Patch Reef (shallow reefs off Tortugas), Soft Bottom, West Ridge- East Slope, West Ridge- Top, West Ridge- West Slope. This category is used to plot the percent cover of benthic macrobiota for each habitat zone at each dive site and to plot the dive track overlay on multibeam sonar maps in ArcGIS.
2. [Geographic Area]: Block locations were classified based on where they occurred along Pulley Ridge: Main Ridge- North, Main Ridge- South, Main Ridge- Middle, Off Main Ridge and West Ridge. The Tortugas sites were all classified as Tortugas (T).
3. [Depth]: Depth range (m) of the transect or dive.
4. [*Relief*]: LR= Low Relief (0- <1.0 m), MR= Moderate Relief (1-3 m), HR= High Relief (>3 m). This is modified from the NOAA Southeast Area Monitoring and Assessment Program (SEAMAP) designations of outer continental shelf benthic habitat. This category is dependent on the distance over which the depth change occurs. We define relief as the relative height of rock ledges, boulders, or rock outcrops in the field of view.
5. [Slope]: Slope was estimated from the ROV video: Flat = 0-5°, Low = 5-30°, Moderate = 30-60°, High (Wall) = 60-90°. Pulley Ridge was mostly flat to low slope. Only on the rims of the red grouper burrows was there low to moderate slope. In areas where multibeam was available, we were able to plot slope in ArcGIS.
6. [*Rugosity*]: LRu= Low Rugosity, HRu= High Rugosity. Rugosity here is defined as a degree of ruggedness of the rock bottom. This is relative to the size of rock ledges, holes, crevices, which tend to provide the greatest fish habitat. High Rugosity on Pulley Ridge is rarely observed, if ever, except in the region of red grouper burrows. Low Rugosity would be the flat rock pavement typically found top of the ridges or at the base of the mounds and ridges. For the present, this will be an unquantified relative term. Most of the multibeam sonar maps that cover Pulley Ridge (Naar 2000) are of relatively low resolution (5-10 m) and cannot be used to quantify rugosity at this scale; high resolution (<2 m) multibeam maps were collected on the Nancy Foster Cruise in 2010 (Reed 2011) and cover 38 km² on the main ridge show the locations of red grouper pits and tilefish mounds. A fairly accurate slope visualization could be created to determine the number and location of higher rugosity and location of these pits and mounds in the future, but this is beyond the scope of this research.
7. [*Substrate*]: SEADESC Habitat Categories (Table 1). This is a modified subset of SEADESC Habitat Categories which was developed by the NOAA Deep-Sea Coral Program for use in analysis of deep-sea coral surveys (Partyka et al. 2007). These categories which are useful for characterizing deep coral habitat were modified to make them useful for these mesophotic habitats. The presence of fauna was not included as it is quantified in the Point Count analyses. In the region of this survey, substrate categories included: soft bottom (unconsolidated sand, mud) and hard bottom which was

subdivided into rock (pavement, boulder, ledge), and rock rubble/cobble (generally, 5-20 cm diameter). This category is also used to plot the dive track overlay on the multibeam sonar maps in ArcGIS.

Table 1. SEADESC Benthic Habitat Category Codes (modified).

ID	Code	Habitat Name	Habitat Description
1	S	Soft Substrate	Unconsolidated sand/mud, unlithified
2	SR	Soft Substrate/Rubble/Rock	Soft substrate (>50% cover) with rubble and/or rock
3	R	Rubble	Rubble/cobble (~5-20 cm sized rock or coral)
4	RL	Rock/Ledges	Rocks and/or ledges
5	P	Pavement	Rock pavement
6	C	Hard Corals	Live and/or dead colonial scleractinian coral; standing individual colonies, bushes, or thickets.
7	TH	Tilefish (blueline or golden; not sand tile)	Soft bottom with visually identifiable burrows
8	A	Artificial Substrate	Any artificial structure that provides habitat for fishes and/or invertebrates

Statistical Analyses

Multivariate analyses were used to determine differences in benthic fauna assemblages and fish assemblages among blocks and regions. All analyses were conducted in PRIMER[®] 6 v 6.1.13 and based on guidelines of Clarke and Warwick (2001) and Clarke and Gorley (2006). The dive sites were compared by Block and their Geographic Location (Pulley Ridge vs Tortugas) and by Geographic Area (e.g., Main Ridge- North, Off Main Ridge, etc.) For the benthic analysis, images were analyzed using CPCe 4.1 for percent cover of benthic biota. The CPCe percent cover data were then averaged by block. Then these data were square root transformed to reduce the dominate influences of copious species to the similarity matrix.

For the fish analysis, fish species were counted within each transect, summed for the entire transect and then divided by the total distance examined with in each transect. This resulted in the sum of each species per km by transect. The counts were then averaged by site and fourth root transformed to reduce the dominate influences of copious species to the similarity matrix.

Similarities between samples for both fish and benthic biota were then calculated separately using S17 Bray-Curtis similarity in PRIMER 6. A non-metric multidimensional scaling ordination (MDS) plot and a dendrogram with group-average linking were created showing the

results of a concurrently run SIMPROF ‘similarities profile’. SIMPER: ‘Similarity Percentages’ was utilized to determine which species contributed to the dissimilarities among group pairs.

RESULTS

Study Areas

Study sites at Pulley Ridge included a total of 28 random 1-km² blocks on the PR HAPC Main Ridge which we defined as Main Ridge-North, -Middle, and -South; some blocks off the Main Ridge, and blocks on the West Ridge outside of the HAPC (Fig. 1). The Tortugas sites (13 random blocks) were just west of the Florida Keys National Marine Sanctuary boundary and adjacent to but outside of the Tortugas Ecological Reserve North and South (Fig. 2).

2012/2013 Pulley Ridge
 R/V Walton Smith – Cruise Nos.
 WS1213 & WS1312
 Overall Pulley Ridge
 NAD_1983_UTM_Zone_17N
 Projection: Transverse_Mercator
 Datum: D_North_American_1983

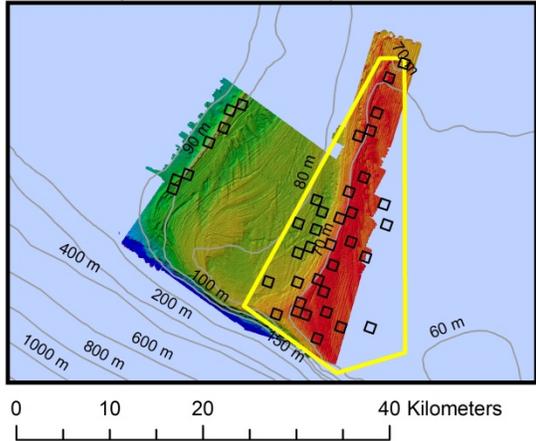
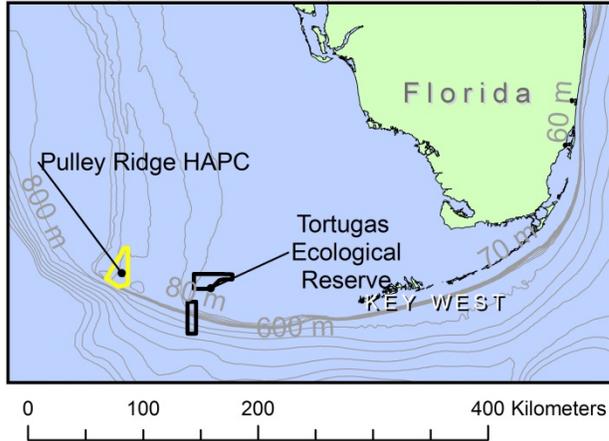
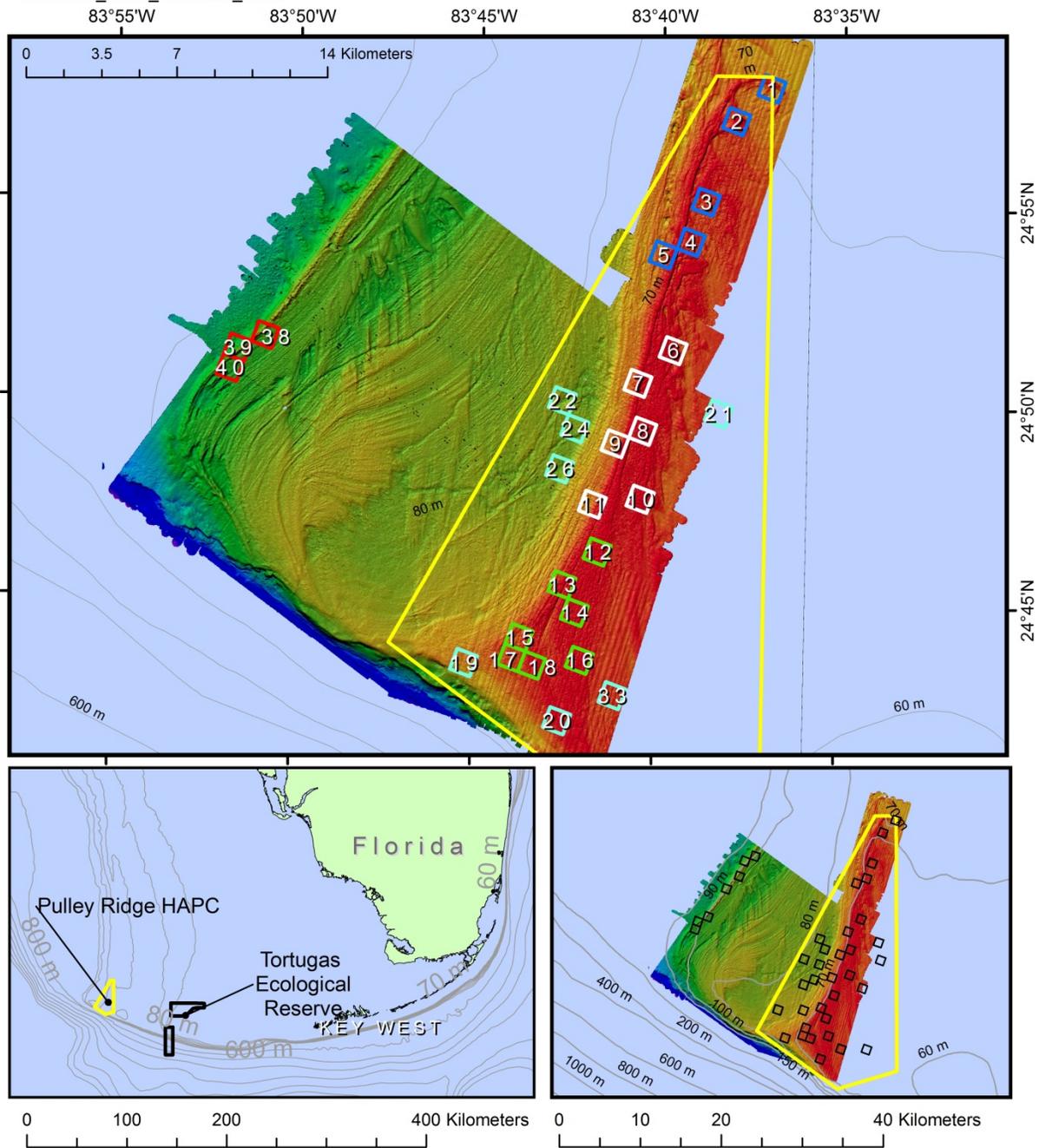


Figure 1. Random 1 km x 1 km blocks (28 total) surveyed on Pulley Ridge with UNCW *Super Phantom* ROV during the 2012/2013 R/V *Walton Smith* cruises, August 14 to 25, 2012 and August 12 to 27, 2013. Blocks are color coded by Geographic Area.

2012/2013 Pulley Ridge
 R/V Walton Smith – Cruise Nos.
 WS1213 & WS1312

Tortugas Sites
 NAD_1983_UTM_Zone_17N
 Projection: Transverse_Mercator
 Datum: D_North_American_1983

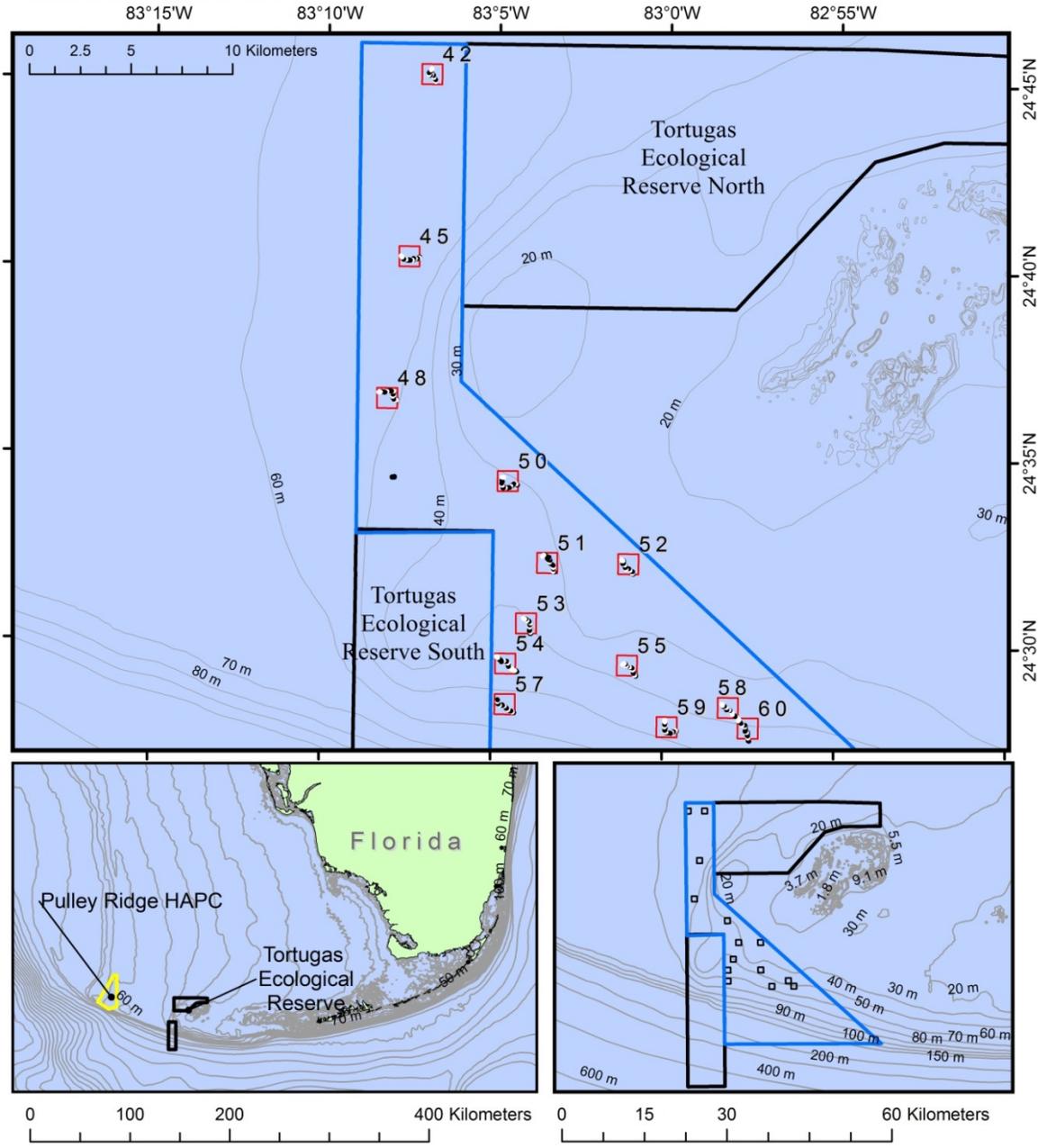


Figure 2. Random 1 km x 1 km blocks (13 total) surveyed at Tortugas region with UNCW *Super Phantom* ROV during the 2013 R/V *Walton Smith* cruise, August 12 to 27, 2013.

ROV Dive Summary

The UNCW *Super Phantom* S2 ROV was used to conduct the quantitative video and photographic transects to characterize the mesophotic benthic habitat, benthic biota, and fish populations at Pulley Ridge (PR) and Tortugas (T) regions. Within each random 1-km² block (“site”), we conducted five random 100-m transects with the ROV (Figs. 3-6). We made two ROV dives per op day, completing 42 random sites during the 2012 and 2013 cruises (Table 2).

A total of 44 ROV dives were conducted, resulting in a total bottom time of 99.75 hours, covering 45.45 km, at depths from 94.5 m to 23.1 m (PR = 94.5 m - 60.3 m; T = 58 m - 23.1 m). A total of 99.75 hours of ROV video were recorded (PR = 74.5; T = 24.5) and 7,563 *in situ* digital images were taken which included quantitative transect images (7,336), general habitat, and species documentation images.

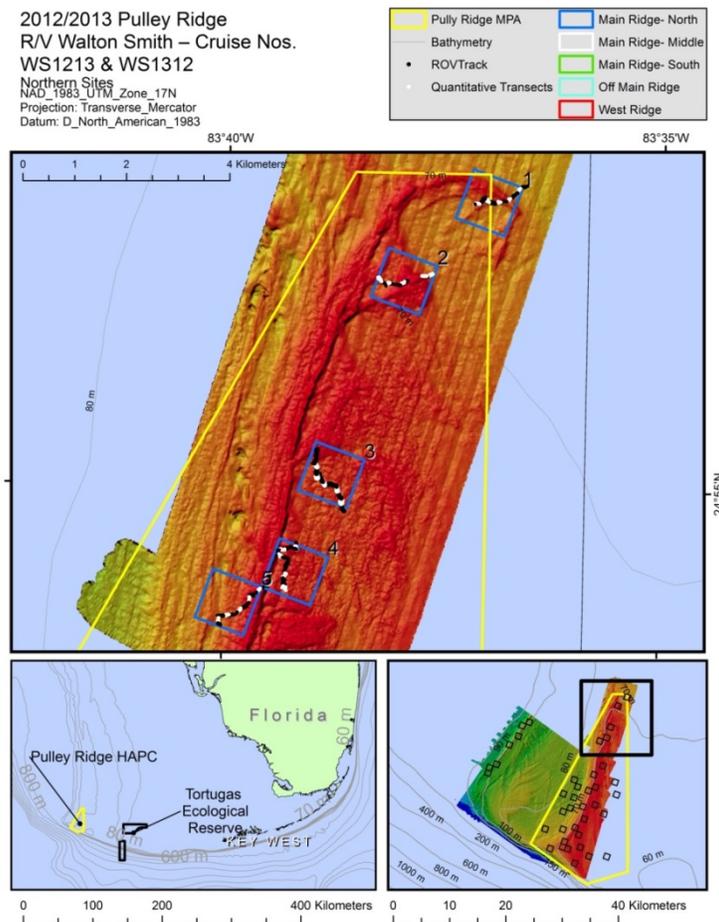


Figure 3. Locations of the Main Ridge northern blocks (blue = Main Ridge- North) and ROV dive transects on Pulley Ridge during the 2012/2013 R/V *Walton Smith* cruises (quantitative transects- white lines, non-quantitative transects- black lines).

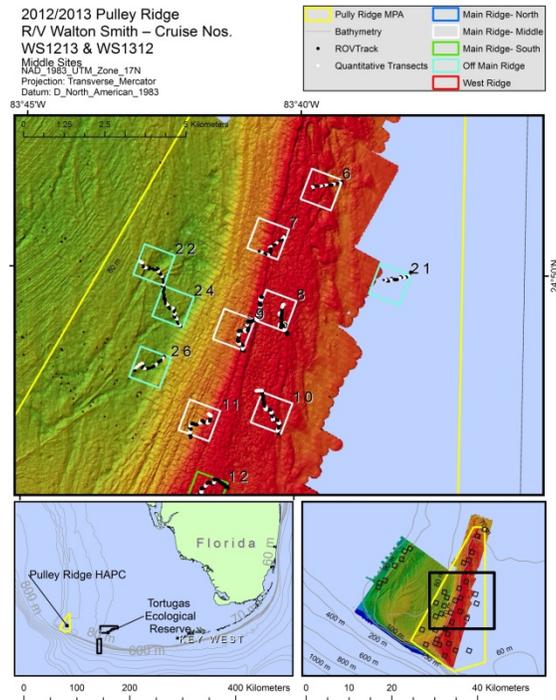


Figure 4. Locations of the Main Ridge middle blocks (white = Main Ridge- Middle; green = Off Main Ridge) and ROV dive transects on Pulley Ridge during the 2012/2013 R/V *Walton Smith* cruises (quantitative transects- white lines, non-quantitative transects- black lines).

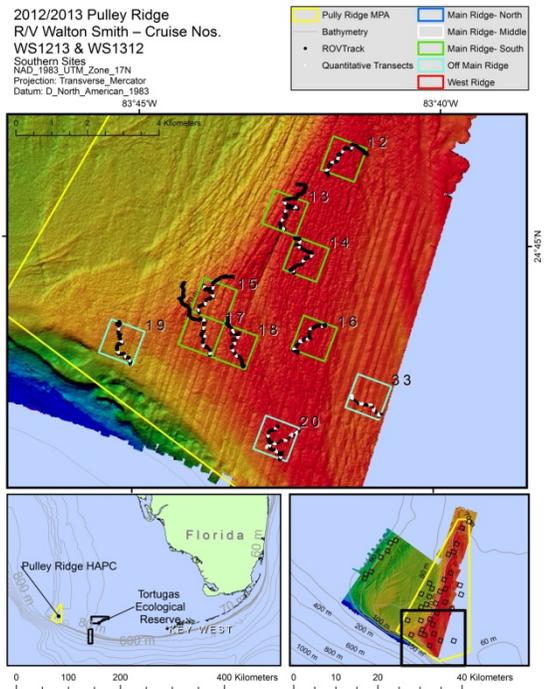


Figure 5. Locations of the Main Ridge southern blocks (green=- Main Ridge- South; white- Off Main Ridge) and ROV dive transects on Pulley Ridge during the 2012/2013 R/V *Walton Smith* cruises (quantitative transects- white lines, non-quantitative transects- black lines).

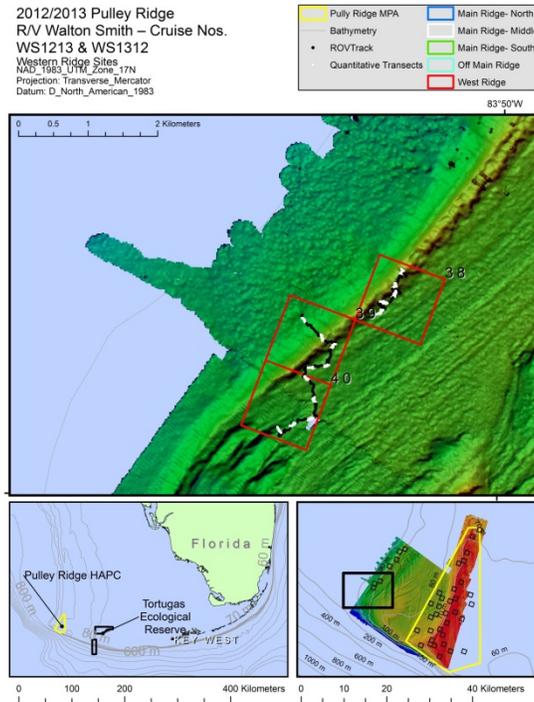


Figure 6. Locations of the West Ridge blocks (red= West Ridge) and ROV dive transects on Pulley Ridge during the 2012/2013 R/V *Walton Smith* cruises (quantitative transects- white lines, non-quantitative transects- black lines).

Table 2. ROV dive sites during 2012/2013 R/V *Walton Smith* cruises, August 14 to 25, 2012 and August 12 to 27, 2013. (Site Number= Day-Month-Year-Site).

Site Number	Method	Latitude (On Bottom)	Longitude (On Bottom)	Latitude (Off Bottom)	Longitude (Off Bottom)	Depth Range (m)	Distance (km)	Block Number
17-VIII-12-2	ROV 12-01	24.72°N	-83.72°W	24.73°N	-83.72°W	67-70	1.43	Block #18
17-VIII-12-4	ROV 12-02	24.72°N	-83.75°W	24.73°N	-83.75°W	73-78	1.04	Block #19
18-VIII-12-2	ROV 12-03	24.73°N	-83.73°W	24.74°N	-83.72°W	68-72	1.36	Block #15
18-VIII-12-4	ROV 12-04	24.69°N	-83.71°W	24.7°N	-83.71°W	66-69	0.93	Block #20
19-VIII-12-3	ROV 12-05	24.72°N	-83.73°W	24.72°N	-83.74°W	69-74	0.68	Block #17
19-VIII-12-5	ROV 12-06	24.72°N	-83.7°W	24.73°N	-83.7°W	67-68	1.1	Block #16
20-VIII-12-3	ROV 12-07	24.75°N	-83.71°W	24.76°N	-83.71°W	65-70	1.29	Block #13
20-VIII-12-5	ROV 12-08	24.79°N	-83.7°W	24.79°N	-83.69°W	68-74	0.91	Block #11
21-VIII-12-3	ROV 12-09	24.74°N	-83.71°W	24.75°N	-83.71°W	66-69	0.98	Block #14
21-VIII-12-5	ROV 12-10	24.77°N	-83.7°W	24.77°N	-83.69°W	65-68	1.24	Block #12
22-VIII-12-3	ROV 12-11	24.79°N	-83.67°W	24.8°N	-83.68°W	64.5-68.5	1.5	Block #10
22-VIII-12-6	ROV 12-12	24.91°N	-83.64°W	24.92°N	-83.65°W	66-71	1.29	Block #03
23-VIII-12-2	ROV 12-13	24.81°N	-83.68°W	24.83°N	-83.68°W	60-70	1.78	Block #09
23-VIII-12-4	ROV 12-14	24.82°N	-83.67°W	24.82°N	-83.67°W	63-67	0.93	Block #08
15-VIII-13-1	ROV 13-01	24.86°N	-83.65°W	24.86°N	-83.65°W	61-66	0	Block #06

15-VIII-13-2	ROV 13-02	24.86°N	-83.65°W	24.86°N	-83.66°W	64-71	0.92	Block #06
15-VIII-13-3	ROV 13-03	24.83°N	-83.63°W	24.83°N	-83.64°W	65-69	0.93	Block #21
16-VIII-13-1	ROV 13-04	24.84°N	-83.67°W	24.84°N	-83.68°W	62-71	0.91	Block #07
16-VIII-13-2	ROV 13-05	24.91°N	-83.65°W	24.9°N	-83.65°W	62-69	0.96	Block #04
17-VIII-13-1	ROV 13-06	24.9°N	-83.66°W	24.89°N	-83.67°W	59-72	1.13	Block #05
17-VIII-13-2	ROV 13-07	24.97°N	-83.61°W	24.97°N	-83.62°W	70-74	1.15	Block #01
17-VIII-13-3	ROV 13-08	24.95°N	-83.63°W	24.95°N	-83.63°W	62-71	0.2	Block #02
18-VIII-13-1	ROV 13-09	24.81°N	-83.71°W	24.8°N	-83.72°W	78-84	1.06	Block #26
18-VIII-13-2	ROV 13-10	24.82°N	-83.7°W	24.84°N	-83.71°W	68-84	2.38	Block #24
19-VIII-13-1	ROV 13-11	24.86°N	-83.85°W	24.86°N	-83.85°W	80-88	0.94	Block #38
19-VIII-13-2	ROV 13-12	24.86°N	-83.86°W	24.84°N	-83.86°W	82-96	1.64	Block #39 & #40
20-VIII-13-1	ROV 13-13	24.95°N	-83.63°W	24.95°N	-83.64°W	63-70	0.58	Block #02
20-VIII-13-2	ROV 13-14	24.7°N	-83.7°W	24.7°N	-83.71°W	64-69	1.05	Block #20
20-VIII-13-3	ROV 13-15	24.71°N	-83.68°W	24.72°N	-83.69°W	68-79	1.32	Block #33
21-VIII-13-1	ROV 13-16	24.67°N	-83.12°W	24.67°N	-83.13°W	55-57	0.94	Block #45
21-VIII-13-2	ROV 13-17	24.61°N	-83.13°W	24.61°N	-83.14°W	44-55	0.96	Block #48
21-VIII-13-3	ROV 13-18	24.57°N	-83.07°W	24.57°N	-83.08°W	25-31	0.7	Block #50
21-VIII-13-4	ROV 13-19	24.57°N	-83.13°W	24.57°N	-83.13°W	50-50	0.12	NR-Block #1082
22-VIII-13-1	ROV 13-20	24.49°N	-83.07°W	24.49°N	-83.08°W	43-48	1.3	Block #54
22-VIII-13-2	ROV 13-21	24.47°N	-83.07°W	24.48°N	-83.08°W	52-56	1.26	Block #57
23-VIII-13-3	ROV 13-22	24.46°N	-82.99°W	24.47°N	-83°W	44-50	0.81	Block #59
23-VIII-13-4	ROV 13-23	24.46°N	-82.96°W	24.47°N	-82.97°W	40-45	1.64	Block #60 & #58
24-VIII-13-2	ROV 13-24	24.75°N	-83.11°W	24.76°N	-83.12°W	46-57	0.69	Block #42
24-VIII-13-3	ROV 13-25	24.75°N	-83.15°W	24.75°N	-83.15°W	58-58	0.42	Block #41
25-VIII-13-1	ROV 13-26	24.53°N	-83.05°W	24.54°N	-83.06°W	32-35	0.87	Block #51
25-VIII-13-2	ROV 13-27	24.53°N	-83.01°W	24.54°N	-83.02°W	29-34	0.91	Block #52
25-VIII-13-3	ROV 13-28	24.5°N	-83.06°W	24.51°N	-83.07°W	43-44	0.86	Block #53
26-VIII-13-1	ROV 13-29	24.49°N	-83.01°W	24.49°N	-83.02°W	40-41	0.86	Block #55
26-VIII-13-2	ROV 13-30	24.47°N	-82.97°W	24.47°N	-82.97°W	34-41	0.5	Block #58

Benthic Macrobiota and Habitat

Appendix 1 lists all of the benthic macro-invertebrates and algal taxa that were identified from the quantitative photo transects at each mesophotic dive site and their percent cover based on CPCe Point Count of the photo images. Table 3 and Figure 7 compare the percent cover of bare substrate and benthic biota for each block and region. Overall, PR had very dense cover of biota ranging from 40.86 to 78.78% cover. Only 7.52% of the bottom of all sites at PR had bare sediment and 35.39% had bare rock (dead coral plates, rubble, cobble, or pavement). The Main Ridge-South had the highest level of biota cover (78.78%) and lowest cover of soft bottom

(0.99%). The Tortugas sites however were predominately soft bottom habitat (63.81% cover of bare sediment; Table 3, Fig. 8). Only six sites at Tortugas encountered hard bottom; three had patch reef habitat (Block 51, 52, 58; depth range 27 to 41 m) but were on the shallow end of the mesophotic zone. Some consider the shallow mesophotic zone as starting at 30 m.

Table 3. Percent cover (from CPCe Point Count) of benthic biota and bare substrate types for ROV sites surveyed at Pulley Ridge and Tortugas from 2012-2013 *Walton Smith* cruises.

Region	No. Blocks	% Cover-Biota	% Cover-Bare Sediment	% Cover-Bare Rock	Grand Total	Depth Range (m)
Pulley Ridge	28	57.09%	7.52%	35.39%	100%	94.5 m - 60.3 m
Main Ridge- North	5	47.63%	8.64%	43.72%	100%	73.5 m - 60.3 m
Main Ridge- Middle	6	61.85%	3.49%	34.65%	100%	73.2 m - 61 m
Main Ridge- South	7	78.78%	0.99%	20.23%	100%	71.8 m - 63.5 m
Off Main Ridge	7	40.86%	16.11%	43.03%	100%	83.2 m - 64.5 m
West Ridge	3	49.75%	9.53%	40.72%	100%	94.5 m - 80.1 m
Tortugas	14	29.37%	63.81%	6.82%	100%	58 m - 23.1 m
Grand Total	42	48.17%	25.64%	26.20%	100%	94.5 m - 23.1 m

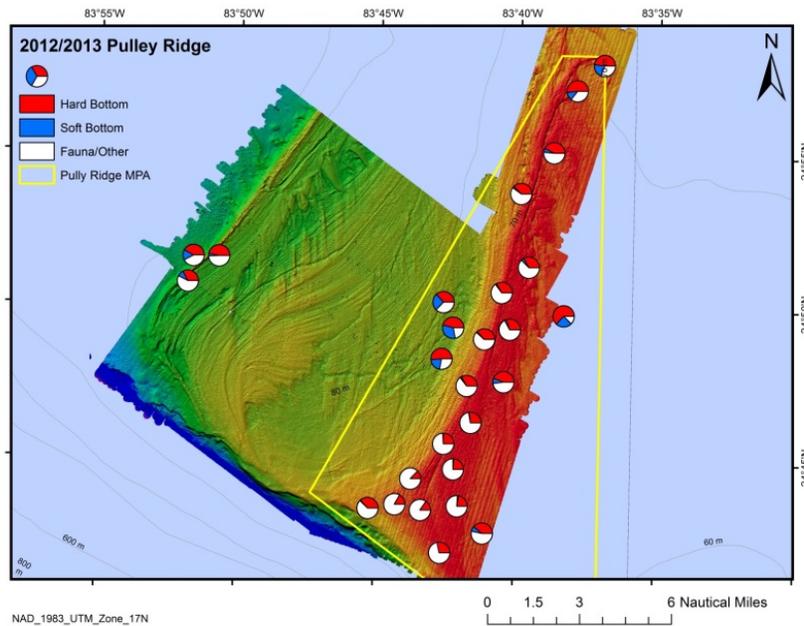


Figure 7. Percent cover of bare substrate and benthic macrobiota at Pulley Ridge from 2012-2013 *Walton Smith* cruises.

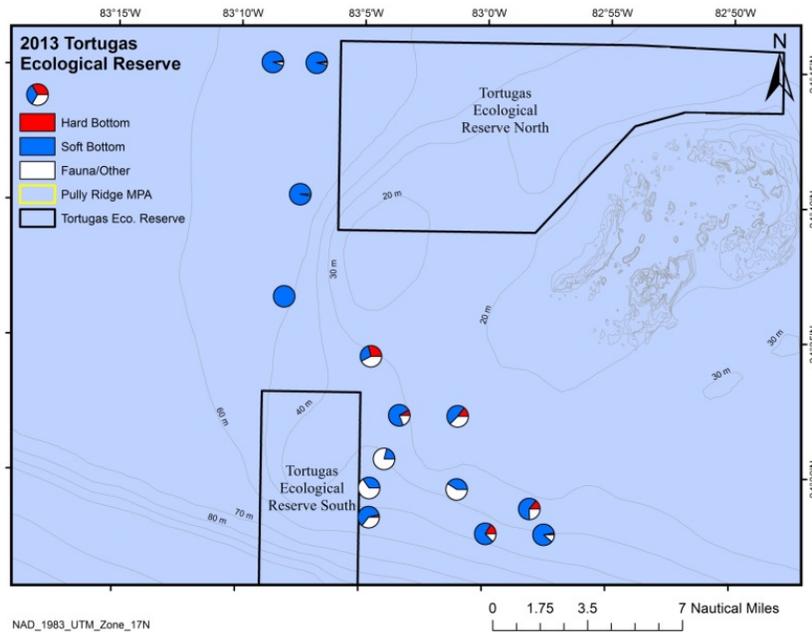


Figure 8. Percent cover of bare substrate and benthic macrobiota at Tortugas from 2012-2013 *Walton Smith* cruises.

A total of 216 and 196 taxa of benthic biota were identified from the quantitative photo transects at Pulley Ridge (PR) and Tortugas (T), respectively, and were used for CPCe percent cover analyses (Appendix 1, Table 4, Figs. 9-10). These included 102/60 taxa (PR and T, respectively) of Porifera and 47/54 Cnidaria which included the following corals: 32 Scleractinia, 27 Alcyonacea- gorgonian, 4 soft corals (Alcyoniina), and 4 black coral (Antipathidae) (Table 5). Other fauna included Annelida, Mollusca, Arthropoda, Bryozoa, Echinodermata, and Ascidiacea. A large part of Pulley Ridge and the Tortugas sites were dominated by macroalgae (Fig. 9): Chlorophyta (11/22 taxa, PR and T, respectively), Phaeophyceae (4/5), and Rhodophyta (7/8). On Pulley Ridge, algae (predominately *Anadyomene menziesii* and crustose corallines) provided an average of 53.8% cover. The greatest cover of algae (75.1%) was on the Main Ridge- South (Fig. 9). *A. menziesii*- lettuce algae were also predominant on the Main Ridge, but were mostly absent from the Off Main Ridge blocks and the West Ridge which was deeper and which was dominated by Rhodophyta, such as fleshy *Kallymenia* and crustose corallines. On Pulley Ridge, sponges contributed an average of 1.6% cover and were fairly consistent in density over the entire Main Pulley Ridge (Table 4). However, they were generally densest at the Off Main Ridge Blocks and on the West Ridge.

Table 4. Percent cover of dominant benthic taxa and species richness for ROV sites at Pulley Ridge and Tortugas from 2012-2013 *Walton Smith* cruises.

Region/General Area	%				# Taxa-Cnidaria	# Taxa-Sponges	# Taxa-Algae	# Taxa-Total
	Hard Coral	% Algae	% Sponges	% Bare Substrate				
Pulley Ridge	0.9%	53.8%	1.6%	42.9%	47	102	23	216
Main Ridge- Middle	0.2%	59.5%	1.3%	38.1%				
Main Ridge- North	0.2%	45.5%	1.2%	52.4%				
Main Ridge- South	1.9%	75.1%	1.2%	21.2%				
Off Main Ridge	0.7%	37.2%	2.3%	59.1%				
West Ridge	1.4%	44.0%	2.5%	50.2%				
Tortugas	0.1%	27.0%	0.9%	69.5%	54	60	37	196
Grand Total	0.6%	45.4%	1.4%	51.2%				

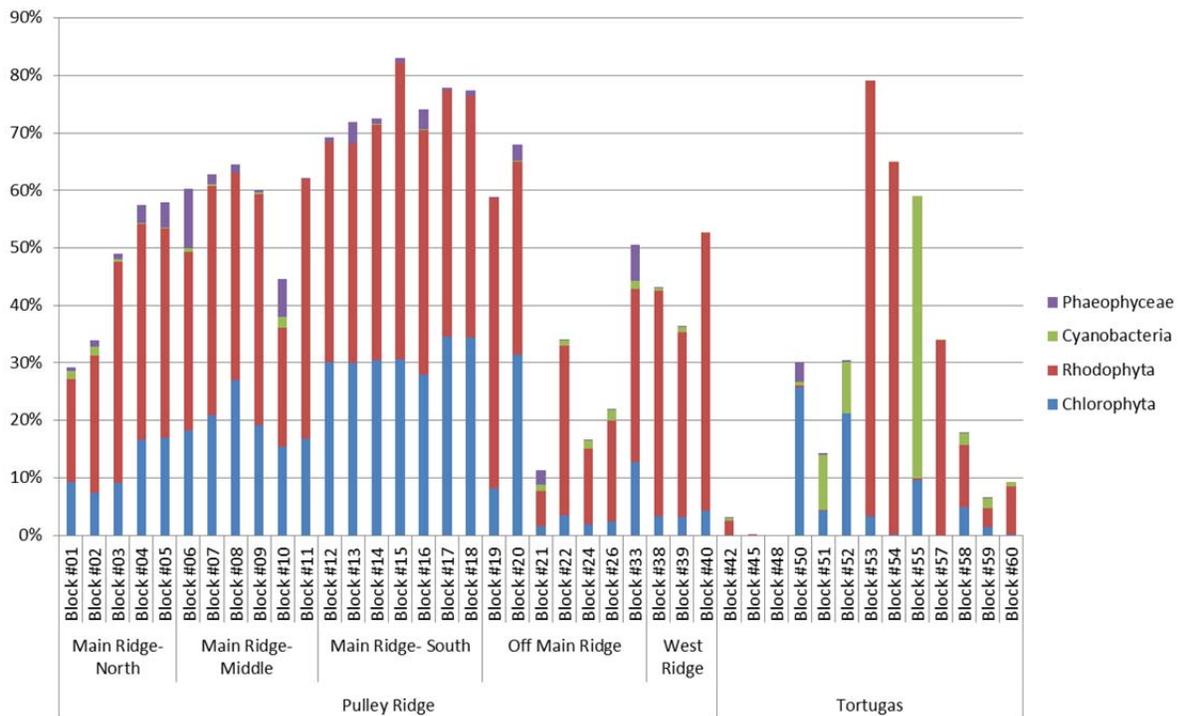


Figure 9. Percent cover of macroalgae at Pulley Ridge and Tortugas by Block and Geographic Area.

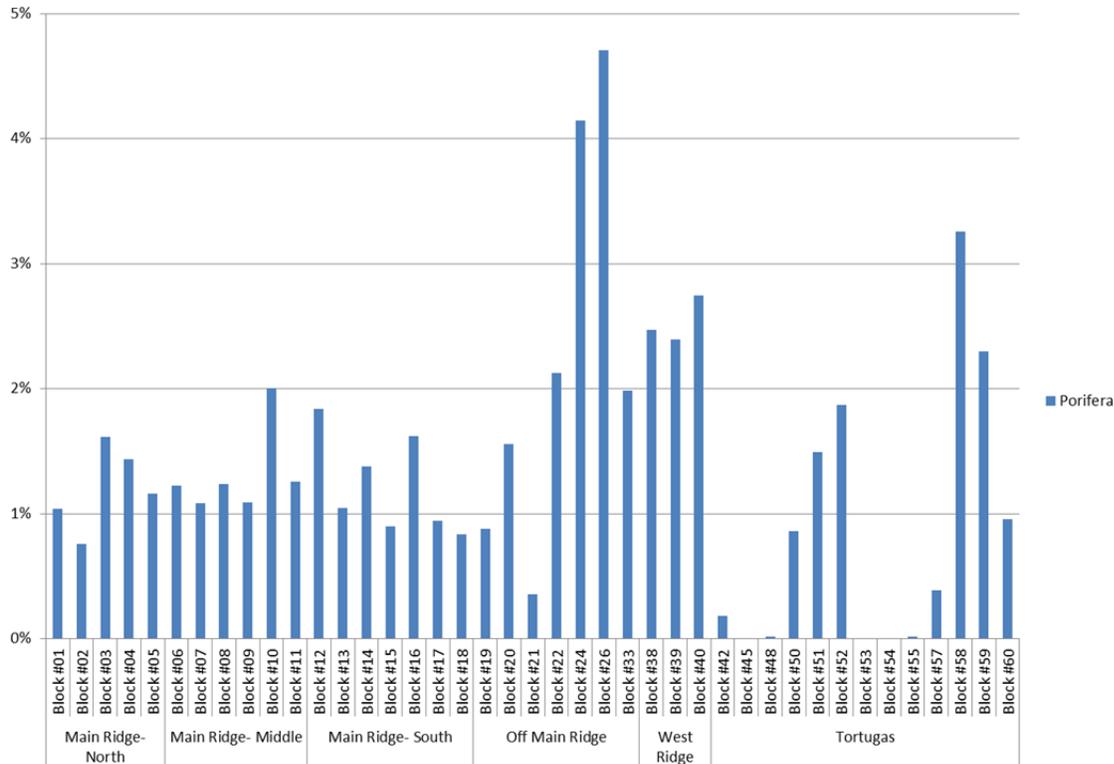


Figure 10. Percent cover of sponges at Pulley Ridge and Tortugas by Block and Geographic Area.

Table 5. Distribution of hard corals, gorgonacea, soft corals, and Antipatharia at mesophotic reef sites at Pulley Ridge and Tortugas from 2012-2012 *Walton Smith* cruises.

<u>Hard Coral</u>	<u>Pulley Ridge</u>	<u>Tortugas</u>
<i>Agaricia</i> sp.	X	X
<i>Agaricia fragilis</i>	X	
<i>Agaricia lamarcki/ grahamae</i>	X	
<i>Colpophyllia natans</i>		X
<i>Eusmilia fastigiata</i>		X
<i>Favia fragum</i>		X
<i>Leptoseris cucullata</i>	X	
<i>Madracis auretenra</i>	X	
<i>Madracis decactis</i>		X
<i>Madracis formosa</i>	X	
<i>Madracis pharensis</i>	X	
<i>Madracis</i> sp.	X	
<i>Manicina areolata</i>	X	X
<i>Meandrina meandrites</i>		X
<i>Millepora alcicornis</i>		X
<i>Montastraea cavernosa</i>	X	X
<i>Mycetophyllia</i> sp.		X

<i>Oculina diffusa</i>	X	X
<i>Orbicella annularis</i>		X
<i>Orbicella faveolata</i>		X
<i>Orbicella franksi</i>		X
<i>Pseudodiploria clivosa</i>		X
<i>Pseudodiploria strigosa</i>		X
Scleractinia- unid colonial	X	X
Scleractinia- unid solitary	X	X
<i>Scolymia</i> sp.	X	X
<i>Siderastrea siderea</i>		X
<i>Siderastrea radians</i>		X
<i>Solenastrea</i> sp.		X
<i>Stephanocoenia intersepta</i>	X	
Stylasteridae (hydrocoral)	X	
<i>Undaria agaricites</i>		X
Gorgonacea		
Alcyonacea- unid gorgonian	X	X
<i>Bebryce</i> sp.	X	
<i>Briareum asbestinum</i>		X
<i>Diodogorgia nodulifera</i>	X	
<i>Ellisella barbadensis</i>		X
<i>Ellisella</i> sp.	X	X
Ellisellidae	X	X
<i>Erythropodium caribaeorum</i>		X
<i>Eunicea</i> sp.		X
<i>Iciligorgia schrammi</i>		X
Isididae	X	
<i>Leptogorgia</i> sp.	X	
<i>Muricea</i> sp.	X	X
<i>Muriceopsis</i> sp.		X
<i>Nicella goreau</i>	X	
<i>Nicella</i> sp.	X	
<i>Plexaura kukenthali</i>		X
<i>Plexaurella nutans</i>		X
<i>Plexaurella</i> sp.		X
Primnoidae	X	X
<i>Pseudoplexaura</i> sp.		X
<i>Pseudopterogorgia</i> sp.	X	X
<i>Pterogorgia anceps</i>		X
<i>Swiftia exserta</i>	X	
<i>Telesto</i> sp.	X	
<i>Thesea</i> sp.	X	
Alcyoniina		
Alcyoniina- unid. sp.	X	

<i>Chironophthya caribaea</i>	X
Nephtheidae	X
<i>Nidalia</i> sp.	X
Antipathidae	
Antipathidae- unid. sp.	X
Antipatharia- white fan	X
<i>Stichopathes lutkeni</i>	X
<i>Tanacetipathes hirta</i>	X

Coral Analyses

At PR, a total of 14 Scleractinia taxa were identified. These included the agariciids, *L. cucullata*, *M. cavernosa*, *Madracis auretenra*, *M. formosa*, *M. pharensis*, *Manicina areolata*, *Oculina diffusa*, *Stephanocoenia intersepta*, *Scolymia* spp., and small unidentified solitary corals (Appendix 1, Table 5). A total of 839 digital still transect images had coral; total individual coral colonies was 2,272, and of these 2,075 were measured for maximum diameter.

At PR, the range of coral cover by block was 0.02 to 5.62%. Site #18 on Main Ridge-South had the greatest cover of all individual blocks with 5.62% and every PR block had some coral (Fig. 11). The average percent cover at PR of all coral species (colonial and solitary Scleractinia) was 0.85% (Table 6). The greatest average coral cover was on Main Ridge-South (1.85%), followed by the West Ridge (1.44%). The lowest cover was Main Ridge-North and -Middle (0.19 and 0.20%, respectively). The plate coral taxa (*Agaricia* spp., *L. cucullata*) were also greatest at the Main Ridge- South (1.69% average cover) and lowest on the Main Ridge- North and West Ridge (0.08%). However, *M. cavernosa* were more prevalent on the Main Ridge- North, and none were counted by Point Count on the Off Ridge and West Ridge regions. The West Ridge was dominated by *Madracis* spp.

Table 6. Average percent coral cover (CPCe point count) and densities by species. Plate coral includes agariciids and *Leptoseris cucullata*. *Montastraea cavernosa* are counted separately. *Madracis* included *M. pharensis*, *M. auretenra*, and *M. formosa*.

Region/ General Area	% Cover- Plate Coral	% Cover- M. cavernosa	% Cover- Madracis	% other Hard Coral	% Cover- All Hard Coral	Density- Plate Coral (#/m2)	Density- M. cavernosa (#/m2)
Pulley Ridge	0.55%	0.01%	0.29%	0.01%	0.85%	0.54	0.024
Main Ridge-North	0.08%	0.04%	0.06%	0.00%	0.19%	0.20	0.004
Main Ridge-Middle	0.17%	0.01%	0.03%	0.00%	0.20%	0.24	0.016
Main Ridge-South	1.69%	0.00%	0.16%	0.00%	1.85%	3.66	0.004
Off Main Ridge	0.30%	0.00%	0.38%	0.01%	0.68%	0.94	0.000
West Ridge	0.08%	0.00%	1.31%	0.05%	1.44%	0.49	0.000
Tortugas	0.00%	0.01%	0.00%	0.06%	0.08%	N/A	N/A
Grand Total	0.38%	0.01%	0.20%	0.02%	0.61%		

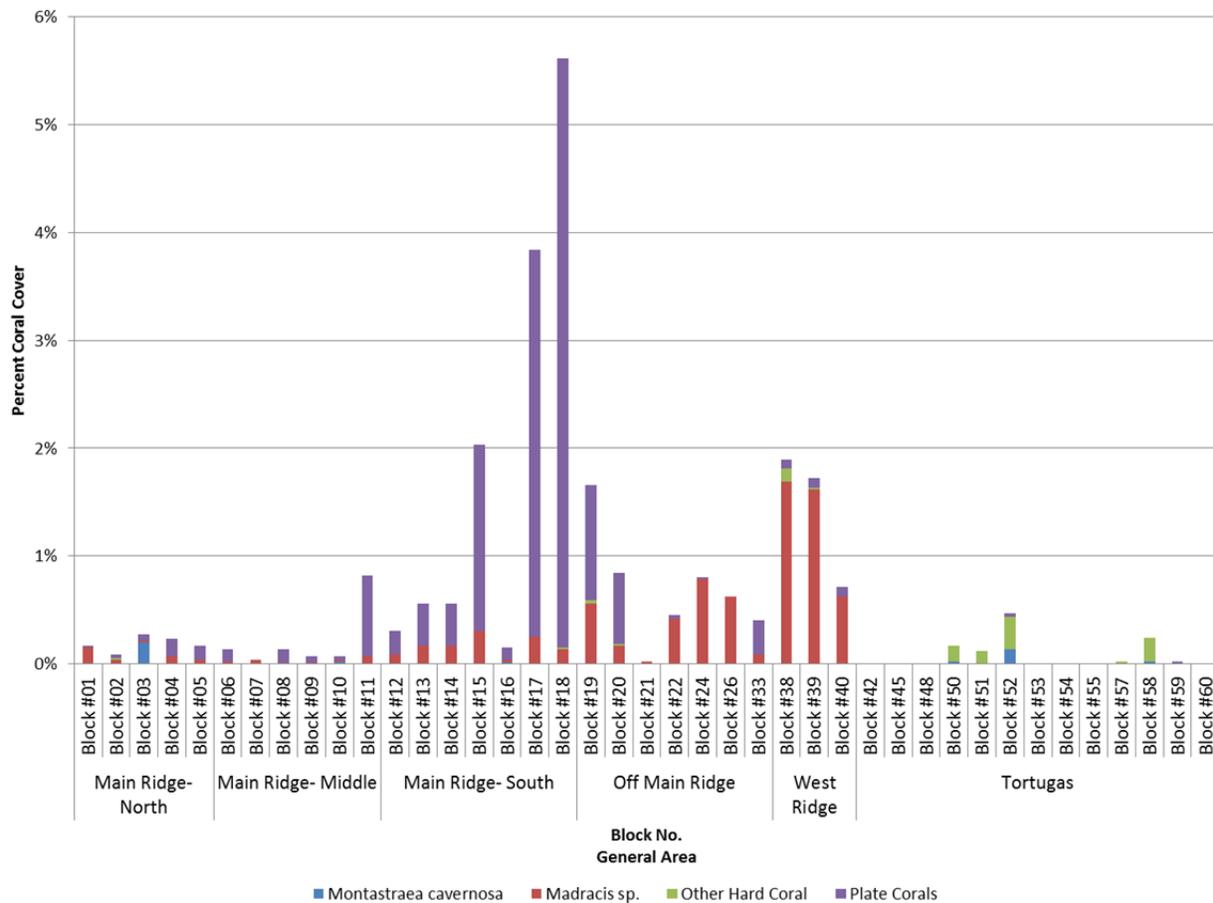


Figure 11. Percent cover of scleractinian coral by Block and Geographic Area.

Coral Density Analysis

A total of 3,528 of the digital still images had visible lasers which allowed for the area to be determined; maximum single image area was = 2.181 m², minimum area = 0.043 m², average = 0.47 m². For 2013 PR there were 193 images with coral; 2012 had 622 images with coral. This difference was primarily due to the fact that the dive sites in 2012 were predominately in the Main Ridge- South which had the most corals (Fig. 12). Table 6 shows the overall density of plate corals for all PR sites was 0.54 colonies m⁻², with the greatest density at the Main Ridge-South (3.66). *M. cavernosa* density was greatest (0.12) at the Main Ridge-Middle.

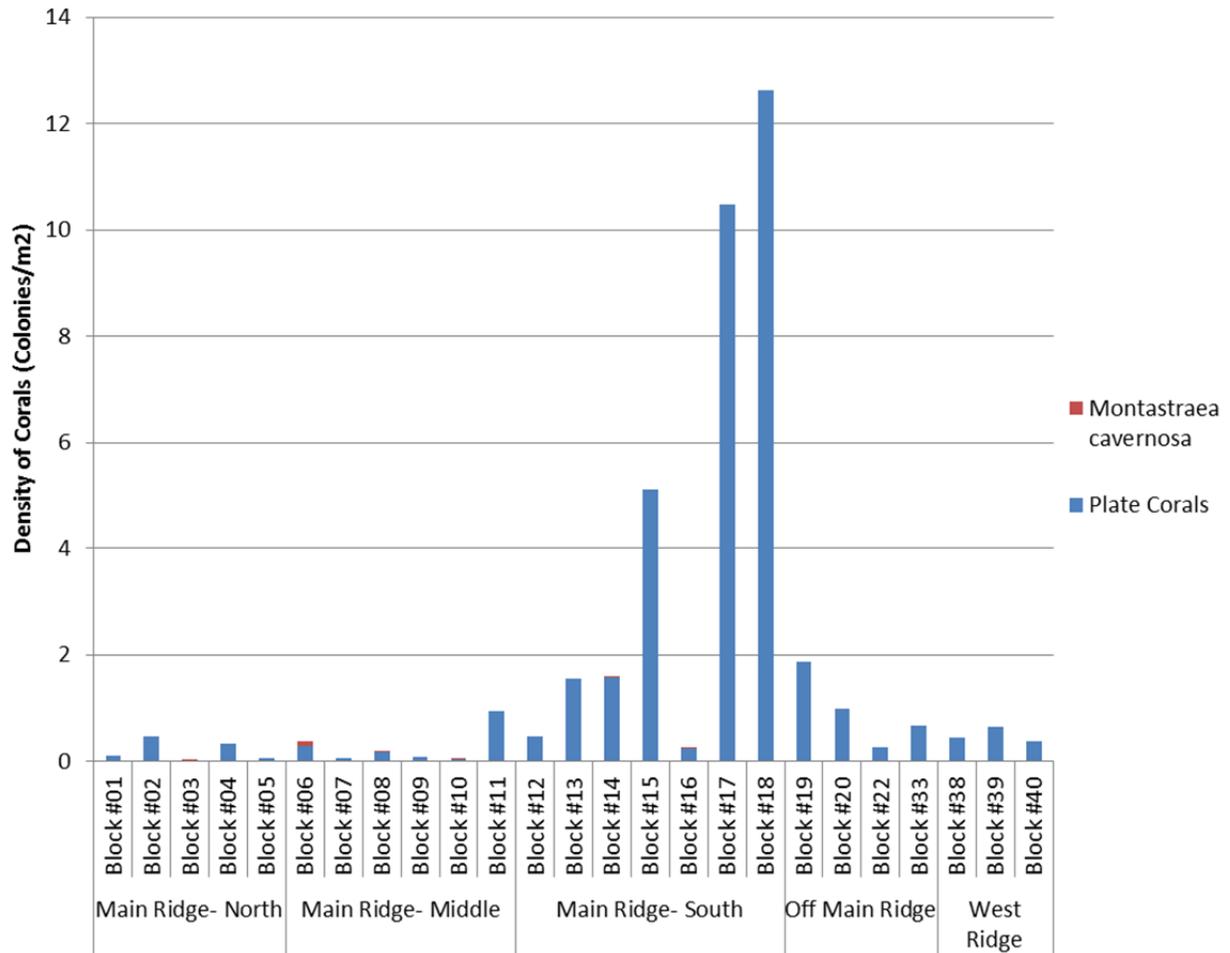


Figure 12. Density of scleractinian plate corals (agariciids and *Leptoseris cucullata*) and *Montastraea cavernosa* at Pulley Ridge HAPC and West Ridge outside of the HAPC from 2012 and 2013 ROV dives by Block and Geographic Area.

Coral Size

Using the data set collected above, a histogram was created by counting the number of each plate coral and *M. cavernosa* that fell inside each 5 cm size class (<5 cm, 5 to 9 cm, 10- 14 cm, 15- 19 cm, etc.) (Fig. 13). The histogram clearly shows the corals are greatly dominated by relatively newly settled colonies; 47.68% were <5 cm diameter and 35.41% were 5 to 9 cm diameter. Although we do not have growth rates for these corals at mesophotic depths, the growth rate of *Montastraea annularis* in shallow water is 6.6-8.9 mm/yr and *Agaricia asteroides* is 3.0-3.5 mm/yr (Gladfelter et al. 1978). So if we assume a maximum growth rate of 10 mm/yr, these small corals of <5 cm diameter are less than 5 years old. Minimum coral measured was 0.476 cm and maximum diameter measured was 52.32 cm. The greatest coral set was in the Main Ridge- South region (Fig. 14).

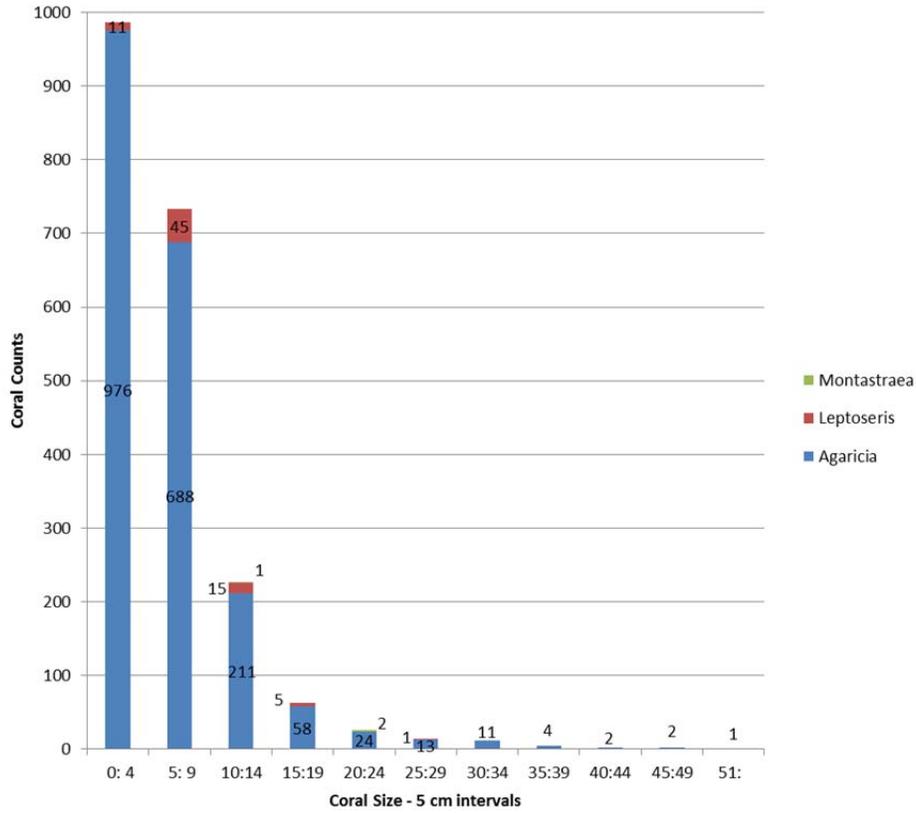


Figure 13. Size distribution of plate corals (agariciids and *L. cucullata*) and *M. cavernosa* for all Pulley Ridge dives. Size in cm of maximum diameter.

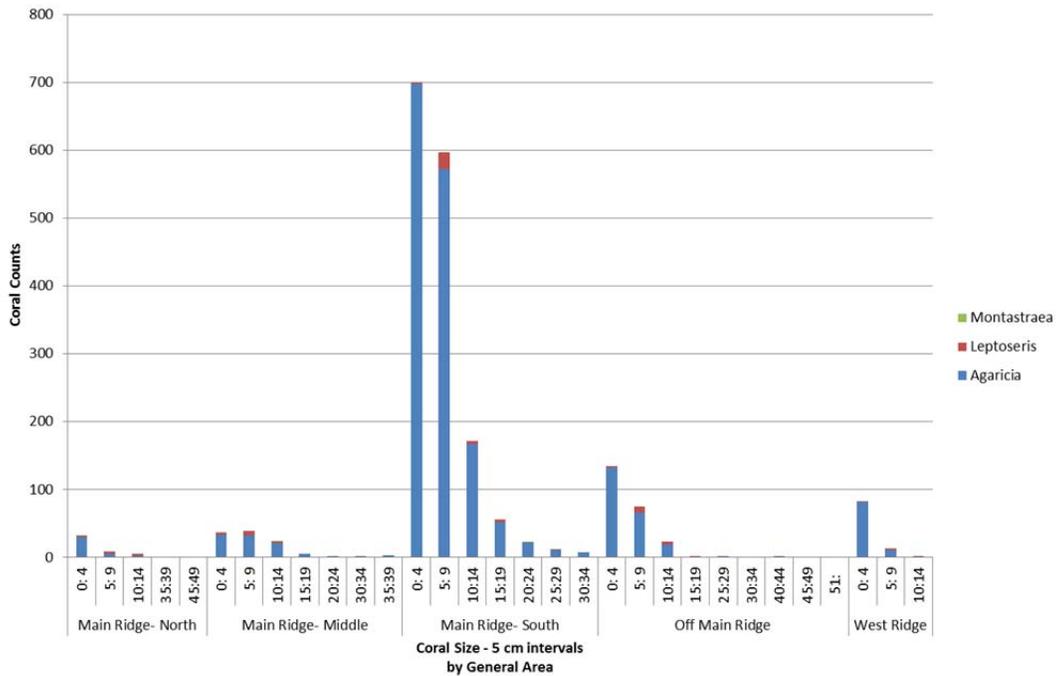


Figure 14. Coral size histogram in 5 cm intervals by Geographic Area at Pulley Ridge. Size in cm of maximum diameter.

Previous Coral Data

In 2003, the USGS surveyed 14 transects, each ~1+ km long, along the main Pulley Ridge (equivalent to our North, Middle and South regions) using a towed sled called the SeaBOSS (Fig. 15) (Cross et al. 2005; Hine et al. 2008). Downward looking digital still images were of comparable area as ours, ~1-2 m². Using the photographs collected from the SeaBOSS, Coral Point Count was used to define 32 classes within 7 major categories (Table 7). The data was analyzed by dive # (Table 8). Overall, coral cover was 11.69%; Northern and Middle transects showed the highest percent cover of coral (12.18% and 14.06%, respectively). Dive 13, in the Middle zone had the greatest coral cover of all dives (23.23%).

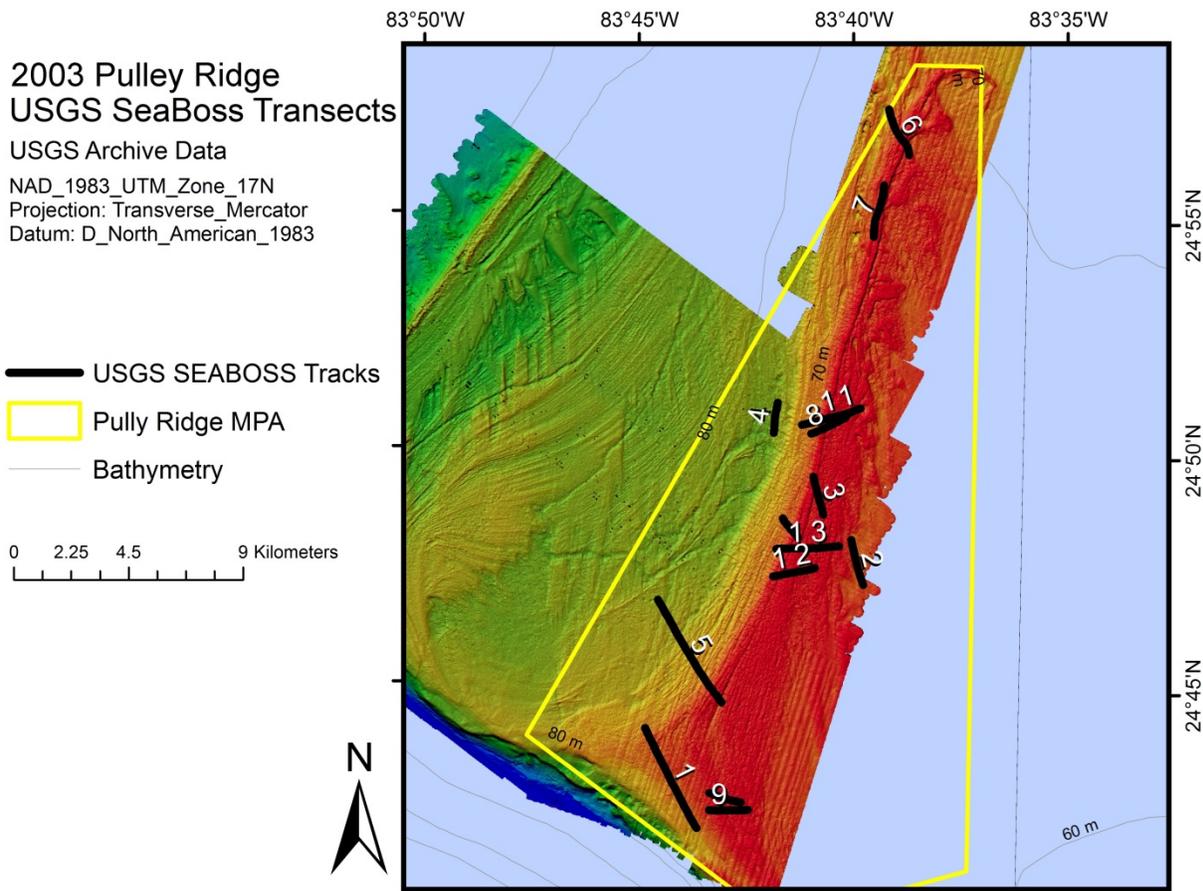


Figure 15. USGS SeaBOSS photographic transects conducted on Pulley Ridge in 2003.

Table 7. Classifications used by USGC in survey of Pulley Ridge in 2003.

USGS CPCe Code list
Chlorophyta
Anadyomene menziesii Chlorophyta undifferentiated Halimeda spp.
Coral
Agaricia spp. Leptoseris cucullata Madracis spp. Montastraea spp. Oculina spp. Porites sp. Scolymia spp.
Hard Bottom
Algal Nodule Bare limestone rock Coral skeleton Gravel/Rubble Limestone Gray Rubble Gray Rubble Green Rubble Red Shell Debris Shell Gray turf covered limestone rock
Phaeophyta
Dictyota divaricata Lobophora variegata Phaeophyta undifferentiated Ventricaria ventricosa
Porifera
Porifera
Rhodophyta
Rhodophyta maroon Rhodophyta pink Rhodophyta red Rhodophyta undifferentiated
Soft Bottom
Sand
Unknown
Unknown

Table 8. Comparison of percent cover of coral, sponges, algae and substrate at Pulley Ridge from 2003 USGS SeaBOSS photographic transects ((Hine et al. 2008) and 2012-2013 R/V *Walton Smith* cruises. Main Ridge-North, -Middle, -South; CCA= crustose coralline algae; *Anadyomene menziesii* (leaf green algae); *Lobophora* (brown algae).

2003 USGS SEABOSS Transects

Region/ Dive Number	Total Coral	Porifera	CCA	<i>A. menziesii</i>	<i>Lobo- phora</i>	Sand	Lime- stone/ Rock	Dead Coral Plate	Rubble/ Shell
North	12.47%	2.18%	26.57%	4.39%	0.46%	5.35%	28.75%	1.94%	10.19%
4	0.67%	6.67%	6.67%	0.33%	2.00%	44.33%	7.00%	0.00%	20.67%
6	1.34%	2.68%	16.83%	7.07%	0.12%	8.90%	29.63%	0.00%	20.00%
7	17.83%	1.59%	28.99%	4.64%	0.14%	0.58%	28.41%	2.46%	7.54%
8	17.74%	2.83%	29.43%	5.28%	0.38%	1.13%	26.60%	2.83%	5.85%
11	15.85%	1.26%	32.46%	3.50%	0.49%	0.38%	32.68%	2.68%	6.34%
Middle	14.15%	1.76%	31.76%	7.55%	0.12%	3.20%	19.39%	1.79%	11.93%
2	0.19%	2.83%	4.72%	2.45%	0.00%	20.94%	5.85%	0.00%	53.21%
3	15.08%	0.95%	38.25%	6.03%	0.32%	0.00%	22.06%	3.49%	6.03%
12	13.04%	1.61%	42.86%	10.36%	0.18%	0.00%	18.57%	2.50%	5.36%
13	23.23%	1.41%	29.70%	6.77%	0.10%	0.00%	20.71%	1.31%	3.84%
14	12.11%	2.24%	39.74%	11.32%	0.00%	0.00%	25.53%	1.71%	3.42%
South	9.04%	1.49%	41.18%	18.11%	0.68%	0.00%	18.08%	1.44%	2.76%
1	5.69%	2.11%	40.28%	24.40%	0.46%	0.00%	19.63%	1.10%	2.39%
5	9.21%	0.48%	40.63%	15.00%	1.51%	0.00%	21.11%	0.71%	3.49%
9	10.00%	2.83%	43.48%	20.22%	0.00%	0.00%	11.30%	3.04%	4.13%
10	13.11%	1.49%	42.03%	12.84%	0.00%	0.00%	14.86%	2.16%	1.22%
Grand Total	11.90%	1.83%	32.82%	9.72%	0.42%	2.98%	22.47%	1.73%	8.37%

2012-2013 R/V Walton Smith Cruises

Region/ General Area	Total Coral	Porifera	CCA	<i>A. menziesii</i>	<i>Lobo- phora</i>	Bare Soft Bottom	Bare Rock	Dead Coral Plate	Bare rubble/ cobble
Pulley Ridge	0.85%	1.63%	29.30%	15.01%	1.00%	7.52%	5.67%	0.42%	29.31%
Main Ridge- North	0.19%	1.20%	22.80%	9.13%	0.44%	8.64%	5.53%	0.23%	37.96%
Main Ridge- Middle	0.20%	1.31%	33.51%	19.11%	2.23%	3.49%	7.23%	0.42%	26.98%
Main Ridge- South	1.85%	1.22%	41.45%	29.93%	0.95%	0.99%	6.86%	1.11%	12.27%
Off Main Ridge	0.68%	2.25%	19.16%	6.64%	0.73%	16.11%	4.48%	0.04%	38.50%

West Ridge	1.44%	2.54%	26.39%	0.64%	0.00%	9.53%	2.51%	0.00%	38.20%
Tortugas	0.08%	0.87%	0.03%	0.00%	0.01%	62.49%	1.98%	0.00%	5.04%
Grand Total	0.61%	1.39%	20.17%	10.33%	0.69%	24.68%	4.52%	0.29%	21.74%

Table 9. Comparison of coral cover at Pulley Ridge from 2003 USGS SEABOSS transects and 2012-2013 R/V *Walton Smith* cruises.

2003 USGS SEABOSS Transects

Area	<i>Agaricia</i> spp.	<i>Leptoseris cucullata</i>	<i>Madracis</i> spp.	<i>Manicina areolata</i>	<i>M. cavernosa</i>	<i>Oculina</i> spp.	<i>Porites</i> sp.	<i>Scolymia</i> spp.	% Cover Range	Total %
North	10.43%	0.67%	0.65%	0.00%	0.43%	0.24%	0.00%	0.05%	0.67-17.83%	12.47
Middle	12.25%	0.17%	0.84%	0.00%	0.81%	0.06%	0.00%	0.03%	0.19-23.23%	14.15
South	7.58%	0.28%	0.82%	0.00%	0.11%	0.20%	0.03%	0.03%	5.69-13.11%	9.04
Grand Total	10.09%	0.39%	0.76%	0.00%	0.45%	0.17%	0.01%	0.04%	0.67-23.23%	11.90

2012-2013 R/V Walton Smith Cruises

Area	<i>Agaricia</i> spp.	<i>Leptoseris cucullata</i>	<i>Madracis</i> spp.	<i>Manicina areolata</i>	<i>M. cavernosa</i>	<i>Oculina</i> spp.	<i>Porites</i> sp.	<i>Scolymia</i> spp.	% Cover Range	Total %
Main Ridge-North	0.07%	0.01%	0.06%	0.00%	0.04%	0.00%	0.00%	0.00%	0.08 - 0.27%	0.19
Main Ridge-Middle	0.16%	0.01%	0.03%	0.00%	0.01%	0.00%	0.00%	0.00%	0.03 - .082%	0.20
Main Ridge-South	1.62%	0.07%	0.16%	0.00%	0.00%	0.00%	0.00%	0.00%	0.15 - 5.62%	1.85
Off Main Ridge	0.29%	0.01%	0.38%	0.00%	0.00%	0.00%	0.00%	0.00%	0.02 - 1.66%	0.68
West Ridge	0.05%	0.03%	1.31%	0.00%	0.00%	0.00%	0.00%	0.00%	0.71 - 1.89%	1.44
Grand Total	0.52%	0.03%	0.29%	0.00%	0.01%	0.00%	0.00%	0.00%	0.02 - 5.62%	0.85

Comparing the 2003 year USGS data with our data of 2012-2013, within 10 years the coral cover has drastically declined on Pulley Ridge (Table 9). The overall mean coral cover went from 11.90% to 0.85% which is a 92.8% loss of coral cover! The average range of total percent cover by region (North, Middle, South) was 9.04 to 14.15% in year 2003, and 0.19 to 1.85% in 2013. Maximum cover of any one transect was 23.23% in 2003 (Dive 13, Middle Region) and 5.62% in 2013 (Block 18, South Region). The Main Ridge-Middle region had the greatest overall lost from an average of 14.15% cover in 2003 to 0.2% in 2013, or a 98.6% loss. All major coral species lost cover; *Agaricia* spp. dropped from 12.25% maximum cover in 2003 to 1.62% in 2012-2013; *Leptoseris* dropped from 0.67% to 0.07%; *Montastraea* from 0.81% to 0.04%; and *Oculina* sp. 0.24 to <0.01%. Our coral size analysis, which is not available for the USGS data, does show some promising recovery in that 47.68% of the corals at Pulley Ridge are fairly newly

settled, <5 cm diameter. Using the Geostatistical Analyst in ArcGIS 10.0, interpolation maps using Kernel smoothing were used to estimate the cover of coral over the entire Pulley Ridge region (Fig. 16). The coral cover hot spot is clearly evident in the middle region of PR in 2003, and is missing by 2013. A slightly higher coral cover is evident in 2013 at the very southern portion of PR.

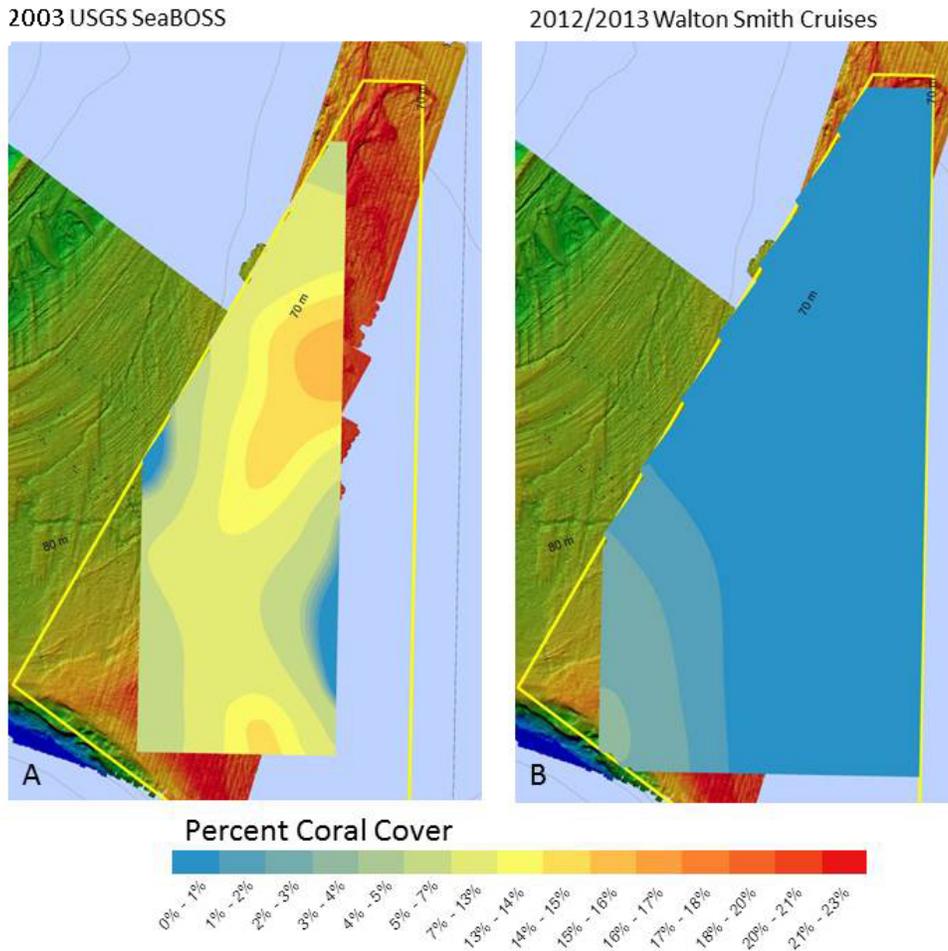


Figure 16. Comparison of percent hard coral cover of the 2003 USGS SeaBOSS photo transect data to the 2012-2013 *Walton Smith* cruise data using Kernel smoothing method in ArcGIS (scale 0-23% cover).

Causes of Coral Loss

What was the cause(s) for this coral loss on Pulley Ridge in the past decade? The usual predictors of coral death on shallow water reefs is typically, global warming (temperature >30°C for some period) causing coral bleaching and death, coral disease, coral predators (fish, mollusks, polychaetes), lack of light (turbidity), smothering (dredging), direct human impact (bottom trawls, traps, oil spill), and natural impact (hurricanes). Part of this research grant is to better understand mesophotic reefs, their comparative health, and connectivity to shallow water reefs. Mesophotic reefs have been considered in general to be more stable and less impacted by warm temperature, and direct human impacts. The majority of corals we have seen on Pulley

Ridge appear to be relatively healthy, although a few showed signs of recent tissue loss or disease. No obvious bleaching has been observed. In 2012 UM deployed an ADCP at the Pulley Ridge site, and now have a year's bottom temperature and current data. Overall the bottom temperature at 69.5 m depth ranged from 19.71 to 27.67°C and averaged 23°C (Figure 17). We have never seen temperatures on Pulley Ridge warm enough to cause bleaching. In general, corals become stressed and may begin bleaching at temperatures exceeding 29°C, but the historical temperature variability is a factor too (Carilli et al. 2012). Also we do not know as yet the genetic connectivity of these mesophotic corals with shallow ones, and their zooxanthellae symbionts may have different stress limitations. There is no evidence of extensive damage from fish traps or trawls, although there have been ghost traps observed primarily along the south drop-off, south of the PR HAPC. We have not observed any predation by fish or invertebrates causing major damage. At depths of 70-80 m, we do not expect damage from hurricanes but it is a possibility. In 2012 Hurricane Isaac went directly over PR, but last year's dives in 2013 did not indicate any obvious damage. Loose leafed *Anadyomene* algae were as abundant as ever. Turbidity and smothering are unlikely in this region that is over 100 miles off the Florida coast. The water is usually crystal clear and usually >20 m visibility on bottom. It is in a region of variable currents, and potential upwelling. The Gulf of Mexico Loop Current meanders along this region and certainly can affect the bottom water conditions and chemistry. Surface currents in the Loop can exceed 3 knots. It is possible a strong cold-water upwelling event could impact the coral. Off the Florida east coast, cold water upwelling events often bleach *Oculina* coral on the mid shelf (Reed 1980), but we never have seen coral death due to that. Could the 2010 oil spill have caused the die off? In the summer of 2010, and during the Deep Horizon oil spill, HBOI-CIOERT conducted a month long research cruise with HBOI-FAU's R/V *Seward Johnson* and the *Johnson-Sea-Link II* submersible to survey the mesophotic reefs all along the west Florida shelf from the Florida Keys to Madison Swanson off Pensacola to see if there was any oil impact on the benthos. We saw no evidence whatsoever. In addition, we saw no recent coral death on Pulley Ridge or any other of the mesophotic reefs that would have been present if the coral had been recently killed by the oil. So this leaves coral disease as the most reasonable culprit.

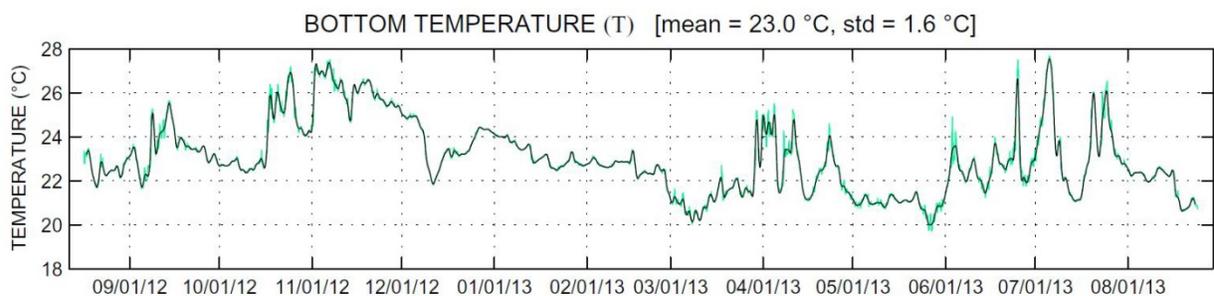


Figure 17. Bottom ADCP temperature records at Pulley Ridge (mooring depth 69.5 m; from Ryan Smith, NOAA/AOML).

Benthic Biota and Habitat Relationships

Dive sites at Pulley Ridge and the Tortugas were compared using a multi-dimensional scaling plot of Bray-Curtis Similarity (square root transformations) for benthic macrobiota percent cover (Fig. 18 and 19). The letter designations in the plot show statistically different groups

(SIMPROF, $p < 0.05$, Fig. 18). These plots clearly show the greatest similarity of sites is by region; i.e., Pulley Ridge clumping together.

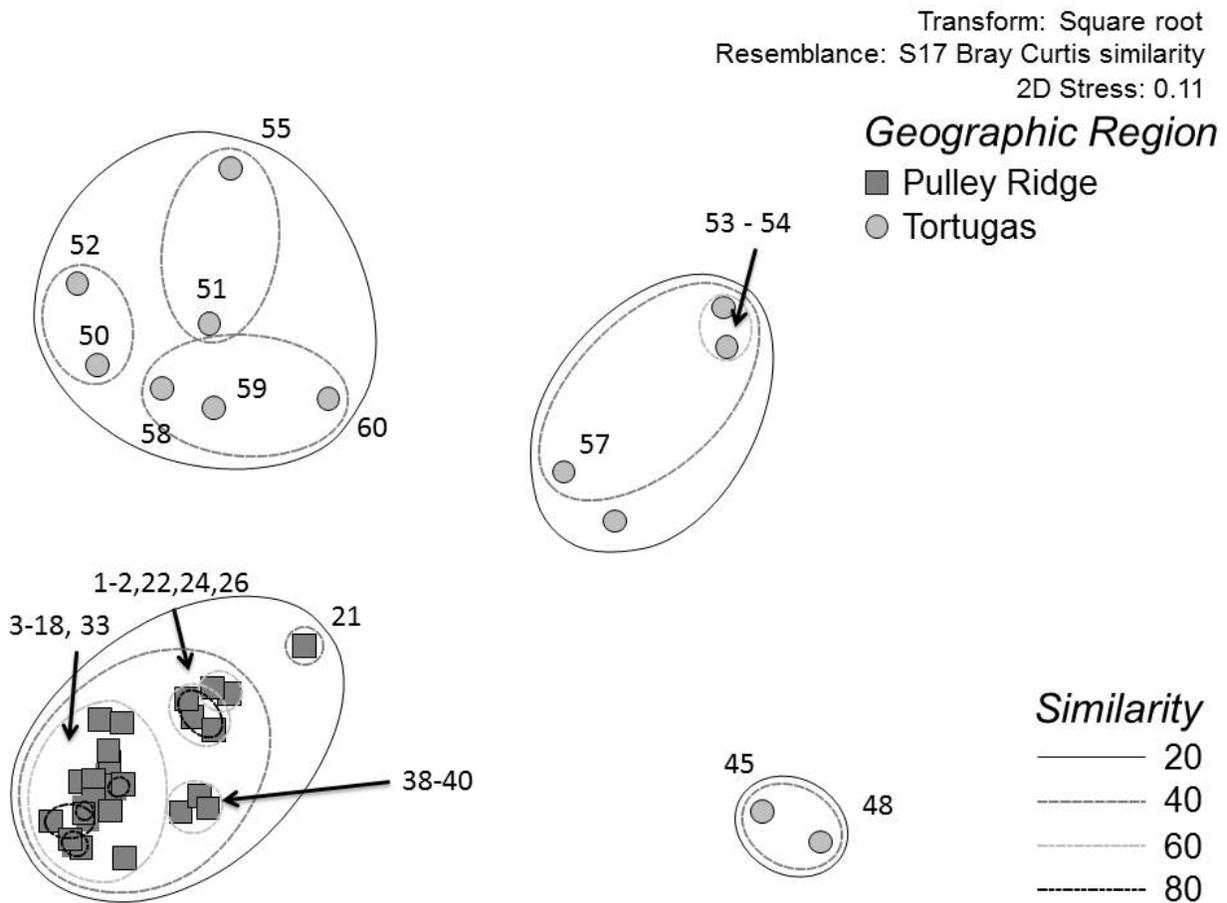


Figure 18. Multi-dimensional scaling (MDS) plot of ROV dive sites at Pulley Ridge and Tortugas regions based on Bray-Curtis similarity matrix of percent cover of benthic macrobiota. Assemblage similarities at 20-80% are indicated.

ANOSIM test between Pulley Ridge (PR) and Tortugas (T) sites shows a global $R: 0.868$ (0.1%) showing there are definite differences in the biota between the two regions. The main differences between the two regions was contributed by following taxa: Corallinales (15.78% contribution; mostly at PR), *Anadyomene* (10.25%, at PR only), *Martensia* (6.37%, mostly at T), 13 other taxa, primarily algae contributed to the remaining differences. Within Pulley Ridge, site 21 is an outlier; it was off the main ridge, and was flat sediment/rubble and mostly barren. Sites 1-18 are all within the PR HAPC on the Main Ridge. The grouping of Sites 38-40 are all outside the HAPC on the West Ridge. For the Tortugas region, sites 45 and 48 were outliers; both were deeper soft bottom habitat (51-57 m). Sites 51, 52 and 58 were the only patch reef sites.

MDS plots of sites within Pulley Ridge shows some clustering (Fig. 19). The West Ridge sites (Groups I, J) are clearly separated as is the Main Ridge South (Groups E, F) and sites off the Main Ridge (A, K).

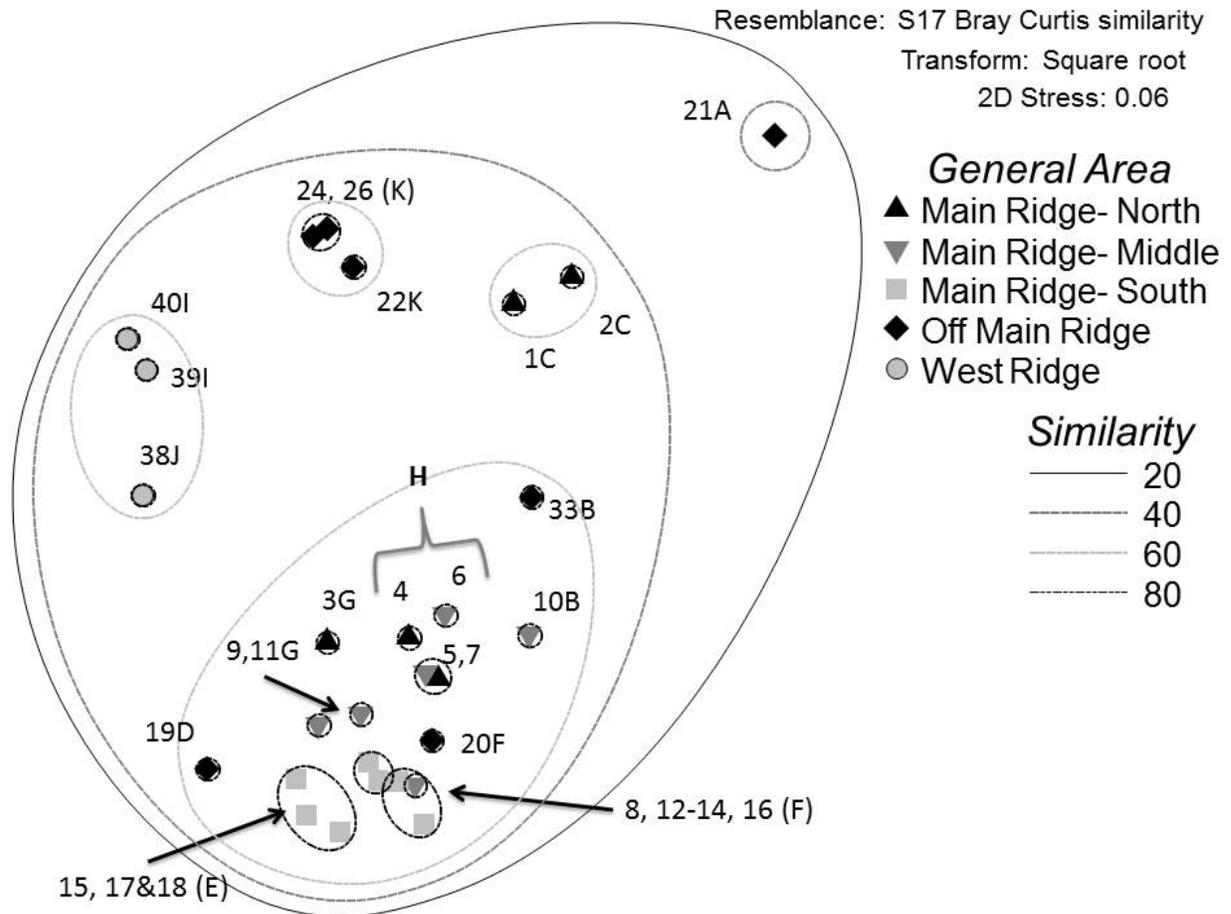


Figure 19. Multi-dimensional scaling (MDS) plot of ROV dive sites within and outside of Pulley Ridge HAPC based on Bray-Curtis similarity matrix of benthic macrobiota percent cover. Assemblage similarities at 20-80% are indicated. Statistically different groups (SIMPROF, $p < 0.05$) are indicated by letters.

Gridded ASCII multibeam sonar data is available for most of the PR sites (but none at Tortugas) and were used to compare benthic macrobiota distribution based on slope, depth and latitude/longitude. Slope was incorporated from the Gridded ASCII in ArcGIS for every dive track data point that fell over available grid data. The slope was then exported out of Arc and into Access. The ROV navigation data points were then connected back to the dive track (using Navigation ID) and then the images were updated with the slope from the grid data. These data were then imported into PRIMER 6, averaged by block, normalized and then compared using BEST (BIOENV). BioEnv shows that longitude, depth and slope correlated with species composition at $\rho = 0.728$, significance: 1%.

Correlation of Coral by Depth

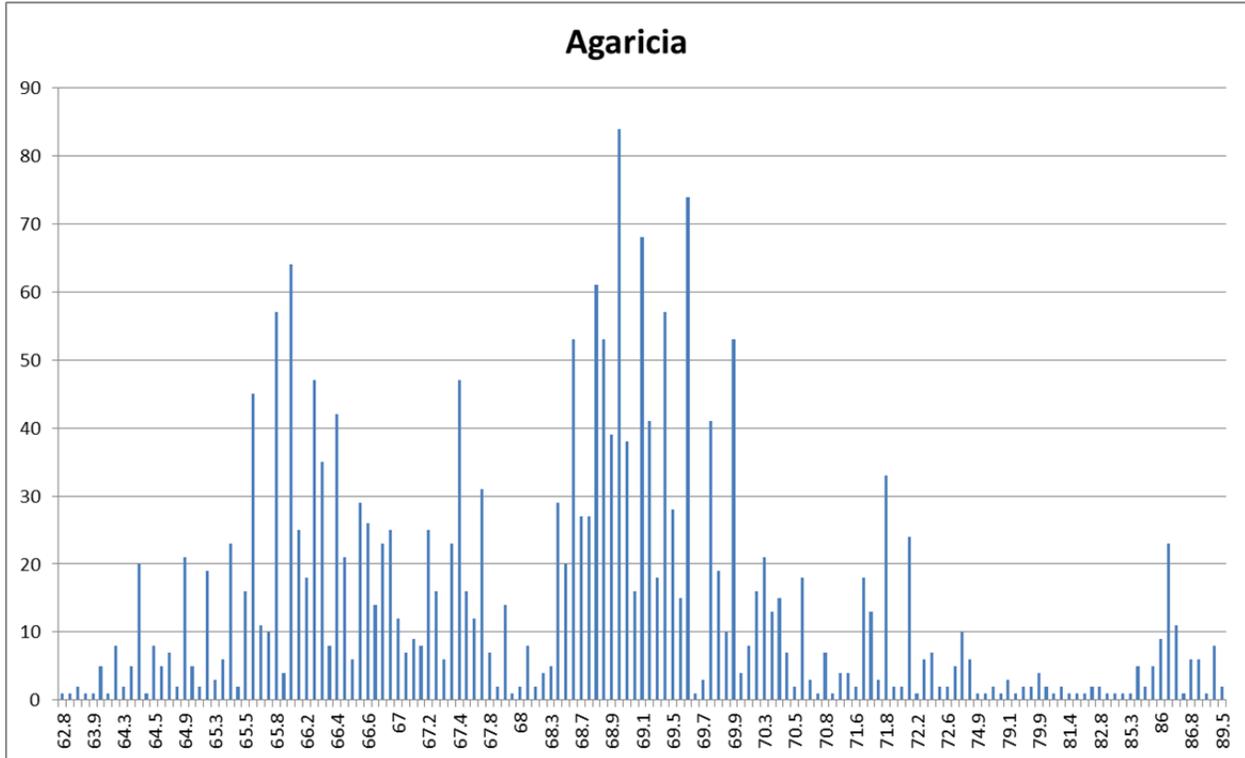


Figure 20. Plot of *Agaricia* spp. coral by depth occurrence at Pulley Ridge in 2012/2013.

The distribution of *Agaricia* spp. corals on Pulley Ridge were strongly correlated with depth (R value = -0.2831 ; $p < 0.00039$). The coral distribution appeared to be multimodal, and peaked at depths of 66 m, 69 m, and 86 m Fig. (20). The depth range was 62.8 to 89.5 m. The distribution of *Montastraea cavernosa* was also plotted but since there were such few specimens there was no significant correlation with depth, but colonies occurred a depths from 63.7 to 67.9 m. The greatest known depth distribution of coral in the western Atlantic is much deeper with *Agaricia* sp. reported to 119 m, *A. grahamae* 115 m, *Leptoseris cucullata* 108 m, and *M. cavernosa* 113 m (Reed 1985). These were all in the Bahamas on the steep slopes of San Salvador Island.

Analysis of Fish Video Surveys

All fish were identified for each ROV dive to species level and counted. The total distance (km) of each dive was used to calculate the linear density (# individuals/km) of each fish species. A total of 111 fish taxa were identified from both Pulley Ridge (78 taxa) and Tortugas (73) dives in 2012 and 2013. The most common species included sharpnose puffer, cherubfish, reef butterflyfish, yellowtail reeffish, sunshinefish, purple reeffish, squirreffish, wrasse bass, orangeback bass, chalk bass, greenblotch parrotfish, and bicolor damselfish. Unfortunately, lionfish were also abundant at Pulley Ridge, mostly associated with red grouper burrows, and present in 72% of the blocks. A total of 703 were counted over both years. Fifteen species of commercially and recreationally important grouper and snapper species were counted (total 681 individuals; Table 10). The dominant species were vermilion snapper (428), black grouper (13), graysby (19), mutton snapper (41), red grouper (88), and scamp (56).

Table 10. Counts of recreationally/commercially important fish species at Pulley Ridge and Tortugas during 2012-2013 *Walton Smith* cruises. “Transects” refer to species observed only while on transect and “Blocks” refer to species observed while the ROV was in a particular block including both on and off transect time.

Snapper Grouper	2012 Transects	2012 Blocks	2013 Transects	2013 Blocks
Black Grouper	6	13	0	0
Cubera Snapper	1	1	0	0
Goliath Grouper	0	0	0	1
Graysby	4	15	1	4
Grey Snapper	0	0	1	1
Mahogany or Lane Snapper	0	0	0	1
Mahogany Snapper	0	0	1	1
Mutton Snapper	19	41	0	0
Red Grouper	20	56	10	32
Rock Hind	0	0	1	2
Scamp	28	53	1	3
Unid Snapper	1	1	4	18
Vermilion Snapper	77	378	0	50
Yellowfin Grouper	0	1	0	0
Yellowmouth Grouper	1	9	0	0
Total	157	568	19	113
Lionfish	100	379	61	324

Pulley Ridge vs Tortugas

The complete mesophotic fish species list and densities are listed by Block and Region in Appendix 2. PRIMER was used to analyze fish assemblages with factors of location (Pulley Ridge, Tortugas), Time (Day, Night) and Year (2012, 2013). A non-metric multi-dimensional scaling (MDS) ordination of ROV transects was constructed from a Bray Curtis similarity matrix of fourth root transformed abundances for all fish species (Fig. 21).

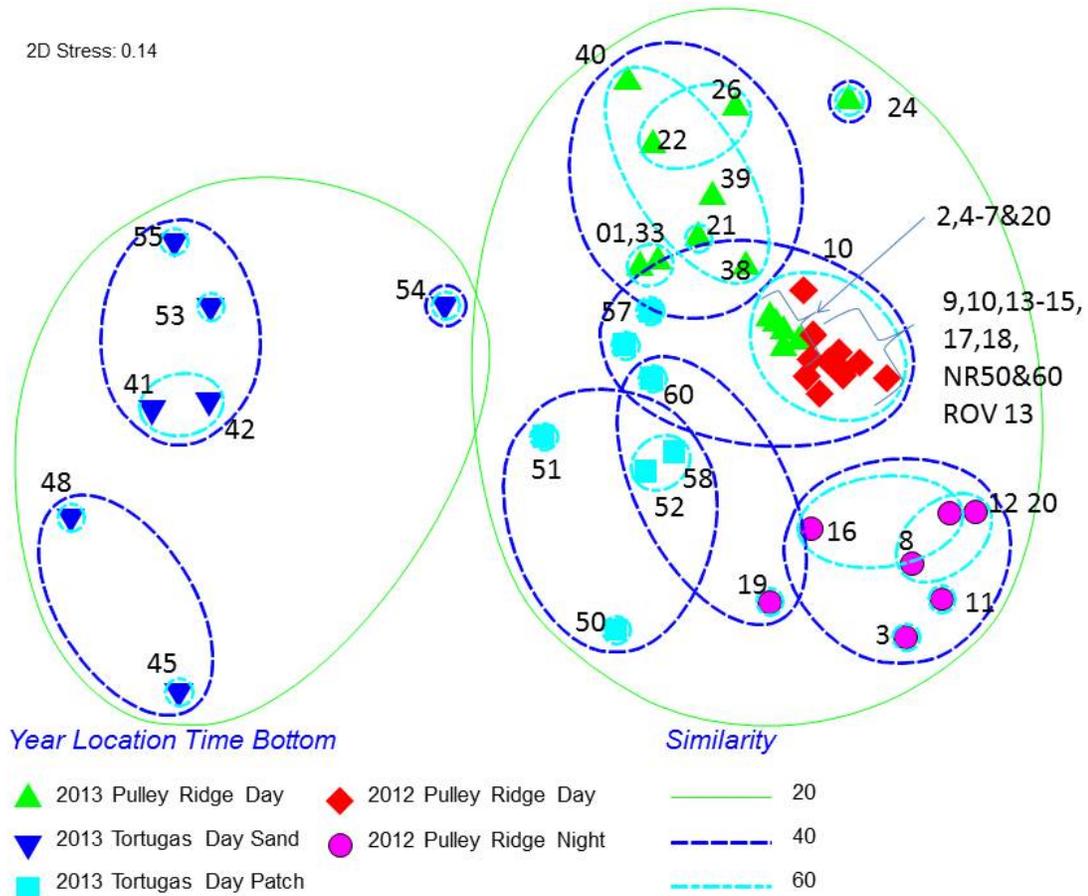


Figure 21. Multi-dimensional scaling (MDS) plot of ROV dives at Pulley Ridge and Tortugas based on Bray-Curtis similarity matrix of fourth root transformed abundances for all fish species from 2012-2013 *Walton Smith* cruises. Assemblage similarities at 20-60% are indicated.

This MDS plot shows that the fish assemblages for night dives (purple circles) conducted in Pulley Ridge in 2012 were very different from all dives conducted during the day (Figure 21). In part, this may be due to the reduced visibility of a few meters at night compared to 5-10 m in the day. But some species were clearly on diurnal or nocturnal cycles. There also appears to be a slight difference in fish abundances when comparing Pulley Ridge Day 2012 and 2013; the 2012 dives (red diamond) were primarily along the Main Ridge- North and formed a distinct cluster. This difference is explored more below. The Tortugas sites and Pulley Ridge sites appear to be different and possibly two different groups within the Tortugas. Further examination to see the differences are explored below. Clearly the Tortugas soft bottom habitat sites (blue triangle) are quite distinct. The Tortugas patch reef sites are more similar to the PR sites but still somewhat distinct.

Pulley Ridge and Tortugas (excluding night dives)

A separate MDS plot was created factoring out the Blocks that were surveyed during the night to get a better understanding of the relationship between species compositions at Pulley Ridge and the Tortugas (Fig. 22). In doing so, the plot showed more clearly the differences between the two locations.

2D Stress: 0.11

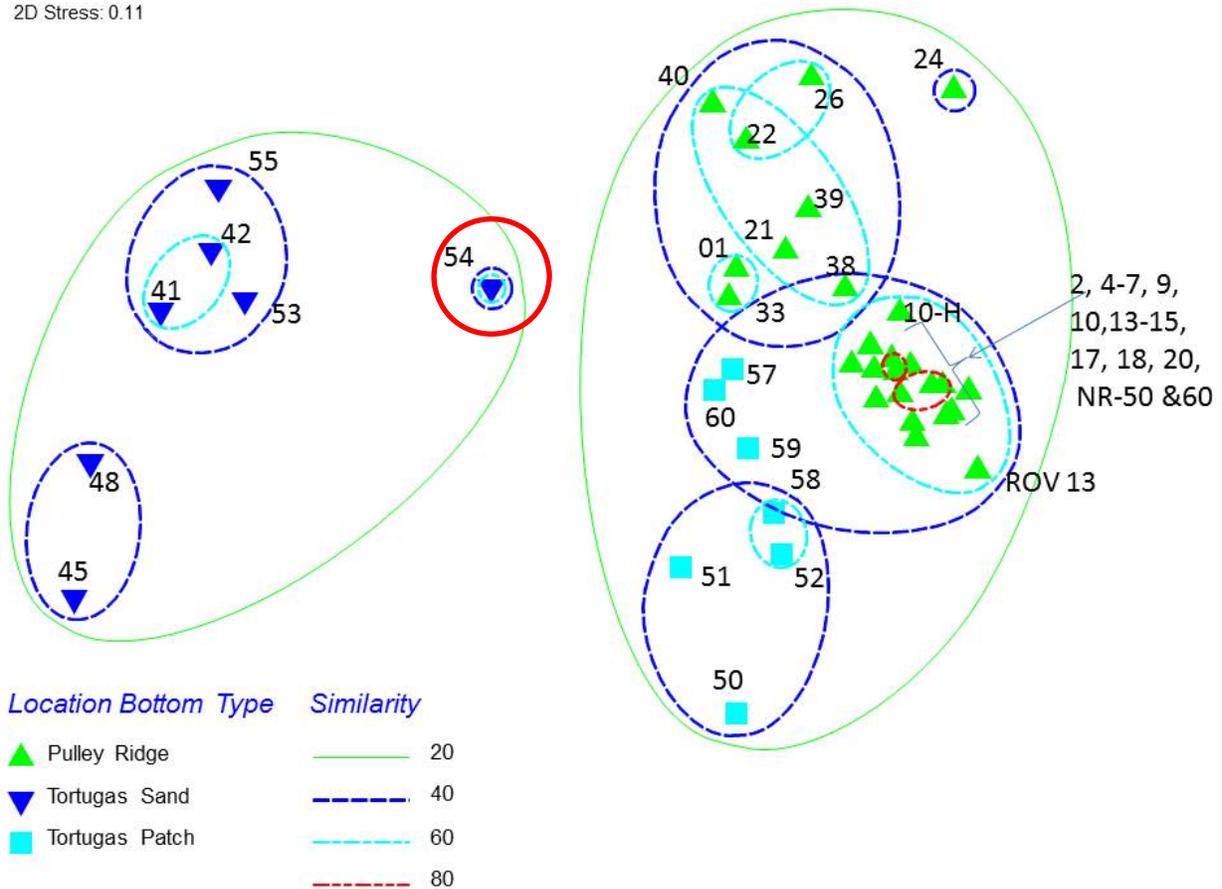


Figure 22. Multi-dimensional scaling (MDS) plot of ROV dives at Pulley Ridge and Tortugas based on Bray-Curtis similarity matrix from 2012-2013 *Walton Smith* cruises with night dives excluded.

This plot suggested that there might be two groupings within the Tortugas. This may be due to the two different habitat types that were observed in the Tortugas, sand and patch reef. Blocks 50, 51, 52, 57, 58, 59, and 60 were classified as patch reef while Blocks 41, 42, 45, 48, 53, 54, and 55 were classified as sand and the MDS plot was rerun. The clustering matched the generalization of bottom habitat. The one slight outlier is Block 54, it is designated “Tortugas Sand” but is tending towards the other groups (circled in red in Fig. 21). This Block was predominantly covered in dense matted algae instead of uncovered sand like the other blocks. The matted algae most likely provided more habitat for the fish to reside.

An ANOSIM and SIMPER analysis was run to compare the groupings in the above MDS plot. The global R value for this was 0.772 suggesting a statistical difference among the groups. Pairwise tests showed significant differences between groups. The species most responsible for the difference between Pulley Ridge and Tortugas Sand are higher densities of chalk bass, yellowtail reeffish, orangeback bass, greenblotch parrotfish, and purple reeffish at Pulley Ridge (SIMPER). The species most responsible for the differences between Pulley Ridge and Tortugas Patch Reef are higher density of wrasse and bicolor damselfish in the Tortugas Patch Reef and

higher density of purple reeffish and Inermiidae (bonnetmouths) in Pulley Ridge (SIMPER). The species most responsible for the difference between Tortugas Sand and Tortugas Patch Reef are higher densities of yellowtail reeffish, bicolor damselfish, and wrasses on the Tortugas Patch Reefs (SIMPER).

Pulley Ridge 2012 vs 2013

Since the night dives were visually quite different from any other set of dives they were taken out of this analysis to further compare the differences between Pulley Ridge 2012 and 2013 (Fig. 23). An ANOSIM and SIMPER analysis were run to compare the two years’ daytime dives. The R value from the ANOSIM test of 0.321 suggests there is only a slight difference between the two groups. The SIMPER test showed that the difference was primarily due to large schools of Inermiidae observed in 2012 that were completely absent in 2013. The other major difference was the higher abundance of purple reeffish and yellowtail reeffish in 2012 then 2013.

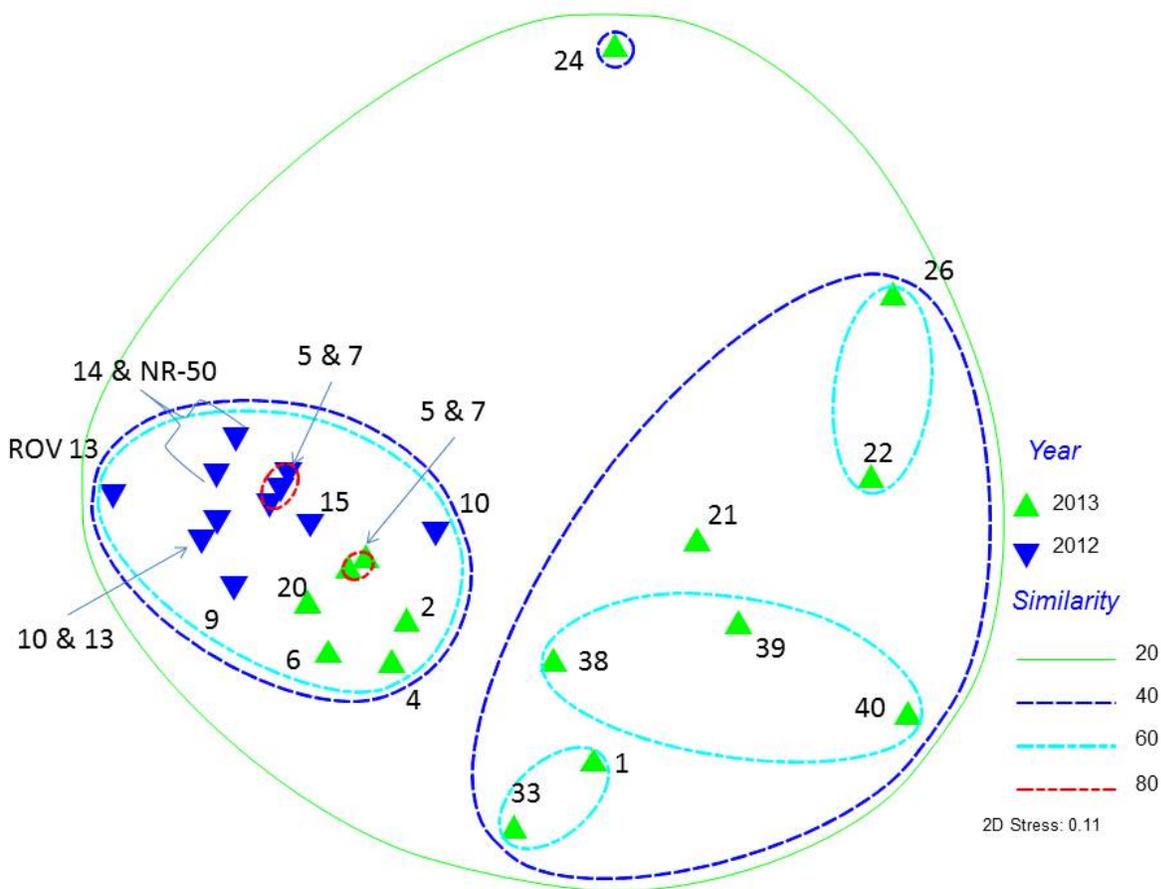


Figure 23. Multi-dimensional scaling (MDS) plot of ROV dives at Pulley Ridge by year based on Bray-Curtis similarity matrix from 2012-2013 *Walton Smith* cruises with night dives excluded.

Lionfish Populations

Lionfish continue to have a strong presence in and around Pulley Ridge HAPC. A total of 703 lionfish were observed at Pulley Ridge during the 2012-2013 ROV dives; densities by block are

presented in Figure 24. A maximum density of 0.04 individuals/km was recorded at Block 10 and mean density overall was 0.0092. The greatest densities were generally along the Main Ridge -Middle and -South, but the second most abundant site was Off Main Ridge at Block 24. Of the 33 1-km² blocks surveyed at Pulley Ridge, 72% had lionfish. Most of the fish were associated with active red grouper burrows. It was not infrequent that several (3-6 or more) would be inside along the rugged rock walls of the burrow and in close proximity to the resident large red grouper and dozens of small reef fish such as bicolor damselfish that assemble in masses over these holes as an oasis. Although we do not have stomach data on what the lionfish are eating here, anecdotal observations since 2010, when we made our first dives here during the oil spill, seems to indicate that some species such as flamefish are less common now in the holes than previously. Also in 2010 we saw only a few dozen lionfish overall.

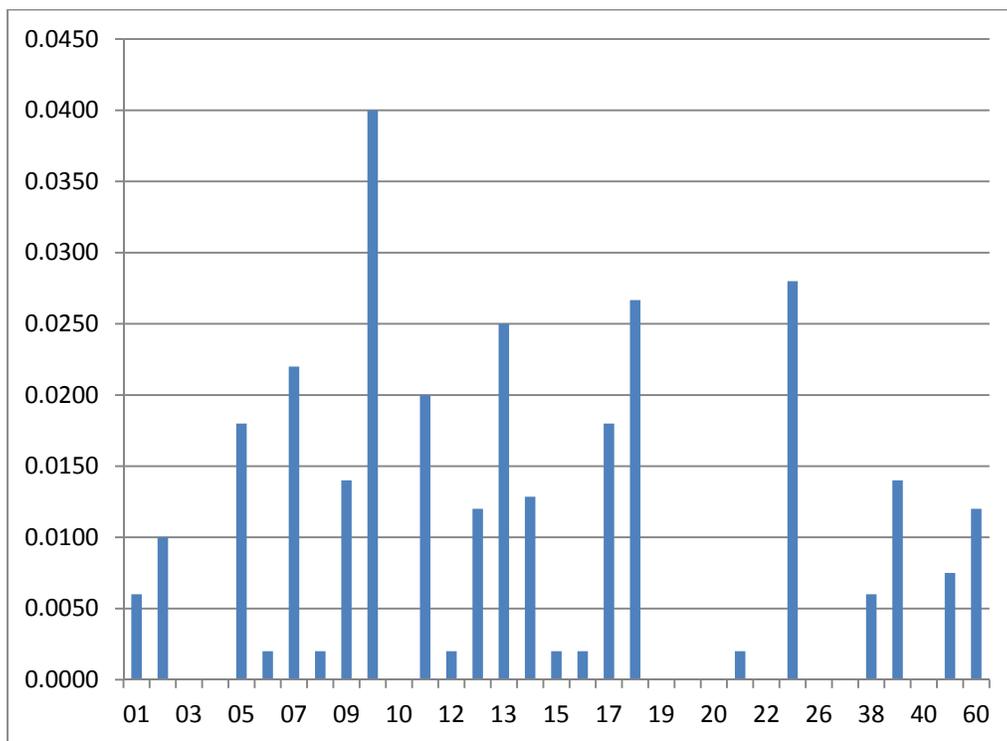


Figure 24. Density (number of individuals/km) of lionfish (*Pterois miles/volitans*) in each 1 km² random block at Pulley Ridge from ROV observations during the 2012-2013 R/V *Walton Smith* cruises.

FUTURE WORK AND CONCLUSIONS

This cruise and research has resulted in a rich set of new data discovering and characterizing the benthic communities and fish populations at mesophotic sites at Pulley Ridge and Tortugas off the southwestern Florida. New sonar maps, ground-truthed by ROV dives, have provided data for characterizing the Pulley Ridge HAPC and adjacent areas. These data will be important for managers and scientists with NOAA Fisheries, Fisheries Management Councils, Florida Keys National Marine Sanctuary, NOAA DSCRTP, NOAA CRCP, and NOAA Mesophotic Reef

Ecosystem Program. These data may then be compared to previous and future research cruises and to areas adjacent to the protected areas to better understand the long-term health and status of these important mesophotic coral/sponge ecosystems.

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APPENDIX 1

Species List and Percent Cover of Benthic Macrobiota

Species list of the benthic macro-invertebrates and algae that were identified from quantitative photo transects for each ROV dive during 2012 and 2013 R/V *Walton Smith* cruises to Pulley Ridge and Tortugas. Still images captured from the photo transects were analyzed using CPCe[®] software to determine relative percent cover of benthic biota and habitat types. (Best viewed in PDF format in order to zoom view)

APPENDIX 2

Species List and Density of Fish Populations

Species list all of fish that were identified and counted from the quantitative video transects for each ROV dive during 2012 and 2013 R/V *Walton Smith* cruises to Pulley Ridge and Tortugas. The total distance (km) of each dive was used to calculate the linear density (# individuals/km) of each fish species. The estimated field of view width was ~10 m, and most fish were identified within a 5 m distance. (Best viewed in PDF format in order to zoom view)

