INTEGRATED STUDIES IN CORAL REEF ECOLOGY: GENETICS, OCTOCORAL PREDATION, AND REMOTE SENSING

by

Matthew Q. Lucas

A dissertation submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY *in*

MARINE SCIENCES (Biological Oceanography) University of Puerto Rico-Mayagüez 2016

Approved by:

Ernesto F. Weil, Ph.D. Chairperson, Graduate Committee

Nikolaos V. Schizas, Ph.D. Member, Graduate Committee

Govind Nadathur, Ph.D. Member, Graduate Committee

Matthew C. Smith, Ph.D. Director, Department of Marine Science

Ernesto Otero, Ph.D. Director, Department of Marine Science

Wilson Ramirez, Ph.D. Graduate School Representative Date

Date

Date

Date

Date

Date

Abstract

This dissertation consists of three peer-reviewed publications that are integrated by their collective contribution to several different scales of information (molecular, community, and landscape) that are important in coral reef ecology. At the molecular level, (1) *Symbiodinium* diversity in a reef-building coral between shallow and mesophotic depths among different islands in the Northern Caribbean, (2) at the community level, we studied the predation by *Cyphoma gibbosum* on the octocoral community among La Parguera's reefs, (3) and at the landscape level we examined the influence of the water column on spectral diversity and its implications for monitoring shallow water marine communities using remote sensing. In Chapter 5, I provide a few concluding comments regarding active and practical conservation goals on the basis that coral reef resilience and sustainable ecosystem services are dependendent on maintaining high biodiversity.

Resumen

Esta tesis consiste en tres artículos científicos especializados que se integran por su contribución colectiva a varias escalas diferentes de información (molecular, la comunidad, y paisaje) que son importantes en la ecología de los arrecifes de coral. A nivel molecular, (1) la diversidad *Symbiodinium* en un arrecife de coral de la capacidad entre las aguas poco profundas y mesophotic entre las diferentes islas en el Caribe Norte, (2) a nivel comunitario, se estudió la depredación por *Cyphoma gibbosum* en la comunidad octocoral entre los arrecifes de La Parguera, (3) y a nivel del paisaje, se analizó la influencia de la columna de agua sobre la diversidad espectral y sus implicaciones para la vigilancia de las comunidades marinas de aguas poco profundas usando sensores remotos. En el capítulo 5, proporciono un par de observaciones finales relativas a los objetivos de conservación activas y prácticas sobre la base de que la capacidad de recuperación de arrecifes de coral y servicios de los ecosistemas sostenibles se dependendent en el mantenimiento de una alta biodiversidad.

Copyright

In presenting this dissertation in partial fulfillment of the requirements for a Doctor of Philosophy degree at the University of Puerto Rico, I agree that the library shall make its copies freely available for inspection. I therefore authorize the Library of the University of Puerto Rico at Mayaguez to copy my dissertation totally or partially. Each copy must include the title page. I further agree that extensive copying of this dissertation is allowable only for scholarly purposes. It is understood, however, that any copying or publication of this dissertation for commercial purposes, or for financial gain, shall not be allowed without my written permission.

© Matthew Q Lucas, 2016

Dedication

To my Aunt, Meridith Lucas, who introduced me to SCUBA diving and the splendor of coral reefs. To my parents, Belinda and Michael Lucas, for their support and love throughout my life. To my loving and dearest wife Natalia and daughter Belinda (BeLu), for showing me the meaning of success. To my brothers Erik, Jason, and David for their support.

Acknowledgements

A special thanks to my adviser Dr. Ernesto Weil for your professional and personal guidance. You made me a better scientist and broadened my understanding of coral reef communities. Likewise, I extend my sincerest gratitude to my graduate committee, Dr. Nik Schizas, Dr. Govind Nadathur, and Dr. Matthew Smith for their professional advice and support over the years. In addition, I thank Dr. James Goodman for his relentless guidance on the remote sensing project. I also want to express my gratitude to Dr. Michael Stat for his advice that improved the *Symbiodinium* manuscript. I would also like to thank my friends and UPRM undergraduate students for helping in the lab and field; Luis Rodriguez, Duane Sanabria, Ramon Vicens, Bethsymarie Soto, and exchange students from France, Tavi Castagnoli, Julia Sanchez, and Jesse LeDu. I would like to thank the Department of Marine Sciences Faculty and Staff especially, Taty, Zulma, and Lillivette for always being so very helpful. Lalo and Negrito for their candid help with boat logistics and Milton Carlo and Orlando Espinoza for dive assistance, thank you. Finally yet importantly, I want to thank all of my family and friends.

Table of Contents

Abstract	ii
Resumen	iii
Copyright	iv
Dedication	v
Acknowledgments	vi
Table of Contents	vii
List of Tables & Figures	ix
Chapter 1: General Introduction	1
References	5
Chapter 2: <i>Symbiodinium</i> (ITS2) diversity in the coral host <i>Agaricia lamarcki</i> (Cnidaria: Scleractinia) between shallow and mesophotic reefs in the Northern Caribbean (20–70 m)	9
2.1 Abstract	9
2.2 Introduction	10
2.3 Methods	12
2.4 Results	14
2.5 Discussion	19
2.6 Acknowledgements	25
2.7 References	26
Chapter 3: Natural prey preferences and spatial variability of predation pressure by Cyphoma	
gibbosum (Mollusca: gastropoda) on octocoral communities off La Parguera, Puerto Rico	32
3.1 Abstract	32
3.2 Introduction	33
3.3 Method	39
3.4 Results	43
3.5 Discussion	55
3.6 Acknowledgements	60
3.7 References	60

Chapter 4: Linking coral reef remote sensing and field ecology: it's a matter of scale			
4.1 Abstract	64		
4.2 Introduction	65		
4.3 Methods	69		
4.4 Results	75		
4.5 Discussion	86		
4.6 Acknowledgements	92		
4.7 References	92		
Chapter 5: Concluding comments	98		
References	105		

List of Tables & Figures

Chapter 2. <i>Symbiodinium</i> (ITS2) diversity in the coral host <i>Agaricia lamarcki</i> (Cnidaria: Scleractinia) between shallow and mesophotic reefs in the Northern Caribbean (20 – 70 m)	
Table 1 : Sampling summary including the coral colony number, locality sampled, number of Symbiodinium ITS2 molecular clones per colony, and depth.	15
Table 2: <i>Symbiodinium</i> (ITS2) OTU representatives, the number of sequences clustered in each OTU, genbank accession numbers, and ITS2 sequence references for novel OTU sequences. * <i>N</i> , refers to novel sequences recovered in this study.	16
Figure 1: The prevalence of all <i>Symbiodinium</i> OTUs occurring in <i>A. lamarcki</i> colonies located at shallow and mesophotic depths for each location. The value n represents the number of colonies sampled in each location and the value in parenthesis represents the number of cloned sequences.	17
Figure 2: Minimum spanning network of <i>Symbiodinium</i> clade C (OTUs) in the coral host <i>A. lamarcki</i> . *Gaps are indicated by blue dashes and mutations are indicated in red.	19
List of Tables and Figures	
Chapter 3. Natural prey preferences and spatial variability of predation pressure by <i>Cyphoma gibbosum</i> (Mollusca: gastropoda) on octocoral communities off La Parguera, Puerto Rico	
Table 1: Total octocoral species and the number of colonies harboring snails. The total number of band-transects (20 m ²) surveyed in the entire study was 72.	41
Table 2: Octocoral species densities (colonies/m ²) for the six most affected octocoral species within each reef along an inshore to offshore gradient.	43
Table 3: Ivlev's prey electivity index for each reef zone for octocoral species harboring snails. Prey election, electivity (e) = ($ri -Pi$)/($ri +Pi$), where ri is the proportion of prey species i utilized and Pi is the proportion of prey species i available.	44
Table 4: Spearman rank correlations between the mean (%) PP and octocoral species density for the six most affected species across all reefs.	45
Table 5: Spearman rank correlations between the mean (%) PP and the pooled densities of all octocoral species within reefs.	46
Table 6: Mean percent (%), standard deviation and standard error of (PP) (K-W; α =0.05) for the six octocoral species showing the highest levels of (PP) within and across reefs.	49

Figure 1: Site map of the La Parguera Natural Reserve located off the southwest coast of Puerto Rico showing the location of the six reefs surveyed in this study.	40
Figure 2A: Photographs showing the snail, <i>Cyphoma gibbosum</i> , grazing on several species of octocoral. Two snails each on a single colony of <i>P. americana</i> , <i>P. homomalla</i> and <i>P. acerosa</i> (A, B, C). A branch of <i>P. porosa</i> with a large portion of tissue eaten by one snail (D). Snail eating tissue from <i>B. asbestinum</i> (E, F) and <i>P. nutans</i> (G). (Photo credit: E.Weil).	50
Figure 2B: Photographs showing a medium size colony of <i>G. ventalina</i> with four snails and scar areas produced by its feeding activity on the main axis and blade (A, B). Close up of the damage produced by the snail feeding (C, D), and a large colony showing the impact of predation activity of one snail along the main axes and the blade (E). These damaged tissue areas are susceptible to infections by pathogens and/or could be rapidly colonized by algae, sponges or <i>Millepora</i> which could increase sea fan tissue mortality (Photo credit: E.Weil).	51
Figure 3: Mean (%) predation pressure by <i>C. gibbosum</i> on the octocoral community (pooled data) for each reef zone (upper left) and reef locality (bottom left) and their associated mean <i>C. gibbosum</i> densities (right column).	52
Figure 4: Mean (%) predation pressure variability by <i>C. gibbosum</i> on the most affected octocoral species across reefs in La Parguera. Error bars represent the standard error. Reef Sites: <i>PL</i> = Pelotas; <i>EN</i> = Enrique; <i>SC</i> = San Cristobal; <i>ML</i> = Media Luna; <i>OB</i> = Old Buoy; <i>WB</i> = Weinberg.	53
Figure 5: Variability in mean (%) PP by <i>C. gibbosum</i> across the six major octocoral prey species within each reef off La Parguera. Error bars represent the standard error.	54
List of Tables and Figures	
Chapter 4. Linking coral reef remote sensing and field ecology: it's a matter of scale	
Table 1: Summary of field reflectance spectra (R) collected from shallow reef areas in southwestern	

х

Puerto Rico. Instrument configuration was set to automatically record and average four samples for each

Figure 1: The GER-1500 spectrometer in underwater housing with attached illumination (left) and a map

Figure 2: (**a**) Flagged rebar was hammered into non-living substrate and tightly tethered to the surface to mark the GPS location for each habitat plot; (**b**) Photoquadrat of seagrass area mixed with soft corals; (**c**) Photoquadrat of from the reef crest area showing soft corals, hard corals, encrusting sponge, algae and

Figure 3: Representative individual spectra measured for Acropora palmata; examples shown here are 10

non-living substrate; (d) Recording the GPS location of a habitat plot (tethered buoy).

of the study location off the coast of southwestern Puerto Rico.

spectra subset from a total sample size = 139 spectra.

75

70

73

77

measurement.

Figure 4: Representative average spectra for ten coral species; examples shown here are subset of the 25 total Cnidarian species sampled (5556 samples; 1389 reflectance spectra).	77
Figure 5: Optical dendrograms illustrating the effect of increasing depth on separability of field spectra grouped into four fundamental reef components: coral, sponge, submerged aquatic vegetation (SAV), and sand.	81
Figure 6: Optical dendrograms for 3 and 10 m water depth illustrating the effects of increasing depth on separability of field spectra from 24 coral species, 10 sponge species, 3 submerged aquatic vegetation (SAV) species, and sand.	82
Figure 7: Optical dendrograms for 3 and 10 m water depth illustrating the effects of increasing depth on separability of field spectra from 24 coral species, 10 sponge species, 3 submerged aquatic vegetation (SAV) species, and sand.	83
Figure 8: Estimates of biodiversity calculated using the exponential of Shannon entropy, exp(H'), illustrating influence of increasing spectral similarity amongst reef species as a function of increasing water depth: 0* is biodiversity obtained from photoquadrats, 0** is biodiversity calculated using only those species considered prevalent or sizable enough to significantly influence the remote sensing signal (i.e., species included in the spectral measurements for this study area), and 0–10 is biodiversity calculated with consideration for optical similarities amongst species (i.e., based on hierarchical clustering of reflectance spectra as influenced by the overlying water column)	85
clustering of renectance spectra as influenced by the overlying water column).	65

85

Chapter 1. General Introduction

Coral reefs are the most biologically diverse and productive marine communities on the planet (Connell 1978; Paulay 1997; Knowlton 2001; Sala and Knowlton 2006). Coral reef communities occupy less than one percent of the ocean benthos but they provide habitat to more than 25% of all marine life (Bryant et al. 1998). Yet, high species diversity (biodiversity) is one of the defining characteristics of coral reef communities, and while past estimates range from 600,000 to greater than 9 million species globally (Reaka-Kudla 1997; Knowlton 2001; Rogers 2013), it is likely, that biodiversity on coral reefs has been seriously underestimated (Small et al. 1998; Plaisance et al. 2011). In addition, coral reefs supply a range of ecological goods and services to marine life and human populations living near coral reefs (Moberg and Folke 1999; Worm et al. 2006). Coral reefs provide three-dimensional structure that serves as an important habitat for spawning, nursery and breeding grounds to numerous reef fish and invertebrates, as well as feeding grounds for large predatory fish, sea turtles and marine mammals. For human well-being, coral reef communities provide a source of food (fishery), coastal protection by dissipating wave energy produced by tropical storms and hold vast potential in supplying natural products and human medicines (Moberg and Folke 1999; Worm et al. 2006). The intrinsic beauty and diversity of life inhabiting coral reefs attracts tourists from around the world creating jobs and income for local communities. Over a decade ago, the total net benefit of the world's coral reefs was estimated at \$30 billion/year (Cesar et al. 2003). In spite of this,

nearly two-thirds of Caribbean coral reefs are currently under threat by climate change and human activities (Bryant et al. 2009; Hoegh-Guldberg et al. 2015).

Historical overfishing in the Caribbean came long before all other human disturbances to coastal ecosystems and predates the arrival of European settlers in the 15th century (Wing and Reitz 1982; Jackson et al. 2001; Jackson et al. 2014; Mumby et al. 2015). Further declines continued well into the 1950-1960s when populations of large predators (i.e., sharks, barracudas, and groupers) were heavily overfished, consequently leaving behind small carnivorous fish (grunts, smaller groupers and snappers), herbivorous fish (parrotfish, surgeonfish) and planktivorous fish (damselfish) (McClenachan 2008; Jackson et al. 2014; Mumby et al. 2015). In the 1970s, coral reefs still appeared relatively healthy, but they began to change and one ecological tragedy followed another throughout coral reefs of the Caribbean. Then, in 1977, White Band Diseased first affected corals in the US Virgin Island and then rapidly spread across the Caribbean. The two most impacted species that served as vital habitat for many fish and other reef inhabitants were Acropora palmata and A. cervicornis (Gladfelter 1982; Aronson and Precht 2001). After the mass mortality of Acropora species in the late 1970s, another disease swept through the Caribbean killing 93% of the herbivorous sea urchin Diadema antillarum in 1983 - 1984 (Lessios 1988). Diadema antillarum once served an important role in maintaining algal growth on reefs (Lessios 1988). Correspondingly, after the die-off of D. antillarum, the few remaining herbivorous reef fish were also over-fished in many locations around the

Caribbean. The loss of *D. antillarum* and subsequent overfishing of herbivorous fish left reefs unchecked and they began to increase in macroalgae and decrease in coral cover, which in turn diminished reef resilience (Hughes 1994; Nyström et al. 2000; Hughes et al. 2003; Hughes et al. 2007; Knowlton and Jackson 2008; Jackson et al. 2014). The identification of the pathogen that caused mass mortality in *Diadema antillarum* remains unknown. In the wake of the catastrophic loss of foundation (*A. palmata, A. cervicornis*) and keystone species (*D. antillarum*), the adverse effects of climate change (thermal anomalies) began to emerge. Thermal anomalies related to climate change induced Caribbean-wide bleaching events in 1995, 1998, 2005, 2010 – and subsequently many colonies suffered mass mortalities due to diseases that followed these bleaching events (Cróquer and Weil 2009; Rogers et al. 2009; Weil et al. 2009; Weil and Rogers 2011).

From the 1990's to present day, the effects of climate change (thermal anomalies, acidification with increasing frequency and severity of hurricanes and storms), marine diseases, African dust, overfishing, coastal development and other forms of pollution (i.e., agricultural runoff, sewage outfall) continue to cause mortality, destruction of reef complexity (habitat), and undermines the natural ability of corals to recover (Hughes 1994; Hughes and Tanner 2000;, Hughes et al. 2003; Wilson et al. 2006; Weil and Rogers 2011; Jackson et al. 2014). Not only in the Caribbean, but around the planet both natural and anthropogenic disturbances have led to significant losses in coral cover, diversity, resilience and physical structure, as well as significant decreases in reef fish size,

abundance, and diversity (Gardner et al. 2003; Knowlton 2001; Hughes et al. 2003; Jones et al. 2004; Sala and Knowlton 2006; Wilson et al. 2006; Worm et al. 2006; Knowlton and Jackson 2008; Paddack et al. 2009; Palumbi et al. 2009; Wild et al. 2011; De'ath et al. 2012; Jackson et al. 2014; Mumby et al. 2015).

From space to deep reefs, great efforts continue to monitor and better understand how future coral reef communities might respond and adapt to the adverse effects of continued environmental degradation and climate change (i.e., Lesser et al. 2009; Eakin et al. 2010). As such, this dissertation, "Integrated studies in coral reef ecology: genetics, octocoral predation, and remote sensing," describes different approaches that investigated the influence of depth on various aspects in coral reef ecology. Each chapter stands alone and includes its own discussion and conclusions. Specifically, these chapters cover several different important research themes in coral reef ecology, including 1) zooxanthellae (genus Symbiodinium) diversity in the coral host Agaricia lamarcki between shallow and mesophotic coral reefs (Lucas et al. 2016), 2) predation pressure by the snail, Cyphoma gibbosum, on a diverse array of octocoral species among La Parguera reefs (Lucas et al. 2014), and 3) the utility of remote sensing in ecology, biodiversity, and conservation (Lucas and Goodman 2015).

References

- Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. *Hydrobiologia*, 460: 25–38.
- Bryant D, Burke L, McManus J, and Spalding M (1998) Reefs at risk—a map-based indicator of threats to the world's coral reefs. Washington DC, USA: World Resources Institute.
- Cesar HJS, Burke L, Pet-Soede L (2003) The Economics of Worldwide Coral Reef Degradation. *Cesar Environmental Economics Consulting (CEEC)*, 6828GH Arnhem, The Netherlands. pp. 23.
- Connell JH (1978) Diversity in tropical rain forests and coral reefs. Science, 199: 1302-1310.
- Cróquer A, Weil E (2009) Changes in Caribbean coral disease prevalence after the 2005 bleaching event. *Diseases of Aquatic Organisms*, 87: 33–43.
- De'ath G, Fabricius KE, Sweatman H, Puotinen M (2012) The 27-yr decline of coral cover on the Great Barrier Reef and its causes. *Proceedings of the National Academy of Sciences*, 109: 17734–17735.
- Eakin CM, Nim C, Brainard R, Aubrecht C, Elvidge C, Gledhill D, Muller-Karger F et al. (2010) Species from space. *Oceanography*, 23: 118–133.
- Gladfelter WB (1982) White-band disease *in Acropora palmata*: implications for the structure and growth of shallow reefs. *Bulletin of Marine Science*, 32: 639–643.
- Gardner TA, Cote IM, Gill JA, Grant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. *Science*, 301: 958–960.
- Hughes TP (1994) Catastrophes, phase shifts and large-scale degradation of a Caribbean coral reef. *Science*, 265: 1547-1551.
- Hughes TP, Tanner JE (2000) Recruitment failure, life histories, and long-term decline of Caribbean corals. *Ecology*, 81: 2250-2263.
- Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, et al. (2003) Climate change, human impacts, and the resilience of coral reefs. *Science*, 301: 929–933.

- Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N et al. (2007) Phase shifts, herbivory and the resilience of coral reefs to climate change. *Current Biology*, 17: 360–365.
- Hoegh-Guldberg O, Eakin CM, Hodgson G, Sale PF, Veron JEN (2015) ISRS consensus statement on climate change and coral bleaching. Prepared for the 21st Session of the Conference of the Parties to the United Nations Framework Convention on Climate Change, Paris, France. http://reefcheck.org/PDFs/ISRSConsensus2015.pdf
- Jackson JBC, Donovan MK, Cramer KL, Lam VV (Eds) (2014) Status and trends of Caribbean coral reefs: 1970-2012. *Global Coral Reef Monitoring Network, IUCN,* Gland, Switzerland.
- Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, et al. (2001) Historical overfishing and the recent collapse of coastal ecosystems. *Science*, 293: 629–637.
- Jones GP, McCormick MI, Srinivasan M, Eagle JV (2004) Coral decline threatens fish biodiversity in marine reserves. *Proceedings of the National Academy of Sciences of the United States of America*, 101: 8251–8253.
- Knowlton N (2001) The future of coral reefs. *Proceedings of the National Academy of Sciences of the United States of America*, 98: 5419–5425.
- Knowlton N, Jackson JBC (2008) Shifting baselines, local impacts, and global change on coral reefs. *PLoS Biology*, 6(2): e54.doi:10.1371/journal.pbio.0060054.
- Lesser MP, Slatter M, Leichter JL (2009) Ecology of mesophotic coral reefs. Journal of Experimental Biology and Ecology, 375: 1-8.
- Lessios HA, Cubit JD, Robertson DR, Shulman MJ, Parker MR, Garrity SD et al. (1988) Mass mortality of *Diadema antillarum* on the Caribbean coast of Panama. *Coral Reefs*, 3: 173-182.
- Lucas MQ, Rodriguez L, Sanabria D, Weil E (2014) Natural prey preferences and spatial variability of predation pressure by *Cyphoma gibbosum* (Mollusca: Gastropoda) on octocoral communities off La Parguera, Puerto Rico. *ISRN Ecology*, 742387: 1-13. doi.org/10.1155/2014/742387.

- Lucas MQ, Goodman J (2015) Linking coral reef remote sensing and field ecology: it's a matter of scale. *Journal of Marine Science and Engineering*, 3: 1-20. doi: 10.3390/jmse3010001
- Lucas MQ, Stat M, Smith MC, Weil E, Schizas N (2016) *Symbiodinium* diversity in the coral host *Agaricia lamarcki* (Cnidaria: Scleractinia) between shallow and mesophotic reefs in the Northern Caribbean. *Marine Ecology*, In Press.
- McClenachan L (2008) Documenting loss of large trophy fish from the Florida Keys with historical photographs. *Conservation Biology*, 23: 636-643.
- Moberg F, Folke C (1999) Ecological goods and services of coral reef ecosystems. *Ecological Economics*, 29: 215–233.
- Mumby PJ, Flower J, Chollett I, Box SJ, Bozec Y, Fitzsimmons C, Forster J et al. (2015) Towards reef resilience and sustainable livelihoods: a handbook for Caribbean coral reef managers. University of Exeter, Exeter. pp. 1-172.
- Nyström M, Folke C, Moberg F (2000) Coral reef disturbance and resilience in a humandominated environment. *Trends in Ecology and Evolution*, 15, 413–417.
- Paddack M, Reynolds J, Aguilar C, Appledoorn R, Beets J, Burkett EW, Chittaro PM et al. (2009) Recent region-wide declines in Caribbean reef fish abundance. *Current Biology*, 19: 590–595.
- Palumbi SR, Sandifer PA, Allan JD Beck M, Fautin DG et al. (2009) Managing for ocean biodiversity to sustain marine ecosystem services. *Frontiers in Ecology and the Environment*, 7: 204–211.
- Paulay G (1997) Diversity and distribution of reef organisms, *in Life and Death of Coral Reefs*, Birkeland C (Eds) Chapman and Hall, New York, NY, USA. pp. 298–353.
- Plaisance L, Caley MJ, Brainard RE, Knowlton N (2011) The diversity of coral reefs: what are we missing? *PLoS ONE*, 6(10): e25026. doi:10.1371/journal.pone.0025026.
- Reaka-Kudla ML (1997) The global biodiversity of coral reefs: a comparison with rain forests, in Biodiversity II: Understanding and Protecting Our Biology Resources (Eds) Reaka-Kudla ML, Wilson DE, Wilson EO), Joseph Henry, Washington D.C. pp. 83– 108.

- Rogers CS, Muller E, Spitzack T, Miller J (2009) Extensive coral mortality in the US Virgin Islands in 2005/2006: a review of the evidence for synergy among thermal stress, coral bleaching and disease. *Caribbean Journal of Science*, 45: 204–214.
- Rogers CS (2013) Coral reef resilience through biodiversity. *ISRN Oceanography*, 739034: 1-18. doi.org/10.5402/2013/739034.
- Sala E, Knowlton N (2006) Global marine biodiversity trends. *Annual Review of Environment and Resources*, 31: 93–122.
- Small A, Adey A, Spoon D (1998) Are current estimates of coral reef biodiversity too low? The view through the window of a microcosm. *Atoll Research Bulletin*, 458: 1–20.
- Weil E, Cróquer A, Urreiztieta I (2009) Temporal variability and impact of coral diseases and bleaching in La Parguera, Puerto Rico from 2003–2007. *Caribbean Journal of Science*, 45: 221–246.
- Weil E, Rogers CS (2011) Coral reef diseases in the Atlantic-Caribbean, in Coral Reefs: An Ecosystem in Transition. Dubinsky Z, Stambler N (Eds) Springer, Dordrecht, Netherlands. pp. 465–491.
- Wild C, Hoegh-Guldberg O, Naumann MS et al. (2011) Climate change impedes scleractinian corals as primary reef ecosystem engineers. *Marine and Freshwater Research*, 62: 205–215.
- Wilson SK, Graham NAJ, Pratchett MS, Jones GP, Polunin NVC (2006) Multiple disturbances and the global degradation of coral reefs: are reef fishes at risk or resilient? *Global Change Biology*, 12: 2220–2234.
- Wing ES, Reitz E (1982) Prehistoric fishing economies of the Caribbean. *Journal of New World Archaeology*, 5: 13–32.
- Worm B, Barbier EB, Beaumont N et al. (2006) Impacts of biodiversity loss on ocean ecosystem services. *Science*, 314: 787–790.

2. *Symbiodinium* (internal transcribed spacer 2) diversity in the coral host *Agaricia lamarcki* (Cnidaria: Scleractinia) between shallow and mesophotic reefs in the Northern Caribbean (20 - 70 m)

Lucas MQ, Stat M, Smith MC, Weil E, & N. Schizas. (2016) Marine Ecology, In press. ISSN: ISSN 0173-9565

2.1 Abstract

This study investigated differences in Symbiodinium diversity in the scleractinian coral species Agaricia lamarcki between shallow (20 – 25 m) and mesophotic (50 – 70 m) depths in the northern Caribbean. Corals were sampled in each of four shallow sites (20 – 25 m; n = 18) and three mesophotic sites (50 - 70 m; n = 18) from Mona Island (Puerto Rico) and the US Virgin Islands during a mesophotic exploratory cruise and from the La Parguera shelf edge, off southwestern Puerto Rico. Symbiodinium diversity was assessed using internal transcribed spacer 2 (ITS2) sequences clustered into Operational Taxonomic Units (OTUs). Clustering resulted in eight clade C OTUs and one clade D OTU. Of these, there were three common Symbiodinium OTUs consisting of C3 and D1a.N14 in shallow reefs and C11.N4 in mesophotic reefs. Statistical tests (PERMANOVA and ANOSIM) showed significant differences between clade C Symbiodinium OTUs in A. lamarcki colonies located at shallow and mesophotic depths, indicating symbiont zonation. Symbiodinium diversity in A. lamarcki from the northern Caribbean is comparable to previous reports in the southern Caribbean for this species. This is the first report of the thermal tolerant species *Symbiodinium trenchii* (D1a) in *A. lamarcki*.

2.2 Introduction

Reef-building corals are energetically dependent on their photosynthetic symbionts (genus Symbiodinium) and the exponential decrease in light with increasing depth is one of the most important factors driving their distributions over wide depth ranges (Rowan et al. 1997; Lesser et al. 2009; Lesser et al. 2010; Cooper et al. 2011). In this regard, depth zonation of genetically distinct Symbiodinium genotypes (symbiont zonation) has been mostly attributed to the differential photoacclimation responses of the different symbiont genotypes to decreasing light levels with increasing depth (Rowan and Knowlton 1995; Iglesias-Prieto et al. 2004; Frade et al. 2008). While there are a growing number of studies investigating Symbiodinium diversity in corals along mesophotic depth gradients (Chan et al. 2009; Lesser et al. 2010; Cooper et al. 2011; van Oppen et al. 2011a; Bongaerts et al. 2013; Bongaerts et al. 2015a, b; Pochon et al. 2015; Ziegler et al. 2015), the role of symbiont zonation influencing the depth distributions in corals is understudied, especially on an ecosystem-wide scale.

In the Caribbean, the most extensive surveys of *Symbiodinium* diversity in mesophotic corals have been carried out in Curaçao (Bongaerts et al. 2013, 2015a, b). These results show that symbiont zonation is common on a reef-wide scale, a dominant trait in species with the widest depth ranges, and is more common in broadcast spawning species (Bongaerts et al. 2015 a). An assessment of the lower mesophotic community (60-100 m) revealed a specialized coral community composed of *Agaricia grahamae, Agaricia undata,*

and Madracis pharensis (90 m), all associating with putative 'deep specialist' Symbiodinium types (Bongaerts et al. 2015b). An earlier study by Bongaerts et al. (2013) used ITS2 Denaturing Gradient Gel Electrophoresis (DGGE) profiles to examine five Agaricia species and their Symbiodinium associations in Curaçao (2-60 m). Four of the five species showed distinct depth distributions and specific symbiont associations, whereas A. lamarcki was the only species showing symbiont zonation (Bongaerts et al. 2013). Even though a "deep specialist" symbiont association is not a universal mechanism to survive at mesophotic depths (Chan et al. 2009; Bongaerts et al. 2010), these studies highlight the important role of symbiont zonation and its influence on the depth distributions in some coral species (Bongaerts et al. 2013; Bongaerts et al. 2015a, b). Because some coral species can have geographic differences in symbiont associations (LaJeunesse et al. 2004; Garren et al. 2006; Jones et al. 2008; Stat et al. 2008; Finney et al. 2010), local assessments of Symbiodinium diversity are needed to further understand coral-symbiont associations on a broader geographic scale.

The coral species *Agaricia lamarcki* inhabits cryptic areas on shallow reefs (10 – 40 m) and is also one of the most abundant species inhabiting mesophotic reefs in the Caribbean (Bongaerts et al. 2013; Sherman et al. 2013), thus representing the ideal species to examine *Symbiodinium* diversity across a wide depth range. In this study, *Symbiodinium* (ITS2) OTUs were constructed to investigate 1) differences in *Symbiodinium* diversity in *Agaricia lamarcki* from shallow (20-25 m) and mesophotic depths (50-70 m) from Mona Island and

La Parguera in Puerto Rico, and St. Croix and St. Thomas in the US Virgin Islands (USVI) and 2) establish if the pattern of symbiont zonation in *A. lamarcki* is comparable to previous studies in the southern Caribbean (Curaçao, Bongaerts et al. 2013; Bongaerts et al. 2015a,b).

2.3 Materials and Methods

Sample collection

Agaricia lamarcki (n = 36) samples were collected in 2010 in each of four shallow sites (20 – 25 m; n = 18) and three mesophotic sites (50 - 70 m; n = 18) from Mona Island (Puerto Rico) and St. Croix, USVI during a mesophotic exploratory cruise and from the La Parguera shelf edge, located off southwestern Puerto Rico. There were no mesophotic samples for comparison from St. Thomas USVI. Study sites are described in Sherman et al. (2013). Shallow water colonies were collected with SCUBA-NITROX while trimix-rebreather systems were used to collect mesophotic colonies. Coral specimens were identified by Dr. Ernesto Weil and preserved in 100% ethanol. Specimens were cataloged in a database by colony number, date, location and deposited in the Museum of Marine Invertebrates at Magueyes Marine Laboratories, University of Puerto Rico, Mayagüez.

DNA extraction, PCR, cloning and sequencing

Total genomic DNA (gDNA) was extracted using a modified cetyltrimethylammonium bromide) (CTAB) protocol after Dempster et al. (1999). The ITS2 region was PCR amplified using standard thermal cycle conditions using the *Symbiodinium* specific primers, ITSintfor2 (5'-<u>GAATTGCAGAACTCCGTG</u>-3') and ITS2 reverse (5'-<u>GGATCCATATGCTTAAGTTCAGCGGGT</u>-3') (LaJeunesse 2002). PCR products for the *Symbiodinium* ITS2 region were purified using QIAquick® PCR Purification Kit (Qiagen, Valencia, CA), ligated overnight into the pGEM® T-Easy Cloning Vector, transformed into JM109 competent cells (Promega, Madison, WI), and grown overnight on selective LB media (ampicillin 50 μ g/ml, isopropyl-ß-D-thiogalactopyranoside (IPTG) 0.1mM, 5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside (X-Gal) 50 μ g/ml). ITS2 clones were sequenced with M13 primers at High-Throughput Genomics Center (htseq.org) (Seattle, WA).

Sequence analysis and OTUs

Symbiodinium ITS2 sequences were edited using CodonCode Aligner (v. 4.2.7, CodonCode Corporation, www.codoncode.com) and aligned to closely related ITS2 sequences from Genbank. Mothur (v. 1.29) (Schloss et al. 2009) was used to generate a distance matrix with each gap treated as a mutation and subsequently clustered into operational taxonomic units (OTUs) using a 97% sequence similarity threshold (SST) with the furthest neighbor algorithm. OTUs represented by a single sequence were not considered in downstream analyses and omitted from the dataset. Novel sequences representing an OTU were named after their closest ITS2 sequence followed by a capital italic N (to represent 'novel'), with the next available integer in GenBank (i.e. C3b.N9)

used to identify novel *Symbiodinium* ITS2 sequences in *Agaricia* spp. from Curaçao (Bongaerts et al. 2013).

Statistical analyses of Symbiodinium OTUs

The frequency of *Symbiodinium* OTUs in each colony was square root transformed and statistical analyses performed using the software Paleontological Statistics (PAST v.3.0) (Hammer et al. 2001). Because of limited sampling, location was not considered. Instead, samples were pooled across locations for statistical analyses. To test for symbiont zonation of Symbiodinium OTUs by depth, a permutational multivariate analysis of variance (PERMANOVA) (Anderson 2005; Anderson 2006) was performed with depth as a factor and colonies as the replicate. Analysis of Similarity (ANOSIM) among Symbiodinium OTUs was also performed using the Bray-Curtis coefficient of similarity matrix to identify differences between shallow and mesophotic Symbiodinium OTUs occurring A. lamarcki (Clarke 1993). Minimum-spanning networks for representative OTUs (ancestral sequences) were inferred in Network v.4.6 with the full median joining algorithm (Bandelt et al. 1999) and illustrated with Network Publisher v.1.3 (Fluxusengineering.com).

2.4 Results

A total of 235 *Symbiodinium* ITS2 sequences were examined among 36 colonies of *A*. *lamarcki* from shallow (n = 18 colonies) and mesophotic depths (n = 18 colonies) (Table 1). Briefly, 29 colonies hosted clade C *Symbiodinium* and 5 colonies hosted clade D, while 1 colony from shallow reefs of St. Thomas USVI hosted both clades C and D simultaneously. The prevalence of *Symbiodinium* OTUs recovered in *A. lamarcki* colonies located at shallow and mesophotic depths is presented for each location (Figure 1). Clustering ITS2 sequences resulted in nine *Symbiodinium* OTUs, eight in clade C and one in clade D (Table 1).

Table 1: Sampling summary by location and depth, number of cloned ITS2 sequences for each *A*. *lamarcki* sample, and the distribution of sequences among *Symbiodinium* OTUs.

Shallow samples	No.	1	2	3	4	5	6	7	8	9
Colony # (id) depth	clones	C3	C11.N4	C3d	C3b.N9	C11.M0	C.M1	C11.M2	C.M3	Dla.M4
122-Cane Bay, St. Croix (20-25 m)	9	7					2			
123-Cane Bay, St. Croix (20-25 m)	7									7
16-Cane Bay, St. Croix (20-25 m)	6	3	2		1					
*2-Mona Island, PR (20-25 m)	8	б		1						
3- Mona Island, PR (20-25 m)	10	9		1						
5-Mona Island, PR (20-25 m)	11	4	3	2		1			1	
79-Mona Island, PR (20-25 m)	2									2
80-Mona Island, PR (20-25 m)	4									3
81-Mona Island, PR (20-25 m)	4									4
*21-St. Thomas (20-25 m)	8	4		2					1	
*22-St. Thomas (20-25 m)	7	2								4
24-St. Thomas (20-25 m)	3	3								
25-St. Thomas (20-25 m)	9	5		1	3					
34-La Parguera, PR (20-25 m)	9									9
33-La Parguera, PR (20-25 m)	3	3								
26-La Parguera, PR (20-25 m)	9	9								
28-La Parguera, PR (20-25 m)	11	9	1	1						
76-La Parguera, PR (20-25 m)	3	3								
		_	_	_		_	_	_	_	_
Mesophotic samples	No.	1	2	3	4	5	6	7	8	9 D1-374
Colony # (id)⁄ depth	clones	C3	CIT.N4	C3d	C303V9	CITZVID	Cavit	CHIMIZ	C2VI3	DIaJVI4
120-Cane Bay, St. Croix (67 m)	10		9					1		
121-Cane Bay, St. Croix (67 m)	8		7					1		
*17-Cane Bay, St. Croix (55 m)	8	5	1					1		
19-Cane Bay, St. Croix (67 m)	8	7			1					
10-Mona Island, PR (67 m)	6	3	3							
6-Mona Island, PR (67 m)	3	3								
7-Mona Island, PR (50 m)	10	3	7							
78-Mona Island, PR (50 m)	3		3							
82-Mona Island, PR (50 m)	2	1	1							
84-Mona Island, PR (72 m)	9	2	7							
*(6A)-La Parguera, PR (60 m)	5	2	1			1				
*43-La Parguera, PR (60 m)	6		5							
44-La Parguera, PR (60 m)	2	1				1				
46-La Parguera, PR (67 m)	8		8							
48-La Parguera, PR (55 m)	6	3	3							
(4A)-La Parguera, PR (60 m)	6	1	4				1			
41-La Parguera, PR (60 m)	3	1				2				
(3A)-La Parguera PR (70 m)	9		9							

*OTUs represented by a single sequence were not considered in downstream analyses and omitted from the data set.

OTU#	OTU-Rep	No. Seqs/OTU	Genbank Accession No.	Reference
1	C3	99	100% match	LaJeunesse 2002 (AF499789)
2	C11.N4	74	100% match	Bongaerts et al. 2013 (KF551188)
3	C3d	8	100% match	LaJeunesse 2002 (AF499792)
4	*C3b.N9	5	KP109696	LaJeunesse 2002 (AF499791)
5	*C11.N10	5	KP109697	Bongaerts et al. 2013 (KF551188)
6	*C.N11	3	KP109698	Thornhill et al. 2007 (EU074889)
7	*C11.N12	3	KP109699	LaJeunesse 2002 (AF499800)
8	*C.N13	2	KP109700	Barbrook et al. 2014 (HG515026)
9	*D1a.N14	29	KP109701	LaJeunesse et al. 2014 (KJ019889)

Table 2: *Symbiodinium* internal transcribed spacer 2 (ITS2) operational taxonomic unit (OTU) representatives (rep), the number of sequences (seqs) clustered in each OTU, GenBank accession numbers and ITS2 sequence references for novel OTU sequences.

The most common or dominant *Symbiodinium* OTUs (i.e., containing the majority of sequences) in *A. lamarcki* across all sampling locations were C3 OTU1, C3d OTU3, and D1a.N14 OTU9 in shallow waters and C11.N4 OTU2 at mesophotic depths (Table 2, Figure 1). Statistical tests (PERMANOVA, P = 0.0001, Pseudo-F = 9.65, df = 1; ANOSIM, P = 0.0001, R = 0.33) showed significant differences between clade C *Symbiodinium* OTUs (i.e., C3 OTU1 and C11.N4 OTU2) located at shallow and mesophotic depths, indicating symbiont zonation in *A lamarcki*.



Figure 1: The prevalence of all *Symbiodinium* OTUs occurring in *A. lamarcki* colonies located at shallow and mesophotic depths for each location. The value n represents the number of colonies sampled in each location and the value in parenthesis represents the number of cloned sequences. USVI = US Virgin Islands.

The minimum spanning network illustrates Symbiodinium C3 OTU1 as the inferred ancestral (ITS2) sequence among Symbiodinium OTUs recovered (Figure 2). The most common clade C OTUs (C3, C3d, and C11.N4) were identical to their reference sequences in Genbank (Table 2). The C11.N4 OTU2 consisted of C11 (40%, LaJeunesse et al. 2002) and C11.N4 sequences (60%, Bongaerts et al. 2013). The C3b.N9 OTU4 sequence is 1 bp different from its reference sequence (C3b, LaJeunesse 2002). The C11.N10 OTU5 sequence is characterized by a 1 bp deletion and a 2 bp insertion, while C11.N12 OTU7 contains an 8 bp deletion from its reference sequence (C11, LaJeunesse 2002). The C.N11 OTU6 is 1 bp different compared to a C1 sequence (culture 152, LaJeunesse 2002), considered to be a PCR chimera (LaJeunesse 2002). Because of this, the C1 sequence (Symbiodinium goureaui, LaJeunesse 2002) was incorporated into the network to illustrate the relationship between C.N11, C3 and C1 sequences (Figure 2). The C.N13 OTU8 has a 4 bp deletion and 1 bp difference compared to the C3 reference sequence (LaJeunesse 2002) (Table 2). The D1a.N14 OTU9 consisted of D1a (42%, LaJeunesse et al. 2014) and D1 sequences (58%, LaJeunesse et al. 2001; LaJeunesse 2002), and is characterized by a single (1 bp deletion) difference compared to its reference sequence (Symbiodinium trenchii, LaJeunesse et al. 2014). Novel ITS2 sequences representative of OTUs were submitted to GenBank (Table 2).



Figure 2: Minimum spanning network of *Symbiodinium* clade C (OTUs) in the coral host *A. lamarcki.* *Gaps are indicated by blue dashes and mutations are indicated in red. ITS2 = internal transcribed spacer 2

2.5 Discussion

The ITS2 region has been the most widely employed phylogenetic marker in the exploration of coral *- Symbiodinium* diversity along shallow and mesophotic depth gradients (Chan et al. 2009; Bongaerts et al. 2010; Lesser et al. 2010; Bongaerts et al. 2011; Bongaerts et al. 2015a, b; Pochon et al. 2015; Ziegler et al. 2015).

Notwithstanding, the multi-copy nature of the ITS2 region with numerous intragenomic variants has invoked considerable debate regarding the interpretation of Symbiodinium diversity and species delineation (Apprill & Gates 2007; Thornhill et al. 2007; Correa & Baker 2009; Stat et al. 2011; Stat et al. 2013; Sampayo et al. 2009; Thornhill et al. 2010). Therefore, to provide a conservative yet meaningful interpretation of these data, this study considers Symbiodinium ITS2 diversity in the context of OTUs. While the OTU approach does not completely alleviate the problems associated with intragenomic variation and PCR artifacts, it does reduce the overall complexity (Sneath and Sokal 1973; Thornhill et al. 2007; Correa and Baker 2009; Stat et al. 2013), and a 97% sequence similarity has gained recent support as the most informative threshold for studies of Symbiodinium using the ITS2 gene (Arif et al. 2014, Stat et al. 2015). In this regard, the C11.N4 sequence reported in A. lamarcki from Curaçao (Bongaerts et al. 2013) is only a single (1 bp) deletion compared to its reference C11 sequence (LaJeunesse 2002). In this regard, the C11.N4/C11 OTU2 (inclusive of C11) is interpreted here as Symbiodinium C11 (LaJeunesse 2002). Likewise, the novel D1a.N14 OTU9 is also characterized by a single (1 bp) deletion difference and therefore is interpreted as Symbiodinium trenchii (sensu D1a, or D1-4; but see LaJeunesse et al. 2014). Furthermore, S. trenchii is the only clade D species inhabiting Atlantic/Caribbean corals (Pettay et al. 2015). In addition, some of the novel clade C OTUs with few sequences are characterized by substantial differences compared to novel sequences identified in DGGE ITS2 profiles in Agaricia lamarcki in Curaçao

(Bongaerts et al. 2013) (Table 2, Figure 2). Although these novel sequences likely represent intragenomic variants of a single rDNA lineage (Thornhill et al. 2007; Bongaerts et al. 2013), it cannot be discounted they may represent a mix of distinct background symbionts (Stat et al. 2011; Bongaerts et al. 2013). However, the taxonomic and ecological significance of this diversity remains uncertain owing to a lack of consensus in the interpretation of ITS2 diversity (Correa and Baker 2009; LaJeunesse and Thornhill 2011; Stat et al. 2011).

Results of this study indicate that *A. lamarcki* associates with a different *Symbiodinium* community across its depth distribution in the northern Caribbean. Furthermore, this is the first report of the thermal tolerant *Symbiodinium trenchii* (LaJeunesse et al. 2014) recovered in shallow water colonies of *A. lamarcki*.

Symbiodinium OTUs C3 OTU1 and C11.N4 OTU2 were present in A. lamarcki colonies located at shallow and mesophotic depths; however C11.N4 OTU2 is most abundant at mesophotic depths indicating that A. lamarcki associates with C11.N4, previously described as a putative 'deep specialist' (Curaçao, Bongaerts et al. 2013). These data are consistent with the C11.N4/C11 DGGE profile recovered by Bongaerts et al. (2013). The Symbiodinium types C11.N4/C11 also occur in the deep-water species Agaricia grahamae and Agaricia undata (Bongaerts et al. 2015a, b). Noteworthy, Symbiodinium C3d (Bongaerts et al. 2013) was also present (C3d OTU3) in a few shallow water colonies of A. lamarcki from Mona Island, La Parguera, and St. Croix (Figure 1). These data parallel Symbiodinium diversity recovered in A. lamarcki from the southern Caribbean where two different DGGE ITS2 profiles were observed, C3/C3d or C3/C11 in shallow

reefs, transitioning to exclusively the C3/C11 profile at mesophotic depths (Bongaerts et al. 2013; Figure 1). The most common or dominant *Symbiodinium* ITS2 sequences representing OTUs in this study are identical to those found in other coral species; including other *Agaricia* spp. (C3), *Montastraea cavernosa, A. lamarcki* (C3d) (Lesser et al. 2010; Bongaerts et al. 2013; Barbrook et al. 2014), as well as *Scolymia cubensis, Mussa angulosa, Mycetophyllia ferox, M. lamarckiana* (C11) (LaJeunesse 2002; Finney et al. 2010), and more recently in *A. lamarcki, Agaricia undata,* and *Agaricia grahamae* (C11.N4/C11) (Bongaerts et al. 2013; Bongaerts et al. 2015a, b).

Depth zonation of genetically distinct Symbiodinium among reef-building corals has long been considered one of the most important factors driving the distribution of corals with wide depth distributions (Rowan and Knowlton 1995). A number of recent studies report host-symbiont depth specialization (Sampayo et al. 2007; Lesser et al. 2010; Bongaerts et al. 2013; Pochon et al. 2015), but a concise explanation as to which symbiotic partner is driving their depth distributions has not yet been provided. Furthermore, why some coral species with wide depth distributions exhibit symbiont zonation and others do not, is presently unclear (Bongaerts et al. 2015a; Pochon et al 2015). Conversely, a recent study combing ecological, physiological, and molecular data examined photoacclimation among four coral genera (Porites, Leptoseris, Pachyseris and Podabacia) and their symbionts along a mesophotic depth gradient in the Red Sea (Ziegler et al. 2015). Overall, general photoacclimation strategies were common among all coral host-symbiont combinations, such that both symbiont cell densities and photoprotective pigments to light harvesting pigment ratios significantly decreased with depth (Ziegler et al. 2015). Interestingly, the authors reported that the coral hosts have an effect on the photosynthetic pigment composition of their symbionts. For example, in a comparison of *Symbiodinium* type C1 in *Podabacia* and *Pachyseris*, the ß-carotene: chl a, peridinin: chl a, and diadinoxanthin: chl a ratios significantly differed over depth among host species. Ziegler et al. (2015) concluded that depth acclimation in corals is facilitated by symbiont physiology (e.g. *Symbiodinium* pigment composition and cell densities) which in turn is host-specific, as demonstrated by their analysis of different coral species associating with the same symbiont type. Their study underpins the importance of the ecological and physiological interactions of both corals and their *Symbiodinium* types to better leverage an understanding of the drivers in their distributions along mesophotic gradients (Ziegler et al. 2015).

This is the first report of physiologically tolerant *Symbiodinium trenchii* (e.g., D1a.N14 OTU9 this study) associating with *A. lamarcki* in shallow reefs. *Symbiodinium trenchii*, is endemic to the Indo-Pacific Ocean and new evidence shows that this species has quickly spread to corals throughout the Greater Caribbean on ecological timescales (Pettay et al. 2015). The authors revealed that Atlantic/Caribbean populations of *S. trenchii* have low genetic diversity and several widespread and genetically similar clones compared to Indo-Pacific populations (Pettay et al. 2015). Physiologically tolerant clade D *Symbiodinium* have received considerable attention for their potential role in mitigating the effects of changing ocean conditions (e.g., thermal anomalies, ocean acidification, and pollution) related to climate change and human activities. While corals hosting *S. trenchii* may be afforded some resilience to various environmental disturbances (Rowan 2004; Berkelmans & van Oppen 2006; Abrego et al. 2008; van Oppen et al. 2011b), there is a

tradeoff between increased survival and reduced growth (calcification) at the ecosystem scale and this has raised concerns over the long-term productivity and reef-building capacity among Caribbean coral reef communities (Ortiz JC et al. 2013; LaJeunesse et al. 2014; Pettay et al. 2015). Nonetheless, the presence of *Symbiodinium trenchii* in *A. lamarcki* could be a result of selection and or acclimation to three high thermal anomalies between 1998 and 2003, when bleaching was more intense at intermediated depths (10-30 m). In this case, *A lamarcki* bleached and recovered with only a few colonies showing partial mortality. During the 2005 intense bleaching event, *A. lamarcki* bleached but was resistant to mortality, in contrast with the other agaricids (i.e. *Undaria* spp.) who suffered high mortality at these depths (Weil et al. 2009). Nonetheless, the finding that *A. lamarcki* can associate with *Symbiodinium trenchii* in shallow reefs may impart some resilience during future environmental disturbance.

Collectively, the ecology of coral-symbiont assemblages from mesophotic studies establishes how the symbiont community is distributed in *A. lamarcki* over its depth range across a larger biogeographic range in the Caribbean. Overall, *Symbiodinium* diversity and the pattern of depth zonation in *A. lamarcki* sampled from Mona Island, La Parguera, and the US Virgin Islands is similar to that found in *A. lamarcki* in the southern Caribbean (Bongaerts et al. 2013). Continuing research into the biology and ecology of mesophotic ecosystems is essential to understanding adaptation and their roles as potential refugia in an era of rapid global environmental degradation. This work serves as an important baseline study for future resilience assessments.

2.6 Acknowledgments

This study was supported by National Oceanic Atmospheric Association's (NOAA) Center for Sponsored Coastal Ocean Research Award (No. NA06NOS4780190) to N. Schizas and E. Weil (Caribbean Coral Reef Institute) and National Science Foundation Grants (OCE 1105201 and IOS # 1017510) to E.Weil. Mesophotic cruises were supported by NOAA grants (NA10NOS4260223, NA11NOS4260157, and NA11NOS4260184) to the Caribbean Coral Reef Institute (CCRI) at the University of Puerto Rico, Mayaguez (UPRM). We thank the Department of Marine Sciences (UPRM) for logistical and lab support and the mesophotic dive team for coral samples (Clark Sherman, Milton Carlo, Michael Nemeth, IvonneBejarano, and Hector Ruiz), as well as David Anderson and Derek Soto for collecting and processing samples.
2.7 References

- Abrego D, Ulstrup KE, Willis BL, van Oppen MJH (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings of the Royal Society B: Biological Sciences*, 275: 2273–2282.
- Anderson MJ (2005) PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Auckland, New Zealand. pp. 1–23.
- Anderson MJ (2006) A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32–46.
- Apprill AM, Gates RD (2007) Recognizing diversity in coral symbiotic dinoflagellate communities. *Molecular Ecology*, 16: 1127–1134.
- Arif C, Daniels C, Bayer T, Banguera-Hinestroza E, Barborrk A, Howe CJ, LaJeunesse TC, Voolstra CR (2014) Assessing *Symbiodinium* diversity in scleractinian corals via nextgeneration sequencing-based genotyping of the ITS2 rDNA region. *Molecular Ecology*, 23: 4418-4433.
- Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology Evolution*, 16:37–48.
- Barbrook AC, Voolstra CR, Howe CJ (2014) The chloroplast genome of a *Symbiodinium* sp. clade C3 isolate. *Protist*, 165: 1–13.
- Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a "nugget of hope" for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences*, 273: 2305–2312.
- Bongaerts P, Ridgway T, Sampayo EM, Hoegh-Guldberg O (2010) Assessing the "deep reef refugia" hypothesis: focus on Caribbean reefs. *Coral Reefs*, 29, 309–327.
- Bongaerts P, Sampayo E, Bridge T, Ridgway T, Vermeulen F, Englebert N et al. (2011) *Symbiodinium* diversity in mesophotic coral communities on the Great Barrier Reef: a first assessment. *Marine Ecology Progress Series*, 439: 117–126.
- Bongaerts P, Frade PR, Ogier JJ, Hay KB, Bleijswijk J, Englebert N, Vermeij MJA, Bak RPM, Visser PM, Hoegh-Guldberg O (2013) Sharing the slope: depth partitioning of agariciid corals and associated *Symbiodinium* across shallow and mesophotic habitats (2-60 m) on a Caribbean reef. *BMC Evolutionary Biology*, 13: 205.

- Bongaerts P, Carmichael M, Hay KB, Tonk L, Frade PR, Hoegh-Guldberg O (2015a) Prevalent endosymbiont zonation shapes the depth distributions of scleractinian coral species. Royal Society Open Science, 2: 140297.
 - Bongaerts P, Frade PR, Hay KB, Englebert N, Latijnhouwers KRW, Bak RPM, Vermeij MJA, Hoegh-Guldberg O (2015b) Deep down on a Caribbean reef: lower mesophotic depths harbor a specialized coral-endosymbiont community. *Scientific Reports*, *5*, 7652.
- Chan YL, Pochon X, Fisher MA, Wagner D, Concepcion GT, Kahng SE, Toonen RJ, Gates RD (2009) Generalist dinoflagellate endosymbionts and host genotype diversity detected from mesophotic (67-100 m depths) coral *Leptoseris*. *BMC Ecology*, 9: 21.
- Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Austral J Ecol*, 18, 117–143.
- Cooper TF, Ulstrup KE, Dandan SS, Heyward AJ, Kühl M, Muirhead A, O'Leary RA, Ziersen BEF, van Oppen MJH. (2011) Niche specialization of reef-building corals in the mesophotic zone: metabolic trade-offs between divergent *Symbiodinium* types. *Proceedings of the Royal Society B: Biological Sciences*, 278: 1840–1850.
- Correa AMS, Baker AC (2009) Understanding diversity in coral-algal symbiosis: a clusterbased approach to interpreting fine-scale genetic variation in the genus *Symbiodinium*. *Coral Reefs*, 28, 81-93.
- Dempster EJ, Pryor KV, Francis D, Young JE, Rogers HJ (1999) Rapid DNA extraction from ferns for PCR-based analyses. BioTechniques 27: 62–64.
- Finney JC, Pettay DT, Sampayo EM, Warner ME, Oxenford HA, LaJeunesse TC (2010) The relative significance of host-habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus *Symbiodinium*. *Microbial Ecology*, 60: 250–263.
- Frade PR, De Jongh F, Vermeulen F, van Bleijswijk J, Bak RPM (2008) Variation in symbiont distribution between closely related coral species over large depth ranges. *Molecular Ecology*, 17: 691–703.
- Garren M, Walsh SM, Caccone A, Knowlton N (2006) Patterns of association between *Symbiodinium* and members of the *Montastraea annularis* species complex on spatial scales ranging from within colonies to between geographic regions. *Coral Reefs*, 25: 503–512.

- Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4: 1–9.
- Iglesias-Prieto R, Beltrán VH, LaJeunesse TC, Reyes-Bonilla H, Thomé PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. *Proceedings of the Royal Society B: Biological Sciences*, 271: 1757–1763.
- Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B: Biological Sciences*, 275: 1359–1365.
- LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology*, 141: 387–400.
- LaJeunesse TC, Bhagooli R, Hidaka M, DeVantier L, Done T, Schmidt G, Fitt W, Hoegh-Guldberg O (2004) Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. *Marine Ecology Progress Series*, 284: 147–161.
- LaJeunesse TC, Thornhill DJ (2011) Improved resolution of reef-coral endosymbiont (*Symbiodinium*) species diversity, ecology, and evolution through psbA non-coding region genotyping. *PLoS ONE*, 6: e29013. 10.1371/journal.pone.0029013.
- LaJeunesse TC, Wham DC, Pettay DT, Parkinson JE, Keshavmurthy S, Chen A (2014) Ecologically differentiated stress-tolerant endosymbionts in the dinoflagellate genus *Symbiodinium* (Dinophyceae) Clade D are different species. *Phycologia*, 53: 305-319.
- Lesser MP, Slattery M, Leichter JJ (2009) Ecology of mesophotic coral reefs. *Journal of Experimental Marine Biology and Ecology*, 375: 1–8.
- Lesser MP, Slattery M, Stat M, Ojimi M, Gates RD, Grottoli A (2010) Photoacclimatization by the coral *Montastraea cavernosa* in the mesophotic zone: light, food, and genetics. *Ecology*, 91: 990–1003.
- Ortiz JC, Gonzalez-Rivero M, Mumby PJ (2013) Can a thermally tolerant symbiont improve the future of Caribbean coral reefs? *Global Change Biology*, 19: 273–281.

- Pettay DT, Wham DC, Smith RT, Iglesias-Prieto R, LaJeunesse TC (2015) Microbial invasion of the Caribbean by an Indo-Pacific coral zooxanthellae. *Proceedings of the National Academy of Sciences of the United States of America*, 112: 7513-7518.
- Pochon X, Forsman ZH, Spalding HL, Padilla-Gamiño JL, Smith CM, Gates RD (2015) Depth specialization in mesophotic corals (*Leptoseris* spp.) and associated algal symbionts in Hawai'i. *Royal Society Open Science*, 2; 1-14. doi: 10.1098/rsos.140351.
- Rowan R, Knowlton N (1995) Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, 92: 2850–2853.
- Rowan R (2004) Thermal adaptation in reef coral symbionts. Nature, 430: 742.
- Rowan R, Knowlton N, Baker AC, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature*, 388: 265–269.
- Sampayo EM, Franceschinis L, Hoegh-Guldberg O, Dove S (2007) Niche partitioning of closely related symbiotic dinoflagellates. *Molecular Ecology*, 16: 3721–7233.
- Sampayo EM, Dove S, LaJeunesse TC (2009) Cohesive molecular genetic data delineate species diversity in the dinoflagellate genus *Symbiodinium*. *Molecular ecology*, 18: 500–519.
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, 75: 7537–7541.
- Sherman C, Appeldoorn R, Ballantine D, Bejarano I, Kesling CM, Nemeth M, Pagan F, Ruiz H, Schizas N, Weil E (2013) Exploring the mesophotic zone: Diving operations and scientific highlights of three research cruises across Puerto Rico and US Virgin Islands. *Proceedings of the Joint International Science Diving Symposium*, AAUS European Science Diving Panel, 297–312 pp.
- Sneath RR, Sokal PHA (1973) *Numerical taxonomy: the principles and practice of numerical classification*. W.H. Freeman and Company, San Francisco, California, United States of America, 573 pp.

- Stat M, Loh WKW, Hoegh-Guldberg O, Carter DA (2008) Symbiont acquisition strategy drives host–symbiont associations in the southern Great Barrier Reef. *Coral Reefs*, 27: 763–772.
- Stat M, Bird CE, Pochon X, Chasqui L, Chauka LJ, Concepcion G, Logan D, Takabayashi M, Toonen RJ, Gates RD (2011) Variation in *Symbiodinium* ITS2 sequence assemblages among coral colonies. *PLoS One*, 6:e15854. doi: 10.1371/journal.pone.0015854
- Stat M, Pochon X, Franklin EC, Bruno JF, Casey KS, Selig ER, Gates RD (2013) The distribution of the thermally tolerant symbiont lineage (*Symbiodinium* clade D) in corals from Hawaii: correlations with host and the history of ocean thermal stress. *Ecology and Evolution*, 3: 1317–1329.
- Stat M, Yost DM, Gates RD (2015) Geographic structure and host specificity shape the community composition of symbiotic dinoflagellates in corals from the Northwestern Hawaiian Islands. *Coral Reefs*, 34: 1075–1086. doi: 10.1007/s00338-015-1320-0.
- Thornhill DJ, LaJeunesse TC, Santos SR (2007) Measuring rDNA diversity in eukaryotic microbial systems: how intragenomic variation, pseudogenes, and PCR artifacts confound biodiversity estimates. *Molecular ecology*, 16: 5326–5340.
- Thornhill DJ, Kemp DW, Sampayo EM, Schmidt GW (2010) Comparative analyses of amplicon migration behavior in differing denaturing gradient gel electrophoresis (DGGE) systems. *Coral Reefs*, 29: 83–91.
- van Oppen MJH, Bongaerts P, Underwood JN, Peplow LM, Cooper TF (2011a) The role of deep reefs in shallow reef recovery: an assessment of vertical connectivity in a brooding coral from west and east Australia. *Molecular Ecology*, 20: 1647–1660.
- van Oppen MJH, Weeks S, O'Leary RA, Canto M, Radford B, Ulstrup KE, Cooper TF, Doyle J, Jones AM, Berkelmans R (2011b) Environmental factors controlling the distribution of *Symbiodinium* harboured by the coral *Acropora millepora* on the Great Barrier Reef. *PLoS One*, 6:e25536. doi: 10.1371/journal.pone.0025536.
- Ziegler M, Roder CM, Büchel C, Voolstra CR (2015) Mesophotic coral depth acclimatization is a function of host–specificity symbiont physiology. *Frontiers in Marine Science*, 2: 1-10.doi: 10.3389/fmars.2015.00004.

Weil E, Cróquer A, Urreiztieta I (2009) Temporal variability and consequences of coral diseases and bleaching in La Parguera, Puerto Rico from 2003-2007. *Caribbean Journal of Science*, 45: 221-246.

Chapter 3. Natural prey preferences and spatial variability of predation pressure by *Cyphoma gibbosum* (Mollusca: gastropoda) on octocoral communities off La Parguera, Puerto Rico

Lucas MQ, Rodriguez L, Sanabria D, Weil E. **ISRN Ecology, 2014** 742387: 1-3, doi: 10.1155/2014/742387

3.1 Abstract

This study evaluated the natural prey preferences and spatial variability of predation pressure (PP = proportion of colonies with snails and/or clear predation signs) by the gastropod Cyphoma gibbosum on octocoral communities off the La Parguera Natural Reserve, Puerto Rico. All octocoral colonies were checked for presence of C. gibbosum and/or clear predation signs in four permanent band-transects (2 X 10 m), along three depth intervals (0-5, 7-12, >15 m deep) in each of six reefs along an inshore offshore gradient. Results indicate that C. gibbosum preys on at least 16 species, six of which (Briareum asbestinum, Gorgonia ventalina, Pseudoterogorgia americana, P. acerosa, Plexaura flexuosa, and Pseudoplexaura porosa) consistently showed significantly higher (K-W,) (17-37%) PP compared to all other species. Plexaura flexuosa, P. americana, and P. porosa had significantly higher PP (11-38%) among inner and mid-shelf reefs, and G. ventalina had higher PP in shelf-edge reefs (16-20%). A combination of differential spatial distributions and octocoral species abundances seems to explain the observed patterns of predation by C. gibbosum. Prey preference and higher abundances of 3-dimensional octocorals providing increased refuge or microhabitats utilized for mating or egg-deposition could

be driving the spatial distribution of *C. gibbosum* and the observed differential predation pressure.

3.2 Introduction

Population outbreaks of coral predators are able to produce significant tissue/colony losses affecting overall productivity, population densities, and reef composition (Kayal et al. 2012) and can be responsible for hindering reef growth by reducing their prey abundances and distribution (Witman 1988). Many predators inhabiting reef environments fully consume their small prey, but for large modular organisms such as corals and octocorals, partial mortality due to predation is more common. Their ability to recover from partial colony mortality has contributed to their ecological and evolutionary success (Jackson and Coates 1986).

Octocorals around the Caribbean thrive in shallow and intermediate depths (1–10 m), usually dominating habitats on exposed reef platforms (Manrique-Rodriguez et al. 2006). They are subject to damage by storms and hurricanes and over the last decades, several species have suffered significant colony and/or partial mortalities due to outbreaks of diseases such as aspergillosis, black-band disease, red-band disease, bleaching, and other undescribed syndromes (Guzman and Cortes 1985; Santavy and Peters 1997; Nagelkerken et al. 1997a, 1997b; Nugues and Nagelkerken 2006; Kim and Harvell 2004; Weil 2004; Flynn and Weil 2009; Weil and Rogers 2011). It seems that prevalence, virulence, and impact of these and other coral diseases have been increasing due to climate warming and bleaching events (Cróquer and Weil 2009; Ruiz-Moreno et al. 2012). Furthermore, increased predation pressure (PP) resulting in scarring or wounds to the colony may lead to infections by potential pathogens compromising the recovery and survivorship of the colony and the population dynamics in the community (Witman 1998; Weil and Rogers 2011).

The dominance of octocorals (Cnidaria: Octocorallia) in shallow Caribbean reefs has been partially attributed to their chemical defenses, which significantly limits predation impacts (Pawlik et al. 1987; O'Neal and Pawlik 2002). As a result, there are only a few specialized predators known to intensely prey on octocorals (Pawlik et al. 1987). The gastropod, Cyphoma gibbosum (Mollusca: Ovulidae), is the main predator of octocorals in the Caribbean and can be found to inhabit shallow water coral reefs in the wider Caribbean and southern Atlantic Ocean from North Carolina, USA, to Brazil (Rehder 1981; Gerhart 1986; Nowlis 1993) [18–20]. A lack of intense predation on octocorals has been attributed to their ability to produce a diverse array of secondary metabolites or allelochemicals that deter predation by fish and other invertebrates (Rodriguez 1995). Octocoral structural and chemical defenses, such as sclerite content (Harvell and Suchanek 1987; Van Alstyne and Paul 1992), and secondary metabolites (i.e., prostaglandins) (Pawlik et al. 1987; Sammarco and Coll 1992), have been investigated, but they do not support the observed patterns of prey preferences in *C. gibbosum*.

Cyphoma gibbosum is considered a trophic generalist because it is known to graze on numerous species representing at least three octocoral families (Lasker et al. 1988) in spite of the toxic chemical defenses (allelochemicals) that they produce. *Cyphoma gibbosum*

moves along the substrate from one octocoral colony to the next grazing mostly on the base and axial areas removing small quantities of live tissue and leaving the exposed endoskeleton and tissue scars. Despite the ability of C. gibbosum to heavily graze on octocorals, their impact has largely been considered modest since it often results in only partial mortality of the colony and tissues can quickly regenerate under normal conditions (Yoshioka and B. B. Yoshioka 1991). In spite of this, immune-compromised or physiologically weak octocoral colonies may not recover effectively, or they may become infected with progressive colony mortality (Gerhart 1989). Harvell and Suchanek (1987) found that predation lesions by C. gibbosum vary from prey species to prey species, with superficial tissue damage in B. asbestinum and more drastic damage fully exposing the octocoral skeleton in Plexaura spp. Octocoral colonies normally harbor from one to three snails (Lasker et al. 1988); however, a population outbreak of *C. gibbosum* has been observed at Mona Island, where more than 150 snails were found grazing on large individual colonies resulting in total colony mortality (Schärer and Nemeth 2010).

A more recent study identified the ability of *C. gibbosum* to consume allelochemically rich octocoral prey may involve inducible biotransformation enzymes, such as cytochrome P450s (CYPs hereafter), that serve to detoxify allelochemicals defenses of octocorals (Whalen et al. 2010). On the other hand, predation on *C. gibbosum* is thought to be rare as most fishes find their mantle unpalatable and is generally ignored as a common prey item (Burkepile and Hay 2007). There are a few natural predators of *C. gibbosum*, such as the Caribbean spiny lobster (*Panulirus argus*), Hogfishes, (*Lachnolaimus maximus*) and

Pufferfishes, Tetraodontidae spp. (Burkepile and Hay 2007). Groupers (Family Epinephelidae) have also been found to prey on *C. gibbosum* but only because they are indiscriminate feeders (Sterrer 1992). Harvell and Fenical (1989) showed that the mantle provides protection through its distasteful or toxic qualities. This distastefulness or toxicity may arise from the sequestering of octocoral chemicals on which the snail feeds (Gerhart 1986; Lasker et al. 1988). A large-scale survey in the Florida Keys revealed that *C. gibbosum* abundances were greater in areas where large predatory fish were regularly harvested, concluding that the removal of top predators results in the release of their prey (Chiappone et al. 2003).

Previous studies have documented *C. gibbosum* grazing on a diverse array of octocoral species, mainly *Pseudoterogorgia* spp., *Pseudoplexaura* spp., *Plexaura homomalla*, and *G. ventalina* (Lasker et al. 1988). The spatial variability in the distributions of *C. gibbosum* foraging on octocorals has often been interpreted as feeding or prey preferences (Lasker et al. 1988). Likewise, *C. gibbosum* may select its octocoral prey for other than just food sources; they could be using it as refuge from predation and sites for mating and/or egg deposition (Gerhart 1986). Previous research exploring prey preferences of *C. gibbosum* on

octocoral communities has yielded disparate results and various authors report different octocoral species as the preferred prey. Kinzie (1970) found no preferences and related prey utilization to octocoral species abundances, whereas Birkeland and Gregory (1975) reported *Gorgonia* spp. and *Eunicea succinea* as preferred prey. At Salt River Canyon, St. Croix USVI, Harvell and Suchanek (1987) identified various species of *Plexaura* harboring snails for longer periods than expected and suggest that feeding preferences alone do not explain the foraging patterns of *C. gibbosum*. Instead, the authors partition the foraging patterns of *C. gibbosum* to various social behaviors (e.g., mating, egg deposition, feeding, and predator avoidance) (Harvell and Suchanek 1987). In Panama, *Pseudoterogorgia* spp.,

P. homomalla, and *P. porosa* were found to be the preferred prey (Lasker et al. 1988). This variability in food preferences seems to reflect sampling variation, temporal and geographical differences in preferences, and/or prey quality (Lasker et al. 1988). Furthermore, different behaviors can lead to misinterpretation of octocoral species as preferred prey including host species selection, residence time on the host, mating, egg deposition, and grazing rates (Lasker et al. 1988). The authors conclude that snail social interactions and predator avoidance behaviors play an important role in *C. gibbosum's* prey preferences, and rather than referring to octocorals as prey they may be best described as hosts (Lasker et al. 1988), serving a variety of the aforementioned ecological roles.

Predation by C. gibbosum on octocoral communities has never been assessed among the reefs of the La Parguera Natural Reserve, located off the southwest coast of Puerto Rico. Populations of most natural predators of C. gibbosum in this area have been overfished (reviewed in Ballantine et al. 2008), resulting in reduced predation pressure on this predatory snail. Coral and octocoral populations have been affected by several bleaching events and disease outbreaks over the last 20 years and a significant increase in octocoral predation signs by C. gibbosum has been observed (Cróquer and Weil 2009) (E. Weil pers. obs.) underpinning the potential impact of increased predation pressure of this snail over several species of common octocorals. The population outbreak of C. gibbosum, with high colony and tissue mortalities reported from the waters of Mona Island (Schärer and Nemeth 2010), serves as an example of the potential impact of this snail on local octocoral communities in the waters of the La Parguera Natural Reserve and other Caribbean localities. Therefore, it is important to assess the current status of populations of C. gibbosum and its common prey species. The objectives of this study were to (1) identify the natural prey preferences of C. gibbosum and (2) characterize the spatial variability of predation pressure by C. gibbosum on octocorals among La Parguera reefs. Furthermore, we address the potential impact of predation pressure on different octocoral species and the community. The results are discussed in light of previous research and the ecological factors potentially contributing to the natural prey preferences and the

spatial variability of predation pressure by *C. gibbosum* on the octocoral communities of southwestern Puerto Rico.

3.3 Materials and Method

Study location

Coral reefs of the La Parguera Natural Reserve are dispersed over an insular shelf extending 8–10 km offshore [36] consisting of three distinct inshore-offshore zones: (1) the inner reef zone that is mainly formed by shoreline fringing mangrove forests as well as fringing and patch reefs close to shore, (2) the mid-shelf zone characterized by reefs fringing along coral rubble and mangrove keys oriented from east to west at about 2-3 km from the coast, and (3) the shelf-edge zone which is characterized by deep spur and groove and bank reef formations (Irizarry-Soto and Weil 2009). For this study two inner reefs; Pelotas (17°57.442 N, 67°04.176 W) and Enrique (17°56.658 N, 67°02.213 W), two mid-shelf reefs; Media Luna (17°56.093 N, 67°02.931 W) and San Cristobal (17°56.501 N, 67°04.509 W), and two shelf-edge reefs; Weinberg (17°53.429 N, 66°59.320 W) and Old Buoy (17°53.380 N, 66°59.090 W) were selected (Figure 1). At each reef site, all octocoral colonies were checked for presence of *C. gibbosum* and/or predation signs in each of four haphazardly placed, permanent 20 m² band transects (10×2 m) in each of three depth habitats (3–5, 5–10, and >15 m) in the inner- and mid-shelf reefs. At the shelf-edge reefs, the 12 band-transects were distributed at depths between 18 and 25 m.



Figure 1: Site map of the La Parguera Natural Reserve located off the southwest coast of Puerto Rico showing the location of the six reefs surveyed in this study.

Predation pressure (PP) by C. gibbosum on octocorals

Predation pressure (PP hereafter) is herein defined as the proportion (%) of octocoral colonies harboring snails with signs of predation and/or octocorals with only clear and recent predation signs. Data collected included the species or genus of the octocoral colony with snails and/or predation signs, the number of snails on each colony, and the total number of octocorals within each band-transect. The proportions of snails per octocoral colony were estimated for each species and for the octocoral community. In

addition, the mean proportion (%) (±standard deviation or standard error) of colonies with predation signs and/or snails (PP) was estimated for each species in each habitat, within each reef, and zone. Octocoral species with no predation signs and/or with low population abundance were not included in downstream analyses (Table 1).

Table 1: Total octocoral species and the number of colonies harboring snails. The total number of band-transects (20 m²) surveyed in the entire study was 72.

Octocoral species	Total colonies	No. Snails	% Snails
Briareum asbestinum	130	16	12.3%
Pseudoterogorgia americana	827	61	7.3%
Plexaura flexuosa	532	34	6.3%
Gorgonia ventalina	1153	67	5.8%
Pseudoplexaura porosa	425	21	4.9%
Plexaura homomalla	90	4	4.4%
Plexaurella nutans	30	1	3.3%
Pseudoterogorgia acerosa	397	6	1.5%
Pterogorgia guadalupensis	2	1	-
Muricea spp.	1	1	-
Eunicea spp.	2	2	-
*Erythropodium caribaeorum	1	1	-
*Pterogorgia anceps	1	1	-
*Pterogorgia citrina	1	1	-
*Gorgonia flabellum	1	1	
*Gorgonia mariae	3	1	-

*Low abundant octocoral species and colonies observed with snails outside of band-transects were not considered in downstream analyses.

Data Analyses

Natural prey preferences of C. gibbosum

The occupancy patterns of *C. gibbosum* were evaluated to determine if the spatial variability in PP by *C. gibbosum* was related to the abundances (octocoral spp. density) of

preferred octocoral species. Ivlev's index of electivity was used to estimate natural prey preferences of *C. gibbosum* on octocoral species [33, 38]. Prey electivity, $(e) = (r_i - P_i)/(r_i + P_i)$, was estimated where r_i is the proportion of prey species *i* utilized and P_i is the proportion of prey species *i* available. Ivlev's index rates species utilization from -1 to +1, with -1indicating total rejection, 0 indicating that prey is taken in proportion to their abundances, and +1 indicating a preference of host species to the exclusion of others [33, 39]. Octocoral species abundances (densities) were estimated for each transect, habitat (depth), and reef by pooling the data. Spearman rank correlation analyses were performed to assess the relationship between octocoral abundances (density) and PP (α =0.05).

Spatial variability of predation pressure (PP)

To test the hypothesis that there is no spatial variability in *C. gibbosum* and octocoral population densities, data was tested for normality and equality of variance with the Shapiro-Wilk test, followed by a one-way ANOVA or Kruskal-Wallis (K-W) tests. To test the hypothesis that PP by *C. gibbosum* on the main octocoral species is similar among habitats within reefs, between reefs within zones, across reefs and reef zones, the mean proportion (%) values for PP for each spatial level (habitat, reefs, and zones) were calculated, arcsine transformed, and independently compared using one-way ANOVA or K-W tests. If significant differences were found, pairwise differences were tested with Tukey test. Statistical analyses were performed (α =0.05) using SigmaStat v.10.0 (Systat Software, San Jose, CA).

3.4 Results

Natural prey preferences of C. gibbosum

A total of 219 snails were found grazing on 16 different octocoral species spanning eight genera and four families (Briaridae, Plexauridae, Gorgonidae, and Anthothelidae). Of the 16 species, *B. asbestinum* (12.3%), *P. americana* (7.3%), *P. flexuosa* (7.1%), *G. ventalina* (5.8%), *P. porosa* (4.7%), and *P. acerosa* (1.5%) were the most frequently observed octocoral species harboring snails (Table 1). However, octocoral species with low abundances and colonies harboring snails outside of the experimental band-transects were not considered in downstream analyses (see Table 1).

Octocoral species densities were relatively uniform from inshore to offshore reefs (Table 2). Ivlev's prey electivity index rating (Brewley and Adey 1982) indicates that *C. gibbosum* preys on various octocoral species relative to their abundance or availability (Table 3).

Reef Zone	Inner-Shelf		Mid-Shelf		Shelf-edge	
Octocoral spp.	Pelotas	Enrique	San Cristobal	Media Luna	Old Buoy	Weinberg
B. asbestinum	0.07	0.09	0.05	0.19	0.04	0.15
G. ventalina	0.73	0.81	1.10	0.86	0.63	0.67
P. americana	0.94	0.98	0.65	0.42	0.18	0.13
P. acerosa	0.24	0.35	0.32	0.49	0.03	0.18
P. flexuosa	0.10	0.55	0.58	0.46	0.20	0.33
P. porosa	0.10	0.32	0.32	0.44	0.26	0.33

Table 2: Octocoral species densities (colonies/m²) for the six most affected octocoral species within each reef along an inshore to offshore gradient.

Inner Reefs	Occupancy		Availability		Electivity index	
Octocoral spp.	f	ri	f	Pi	(e=ri-Pi/ri+Pi)	Spp. utilization
B. asbestinum	0	0.000	25	0.019	-1.000	total rejection
G. ventalina	10	0.156	370	0.280	-0.284	total rejection
P. americana	33	0.516	496	0.375	0.158	prop. abundance
P. acerosa	4	0.063	141	0.107	-0.259	total rejection
P. flexuosa	8	0.125	154	0.117	0.033	prop. abundance
P. porosa	8	0.125	101	0.076	0.244	prop. abundance
P. homomalla	1	0.016	34	0.026	-0.238	total rejection
Total	64	1.0	1321	1.0		
Mid-Shelf Reefs	Occupancy		Availability		Electivity index	
Octocoral spp.	f	ri	f	Pi	(e=ri-Pi/ri+Pi)	Spp. utilization
B. asbestinum	5	0.125	58	0.040	0.515	prop. abundance
G. ventalina	9	0.225	470	0.327	0.185	prop. abundance
P. americana	10	0.250	255	0.177	0.171	prop. abundance
P. acerosa	0	0.000	194	0.135	-1.000	total rejection
P. flexuosa	10	0.250	250	0.174	0.179	prop. abundance
P. porosa	3	0.075	182	0.127	-0.257	total rejection
P. homomalla	3	0.075	28	0.019	0.596	prop. abundance
Total	40	1.0	1437	1.0		
Shelf-Edge Reefs	Occupancy		Availability		Electivity index	
Octocoral spp.	f	ri	f	Pi	(e=ri-Pi/ri+Pi)	Spp. utilization
B. asbestinum	11	0.105	47	0.059	0.280	prop. abundance
G. ventalina	48	0.457	313	0.393	0.075	prop. abundance
P. americana	18	0.171	76	0.095	0.286	prop. abundance
P. acerosa	2	0.019	62	0.078	0.603	prop. abundance
P. flexuosa	16	0.152	128	0.161	-0.029	total rejection
P. porosa	10	0.095	142	0.178	-0.304	total rejection
P. homomalla	0	0.000	28	0.035	-1.000	total rejection
Total	105	1.0	796	1.0		

Table 3: Ivlev's prey electivity index for each reef zone for octocoral species harboring snails. Prey election, electivity (*e*) = $(r_i - P_i)/(r_i + P_i)$, where r_i is the proportion of prey species *i* utilized and P_i is the proportion of prey species *i* available.

Spearman rank correlations (*Rs* coefficient) between octocoral occupancy (colonies with snails) and octocoral availability (palatable prey) covaried significantly at inner (*Rs*=0.94, p<0.05, P=0.006) and shelf-edge reefs (*Rs*=0.68, p<0.05, P=0.003), suggesting that as palatable octocoral availability increases, snail occupancy also increases (Table 3). In contrast, there was no significant correlation in the mid-shelf reefs. Mid-shelf reefs had the fewest snails (n=40) and the highest number of octocorals surveyed (n=1421 colonies) of all reefs (Table 3).

Spearman rank correlations between mean (%) PP and densities of each octocoral species showed no significant covariation for most species (Table 4). Although, there was a significant (Rs=0.09, p<0.05, P=0.02) positive covariation between mean (%) PP and octocoral density for *P. porosa* and significant negative (Rs= -0.94, p<0.05, P=0.02) covariation for *G. ventalina* (Table 4). No significant correlations were found between mean (%) PP and the pooled densities for all octocoral species within each reef (Table 5). These results indicate that along an inshore-offshore gradient, *C. gibbosum* largely preys on six different octocoral species relative to the proportion of their abundance in the communities off La Parguera, Puerto Rico.

Octocoral <i>spp</i> .	Rs	P-value			
B. asbestinum	0.09	0.80			
*G. ventalina	-0.94	0.02			
P. americana	0.14	0.80			
P. acerosa	0.09	0.92			
P. flexuosa	-0.26	0.66			
<u>*P. porosa</u>	0.90	0.02			
*Significant values (p< 0.05)					

Table 4: Spearman rank correlations between the mean (%) PP and octocoral species density for the six most affected species across all reefs.

			<u>Reefs</u>			
Spearman Rank	Pelotas	Enrique	San Cristobal	Media Luna	Old Buoy	Weinberg
*Rs (rho)	0.49	0.26	-0.73	-0.20	0.54	0.12
P-value	0.36	0.66	0.10	0.66	0.30	0.80

*Rs coefficient = Spearman Rank Coefficient

*No. Samples / correlation (n = 6 reefs)

Spatial Variability of Predation Pressure (PP) by C. gibbosum

Snail densities reached (0.15 ind. /m²) among 3,589 octocoral colonies (2.5 ind. /m²) within the 72 band-transects surveyed in this study (Table 1). Overall, six octocoral species (Briareum asbestinum, Pseudoterogorgia americana, Plexaura flexuosa, Gorgonia ventalina, Pseudoterogorgia acerosa and Pseudoplexaura porosa) were the most affected by snail predation (Table 6, Figure 2A). Snails were most often found at the base of the colonies or along the main axis, with few along the branches of stiliform, plume-like or candelabra-like growth forms. For the species G. ventalina, snails were generally found at the base and along the main axes with fewer snails eating on the open fan structure (Figure 2B). When pooling all octocorals sampled, no significant differences in C. gibbosum densities and/or PP were found among depth habitats within reefs (data not shown). Similarly, no significant differences in snail densities and/or PP were found between reefs within each zone (data not shown). In contrast, the shelf-edge zone had significantly higher (K-W, p<0.05, P=0.001) snail densities and PP compared to the innerand mid-shelf reefs (Table 6, Figure 3).

In general, there was high variability in PP within each of the preferred species across reefs. Briareum asbestinum and G. ventalina were the only species with significant differences in PP across reefs. Briareum asbestinum had significantly (K-W, p<0.05, P=0.024) higher PP at Weinberg (21.9%) compared to the other five reefs, while G. ventalina was significantly (K-W, p<0.05, P=0.001) higher PP at Weinberg and the Old Buoy (16-20%) compared to reefs in the inner and mid-shelf zones (2-4%) (Table 6, Figure 4). Pseudoterogorgia americana had the highest PP in Pelotas (32.9%) compared to Enrique (lowest) (6.7%), and PP was similar in all the other reefs (Table 6, Figure 4). Pseudoplexaura porosa (13.7-37.3%), P. flexuosa (16-26.7%), and P. acerosa (2.8-17.4%) showed high, but not significant variability in PP across reefs (Table 6, Figure 4). This lack of statistical significance is mainly due to the high variance around the means produced by differential levels of PP in the sampling units (i.e., some reefs had transects with few or no colonies with PP, while others had numerous colonies with high PP).

No species-specific patterns were observed when comparing PP among species within reefs (Table 6, Figure 5). *Briareum asbestinum* had significantly (K-W, p<0.05) lower PP (5.6%) in the Old Buoy compared to *P. flexuosa, P. americana* and *P. porosa* in Media Luna (Table 6, Figure 5). *Plexaura flexuosa* and *P. porosa* had significantly (K-W, p<0.05) higher PP compared to *G. ventalina, P. acerosa,* and *B. asbestinum* in Enrique, Media Luna and San Cristobal, but were similar to the other species in the two shelf-edge reefs (Table 6, Figure 5). *Pseudoterogorgia americana* had significantly higher (K-W, p<0.05) PP compared *G.*

ventalina and *B. asbestinum* in Pelotas and Media Luna reefs. *Pseudoplexaura porosa* and *P. flexuosa* had significantly higher (K-W, p<0.05) PP than *G. ventalina* and *P. acerosa* in the mid-shelf reefs and no significant differences in PP were found across species in both shelf-edge reefs (Table 6, Figure 5).

Colonies # Colonies (PP) Species Reef **# Transects** Mean % SD SE G. ventalina Pelotas 12 175 9 4.0% 0.04 0.01 9 G. ventalina 12 195 3.8% 0.05 Enrique 0.01 9 G. ventalina San Cristobal 12 263 2.8% 0.04 0.01 G. ventalina Media Luna 12 207 3.2% 0.05 11 0.01 G. ventalina Old Buoy 12 152 32 20.7% 0.19 0.05 G. ventalina Weinberg 12 161 25 16.3% 0.12 0.04 # Colonies (PP) <u># Transects</u> # Colonies Species <u>Reef</u> Mean % <u>SD</u> SE Pelotas 12 58 3 17.4% 0.39 0.11 P. acerosa 3 12 83 6.3% 0.19 0.05 P. acerosa Enrique 12 1 P. acerosa San Cristobal 118 2.8% 0.10 0.03 P. acerosa Media Luna 12 76 1 4.2% 0.14 0.04 12 0 0.0% 0.00 P. acerosa Old Buoy 18 0.00 P. acerosa Weinberg 12 44 3 9.5% 0.19 0.06 **Species** Reef **# Transects # Colonies** # Colonies (PP) Mean % SD <u>SE</u> Pelotas 12 25 13.7% P. porosa 6 0.28 0.08 P. porosa Enrique 12 76 15 23.6% 0.25 0.07 12 106 14 15.7% 0.14 P. porosa San Cristobal 0.04 12 76 0.35 P. porosa Media Luna 21 37.3% 0.10 12 0.22 P. porosa Old Buoy 62 11 15.0% 0.06 Weinberg 12 80 9 19.5% 0.19 0.05 P. porosa Species Reef **# Transects # Colonies** # Colonies (PP) Mean % SD SE P. flexuosa Pelotas 12 23 3 26.7% 0.39 0.11 23 24.2% P. flexuosa Enrique 12 131 0.32 0.09 P. flexuosa San Cristobal 12 110 14 20.9% 0.28 0.08 12 0.25 P. flexuosa Media Luna 140 12 26.0% 0.07 P. flexuosa 12 48 16.7% 0.26 Old Buoy 8 0.08 12 P. flexuosa Weinberg 80 21.0% 0.20 0.06 11 # Colonies # Colonies (PP) Species Reef # Transects Mean % SD SE P. Americana Pelotas 12 261 18 32.9% 0.32 0.09 6.7% 12 14 0.19 P. Americana Enrique 235 0.05 P. Americana San Cristobal 12 155 14 11.2% 0.17 0.05 12 21 0.32 P. Americana Media Luna 100 21.6% 0.09 P. Americana Old Buoy 12 45 10 22.9% 0.29 0.08 P. Americana Weinberg 12 31 10 14.0%0.27 0.07 Reef # Transects # Colonies # Colonies (PP) Mean % SD SE Species 12 0.0% B. asbestinum Pelotas 4 0 0.0 0.00 0 B. asbestinum Enrique 12 21 0.0% 0.0 0.00 5 B. asbestinum San Cristóbal 12 12 16.7% 0.31 0.09 B. asbestinum Media Luna 12 0 5.6% 0.15 0.04 46 B. asbestinum Old Buoy 12 10 2 5.6% 0.13 0.04 9 B. asbestinum Weinberg 12 37 21.9% 0.28 0.08

Table 6: Mean percent (%), standard deviation and standard error of (PP) (K-W; α =0.05) for the six octocoral species showing the highest levels of (PP) within and across reefs.



Figure 2A: Photographs showing the snail *Cyphoma gibbosum* grazing on several species of octocoral. Two snails each on a single colony of *P. americana, P. homomalla* and *P. acerosa* (A, B, C). A branch of *P. porosa* with a large portion of tissue eaten by one snail (D). Snail eating tissue from *B. asbestinum* (E, F) and *P. nutans* (G). (Photo credit: E.Weil).



Figure 2B: Photographs showing a medium size colony of *G. ventalina* with four snails and scar areas produced by its feeding activity on the main axis and blade (A, B). Close up of the damage produced by the snail feeding (C, D), and a large colony showing the impact of predation activity of one snail along the main axes and the blade (E). These damaged tissue areas are susceptible to infections by pathogens and/or could be rapidly colonized by algae, sponges or *Millepora* which could increase sea fan tissue mortality (Photo credit: E.Weil).



Figure 3: Mean (%) predation pressure by *C. gibbosum* on the octocoral community (pooled data) for each reef zone (upper left) and reef locality (bottom left) and their associated mean *C. gibbosum* densities (right column).



Figure 4: Mean (%) predation pressure variability by *C. gibbosum* on the most affected octocoral species across reefs in La Parguera. Error bars represent the standard error. **Reef Sites:** *PL*= Pelotas; *EN*= Enrique; *SC*= San Cristobal; *ML*= Media Luna; *OB*= Old Buoy; *WB*= Weinberg.



Figure 5: Variability in mean (%) PP by *C. gibbosum* across the six major octocoral prey species within each reef off La Parguera. Error bars represent the standard error.

3.5 Discussion

This study found no consistent patterns in the levels of PP across the inshoreoffshore gradient for all octocoral species surveyed. Nonetheless, the sea fan *G. ventalina* showed the highest PP in the two offshore reefs where its own densities and that of the other branching and plume-like species were lower than in mid- and inner- shelf reefs. However, *G ventalina* was the most abundant octocoral (accounting for 40%) of all octocorals surveyed in the spur and groove formations of the shelf-edge zone.

Predation on C. gibbosum by fish and other invertebrates has been proposed as one of the mechanisms controlling its abundances and distribution (Chiappone et al. 2003; Burkepile and Hay 2007), and therefore, may be influencing the differential distribution of C. gibbosum's PP on octocorals. The high abundances of snails on sea fans in the two offshore reefs may indicate a lack of significant PP on the snail due to overfishing in combination with low densities of the snail's other preferred octocoral prey. Results for the other reefs suggests that if G. ventalina and the other preferred species have similar and/or high densities, C. gibbosum prefers them over G. ventalina, which showed significantly lower PP in these reefs. This raises the question as to whether prey preference is driven by the quality of the food supply, the refuge provided by the structurally more complex plume and branching species, or other ecological functions, such as mating or egg deposition sites that the octocoral host provides the snail (Lasker et al. 1988) and how is it regulated by natural predation pressure on the snail. Burkepile and Hay (2007) investigated the impact of snail predators using cage and uncaged treatments and showed that, when large predators were excluded, there were

significant increases in snail densities and predation on octocorals. The removal of large predators allowed an increase of *C. gibbosum* abundances (19x) consequently resulting in an increase in predation on octocorals. They further stated that a (0.8 meter) tall colony of *Eunicea calyculata* displayed approximately 75% live tissue mortality (Burkepile and Hay 2007).

While the impact of C. gibbosum is considered minimal, given the intense fishing pressures and the rapid removal of their predators, there is precedent for concern of outbreaks negatively affecting octocoral communities. Reefs in La Parguera continue to be heavily overfished with significant changes in species composition and fish community structure in coral reefs (Ruiz-Moreno et al. 2012; Ballantine et al. 2008), which may explain the observed increase in population densities of the snail in the area over the years (E. Weil pers. obs.). This (overfishing), in combination of successful reproductive events could increase the likelihood of snail population outbreaks, such as the one observed at Mona Island in (Schärer and Nemeth 2010), which could lead to significant increase in PP on the octocoral community of La Parguera's reefs. A similar condition has been observed in another coral predator by the gastropod Coralliophila abbreviata, which has a wide list of scleractinian coral prey (Brawley and Adey 1982; Ott and Lewis 1972; Bruckner et al. 1997). Bruckner et al. (1997) followed the movements and feeding rates of *C. abbreviata* on *Acropora palmata* off La Parguera. Their findings showed that a combination of reduced A. palmata densities due to the white band disease epizootic, environmental disturbances, and overfishing of natural predators of the snail could increase abundances and significant predation damage by C. abbreviata, contributing

to the demise of *A. palmata* colonies inhabiting the inshore reefs of southwestern Puerto Rico (Baums et al. 2003). Similarly, a 75% decrease in *A. palmata* colonies in the Florida Keys occurred after Hurricane Georges (1998), followed by a doubling of the proportion of colonies infested with snails (from 19% to 46%), and an increase in snail density per infested colony as snails concentrated on surviving *A. palmata*, which significantly increased the predation impact on *A. palmata* populations (Baums et al. 2003).

Overall, the spatial variability of PP and Ivlev's index of electivity suggests that *C. gibbosum* naturally preys on *B. asbestinum, P. americana, P. acerosa, P. flexuosa, G. ventalina,* and *P. porosa* relative to their abundances or availability across reefs. Although, Ivlev's index weights electivity for rare species disproportionately, the combined results of PP and electivity in this study corroborate the notion that prey preference seem to be related to octocoral abundances, rather than feeding preferences alone. In addition, correlation analyses between mean (%) PP and the density of individual octocoral species within each reef revealed no significant covariation for most species, however. In contrast, there was a weak negative correlation between the mean (%) PP and the densities of *G. ventalina*. Similarly, there was a strong positive correlation between the mean (%) PP and the densities of *P. porosa* across all reefs, inshore to offshore reefs.

These data indicate that PP by *C. gibbosum* predation increases as octocoral abundances decrease. The result corresponds with the observation that *C. gibbosum* differentially preys or utilizes different octocoral species relative to their abundances. For example, when stiliform and plume-like or candelabra-like growth forms are abundant

and readily available they are preyed on more frequently than the openly exposed fans of *G. ventalina*, which also renders them vulnerable to predation by fish, lobsters, etc. In addition, there were no significant correlations between the mean (%) PP and the pooled snail densities for all octocoral species within each reef, thus supporting the notion that when octocoral species abundances vary, predation by *C. gibbosum* will vary accordingly. In general, these results suggest that the natural prey preferences of *C. gibbosum* might be linked to a combination of differential spatial distributions and octocoral species abundances, feeding preferences of *C. gibbosum*, and predation pressure on snail populations.

Chiappone et al. (2003), suggested for spur and groove reefs in Florida that most octocoral hosts are readily available and probably not food limited due to C. gibbosum's ability to tolerate high levels of octocoral chemical defenses (Vrolijk and Targett 1992). Whalen et al. (2010) investigated the genetic diversity, transcriptional responses, and enzymatic activities of cytochrome P450s (CYPs) in seven octocoral species potentially linked to detoxification of allelochemicals in C. gibbosum. The authors link the induction of specific CYP transcript expression and corresponding enzymatic activity in C. gibbosum to differences in octocoral prey (Whalen et al. 2010). Specifically, their results show that only snails consuming *P. homomalla* demonstrated greater induction of CYP transcripts (2.7- to 5.1-fold), as well as a corresponding increases in the metabolic activity of eicosanoid LTB4 (i.e., a prostaglandin that serves as a feeding deterrent molecule) in the digestive gland of snails (Whalen et al. 2010). Their findings are consistent with previous research indicating that the tissues of the Caribbean genus *Plexaura* contain high concentrations of prostaglandins (Whalen et al. 2010; Schneider et al. 1977). The authors conclude the possibility that allelochemicals in *P. homomalla* induce *Cyphoma* CYP4 enzymes and may serve to detoxify chemical defenses of octocorals. Although their findings are no indication of prey preferences in *C. gibbosum*, predation on *Plexaura* species rich in unique prostaglandins may serve as an evolutionary innovation in *C. gibbosum*'s ability to tolerate allelochemically rich octocoral prey (Whalen et al. 2010), as well as securing prey that is not readily available to other predators. Future *in-situ* and laboratory experiments may provide better insight on more specific prey preferences and increase our understanding of the feeding behavior, ecological dynamics, and the potential of *C. gibbosum* to regulate octocoral community structure in Caribbean reef communities.

Overall, the predatory snail *C. gibbosum* was observed on 16 different octocoral species spanning eight genera and four families (Briaridae, Plexauridae, Gorgonidae, and Anthothelidae). Although this study lacks temporal observations, the spatial distribution (differential abundances) of preferred octocoral species, local ecological conditions, and reduced snail predation pressure are the most parsimonious factors explaining predation patterns of *C. gibbosum* on the octocoral communities off the coast of La Parguera. The predatory or foraging behavior of *C. gibbosum* is not likely to have detrimental impact on octocoral populations under low snail population densities and high prey densities; however, population outbreaks of the snail or high mortalities of preferred prey or a combination of these are bound to significantly increase the impact of predation by the snail on octocoral communities. In La Parguera, overfishing has

removed most snail predators and there is concern over population outbreaks of invertebrate corallivorous species, such as *C. gibbosum* and *C. abbreviata*, resulting in increased predation to octocoral and coral colonies, loss of reproductive tissue and a source of vectoring diseases. Future work should include long-term laboratory and *in situ* studies that investigate the partitioning of ecological behaviors, including mating, egg deposition, as well as foraging patterns and prey preferences across octocoral communities. Monitoring octocoral communities and their predators will be increasingly important in broadening our understanding of the complex trophic interactions among Caribbean coral reef communities.

3.6 Acknowledgements

The authors thank the Department of Marine Sciences, UPR-Mayaguez for funding and logistics. Ernesto Weil also provided partial funding. We also thank Meridith Lucas and Diane Wiel for providing helpful comments that improved the manuscript.

3.7 References

- Ballantine DL, Appeldoorn RS, Yoshioka P et al. (2008) Biology and ecology of Puerto Rican coral reefs. In *Coral Reefs of the USA*, Eds., Riegl BM, Dodge RE, Springer: Berlin, Germany, pp. 375-406.
- Baums IB, Miller MW, Szmant AM (2003) Ecology of a corallivorous gastropod, *Coralliophila abbreviata*, on two scleractinian hosts, 1: population structure of snails and corals. *Marine Biology*, 142: 1093-1101.
- Birkeland C, Gregory B (1975) Foraging behavior and rates of feeding of the gatropod, *Cyphoma gibbosum* (Linnaeus). *Bulletin of Natural History Museum Los Angeles*, 20: 57-67.
- Brewley SH, Adey W (1982) *Coralliophila abbreviata:* a significant corallivore. *Bulletin of Marine Science*, 32: 595-599.

- Bruckner RJ, Bruckner AW, Williams Jr EH (1997) Life history strategies of *Coralliophila abbreviata* Lamarck (Gastropoda: Coralliophilidae) on the southwest coast of Puerto Rico. *Proceedings 8th Coral Reef Symposium*, 1: 627-632.
- Burkepile DE, Hay ME (2007) Predator release of the gastropod *Cyphoma gibbosum* increases predation on gorgonian corals. *Oecologia*, 154: 167-173.
- Chiappone M, Dienes H, Swanson DW, Miller SL (2003) Density and gorgonian hostoccupation patterns by flamingo tongue snails (*Cyphoma gibbosum*) in the Florida Keys, *Caribbean Journal of Science*, 39: 116-127.
- Cróquer A, Weil E (2009) Change in Caribbean coral disease prevalence after the 2005 bleaching event. *Diseases of Aquatic Organisms*, 87: 33-43.
- Flynn K, Weil E (2009) Variability of aspergillosis in *Gorgonia ventalina* in La Parguera, Puerto Rico. *Caribbean Journal of Science*, 45: 215-220.
- Gerhart DJ (1986) Gregariousness in the gorgonian-eating gastropod *Cyphoma gibbosum*: tests of several possible causes. *Marine Ecology Progress Series*, 31: 255-263.
- Gerhart DJ (1989) Fouling and gastropod predation: consequences of grazing for a tropical octocoral. *Marine Ecology Progress Series*, 62: 103-108.
- Guzmán HM, Cortés J (1985) Mortality of *Gorgonia flabellum* Linneaus (Octocorallia: Gorgoniidae) on the Caribbean coast of Costa Rica. *Revista de Biologia Tropical*, 60: 304-308.
- Harvell CD Fenical W (1989) Chemical and structural defenses of Caribbean octocorals (*Pseudopterogorgia* spp.): Intra colony localization of defense. *Limnology and Oceanography*, 34: 382-389
- Harvell CD, Suchanek TH (1987) Partial predation on tropical octocorals by *Cyphoma* gibossum (Gastropoda). *Marine Ecology Progress Series*, 38: 37-44.
- Irizarry-Soto E, Weil E (2009) Spatial and temporal variability in juvenile coral densities, survivorship and recruitment in La Parguera, southwestern Puerto Rico. *Caribbean Journal of Science*, 45: 269-281.
- Jackson JB, A.G Coates (1986) Life cycles and evolution of clonal (modular) animals. *Philosophical Transactions of the Royal Society*, 313: 7-22.
- Kayal M, Vercelloni V, Lison de Lomi T, et al. (2012) Predator crown-of-thorns starfish (*Acanther planci*) outbreak, mass mortality of corals, and cascading effects on reef fish benthic communities. *PLoS One*, 7: e47363
- Kim K, Harvell CD (2004) The rise and fall of a 6 year coral-fungal epizootic. *American Naturalist*, 164: 52–63.
- Kinzie RA (1970) The ecology of the gorgonians (Cnidaria, Octocorallia) of Discovery Bay, Jamaica. *Ph.D. Dissertation*. Yale University, New Haven, CT, USA.
- Lasker HR, Coffroth MA, Fitzgerald LM (1988) Foraging patterns of *Cyphoma gibbosum* on octocorals: the roles of host choice and feeding preference. *Biological Bulletin*, 174: 254-266.
- Manly BF, McDonald LL, Thomas DL (1993) Resource selection by animals: Statistical design and analysis for field studies. Chapman & Hall, NY, pp. 177.
- Manrique-Rodriguez N, Bejarrano Chavarro S, Garzon-Ferreira J (2006) Growth of the sea fan *Gorgonia ventalina* (Linnaeus, 1758) (Cnidaria Gorgoniidae) of Santa Marta, Colombian Caribbean. *Bulletin of Marine and Coastal Research*, 35: 77-90.
- Nagelkerken I, Buchan K, Smith GW et. al. (1997 a) Widespread disease in Caribbean sea fans I. Spreading and general characteristics. *Proceedings of the 8th Int Coral Reef Symposium*, 1: 679-682.
- Nagelkerken I, Buchan K, Smith GW, et al. (1997 b) Widespread disease in Caribbean sea fans II. Patterns of infection, and tissue loss. *Marine Ecological Progress Series*, 160: 255-263.
- Nowlis JP (1993) Mate-and oviposition-influenced host preferences in the coral-feeding snail *Cyphoma gibbosum*. *Ecology*, 74: 1959-1969.
- Nugues MM, Nagelkerken I, (2006) Status of aspergillosis and sea fan populations in Curaçao ten years after the 1995 Caribbean epizootic. *Revista Biologia Tropical*, 54: 153-160.
- O'Neal W, Pawlik JR (2002) A reappraisal of the chemical and physical defenses of Caribbean gorgonian corals against predatory fishes. *Marine Ecology Progress Series*, 240: 117-126.
- Ott B, Lewis JB (1972) The importance of the gastropod *Coralliophila abbreviata* (Lamarck) and the polychaete *Hermodice carunculata* (Pallas) as coral reef predators. *Canadian Journal of Zoology*, 50: 1651-1656.
- Pawlik JR, Burch MT, Fenical W (1987) Patterns of chemical defense among Caribbean gorgonian corals: a preliminary survey. *Journal of Experimental Marine Biology and Ecology*, 108: 55-66.
- Rehder HA (1981) Field Guide to Shells: North America. *National Audubon Society,* Alfred Knopf. NY, pp. 483.
- Rodriguez AD (1995) The natural products chemistry of west indian gorgonian octocorals. *Tetrahedron*, 16: 4571-461.

- Ruiz-Moreno D, Willis BL, Page AC et al. (2012) Global coral disease prevalence associated with sea temperature anomalies and local factors. *Disease of Aquatic Organisms*, 100: 249-261.
- Sammarco PW, Coll JC (1992) Chemical adaptations in the Octocorallia: evolutionary considerations. *Marine Ecology Progress Series*, 88: 93-104.
- Santavy DL, Peters EC (1997) Microbial Pest: Coral Disease in the Western Atlantic. *Proceedings of the 8th Int Coral Reef Symposium*, 1: 607-612.
- Schärer MT, Nemeth MI, "Mass mortality of gorgonians due to a *Cyphoma gibbosum* (Linnaeus) population outbreak at Mona Island, Puerto Rico. *Coral Reefs*, 29: 533, 2010.
- Schneider WP, Bundy GL, Lincoln FH, Daniels EG, Pike JE (1997) Isolation and chemical conversions of prostaglandins from *Plexaura homomalla*: preparation of prostaglandin E2, prostaglandin F2 and their 5,6-trans-isomers. *Journal of the American Chemical Society*, 99: 1222-1232.
- Sterrer W, Bermuda's Marine Life, Bermuda Island Press, v: 133.
- Van Alstyne KL, Paul VJ (1992) Chemical and structural defenses in the sea fan *Gorgonia ventalina*: effects against generalist and specialist predators. *Coral Reefs*, 11: 155-159.
- Vrolijk NH, Targett NM (1992) Biotransformation enzymes in *Cyphoma gibbosum* (Gastropoda: Ovulidae): implications for detoxification of gorgonian allelochemicals. *Marine Ecological Progress Series*, 88: 237-246.
- Weil E (2004) Coral reef diseases in the wider Caribbean, *in Coral Reef and Disease*, E. Rosemberg and Y. Loya, Eds., Springer-Verlag, pp. 35-68.
- Weil E, Rogers C (2011) Coral Reef Diseases in the Atlantic-Caribbean, in Coral Reefs: An Ecosystem in Transition, Eds., Z. Dubinski and N. Strambler, Springer-Verlag, pp. 465-491.
- Whalen KE, Starczak VR, Nelson DR et al. (2010) Cytochrome P450 diversity and induction by gorgonian allelochemicals in the marine gastropod *Cyphoma gibbosum*. *BMC Ecology*, 10: 24.
- Witman JD (1988) Effects of predation by the fire worm *Hermodice carunculata* on Milleporid hydrocorals. *Bulletin of Marine Science*, 42: 446-458.
- Yoshioka PM, Yoshioka BB (1991) A comparison of the survivorship and growth of shallow water gorgonian species of Puerto Rico. *Marine Ecology Progress Series*, 69: 253–26.

Chapter 4. Linking coral reef remote sensing and field ecology: it's a matter of scale. Lucas MQ & Goodman JG (2015) **J. Mar. Sci. Eng.** 3(1), 1-20; doi: 10.3390/jmse3010001

4.1 Abstract

Remote sensing shows potential for assessing biodiversity of coral reefs. Important steps in achieving this objective are better understanding the spectral variability of various reef components and correlating these spectral characteristics with field-based ecological assessments. Here we analyze >9400 coral reef field spectra from southwestern Puerto Rico to evaluate how spectral variability and, more specifically, spectral similarity between species influences estimates of biodiversity. Traditional field methods for estimating reef biodiversity using photoquadrats are also included to add ecological context to the spectral analysis. Results show that while many species can be distinguished using in situ field spectra, the addition of the overlying water column significantly reduces the ability to differentiate species, and even groups of species. This indicates that the ability to evaluate biodiversity with remote sensing decreases with increasing water depth. Due to the inherent spectral similarity amongst many species, including taxonomically dissimilar species, remote sensing underestimates biodiversity and represents the lower limit of actual species diversity. The overall implication is that coral reef ecologists using remote sensing need to consider the spatial and spectral context of the imagery, and remote sensing scientists analyzing biodiversity need to define confidence limits as a function of both water depth and the scale of information derived, e.g., species, groups of species, or community level.

4.2 Introduction

Remote sensing has become increasingly important among the fields of ecology, biodiversity, and conservation, appealing to scientists by providing repeat temporal observations over broad spatial scales and offering relative simplicity for acquiring data over large areas, as compared to extensive fieldwork (Kerr et al. 2003; Turner et al. 2003; Wang et al. 2010). Given numerous anthropogenic stressors, in conjunction with recent climate change, the species range and composition of various marine and terrestrial communities are rapidly declining (Walther et al. 2002; Hoegh-Guldberg et al. 2002; Ateweberhan et al. 2013). Thus, rapid and repeatable assessment of coral reef ecosystems has become an important objective for monitoring the associated impacts of climate change on coral reef communities (Scopélitis et al. 2009, 2010; Goodman et al. 2007; Joyce et al. 2013). In this regard, remote sensing technologies provide valuable information related to the spatial analysis of ecosystem properties, including habitat composition, biodiversity, and the design of marine protected areas (Green et al. 2009; Dalleau et al. 2010; Eakin et al. 2010). Further, the available remote sensing tools and analysis techniques are ever evolving, and new capabilities continue to emerge, including sensors with improved spatial and spectral resolution (Wang et al. 2010; Medina et al. 2013; Goodman et al. 2013).

Hyperspectral remote sensing (imaging spectrometry) in particular shows strong potential for developing enhanced analysis tools to assess patterns and processes of reef composition and biodiversity (Mumby et al. 2004; Hochberg et al. 2003; Goodman and Ustin 2007; Lesser and Mobley 2007; Lucas et al. 2008; Herkül et al. 2013; Joyce et al. 2013). However, to better leverage this technology, it is important to improve our understanding of the spectral characteristics and relationships of the various reef components. It is the variability in composition and structure of different components that manifests as variability in spectral reflectance, and inverting this relationship through image analysis facilitates the identification and classification of coral reef biota and substrates using remote sensing. Past research indicates that reef components can be differentiated according to general categories, e.g., live coral, carbonate sand, macroalgae, and seagrass (Hochberg and Atkinson 2003; Lesser and Mobley 2007; Herkül et al. 2013 Joyce et al. 2013; Hochberg et al. 2004; Karpouzli et al. 2004; Hedley et al. 2004; 2012). This knowledge has assisted with the development of reef classification schemes, but further research is required to evaluate how the spectral variability between categories and within categories affects image-derived estimates of biodiversity.

Additional research is also required to identify and specify limits on our ability to differentiate species according to their spectral characteristics. More specifically, it has been shown that reef species can be generally categorized into a discrete number of categories with many species exhibiting similar spectral characteristics (Hochberg et al. 2003). The inability to classify or distinguish distinct coral species as a function of spectral reflectance results because many coral species share a common suite of pigments that are conserved over geographic and taxonomic boundaries (Hedley et al. 2002; Hochberg et al. 2004). Additionally, only limited research has been conducted to examine how species separability, and hence detectability via remote sensing, changes with varying water properties and water depth (e.g., (Hedley et al. 2008; 2009; Botha et al. 2013). As such,

detailed spectral taxonomic classification and further environmental details are needed at appropriate spatial and temporal resolutions to improve ecological assessments (e.g., estimates of abundance, distribution, patchiness, and biodiversity) and better inform reef management decisions (Lubin et al 2001; Turner et al. 2003; Scopélitis et al. 2010; Dalleau et al. 2010; Pettorelli et al. 2014).

Numerous studies have successfully integrated fieldwork and various remote sensing techniques that have identified coral morphologies, habitat maps, live coral indices, coral reef resilience indicators, and other general reef categories (Hochberg and Atkinson 2003; Scopélitis et al. 2010; Mumby et al. 2004; Joyce et al. 2013; Karpouzli et al 2004; Knuby et al. 2007; 2013). Likewise, the ability to detect coral bleaching (Holden 2000; Yamano and Tamura 2004; Ziskin 2011; Hedley et al. 2012) and diseased states in corals (Anderson et al. 2013), continues to improve with recent advances in remotes sensing. Even so, coral reef managers are yet calling for an improved understanding of biodiversity (species) estimates derived through remote sensing (Kerr et al. 2003; Turner et al. 2003; Pettorelli et al. 2014). Towards that end, it is necessary to further examine detailed spectral variability of the spatially dominant reef species (i.e., those species that significantly contribute to the remote sensing signal), as well as analyze how inter- and intra-species spectral relationships vary as a function of environmental characteristics and water depth. Also of fundamental importance is determining the appropriate spatial scale for a given investigation as well as being able to effectively interpret and put that information in context across different spatial scales (Wiens 1989; Marceau 1999; Blaschke and Geoffrey

2001; Knudby et al. 2007; Roelfsema et al. 2010). To objectively evaluate these requirements, locally optimized projects (i.e., fine spatial scale reef habitat plots ~5 m²), where high spatial and high spectral resolution imagery (i.e., hyperspectral) coincide with detailed in situ spectral measurements and ecological field data, are needed to assess the spectral processes that reflect the composition, distribution, and biodiversity found on coral reefs.

The objective of this study is to provide a broader understanding of reef spectral variability and the effect of depth on our ability to distinguish species, groups of species, or even major reef components (i.e., sand, algae, seagrass, dead coral, live coral). We also aim to invoke further discussion and additional research on the relationships between spectral diversity and species diversity. Here we describe methods that provide the opportunity to correlate spectral characteristics with localized in situ measurements of biodiversity (species) that can be scaled-up to the spatial resolution and geographic extent provided through remote sensing image analysis. Specifically, the goals of this study are to: (1) identify the spectral characteristics of the spatially dominant taxa for a coral reef system in southwest Puerto Rico; (2) assess the ability to differentiate species based on spectral characteristics; and (3) examine how spectral separability and varying water depth impact our ability to estimate biodiversity and classify species, or groups of species, using remote sensing data.

4.3 Methods

To evaluate spectral characteristics of different species and different groups of species, an extensive sample of in situ spectral measurements were acquired from the spatially dominant reef and seagrass species in southwestern Puerto Rico. A semi-analytical algorithm for the water column was next used to model the influence of increasing water depth on the average spectral reflectance of each species. The resulting spectra at each depth were then grouped according to spectral similarity using hierarchical clustering. In the same study area, corresponding photoquadrats were collected from small habitat plots and visually analyzed to determine field estimates of biodiversity. These field estimates were then recalculated at each depth using the results of the spectral clustering analysis to demonstrate the influence of increasing water depth and spectral similarity on remote sensing derived biodiversity.

Reef Field Spectra

Field reflectance spectra were collected in situ from amongst the inner shelf reefs of southwestern Puerto Rico, focusing predominantly on Enrique Reef, using a GER-1500 spectrometer in an underwater housing with attached illumination source (Spectra Vista Corporation, Poughkeepsie, NY, USA; Figure 1) (Goodman 2004; 2007). The GER-1500 measures 512 spectral bands from 350 to 1050 nm at a 1.5 nm sampling interval. The unit is self-contained and can be easily operated using external controls on the underwater housing. The attached quartz halogen light provided steady, spectrally consistent, light with which to acquire measurements, and thus effectively eliminated uncertainties associated with the inherent variability of the natural underwater light environment. Field methodology followed standard spectral collection protocols, where target measurements of species and substrate were normalized to reflectance using contemporaneous in situ reference measurements of a 99% Spectralon panel (Labsphere, North Sutton, NH, USA). Included in the final data were measurements of 40 different marine species, representing Cnidaria, Porifera, and submerged aquatic vegetation (e.g., seagrass and algae), as well as representative samples of numerous sand areas. The resulting coral reef spectral library was organized by taxa, size, and spatial dominance, and cataloged using SAMS (Spectral Analysis and Management System) [46]. Data was analyzed to calculate the mean and standard deviation for each individual species plus sand, resulting in representative reflectance spectra for 41 different reef components.



Figure 1: The GER-1500 spectrometer in underwater housing with attached illumination (**left**) and a map of the study location off the coast of southwestern Puerto Rico (**right**).

Separability Analysis and Water Column Modeling

Measured field spectra represent reflectance at zero water depth, where the artificial

illumination and reference Spectralon measurements serve to effectively remove influences

of varying water properties, fluctuating natural light, and differing water depths. While useful for in situ spectral analysis and remote sensing studies with robust water column correction schemes (which are difficult to achieve and not typically the norm), it is informative to investigate how the water column influences spectral relationships at different depths.

To introduce a simulated water column to the average reflectance spectra for each of the measured 41 reef components, water column modeling was performed using the semi-analytical algorithm developed by Lee et al. 1998, 1999). This is a widely accepted model in the remote sensing community that is commonly used as an "inverse" model to derive coastal water properties from hyperspectral imagery (Lucas et al. 2008), whereas here it is used in an equally applicable role as a "forward" water column model. Here the model is used as a discrete example of how the water column can influence spectral characteristics at the water surface; however, analysis could also be adapted to other water properties, models and measurements, or extended to also consider the additional confounding impact of variations in water properties. In this study, a set of default values were used to parameterize the model for clear tropical water: phytoplankton absorption at 440 nm = 0.05 m⁻¹; detritus/gelbstoff absorption at 440 nm = 0.05 m⁻¹; and particle backscattering at 440 nm = 0.01 m⁻¹. Output from the model, which was replicated at 1, 3, 5 and 10 m water depths, represents reflectance spectra at the water surface (i.e., as if viewing each reef component through the water column).

A quantitative analysis was used to calculate the similarities and differences, and thereby assess the ability to spectrally differentiate, between the various reef components at each depth. The metric selected for quantifying the level of separability was spectral angle, which calculates the "angle" between two spectra in n-dimensional space (Kruse et al. 1993). The smaller the angle, the less separable two spectra are considered. Spectral angle was calculated pairwise at each depth (0, 1, 3, 5 and 10 m) for all possible combinations of reef components. A weighted hierarchical clustering scheme (cluster distance is the average distance of pairs between each cluster weighted by number of members in each cluster) was used to generate optical dendrograms (as opposed to taxonomic) that define how components are progressively grouped as a function of increasing spectral angle (Exelis Visual Information Solutions, 8.3).

Field estimates of biodiversity

To correlate the spectral analysis with reef composition and biodiversity, photoquadrat images were obtained from selected habitat plots on Enrique Reef. Photo acquisition coincided with a 2013 airborne hyperspectral mission conducted over the same area, which is being used in a related project to investigate and develop remote sensing estimates of biodiversity. The particular study location, Enrique reef, is an important component of the CenSSIS-SeaBED project (Goodman et al. 2008); where over the last decade UPRM researchers have compiled an extensive array of image and field data as support for testing and validating remote sensing algorithms for benthic habitat mapping. Habitat types on Enrique Reef include large areas of seagrass, patches of pure carbonate sand, and a diverse coral reef community including areas of both hard and soft coral. The reef measures approximately 1 × 0.5 km, and includes water depths ranging from >10 m along the fore and back reef, 1–3 m along the reef flat, and <1 m along the reef

crest, with areas of exposed sand and rubble and emergent mangroves.

Photoquadrat images were obtained from selected plots within the seagrass (n = 44) and coral reef (n = 26) habitat areas on Enrique Reef. For each habitat plot, a 1 × 1 m quadrat was randomly placed five times within a 2 m distance around a central point (marked with buoys), acquiring high quality photographic images of each quadrat location using a Nikon P7000 digital camera (Figure 2). The five photoquadrats sampled for each plot were used to statistically represent the 4 × 4 m (16 m²) habitat characteristic for each plot. Individual plots were randomly located within each habitat type a minimum distance 10–15 m apart, and a Magellan Mobile Mapper 6 GPS was used to record the central location of each plot.



Figure 2: (a) Flagged rebar was hammered into non-living substrate and tightly tethered to the surface to mark the GPS location for each habitat plot; (b) Photoquadrat of seagrass area mixed with soft corals; (c) Photoquadrat of from the reef crest area showing soft corals, hard corals, encrusting sponge, algae and non-living substrate; (d) Recording the GPS location of a habitat plot (tethered buoy).

Summary statistics of habitat composition were derived from each group of five photoquadrats for each plot (pooled photoquadrats) using on-screen visual classification and identification (Dethier et al 1993; Pante and Dustan 2012). The resulting statistics were used to calculate diversity using the Shannon Weiner Index, where the exponential of Shannon entropy (Whittaker 1972; Jost 2007), "diversity of order one", was utilized to provide meaningful comparisons of biodiversity across this single marine landscape (inclusive of seagrass beds, sand patches, and coral communities). Considering that estimates of alpha diversity (habitat) and beta diversity (extent of differentiation among those habitats) (Whittaker 1972) are independent, the exponential of Shannon entropy accounts for all species according to frequency, and rare or common species are not favored disproportionately, therefore making it the "fairest" index for the purposes of the differentiation among given coral reef habitats (Equation 1): $D1=\exp(-\sum i=1Rpi\ln(pi))=\exp(H')$, where ¹D is diversity of order one, p_i is the relative proportion of species i relative to the total number of species R, and H' is the Shannon index value calculated using natural logarithms (Jost 2007).

Utilizing results from the separability analysis, which provided an indication of how reef components group together spectrally (not taxonomically), calculations of the Shannon index were repeated for different water depths using the indicated spectral groupings. In other words, wherever analysis revealed certain species could not be reasonably differentiated spectrally, these species were grouped together and the Shannon index was recalculated with a reduced number of total "species". Using output from the hierarchical clustering scheme at each depth, species were deemed spectrally inseparable if the spectral angle was <0.1; and conversely, separable if the spectral angle was \geq 0.1. The 0.1 threshold is a common default value in spectral analysis; however, the threshold could be adjusted to reflect the specific variability of any given spectral dataset. The separability analysis allowed field measurements of biodiversity to be adapted to the remote sensing perspective, demonstrating how spectral characteristics influence what can be resolved in remote sensing imagery.

4.4 Results

Reef Field Spectra

A total of 9400 in situ spectra samples (after removing erroneous or noisy spectra) were collected from 41 reef components in five categories: hard corals, soft corals, sponges, submerged aquatic vegetation (SAV; i.e., seagrass and algae), and carbonate sand (Table 1; Figure 3 and Figure 4). Not intended to be inclusive of all species in the study area, these components represent a comprehensive sampling of those species that are individually or aggregately sizeable enough to contribute significantly to the remote sensing signal. Hence, these components also represent the feasible upper limit of taxonomic detail that can be achieved for remote sensing of biodiversity in this area.

Table 1: Summary of field reflectance spectra (R) collected from shallow reef areas in southwestern Puerto Rico. Instrument configuration was set to automatically record and average four samples for each measurement.

Phylum	Individuals	Species/Type	Samples	Spectra R
Cnidaria	73	25	5556	1389
Porifera	34	11	2028	507
SAV (seagrass/algae)	Numerous	4	1268	317
Sand/Substrate	Numerous	1	548	137
Totals	107	41	9400	2350

The individual reflectance spectra exhibit smooth curves with negligible noise from 400 to 800 nm, demonstrating the advantages of using artificial light. More specifically, given the challenges of acquiring in situ underwater measurements using natural sunlight, which are susceptible to fluctuations in the incident downwelling light (e.g., associated with light refraction, wave focusing, variable wave height, and instrument and diver selfshading), utilizing artificial light minimizes the uncertainty associated with these unwanted natural light variations. As a result, observed variations in spectra can be confidently attributed to actual physical variations in species and substrate reflectance characteristics. Figure (3) illustrates an example subset of spectra acquired from a single coral species (A. palmata), where it is evident that while variability exists within the individual measurements for this species there is also a strong degree of similarity. Considering that similar relationships are observed amongst all of the measured species (Figure 4), this indicates that spectral similarities can exist both within and between different species.



Figure 3: Representative individual spectra measured for *Acropora palmata;* examples shown here are 10 spectra subset from a total sample size = 139 spectra.



Figure 4: Representative average spectra for ten coral species; examples shown here are subset of the 25 total Cnidarian species sampled (5556 samples; 1389 reflectance spectra).

Spectral Separability

Analysis of spectral separability, and correspondingly spectral similarity, was performed at two different taxonomic levels: (i) as groups of related species; and (ii) ungrouped as individual species. Calculations were performed using 38 of the 41 measured components, eliminating those with fewer than 10 spectral measurements and thereby retaining only those components with sufficient sampling to be considered representative of each species' reflectance characteristics.

The first level of analysis grouped the reef spectra into four fundamental components: coral, sponge, submerged aquatic vegetation (SAV), and sand. Average spectra were generated, and results were used to calculate spectral angles between the components as well as generate optical dendrograms for each of five water depths: 0, 1, 3, 5 and 10 m. Using a reasonable spectral angle threshold of 0.1 it is evident that the average in situ field spectra of these components exhibit unique reflectance characteristics and can be readily differentiated at 0 and 1 m water depth (Figure 5). However, with increasing water depth the relative separability of these four components decreases. For example, even when considering a lower spectral angle threshold, it is more difficult to differentiate sand from SAV and coral from sponge at 3 m water depth, and at 10 m water depth the system is essentially reduced to two differentiable components: sand vs. coral, sponge and SAV (Figure 5). It is also instructive to observe that the hierarchical relationships can change with depth (e.g., SAV is most closely grouped with sand up to a

depth of 5 m and then shifts to coral as its closest spectral component at 10 m), an observation that is even more apparent in the results that follow for the individual species.

When applying the same pairwise spectral angle calculations and optical dendrogram creation using the average individual spectra, results reveal that even at 0 m water depth there are already many species that are difficult to separate spectrally (Figure 6). In some cases species group together within categories, such as coral with coral (e.g., D. labyrinthiformis and D. cylindricus), but in other cases species are grouped outside their respective categories, such as coral with algae (e.g., Porites spp. and Dictyota spp.). While biologically different, this spectral similarity across categories may be explained by the presence of common photosynthetic pigments between species. For example, Hochberg et al. (2006) and Torres et al. (2012) both provide evidence that certain corals contain the same pigments (e.g., carotenes and xanthophyll) that also characterize brown algae. When the effects of the water column are included, the ability to distinguish individual species diminishes significantly with increasing water depth (Figure 7). [Note: for brevity only the optical dendrograms from 3 and 10 m water depth are displayed here]. Furthermore, varying water depth is also accompanied by substantial variations in how spectra are grouped, regardless of taxonomic relationship. For example, *Dictyota* spp. is most closely grouped with Porites spp. at 0 m depth, whereas at 3 m, it is grouped with *Halimeda* spp. and at 10 m, it is grouped with *Diploria* spp. These changing spectral relationships are

attributed here to the inherent nonlinearity of light interaction and attenuation in the water column; but when also considering intra-species spectral variations these relationships can be further influenced by depth dependent differences in photosynthetic pigment content within species (Segura 1981). This indicates that unless such variations are accounted for, significant uncertainty can be associated with species-level spectral and image analysis. Furthermore, considering the correlation between increasing depth and decreasing spectral separability, these results also affirm the importance of compensating for variable water depth (e.g., using a depth invariant index, semi-analytical algorithm or radiative transfer model) when deriving habitat classifications and biodiversity estimates using remote sensing.



Figure 5: Optical dendrograms illustrating the effect of increasing depth on separability of field spectra grouped into four fundamental reef components: coral, sponge, submerged aquatic vegetation (SAV), and sand.



Figure 6: Optical dendrogram for 0 m water depth illustrating separability of field spectra from 24 coral species, 10 sponge species, 3 submerged aquatic vegetation (SAV) species, and sand.



Figure 7: Optical dendrograms for 3 and 10 m water depth illustrating the effects of increasing depth on separability of field spectra from 24 coral species, 10 sponge species, 3 submerged aquatic vegetation (SAV) species, and sand.

Field Estimates of Biodiversity

Photoquadrats were acquired from a total of 90 plots distributed across three different habitat categories: coral reef community (n = 26), dense seagrass (n = 44), and pure sand (n = 20). As expected, field estimates of biodiversity derived from photoquadrats using the Shannon index (order one diversity) $\exp(H')$, revealed different magnitudes of biodiversity corresponding to each of the three habitat areas. Estimates ranged from no biodiversity $\exp(H') = 1$ in areas of pure carbonate sand, low/moderate diversity $\exp(H') = 1-3$ in seagrass areas, and high biodiversity $\exp(H') = 10$ in reef communities. After using results from the separability analysis to group spectrally similar species and recalculate the Shannon index for different depths, it is observed that estimated biodiversity, as well as the range and standard deviation, decreases with increasing water depth (Figure 8).



Figure 8: Estimates of biodiversity calculated using the exponential of Shannon entropy, exp(H'), illustrating influence of increasing spectral similarity amongst reef species as a function of increasing water depth: 0* is biodiversity obtained from photoquadrats, 0** is biodiversity calculated using only those species considered prevalent or sizable enough to significantly influence the remote sensing signal (i.e., species included in the spectral measurements for this study area), and 0–10 is biodiversity calculated with consideration for optical similarities amongst species (i.e., based on hierarchical clustering of reflectance spectra as influenced by the overlying water column).

This indicates that depth-influenced estimates of biodiversity derived from remote sensing (i.e., optical diversity) can significantly underestimate biodiversity and represents the lower limit of actual species diversity. For example, depending on water depth, species composition, and the associated spectral relationships, a hypothetical optical diversity index of 1 for seagrass could indicate an actual biodiversity index of 1–3, whereas and optical diversity index of 1 for coral could indicate a biodiversity index as high as 10. Based on this analysis, it appears feasible to derive mathematical relationships for each habitat type that correlate optical diversity with biodiversity as a function of water depth, and then utilize these empirical functions to normalize remote sensing derived estimates of biodiversity. Although implementation of such a technique would require knowledge of water depths for a given study area, these values are often available from bathymetric charts or can alternatively be estimated directly from the imagery.

The overall results of this investigation suggest three important implications for remote sensing of biodiversity: (1) unless the influence of the water column is accounted for, then estimates of biodiversity for any given area will be depth-biased; (2) given the occurrence of spectral similarity amongst reef species, even without influence of the water column, biodiversity estimates should not be considered absolute but rather the minimum or lower limit of true biodiversity; and (3) even small or moderate differences in remote sensing derived optical diversity may indicate substantial differences in actual species diversity.

4.5 Discussion

This study explored spectral relationships of reef components in southwestern Puerto Rico and illustrates how the overlying water column can impact these relationships. Results indicate that the ability to distinguish individual species significantly diminishes with increasing water depth, thereby contributing a level of uncertainty to any spectrally derived estimates of biodiversity, such as through remote sensing. Additional considerations not addressed in this analysis, but that would contribute further uncertainty, include: variable water properties and water surface conditions, differing sensor spectral and spatial characteristics, within-species spectral variations, and overly simplified or imperfect water correction schemes. Thus, it is expected that the observations illustrated here for Puerto Rico would be equally applicable to reef remote sensing elsewhere around the globe. So, if species level distinctions are not feasible with remote sensing imagery, then what can be detected in a remote sensing pixel? At what level can biodiversity be measured? Moreover, can a biodiversity index for coral reefs be conceived using remote sensing? And if so, how do we correlate spectral diversity measured in remote sensing with biodiversity and reef composition values generated from field data? The answers to these questions lie in continuing to improve our understanding of the complex relationships and environmental drivers that govern species distribution, continuing to investigate relationships between optical diversity and species diversity, and utilizing this knowledge to improve our capacity for monitoring biodiversity using remote sensing imagery.

Remote sensing is a powerful tool for assessing reef characteristics over large spatial areas, including the estimation of biodiversity, but interpretation of image-derived output requires informed decision making from remote sensing specialists and ecologists alike regarding physical constraints on what is being measured and what the confidence levels are for those measurements. An important aspect of consideration for remote sensing users and producers is to understand the scale of observation, inclusive of spatial extent, observation detail, and taxonomic level. For example, the *Pyramids of Observation* illustrate the different scales and relationships that exist in remote sensing and field-based studies of biodiversity (Figure 9).



Figure 9. The *Pyramids of Observation* relating the different scales at which biodiversity is measured from the perspective of both remote sensing and field-based analysis.

The lower levels of the pyramids represent the finest scale of observation, such as where the marine field ecologist records detailed habitat information and species composition within a local study area. Moving up through the pyramids depicts decreasing levels of habitat information acquired at a coarser observation scale, but with increasing magnitude in spatial coverage. Remote sensing is represented at two levels within the context of these pyramids: high resolution (<5 m pixel scale, e.g., WorldView-2) and moderate resolution (5–100 m pixel scale, e.g., Landsat). A caveat here is that the distinction between moderate and high-resolution spatial coverage is becoming more a function of processing capacity, and less an issue of acquisition extent, as more and higher resolution imagery becomes available globally. In this situation, even with high spatial resolution imagery (e.g., 30 cm WorldView-3) the primary challenge remains overcoming radiometric and spectral resolution. Nonetheless, increasing from moderate to high resolution remote sensing facilitates detection of an increasing number of general habitat categories, such as differentiating more variations in seagrass density and coral community type. However, as shown here, except for situations where certain species exhibit unique spectral characteristics, even with high spectral, high spatial resolution imagery it is currently not possible to spectrally differentiate all individual species. This constraint is a function of both spectral similarity amongst species and spatial size or extent, where many species are not prevalent or sizable enough to significantly influence the remote sensing signal. This in turn has direct relevance to remote sensing estimates of biodiversity, which become inherently limited by these spectral characteristics. For example, a large expanse of seagrass can appear as a relatively uniform dark area in moderate resolution imagery and as a collection of different seagrass densities in high resolution imagery; however, a field ecologist surveying the same area can identify a patchy distribution of numerous benthic groups and species, such as scleractinian corals, octocorals, sponges, macroalgae, and even different seagrass species. In other words,

there is typically an intrinsic tradeoff between the scale of observation and the scale of information.

To better understand the implications of this observation tradeoff there is a need to more explicitly define the relationships between optical diversity and species diversity. A common challenge when linking remote sensing applications and coral reef ecology is the calibration and validation of field data with data from remote sensing imagery (Joyce et al. 2013), especially in marine environments (Roelfsema et al. 2009). For example, field data and remote sensing image acquisitions are not always coincident (Fuller et al. 1998; Scopélitis et al. 2009, 2010; Joyce et al. 2013), and in some cases, there are even years of difference between the acquisition of field and image data (Fuller et al. 1998; Scopélitis et al. 2010). Additionally, spectral libraries for marine species remain undersampled, and significant opportunity exists for detailed analysis of intra- and inter-species similarities. While there are many informative coral reef remote sensing applications, there is yet a need for more studies that incorporate specific consideration for calibrating and validating remote sensing estimates of biodiversity. This includes directly correlating small-scale localized field estimates of biodiversity and species composition in different habitat areas with estimates derived from corresponding high spectral, high spatial resolution imagery. It is also instructive to consider information and measurements derived from other related disciplines, such as climate patterns, physical processing, ocean chemistry, larval dispersal and population connectivity, which can further

contribute to the overall assessment of biodiversity (Andréfouët et al. 2001; Kerr et al. 2003; Pettorelli et al. 2014). Once the variability in spectral diversity is better understood at these scales, it then becomes feasible to extend the observed correlations to other spatial and spectral scales, and thereby develop more effective tools for linking remote sensing biodiversity estimates with field ecology.

This study demonstrated that although average field spectra can be used to differentiate many coral reef species, once the overlying water column is considered the ability to distinguish species significantly declines with increasing water depth. These results illustrate the challenges associated with developing a depth-invariant biodiversity index using remote sensing, and reveal that there is important groundwork ahead for reef ecologists and remote sensing specialists to better understand the relationships between optical diversity and species diversity.

Remote sensing will no doubt continue to serve as an important tool for ecology, conservation, and biodiversity, particularly considering the current predictions of continued environmental degradation related to climate change on a global scale. Consequently, it is imperative that we continue to improve our abilities for rapid ecological monitoring using remote sensing, including the capacity to assess and monitor changes in coral reef biodiversity. As remote sensing technologies improve in both coverage and resolution (spatially, spectrally and temporally), there is increasing opportunity, given development of a strong foundation in spectral knowledge, to realize these improvements and put the resulting analysis tools into practice.

4.6 Acknowledgments

This project was supported by NASA EPSCoR Project: Hyperspectral Imaging for Biodiversity Assessment of Coastal and Terrestrial Ecosystems (NASA Grant NNX09AV03A). Additional project support was provided courtesy of HySpeed Computing. M.L. was supported by NASA PR Space Grant Fellowship Award (2013– 2015). We thank UPRM undergraduate students that provided field support for the marine fieldwork: Ramon Vicens, Bethsymarie Soto, Tavi Castagnoli, Julia Sanchez, and Jesse LeDu. Jan Vicente provided assistance with difficult sponge identifications. Sam Rosario provided invaluable logistics support.

4.7 References

- Anderson DA, Armstrong RA, Weil E (2013) Hyperspectral sensing of disease stress in the Caribbean reef-building coral, *Orbicella faveolata*—perspectives for the field of coral disease monitoring. *PLoS ONE*, 8: e81478. doi: 10.1371/journal.pone.0081478.
- Andréfouët S, Muller-Karger F, Mumby J, McField M, Hu C (2002) Revisiting coral reef connectivity. *Coral Reefs*, 21: 43–48.
- Ateweberhan M, Feary DA, Keshavmurthy S, Chen A, Schleyer MH, Sheppard CR (2013) Climate change impacts on coral reefs: synergies with local effects, possibilities for acclimation, and management implications. *Marine Pollution Bulletin*, 74: 526–539.
- Blaschke T, Geoffrey J (2001) Object-oriented image analysis and scale-space: theory, methods, and evaluating multiscale landscape structure. *International Archives of Photogrammetry, Remote Sensing and Spatial Information Sciences*, 34: 22–29.

- Botha EJ, Brando VE, Anstee JM, Dekker AG, Sagar S (2013) Increased spectral resolution enhances coral detection under varying water conditions. *Remote Sensing of Environment*, 131: 247–261.
- Dalleau M, Andréfouët S, Wabnitz CC, Payri C, Wantiez L, Pichon M, Friedman K, Vigliola L, Benzoni F (2010) Use of habitats as surrogates of biodiversity for efficient coral reef conservation planning in Pacific Ocean islands. *Conservation Biology*, 24: 541– 552.
- Dethier M, Graham E, Cohen S, Tear L (1993) Visual versus random-point percent cover estimations: "objective is not always better". *Marine Ecology Progress Series*, 96: 93–100.
- Eakin CM, Nim C, Brainard R. Aubrecht C, Elvidge C, Gledhill D, Muller-Karger F, Mumby P, Skirving W, Strong A, et al. (2010) Monitoring coral reefs from space. *Oceanography*, 23: 118–133.
- Exelis Visual Information Solutions. Interactive Data Language 8.3 Reference Guide. Available online: http://www.exelisvis.com/docs/using_idl_home.html (accessed on 14 October 2014).
- Fuller RM, Groom GB, Mugisha S, Ipulet P, Pomeroy D, Katende A, Bailey R, Ogutuohwayo R (1998) The integration of field survey and remote sensing for biodiversity assessment: a case study in the tropical forests and wetlands of Sango Bay, Uganda. *Biological Conservation*, 86: 379–391.
- Goodman J (2004) Hyperspectral remote sensing of coral reefs: deriving bathymetry, aquatic optical properties and a benthic spectral unmixing classification using AVIRIS data in the Hawaiian Islands. Ph.D. Dissertation, University of California, Davis, Davis, CA, USA.
- Goodman J (2008) The 2007 Puerto Rico hyperspectral mission: image acquisition and field data collection. University of Puerto Rico, San Juan, PR-USA. pp. 56.
- Goodman J, Purkis S, Phinn S (2013) Coral Reef Remote Sensing: A Guide for Mapping, Monitoring, and Management. Eds., Goodman JA, Purkis SJ, Phinn SR. Springer Netherlands: Dordrecht, Netherlands, pp. 1–436.
- Goodman J, Ustin S (2007) Classification of benthic composition in a coral reef environment using spectral unmixing. *Journal of Applied Remote Sensing*, 1: 011501. doi:10.1117/1.2815907

- Goodman JA, Vélez-Reyes M, Rosario-Torres S (2008) An update on SeaBED: A TestBED for validating subsurface aquatic hyperspectral remote sensing algorithms. *SPIE Remote Sensing*, 2: doi:10.1117/12.803785.
- Green A (2009) Designing a resilient network of marine protected areas in Kimbe Bay, West New Britain, Papua New Guinea. *Oryx*, 43: 488–498.
- Hedley JD (2008) A three-dimensional radiative transfer model for shallow water environments. *Optics Express*, 16: 21887–21902.
- Hedley JD, Mumby PJ (2002) Biological and remote sensing perspectives of pigmentation in coral reef organisms. *Advances in Marine Biology*, 43: 277–317.
- Hedley JD, Mumby PJ, Joyce KE, Phinn SR (2004) Spectral unmixing of coral reef benthos under ideal conditions. *Coral Reefs*, 23: 60–73.
- Hedley JD, Roelfsema C, Koetz B, Phinn S (2012) Capability of the Sentinel 2 mission for tropical coral reef mapping and coral bleaching detection. *Remote Sensing of Environment*, 120: 145–155.
- Hedley JD, Roelfsema C, Phinn SR (2009) Efficient radiative transfer model inversion for remote sensing applications. *Remote Sensing of Environment*, 113: 2527–2532.
- Hedley JD, Roelfsema CM, Phinn SR, Mumby PJ (2012) Environmental and sensor limitations in optical remote sensing of coral reefs: Implications for monitoring and sensor design. *Remote Sensing*, 4: 271–302.
- Herkül K, Kotta J, Kutser T, Vahtmäe E. (2013) Relating remotely sensed optical variability to marine benthic biodiversity. *PLoS ONE*, 8(2): e55624. doi:10.1371/journal.pone.0055624
- Hochberg E, Atkinson M (2003) Capabilities of remote sensors to classify coral, algae, and sand as pure and mixed spectra. *Remote Sensing of Environment*, 85: 174–189.
- Hochberg E, Atkinson M, Andréfouët S (2003) Spectral reflectance of coral reef bottomtypes worldwide and implications for coral reef remote sensing. *Remote Sensing of Environment*, 85: 159–173.
- Hochberg EJ, Apprill AM, Atkinson MJ, Bidigare RR (2006) Bio-optical modeling of photosynthetic pigments in corals. *Coral Reefs*, 25: 99–109.
- Hochberg EJ, Atkinson MJ, Apprill A, Andréfouët S (2004) Spectral reflectance of coral. *Coral Reefs*, 23: 84–95.

- Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. *Science*, 328: 1523–1528.
- Holden H (2000) Accuracy assessment of hyperspectral classification of coral reef features. *Geocarto International*, 15: 7–14.
- Jost L (2007) Partitioning diversity into independent alpha and beta components. *Ecology*, 88: 2427–2439.
- Joyce K, Phinn S, Roelfsema C (2013) Live coral cover index testing and application with hyperspectral airborne image data. *Remote Sensing*, 5: 6116–6137.
- Joyce KE (2013) Spectral index development for mapping live coral cover. *Journal of Applied Remote Sensing*, 7(1):073590. doi: 10.1117/1.JRS.7.073590
- Karpouzli E, Malthus TJ, Place CJ (2004) Hyperspectral discrimination of coral reef benthic communities in the western Caribbean. *Coral Reefs*, 23: 141–151.
- Kerr JT, Ostrovsky M (2003) From space to species: ecological applications for remote sensing. *Trends in Ecology and Evolution*, 18: 299–305.
- Knudby A, Jupiter S, Roelfsema C, Lyons M, Phinn S (2013) Mapping coral reef resilience indicators using field and remotely sensed data. *Remote Sensing*, 5: 1311–1334.
- Knudby A, LeDrew E, Newman C (2007) Progress in the use of remote sensing for coral reef biodiversity studies. *Progress in Physical Geography*, 31: 421–434.
- Kruse FA, Lefkoff AB, Boardman JW, Heidebrecht KB, Shapiro AT, Barloon PJ, Goetz AF (1993) The spectral image processing system (SIPS)-interactive visualization and imaging spectrometer data. *Remote Sensing of Environment*, 44: 145–163.
- Lee Z, Carder K, Mobley CD, Steward RG, Patch JS (1998) Hyperspectral remote sensing for shallow waters. I. A semi-analytical model. *Applied Optics*, 37: 6329–6338.
- Lee Z, Carder KL, Mobley CD, Steward RG, Patch JS (1999) Hyperspectral remote sensing for shallow waters. 2. deriving bottom depths and water properties by optimization. *Applied Optics*, 38: 3831–3843.
- Lesser MP, Mobley CD (2007) Bathymetry, water optical properties, and benthic classification of coral reefs using hyperspectral remote sensing imagery. *Coral Reefs*, 26: 819–829.
- Lubin D, Li W, Dustan P, Mazel CH, Stamnes K (2001) Spectral signatures of coral reefs: features from space. *Remote Sensing of Environment*, 137: 127–137.

- Lucas R, Mitchell A, Bunting P (2008) Hyperspectral remote sensing of tropical and subtropical forests. In
- Hyperspectral Remote Sensing for Assessing Carbon Dynamics and Biodiversity of Forests. Eds., Kalacska M, Sanchez-Azofeifa GA. CRC Press: London, United Kingdom, pp. 47–86.
- Marceau DJ (1999) The scale issue in social and natural sciences. *Functional Ecology*, 25: 347–356.
- Medina O, Manian V, Chinea JD (2013) Biodiversity assessment using hierarchical agglomerative clustering and spectral unmixing over hyperspectral images. *Sensors*, 13: 13949–13959.
- Mumby PJ, Skirving W, Strong AE, Hardy JT, Ledrew EF, Hochberg E, Stumpf RP, David LT (2004) Remote sensing of coral reefs and their physical environment. *Marine Pollution Bulletin*, 48: 219–228.
- Pante E, Dustan P (2012) Getting to the point: accuracy of point count in monitoring ecosystem change. *Journal of Marine Biology*, doi:10.1155/2012/802875.
- Pettorelli N, Safi K, Turner W (2014) Satellite remote sensing, biodiversity research and conservation of the future. *Philosophical Transactions of the Royal Society of London B*, 369: 20130190. doi: 10.1098/rstb.2013.0190.
- Roelfsema CM, Phinn S, Jupiter S, Comley J, Beger M, Patterson E (2010) The application of object based analysis of high spatial resolution imagery for mapping large coral reef systems in the west pacific at geomorphic and benthic community spatial scales. Proceedings of the IEEE International Ultrasonics Symposium (IUS), San Diego, CA, USA, pp. 4346–4349.
- Roelfsema CM, Phinn SR, Udy N, Maxwell P (2009) An integrated field and remote sensing approach for mapping Seagrass cover, Moreton Bay, Australia. *Journal of Spatial Science*, 54: 45–62.
- Rueda C, Wrona A (2003) SAMS spectral analysis & management system user's manual. University of California, Davis, USA.
- Scopélitis J, Andréfouët S, Phinn S, Arroyo L, Dalleau M, Cros A, Chabanet P (2010) The next step in shallow coral reef monitoring: combining remote sensing and *in situ* approaches. *Marine Pollution Bulletin*, 60: 1956–1968.

- Scopélitis J, Andréfouët S, Phinn S, Chabanet P, Naim O, Tourrand C, Done T (2009) Changes of coral communities over 35 years: integrating *in situ* and remote-sensing data on Saint-Leu Reef (la Réunion, Indian Ocean). *Estuarine Coastal Shelf Science*, 84: 342–352.
- Segura J (1981) Water depth effects in photosynthetic pigment content of the benthic algae *Dictyota dichotoma* and *Udotea petiolata. Aquatic Botany*, 11: 373–378.
- Torres-Pérez J, Guild L, Armstrong R (2012) Hyperspectral distinction of two Caribbean shallow-water corals based on their pigments and corresponding reflectance. *Remote Sensing*, 4: 3813–3832.
- Turner W, Spector S, Gardiner N, Fladeland M, Sterling E, Steininger M (2003) Remote sensing for biodiversity science and conservation. *Trends in Ecology and Evolution*, 18: 306–314.
- Walther G, Post E, Convey P, Menzel A, Parmesan C, Beebee T, Fromentin J, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. *Nature*, 416: 389–395.
- Wang K, Franklin SE, Guo X, Cattet M (2010) Remote sensing of ecology, biodiversity and conservation: A review from the perspective of remote sensing specialists. *Sensors*, 10: 9647–9667.
- Whittaker R (1972) Evolution and measurement of species diversity. Taxon, 22: 213–251.
- Wiens, J. Spatial scaling in ecology. Funct. Ecol. 1989, 3, 385–397.
- Yamano H, Tamura M (2004) Detection limits of coral reef bleaching by satellite remote sensing: Simulation and data analysis. *Remote Sensing of Environment*, 90: 86–103.
- Ziskin D, Aubrecht C, Elvidge CD, Tuttle B, Eakin M, Strong AE, Guild LS (2011) Describing coral reef bleaching using very high spatial resolution satellite imagery: experimental methodology. *Journal of Applied Remote Sensing*, 5: 053531. doi: 10.1117/1.3595300.
Chapter 5. Concluding comments

These studies are integrated by their collective contribution to several different research themes that are important in coral reef ecology today. In the remaining discussion I provide a few concluding comments regarding active and practical conservation goals on the basis that coral reef resilience and sustainable ecosystem services are dependendent on biodiversity (Daily et al. 1997; Covich et al. 2004; Sala and Knowlton 2006; Hector and Bagchi 2007; Nyström et al. 2008; Palumbi et al. 2009; Baskett et al. 2010; Hughes et al. 2010; Rogers 2013; Dalton et al. 2015). Management decisions that would assist in increasing the resilience and survivorship of coral reefs and their overall biodiversity should include strict and active law enforcement, environmental and ecological restoration, as well as addressing poverty and improving educational outreach with an emphasis on climate change, natural resources, sustainability, as well as the legal consequences of violating conservation laws.

The most extensive and developed coral reefs of Puerto Rico are located in the waters off La Parguera, however, human activities (i.e., pollution, overfishing, recreational activities) continue to severely degrade both terrestrial and marine environments in the region (Ballantine et al. 2008; Valdés-Pizzini and Schärer-Umpierre 2014). Marine Protected Areas (MPAs) are regarded as the best strategy to conserve coral reefs and their biodiversity (Dalton et al. 2015). In this regard, designating No Take Zones (NTZs; where fishing and all other extractive activities are prohibited) within the La Parguera Natural Reserve would be ideal (Dalton et al. 2015), although like many social and political problems on the island, environmental management and legislative

decisions are a complicated endeavor due to Puerto Rico's political status with the United States (Aguilar-Perera et al 2006; Valdés-Pizzini and Schärer-Umpierre 2014). However, in my opinion, the most important action to prevent illegal activities, protect fish stocks and biodiversity, would be to increase the number of active Federal Law Enforcement Agents (National Oceanic and Atmospheric Association; NOAA). This is especially important for La Parguera (a pirate's paradise), since the local Department of Natural and Environmental Resources of Puerto Rico (DNER) Ranger Core is plagued by local politics, corruption, and a lack of leadership, training, and expertise from the top-down as to how to effectively manage a conservation police unit. The simple presence of federal conservation officers would certainly deter illegal behavior. Unfortunately, there is a single federal agent covering Puerto Rico and the United States Virgin Islands that has jurisdiction over the protection, management and conservation of our fisheries, Endangered Species Act (ESA) species, marine mammals, marine environments and ecosystems (pers. comm. Lynn Rios, NOAA Federal Agent). Active federal protection will require significant resource allocation, but if federal funding agencies are willing to provide significant financial resources into basic fisheries research, then allocating resources into conservation police protection in Puerto Rico's waters should be considered equally important (pers. comm. Lynn Rios, NOAA Federal Officer).

Environmental restoration is another way to protect coral reefs and their biodiversity. In this respect, improving local water quality should become a priority. Over the last 20 years water quality in La Parguera has been declining (Weil E unpublished data; Otero 2009; Wade et al. 2015). Secchi disc measurements show that the average annual water visibility has been decreasing (E Weil unpublished data). A comprehensive study by Otero (2009) used an array of water quality indicators (salinity, temperature, chlorophyll a, turbidity, fluorescence of dissolved organic matter, bacteriophytoplankton, bacterial abundance and productivity, sedimentation and stable isotopes of particulate organic matter) in southwestern Puerto Rico (2003 – 2005) and reported significant spatial and temporal changes in water quality in reefs and associated habitats of La Parguera as indicated by fluctuations in turbidity, microbial biomass, and production. The author recommended preventive measures to decrease terrestrial inputs to near shore coastal systems, such as coral reefs (Otero 2009). Simply improving water quality by reducing excess nutrients and controlling local stressors increases coral resistance by raising the upper thermal threshold of coral bleaching (Wooldridge 2009; Wooldridge and Done 2009; Wooldridge et al. 2015). To that end, reef-building corals require clear, nutrient free water, low sedimentation, sufficient light for photosynthesis and water temperatures between (23-30°C) to grow, reproduce and survive therefore, it is imperative there are improvements in water quality and increased protection from human activities along Puerto Rico's coastlines.

Ecological restoration has become an important component of managing for reef resilience and coral survivorship. However, while ecological restoration (*Acropora* spp. in the Caribbean) is helpful on a local scale, it is not the solution at the ecosystem-wide scale because the same environmental pressures still exist and are predicted to worsen in the coming decades (Hughes 1994; Hughes and Tanner 2000; Hughes et al. 2003; Jackson et al. 2014; Hoegh-Guldberg et al. 2015). Additionally, Caribbean *Acropora* species are highly susceptible to bleaching and disease (Weil and Rogers 2011; Lucas and Weil 2015) and whether growing in a restoration site or naturally on a back reef, they cannot escape the impact of acidification and ocean warming. Incidentally, the future of reef building corals (even the most fit of genotypes) run the risk of reduced rates of calcification (growth), fecundity, and genetic diversity and therefore, reduced effective dispersal (Knowlton 2001; Anthony et al. 2008; Carpenter et al. 2008; Jackson et al. 2014; Hoegh-Guldberg et al. 2015). Furthermore, there is no support for differing disease quality, quantity or dynamics and health management strategies between wild and restoration colonies of *A. cervicornis* in Florida (Miller et al. 2014). Notwithstanding, restoration areas create essential habitat and should not continue without caution since there is a growing body of evidence demonstrating that coral transplantation alters the corals microbial community and increases disease susceptibility (Casey et al. 2015; Certner and Vollmer 2015).

Coral diseases have played a significant role in the decline of Caribbean corals and have not only caused significant declines in the *Acropora* spp. but almost all coral species have been inflicted with various diseases (Gladfelter 1982; Aronson and Precht 2001; Weil 2004; Cróquer and Weil 2009; Rogers et al. 2009; Weil 2004; Weil et al. 2009; Weil and Rogers 2011). Factors such as lower biomass, loss of three-dimensional structures provided by acroporid corals, and fast growing macroalgae are factors associated with diminished reef resilience in the Caribbean (Roff and Mumby 2012). Likewise, a recent study indicates that White Band Disease in *A. cervicornis* may be caused by opportunistic pathogenesis of the corals resident microbial community (Certner and Vollmer 2015). Thus, continuing to identify the potential reef invertebrates (i.e., *Coralliophila abbreviata*,

Gignoux et al. 2012) and fish species that are vectors of disease would provide a broader understanding of disease dynamics and trophic cascades on Caribbean reefs (Weil 2004; Kline and Vollmer 2011; Weil and Rogers 2011; Miller et al. 2014; Lucas et al. 2014; Casey et al. 2015).

Another important aspect of protecting coral reef biodiversity that is often overlooked is addressing poverty (Cinner et al. 2009a, b). Poverty causes fisherman to use ecologically destructive fishing practices and encourages large takes for short-term economic gain (Cinner 2010). There is a need to explore new ways of improving the livelihoods of the people living and fishing near La Parguera's reefs in order to reduce their dependence on the fishery (Cinner 2011). This will require careful, social, ecological, and economic planning, as well as personal sacrifices to reach this goal in La Parguera. However, I maintain that increased *federal* law enforcement on the waters at all time will highly deter illegal activities and overfishing that plague La Parguera's natural resources.

Lastly, it is important that we address global climate change and strive to reduce CO₂ emissions. This presents serious challenges on a global scale and will take cooperation from individuals – communities – states – and nations. The adverse effects of global climate change (warming oceans and acidification) related to increasing atmospheric CO₂ acting synergistically with other anthropogenic disturbances have been responsible for the 33-50% decline of all coral reefs worldwide in only under a few decades (Hoegh Guldberg et al. 2015). Additionally, continued environmental degradation and the current rate of ocean warming has raised concerns that corals may not be able to adapt fast enough under current climate scenarios (Hoegh Guldberg et al. 2015). Under existing

patterns of human activity, the global average land and sea surface temperatures will soon reach 2° C above pre-industrial levels and by the year 2100, tropical waters could be 3 - 4° C warmer. The scientific consensus (Hoegh-Guldberg et al. 2015) predicts that if there is not a significant reduction in CO₂ emissions immediately, then the impacts of climate change will have decimated most functioning coral reefs by the middle of the century. Increasing ocean temperatures will result in more coral bleaching, disease outbreaks, reduced fecundity and survivorship in reef corals (Brown 1997; Knowlton 2001; Carpenter et al. 2008; Weil and Rogers 2011; Jackson et al. 2014; Hoegh-Guldberg et al. 2014). The influence of climate change on ocean-atmosphere interactions is also expected to have negative effects on weather patterns, ocean current patterns, as well as increasing the frequency and severity of storms, all of which can damage coral reef structure, change connectivity patterns (ocean currents), and reduces their overall fecundity and survivorship (if severe and frequent) (Brown 1997; Hoegh-Guldberg et al. 2014; Hoegh-Guldberg et al. 2015). Likewise, ocean acidification (CO₂ emissions being absorbed into the oceans) is altering seawater chemistry and lowering the pH of the ocean (Anthony et al. 2008; Kleypas and Yates 2009; Hoegh-Guldberg et al. 2014; Hoegh-Guldberg et al. 2015). Similar to ocean warming, the rate of ocean acidification has raised concerns over the survival of reef-building corals due to their long generation times and their capacity to adapt fast enough to changing seawater chemistry (Hoegh-Guldberg et al. 2015). Furthermore, acidification lowers aragonite saturation state that limits calcifying organisms' ability to construct their skeleton and run the risk of dissolution (Anthony et al. 2008).

103

Additionally, there is evidence that acidification has adverse effects on metabolism, sensory systems, and reproductive stages (Kleypas and Yates 2009; Hoegh-Guldberg et al. 2014). Overall, the principle concerns are that coral reefs experiencing ocean acidification will lead to lower recovery potentially with increasing susceptibility to coral bleaching and disease (Anthony et al. 2008; Kleypas and Yates 2009; Hoegh-Guldberg et al. 2014; Hoegh-Guldberg et al. 2015).

Climate change presents serious challenges on a global scale and it should be apparent that there are limited options beyond our immediate control. If the overall goal is to protect biodiversity to maintain ecosystem goods and services then perhaps our efforts should focus on reducing or eliminating local disturbances to coastal areas. This can be achieved to some extent by establishing *serious* federal conservation law enforcement within marine reserves, environmental / ecological restoration, addressing poverty, and increasing community awareness of natural resources and sustainability are good first steps in effectively increasing coral reef resistance and resilience to future disturbances.

References

- Aguilar-Perera, Schäerer M, Valdés-Pizzini M (2006) Marine protected areas in Puerto Rico: Historical and current perspectives. *Ocean & Coastal Management*, 49: 961–975.
- Anthony KRN, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proceedings* of the National Academy of Science USA, 105: 17442–17446.
- Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. *Hydrobiologia*, 460: 25–38.
- Baskett ML, Nisbet RM, Kappel CV, Mumby PJ, Gaines SD (2010) Conservation management approaches to protecting the capacity for corals to respond to climate change: a theoretical comparison. *Global Change Biology*, 16: 1229–1246.
- Ballantine DL, Appeldoorn RS, Yoshioka P, Weil E, Armstrong R, Garcia JR, Otero E, et al. (2008) Biology and ecology of puerto rican coral reefs. *in Coral reefs of the USA*. Riegl B, Dodge RE (Eds). Springer, Berlin. pp. 375-406.
- Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs, 16: 129–138.
- Carpenter KE, Abrar K, Aeby G, Aronson RD, Banks S, et al. (2008) One- third of reefbuilding corals face elevated extinction risk from climate change and local impacts. *Science*, 321: 560–563.
- Casey JM, Connolly SR, Ainsworth TD (2015) Coral transplantation triggers shift in microbiome and promotion of coral disease associated potential pathogens. *Scientific Reports*, 5: 11903. doi: 10.1038/srep11903.
- Certner RH, Vollmer SV (2015) Evidence for autoinduction and quorum sensing in White Band Disease-Causing microbes on *Acropora cervicornis*. *Scientific Reports*, 5: 11134. doi:10.1038/srep11134.
- Cinner JE, Daw T, McClanahan TR (2009a) Socioeconomic factors that affect artisanal fishers' readiness to exit a declining fishery. *Conservation Biology*, 23: 124–130.
- Cinner JE, McClanahan TR, Daw TM, Graham NAJ, Maina J, Wilson SK, Hughes TP (2009b) Linking social and ecological systems to sustain coral reef fisheries. *Current Biology*, 19: 206–212.
- Cinner J (2010) Poverty and the use of destructive fishing gear near east African marine protected areas. *Environmental Conservation*, 36: doi:10.1017/S0376892910000123.

- Cinner J (2011) Social-ecological traps in reef fisheries. *Global Environmental Change*, 21: 835–839.
- Covich AP, Austen MC, Barlocher F et al. (2004) The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. *BioScience*, 54: 767–775.
- Cróquer A, Weil E (2009) Changes in Caribbean coral disease prevalence after the 2005 bleaching event. *Diseases of Aquatic Organisms*, 87: 33–43.
- Daily GC, Alexander SE, Ehrlich PR, Goulder L, Lubchenco J, Matson PA, Mooney HA et al. (1997) Ecosystem services: benefits supplied to human societies by natural ecosystems. *Issues in Ecology*, 2: 1–18.
- Dalton T, Forrester G, Pollnac R (2015) Are Caribbean MPAs making progress toward their goals and objectives? *Marine Policy*, 54: 69-76.
- Gignoux-Wolfsohn SA, Marks CJ, Vollmer SV (2012) White band disease transmission in the threatened coral, *Acropora cervicornis*. *Scientific Reports*, 2: 804. doi:10.1038/srep00804.
- Gladfelter WB (1982) White-band disease in Acropora palmata: implications for the structure and growth of shallow reefs. *Bulletin of Marine Science*, 32: 639–643.
- Hector A, Bagchi R (2007) Biodiversity and ecosystem multifunctionality. *Nature*, 448: 188–190.
- Hoegh-Guldberg O, Skirving W, Stone D, Burrows MT, Bell J, Cao L, Donner S et al.
 (2014) The Ocean, *in Climate Change* 2014: *Impacts, Adaptation, and Vulnerability*.
 Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
 pp. 1655-1731.
- Hoegh-Guldberg O, Eakin CM, Hodgson G, Sale PF, Veron JEN (2015) ISRS consensus statement on climate change and coral bleaching. *Prepared for the 21st Session of the Conference of the Parties to the United Nations Framework Convention on Climate Change*, Paris, France. http://reefcheck.org/PDFs/ISRSConsensus2015.pdf
- Hughes TP (1994) Catastrophes, phase shifts and large-scale degradation of a Caribbean coral reef. *Science*, 265: 1547-1551.
- Hughes TP, Tanner JE (2000) Recruitment failure, life histories, and long-term decline of Caribbean corals. *Ecology*, 81: 2250-2263.
- Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, et al. (2003) Climate change, human impacts, and the resilience of coral reefs. *Science*, 301: 929–933.

- Hughes TP, Graham NAJ, Jackson JBC, Mumby PJ, Steneck RS (2010) Rising to the challenge of sustaining coral reef resilience. Trends in Ecology and Evolution, 25: 633–642.
- Jackson JBC, Donovan MK, Cramer KL, Lam VV (2014) Status and trends of Caribbean coral reefs: 1970-2012. *Global Coral Reef Monitoring Network*, IUCN, Gland, Switzerland.

Kleypas and Yates (2009) Coral reefs and ocean acidification. Oceanography, 22: 108-117

- Kline DI, Vollmer SV (2011) White Band Disease (type I) of endangered Caribbean acroporid corals is caused by pathogenic bacteria. *Scientific Reports*, 1:7. doi:10.1038/srep00007.
- Knowlton N (2001) The future of coral reefs. *Proceedings of the National Academy of Sciences of the United States of America*, 98: 5419–5425.
- Lucas MQ, Rodriguez L, Sanabria D, Weil E (2014) Natural prey preferences and spatial variability of predation pressure by *Cyphoma gibbosum* (Mollusca: Gastropoda) on octocoral communities off La Parguera, Puerto Rico. *ISRN Ecology*, 742387: 1-13. doi.org/10.1155/2014/742387.
- Lucas MQ, Goodman J (2015) Linking coral reef remote sensing and field ecology: it's a matter of scale. *Journal of Marine Science and Engineering*, 3: 1-20. doi: 10.3390/jmse3010001.
- Lucas MQ, Weil E (2015) Recent recovery in *Acropora cervicornis* and abundance of *A. prolifera* off La Parguera, Puerto Rico. *Marine Biodiversity*, doi: 10.1007/s12526-015-0399-4.
- Lucas MQ, Stat M, Smith MC, Weil E, Schizas N (2016) *Symbiodinium* diversity in the coral host *Agaricia lamarcki* (Cnidaria: Scleractinia) between shallow and mesophotic reefs in the Northern Caribbean. *Marine Ecology*, In Press.
- Miller MW, Lohr KE, Cameron CM, Williams DE, Peters EC (2014) Disease dynamics and potential mitigation among restored and wild staghorn coral, *Acropora cervicornis*. *PeerJ*, 2: e541. doi:10.7717/peerj.541.
- Nyström M, Graham NAJ, Lokrantz J, Norström AV (2008) Capturing the cornerstones of coral reef resilience: linking theory to practice. *Coral Reefs*, 27: 795–809.
- Otero E (2009) Spatial and temporal patterns of water quality indicators in reef systems of southwestern Puerto Rico. *Caribbean Journal of Science*, 45: 168-180.

- Palumbi SR, Sandifer PA, Allan JD Beck M, Fautin DG et al. (2009) Managing for ocean biodiversity to sustain marine ecosystem services. *Frontiers in Ecology and the Environment*, 7: 204–211.
- Roff G, Mumby PJ (2012) Global disparity in the resilience of coral reefs. *TREE*, 27: 404–413.
- Rogers CS, Muller E, Spitzack T, Miller J (2009) Extensive coral mortality in the US Virgin Islands in 2005/2006: a review of the evidence for synergy among thermal stress, coral bleaching and disease. *Caribbean Journal of Science*, 45: 204–214.
- Rogers CS (2013) Coral reef resilience through biodiversity. *ISRN Oceanography*, 739034: 1-18. doi.org/10.5402/2013/739034.
- Sala E, Knowlton N (2006) Global marine biodiversity trends. *Annual Review of Environment and Resources*, 31: 93–122.
- Valdés-Pizzini M, Schärer-Umpierre M (2014) People, habitats, species, and governance: an assessment of the social-ecological system of La Parguera, Puerto Rico. *Interdisciplinary Center for Coastal Studies*, University of Puerto Rico, Mayagüez, Puerto Rico, USA.
- Wade C, Otero E, Poon-Kwong B, Rozier R, Bachoon D (2014) Detection of humanderived fecal contamination in Puerto Rico using carbamazepine, HF183 Bacteroides and fecal indicator bacteria. *Marine Pollution Bulletin*, 101: 872-7. doi: 10.1016/j.marpolbul.2015.11.016.
- Weil E (2004) Coral reef diseases in the wider Caribbean, *in Coral Reefs and Disease*, Rosemberg E, Loya Y (Eds) Springer-Verlag. pp. 35-68.
- Weil E, Cróquer A, Urreiztieta I (2009) Temporal variability and impact of coral diseases and bleaching in La Parguera, Puerto Rico from 2003–2007. *Caribbean Journal of Science*, 45: 221–246.
- Weil E, Rogers CS (2011) Coral reef diseases in the Atlantic-Caribbean, in Coral Reefs: An Ecosystem in Transition. Dubinsky Z, Stambler N (Eds) Springer, Netherlands. pp. 465–491.
- Wooldridge SA, Done TJ (2009) Improved water quality can ameliorate effects of climate change on corals. *Ecological Applications*, 19: 1492–1499.

- Wooldridge SA (2009) Water quality and coral bleaching thresholds: formalising the linkage for the inshore reefs of the Great Barrier Reef, Australia. *Marine Pollution Bulletin*, 58: 745–751.
- Wooldridge SA, Brodie JE, Kroon FJ, Turner RDR (2015) Ecologically based targets for bioavailable (reactive) nitrogen discharge from the drainage basins of the Wet Tropics region, Great Barrier Reef. *Marine Pollution Bulletin*, 97: 262–272. doi.org/10.1016/j.marpolbul.2015.06.007.