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Use of a Towed Camera System along the west Florida shelf: A Case Study of the Florida Middle Grounds Benthic Marine Communities

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Use of a Towed Camera System along the west Florida shelf:

A Case Study of the Florida Middle Grounds Benthic Marine Communities

by

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

As technologies advance the study of ocean dynamics, new approaches to vexing problems of scale and process are becoming more widely available. Originally conceived as a tool primarily for indexing the abundance of near-bottom fishes, the Camera-based Assessment and Survey System (C-BASS) may also be an effective tool for monitoring benthic invertebrate resources vulnerable to natural and anthropogenic perturbations, and for characterizing the composition of benthic communities to inform spatial management. Using still images derived from the C-BASS video of benthic transects within the Florida Middle Grounds, I documented the abundance of benthic habitat-forming functional groups—sponges, algae, and corals—and noted taxa that were present in a SCUBA and ROV study conducted a decade earlier. Images were pre-processed using MATLAB computer programming language to correct for light attenuation and scattering in seawater at depth, and examined using ImageJ software and Coral Point Count software or rapid visual assessment methodology to assess image quality and percent cover, respectively. Exploratory data analysis (dissimilarity profile) delineated five habitat types in the northern Florida Middle Grounds, and discriminating benthic cover was identified using similarity percentage analysis: soft corals, fleshy macroalgae, low-relief algae, encrusted rubble, and sand. Hard corals and sponges represented relatively low area cover. A canonical analysis of principle components of in situ environmental measurements, chlorophyll a, turbidity, salinity, slope, and depth highlighted the association of the sand habitat type with greater depths and least amount of slope. Fleshy macroalgae were associated with greater slope, which reflected its presence in transitional areas between sand and reef. Soft coral habitat type

was correlated with shallower depths, but also to lower temperature and lower salinity, highlighting the limitations of one-time environmental measurements to the condition of that time and space. A distance-based redundancy analysis of fish species abundance revealed that sponges, soft corals, and hard corals explained some of the variation of Holocentridae spp., angelfishes, and porgy, and that gray snapper appeared to associate with higher measurements of chlorophyll *a*. A comparison of C-BASS measurements with a coincidental stationary camera survey revealed that a slight shift in view, either from the seafloor to the water column, or from two slightly different positions in the water column, can obscure or reveal benthic cover to varying degrees, suggesting that more imaging could provide more complete representations of the benthic cover. Continued surveys of the benthic composition of the west Florida shelf could elucidate the range of environmental conditions and facilitate further investigations into the fish species associations with biotic cover in these benthic communities.

CHAPTER 1: INTRODUCTION

The abundance and composition of corals and other biota that produce high-relief marine habitats affect the abundance and distribution of economically important marine resources. While tropical, shallow-water reefs are widely recognized for their relatively high species diversity, the benthic features along the northeastern Gulf of Mexico's continental shelf (the west Florida shelf) host diverse habitats that are rich in mesophotic reef organisms and healthy populations of commercial reef fishes (Darnell 2015). The west Florida shelf edge includes an aggregate of geologic features, including hard substrate necessary for sessile organisms to attach and grow, creating hard-bottom communities composed of a variety of hard and soft corals, sponges, and algae that provide shelter and food for communities of fishes and invertebrates (Mallinson et al. 2014; Coleman et al. 2004a). Several discrete areas along the west Florida shelf are federally-designated marine protected areas (MPAs), including habitat areas of particular concern (HAPCs), established to protect habitat and ecological structure and function by limiting fishing and other activities. However, lack of monitoring hinders the ability of managers to assess the effectiveness of such designations (Coleman et al. 2004a). The development of enhanced long-term monitoring programs for both the fishes and their habitats necessarily would involve the expansion of temporal and spatial data collections, requiring the use of advanced technological resources such as cameras and acoustics.

The purpose of the research presented in this thesis was to develop methodologies and collect data to provide finer-scale spatial records of species habitats. Still images of benthic habitat from the Florida Middle Grounds were collected on the west Florida shelf using a towed video system (Lembke et al. 2017) and were used to quantify habitat composition and percent cover of dominant flora (algae) and fauna (stony corals, soft corals, and sponges). When recognized, high-order taxa (e.g., families) were noted. Benthic community composition was tested for statistically significant associations with environmental parameters including depth and slope, and with fish species abundances. Associations between biotic and abiotic variables were tested to detect associations between variables potentially sensitive to natural and anthropogenic perturbations. Such associations would help characterize responses to adverse effects on particularly vulnerable species, and to discover additional sites with habitat components that provide important ecological functions. The overall objective of this work is to contribute to the development of fine-scale, comprehensive benthic maps of the west Florida shelf that can inform ecosystem-based management in the larger Gulf of Mexico (Karnauskas et al. 2017).

CHAPTER 2: LITERATURE REVIEW

Effective management of marine resources incorporates knowledge of their composition and abundance over time to both establish current conditions and develop goals for restoration if required. Without a detailed understanding of the dynamics that characterize ecosystems and communities, it is difficult to anticipate or respond to perturbations that may have negative impacts on habitat and associated populations of marine species.

In 2010, an explosion caused the oil rig *Deepwater Horizon* (DWH) to sink on the continental slope at Mississippi Canyon 252, offshore of southeastern Louisiana. The rig's blowout preventer was unable to stop the flow of oil from the prospect well into the Gulf of Mexico, releasing an estimated 4.9 million barrels of oil from a depth of 1,525 m (Lubchenco et al. 2010). Much of the multi-organization response to the event was focused on its cause, the amount and fate of the oil, and its effect on the fishing and tourism industries. Insufficient pre-spill information was available to predict how pelagic and benthic marine ecosystems would be affected, as a spill of that magnitude and depth was unprecedented, and organisms at all trophic levels could have been adversely affected by the oil (Lubchenco et al. 2010).

Oil components that were not collected, burned, naturally evaporated/dissolved, or chemically dispersed, formed a deep-water plume traveling at a depth between 900 and 1,500 m with the currents of the Gulf waters (Murawski et al. 2016; Romero et al. 2017). Studies have since found a number of adverse effects on biota that came into contact with the chemical compounds released at the DWH well, including fish lesions and compromised reproductive systems, degradation of long-lived octocorals, and deposition of oil-laden sediments posing a continued health risk to burrowing animals (White et al. 2012; Murawski et al. 2012; Deak 2014; Snyder 2014).

Researchers continue to assess the impacts of the DWH event and the vulnerability of marine species to natural or anthropogenic perturbations. Findings from these studies provide baseline information from which long-term monitoring programs could be developed to better prepare a response to future disasters. The shelf-edge of the northeastern Gulf of Mexico, however, is relatively shallow compared to the focal areas for many DWH response studies. Developing knowledge of offshore marine resources of all depths is important because benthic ecosystems provide food and shelter for marine fish populations. Other factors may impact the species composition and abundance in benthic communities, which may resonate at higher trophic levels, especially in a system like the Gulf of Mexico, where even some highly migratory and economically important fish species have been known to remain throughout their life history.

One of the objectives of research and monitoring in marine communities is to provide information that can be used to protect the ecosystem services they provide. An example of an ecosystem service, sponges in the Gulf of Mexico can be used for washing. While once abundant, the production of sponges in the Gulf of Mexico was greatly impacted by a widespread sponge-disease epidemic in the late 1930s, causing the commercial sponge fishery of the west Florida shelf to experience a severe decline (97%) in harvest from 1935 to 1936 (Felder and Camp 2009). One focus of my study was to summarize observations from a relatively well-documented area, to record current measurements of such benthic species' abundance on the west Florida shelf.

Occupying over 1,500 km^2 of the west Florida shelf (Figure 1), the Florida Middle Grounds are considered the latitudinal extent of hermatypic coral communities in the United

States (Puglise and Kelty 2007). The majority (97 %) of the Florida Middle Grounds are deeper than 30 m, with nearly 50 percent between 35 and 40 m, and nearly 12 percent deeper than 45 m (Coleman et al. 2004a). Along portions of the west Florida shelf between depths of 30 and 40 m, light-dependent corals have adapted to live in low-light conditions, and, with sponges and algae, dominate outcrops.[1] Previous studies of the geologic and biotic components of the Florida Middle Grounds (e.g., Koenig et al. 2000; Mallinson et al. 2000, 2014; Coleman et al. 2004a, b; Gledhill and David 2004) have provided baseline summaries of this diverse area, which is identified as essential habitat for corals, viz: "for all species of the class Hydrozoa and the class Anthozoa," as well as for sponges (GMFMC 2004).

The uniqueness of the Florida Middle Grounds' habitats within the Gulf of Mexico warranted its designation as a Habitat Area of Particular Concern (HAPC) in 1982. HAPCs may have seasonal or year-round closures to certain fishing gears or other human activities, depending on the purpose of designation, and the Florida Middle Grounds is closed to bottom longline, bottom trawl, dredge, pot, and trap activities year-round to protect corals of the Gulf of Mexico [Title 50 Code of Federal Regulations 622.74(b)].

Grimm and Hopkins (1977) and Coleman et al. (2004b) provided historical records of habitat types and related biotic communities of the Florida Middle Grounds. Coleman et al. (2004a) collected images and specimens using remotely operated vehicles (ROVs) and SCUBA divers to compare findings with earlier records of Grimm and Hopkins (1978) from overlapping sites. Coleman et al. (2004a) identified benthic cover and associated fishes to establish a baseline description ("snapshot") and historical comparison of the sites, and were the main source of

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[1] http://oceanexplorer.noaa.gov/explorations/13pulleyridge/background/mce/mce.html

species records for my study. Coleman et al. (2004b) recommended that the site be surveyed on a 10-year basis following their assessment.

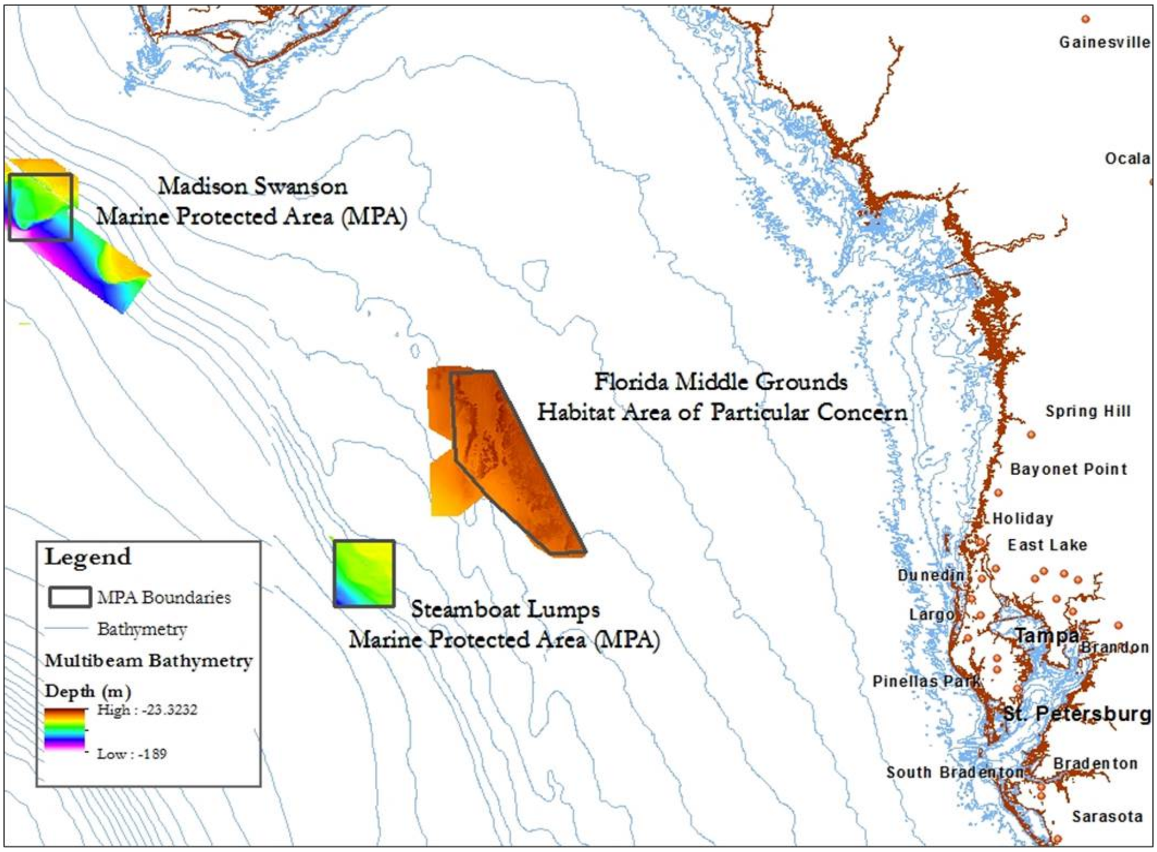

Figure 1 Location of the Florida Middle Grounds Habitat Area of Particular Concern

Previous studies in the Florida Middle Grounds have identified the principle benthic species groups to be algae, sponges, octocorals (soft corals), scleractinian corals (stony corals), *Millepora* (fire corals), and anemones (Coleman et al. 2004a). Coleman et al. (2004b) described seven habitat types and their co-occurring benthic species at overlapping depth strata: (1) shallow reef flat, (2) reef crest, (3) reef slope, (4) reef base, (5) patch reef, (6) rubble, and (7) sand bottom. These descriptions were the basis for the four fish habitat strata defined by Grasty (2015) and five geomorphic habitats across three geographic areas of the Florida Middle Grounds described by Mallinson et al. (2014). Coleman et al. (2004a) expanded the description of habitat types given by Grimm and Hopkins (1977). C-SCAMP has adapted CMECS classifications into categories appropriate for their analysis of the west Florida shelf. These descriptions are summarized in Table 1.

Table 1 **Habitat types of the Florida Middle Grounds from Coleman et al. (2004); adaptations by from Grasty (2015) and Mallinson et al. (2014); and CMECS (FGDC 2012) categories adapted by C-SCAMP**

Type & sites	Geology	CMECS categories adapted for C-SCAMP[1]	Geological classes in Mallinson et al. (2014)	Biota	Fish habitat in Grasty (2015)
Shallow reef flat (25–30 m depth) (0–1 m relief)	Gentle slope; scattered sand patches	Seafloor induration: Hard – Geoform: Rock outcrop – Low relief hardbottom – Attached biota (varied)	Class extended to 35 m and deeper due to similarity to sand bottom classes	Sponges, gorgonians *(Muricea* spp.), and scleractinians *(Dichocoenia* and *Porites);* at 28–30 m, gorgs replaced by *Dichocoenia* and *Madracis*	(1) Shallow reef flat: 22.3 % of area sampled
Reef crest (26–34 m depth) (1–6 m relief) FMG 247 FMG 491	Transition between flat and slope; sharp break along upper reef surface with near-vertical escarpment of exposed rubble; rough habitat incised by numerous valleys; resembles spur and groove of shallow reefs	Seafloor induration: Hard – Geoform: Rock outcrop – High relief hardbottom – Attached biota (varied)	Areas with greatest relief above adjacent areas, and are typically ridge-like and transitional between slope/flat.	*Millepora alcicornis* and *Madracis decactis* scleractinians dominate	
Reef slope (29–38 m depth) (0–6 m relief)	Steeply inclined (~45–75 degrees); numerous erosional sand-filled spillways traversing down reef face interspersed with rubble outcrops; occasionally interrupted by narrow horizontal terraces; patchy biota	Seafloor induration: Hard – Geoform: Beach rock (orthogonal formation) – Sand veneer – Reef biota (varied)	Characterized by steepest slopes (up to 75°) and transition between reef crest and base. Seismic data indicate sediment aprons onlapping individual carbonate banks.	*Millepora* and *Madracis* dominate; hard and soft corals and sponges patchily distributed	

Table 1 (Continued)

Type & sites	Geology	CMECS categories adapted for C-SCAMP[1]	Geological classes in Mallinson et al. (2014)	Biota	Fish habitat in Grasty (2015)
Reef base (37–40 m depth) (>1 m relief)	Transition between slope and surrounding sand bottom; small rock outcrops interspersed with clumps of exposed rubble and coarse sand	Seafloor induration: Hard – Geoform: Coral reef substrate – Reef biota (varied)	Transition between reef slope and sand bottom classes; characterized by scoured troughs, patchy outcrops and course sand.		(2) Deep reef flat: 50.3 % of area sampled
Patch reef (25–50 m depth) (0.5 m relief) FMG 491	Low to moderate slope; large rubble outcrops and coral formations separated by sand; seaward side slopes gently to shelf edge; low relief hardbottom exposed through coarse sand and rubble; similar to reef flat w/different biota	Seafloor induration: Hard – Geoform: Rock outcrop – Moderate relief hardbottom – Attached biota (varied)	[Reef crest]	Epibiota dominated by coralline algae, encrusting sponges, and azooxanthellate gorgonians; coral formations	
Rubble (0–1 m relief)	Reef-derived; at reef base with coarse sand; large rubble areas in deep water provide unique biotype for fishes	Seafloor induration: Hard – Geoform: Rock outcrop – Low relief hardbottom – Attached and Reef biota (encrusting)	[Shallow reef flat]	Corals and sponges attached to rubble	(3 and 4) Deep sand bottom and sand bottom: 6.1 and 21.3 % of area sampled, respectively.
Sand bottom (0–0.3 m relief)	Away from reefs; bottom consists of carbonate sands; primarily rubble and sand waves	Seafloor induration: Hard – Geoform: Soft – Gravel – Encrusting biota			

[1]Physiographic setting: Continental shelf

The primary algal composition of reefs was described by Hochberg et al. (2003), based on Berner (1990)'s three basic forms: turf algae, crustose calcareous algae, and fleshy macroalgae. Fleshy macroalgae and turf algae are subject to grazing, and therefore less prominent on coral reefs (Dawes 1998). Coleman et al. (2004) characterized red algae (Rhodophyta) as the most widespread and diverse algae found in the Florida Middle Grounds, with *Champia salicornioides* as the most commonly found, as well as *Dictyota menstrualis* (brown algae, or Phaeophyta). Even on the sand flats between and among the carbonate banks, rubble is typically encrusted with calcareous red algae and sponges.

Jaap (2015) defined the four most common species of stony corals in the Florida Middle Grounds as *Millepora alcicornis*, *Dichocoenia stokesii*, *Madracis decactis*, and *Oculina diffusa*. In his study, SIMPER analysis identified these species as the most responsible for the difference between the Florida Middle Grounds and other stony coral communities in the eastern Gulf of Mexico, based on a review of existing data and literature (in Felder and Camp 2009) with a focus on the "Hourglass Collections" of corals in the late 1960s.

Rützler et al. (2009) reported that all three classes of sponges, Desmospongiae, Calcarea, and Hexactinellida, occur in the Gulf of Mexico. Sponges have not been widely studied, other than those coastal species that were commercially exploited in the late 19th and early 20th centuries, so identification prior to the early 2000s was difficult (Coleman et al. 2004a; Felder and Camp 2009). The most comprehensive revision of sponge genera (Hooper and van Soest 2002) was published the year prior to the collections Coleman et al. (2004a) reported, and prior work by Harper and van Soest (1974) was referenced in their report only as an example of correct classification. Because of their confusing and incomplete taxonomy, sponges were listed in the appendices of Coleman et al. (2004a) and the collections were sent to the Smithsonian

NMNH for identification; however, they were never archived online. Further, the Smithsonian's online archives have few (40) records of sponge species observed in the Florida Middle Grounds, and only one of which is accompanied by an image. Images of live organisms to compare with the benthic images are often scarce, as samples have historically been dead when provided for the archives. Because ethanol-preserved and dried specimens lack the color and shape characteristics of the living organism, these records may not be useful in analysis of living organisms found in images of benthic communities (Felder and Camp 2009).

Coleman et al. (2004) did not have an octocoral taxonomist, citing Grimm and Hopkins (1977) as a more in-depth source for octocoral records at the Florida Middle Grounds sites; however, they did positively identify 13 taxa to species level. *Muricea spp.* was the genus most prevalent at all sites.

These baseline data will be used to inform this study and C-SCAMP's examinations of the area's benthic-community trends and responses to perturbations. Modern-day surveys of coastal and pelagic fish populations are conducted regularly throughout the Gulf of Mexico; however, benthic habitat is generally not examined. SCUBA diving is an effective method of assessing habitat and species, although it is depth-, area-, and time-limited, may affect the behavior of fishes, and can be intrusive if it involves the collection of biological samples for further analysis in the lab. Less intrusive *in situ* methods of survey, such as autonomous underwater vehicles (AUVs) or in some ways ROVs, are expensive to employ, limiting the frequency and geographic expanse of surveys. Stationary (or "drop") cameras collect community structure, abundance, and health data without affecting the behavior of mobile species, but are limited by their range of view (distance and perspective) at each site (K. Rademacker, NOAA, pers. comm.). A camera system towed behind a research vessel, on the other hand, has the potential to provide cost-effective, expansive imaging of the sea floor (Lembke et al. 2013; 2017).

Two of the most common digital imaging formats are Joint Photographic Expert Group (JPEG) and bitmap (BMP) (Abramoff et al. 2004). Termed “lossy” compression, JPEG image data are the result of an algorithm that separates color and grayscale data in an image, divides the information into squares on which a 2D discrete cosine transform algorithm is applied, and discarding imperceptible color and grayscale data for default values derived from human perception of light frequencies (Murray and Van Ryper 1996). The result is an image composed of lossy pixel data, which lack the intensity of the pixels in a non-lossy (“lossless”) image, and the inclusion of artifacts in otherwise more heterogeneous areas of an image. The issue of lossy images, as described, can negatively impact the recognition of benthic features when using image-analysis programs such as ImageJ.

BMP images are compressed but lossless, employing an encoding algorithm that identifies patterns of data and rewrites them as a phrase value that is physically shorter than the original, thereby decreasing the space required to store the data, which is then translated by phrase when re-opened (Murray and Van Ryper 1996).

Additionally, images lose contrast and have a narrower color spectrum due to light attenuation in seawater at depth. For image data, loss of contrast is loss of pixel intensity, or amount of gray represented by numerical values that define how a pixel is rendered in an image. Gray is a slice of the color spectrum where all color values are equal, and is responsible for rendering contrast (Murray and Van Ryper 1996). Light photons scatter in seawater due to water molecules (Rayleigh scattering) and macroscopic particulate matter (Mie scattering) (Papaikonomou et al. 2014). By 10 m deep, about 50 percent of long-wave visible light from the surface, including red and orange wavelengths, has been absorbed (Hitam et al. 2013). RGB (Red-Green-Blue) is a very commonly-used image color format in which each image pixel contains numerical values from 0 to 255 for each R, G, and B band. Used most often in remote sensing, cameras that record at 32-bit have separate sensors for each wavelength. Generally, RGB = (0,0,0) is perceived as black, and RGB = (255,255,255) is perceived as white (Murray and Van Ryper 1996).

The enhancement of underwater images is common among professional photographers and recreational divers. Capturing and modifying images in the scientific community differs from general photography, however, because it follows industry standards that minimize alteration of data while enhancing the ability to collect it. These standards have long been in place, as the broadest area of scientific imaging is in the biomedical field of microscopy. Image manipulation is only effective on files containing pixels with grayscale (contrast) values high enough to be detected. With advancements in instrumentation and standardized image processing to reconcile color and contrast, seafloor imaging can be an effective and efficient tool for benthic community assessments.

The importance of species-habitat associations is reflected in the Magnuson-Stevens Fishery Conservation and Management Act (Magnuson-Stevens Act)'s National Standards, which require the designation of essential fish habitat for species within each fishery management plan (50 Code of Federal Regulations 600.815). Essential fish habitat is defined as "those waters and substrate necessary to fish for spawning, breeding, feeding or growth to maturity" (USC 16 §1802(10)). These areas are determined by information categorized in tiers, wherein "species distribution data" is the lowest tier, and "species production rate by habitat" (where production refers to biomass) is the highest tier. Surveys conducted using C-BASS could provide top-tier essential habitat information. Under the National Standard Guidelines, scientists and policy-makers are preparing the framework for a near-future shift from species-based to ecosystem-based fishery management (Levin et al. 2009; NMFS 2016). Research using C-BASS into the associations between fishes and benthic biota, as well as environmental variables recorded *in situ*, could provide a solid foundation for long-term monitoring that would address, at least in part, some ecosystem-based fishery management goals. By observing the presence and health of species within their habitats, researchers can further examine the processes that support the ecosystems, and provide management advice on ecosystem-level planning (e.g., the effect a proposed management measure for one species may have on a community of species).

The increased designation of MPAs, such as the 2009 and 2014 Presidential Proclamations to designate (Proclamation 8336, 74 FR 7) and expand (Proclamation 9173, 79 FR 188), respectively, the Pacific Remote Islands Marine National Monument, illustrates that interest in planning and implementing MPAs has existed for some time (Gell and Roberts 2002). Fishery managers often view MPAs as an effective form of insurance against the modern increase of anthropogenic stressors on the ocean, including technologically-enhanced fishing pressure, drilling, coastal development, and dumping. Both proponents and skeptics of the utility of MPAs recognize the need for improved ocean planning and monitoring capabilities (Levin et al. 2009; Murawski et al. 2010; Sale et al. 2005). Criteria for monitoring protected areas would depend on a number of factors, including the essential features identified at the initial designation, level of disturbance before designation, type of environment, and scope of protection (Lester et al. 2009).

The Continental Shelf Characterization, Assessment, and Mapping Project (C-SCAMP) is the greater project of which this research is a part, and includes bathymetric and backscatter data. Detailed images of seafloor features are produced and ground-truthed using C-BASS and an expanded version of the Coastal and Marine Ecological Classification Standard (CMECS). C-SCAMP's goal is to contribute high-resolution bathymetric maps covering at least 4% (to the existing 5%) of the west Florida shelf by the end of 2017. Detailed maps that characterize the substrate and other bottom features along the west Florida shelf are essential to identifying areas of rugosity that may host habitat in need of protection (Jordan et al. 2005). C-BASS will allow scientists to examine the biotic composition on a finer scale to assess the area's functional importance to the shelf ecosystem.

CHAPTER 3: METHODS

Survey Design and Data Collection

C-BASS Instrumentation and Operation

The USF Center for Ocean Technology's construction and initial deployments of the Camera-Based Assessment Survey System (C-BASS; Figure 2) were described in Lembke et al. (2013; 2017). Grasty (2015) used video from the C-BASS to assess fish species abundances and behavior in three Marine Protected Areas (MPAs) along the west Florida shelf, including the Florida Middle Grounds HAPC. Those fish abundance data are included in this examination of community-habitat relationships within the Florida Middle Grounds.

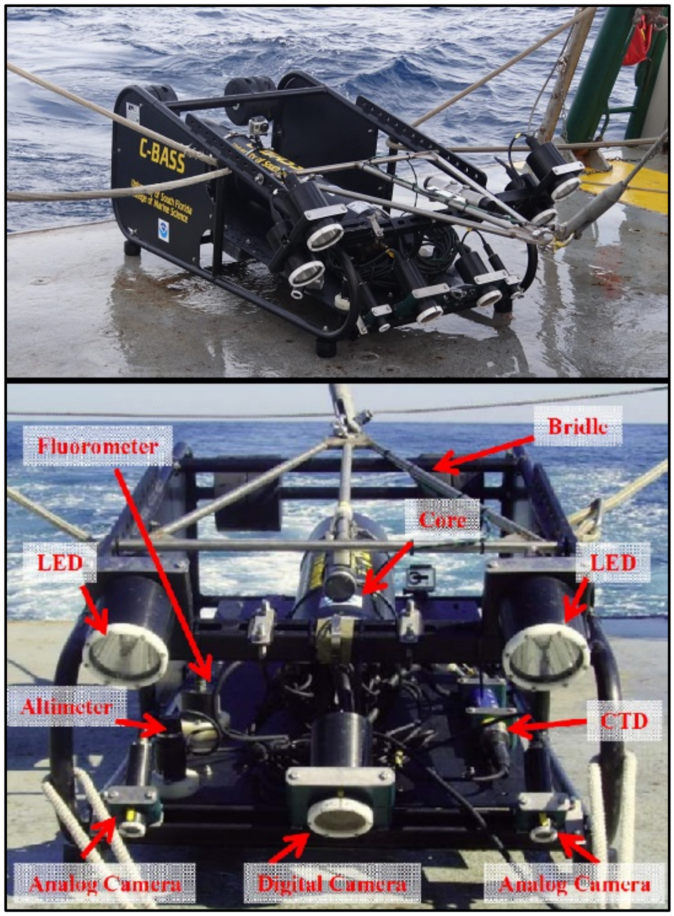

Figure 2 **Profile and instruments of the Camera-Based Assessment and Survey System (C-BASS) sled on deck of the R/V *Weatherbird II* (Source: Grasty 2014)**

The C-BASS could be towed from any ship outfitted with an A-frame. The system is typically towed at 3.5–4 kn and the instruments are rated to a depth of 200 m. The deepest video taken so far has been at a depth of 180 m (C-SCAMP, unpublished data). The C-BASS is not without limitations, however. The video array produces Moving Pictures Expert Group (MPEG) files, and at an average ship speed of 3.5 knots [relatively fast for underwater imaging systems (see Shortis et al. 2008)], the resolution of images is coarse, revealing habitat structure but limiting classification of most organisms to broader taxa; particularly those that are sessile, exhibiting no unique and identifiable movement patterns in response to the presence of C-BASS. MPEG recordings were selected in the initial design because they require the least amount of data storage space; however, recordings in this format produce JPEG images, which use a method of compression that directly impacts the color and contrast produced in an image.

The C-BASS continuously measured chlorophyll *a* (mg/m^3) and turbidity (NTU) with a WET Labs FLNTU fluorometer, distance above the seafloor with a Tritech PA 200/20 altimeter (m), salinity (PSU), temperature (°C), and depth (m) with an RBR XR-420 CTD, and recorded video using four PC887WR/PC 88WR analog cameras, an Arecont AV10005 HD Camera with a Lensagon CY0316 lens, and an AVT Prosilica GT1920 HD camera with a Schneider 3 Mega Pixel Cinegon 1.8/4.8 C-Lens. The platform was powered by the Tyco A301592 winch hydrowire. Temperature and leak detection were monitored by an ATMEGA32u4 arduino microcontroller, and low-resolution video, along with environment and compass measurements (pitch, yaw, and roll), were streamed through a DSL connection to the ship at 1.5 Mbps.

The C-BASS was operated using a shipboard program written by the Center for Ocean Technology using Python and MySQL databases serverside, and HTML and JavaScript on client side (Figure 3), where the operator monitored the compass, single-axis analog video, altimetry, and depth of the sled (Lembke et al. 2017). The C-BASS operator watched forward-facing DIDSON 300M sonar to anticipate changes in bathymetric features and adjust the sled's altitude through radio communication with the winch operator. Shipboard global positioning system (GPS) communicated time (UTC), location, and speed to the lab, where a towbody onboard computer with two hard drives was used for data storage and future analysis. Notable objects (e.g., anchors, tires) or fauna (e.g., echinoderms, turtles, fishes) observed in the video were recorded in an event log for reference during post-tow video analysis.

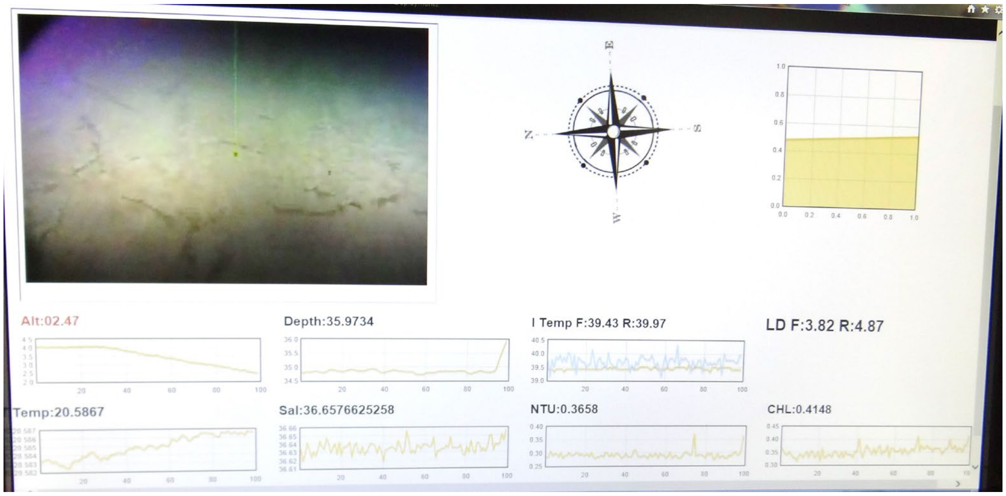

Figure 3 C-BASS user interface for live video and measurement feeds

Data Processing

For this study, C-BASS and ship data were summarized into readings per second. Shipboard GPS data were adjusted for layback (lag distance and time) between the vessel and the C-BASS in Microsoft Excel based on the regression equation provided in Brizzolara (2017):

$$\textit{Equation 1:} \quad L = 0.0003d^2 + 0.01d + 0.7168$$

where L is layback in seconds and d is depth in meters.

Still images were extracted and saved as the shipboard corresponding date and time (UTC) in “DDMMYYY HH:MM:SS.jpg” format. Images taken higher than four meters above the seafloor at the beginning and end of each transect (deployment and retrieval periods, respectively) were removed from this analysis because they were generally unusable for benthic habitat analysis. Layback-corrected environmental data for each image were indexed by time in Excel and, where time was reported, UTC was converted to EDT (surveys were conducted in May, August, and October daylight-saving time) by subtracting four hours.

Average ship speed was converted from knots to meters per second (1 kn = 1.15 mi/h; 1 mi = 1609.34 m; 1 h = 3600 s), and average distance between images was calculated as meters per 15 seconds.

Slope was calculated as the angle of difference of depth over distance between images (i = 1:n):

$$\text{Equation 2:} \quad m = tan^{-1}[(d_n - d_{n-1})/D]$$

where m is slope, d is depth in meters, and D is average distance between images.

Width of area viewed was estimated using the regression equation from Grasty (2015):

$$\text{Equation 3:} \quad W = 1.6877A + 1.4905$$

where W is the transect width in meters and A is the altitude of C-BASS in meters.

C-BASS Deployments (2014–2016) Revisited in this Study

Florida Middle Grounds C-BASS surveys used in this study were completed prior to 2017. Total time and extent of each survey, and the subject for which they were used in this study are summarized in Table 2.

Table 2 Summary of C-BASS deployments sampled for this study (2014–2016)

Area Code	Year	Month	Total Tow Time (h)	Total Transect Length (km)	Subject Studied	Vessel
FMG*	2014	May	20.50	133	Benthic composition and Image quality	R/V *Weatherbird II*
FMG**	2014	Aug	6.20	40	SEAMAP stationary cameras	R/V *Pelican*
FMG	2015	Aug	-	-	Image quality	R/V *Weatherbird II*
SWFMG***	2016	Oct	37.40	299	Image file compression (JPEG vs BMP)	R/V *Weatherbird II*

*Images and environmental data are subjects of benthic examination in this study. **Stationary camera images were available from SEAMAP; site locations estimated by iPhone App at deployment. ***Images (n = 9) analyzed for relationship between size and quality; total tow time and transect length not considered in this analysis. ****Southwest Florida Middle Grounds; Total transect length included areas outside of the FMG and areas that excluded C-BASS; Images outside the FMG were recorded using two file compression techniques.

The May 2014 survey collected benthic images across six transects of the Florida Middle Grounds (Figure 4), which coincided with several Coleman et al. (2004a) study sites, and produced the greatest range of quality of all of the benthic images; therefore, these images were the subject of habitat analysis and image quality analysis. Fishes were quantified per 100 m^2 by Grasty (2015) from video collected in the May 2014 survey, including fishes at five sites that overlapped sites characterized by Hopkins and Grimm (1981) and Coleman et al. (2004a). My study sampled benthic images from the May 2014 survey at 15-second intervals (4 per minute) to examine benthic cover and compare to fish quantities in Grasty (2015) and benthic species recorded by Coleman et al. (2004a) at SCUBA sites.

In August 2014, C-BASS was towed over four Southeast Area Monitoring and Assessment Program (SEAMAP) stationary cameras (modular optics underwater stereo systems, MOUSS; Stations 14 – 17; Figures 4 and 5). The estimated time that C-BASS passed each camera was recorded in the cruise log and used in this study to extract images from C-BASS and SEAMAP cameras' video recordings. The present study compared the differences in perspective of benthic species from stationary, horizontally-oriented monochrome videos to moving, above-bottom C-BASS videos.

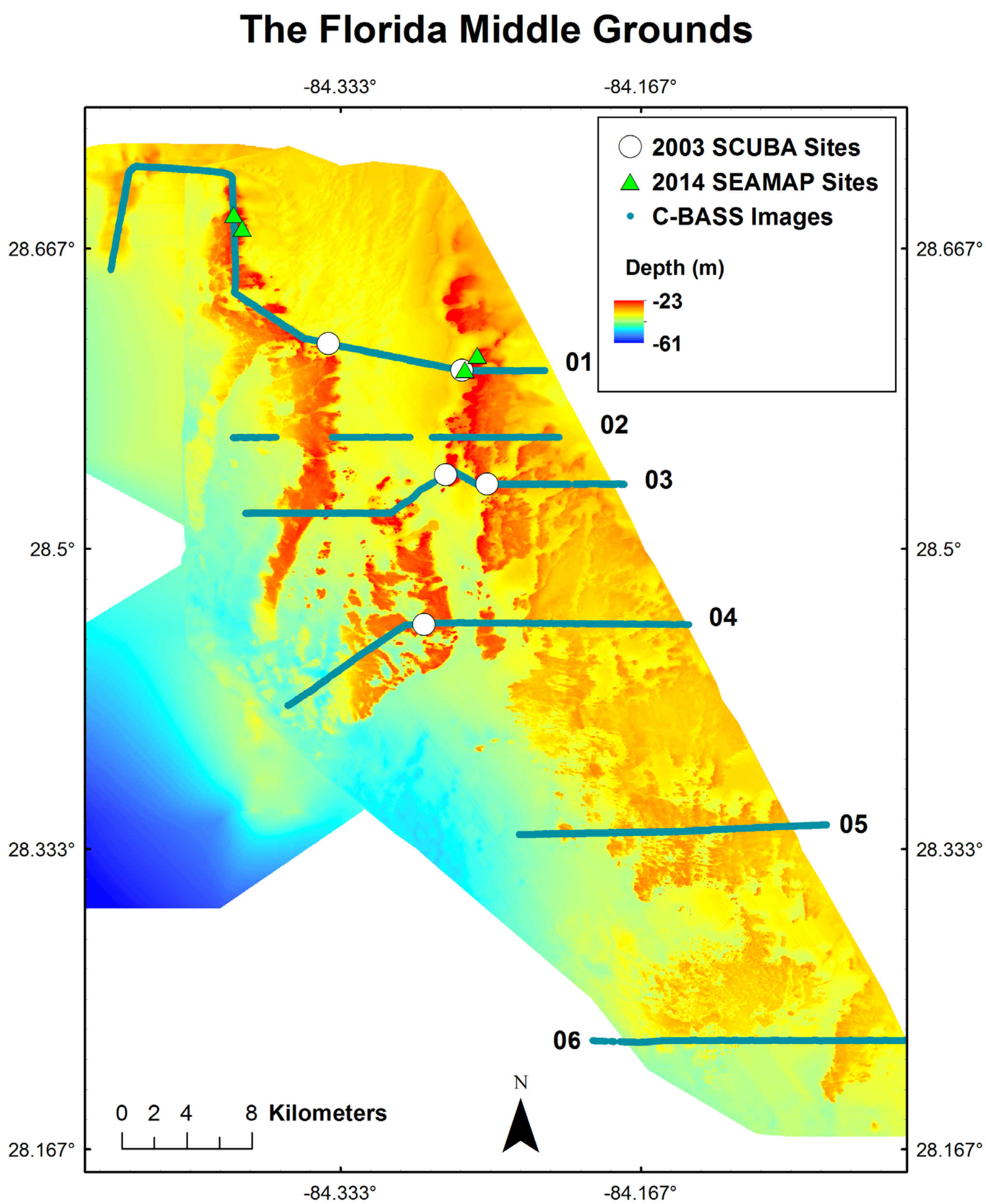

Figure 4 **Map of the Florida Middle Grounds surveys and sites sampled in this study; C-BASS images are point-samples of transects (01 – 06) from May 2014. Base map bathymetry of the Florida Middle Grounds is courtesy of C-SCAMP.**

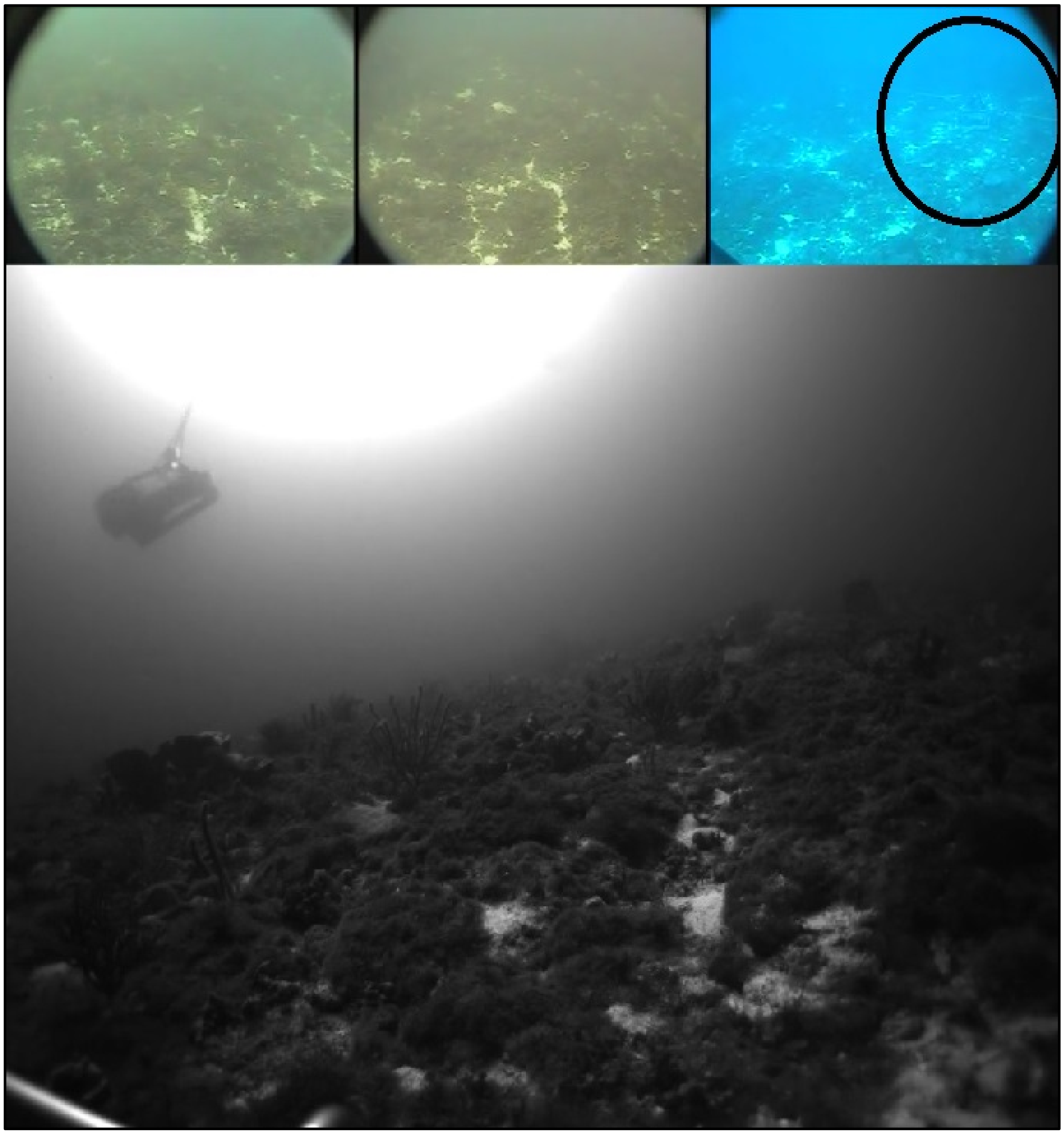

Figure 5 **View from C-BASS (top three images) and SEAMAP (bottom image) at same site; Stationary drop camera is circled. C-BASS images have not been corrected for color or contrast.**

In August 2015, C-BASS collected images that proved too poor quality for benthic habitat analysis, and were therefore the subject of image quality analysis in this study.

In October 2016, C-SCAMP collected sonar data and C-BASS images from additional territory southwest of the Florida Middle Grounds to produce bathymetric products. Images were recorded in BMP simultaneously with JPEGs at the standard interval of 15 seconds. Image values were summarized in this study to compare lossy and loss-less image compression techniques.

Image Processing

Contrast-Limited Adaptive Histogram Equalization

The images collected by C-BASS were 32-bit RGB. In the Florida Middle Grounds, which ranges from 20 to 50 m deep (< 25% light penetration) the perception of color is limited mostly to green and blue. MATLAB's Image Processing Toolbox was used to correct for the loss of contrast and color associated with light attenuation at depth (Appendix B). A batch-processing function (RGB_CLAHE.m) was adapted from the contrast-limited adaptive histogram equalization (CLAHE) technique using Rayleigh distribution, as pixel intensity and light distribution underwater is Rayleigh scattered (Andono et al. 2013).

The RGB_CLAHE function processed the image in several steps. First, it applied histogram equalization across the separate R, G, and B pixel values to enhance contrast based on their relative intensities across the image in its original state. It then performed Rayleigh CLAHE on the RGB components. Each image was broken into a 20 × 20 grid of "tiles," so that the function could enhance contrast and adjust the color histograms for each tile to match, approximately, that of Rayleigh distribution. The number of tiles was chosen after observing the results of testing several different dimensions within images having different benthic compositions. The CLAHE function uses bilinear interpolation to recombine the tiles after adjustment, eliminating any byproducts that might make the image appear gridded (Mathworks 2016).

Additional functions were considered, including mixed color planes RGB and HSV (adjusting H, or hue) CLAHE from Hitam et al. (2003), and RGB and LAB (adjusting L, or luminance) CLAHE from Anuranda and Kaur (2015), who found their techniques best suited for underwater image enhancement. However, any additional image manipulations, especially conversion from one image plane to another (e.g., RGB to LAB), result in loss of detail. The output images of RGB_CLAHE compared to those that included additional manipulation produced less contrast in the output image; however, over-enhancement of non-target or less detailed areas of an image could amplify noise, creating the appearance of features that were not present. For these reasons, RGB_CLAHE was chosen to process images from the Florida Middle Grounds in this study. As a result, the function carried out fewer steps, making the average processing rate one image per second.

To read and summarize pixel values, the ImageJ Analyze function was used for gray pixel values (intensity) that produced contrast and, likewise, ImageJ RGB Measure was used for the R, G, and B color values. Pixel measurements were taken before and after RGB_CLAHE to quantify the effects of image enhancement on an image. Image intensity data were matched to co-occurring environmental data in Excel to perform tests of significant relationships between image quality and environmental measurements.

Investigating Variability of Image Quality

The clarity of the seafloor varied among images across transects and surveys, limiting the utility of the images for benthic habitat analysis. I used ImageJ to summarize pixel intensity as a quantifiable measure of image quality, and Excel to perform ANOVA between image quality and file size to determine if file size is a significant indicator of image quality, prior to processing or visual examination of the image. Images from August 2015 were measured and tested, as they had poorest clarity and smallest file size. August 2015 image values were sampled from each 10-KB file size tier from 20 to 100 KB ($n = 9$). Original (raw) images from the May 2014 survey were also examined, as they exhibited the greatest range of clarity among all of the surveys. Considering that the variability of image quality between surveys could be caused by an increase in phytoplankton or other particulate concentrations in the water column in spring and summer, I performed a linear regression between image quality (using image file size as proxy) and co-occurring environmental variables such as Chl *a* and turbidity to determine a measureable causal relationship. The null hypotheses were that there are no relationships between file size and chlorophyll or turbidity measurements. If a null hypothesis was rejected, then an indicator variable might be identified and inform C-BASS operators in future deployments, who might avoid areas of higher concentrations in order to produce higher-quality imagery.

Images in 2016 were captured in JPEG and bitmap (BMP) formats to consider potential issues with lossy image compression. Visual observations were made of the quality between post-processed JPEG and BMP images, and the pixel intensity data were measured. ANOVA was performed on the mean (per image) intensity values of JPEG and BMP images. Two-sample t-tests were performed on the mean range of gray values of the JPEG and BMP images. BMP images examined in this study were expected to contain significantly more data and, therefore, potentially greater post-processing noise than JPEG images. The null hypothesis was that no significant difference existed in data means between formats (treatments). If the null hypothesis were rejected, then future image collection by C-BASS should use the format that would allow for post-processing data enhancement, not distortion.

Benthic Habitat and Species Assessment

Benthos Identification

Original images from the 1978 Hopkins and 2003 SCUBA and ROV sites were provided by F. Coleman and C. Koenig (personal communication). Geographic references were derived from the collection records from images. Several sources such as the World Porifera Database[2] and The Sponge Guide[3] were used to identify sponges for which there were reports in the literature (Coleman et al. 2004a) but no images available from the 1978 or 2003 records. Likewise, Coralpedia[4] and Algae Base[5] were used to check assumed identification of unlabeled images of corals and algae from the study sites of record.

Habitat

The C-BASS was primarily built to contribute to the assessment of economically important reef-fish stocks (Lembke et al. 2013); however, more comprehensive assessments are necessary to better predict a species' relationship with habitat characteristics (Hine et al. 2008). In this study, still images from videos were examined to calculate the percent cover of habitat-forming sessile benthic biota such as corals, sponges, and algae for comparison with environmental variables and fish co-occurrences; and to compare this methodology with other methods of benthic habitat assessment.

For this analysis, habitats were classified by the dominant covers of benthic taxa and substrate in the May 2014 images from 1-min videos (sites) in which there were more than one fish present (n = 79). Percent cover categories of sponge, macroalgae, low-relief algae, hard coral, soft coral, encrusted rubble, and sand were assigned based on the rapid visual assessment protocols for towed diver surveys at the NOAA Pacific Islands Fisheries Science Center (Lino et al. 2018; Figure 6 and Table 3).

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[2] http://www.marinespecies.org/porifera/
[3] http://www.spongeguide.org/
[4] http://coralpedia.bio.warwick.ac.uk/
[5] www.algaebase.org/

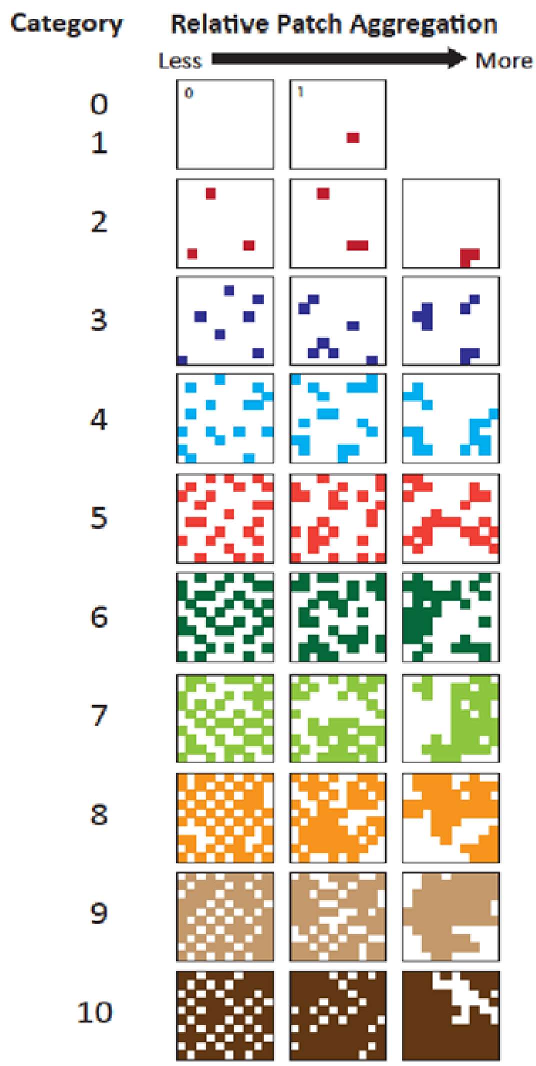

Figure 6 **Visual guide for rapid visual assessment of benthic cover category, illustrating appearances of less and more aggregated patches (developed by the Pacific Islands Fisheries Science Center and reproduced here with permission; Lino et al. 2018).**

Multivariate Statistics

Percent cover categories (0–10) were converted to the mean values in each range (Table 3) for statistical analyses. The semi-metric Bray-Curtis dissimilarity was used in Matlab to create a matrix of the benthic cover measurements. A dissimilarity profile analysis was conducted to test the null hypothesis of no structure present in the data (alpha < 0.05). Cluster analysis was performed to evaluate the number of dissimilar groups (habitat types, or habitats) present within the data, and the resulting cluster diagram was used to identify the habitat type at each site.

Table 3 Percent cover ranges and corresponding categories (from Lino et al. 2018). Categories were converted to numerical mean value of the percent range for statistical analysis.

Range (%)	Category	Mean Value
0.0–0.0	0	0.00
0.1–1.0	1	0.01
1.1–5.0	2	0.03
5.1–10.0	3	0.07
10.1–20.0	4	0.15
20.1–30.0	5	0.25
30.1–40.0	6	0.35
40.1–50.0	7	0.45
50.1–62.5	8	0.56
62.6–75.0	9	0.69
75.1–100.0	10	0.88

Nonparametric multidimensional scaling (nMDS) was performed to visualize these groups and consider further dimension reduction based on the dissimilarity profile. A similarity percentage analysis (SIMPER) was used to identify the discriminating benthic covers among these groups (Clarke and Warwick 1994)).

To test for significant variation of environmental variables between habitats, PERMANOVA (Anderson 2017) was performed on the z-scores (standard score) of the environmental measurements (depth, slope, temperature, salinity, chlorophyll *a*, and turbidity) among the habitats. The null hypothesis was that environmental measurements were not associated with habitat. If the null hypothesis was rejected, certain environmental variables might be considered characteristic of habitat types. A canonical analysis of principle coordinates (CAP) was performed to visualize the most important environmental variables across habitat types, and leave-one-out cross validation was performed to identify where classification of environmental variables was most successful (Legendre and Legendre 1998; Anderson and Robinson 2003).

To test for a relationship between habitat type and the presence of fishes, PERMANOVA was performed on total fish abundance and abundance of each fish species counted within the habitats, respectively. Considering fish species may have associations with benthic cover irrespective of the habitat type classifications, a distance-based redundancy analysis (RDA) was performed on fish species counts with respect to benthic cover values (both log-transformed) and environmental variables (standard scores) (Legendre and Anderson 1999).

Species associations were investigated with ANOSIM for the most abundant fish taxa across the sites: angelfish spp. (n = 57), gray snapper (n = 166), porgy spp. (n = 24), other snapper spp. (n = 27), and Holocentridae spp. (n = 14) (Anderson 2001). Sites were removed for which there were no identified fishes (fishes counted were not identified by Family or a lower-order taxon). Species counts were pre-treated with dispersion-based weighting to account for natural clustering of fish species between replicate sites within habitats (Clarke et al. 2006).

Comparing Methodologies

Areas sampled by C-BASS that coincided with previously assessed SCUBA sites, and images of the benthos surrounding SEAMAP stationary cameras deployed in tandem with C-BASS, were used for comparative assessments. To obtain the most accurate measurements of benthic cover possible, several programs were considered for detailed, single-image analyses, including CPCe (Coral Point Count with Excel extensions; Nova Southeastern University), Vidana (University of Queensland), and ImageJ (National Institute of Health). I compared these various packages based on ease of access, ease of use, and quality of product.

Vidana is a free and simple method of calculating percent cover when the user color-codes areas of the image as different types; however, it can only measure up to four benthic types at one time, is not capable of batch (multiple image) analysis, records results in TSV (requiring Excel conversion), and is not cross-platform (e.g., does not work on Macintosh computers).

Although also not Macintosh-enabled, CPCe is more advanced, enabling the user to measure percent coverage of benthic groups, as well as length and area of specific features for one image or multiple images in sequence (Kohler and Gill 2006). The measurements are recorded in Excel as a function of the program, requiring only defined parameters based on user needs. The advanced platform is developed for in-depth examination of downward-facing stationary imaging of shallower-water species, where species identification and measurement is the primary focus.

A manual frame-by-frame basis is currently necessary for C-BASS due to the need for visual recognition in JPEGs as previously described, but the level of identification is still coarse, so a combination of CPCe and ImageJ were used to measure percent cover, as they were both capable of providing the simple, manual task for the portion of this study. ImageJ is an open-source, cross-platform, Java-based image analysis and processing software supplemented by plug-ins developed and made publicly available by users (Abramoff et al. 2004). ImageJ has been used widely in the biomedical sciences (Abramoff et al. 2004) and more recently in others such as astronomy (pers. obs.). Image data, such as intensity, were also analyzed using ImageJ.

In this portion of my study, hard-bottom taxa were identified and delineated in each image using Coral Point Count with Excel Extensions (Kohler and Gill 2006) (CPCe; Figure 7). The width (m) of each image was calculated by equation 2 and used to calibrate the scale of each image in ImageJ. This program's "Find Edges" function was used to detect the area of the image with visible features (e.g., excluding the water column and blurry or dark areas). Usable areas were cropped and saved for analysis, and the revised width measurements were recorded. In

CPCe, the total area (m^2) of each image was calculated based on the revised width and number of pixels in the image. Benthic groups were classified as soft coral, hard coral, encrusted [sponges, algae] rubble, sand/mud/bare substrate (rock), macroalgae, or sponges. Because sand is the most obvious feature in each image, 100% of the sand was classified. In contrast, not all of the biogenic groups were visibly clear, so it was assumed that the percent identified was representative of all non-sand area (Figure 7).

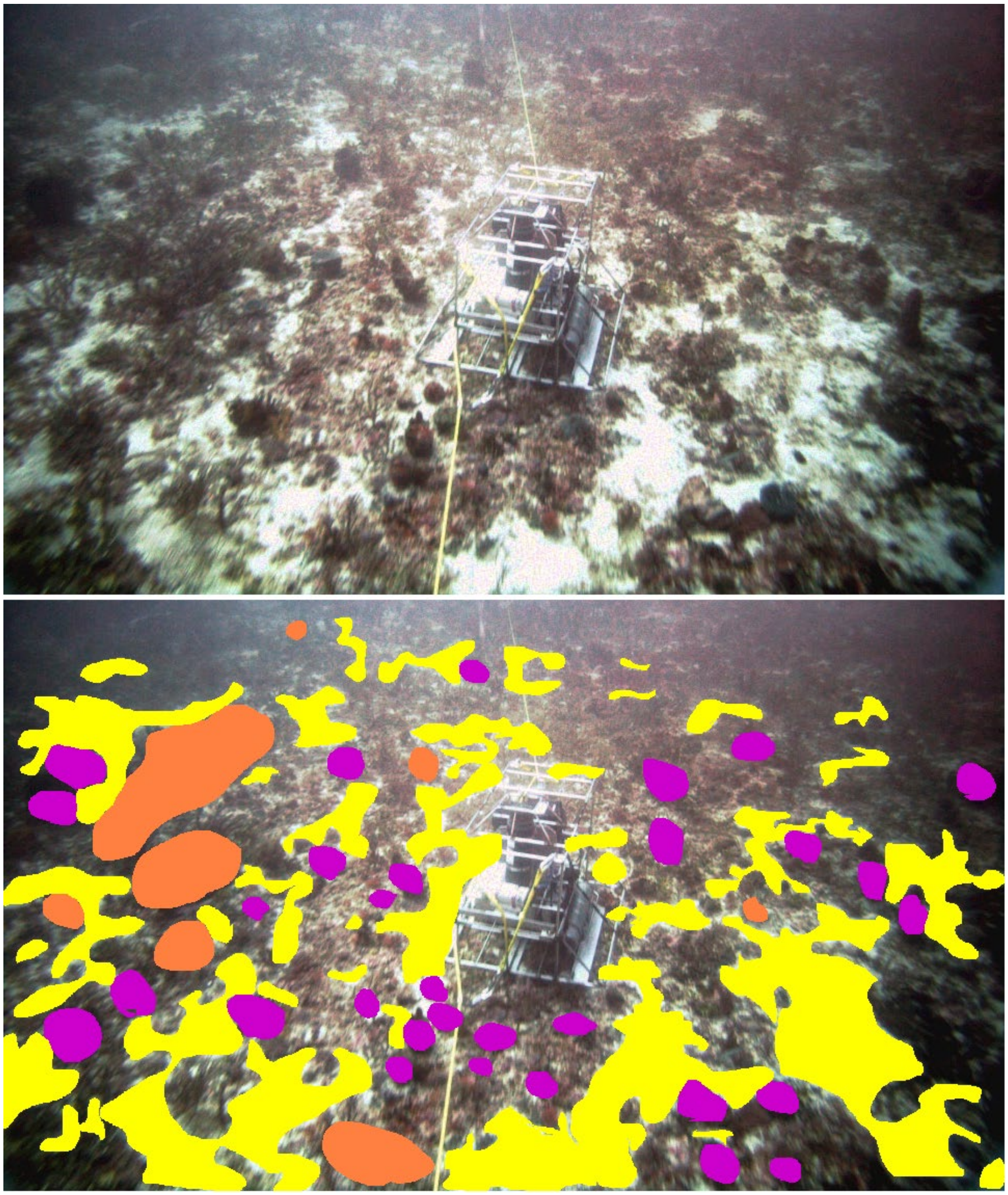

Figure 7 Benthic Cover Classification of SEAMAP Station 17 using Coral Point Count with Excel Extensions Software; yellow is sand, purple is sponge, and orange is octocoral (not shown: green is macroalgae)

CHAPTER 4: RESULTS

The Marine Environment

The C-BASS images from the Florida Middle Grounds in May 2014 were examined for notable environmental features. The transects averaged 3.5 hours and totaled 133 km in length (Table 2). Layback ranged from 25 s (43.8 m) to 42 s (73.5 m) and averaged 33 s (57.8 m). The easternmost C-BASS imagery was recorded in Transect 6 at -84.0156 longitude, and the westernmost C-BASS images were captured in Transect 1 at -84.4608 longitude (Table 4). The highest average Chl *a* concentrations were found in Transects 1 (~28.6500° N) and 4 (~28.4502° N), with Transect 4 containing a wider range of Chl *a* (0.67 mg/m^3) than Transect 1 (0.63 mg/m^3). Transect 4 also exhibited a greater depth range than any other transect (22 m), showing a slight positive linear relationship with Chl *a* concentration ($r^2 = 0.1$, $p < 0.001$). The greatest change in depth between images was 6.9 m in Transect 4 at 28.454° N latitude and -84.303° W longitude, where depth decreased relatively quickly from 34 to 28 m over a 26-m distance.

Table 4 **Mean and range measurements across transect (T) 1 in the Florida Middle Grounds. Latitude, longitude, and tow speed collected by ship and environmental measurements taken in situ by C-BASS**

T	Latitude (°N)	Longitudes: Westernmost Easternmost (°W)	Altitude (m)	Temperature (°C)	Depth (m)	Salinity (PSU)	Chlorophyll *a* (mg/m^3)	Turbidity (NTU)	Speed (kn)
1	28.6500	-84.4608 -84.2205	3.1 (1.6–9.3)	20.95 (20.34–21.44)	32.8 (22–39)	36.31	0.89 (0.57–1.20)	97.8 (83–159)	3.47
2	28.5620	-84.3929 -84.2122	3.4 (2.3–8.3)	20.91 (20.44–21.29)	33.6 (21–41)	36.27	0.85 (0.64–1.18)	96.9 (86–117)	3.40
3	28.5295	-84.2799 -84.1760	3.3 (0.5–11.1)	20.96 (20.45–21.32)	32.8 (22–42)	36.25	0.74 (0.56–0.91)	92.6 (81–137)	3.52
4	28.4502	-84.3626 -84.1402	3.2 (1.5–11.2)	20.80 (20.58–21.12)	35.4 (24–46)	36.27	0.89 (0.66–1.33)	95.9 (83–130)	3.43
5	28.3437	-84.2341 -84.0636	3.5 (1.2–8.6)	20.83 (20.69–20.95)	35.0 (31–44)	36.31	0.87 (0.77–1.15)	95.9 (90–106)	3.41
6	28.2269	-84.1933 -84.0156	3.3 (1.8–6.1)	20.75 (20.70–20.80)	37.6 (32–41)	36.28	0.87 (0.75–0.98)	95.0 (89–114)	3.41

Image Enhancement and Format

Reconciliation of Contrast and Color

The R, G, and B pixel values exhibited dissociation in C-BASS benthic images (Figure 8A) because of light attenuation and loss of color and contrast at depth. Figure 8 illustrates the most extreme dissociation among the red pixel values; the first color to be absorbed at depth (mean pixel intensity = 56; maximum pixel intensity = 216, out of a possible 255). The blue-green coloring of the original image is shown in Figure 9 (“Before [color correction]”). Using the MATLAB function RGB_CLAHE, the R, G, and B pixel values were redistributed across the image color space (Figure 8B), making features visible that were camouflaged in the original image (Figure 9; “After [color correction]”).

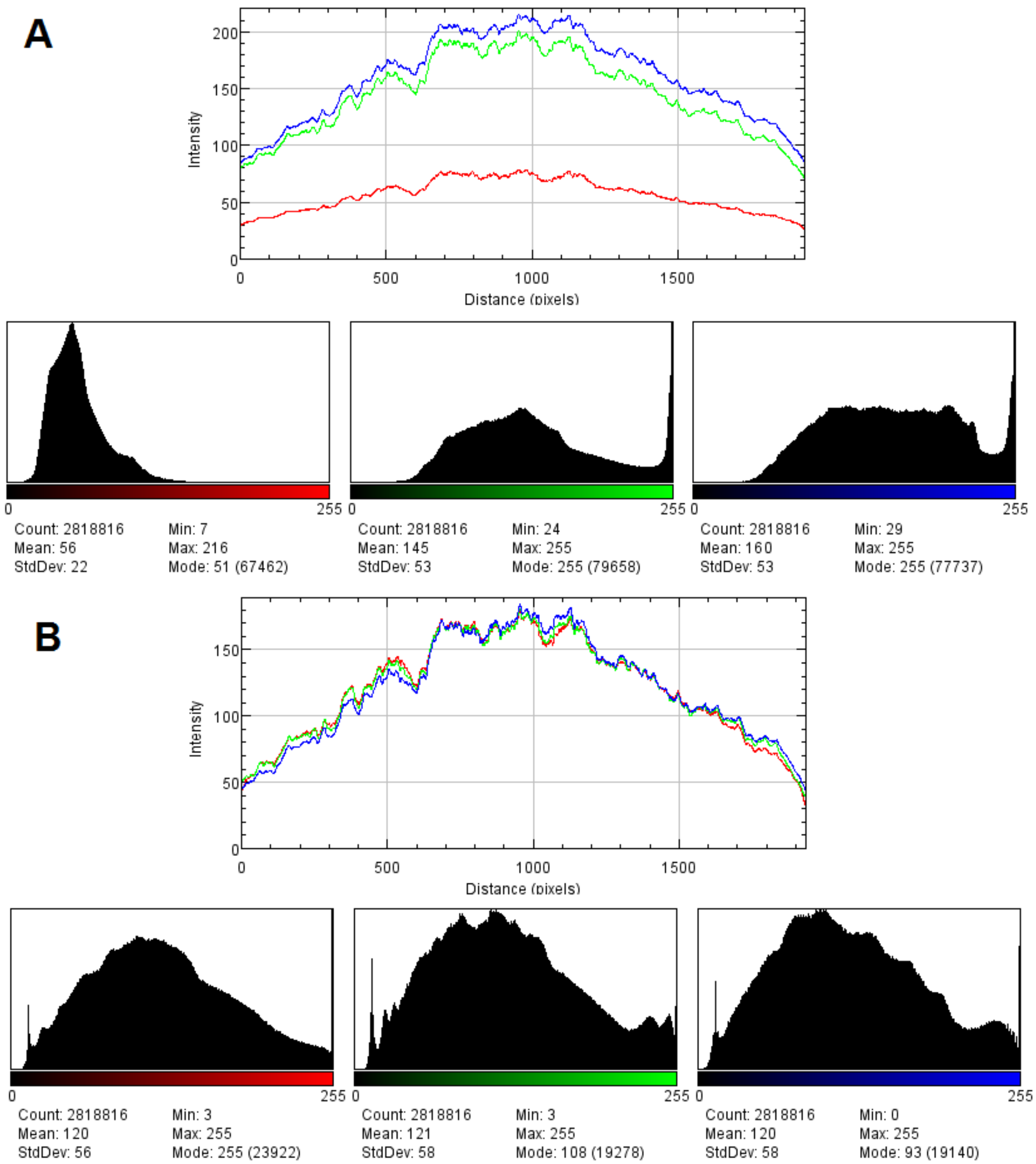

Figure 8 **Example of R, G, and B pixel intensity values in RGB color space for (A) original image with light attenuation and (B) processed image, with color and contrast correction. Image taken along FMG transect 3 at 1730 hours on May 6, 2014.**

Figure 9 **Image in its original state (before) and image processed with color and contrast correction (after). Circles have been made to show improved visibility of sponges. Image taken along FMG transect 3 at 0420 hours on May 7, 2014.**

Histogram equalization returned red, yellow, and orange to the images. It enhanced specific features in the images, including sponges and algae encrusted on rubble or other substrate (Figure 11).

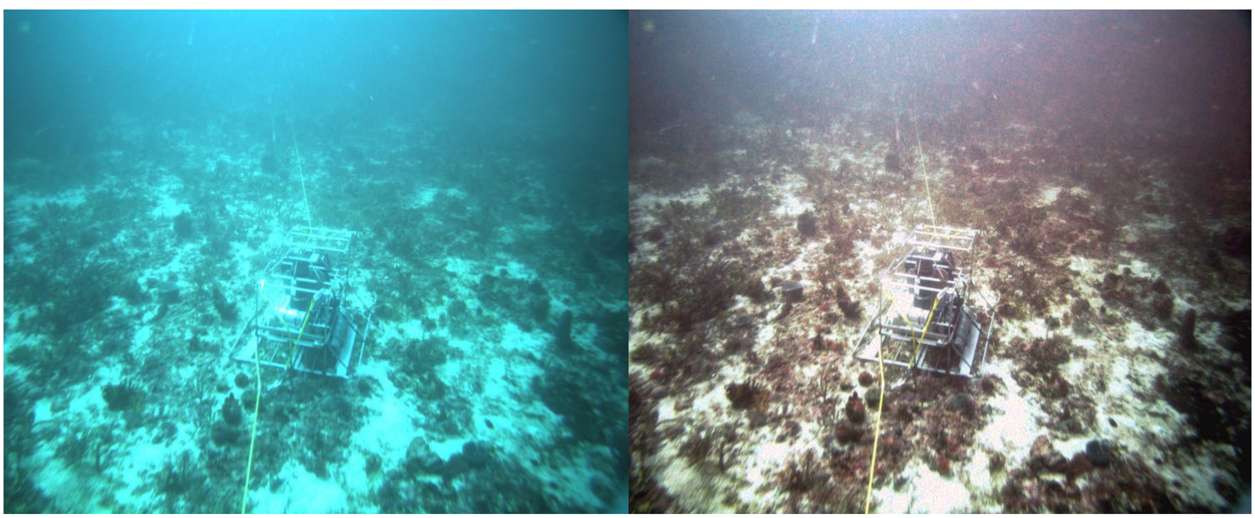

Figure 10 **Before-and-After: SEAMAP Stationary Camera at Station 17**

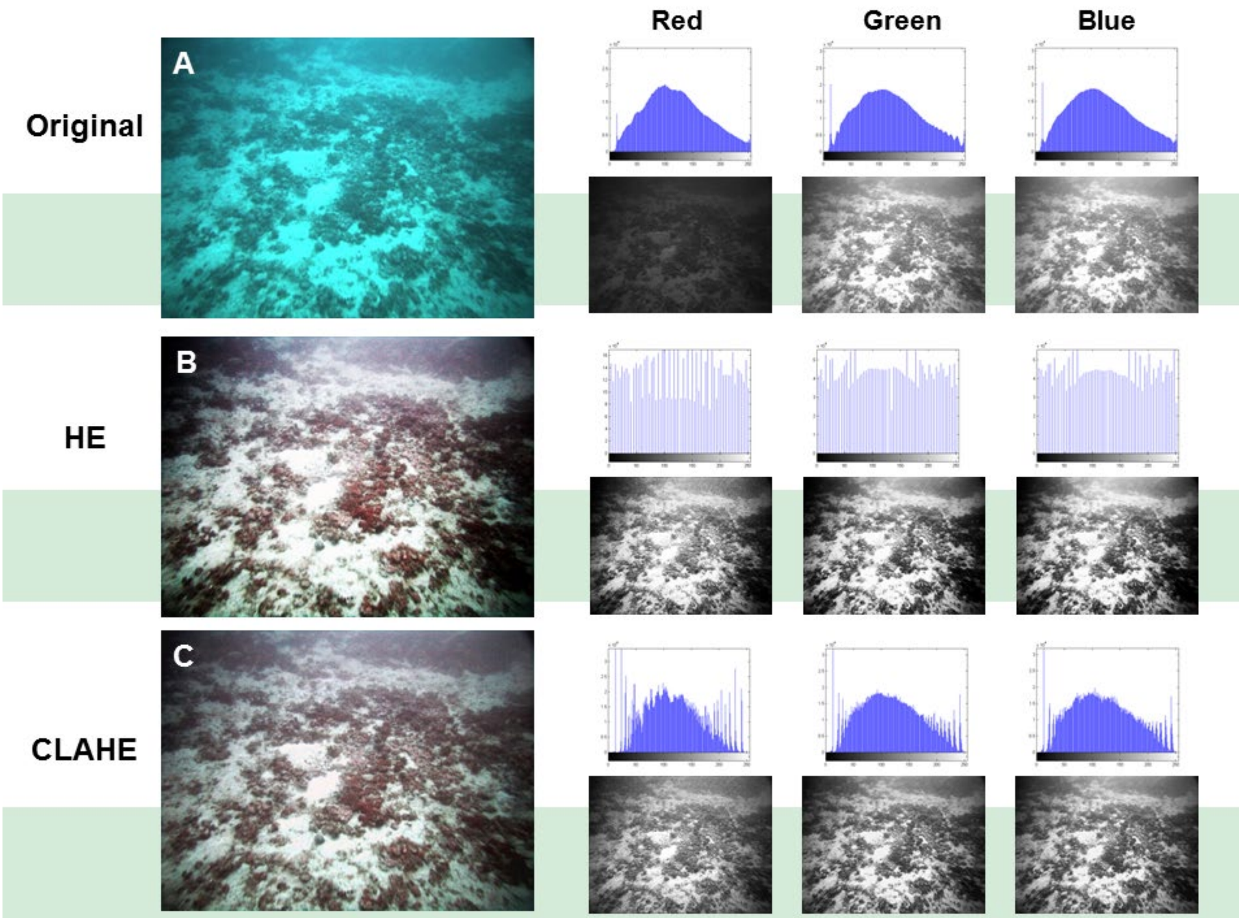

Figure 11 Progression of contrast-limited histogram equalization on an image taken in the Florida Middle Grounds at 2330 hours on May 6, 2014. (A) Original Image (left), and Original image separated into red, green, and blue components (histograms top and contrasts below); (B) Image after RGB histogram equalization (HE; left), and HE image histograms and contrasts; and (C) Fully-processed image, with post-process histograms and contrasted RGB components.

Variations in Image Quality

Prior to processing, images from the May 2014 survey (n = 4617) ranged in size from 88 to 529 KB and mean pixel intensity values per image ranged from 15 to 207. In general, images smaller than 100 KB had a lower resolution (fewer pixels per area) than images greater than 100 KB. Image values sampled from each 10-KB size tier from 20 to 100 KB (n = 9) collected in the August 2015 survey indicated that image size accounted for more than half of the variability of mean pixel value ($r^2 = 0.53$, df = 8, $P < 0.001$). While a relationship was detected between image size and turbidity ($r^2 = 0.21$), the relationship was not significant ($P = 0.2$), perhaps due to small sample size; therefore, the null hypothesis that environmental variables do not affect image quality was not rejected for these data.

The BMP and JPEG images collected simultaneously (n = 3621) in October 2016 produced mean (per image) intensity values that were positively correlated ($r^2 = 0.99$, $P < 0.001$). A significant difference was found, however, between the mean range of gray values in BMPs and the mean range of gray values in JPEGs prior to processing (3620 d.f., $P < 0.001$). The mean range showed a greater difference in the BMPs ($t = 6.35$, $P < 0.001$) and both formats combined ($t = 1.96$, $P < 0.001$) than the mean range of the JPEGs alone ($t = 1.65$, $P < 0.001$). Overall, JPEGs exhibited a greater range of values (mean = 150) than BMPs (mean = 148).

After processing, the difference in quality between an image captured in BMP and an image captured in JPEG format was easily observed by the human eye. Within the water column, for example, light penetration resulted in a halo of red coloring in the JPEG images (Figure 12). The images were not captured in an area of varying relief like the Florida Middle Grounds, however, so visual examination was limited to observable features between images and not between areas. The mean intensity values between BMP and JPEG images post-processing remained positively correlated, although with a lower value ($r^2 = 0.56$, $P < 0.001$). The difference in mean range of gray values grew significantly (3620 d.f.), and was most pronounced in BMPs ($t = 228$, $P < 0.001$), although the difference in mean range of both formats combined and JPEGs alone remained the same ($t = 1.96$ and 1.65, $P < 0.001$, respectively). The overall range of average intensity values grew by almost 100 for both formats; however, JPEGs again exhibited a greater range of values (mean = 251) than BMPs (mean = 245).

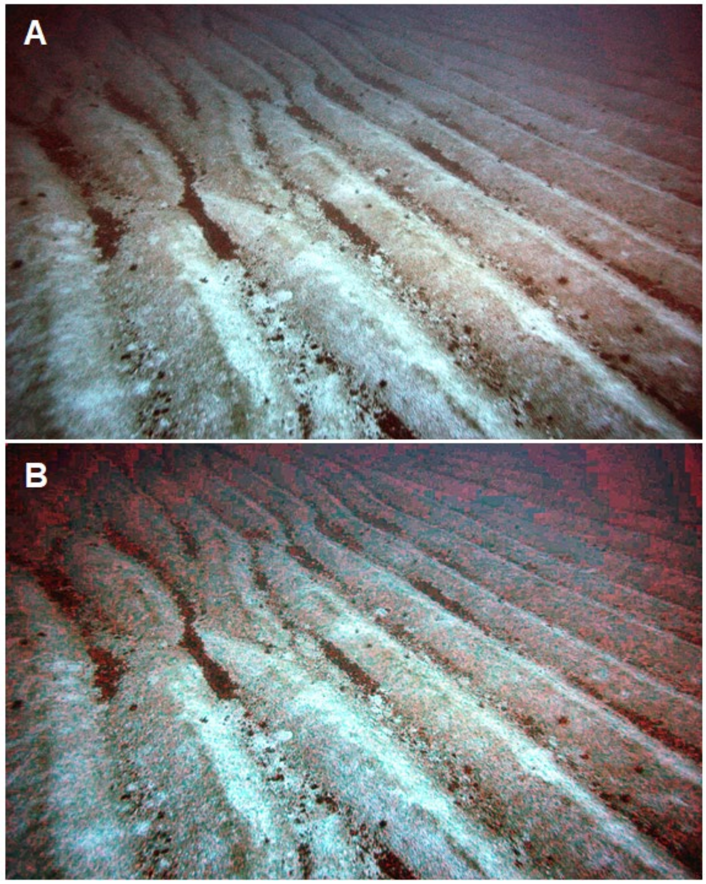

Figure 12. **Difference in quality between images processed from (A) bitmap format and (B) JPEG format. Image collected southwest of the Florida Middle Grounds in October 2016.**

Benthic Cover and Species Associations

Habitat varied across the surveyed transects in the Florida Middle Grounds, ranging from high relief hardbottom to flat sand. The shallowest areas (<30 m) had the most biotic cover, while the deepest areas (>37 m) were sandy with 0–25% algae cover. Rocky outcrops or macroalgae dominated transitional (“reef slope”) areas.

Habitat Types

Two ordination techniques were used to determine the final number of significant and distinct habitat types among the sites sampled along Florida Middle Grounds transect 1 (sites where >1 fish were observed). Dissimilarity cluster analysis identified eight habitats (Figure 13).

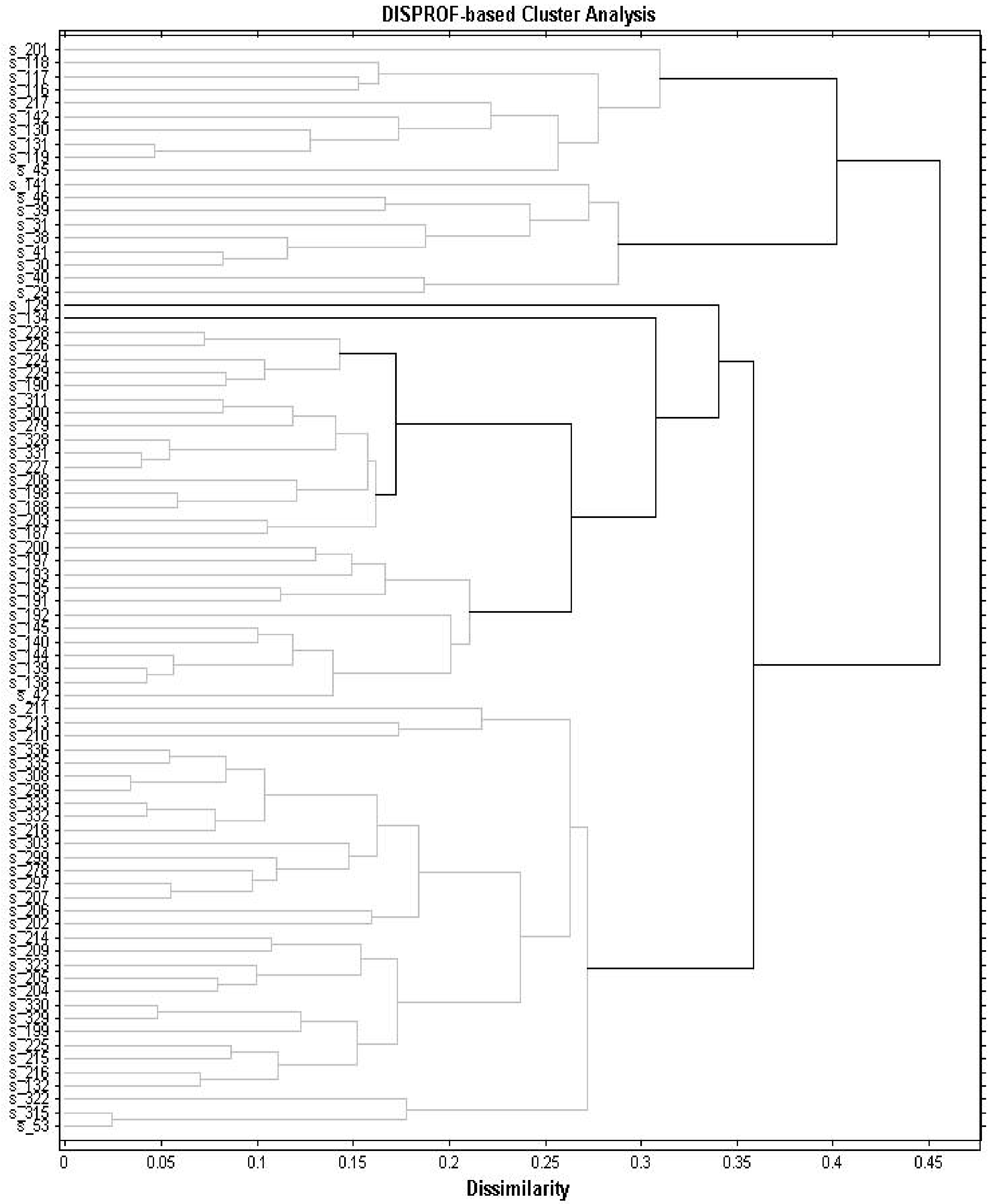

Figure 13 **Dendrogram showing dissimilarity of sites (s) based on benthic cover (n = 79). Dark lines indicate significant groupings (8; P < 0.05). Gray lines indicate homogeneity of benthic composition.**

Spatial differences were apparent in nMDS ordination diagrams corresponding to grouping among sites; however, some numbers that showed no apparent grouping were joined with clusters resulting in further reduction to five habitat types (stress = 0.18; Figure 14a). Strong signals were apparent from encrusted rubble, low-relief algae, macroalgae, soft corals, and sand, while weak signals were detected for sponges and hard corals (Figure 14b).

Figure 15 shows the percent of each benthic cover across the five habitat types, and the dominant benthic cover in each habitat are summarized in Table 5. Visual examples of site composition for each habitat are given in Figure 16–Figure 20.

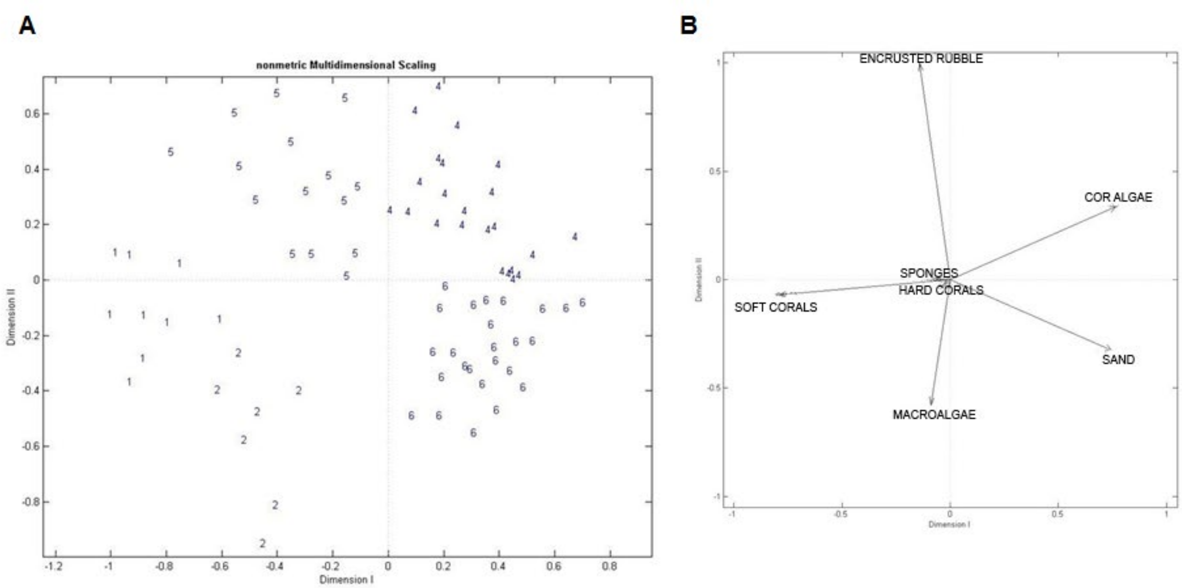

Figure 14 **Two-dimensional nMDS ordination diagrams (stress = 0.18) of (a) five habitat types and (b) benthic cover. Note that there is no habitat type "3" after sites were reassigned to habitat type "4" after initial nMDS ordination.**

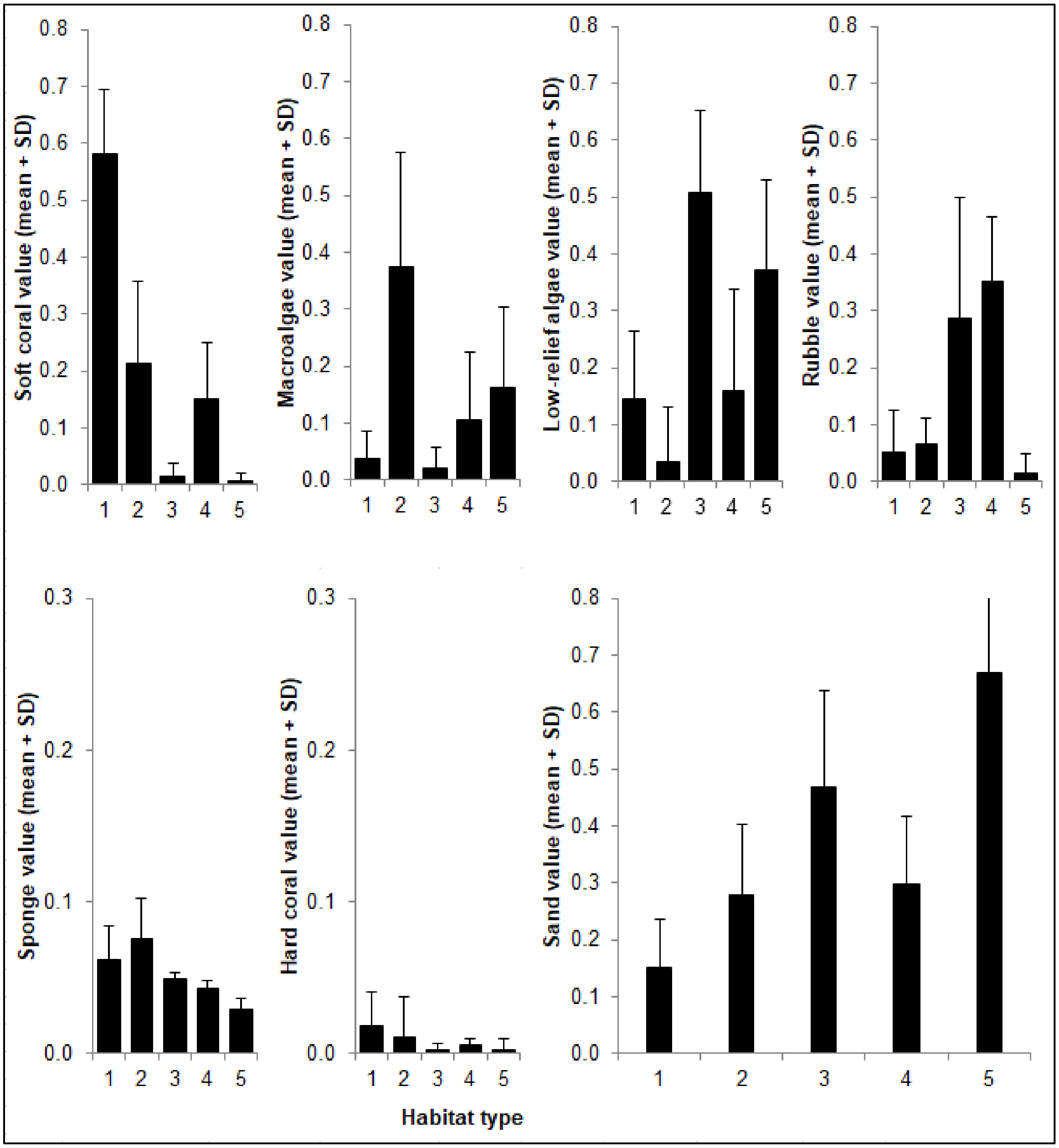

Figure 15 Benthic cover value (mean + 1 SD) of soft coral, macroalgae, low-relief algae, rubble, sponge, hard coral and sand in each habitat type. Note difference in scale for sponge and hard coral cover.

Table 5 Summary of habitat types (n = 5) identified among sample sites (n = 79). Discriminating benthic cover identified in SIMPER procedure.

Habitat	Sites (%)	Shannon Diversity Index (H ± SE)	Discriminating benthic cover and mean value ± SE	
1	11	1.39 ± 0.10	Soft coral	0.58 ± 0.04
2	9	1.27 ± 0.13	Macroalgae	0.37 ± 0.08
3	29	0.93 ± 0.04	Low-relief algae	0.51 ± 0.03
4	19	1.23 ± 0.06	Rubble	0.35 ± 0.03

5	32	0.74 ± 0.04	Sand	0.67 ± 0.03

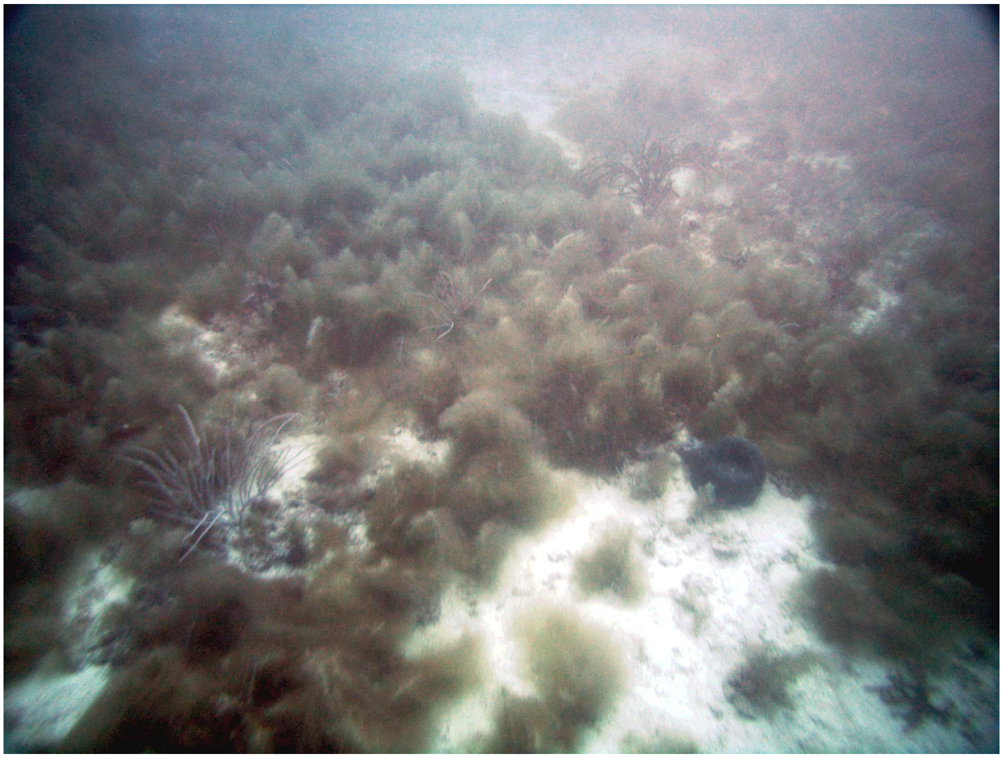

Figure 16 Fleshy macroalgae habitat type in image 05072014_215252 (site 40)

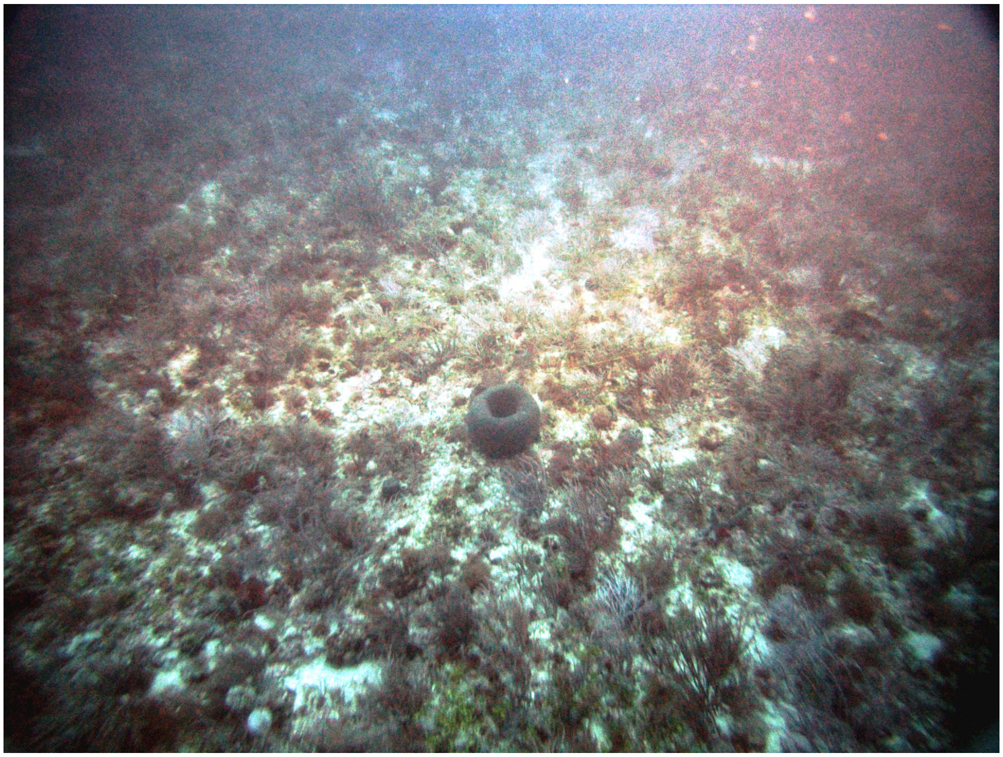

Figure 17 Soft coral habitat type in image 05072014_232211 (site 130)

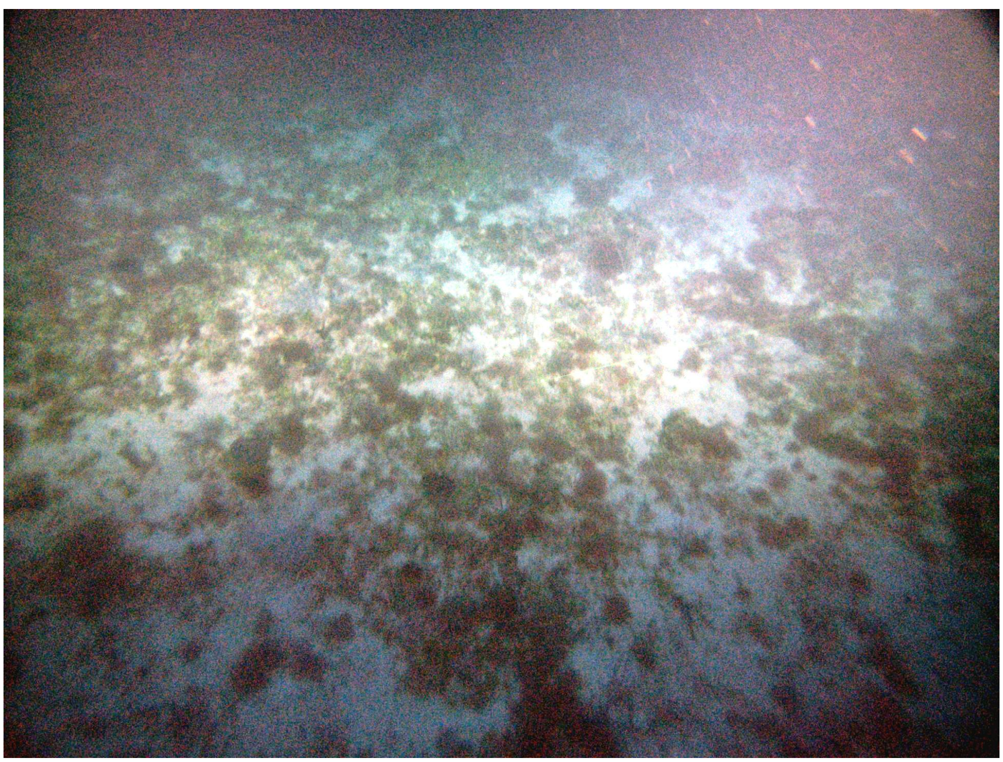

Figure 18 Low-relief algae habitat type in image 05082014_001956 (site 187)

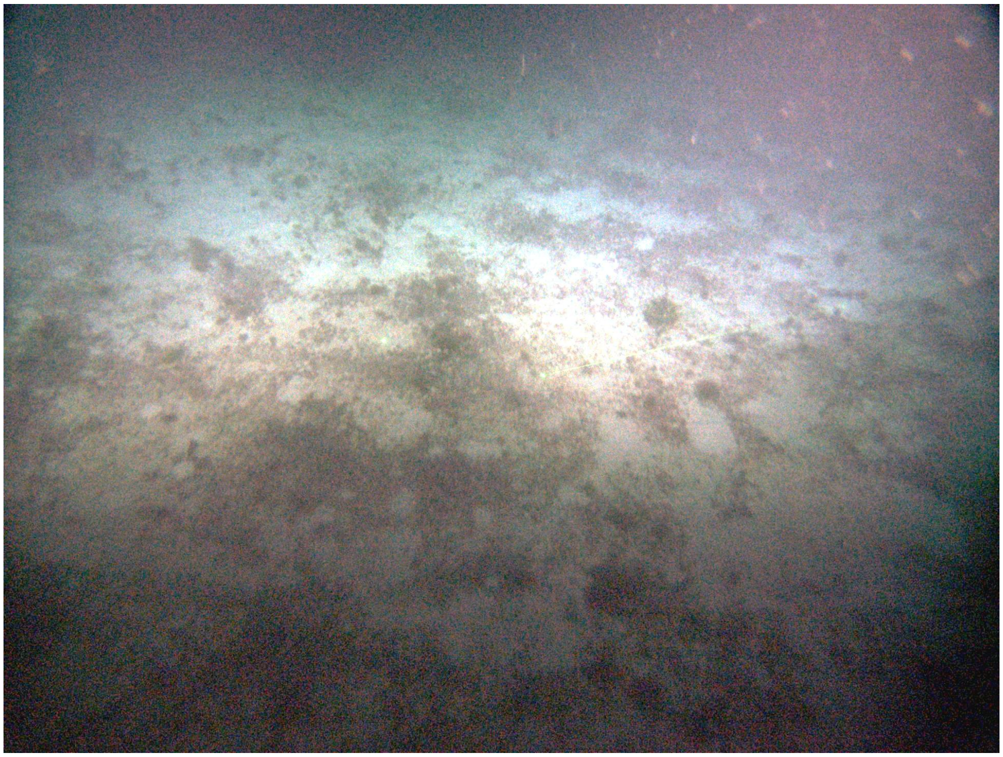

Figure 19 Sand habitat type in image 05082014_004556 (site 213)

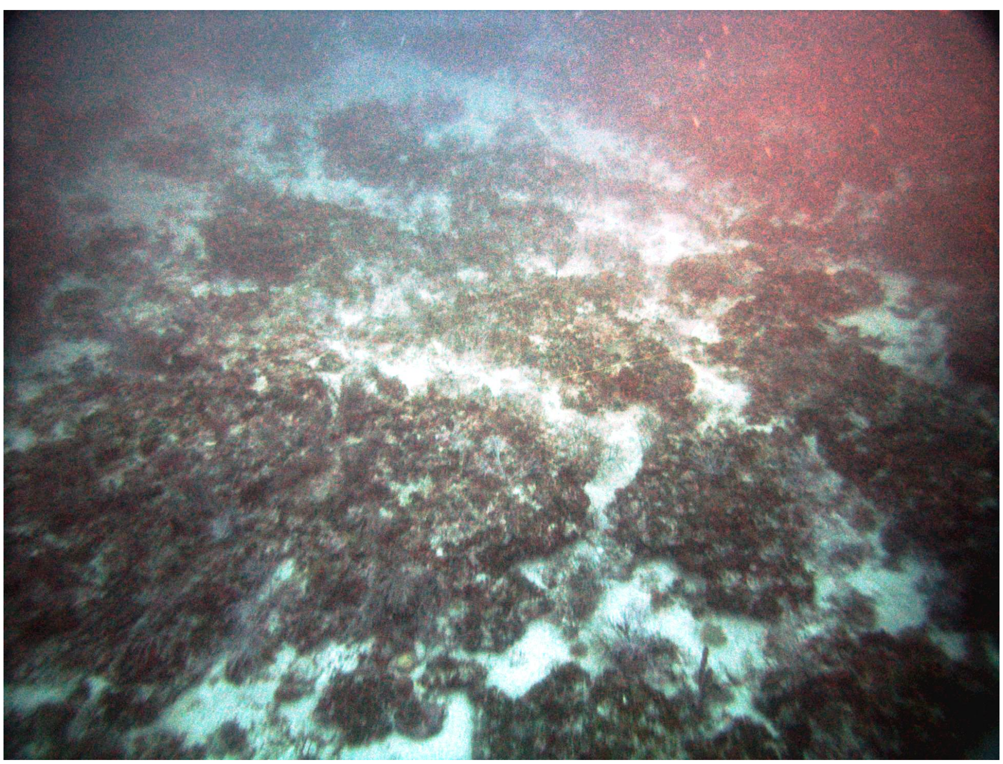

Figure 20 Rubble habitat type in image 05072014_233226 (site 140)

Environmental Characteristics of Habitat Types

A summary of temperature, depth, slope, salinity, chlorophyll, and turbidity measurements (mean and standard error), which were sampled in situ while collecting the video used to delineate each habitat type, are provided in Table 6. Sand habitat exhibited the greatest average depth (31.4 m), and rubble habitat the shallowest (25.3 m).

Table 6 Summary of mean environmental measurements (±SE) from sites sampled along Florida Middle Grounds Transect 1 (n = 79).

Habitat type	Temp (°C)	Depth (m)	Slope, m (m)	Salinity (PSU)	Chlorophyll (mg/m^3)	Turbidity (NTU)
(1) Soft coral	20.83 ± 0.05	26.28 ± 0.53	0.013 ± 0.003	36.21 ± 0.01	87.54 ± 1.56	93.69 ± 0.32
(2) Macroalgae	21.17 ± 0.06	26.34 ± 1.70	0.026 ± 0.009	36.27 ± 0.02	75.30 ± 4.75	90.61 ± 1.69
(3) Low-relief algae	21.15 ± 0.02	28.16 ± 0.78	0.014 ± 0.003	36.32 ± 0.01	81.91 ± 1.13	93.23 ± 0.64
(4) Rubble	21.15 ± 0.02	25.29 ± 0.26	0.011 ± 0.004	36.29 ± 0.01	86.17 ± 1.86	92.87 ± 0.58
(5) Sand	21.01 ± 0.03	31.38 ± 0.97	0.008 ± 0.001	36.36 ± 0.01	90.99 ± 1.60	96.96 ± 0.76

The differences in environmental measurements were globally significant across the habitat types (PERMANOVA; $P < 0.001$); however, a pairwise PERMANOVA indicated that habitats 2, 3, and 4 (macroalgae, low-relief algae, and rubble) did not have significantly different environments (Holms-adjusted $P = 0.1014$). CAP ordination performed on these data illustrated this point further, as those habitats were either clumped together or scattered without apparent association (Figure 21). Strong correlations with the canonical axes (6 eigenvalues; $P = 0.001$) were exhibited in a CAP biplot of the environmental variables, with weaker correlations with the second canonical axis presented for depth, slope, and salinity than for temperature, chlorophyll, and turbidity (Figure 21). Globally, 63% of the samples were correctly assigned to habitat type, which was significantly better than randomized classification success (24%) from a proportional chance criterion ($P = 0.001$).

Table 7 Confusion matrix of percent misclassification of environmental measurements across habitat types. Numbers in gray represent leave-one-out cross-validation

classification success showing the percent of the environmental samples that were assigned to their correct group (habitat type) based on 6 eigenvalues.

		Predicted Group				
		1	2	3	4	5
Actual Group	1	89	0	11	0	0
	2	14	43	14	14	14
	3	0	13	57	9	22
	4	7	20	7	67	0
	5	0	0	8	28	64

The macroalgae habitat, exhibiting the greatest mean slope, also consistently exhibited the largest standard deviation across all variables (Table 6). This habitat type was not significantly different from low-relief algae or rubble habitat in pairwise PERMANOVA tests (Holms-adjusted P = 0.1014). Classification success of the environmental samples was lowest for macroalgae habitat (43% in leave-one-out cross-validation; Table 7), which was reflected in CAP ordination, where macroalgae habitat (2) is spread across at least two other habitat clusters (Figure 21).

Low-relief algae habitat was found in the second-deepest areas (mean 28 m), with second-highest salinity (mean 36.32 PSU) (Table 6). The mean temperature in low-relief algae habitat was similar to macroalgae and rubble habitats, perhaps because low-relief algae was present at the most (89%) of the sites, second only to the presence of sand (100% of sites). This environmental variation is illustrated in CAP ordination, where low-relief algae habitat (3) is clustered among sand and rubble habitats (5 and 4, respectively), and the correlation with salinity, depth, and temperature relationships are visualized in the environmental variable biplot (Figure 21).

The greatest classification success in CAP discriminant analysis (89%) was for the soft coral habitat (Table 7). Soft coral habitat exhibited the lowest mean temperature and salinity (Table 6). CAP biplots for those variables exhibited strong associations negatively correlated with canonical axis I, unlike the soft coral habitat type, which was positively correlated with that axis (Figure 21). Soft coral habitat had a similar mean depth to macroalgae habitat (26.28 and 26.34 m, respectively), although the standard deviation was 31% that of the macroalgae habitat, indicating a smaller depth range (Table 6).

Rubble habitat was found in the shallowest depths (mean 25.29 m; Table 6). CAP ordinations indicated clustering among rubble habitat (4) and low-relief algae habitat (3) (Figure 21).

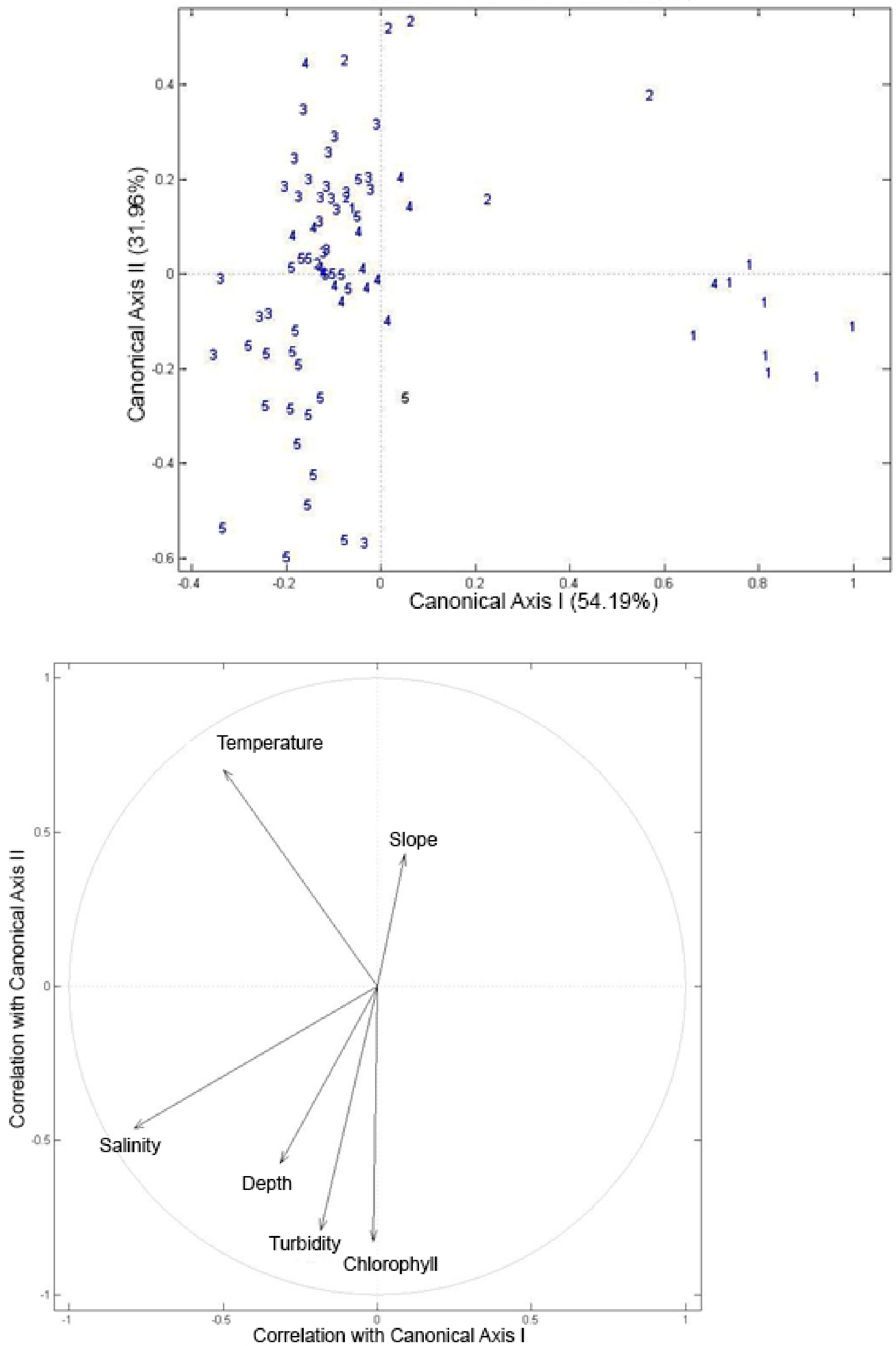

Figure 21 **CAP ordination (top) of habitat type (1 soft coral, 2 macroalgae, 3 low-relief algae, 4 rubble, 5 sand). Similarity of environmental variables and biplot of environmental variables (bottom).**

Sand habitat occupied sample sites with the greatest mean depth (31.38 m), salinity, chlorophyll, and turbidity (Table 6). Sand habitat also exhibited the smallest mean slope

(0.008 m). While all sample sites had sand present, 32% of all sites were classified as sand habitat (sand mean value 0.67; Table 5). CAP ordination presented a clear cluster of sand habitat (5), with some low-relief algae association, which is expected as low-relief algae was often present (mean value 0.37) in the sand habitat type. Sand habitat's correlation with environmental variables depth, salinity, chlorophyll, turbidity, and slope are illustrated in the CAP biplot (Figure 21).

Fish Abundance across Habitat Types

There was no globally significant difference in abundance of all identified fish species among habitat types found from PERMANOVA (P = 0.07); however, pairwise PERMANOVA of habitat types showed dissimilarity of fish species abundances among soft coral habitat (habitat 1) and macroalgae habitat (habitat 2) (P = 0.02), and macroalgae habitat and sand habitat (habitat 5) (P = 0.03). The strengths of these relationships were investigated further with ANOSIM, where R = 0.30 among soft coral and macroalgae habitat (P = 0.02), and R = 0.18 among macroalgae and sand habitats. Soft coral habitat had the highest mean fish diversity (Simpson Index = 0.53), although macroalgae and sand habitats' highest diversity values were comparable (Table 8).

The most abundant fishes identified across all habitat types were angelfish spp., gray snapper, porgy spp., grouper spp., snapper spp., and Holocentridae spp. (Table 9). A global test showed a slight but significant difference in the abundance of these fishes among habitat types (ANOSIM; R = 0.06, P = 0.03). The strengths of the relative fish composition among habitats was investigated further with pair-wise ANOSIM to find significance between soft coral and macroalgae habitat (R = 0.29, P = 0.03) and macroalgae and sand habitats (R = 0.24, P = 0.005).

Table 8 **Summary of fish abundance and diversity in each habitat type. Simpson Diversity Index does not include fish abundance for which the species were not identified.**

Habitat Type (n)	Total Fish Abundance (with ID)	Total Fish Abundance (with + without ID)	Mean Fish Abundance ± SD (with + without ID)	Simpson Diversity Index (1 – λ) Mean ± SD
Soft coral (9)	43	71	7.89 ± 0.93	0.53 ± 0.34
Macroalgae (7)	15	56	8.00 ± 1.51	0.47 ± 0.41
Low-relief algae (23)	98	225	9.78 ± 1.04	0.47 ± 0.29
Rubble (15)	68	347	23.13 ± 1.32	0.50 ± 0.20
Sand (25)	98	271	10.84 ± 0.76	0.50 ± 0.31

Table 9 **Abundance (number of individuals) of fish species identified in each habitat type (1 soft coral, 2 macroalgae, 3 low-relief algae, 4 rubble, 5 sand). Fish counted but not identified are excluded from this table.**

	Habitat						
Species	(1) Soft coral	(2) Macroalgae	(3) Low-relief algae	(4) Rubble	(5) Sand	Total	Fish Abundance Mean ± SE
Angelfish spp.	11	6	16	13	11	57	11.40 ± 0.35
Gray Snapper	21	3	52	40	50	166	33.20 ± 0.91
Porgy spp.	5	3	5	4	7	24	4.80 ± 0.20
Grouper spp.	1	-	2	1	-	4	0.80 ± 0.07
Snapper spp.	-	-	13	3	11	27	5.40 ± 0.37
Lionfish	-	1	1	-	1	3	0.60 ± 0.06
Holocentridae spp.	5	2	3	4	-	14	2.80 ± 0.18
Boxfish spp.	-	-	1	-	7	8	1.60 ± 0.13
Hogfish	-	-	-	2	-	2	0.40 ± 0.08
Jack spp.	-	-	-	-	3	3	0.60 ± 0.08
Surgeonfish spp.	-	-	2	-	1	3	0.60 ± 0.06
Butterfly fish spp.	-	-	1	1	3	5	1.00 ± 0.10
Filefish spp.	-	-	2	-	4	6	1.20 ± 0.09

Correlation with Fish Taxa and Environmental Variables

Distance-based redundancy analysis (RDA) of fish species abundances and both the benthic cover and environmental variables revealed 16% explanation of variation in fish species abundance across two axes ($r^2 = 0.22$, adjusted $r^2 = 0.07$, $P = 0.04$; Figure 22). Canonical axis I explained 9% of the variation in fish species abundances. Along this axis, variation in gray snapper abundance was positively related to sponge, soft coral, and hard coral cover, and chlorophyll, and negatively related to encrusted rubble cover, macroalgae cover, and temperature. Holocentridae spp., angelfishes, and filefish exhibited similar relationships to these variables, but to a lesser degree.

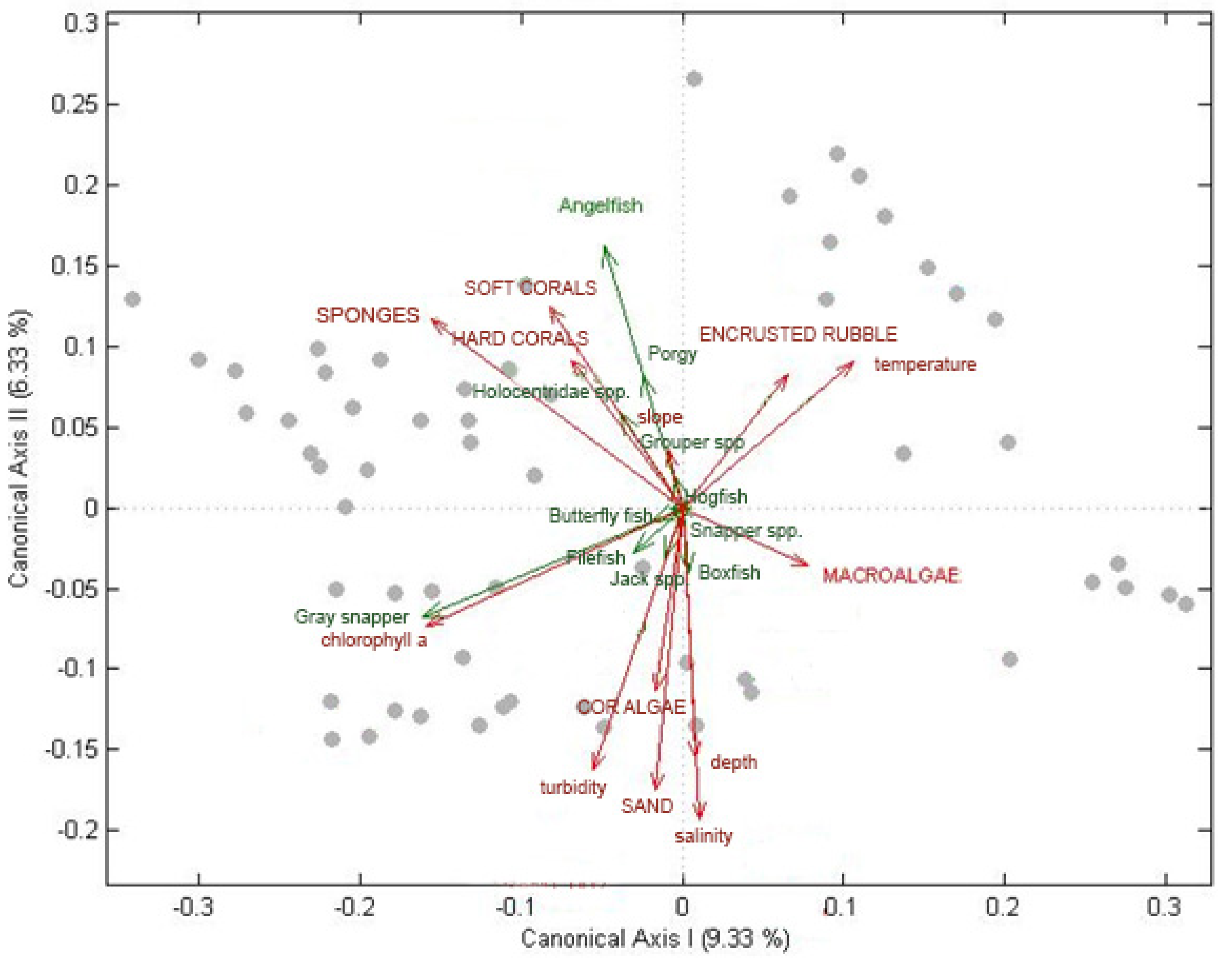

Figure 22 **Distance-based redundancy analysis (RDA) of environmental measurements (chlorophyll a, temperature, turbidity, depth, slope, and salinity) and benthic cover (soft corals, hard corals, encrusted rubble, sponges, macroalgae, coraline algae, and sand) on fish species abundances (Holocentridae spp., angelfish, porgy, grouper spp., gray snapper, [other] snapper spp., jack spp., hogfish, butterflyfish, boxfish, and filefish). Gray dots represent sites.**

The second canonical axis explained 6.33% of the variation observed in fish species abundances, showing relationships with nearly all of the benthic cover and environmental measurements. Most notably, angelfishes, porgy, and Holocentridae spp. abundances exhibited positive relationships with sponge, soft coral, hard coral, and encrusted rubble cover, as well as with slope and temperature. Their variations were also negatively associated with sand and low-relief algae cover, as well as turbidity, salinity, and depth. Gray snapper and chlorophyll exhibited the same relationship on axis II as they did for axis I, although to a lesser degree.

Pairwise ANOSIM revealed significant differences in three fish species abundances among sponge cover values. Angelfish spp. abundance differed between the sponge cover 0 and 0.15 ($R = 0.39$, $P = 0.01$). Porgy spp. abundance differed between sponge cover values 0 and 0.15 ($R = 0.41$, $P = 0.006$) and 0.07 and 0.15 ($R = 0.18$, $P = 0.01$). The greatest number of significant associations among sponge cover values were exhibited by the abundance of Holocentridae spp., with $P < 0.05$ for cover values 0 and 0.15 ($R = 0.40$), 0.01 and 0.15 ($R = 0.28$), 0.03 and 0.15 ($R = 0.29$), and 0.07 and 0.15 ($R = 0.43$).

Globally significant differences in the abundances of three fish taxa among soft coral cover values were found in ANOSIM ($P < 0.05$). These fishes were angelfish spp. ($R = 0.18$), porgy spp. ($R = 0.12$), and Holocentridae spp. (0.19). Pairwise ANOSIM also found that snapper spp. differed significantly among soft coral values 0.03 and 0.07 ($R = 0.29$, $P = 0.01$), and 0.03 and 0.15 ($R = 0.22$, $P = 0.008$). Soft coral was present in 65% of the sample sites; however, 100% of the Holocentridae spp. were observed within those sites.

SCUBA Sites Revisited

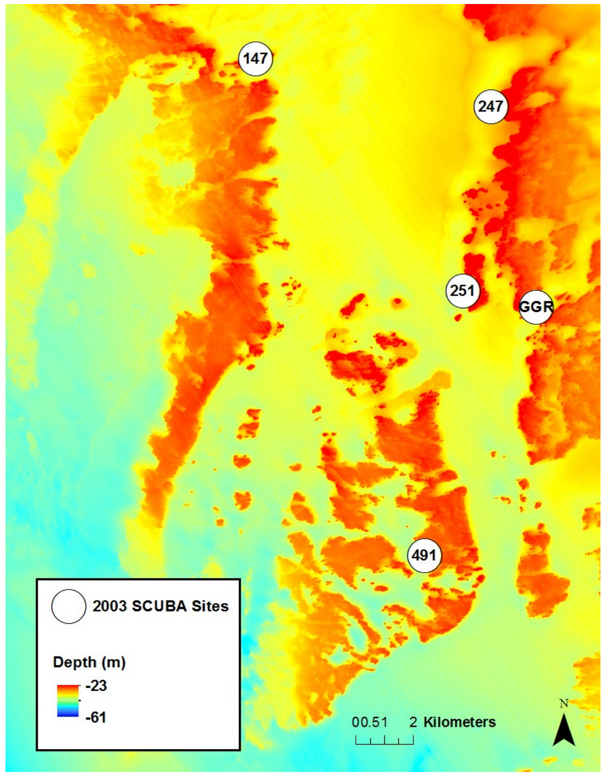

Figure 23 SCUBA Sites from 2003 [in Coleman et al. (2004a)] Revisited by C-BASS in 2014

C-BASS transited five locations where SCUBA surveys were conducted in 2003; however, the relief at FMG 491 was so great that the C-BASS approached the substrate too quickly to capture a clear image; therefore, that site was excluded from this analysis. Sponges, soft corals, hard corals, and substrate (sand, rubble, and rock) were present in C-BASS images as they were in reports from the 2003 SCUBA surveys. Taxa that were reported by Coleman et al. (2004a) from the 2003 SCUBA surveys of the FMG sites were observed in C-BASS imagery of those sites (Appendix B). The most prominent sponges were vase and tube, and appeared to be from the Families Nephatidae, Ircinidae, and Callyspongiidae. Stony corals from the Orders Scleractinia and Milleporina were observed. Several taxa of soft corals from the Families Gorgonidae, Anthothelidae, and Plexauridae were prominent in the images. Coralline algae (*Halimeda* or *Udotea*) were present in both flat sand and areas with abundant sponges, soft corals, and hard corals, where *Halymenia* also appeared. The most prominent macroalgae (*Sargassum and Dictyota*) formed tall, forested areas between sand flats and reefs, and were often accompanied by sponges and soft corals.

The relative percent cover of the benthic species groups was not consistent with historical reports, however. C-BASS data showed that sponges were less prominent and soft corals were more prominent than the historical data indicated (Figures 13 and 14).

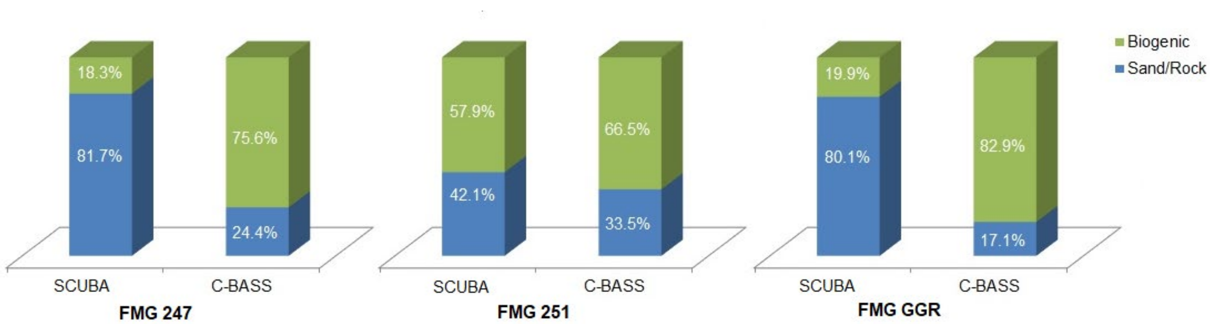

Figure 24 **Percent biogenic and sand/rock (of total cover) observed by C-BASS (2014) compared to SCUBA survey (2003) for FMG 247, FMG 251, and Goliath Grouper Rock (FMG GGR).**

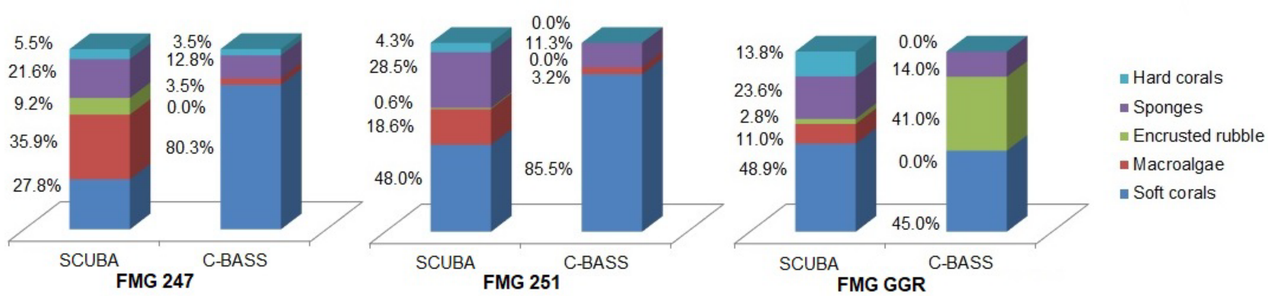

Figure 25 **Percent benthic cover (biogenic) observed by C-BASS (2014) compared to SCUBA survey (2003) for FMG 247, FMG 251, and Goliath Grouper Rock (FMG GGR). (Note: Percent benthic cover was not reported for FMG 147 in Coleman et al (2004a).)**

C-BASS Perspective

Of the five SEAMAP stations over which C-BASS passed, three were the sources of still images from C-BASS and MOUSS videos that captured both platforms simultaneously: stations 15, 16, and 17.

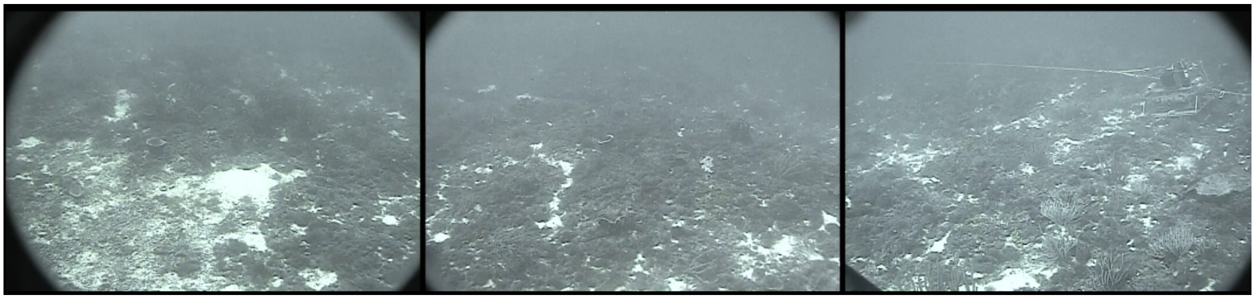

Figure 26 C-BASS monochrome view of SEAMAP station 14 stationary camera (far right)

C-BASS was captured twice at station 16, which allowed this study to summarize benthic cover of the same location from an only slightly different angle of perspective from the water column. I measured 2% sponge cover and 38% algae cover in the first transit, consistent with that of the MOUSS observation. This slight shift of view resulted in no detection of sponges in the second transit. I also measured 16% more encrusted rubble cover and a 17% less algae cover in the second transit (Figure 27b).

Across all three stations, the MOUSS analyst reported more algae than was measured from C-BASS. At station 15, MOUSS detected soft corals, which were not observed from C-BASS. Similarly, at station 17, soft coral cover measured from C-BASS was 23% less than measured from MOUSS.

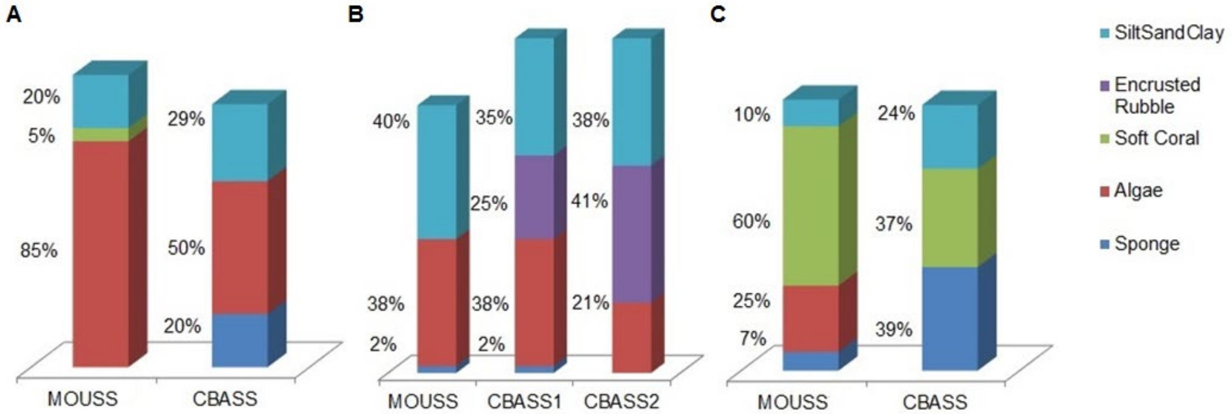

Figure 27 Percent benthic cover observed from C-BASS compared to SEAMAP stationary cameras (MOUSS) at (A) station 15; (B) station 16, showing the results of two different C-BASS transits; and (C) station 17

CHAPTER 5: DISCUSSION

Measuring and Interpreting Environmental Data

Environmental data we collected are useful for comparison between sites. Small-scale studies such as this one can examine water quality for correlation with habitats and fish species presence; however, it would be useful to compare in situ environmental measurements (e.g., chlorophyll *a*, temperature) with satellite-derived surface values to examine if benthic habitat composition is indeed a product of environmental variation. One-time sets of measurements like these are of limited use in the context of habitat variation, as they are snapshots of the conditions of the days in which they were taken, and not necessarily indicative of the range of conditions the community experiences seasonally and interannually. Such measurements may also be confounded by oceanographic conditions such as upwelling. This may have been the case in the CAP ordination and biplot (Figure 21), which showed a correlation between the soft coral habitat type, lower temperature, and shallower depth. We can assume, then, that although statistically significant differences were found in environmental measurements among the habitats, application of these findings is therefore limited within this study.

Correcting ship-position data for C-BASS layback was not as important in the Florida Middle Grounds as it would be in areas greater than 100 m deep (e.g., Madison-Swanson). Increased depth resulting in more than one minute of layback, at an average 1.75 m/s between the ship and the C-BASS, would result in over 100 m between the ship-board GPS and the sled. Greater layback would directly impact mapping results and the ability of researchers to relocate any features reported at that site. This is especially true given the contrasts in benthic habitat and species composition found between depth strata. C-BASS was built to tow in deeper waters of the shelf, providing data at resolutions that do not change drastically with depth. Water column current and ship movement affect the location of C-BASS behind the vessel. Indicators of C-BASS location such as the angle at which the hydrowire is towing above water, the angle at which it is towing below water, and the curvature of the hydrowire due to drag in each environment, could be measured and regressed to continue narrowing its location in tow. Other measurements such as C-BASS yaw and ship heading may be insightful. Although underwater navigation systems are cost-prohibitive, the benefits may outweigh the cost.

Image Enhancement and Analysis

Without the reconciliation of color and contrast in images, benthic features and attached biota would be visually obscured by light attenuation at depth, turbidity, chlorophyll, and other flocculence in the water column. In uncorrected images, the appearance of scattered dark sponges, for example, would lack natural contrast, and that of encrusting or tubular sponges would lack red, one of many of these species' most visually identifiable features. Without color correction, all algae would look green.

The strong positive relationship found between the storage size of an image and its mean pixel value, combined with the greater difference in mean range of pixel values of BMPs when compared to JPEGs, illustrated that lossy and loss-less image compressions have measurable differences. The standards for processing biomedical images include loss-less image compression prior to manipulation of the pixel values to avoid enhancing features rendered as a result of data extraction. The images captured and analyzed in this study were lossy, which was most apparent in the processed white sandy areas, which rendered noise in the form of pink spots. This noise could be mistaken for cyanobacterial growth, for example. The more complex images of high diversity were more prone to this effect; however, the general structures and hues were not overtly obscured. This made it possible to continue to identify organisms at the taxonomic levels adapted from previous studies. The results of comparing BMP and JPEG formats illustrate that using image enhancement techniques (e.g., RGB_CLAHE) will result in significant difference in the mean range of pixel values in JPEGs, likely due to the enhancement of noise. Moving forward, it is recommended that a lossless format be employed during towed video image collection.

Measuring Benthic Cover and Community

The macroalgae habitat type I delineated in this study exhibited the greatest mean slope and standard deviation across all environmental variables, suggesting this habitat was found in the widest range of environments. This, coupled with the lowest leave-one-out classification success, supports the observation that it occupies transitional areas between sand (deeper) and reef (shallower) areas in the Florida Middle Grounds. This is further supported by pairwise PERMANOVA, which showed no significant difference between the macroalgae and low-relief algae or encrusted rubble habitat types. Lack of specificity in environmental requirements may be illustrative of this benthic cover's seasonal variability.

Cheney and Dyer (1974) characterized the algal composition of the Florida Middle Grounds as having strong variations between seasons; most notably, abundant in the summer months. Collecting benthic community and environmental data (temperature, chlorophyll a, and turbidity) throughout the year would provide a temporal perspective that might allow the quantification of algal variation. Such examination of algal variability would provide insight into predicting presence and absence, and detecting perturbations in annual cycles. Observations of variations in algal cover would provide opportunities to investigate the variability and its causes. Temporal monitoring of percent cover could lead to further research into how benthic communities respond to a low-algal-cover year, such as the impact on secondary food sources such as sponges and reef-building corals from shifting forage behaviors of herbivorous fish species (Pawlik 2011).

Soft coral habitat had the most pronounced relationship to environmental variation explained by the canonical axes, owing mostly to lower temperature, salinity, and to some degree, depth. These are not variables that we would expect to have positive relationships, and yet here we saw that they did. While the temperature may be explained by an upwelling of colder water from depth, salinity and temperature combined may be better explained by their lack of variation across all of the habitat types. The slight differences across habitat types may equate to mathematical significance, but are likely negligible in ecological terms.

Rubble habitat's shallow mean depth is characteristic of the hermatypic reef structures in the Florida Middle Grounds that rubble benthic cover was used to describe, including some rocky structures where live biotic cover was not prevalent. CAP ordination clustering with low-relief algae habitat was not surprising, as rubble habitat most often included rubble encrusted with unidentified biotic material—likely algae, sponge, or some combination of both.

The relationships found in the distance-based RDA suggested sponge, hard coral, and soft coral cover attract Holocentridae spp., angelfishes, and porgy. Because Holocentridae spp. (squirrelfishes) are usually associated with ledges and rocky structures under which they take shelter during the day, it may be that their most pronounced association with benthic cover in RDA (on both axes) and ANOSIM, soft coral, is due in part to the shelter-like structure of soft corals. While the same behavior may make the negative relationship between Holocentridae spp.

abundance and encrusted rubble on the first canonical axis surprising, that may be representative of its low-relief form. The high-relief form of rubble (rock) may then be captured in canonical axis II, where we see a correlation with Holocentridae spp. abundance. On the other hand, the second axis may reflect the biotic components of encrusted rubble, and therefore its positive correlation with this fish species associated with encrusting sponges.

The correlation between angelfishes and sponges along both canonical axes in the RDA was expected, as angelfish graze on sponges. The apparent aversion to macroalgae by angelfishes, as well as Holocentridae spp., porgies, and groupers to macroalgae was not expected, however, as areas with macroalgal cover were not exclusive of sponges. In fact, the areas identified as the macroalgae habitat type also exhibited the highest mean value of sponge cover (0.08) of all of the habitat types. The reason for the negative relationship between these fish and macroalgae may warrant further investigation.

The RDA plot also showed a negative relationship between gray snapper and encrusted rubble cover across both axes. Gray snapper appear to be found mostly in deeper waters over sand, where the CAP plots illustrated a correlation with chlorophyll concentrations, and where the highest mean chlorophyll concentrations were measured. Gray snapper abundance and chlorophyll concentrations were nearly exactly correlated across both RDA axes, with slight relationships to sand and low-relief algae along the second canonical axis, possibly illustrating the fish's preference for those benthic characteristics, or a preference for the prey items that reside in them.

Further observation of habitat use by fishes could aid in the detection of habitat features important to the resilience of populations in times of perturbation [e.g., sponge ability to filter water column pollutants or strengthen the attachment of corals to hard substrate (Diaz and

Rützler 2001)] and produce sufficient evidence to warrant expanded areas of protected marine habitat, or designation of essential fish habitat. A key consideration in future considerations of benthic cover and fish species abundances measured from C-BASS could be to standardize the intervals at which these measurements are made. For this study, fish abundance data were acquired from previous work (Grasty 2015), wherein fishes were counted per minute. In contrast, the images I used for benthic cover measurement were collected at a rate of 4 images per minute, or an image every 15 s. One approach could be to count fishes per 15 s; however, C-BASS is towed continuously at an average speed of 2.5 kn, averaging 26 m between images. Fishes counted within even that 26 m may not associate with the benthic cover imaged at the end of that interval. Therefore, I am suggesting a rapid visual assessment of benthic cover at the time of each fish sighting. If schools or shoals of fishes are continuous along a portion of C-BASS transect, then rapid benthic assessment could take place at a standard interval of every 5 seconds (or 10 m) during fish presence. This survey methodology would evidence a direct interaction of fish and benthic cover, and could produce more robust analyses of species associations.

Comparisons with Other Surveys

In August 2014, the C-BASS was towed over MOUSS stations within hours of their placement, yet it took several passes of each station to locate the MOUSS in the C-BASS video feed. Layback and oceanographic conditions likely played a large role, and these factors were eventually overcome to ascertain the MOUSS stations in the C-BASS video. The difference in percent cover calculations between the SEAMAP stationary cameras and C-BASS were a result of two factors—methods and perspective. My methods included "encrusted rubble" as a biotic component, as it appears to be the location of sponge or algal growth. SEAMAP surveys estimate silt/sand/clay, shell/gravel, and rock cover as total substrate (must sum to 100%), and exclude these components from the measure of “attached epifauna” (which need not sum to 100%) (K. Rademacher, personal communication). This is worth noting as well because the percent of attached epifauna reported by the MOUSS analyst summed to more than 100% at station 15 (Figure 27a).

The consistent difference in algal cover could be explained as a function of the MOUSS’s bottom-seated position, which affords it a closer, clearer view of the smaller habitat components. Such components may be obscured from the C-BASS’s downward-facing position in the water column by taxa that occupies the vertical space, such as soft corals. Notes from the MOUSS analyst at these sites described *Halimeda* and “low relief algae,” which were less likely to be observed by C-BASS in areas that included dense aggregations of high-relief epifauna such as soft corals.

These factors would have been similar in a comparison of the 2003 SCUBA survey and C-BASS, if they were conducted simultaneously. At the fine perspective obtained in the 2003 SCUBA survey, the base of octocoral and sizes of sponges were measurable. In the C-BASS survey, the broad above-substrate canopy of the octocorals inflated their apparent abundance and may have obscured other features. The SCUBA surveys were recorded at 2-m intervals of ~10 m^2, keeping cameras 40 cm above the substrate within a 50-m strip transect (Coleman et al. 2004a). This methodology is comparable to C-BASS in that it was performed in a strip. The scale of the C-BASS product was nearly tenfold that of the SCUBA; however, image intervals averaged 26 m, the average area of each image was 20 m^2, and the seafloor was captured from an average distance of about 3 m. SCUBA surveys transected the same spot for 30 minutes to capture it in its entirety, while C-BASS passed over the area once at 3.4 kn.

These differences in methodology, as well as the time between the surveys, confound comparisons of the 2003 SCUBA and May 2014 C-BASS surveys. However, the comparison was made because the 2003 survey was the closest to baseline biological data available for analysis of benthic cover in this area, and it provided a guide to the taxa that may be present. The comparative approach in this study relied on several assumptions. In this comparison, I assumed that the 2014 images were captured at the same location in which the 2003 SCUBA survey took place, based on the positioning datum of the ship recorded at the time the image was collected, which I corrected for layback of the towed system. This methodology assumes that both the ship's GPS and the layback corrections for the C-BASS position were highly accurate.

To ascertain the differences between the benthic community composition of the sites in the 2003 and 2014, or to compare methodologies, a designed experiment is required. The 2003 SCUBA sites revisited in this study could be examined by combining C-BASS efforts with SCUBA or a remotely operated vehicle (ROV). Divers or ROV could collect samples to validate taxonomic identification, and provide a detailed measure of the benthic cover for comparison with simultaneous C-BASS images. For temporal examination, the site could be "marked" with an installation that serves as a visual site identifier for C-BASS in future surveys. Such a marker could also assist researchers in further validating layback calculations, as the ship's location and other oceanographic conditions would be recorded when it is observed in the C-BASS video feed aboard the ship, and the marked location recorded upon deployment.

Assuming C-BASS was accurately aligned over the 2003 SCUBA sites, and differences in percent cover between the Coleman et al. (2004a) report and this study could have been products of natural or temporal variability over the decade between the two surveys, the abundances of sponges and corals were not expected to exhibit such extensive variations from these factors because they are slow-growing and long-lived organisms. On the other hand, studies into the growth of one prevalent sponge (*Callyspongia vaginalis)* showed that not only did tube length increase by more than 10 cm/yr, but specimens at depths greater than 23 m grew two to three times as much due to increased food availability at depth (Lesser and Slattery 2013). At that rate, it is possible that the specimens observed by C-BASS were not present in the 2003 study. This species' rapid growth is an apparent trade-off because it does not produce a chemical defense and is heavily grazed by angelfishes (Pawlik 2011), which were observed by Grasty (2015) within my study area. Some of the other sponges reported by Coleman et al. (2004a), such as *Amphemidon compressa*, produce a chemical defense, and therefore may not experience the same grazing pressure.

Further investigation into these taxa could provide more insight into the decadal changes of the Florida Middle Grounds benthic communities. Anthropogenic factors could also affect the benthic composition since the Florida Middle Grounds was not designated as a HAPC and had no Federal prohibition from bottom trawling and other benthic fishing gears until the year following the SCUBA survey (2004). The high relief of the benthic features, however, was not conducive to successful trawling, and fishermen likely did not risk the time and expense of lost gear by attempting to trawl the area. These characteristics of the Florida Middle Grounds provide a natural protection which, coupled with its relatively large amount of historic information, support its utility as a baseline data reservoir, and a suitable location for rapid surveys of benthic cover using a towed camera system, allowing for spatial contrasts and well as examinations of gross changes in composition over time.

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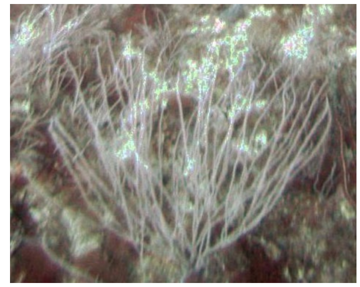
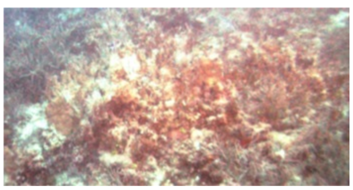
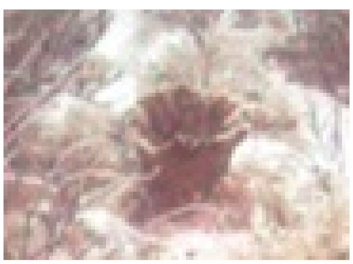
APPENDIX A:

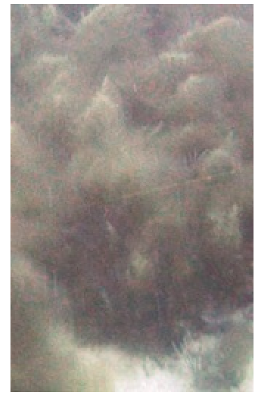
MATLAB CODE FOR MARINE IMAGE ENHANCEMENT FUNCTION RGB_CLAHE.M

```
%%% final_rgb_clahe.m
%%% Image RGB and CLAHE %%%

files=dir('*.jpg');      % Change file type '*.bmp','*.tif', as required
for file=files'

% Input image and enhance RGB
img=imread(file.name);

% Apply histogram equalization to each of the RGB components
r_img=histeq(img(:,:,1));
g_img=histeq(img(:,:,2));
b_img=histeq(img(:,:,3));

% CLAHE
clahe_r = adapthisteq(r_img,'NumTiles',[20,20],'Cliplimit',0.005,...
    'Distribution','rayleigh');
clahe_g = adapthisteq(g_img,'NumTiles',[20,20],'Cliplimit',0.005,...
    'Distribution','rayleigh');
clahe_b = adapthisteq(b_img,'NumTiles',[20,20],'Cliplimit',0.005,...
    'Distribution','rayleigh');

% Return the RGB components to a single 3 dimensional array
out_img=cat(3,clahe_r,clahe_g,clahe_b);

% Save image
out_img_name=strcat('rgb_clahe_',file.name);
imwrite(out_img,out_img_name);

end;
```

APPENDIX B:

C-BASS OBSERVATIONS IN 2014 OF SOME PROMINENT SPONGE AND CORAL TAXA AT FLORIDA MIDDLE GROUNDS 2003 SCUBA SITES

Taxa	**C-BASS Image**	**Taxa**	**C-BASS Image**
Siderastrea sp.		*Muricea sp.*	
Millepora alcicornis		Family Plexauridae	
Cribochalina vasculum		*Pseudopterogorgia sp.*	
Family Ircinidae		*Sargassum sp.*	

Taxa	C-BASS Image	Taxa	C-BASS Image
Callyspongia vaginalis		*Dictyota sp.*	
Halimeda or *Udotea*