PROCEEDINGS

JOINT INTERNATIONAL SCIENTIFIC DIVING SYMPOSIUM

AMERICAN ACADEMY OF UNDERWATER SCIENCES

and

EUROPEAN SCIENTIFIC DIVING PANEL

Michael A. Lang and **Martin D.J. Sayer**

Curaçao, October 24-27, 2013

The American Academy of Underwater Sciences (AAUS) was formed in 1977 and incorporated in the State of California in 1983 as a 501c6 non-profit corporation. Visit: www.aaus.org.

The AAUS Foundation was incorporated in the State of Alabama in 2012 as a 501.c.3 not-for-profit organization.

The mission of AAUS is to facilitate the development of safe and productive scientific divers through education, research, advocacy, and the advancement of standards for scientific diving practices, certifications, and operations.

Divers Alert Network provided major financial support.

Published by American Academy of Underwater Sciences
Dauphin Island Sea Lab, 101 Bienville Boulevard, Dauphin Island, AL 36528
www.aaus.org

Lang, M.A., and M.D.J. Sayer, editors. 2013. *Proceedings of the 2013 AAUS/ESDP Curaçao Joint International Scientific Diving Symposium*, October 24-27, 2013, Curaçao. Dauphin Island, AL: American Academy of Underwater Sciences.

ISBN XXX-X-XXXXXXX-X-X

EXPLORING THE MESOPHOTIC ZONE: DIVING OPERATIONS AND SCIENTIFIC HIGHLIGHTS OF THREE RESEARCH CRUISES ACROSS PUERTO RICO AND US VIRGIN ISLANDS

***Clark Sherman*[1*]**
***Richard Appeldoorn*[1]**
***David Ballantine*[1]**
***Ivonne Bejarano*[1]**
***Milton Carlo*[1]**
***Doug Kesling*[2]**
***Michael Nemeth*[1]**
***Francisco Pagan*[1]**
***Hector Ruiz*[1]**
***Nikolaos Schizas*[1]**
***Ernesto Weil*[1]**

[1]Department of Marine Sciences
University of Puerto Rico at Mayagüez
PO Box 9000, Mayagüez 006681 PUERTO RICO
clark.sherman@upr.edu

[2]Aquatic Training Systems, LLC
5701 Wood Duck Circle
Wilmington, NORTH CAROLINA 28409 U.S.A.

Mesophotic coral ecosystems (MCEs) occur along insular shelf margins and isolated banks at depths of ~50 to 100 m. Given their depth and typical distance from shore-based facilities, specialized diving techniques and logistical arrangements are required to conduct in situ surveys and sampling of these unique settings. From 2010 to 2012, the University of Puerto Rico at Mayagüez Caribbean Coral Reef Institute and Department of Marine Sciences conducted three research cruises in order to characterize MCEs along an east-west trending transect extending from Isla Mona eastward along the southern insular margin of Puerto Rico into the Virgin Islands to Lang Bank off the eastern end of St. Croix. Ship-based operations allowed access to remote locations as well as put a multidisciplinary team of scientists on site to aid in final dive-site selection and conduct surveys, collections and preliminary processing and assessment of data. The application of technical closed-circuit rebreather (CCR) diving made extended, self-supported research cruises possible and practical. Over the course of three cruises, over twenty different sites were surveyed. CCR dives were conducted at depths of ~ 50 to 90 m with most work occurring at depths of ~50 to 70 m. Remotely-operated vehicle (ROV) surveys complemented diving operations by exploring sites prior to CCR dives, surveying beyond CCR working depths and examining sites not surveyed by divers. These cruises help to establish protocols for safely and efficiently conducting extended, self-supported, technical-diving cruises and demonstrate the scientific utility of such operations. Important scientific results from these cruises include the ecological characterization of MCEs in Puerto Rico and US Virgin Islands, documentation of systematic patterns in geomorphology of mesophotic habitats, identification of new species, extension of species

ranges, new information on genetic connectivity between mesophotic coral populations and between mesophotic and shallow coral populations, and documentation of well-established lionfish populations at mesophotic depths.

Introduction

Compared to their shallower counterparts, reef systems deeper than ~50 m remain largely unexplored. Primarily, this has been because of the depth limits of traditional scuba techniques of ~40 m. Accordingly, much of our knowledge of reef environments, including the depth ranges of organisms and of reefs themselves, is limited to this relatively shallow range. Recently, advances in diving technology and techniques and their application to scientific research has opened a new realm for study, the mesophotic zone (water depths of ~50-100 m; Pyle, 1996; Sherman et al., 2009; Hinderstein et al., 2010). Mesophotic coral ecosystems (MCEs) are defined as light-dependent corals and associated benthic communities found at depths of ~50-100 m (Hinderstein et al., 2010). There is renewed scientific interest in these ecosystems (*ibid*.) as they often contain unique flora and fauna (Ballantine and Ruiz, 2010, 2011; Corgosinho and Schizas, 2013) and may have a potential areal extent rivaling that of shallow reefs (Locker et al., 2010). Being further removed from terrestrial and anthropogenic influences than shallower settings (e.g., Armstrong et al., 2006) MCEs may potentially serve as refugia for certain corals and other organisms during times of environmental stress and a possible source of larvae that could boost the resiliency of shallower reefs (Riegl and Piller, 2003; Lesser et al., 2009; Bongaerts et al., 2010). While the depth and typical distance from shore of MCEs may offer refugia for marine organisms, these same qualities present unique challenges to investigators; specialized diving techniques and logistical arrangements are required to conduct *in situ* surveys and sampling of these unique settings (e.g., Pyle, 1996; Sherman et al., 2009).

Technical diving has been crucial to the study of MCEs (e.g., Sherman et al., 2009; Lesser et al., 2010; Sherman et al., 2010; Smith et al., 2010). While submersibles, ROVs and AUVs have important applications (e.g., Armstrong et al., 2006; Bare et al., 2010; Rooney et al., 2010; Bridge et al., 2011; Bridge et al., 2012), they also have limitations. Submersibles are obviously costly and logistically complex, placing limits on study locations and the frequency that sites can be visited. ROVs and AUVs offer greatly reduced cost and increased flexibility versus submersibles but can be limited by on-site conditions such as sea state, currents and bottom topography (Singh et al., 2004). Additionally, in submersible-, ROV- and AUV-based research, the observer is removed from the surrounding environment making detailed observations and sampling difficult. Only diver-based research has the capability and flexibility to make detailed, high-resolution observations (including photography) and conduct carefully targeted collections. Mixed-gas, closed-circuit rebreathers (CCRs) provide scientific divers unparalleled access to mesophotic habitats. CCRs provide significant advantages over open-circuit diving with respect to gas use, flexibility and equipment needs (Sherman et al., 2009).

In the fall of 2007, scientists from the University of Puerto Rico at Mayagüez (UPRM) Caribbean Coral Reef Institute (CCRI) and Department of Marine Sciences (DMS) began an intensive study of the MCEs off of La Parguera, southwest Puerto Rico. Using a combination of technical rebreather diving (cf. Sherman et al., 2009) and ROV observations, this study was focused primarily on characterizing mesophotic communities, understanding their distribution with respect to depth and geomorphology, and determining the extent of connectivity with shallow reef systems. This work has resulted in a thorough characterization of MCEs off of La Parguera and the development of conceptual models of the physical and biological factors controlling their makeup and distribution. From 2010 to 2012, UPRM-CCRI-DMS conducted three research cruises to expand these studies over a broader geographic range within Puerto Rico and US Virgin Islands. The cruises took place from 9-23 January 2010, 15 April – 5 May 2011 and 24 April – 10 May 2012. General objectives of the three cruises were to 1) further characterize MCEs over a broader geographic range and in a variety of geomorphic and oceanographic settings; 2) collect data to test and refine conceptual models describing the character and distribution of MCEs; and, 3) determine the horizontal genetic connectivity between mesophotic coral populations and vertical connectivity between mesophotic and shallow coral populations. This paper seeks to: 1) provide a descriptive summary of cruise diving operations that can help to establish protocols for safely and efficiently conducting extended, self-supported, technical-diving cruises; 2) demonstrate the scientific utility of such operations; and, 3) provide a brief summary of important scientific results and accomplishments.

Study Area

The islands of Puerto Rico and the US Virgin Islands lie along the northeastern margin of the Caribbean between approximately 64° 33’ and 67° 57’ W longitude and 17° 40’ and 18 °31’ N latitude. It is a tectonically active region with generally narrow insular shelves and steep margins that plunge to oceanic depths (Hubbard et al., 2008). The main islands of Puerto Rico and the northern Virgin Islands are connected by the Puerto Rico-Virgin Islands (PR-VI) Platform, while St. Croix and Isla Mona are separated from the PR-VI Platform by deep basins. Dive sites extend along an east-west trending transect from Isla Mona eastward along the southern insular margin of Puerto Rico into the Virgin Islands to Lang Bank off the eastern end of St. Croix (Fig. 1).

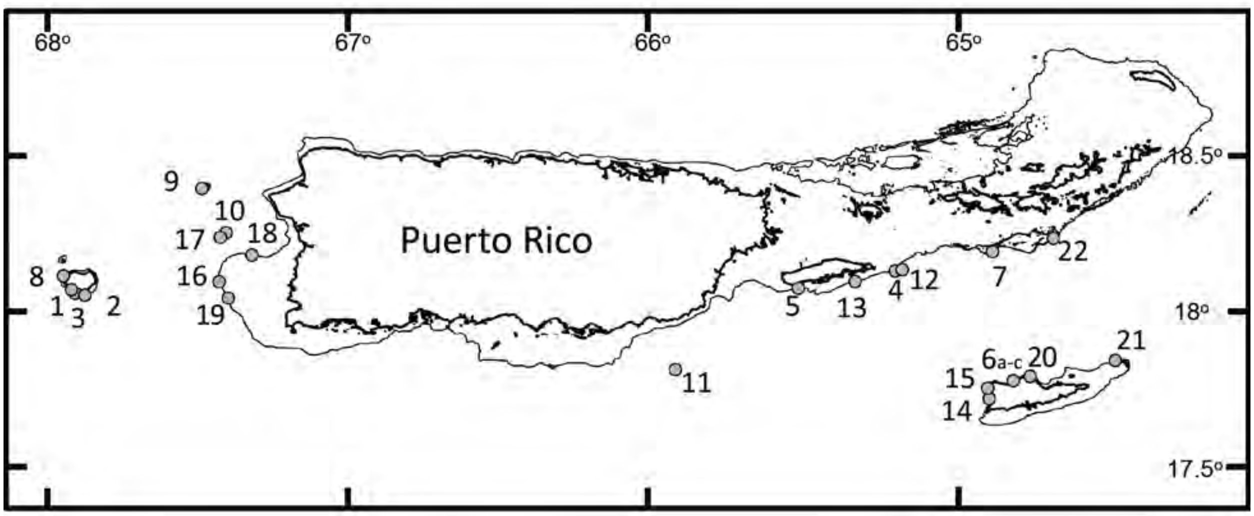

Figure 1. Map showing locations of dive sites for the 2010-2012 UPRM-DMS research cruises. Numbers correspond to site locations listed in Table 1.

Dive sites were chosen to cover a broad geographic range across Puerto Rico and the US Virgin Islands. They were also selected to cover a broad range of geomorphic and oceanographic settings with regard to factors such as slope gradient, bottom topography and degree of exposure to prevailing seas. Preference was given to locales where NOAA multibeam bathymetry was available as this aided in site selection and dive planning and allowed for a more quantitative and systematic assessment of the physical characteristics of each site.

Methods

Cruise platforms

Each cruise lasted from 2 to 3 weeks and consisted of 14 to 18 participants in the scientific party, including divers. Appropriate vessels had to have the capability of accommodating groups of this size, supporting technical diving operations and operating self-sufficiently for at least a week at a time. There were opportunities for resupply between successive (week-long) legs of each cruise. Private live-aboard dive charters were contracted for each cruise as they offered the best combination of cost and flexibility. For the first cruise in 2010 a 24 m by 12 m SWATH (Small Waterplane Area Twin Hull)/catamaran hybrid was chartered and made an exceptional cruise platform. The twin-hull design resulted in a smooth ride and copious space for the scientific party and equipment. A dive platform along the stern of the vessel that could be raised and lowered facilitated recovery of technical divers laden with gear. Large deck space, both covered and uncovered, provided an area for initial processing of samples. The stability of the vessel and large dining area also allowed for setup of a temporary "lab" with computers and a microscope for additional description, photography and cataloging of samples and data. Unfortunately, this same vessel was not available for the later cruises. For the 2011 and 2012 cruises, a 30-m aluminum crew boat refit for open-ocean diving was chartered and made another exceptional cruise platform. It had several large dive benches on the stern deck that could accommodate technical divers, along with boarding ladders and a large swim platform that facilitated recovery of the divers.

Safety considerations

The combination of technical diving and remote locations required that extra steps be taken to ensure the safety of the divers and all participants. This included having the appropriate equipment and personnel on hand to respond to emergency situations as outside assistance would not be readily available. To help achieve this, personnel from the University of North Carolina at Wilmington (UNCW), Advanced Diving Technology Program (ADTP) were brought in to oversee all scientific diving operations and provide first responder support in the event of a diving emergency at sea. Members of the UNCW-ADTP team were fully trained and certified in CCR operations and able to participate actively as members of the scientific dive team. UNCW-ADTP staff also served as the topside dive supervisors when not participating in the dive rotation. They were specifically trained in conducting extended shipboard operations and oversaw all the day-to-day scientific diving operations. Each assigned UNCW-ADTP staff member was also certified as a Diver Medical Technician (DMT-A) with the National Board of Diving and Hyperbaric Technology. They also held certifications as *Hyperlite*™-Hyperbaric Stretcher operators.

Because of the remote nature of these diving operations, an emergency assistance plan (EAP) was developed and posted that outlined the accident management plan including the location of the nearest hospital(s) and hyperbaric chamber(s) for each dive site. Additionally, because the first two cruises (2010 and 2011) were partially supported by UNCW-ADTP through NOAA's Cooperative Institute for Ocean Exploration, Research and Technology, an SOS *Hyperlite*™-Hyperbaric Stretcher for field use was allocated. The SOS *Hyperlite*™ is a portable pressure vessel (or hyperbaric chamber), that provides immediate treatment for different medical conditions by supplying 100% oxygen to the patient at above atmospheric pressures. This on-site chamber allowed for complete self-sufficiency of the dive team in the event that a diving emergency occurred while at sea. Emergency response would be immediate because of the proximity of this portable chamber system. The SOS *Hyperlite*™ and UNCW personnel were not available for the third cruise (2012) because of the unfortunate termination of the UNCW Advanced Diving Technology Program. For the 2012 cruise, appropriate personnel were privately contracted to serve as dive supervisors and first responders.

Diving operations

Diving operations were based on protocols established at our shore-based facility, the Magueyes Island Marine Laboratories in La Parguera, Puerto Rico (cf. Sherman et al., 2009). The technical CCR dive team consisted typically of 5 divers. Divers used Ambient Pressure Diving Ltd./Silent Diving Systems LLC *Inspiration* closed-circuit rebreathers with *Vision Electronics*. Most dives were made to depths of ~50 to 70 m, with a few dives to just over 90 m (Table 1).

Table 1. Generalized dive log of three UPRM-DMS mesophotic research cruises (2010-2012).

Site #	Date	Site Name	Coordinates (Latitude°) (Longitude°)	Depth (m)	Accomplishments
2010 UPRM-DMS Mesophotic cruise					
Leg 1 - Mona					
1	10 Jan 2010	SW Mona	18.05148 -67.90917	71	• Geomorphic characterization • Fish survey • Sample collection: o Corals, algae, lithic substrates
1	11 Jan 2010	SW Mona	18.05148 -67.90917	71	• Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lionfish
2	12 Jan 2010	SE Mona	18.04553 -67.87821	62	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
2	13 Jan 2010	SE Mona	18.04553 -67.87821	75	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, black coral, lithic substrates
3	14 Jan 2010	Carabinero, Mona	18.06250 -67.92223	68	• Geomorphic characterization • Fish survey • Sample collection: o Corals, algae, lithic substrates
3	15 Jan 2010	Carabinero, Mona	18.06250 -67.92223	53	• Benthic photo transect • Fish survey • Sample collection: o Corals
Leg 2 – Vieques and USVI					
4	17 Jan 2010	El Seco, Vieques (west)	18.12357 -65.20160	71	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Lithic substrates, algae
5	18 Jan 2010	SW Vieques	18.07065 -65.52224	71	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, lithic substrates
6a	19 Jan 2010	Cane Bay, St. Croix	17.77327 -64.81383	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Algae, lithic substrates
6a	20 Jan 2010	Cane Bay, St. Croix	17.77327 -64.81383	55	• Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates
6a	20 Jan 2010	Cane Bay, St. Croix	17.77327 -64.81383	70	• Sample collection: o Sclerosponges
7	21 Jan 2010	Grammanik Bank	18.18200 -64.87860	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates

2011 UPRM-DMS Mesophotic Cruise

Leg 1 – West Puerto Rico

8	16 Apr 2011	NW Mona	18.10627 -67.94888	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, sponges, lithic substrates
8	17 Apr 2011	NW Mona	18.10627 -67.94888	50	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
9	18 Apr 2011	W Desecheo	18.38588 -67.49560	73	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates
9	19 Apr 2011	W Desecheo	18.38588 -67.49560	57	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates
10	20 Apr 2011	Bajo de Sico (east)	18.24491 -67.41272	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates
10	21 Apr 2011	Bajo de Sico (east)	18.23105 -67.42367	51	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, lithic substrates

Leg 2 – Grappler, Vieques and USVI

11	23 Apr 2011	Grappler Bank	17.81460 -65.92705	71	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
11	24 Apr 2011	Grappler Bank	17.79458 -65.90825	65	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
12	25 Apr 2011	El Seco, Vieques (east)	18.12723 -65.17777	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, algae, sponges, lithic substrates
13	26 Apr 2011	SE Vieques	18.09117 -65.33367	55	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, sponges, lithic substrates
14	27 Apr 2011	W St. Croix (Sub Mooring)	17.71743 -64.89382	81	• Sample collection: o Sclerosponges
14	27 Apr 2011	W St. Croix (Sub Mooring)	17.71743 -64.89382	57	• Fish survey • Coral colony characterization • Sample collection: o Corals
15	28 Apr 2011	W St. Croix (Armageddon)	17.75062 -64.8978	52	• Geomorphic characterization • Benthic photo transect • Fish survey

					• Sample collection: o Corals, sponges, lithic substrates
6a	30 Apr 2011	Cane Bay, St. Croix	17.77398 -64.81403	83	• Sample collection: o Sclerosponges
6a	30 Apr 2011	Cane Bay, St. Croix	17.77398 -64.81403	60	• Fish survey • Sample collection: o Corals, sponges, lithic substrates
6b	1 May 2011	North Star, St. Croix	17.76985 -64.82173	52	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, black corals, lithic substrates
6b	2 May 2011	North Star, St. Croix	17.76985 -64.82173	62	• Fish survey • Sample collection: o Corals, sclerosponges, lithic substrates
6a	3 May 2011	Cane Bay, St. Croix	17.77398 -64.81403	55	• Sample collection: o Corals

2012 UPRM-DMS Mesophotic Cruise

Leg 1 – West Puerto Rico

16	25 Apr 2012	Abrir la Sierra, PR	18.09083 -67.43467	52	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
16	26 Apr 2012	Abrir la Sierra, PR	18.76197 -67.15696	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
17	27 Apr 2012	Bajo de Sico (west)	18.23075 -67.43177	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
17	28 Apr 2012	Bajo de Sico (west)	18.23075 -67.43177	52	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
18	29 Apr 2012	Tourmaline, PR	18.17530 -67.32730	54	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Sponges, octocorals, lithic substrates
19	30 Apr 2012	N of Buoy 4, PR	18.03939 -67.40445	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, sponges, lithic substrates

Leg 2 – USVI

20	4 May 2012	Salt River Canyon, St. Croix	17.78689 -64.75856	70	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, sponges, lithic substrates
20	5 May 2012	Salt River Canyon, St. Croix	17.78689 -64.75856	54	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, black corals, lithic substrates
21	6 May 2012	Lang Bank, St. Croix	17.83421 -64.47584	55	• Geomorphic characterization

					• Benthic photo transect • Fish survey • Sample collection: o Corals, black corals, lithic substrates
6c	7 May 2012	Davis Bay, St. Croix	17.76600 -64.83100	92	• Geomorphic characterization • Fish survey • Sample collection: o Sclerosponges, black corals, octocorals, sponges, lithic substrates
22	8 May 2012	E St. John	18.22186 -64.67596	54	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates
22	9 May 2012	E St. John	18.22389 -64.66849	71	• Geomorphic characterization • Benthic photo transect • Fish survey • Sample collection: o Corals, lithic substrates

Equipment setups were centered on two-person buddy teams, with each team carrying enough open-circuit (OC) bailout gas to safely bring one diver to the surface in the event of CCR failure. Given the high reliability of the equipment being used and the built-in redundancy within the CCR unit (e.g., dual oxygen controllers, dual displays, dual batteries, etc.), it was thought to be highly unlikely that both CCRs on a buddy team would completely fail on a given dive. Both members of a buddy team carried OC bottom bailout mix that matched the diluent being used in the rebreather. In addition to the bottom mix, one member of the team carried EAN 32% deco mix, while the other member of the team carried 100% O_2. In the event of a three-person buddy team an additional EAN 32% deco mix was carried by the third member of the team. Based on calculations, the EAN 32% deco mix was the gas needed in highest volume in case of a bailout. Two standard mixes for diluent/OC bailout were used depending on the planned dive. For dives from 49 m to 61 m (160 ft to 200 ft) an 18/30 trimix was used (i.e., 18% O_2/30% He/52% N_2). This mixture has a maximum operating depth (MOD) of 68 m (224 ft) at a PO_2 of 1.4 atmospheres absolute (ATA). At 61 m (200 ft) and a rebreather PO_2 setpoint of 1.3 ATA, the mixture provides an equivalent air depth (EAD) of 37 m (120 ft). For dives from 61 m to 91 m (200 ft to 300 ft), a 12/50 trimix was used, which has a MOD of 107 m (352 ft) at 1.4 ATA PO_2 and an EAD of 38 m (126 ft) at a rebreather setpoint of 1.3 ATA PO_2. Divers used two decompression computers, the integrated *Inspiration* computer with a peak PO_2 setpoint of 1.3 ATA and high and low gradient factors set at 85 and 15, respectively and a *VR3* set at a constant PO_2 of 1.25 ATA using the Bühlmann decompression algorithm and a safety factor of 10. At these settings the *VR3* was the more conservative of the two decompression computers and would always clear after the *Inspiration* computer. Divers always cleared both computers before exiting the water.

Both cruise platforms were well-equipped liveaboard dive vessels with their own compressors and were able to provide EAN 32% for the duration of the cruises. All other breathing gases, e.g., oxygen and trimix, needed to be brought onboard. For each approximately two-week cruise, the following complement of diving gases was carried onboard: three (3) Type-H 337 ft^3 cylinders of diving-grade oxygen, two (2) HC4500 434 ft^3 cylinders of premixed 18/30 trimix, and two (2) HC4500 434 ft^3 cylinders of premixed 12/50 trimix. This was more than enough gas to top off the CCR onboard cylinders after each day of diving for a CCR team of 5-6 divers. Premix diving gases were prepared at the technical dive facilities of the DMS Magueyes Island Marine Laboratories. During the cruises, CCR onboard cylinders were topped off from the storage cylinders with the aid of a portable pneumatic booster pump. For the most part, OC bailout gases were never used and only needed occasional topping off caused by minor incidental loss of gas during dive operations. The OC EAN 32% was regularly used to fill lift bags but could be easily topped off using the ship's onboard compressor.

All dives were conducted as live-boat drift dives. Most dives were made to depths of approximately 50 or 70 m with a planned bottom time of 20 minutes. Representative dive profiles are shown in Figure 2.

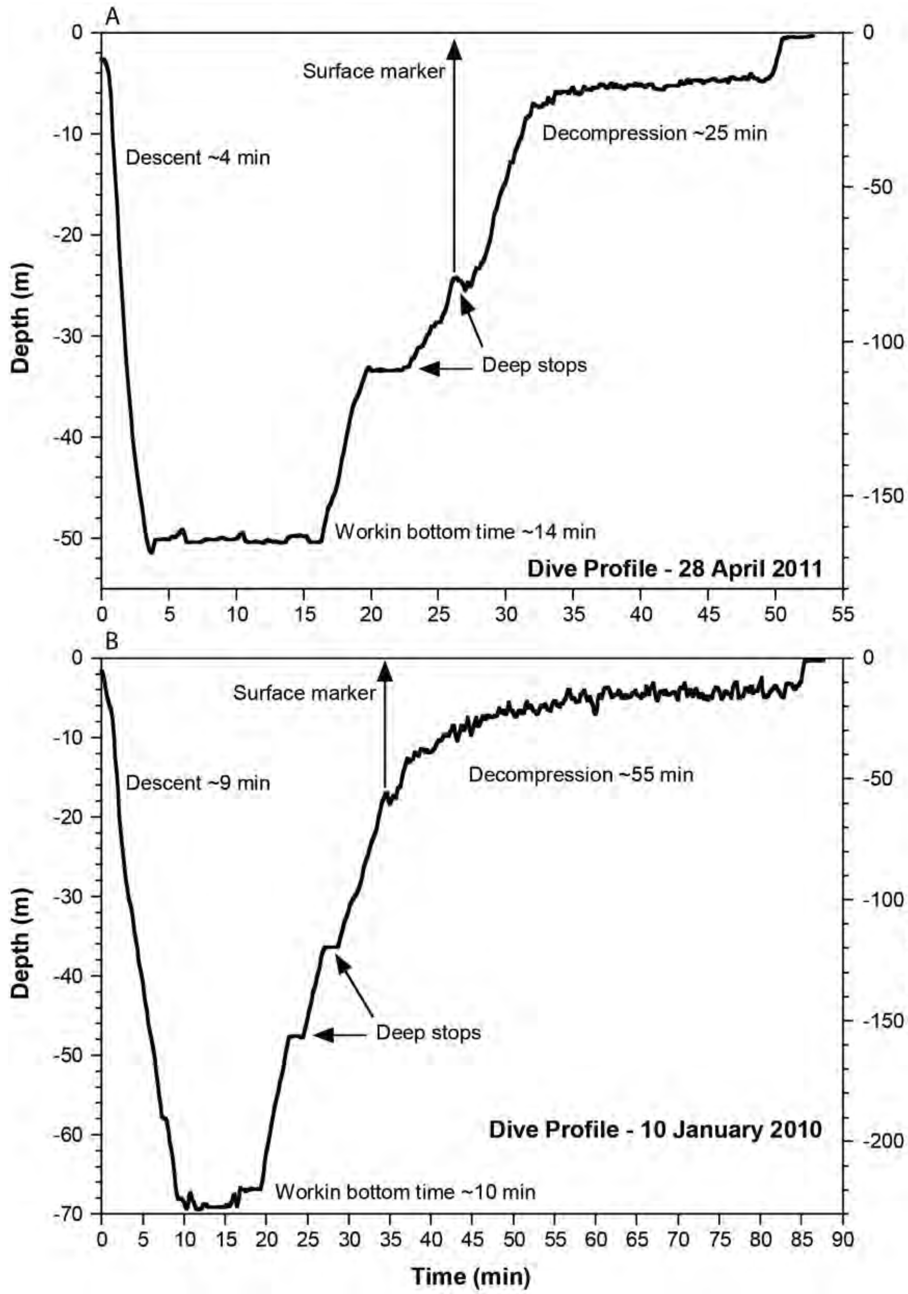

Figure 2. Typical CCR dive profiles for a dive to 52 m (A) and 71 m (B).

Divers were dropped at a suitable location above the target site to minimize descent times to the target depth. This typically entailed dropping in at shelf/slope breaks and descending down steep insular slopes to the target depth. Descents generally took from 5-10 minutes leaving approximately 10-15 minutes of working bottom time before beginning the ascent at a run time of ~20 minutes. During the ascent, two two-minute deep stops were conducted according to the *VR3* dive computer. At an appropriate time during the ascent, typically at a run time of ~25-30 minutes, a surface marker was deployed to alert topside support of the divers' location. Divers would then drift along with the surface marker and complete the remaining decompression. Upon surfacing, divers were recovered by the cruise vessel. Total run times were ~60 minutes for a dive to 50 m and 90 minutes for a dive to 70 m. One decompression dive was conducted each day for up to six consecutive days followed by one offgassing day. Following deep CCR dives, the remainder of each day was used for ROV operations and, if possible, shallow-water dives (<30 m water depth) by a second team of divers.

Sample and data collection

Each member of the CCR dive team had a specific task on each dive. These tasks included geomorphic characterization of the site, photo documentation (including general photography and benthic transect), fish surveys, sampling (live benthic cover and lithic substrates) and in-water divemaster. The geomorphic characterization took place typically during the descent and involved a general description of site topography and nature of the seafloor and benthic cover. Upon reaching the target depth, this same diver would assist the photographer in completing a continuous high-resolution benthic photo-transect ~10 m long by 40 cm wide (cf. Sherman et al., 2009; Sherman et al., 2010). Individual photographs from the transects cover an area of ~40 by 60 cm and were analyzed using CPCe point-counting software to determine the relative abundances of major groups of benthos and substrate types (Kohler and Gill, 2006). Fish surveys were patterned after the techniques of Brock (1954). Fish were identified and counted for ~15 minutes along 10 m long by 3 m wide (30 m^2) belt transects. In addition, in order to quantify target species observed within MCEs but outside of the transects, large predators and important (commercially or ecologically) fish species observed during each dive were recorded (cf. Bejarano, 2013). Divers also made careful and targeted collections of lithic substrates and benthic macrofauna, including corals, algae and sclerosponges, for more detailed studies (e.g., genetics and morphometrics) and positive species identifications in the laboratory. Once samples were collected, they were sent to the surface with a lift bag to be recovered by the cruise vessel. This allowed sample processing and cataloging by topside scientists to begin immediately while the divers completed decompression. The in-water divemaster observed the rest of the team and was able to act in the event of any difficulties and lend assistance with science objectives when needed.

ROV operations

Additional surveys were conducted using a Seabotix LBV200 remotely operated vehicle (ROV) rated to 200 m and equipped with color video camera (570 lines), LED lights, parallel lasers for size estimation, a sampling arm and tracking unit. The purpose of these surveys was to characterize mesophotic communities and geomorphology beyond typical depths reached by the CCR divers and over the full depth range of MCEs down to 100+ m, taking advantage of the greater bottom time available. ROV dives were scheduled to last at least 1 hr. All ROV dives were scheduled after deep CCR diving activities for the day were completed. Video documentation of key components of the ecosystem, especially corals, provided additional information on species composition, size and depth distribution. All video was recorded to digital tape for permanent archiving. During each dive a running log was also kept of key observations (e.g., species, depths, geomorphology), based on expert observers. Matched with the video recordings, these key observations could be quickly referenced and reviewed as necessary. While the sampling capability of the ROV was limited to only one specimen per dive, even this could be significant when the divers were time-limited and tasked with multiple objectives. Weather permitting, ROV dives were made at each location sampled by divers. In addition, five ROV dives were made at sites not visited by divers: (2010) Elbow – Isla Mona PR, Buck Island St. Croix, (2012) Tourmaline PR, eastern Lang Bank St. Croix, and western Grammanik Bank, St. Thomas.

Results

Diving statistics and accomplishments

Over the course of three cruises 24 different sites were surveyed along an east-west trending transect that stretches ~370 km from Isla Mona to Lang Bank, St. Croix (Fig. 1). A total of 42 decompression dives were made to depths ranging from 50 to 92 m, with most dives made to ~50 and 70 m (Tables 1, 2).

Table 2. Summary dive statistics for three UPRM-DMS mesophotic research cruises (2010-2012).

		Cruise I-2010	Cruise II-2011	Cruise III-2012	Total
Number of Sites		7	10	8	24*
Dive Days		11	16	12	39
No. of Team Dives#	Depth 50-59 m	2	8	6	16
	60-69 m	2	3		5
	70-79 m	8	5	5	18
	80-89 m		2		2
	90-99 m			1	1
Total Number of Team Dives#		12	18	12	42

* Does not include sites revisited on multiple cruises.
\# Teams consisted of two to six divers.

Dive totals reflect the number of team dives (not individual dives), with teams consisting of two to six CCR divers. In most cases, these surveys represent the first *in situ* characterization of mesophotic habitats (>50 m water depth) at these sites. To our knowledge, the dives on Grappler Bank were the first documented dives on this isolated bank south of Puerto Rico. A total of 32 continuous, high-resolution, 10-m long, benthic photo-transects were completed, with most done at depths of ~50 and 70 m. An attempt was made to conduct transects at depths of 50 and 70 m at each site. However, topography and conditions at some sites did not allow for this. Transects were analyzed to identify species present and determine the relative abundances of major groups of benthos and substrate types. Numerous specimens of stony corals (primarily *Agaricia* sp. and *Montastrea cavernosa*), macroalgae, sponges and antipatharians were collected and are currently being described, cataloged and further analyzed for genetic and morphometric studies. Sclerosponges (*Ceratoporella nicholsoni*) were collected from depths of ~70-90 m. These will serve as important paleoceanographic archives of factors such as water temperature and water column structure over centennial time scales.

ROV accomplishments

The use of the ROV provided important information both from sites visited and not visited by the CCR divers. Examples of the former include establishing the depth limits of corals and other benthic macrofauna beyond the range of the divers and the observation of unique features, such as a zone of diverse coral development located at 80–90 m at eastern Grammanik Bank, St. Thomas. At slightly shallower depths (70 m) visited by divers live coral cover was sparse. A pre-CCR dive survey off Sub Mooring, western St. Croix discovered one of the largest coral colonies (*Agaricia undata*) observed at a depth of 57 m. This same colony was subsequently documented and sampled by divers to assess its genetic composition. Notable observations made at sites not visited by divers include (1) the broad plain off Tourmaline, western Puerto Rico, which contained adult queen conch down to at least 59 m and a small outcrop at 73 m that supported over 100 lionfish, (2) the use of ledges and ghost traps between 60 – 70 m as key nursery habitat for blackfin snappers on the otherwise featureless sandy slope off Buck Island, St. Croix, and (3) the spectacular extent off western Grammanik Bank characterized by 100% live cover of *A. undata*.

Scientific highlights

Important scientific results from these cruises include the ecological characterization of MCEs in Puerto Rico and US Virgin Islands over a broad geographic range and over a broad range of geomorphic and oceanographic settings. These surveys are allowing for further documentation of systematic patterns in the occurrence and distribution of MCEs related to geomorphology and oceanographic factors. Well-developed MCEs are shown to be distributed patchily and dominated by agariciid corals. Surveys have expanded the number of scleractinian species for Puerto Rico, with *Leptoseris cailleti* and *Agaricia undata* as new reports for the area. Collections are resulting in the identification of new species of algae and invertebrate meiofauna (Pesic et al., 2012; Petrescu et al., 2013) as well as new information on genetic connectivity between mesophotic coral populations and between mesophotic and shallow coral populations. Paleoceanographic sea-surface temperature records have been generated from collected sclerosponges that provide important information on factors controlling regional climate variation over the last 500 years (Estrella Martinez, 2013). Both diver and ROV surveys have documented well-established lionfish populations at mesophotic depths.

Discussion

Scientific utility of cruises to study MCEs

Extended, self-supported diving cruises provide numerous benefits for the study of mesophotic habitats. Extended cruises provide unparalleled access to remote and varied settings that would be difficult or impossible to reach via day-use shore-based facilities. The mobile cruise platform also facilitates visiting multiple sites over a broad area thereby simplifying logistics for regional studies relative to shore-based work. Cruises place a multidisciplinary team of scientists on-site that can help with final site selections, conduct surveys and sampling, and provide immediate insight on and assessment of data and sample collections. This allows for significant processing and interpretation of data to be completed over the course of the cruise. Field operations are necessarily compressed during a cruise resulting in an efficient use of time and resources. The visiting of multiple sites within a short time also facilitates site comparisons. Technical CCR diving requires considerable equipment, including both in-water dive gear and topside support equipment (e.g., custom-mixed breathing gases, CO_2 absorbent, replacement parts, etc.). A cruise vessel can easily transport equipment and personnel from site to site. It would not be practical to transport this equipment and personnel from one shore facility to another, especially in an island setting. The extent of diving and number of sites surveyed on the UPRM-DMS mesophotic cruises would not have been accomplished from shore-based facilities. Samples collected on the cruises to date have formed the basis of one PhD dissertation and two MS theses. The copious data and samples collected over the cruises will continue to generate numerous studies for some time to come.

Value of CCR diving

Previous work has demonstrated the value of technical diving in the study of MCEs (e.g., Sherman et al., 2009; Lesser, 2010; Sherman et al., 2010; Smith et al., 2010) as well as the advantages of CCR diving over open-circuit (OC) techniques (Sherman et al., 2009). The UPRM-DMS research cruises further demonstrate the value and advantages of CCR diving in the study of MCEs (Fig. 3).

The UPRM-DMS CCR divers were able to make detailed *in situ* observations of mesophotic habitats, collect high-resolution photographs and make precise and targeted collections of biological and geological samples. This level of precision and detail is not possible using remote techniques. The advantage of CCR versus OC techniques is especially apparent on extended cruises. On a given dive, a CCR diver uses about an order of magnitude less gas than an OC diver. After a day’s diving during the cruises, the 3 L CCR diluent and oxygen cylinders could be easily topped off from the bank of storage cylinders brought along for the cruise (see above). OC bailout cylinders only needed occasional topping off caused by incidental loss of gas during diving operations. If these cruises were done using OC techniques, more than ten times the amount of breathing gas would have been required to support the diving. This would have greatly inflated the cost of the cruises and may have required larger cruise platforms to accommodate storage of the additional breathing gas. CCR also offers more flexibility than OC diving. During the cruises, the CCR divers switched between two standard breathing mixtures depending on the planned depth of a dive. This was accomplished by each diver having two sets of cylinders, one for each gas mixture. Each cylinder set consisted of a CCR diluent cylinder and corresponding OC bailout cylinder. The CCR diver simply used the appropriate cylinder set/breathing mixture for the planned dive. Making similar changes using OC setups is much more involved and costly and would require either having multiple double-tank setups for each diver, or having to empty and refill a set of double cylinders each time a different breathing mixture is used. Either option would result in increased equipment needs, increased costs and increased setup time. CCR equipment is less cumbersome making both entry into the water and, more importantly, reboarding the cruise vessel following a dive much easier. CCR techniques offer distinct scientific advantages as well. Because bubbles are not continuously released by the CCRs, marine life behavior (e.g., fish, marine turtles, etc.) in the presence of divers is more natural and surveys can provide more accurate information on the composition and behavior of the community.

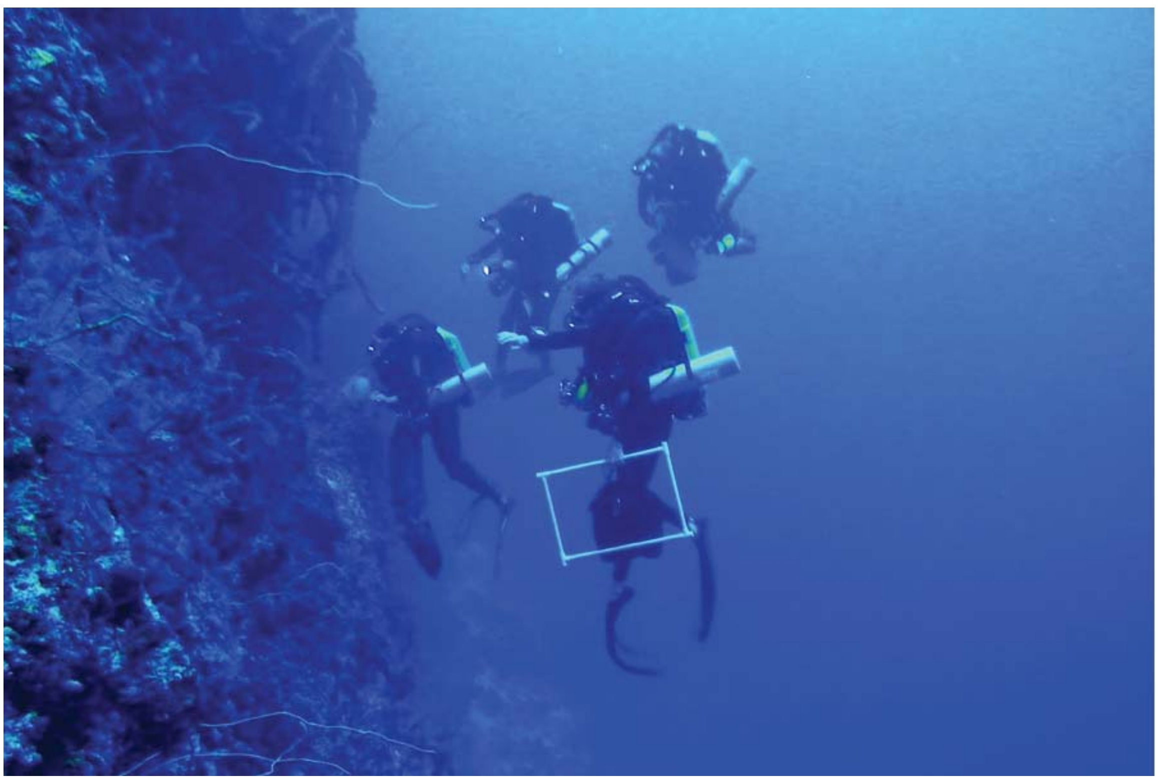

Figure 3. UPRM-DMS CCR divers explore a steep wall off Isla Mona, Puerto Rico.

Difficulties encountered

As with all marine field operations, weather and sea conditions represented the greatest obstacle to completing the planned operations and frequently dictated final site selection and scheduling. As a whole, the majority of sites are located along the more sheltered southern and western margin of the PR-VI Platform. The northern margin of the Platform remains to be explored. ROV operations were especially limited by weather and sea conditions. Strong winds prevented the ship from holding position over the ROV, while strong currents drove both the ship and the ROV too rapidly over the bottom to conduct operations. In moderate conditions, the ROV was deployed with the cable secured to a weighted line at depth to reduce the effects of cable drag. Use of the ROV in high seas was avoided to reduce the chance of impact on the hull of the ship during deployment and recovery. CCR divers were able to operate over a broader range of sea conditions than the ROV.

Mandatory off days were planned into the cruise schedule for divers to relax and reenergize between each subsequent at-sea research legs. Successive deep, decompression dives whether on open-circuit or closed-circuit apparatus is physically demanding and mentally taxing. The mandatory day off allowed divers to rest and be better prepared for the next series of research dives.

There are inherent risks in all types of diving. Deep, decompression diving compounds these risks. Even when diligently following conservative and well-established decompression dive profiles, accidents can occur. During the 2010 cruise, a UPRM CCR diver suffered a mild case of decompression illness (DCI) following a dive to a depth of 71 m with a bottom time of 20 minutes and a total run time of 99 minutes. The dive was uneventful, with a normal ascent. The diver reported severe right shoulder pain immediately upon surfacing and was placed on 100% oxygen for 30 minutes. Vital signs were stable and a complete neurological exam was conducted with negative findings. During the course of the 100% surface-oxygen breathing period, the patient reported his symptoms improving. The decision was then made by the Lead DMT to initiate treatment in the *Hyperlite,* Hyperbaric Stretcher for a USN Treatment Table 6 (USN TT6). The patient received 100 % oxygen therapy with intermittent air breaks throughout the treatment. When the chamber was returned to ambient atmospheric pressure and the patient removed, he was examined and the Lead DMT felt that the recompression treatment was successful and the diver continued to remain asymptomatic, post-treatment. No further treatment was warranted at that time. Working diagnosis was a case of undeserved - Decompression Sickness Type I, with full symptom resolution with recompression therapy. Diving activity was suspended for this individual for the remainder of the cruise. He was instructed to follow-up with a diving physician following the cruise for a thorough examination and determination of return to diving status. The cruise was able to continue on its original at-sea operational schedule.

This incident highlights several important points. Even with the utmost planning and precautions, accidents can occur. Thus, it is critical to have appropriate personnel and equipment on hand to deal with a variety of situations. If the DMTs and *Hyperlite* had not been onboard, this situation would have been very different. The diver would not have been able to receive immediate treatment, significantly increasing his discomfort level and jeopardizing full and timely recovery. He would have required immediate transport to the nearest hyperbaric facility (in this case San Juan, PR), which would have been logistically complex and would have jeopardized the continuation of the cruise. It is, therefore, crucial for those planning extended or remote diving operations and for the agencies supporting these operations to recognize the critical need of having appropriate equipment and personnel to deal with emergency situations.

Conclusions

1. Over the course of three UPRM research cruises from 2010-2012, 24 sites were surveyed along an east-west trending transect stretching ~370 km from Isla Mona, PR to Lang Bank, St. Croix.
2. A total of 42 decompression dives were made to depths ranging from 50 to 92 m, with most dives made to ~50 and 70 m.
3. In many cases, these represent the first *in situ* observations and samplings of mesophotic habitats (>50 m water depth) at these sites.
4. Scientific accomplishments include the ecological characterization of MCEs in Puerto Rico and US Virgin Islands across a broad geographic range, documentation of systematic patterns in geomorphology of mesophotic habitats, identification of new species, broadened geographical and depth range information for several species, new information on genetic connectivity between

mesophotic coral populations and between mesophotic and shallow coral populations and documentation of well-established lionfish populations at mesophotic depths.

Acknowledgements

These research cruises were made possible through the following NOAA grants to the Caribbean Coral Reef Institute, UPRM: NA06NOS4780190, NA10NOS4260223, NA11NOS4260157, NA11NOS4260184. Additional support for UNCW-ADTP personnel came from the NOAA Cooperative Institute for Ocean Exploration, Research and Technology. We are especially grateful to Thor Dunmire, Scott Fowler and Michael Winfield of UNCW-ADTP.

References

Armstrong, R.A., H. Singh, J. Torres, R.S. Nemeth, A. Can, C. Roman, R. Eustice, L. Riggs, and G. Garcia-Moliner. 2006. Characterizing the deep insular shelf coral reef habitat of the Hind Bank marine conservation district (US Virgin Islands) using the Seabed autonomous underwater vehicle. *Continental Shelf Research*, **26:** 194-205.

Ballantine, D.L., and H. Ruiz. 2010. Two new deepwater *Peyssonnelia* species, *Peyssonnelia iridescens* and *Peyssonnelia gigaspora* (Peyssonneliaceae, Rhodophyta) from Puerto Rico, Caribbean Sea. *Phycologia*, **49:** 537-544.

Ballantine, D.L., and H. Ruiz. 2011. A new encrusting deep-water coral reef alga, *Peyssonnelia incomposita* (Peyssonneliaceae, Rhodophyta), from Puerto Rico, Caribbean Sea. *Cryptogamie, Algologie***, 32:** 19-26.

Bare, A., K. Grimshaw, J. Rooney, M. Sabater, D. Fenner, and B. Carroll. 2010. Mesophotic communities of the insular shelf at Tutuila, American Samoa. *Coral Reefs*, **29:** 369-377.

Bejarano, I. 2013. Deep Reef Fishes off La Parguera Insular Slope, Puerto Rico, and their Connectivity with Shallow Reefs, PhD Dissertation, Department of Marine Sciences. Mayaguez, PR: University of Puerto Rico.

Bongaerts, P., T. Ridgway, E. Sampayo, and O. Hoegh-Guldberg. 2010. Assessing the 'deep reef refugia' hypothesis: focus on Caribbean reefs. *Coral Reefs*, **29:** 309-327.

Bridge, T., T. Done, R. Beaman, A. Friedman, S. Williams, O. Pizarro, and J. Webster. 2011. Topography, substratum and benthic macrofaunal relationships on a tropical mesophotic shelf margin, central Great Barrier Reef, Australia. *Coral Reefs*, 30: 143-153.

Bridge, T., K. Fabricius, P. Bongaerts, C. Wallace, P. Muir, T. Done, and J. Webster. 2012. Diversity of Scleractinia and Octocorallia in the mesophotic zone of the Great Barrier Reef, Australia. *Coral Reefs*, **31:** 179-189.

Brock, V.E. 1954. A preliminary report on a method of estimating reef fish populations. *Journal of Wildlife Management*, **18:** 297-308.

Corgosinho, P.H.C., and N.V. Schizas. 2013. *Archeolourinia shermani*, a new genus and species of Louriniidae (Copepoda: Harpacticoida) from a Caribbean mesophotic zone. *Journal of the Marine Biological Association of the United Kingdom*, 93: 651-657.

Estrella Martinez, J. 2013. Caribbean Sea water temperatures of the last 500 years as derived from sclerosponges, MS Thesis, Department of Marine Sciences. Mayaguez, PR: University of Puerto Rico at Mayaguez.

Hinderstein, L., J. Marr, F. Martinez, M. Dowgiallo, K. Puglise, R. Pyle, D. Zawada, and R. Appeldoorn. 2010. Theme section on "Mesophotic Coral Ecosystems: Characterization, Ecology, and Management". *Coral Reefs*, **29:** 247-251.

Hubbard, D.K., R.B. Burke, I.P. Gill, W.R. Ramirez, and C.E. Sherman. 2008. Coral-reef geology: Puerto Rico and the US Virgin Islands, In: Riegl, B.M., and R.E. Dodge, eds. *Coral Reefs of the USA*. pp. 263-302. New York: Springer Verlag.

Kohler, K.E., and S.M. Gill. 2006. Coral Point Count with Excel extensions (CPCe): A Visual Basic program for the determination of coral and substrate coverage using random point count methodology. *Computers and Geosciences*, **32:** 1259-1269.

Lesser, M.P. 2010. Interactions between stressors on coral reefs: analytical approaches, re-analysis of old data, and different conclusions. *Coral Reefs*, **29:** 615-619.

Lesser, M.P., M. Slattery, and J.J. Leichter. 2009. Ecology of mesophotic coral reefs. *Journal of Experimental Marine Biology and Ecology*, **375:** 1-8.

Lesser, M.P., M. Slattery, M. Stat, M. Ojimi, R.D. Gates, and A. Grottoli. 2010. Photoacclimatization by the coral Montastraea cavernosa in the mesophotic zone: light, food, and genetics. *Ecology*, **91:** 990-1003.

Locker, S., R. Armstrong, T. Battista, J. Rooney, C. Sherman, and D. Zawada. 2010. Geomorphology of mesophotic coral ecosystems: current perspectives on morphology, distribution, and mapping strategies. *Coral Reefs*, **29:** 329-345.

Pesic, V., Chatterjee, T., Schizas, N.V., 2012. A new species of *Pontarachna* (Acari, Hydrachnidia, Pontarachnidea) from a mesophotic coral ecosystem off Vieques Island, Puerto Rico, Caribbean Sea. *Zootaxa*, **3440:** 63-67.

Petrescu, I., Chatterjee, T., Schizas, N.V., 2013. Two new species of the genus *Cumella* (Crustacea: Cumacea:Nannastacidae) associated with mesophotic reefs of Puerto Rico and St. Croix, Caribbean Sea. *Cahier de Biologie Marine*, **54:** 257-262.

Pyle, R.L. 1996. A learner's guide to closed-circuit rebreather operations. In: *Proceedings of the Rebreather Forum 2.0*, Richardson, D., ed. pp. P45-P67. Rancho Santa Marguerita, CA; Professional Association of Diving Instructors.

Riegl, B., and W.E. Piller. 2003. Possible refugia for reefs in times of environmental stress. *International Journal of Earth Science*, **92:** 520-531.

Rooney, J., E. Donham, A. Montgomery, H. Spalding, F. Parrish, R. Boland, D. Fenner, J. Gove, and O. Vetter. 2010. Mesophotic coral ecosystems in the Hawaiian Archipelago. *Coral Reefs*, **29:** 361-367.

Sherman, C., R. Appeldoorn, M. Carlo, M. Nemeth, H. Ruiz, and I. Bejarano. 2009. Use of technical diving to study deep reef environments in Puerto Rico. In: Pollock, N.W. (ed.), 28th Annual AAUS Scientific Symposium, Atlanta, Georgia, pp. 58-65. Dauphin Island, AL: American Academy of Underwater Sciences.

Sherman, C., M. Nemeth, H. Ruíz, I. Bejarano, R. Appeldoorn, F. Pagán, M. Schärer, and E. Weil. 2010. Geomorphology and benthic cover of mesophotic coral ecosystems of the upper insular slope of southwest Puerto Rico. *Coral Reefs*, **29:** 347-360.

Singh, H., R. Armstrong, F. Gilbes, R. Eustice, C. Roman, O. Pizarro, and J. Torres. 2004. Imaging Coral I: Imaging Coral Habitats with the SeaBED AUV. *Subsurface Sensing Technologies and Applications*, **5:** 25-42.

Smith, T., J. Blondeau, R. Nemeth, S. Pittman, J. Calnan, E. Kadison, and J. Gass. 2010. Benthic structure and cryptic mortality in a Caribbean mesophotic coral reef bank system, the Hind Bank Marine Conservation District, U.S. Virgin Islands. *Coral Reefs*, **29:** 289-308.