OMNIFARIOUS OCTOCORAL OBSERVATIONS:

ECOLOGY AND GENETICS OF OCTOCORAL COMMUNITIES FROM ÚTILA, BAY ISLANDS, HONDURAS



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A thesis submitted for the degree of
Doctor of Philosophy
Michaelmas 2016

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Abstract

The Oxford English Dictionary defines 'omnifarious' as 'comprising or relating to all sorts or varieties', which quite accurately captures the very nature of octocorals and this thesis. The research reported here, aims to describe undocumented communities of coral reef organisms – the octocorals – which are an emergent dominant component within their threatened ecosystem of the Caribbean. Within the last four decades, coral reefs worldwide have experienced a precipitous plunge in many ecosystem services they provide, and most notably in the Caribbean. The foundation to reef resilience is structured on the ecosystem's ability to repair and restructure itself in the face of environmental shifts. These intricately complex strategies of resilience depend on repair mechanisms provided by a source of biodiversity, much of which remains poorly understood. This work explores many facets of the functioning within this potential future coral reef ecosystem. These reports are one of the most significant contributions to documenting and describing octocoral biodiversity (e.g. species, genetic, and community diversity) of the wider ecoregion of the Mesoamerican Barrier Reef System within the last three decades.

Declaration of Originality

I, Vanessa Blair Lovenburg, do declare that the work presented here is my own, through the supervision of Alex D Rogers and Martin Speight. In the instances of collaboration with the data collected by the research programme of Operation Wallacea volunteers and students, this work is noted. Manuscripts for each chapter are in preparation with collaborators and intended for submission to journals in due time. No part of my thesis has been accepted or is currently being submitted for any degree, diploma or certificate or other qualification in this University or elsewhere.

Dedication

I dedicate this thesis to my Dad.

Acknowledgements

I am overwhelmed with gratitude for the numerous people that have helped me during the process of this thesis. I will attempt to thank you in your order of appearance. Therefore, mom, dad, thanks for the conception...

In all seriousness, I would like to acknowledge my primary supervisor, Alex D Rogers for his unfailing support and belief in me through every dramatic turn in my academic and personal – and seemingly soap-operatic – life. Michelle Taylor. My octocoral guru. What can I say? You led me into the wonderful world of octocorals (admittedly kicking and screaming at first) and molecular genetics with an enviable zest and mastery. I am continually humbled by your dedication to your science, your students, your family, and your friends, of which I consider myself very lucky to be counted amongst (we are still friends after all this, right?!). I would next like to acknowledge the amazing support and superb tutelage of Nicolai Roterman, my PCR Yoda. "Always two there are, no more, no less. A master and an apprentice." Thank you for being both my rock and my kite...

Keeping to the rough structure of acknowledging in order of appearance: Kate Reynolds, my first real and closest friend in Oxford, you are simply the best. You really have been through it all with me. Thank you for 3:33. My wonderful lab, the ORCs (Ocean Research and Conservation), who grow faster than an asexually reproducing coral, thank you for being most excellent. More specifically, I wish to acknowledge Anni Djurhuus. I can't image my time through this without you. We've been side by side the whole way (until the end there where you took off in a sprint like you do on our runs...show-off). You and your amazing husband, Philipp Boersch-Supan are my most favourite of power -couple duos ever. Always be yourself. Unless you can be a unicorn. Then always be a unicorn. Penguin Tom, dude...Your enthusiasm for birds (puns implied) is unparalleled. Thank you for the cute-fury you impart to me every time you come back from Antarctica and for all the caffeinated deliveries. Jesse, thank you for inspiring me to battle R and holding my hand when I needed your sage guidance. Lynne Richardson, your DNA sequencing work made this thesis possible. Thank you to the sequencing department team who ran my samples and the shop team who made my secchi disks like bosses. Thank you to all of the lovely team in Darwin's canteen who kept my belly and coffee pots full and my spirits high.

I wish to thank my supervisor, Martin Speight (fairly sure you're officially my supervisor now?). I am firstly, eternally grateful for your guidance into the amazing programme that Operation Wallacea (I'll get to you, hang on) afforded me for my thesis fieldwork. Thank you for always managing to clear my dark clouds and help me see the sunshine and humour. I would like to thank you for introducing me to Dan Exton, whose advice and support, both personally and through the auspices of Operation Wallacea, has been paramount to this work. In spite of numerous field-meltdowns, you have always been there for me. I will always be your first doctorate advisee, don't forget that.

Max Bodmer, you have your own paragraph. If sunshine could come from anyone, it is most definitely you. Like a limpet, I will cling to you (why hasn't anyone studied limpets in Útila? Get on it people)...forever (if it sounds like a threat it probably is one). You just get me. I would not have survived the field if it weren't for you, and now that I am aware of your existence (and oh what a 'fine!' one it is), I am afraid I will continue to need you for my survival skills. Thank you? Thank you!

From the field seasons in Útila, there are just too many people to properly acknowledge. I'd like to thank Coral View and the staff who provided the facilities and the family-like hospitality. Miss Tonya, yours hugs kept me going. The most awesome of boat captains Romel; and Makine, thank you, your honey is the sweetest of them all (he's a bee keeper, calm down, mom). My wonderful dissertation students: Team Benthos! You were the best team I could have possibly asked for, and I still owe you t-shirts, which I now finally have the time to make. I must single out JENNAY (J

Murray took a lot of the photos during the transects!) and Eric who I managed to convert to Team Octocoral. I literally could not have done all of this without your help. Every data entry and sample is imprinted with your loving memory. Another shining beacon of joy, Willco, my Secchi Sunshine King. MOO MOO! What would I have done without you? Eat the babies. Dom, my field brother, we can be heroes...Thank you to Marvin the Monkey and your Marvellous Mangos. Alicia Hendrix, this is a cashew? I love you.

I would like to acknowledge also the unparalleled support from Coral Morphologic. The human embodiment of corals (the coral Jesus?), Colin Foord, thank you for always for inspiring awe and wonder in me. You, my friend, are the future key to saving the corals. I look forward to helping you turn that key. Now we can finally focus on all of our plans for the Coral Morphologic Foundation. However, can I also propose the 'Institute of Boring Science and Failed Experiments'? Because that really should be a thing.

Genny Anderson, you were the first professor to introduce me to marine biology. My last elective science course at Santa Barbara City College on my way to Hollywood (waitress/ 'actress') and you made my head spin. You reinvigorated my love for science and inspired me to become just like you. I wrote my final chapter in under you and your wonderful husband Shane's gracious roof, completing my academic life cycle that you instigated. Thank you for the oh-so pleasing symmetry...(radial symmetry that is...it's a cnidarian joke, you'll get it if you read this thesis).

Thank you to the deities that helped me along the way: the molecular deities and the PCR gods specifically. But in reality, thank you to God, who I have very recently reconnected with thanks to Hans Cosmas Ngoteya (my next book will be dedicated to you, my love). You've always been there for me, even when I didn't believe in your existence. What's this, how can I be a scientist AND believe in an almighty deity?! Madness! Get over it people and quit acting like you know so much...you know nothing (on that note, I'd like to thank Jon Snow for being an entertaining idiot that won't die...kids, you're not going to get this one).

Thank you to aliens that challenge and inspire me. Thank you to lima beans, coffee beans, and baked beans. All the beans. And human beans. Kevin Benzie, are you finished reading my thesis yet? I can't even begin...so I just won't. You know. Clearly, you're out of order and to blame for everything here...

And finally, saving the best for last, my family. Both past and present, you are always with me...Mom, Lin, Sarah, Auntie D, Bumble Bee, Gelfling Fortytwo (live long and prosper) and all the rest of you crazy awesome mishpocha. John, Julie, Vickie and all of your clans, I love you more than I can ever express. I have truly the best family ever. You are all so special and inspiring and wonderful and joyful and supportive and understanding and kind. ...Was I adopted?

"It's beautiful beneath the sea, but if you stay too long, you'll drown"

-Three Eyed Raven, Game of Thrones

"A PhD is the slowest extreme sport ever"

- Dr. Tom Hart

"Whoever loves instruction loves knowledge,

But he who hates correction is stupid"

-Proverbs 12:1

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1 Chapter One:

Introduction

1.1 Biodiversity of coral reefs

Wholly encompassing the ecosystem, species and genetic diversities, biodiversity is the framework for biological pattern recognition. Within these patterns are potential big-picture solutions for threats to the natural world (UNEP-WCMC, 2015). The universal language of biodiversity connects all disciplines and structures, and acts as the very foundation for tackling global climate change, disease spread, and inspiring medical advances (May, 1986; UNEP, 2000; Costello *et al.*, 2010).

Coral reefs are considered one of the most important ecosystems for conservation and study because of their uniquely high concentration of biodiversity, which is critically threatened by climate change and spread of disease (Woodley *et al.*, 2003; Birkeland, 2015). These highly diverse ecosystems have been referred to as 'evolutionary cradles' that function like 'species pumps' (Brusca *et al.*, 2016) and the lives of nearly half a billion people depend on their continued functioning (TEEB, 2010). The high species richness of corals (over 6,200 described anthozoan species) represent an extraordinary ecological importance (Birkeland, 2015). Additionally, corals are abundant sources of diverse bioactive compounds with antimicrobial and cancer-fighting properties (mostly derived from octocorals) (Cinel *et al.*, 2000b; Gross and König, 2006; Imbs, 2011; Rocha *et al.*, 2011).

Yearly economic value provided by coral reef ecosystem services estimates that a single hectare of reef is worth upwards of $2,129,122 (in international dollars)(Costanza *et al.*, 2014). A study on the economics of surfing calculated that viable surfing waves contribute approximately US$50 billion to the global economy

and reef breaks are one of the best producers of quality waves (McGregor and Wills, 2016). Between 1997 and 2011, nearly 50% of coral cover on global reefs was lost (i.e. from 62 to 28 hectares). It is difficult to assess how the continued loss of coral cover from reefs worldwide has impacted ecosystem services and incomes reliant on its provisioning, i.e. fishing and tourism. The complexity of social-ecological relationships of coral reefs is vast and challenging to model, even through a well-studied reef system such as Mo'orea, French Polynesia (Leenhardt *et al.*, 2017). However, the loss of the unique biodiversity and natural wonder a coral reef contributes cannot be measured in dollars.

1.1.1 Cnidarians

Corals are classified within the phylum Cnidaria, which comprise roughly 13,400 described species living today. These organisms are a diverse group, united by the unique ability to manufacture complex stinging cells known as cnidae, which take on many roles such as movement, defense, feeding, and attachment to substrate. Cnidarians are also widely known for their asexual abilities which produce large colonies (Brusca *et al.*, 2016).

Cnidarians typically exist either as solitary or colonial polyploid forms (e.g. anemones and solitary cup corals or hydroids and corals) or medusoid forms (e.g. jellyfish). Often during cnidarians dimorphic life cycle, individuals will possess both a polyploid and medusoid form (exception seen in Anthozoa) (Brusca *et al.*, 2016). Additionally, they contain simple nerve nets, have two germ layers (i.e. diploblastic) which form musculature from myoepithelial cells, lack cephalization (typically radial symmetrical), and have unique planula larvae, typically motile (Brusca *et al.*, 2016).

Members of the Cnidaria have one of the longest histories in the metazoan fossil record and have putative representatives from Ediacarian fauna before the Cambrian explosion 542 million years ago (Chen *et al.*, 2002). The stem-group cnidarian *Cambroctoconus orientalis,* possessed octoradial symmetry, which supports the hypothesis that octocorals are the most basal group in the cnidarians (Bridge *et al.*, 1995; Berntson *et al.*, 2001; Won *et al.*, 2001; Collins, 2009; Park *et al.*, 2012). Recent mitochondrial sequencing brought the monophyly (i.e. common origin) of the class Anthozoa under question, placing octocorals with Medusozoa (Kayal *et al.*, 2013). However, the scientific consensus supports two major radiations of cnidarians: the Medusozoa, comprising hydrozoans and jellyfish, and the Anthozoa, comprising hexacorals (six-fold radial symmetry) and octocorals (eight-fold radial symmetry) (Daly *et al.*, 2007; Collins, 2009; Van Iten *et al.*, 2014) (see Fig. 1.1 for a postulated phylogenetic relationship within Cnidaria).

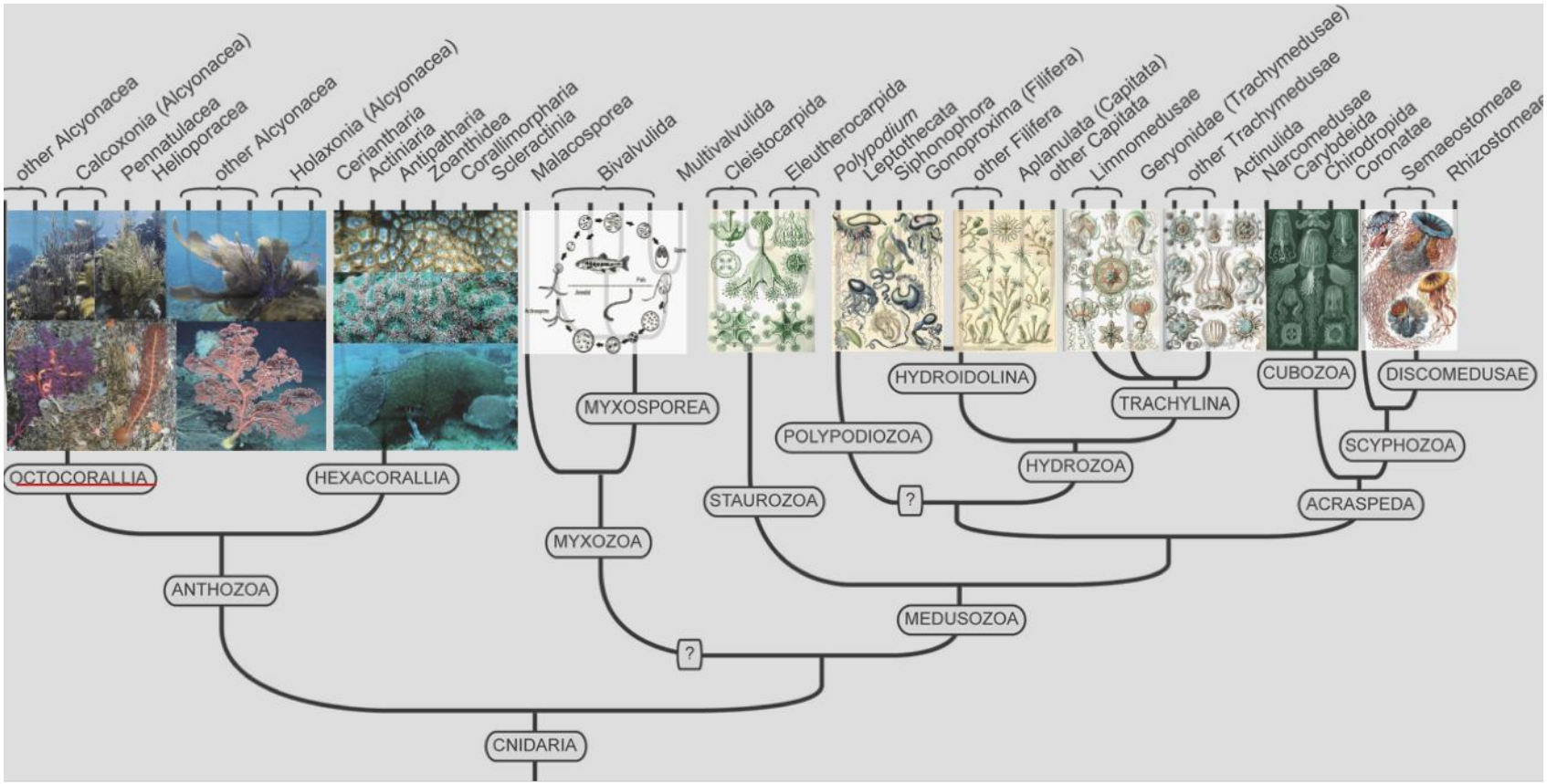


Figure 1.1 Proposed cnidarian phylogeny from (Collins, 2009). Photo credit: Octocorallia) Caribbean shallow: AD Rogers, & SW Indian Ocean deep-sea: JC066 expedition; Hexacorallia) VB Lovenburg & C Foord; Myxozoa life cyle) (Behrmann-Godel and Brinker, 2015); Staurozoa, Hydrozoa, Cubozoa, Scyphozoa: E Haeckel.

1.1.2 Octocorallia

Octocorallia commonly referred to as the soft corals, which includes sea fans, sea whips, sea pens, blue corals and precious corals (family Corallidae) is a well-supported monophyletic subclass within the class Anthozoa. Octocorallia consists of over 3,400 extant species and is considered the sister taxon to Hexacorallia (e.g. scleractinian corals, anemones), the other anthozoan subclass that contains 4,300 extant species (Daly *et al.*, 2007; Williams and Cairns, 2011). It is estimated that only 65% of the diversity within Octocorallia has been described as of 2012 (Appeltans *et al.*, 2012). Octocorals are found in every ocean, pole to pole. They can be found from the shallow sunlit waters of coral reefs to thousands of metres in the deep sea creating large gardens on seamounts and other vulnerable marine ecosystems (France *et al.*, 1996; Berntson *et al.*, 2001).

Octocorallia, as the nomenclature implies, has its basic elements (polyps and mesenteries) arranged in an eight-fold radial symmetry. All octocoral polyps have eight tentacles and eight mesenteries: the only true apomorphic character uniting the group (Daly et al. 2007). These octoradial polyps can be solitary, or more commonly part of an interconnected, genetically identical network that form a colony (Bayer *et al.*, 1983). Another diagnostic character of the group is the presence of lateral extensions on the tentacles known as pinnules; however, this is not strictly universal within octocoral taxa (Alderslade and McFadden 2007).

Like the scleractinians (reef-building corals), octocorals typically have a calcium-carbonate based skeletal support structure (Bayer, 1981; Fabricius and Alderslade, 2001; Miyazaki and

Reimer, 2015). In octocorals however, this calcium-carbonate skeleton typically does not fuse to form a continuous reef-building matrix[1]. The primary uniting skeletal elements, if present[2], are tiny hardened elements called sclerites that are integrated into a fleshy matrix (Bayer, 1981) (Fig. 1.2). These elements are sometimes completely fused into an internal skeletal axis, (e.g. Corallidae) however, many families (e.g. Plexauridae and Gorgoniidae) incorporate the calcium-carbonate with solidified proteins such as gorgonin (Bayer, 1981; Coll, 1992; Fabricius and Alderslade, 2001).

[1] Some species of *Sinularia* are able to fuse their basal sclerites into a matrix known as spiculite that has been found to contribute up to 30% of studied reefs in Taiwan (Jeng *et al.*, 2011).

[2] Some species have no sclerites such as *Malacacanthus* (Bayer, 1981) A new zooxanthellate genus (*Phenganax*), recently described from Indonesisa, also contains no sclerites. It was found to have an encrusting growth form on dead fragments of scleractinian coral (*Acropora* sp.) Alderslade and Mcfadden (2011).

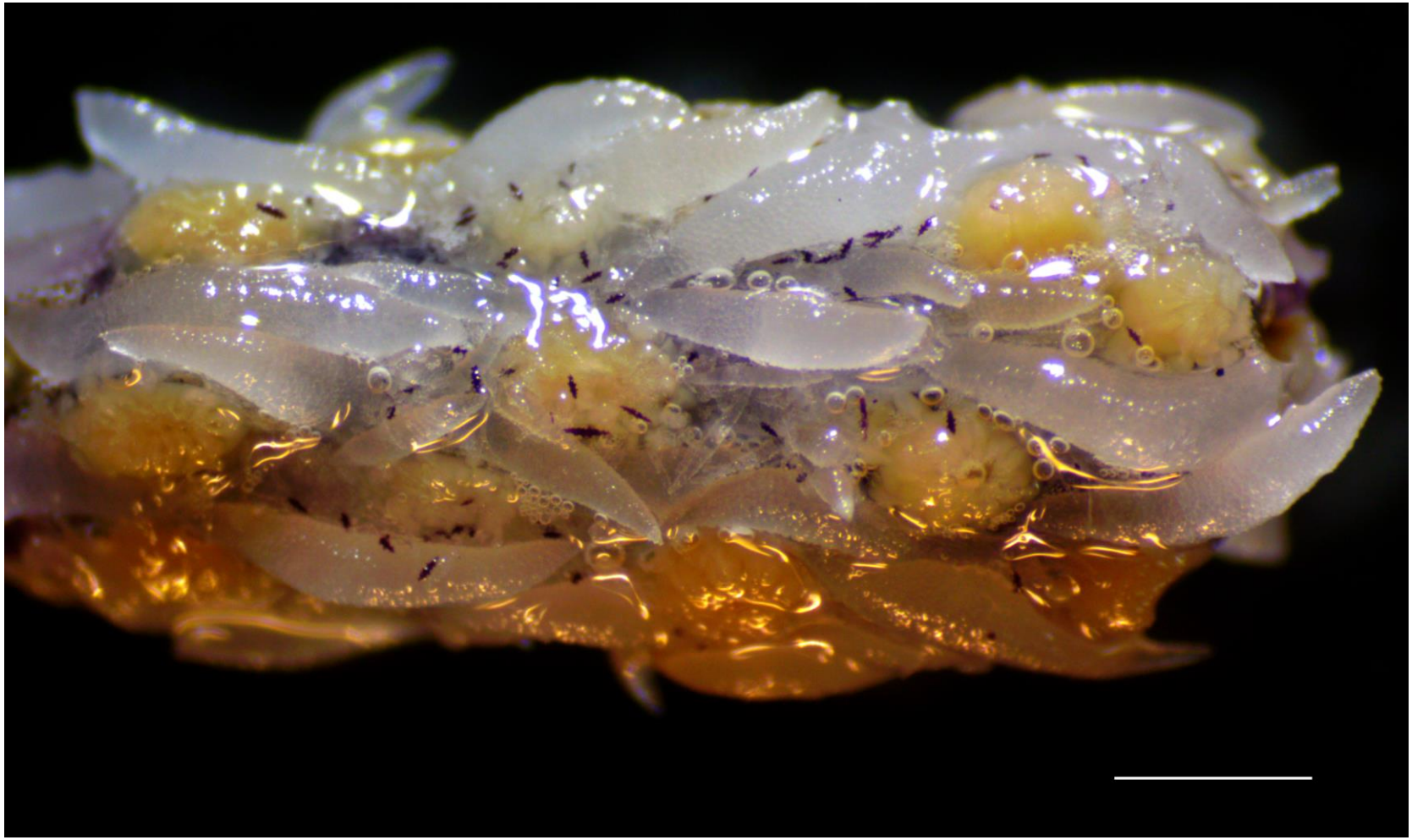


Figure 1.2 A partially dissolved branch from the octocoral, *Eunicea* sp. The top layer of sclerites (i.e. clubs) has been dissolved to reveal larger sclerites underneath (i.e. the large spindles and small purple capstans), which are surrounding the polyps. Scale bar = 1cm.

Symbiotic single-celled algae, called zooxanthellae, are another integral component of octocoral tissues for species living within the photic zone (occurring in approximately 30% of 3,400 described species) (Cairns, 2007). Zooxanthellae are a group of endosymbiotic dinoflagellates of the genus *Symbiodinium.* Often residing sub-dermally in many cnidarians, *Symbiodinium* typically inhabit warm, sunlit oligotrophic (nutrient deficient) waters (Freudenthal, 1962; Roberts *et al.*, 2009; Brusca *et al.*, 2016). The precise dependency between hosts and their symbiotic partner (i.e. nutritional and physiological relationship) is not completely understood. However, studies have shown that calcium carbonate production is positively correlated with this association and the removal (experimental and observed) of the photosyntheic symbionts often results in bleaching and death (Prada *et al.*, 2010; Brusca *et al.*, 2016; Parrin *et al.*, 2016). This autotrophic energy mechanism provided by the *Symbiodinium*, is combined with the heterotrophic suspension feeding by the animal polyps allowing octocorals (as well as other anthozoans) a uniquely dual mixotrophic carbon capture system (Fabricius and Klumpp, 1995).

1.1.3 Ecology

Despite the abundance of octocorals in many sessile benthic communities in all the world's oceans, there remain large knowledge gaps in the basic ecology of many species (Fabricius and Klumpp, 1995; Roberts *et al.*, 2009). As sessile organisms, environmental conditions are integrally influential to their biology and play a critical role in structuring communities both in coral reefs and the deep sea (Wainwright and Dillon, 1969; Kinzie, 1970; Muzik, 1982; Dahlgren, 1989; Fabricius and Alderslade, 2001; Fabricius and De'ath, 2008; Smith *et al.*,

2008; Yesson *et al.*, 2012).

1.1.3.1 Reproduction

The relationship octocorals have with their environment begins with creative reproductive strategies adapted for a sedentary life. The variability of sexuality and reproduction in octocorals is large and varied (Simpson, 2009). There are gonochoric, hermaphroditic and parthenogenetic representatives, with sliding scales of sexuality within each category (Kahng *et al.*, 2011). Reproduction can occur asexually through multiple means: the detachment of branches, as seen in *Plexaura kuna* (Lasker, 1984) and *Eunicea fusca* (Sánchez, 2009); the vegetative propagation of stolons in *Briareum asbestinum* (Lasker, 1983); and the propagation of hundreds of clones simultaneously every other day in *Dendronephthya hemprichi* (Dahan and Benayahu, 1997). Sexual reproduction also has multiple strategies, each with variations within, such as internal brooding seen in *Briareum asbestinum* (Kahng *et al.*, 2011), surface brooding in *Antillogorgia elisabethae* (Lasker, 2004), and – the method most associated with coral reefs – broadcast spawning such as sea fans *Gorgonia* spp. and many others (Fitzsimmons-Sosa *et al.*, 2004; Pakes and Woollacott, 2008; Kahng *et al.*, 2011; Andras *et al.*, 2013). Modes of reproduction, which limit dispersal potential of species, are essential in understanding ecosystem functioning, and population recovery from disturbance (Simpson, 2009).

1.1.3.2 Biochemical

Planula larvae have been shown to use biochemical-settling cues (Pawlik, 1992). As sessile

organisms with slow regenerative properties, especially those that lack substantial physical defense systems, they tend to evolve a complex amalgamation of bioactive weaponry (Brusca *et al.*, 2016). Marine organisms in general have been found to be prolific in bioactive products (Ciereszko *et al.*, 1968 ; Weinheimer and Spraggins, 1969 ; Moncada *et al.*, 1975 ; Gerhart, 1984; Guerriero *et al.*, 1988; Rodríguez and Ramîrez, 1994; Iguchi *et al.*, 1995; Rodríguez *et al.*, 1999; Tomono *et al.*, 1999; Rueda *et al.*, 2001; Mydlarz *et al.*, 2003; Wei *et al.*, 2004; Bhakuni and Rawat, 2005; Epifanio *et al.*, 2007; Rocha *et al.*, 2011; Pawlik, 2012). Octocorals use these chemicals to combat fouling, predators, and disease (Gerhart and Coll, 1993; Pawlik, 1993). Levels of some toxins have been shown to correlate with levels of UV radiation, which suggests an inherent sunscreen-like property (Thornton and Kerr, 2002).

Incidentally, many of these biochemicals have important medical applications for humans (Gross and König, 2006). Prostaglandins, which have wide-ranging effects on smooth muscle tissue and blood pressure (Weinheimer and Spraggins, 1969; Kinzie, 1974; Pike, 1974; Weinheimer, 1974; Carmely *et al.*, 1980), have been isolated from octocoral species in Japan (Iguchi *et al.*, 1995), the Indian Ocean (Guerriero *et al.*, 1988), the Bering Sea (Imbs *et al.*, 1989) and the Caribbean (Imbs, 2011). Eleutherobin, a type of diterpene, originally extracted from the soft coral *Eleutherobia* (Lindel *et al.*, 1997), and subsequently *Erythropodium caribaeorum* (Cinel *et al.*, 2000b), has significant cancer-fighting properties (Gross and König 2006).

The vast medical potential inherent in many octocoral species has led to many chemical screening studies and large-scale harvesting efforts for synthesis research (Weinheimer and Spraggins;

Weinheimer, 1974; Carmely *et al.*, 1980; Roussis *et al.*, 1990; Cinel *et al.*, 2000a; Cinel *et al.*, 2000b; Changyun *et al.*, 2008; Lasker and Porto-Hannes, 2015). Compounds with anti-proliferative (reduction of malignant cell growth, characteristic of cancer cells) properties found in *Anthogorgia* spp. add valuable information to high throughput screening (Zheng *et al.*, 2014). In turn, high throughput screening has also uncovered perezone in *Antillogorgia (=Pseudopterogorgia) rigida,* a compound initially discovered in mangrove plants (*Accourtia*) that induced oxidative stress in human leukemia cells (Abreu *et al.*, 2015).

For the last 18 years in the Bahamas, colonies of *A. elisabethae* have been harvested for pseudopterosins, bioactive compounds with analgesic and anti-inflammatory properties (Look *et al.*, 1986; Lasker and Porto-Hannes, 2015). The drug has been called a 'life-saving' product and the market value of the Bahamian *A. elisabethae* fishery was estimated to be between three to four million USD per annum (Bruckner, 2002).

This particular substance found in *A. elisbethae* originates from the zooxanthellae and not the octocoral tissue (Mydlarz *et al.*, 2003), however, other biochemicals such as oxylipins, that also regulate physiological processes (e.g. inflammation), have been isolated from both zooxanthellate and non-zoxanthellate corals (Imbs, 2011). Microbial biotransformative abilities have also been elucidated in extremophiles (Kim and Peeples, 2006). Such works highlight the necessary inclusion of the entire ecosystem's service potential especially in the face of extreme environmental pressures brought about by climate change (Demirjian *et al.*, 2001). The characterisations of biochemicals has even been used to infer phylogenetic relationships between octocorals (Gerhart, 1983). As the current literature is far from

exhaustive, presumably many more biochemicals with medical potential are awaiting discovery. This illustrates the importance of the study of these organisms in terms of ecosystem services not usually considered in the context of marine invertebrates.

1.1.3.3 Physical

It is often challenging to parse apart the many interacting conditions that define the ecology of an organism, especially for those species for which identification requires expertise (Bayer, 1961). Species-specific ecologies can also be geographically determined, further complicating studies attempting to define their ecological requirements (Opresko, 1973). However, many studies have successfully outlined some of the key elements determining octocoral communities which include suitable substrata, water flow, temperature, salinity, sedimentation, depth, and light depending on whether species are zooxanthellate or azooxanthellate (Bayer, 1961; Dahlgren, 1989; Yoshioka and Yoshioka, 1989; Sánchez *et al.*, 1997; Fabricius and Alderslade, 2001).

The availability and diversity of suitable stable substrata for attachment and growth of colonies is one of the primary factors affecting relative abundance of octocorals (Kinzie, 1970; Kinzie, 1973; Jordan, 1978; Yoshioka and Yoshioka, 1989; Sánchez *et al.*, 1997). Early settlement cues of planulae larvae are based on these restrictive requirements, and larvae tend to prefer rocky benthos with particular amounts of sand (Gotelli, 1988). Habitat complexity and topographic relief of benthic environment is a commonly cited determinant of greater species richness and abundance on reefs (Gratwicke and Speight, 2005; Alvarez-Filip *et al.*, 2009;

Newman *et al.*, 2015).

Wave energetics and the movement of water, intrinsic to the architecture of the substratum, has also been demonstrated to shape octocoral assemblages (Wainwright and Dillon, 1969; Kinzie, 1970; Kinzie, 1973; Yoshioka and Yoshioka, 1989; Fabricius and Alderslade, 2001). The perpendicular positioning of sea fans (*Gorgonia* spp.) into currents is reliable enough to predict prevailing current directions in their environment (Wainwright and Dillon, 1969; Opresko, 1973).

Although octocorals are confined to marine environments where salinities are approximately 35‰, reports have found communities able to tolerate periodically elevated and diminished levels ranging from 14.4‰ (Fabricius and McCorry, 2006) in Hong Kong to over 45‰ in the Red Sea (Fabricius and Alderslade, 2001) and certain species such as *Leptogorgia setacea* has been found in Chesapeake Bay where salinity averages 17.2‰ (Bayer, 1961). However, consistent levels below 25‰ are typically lethal for most species (Fabricius and Alderslade, 2001).

Azooxanthellate species, living at greater depths or temperate climates, generally have broader ranges than zooxanthellae corals for most environmental conditions (Fabricius and Alderslade, 2001) where tropical species require a stable range between 18˚C and 31˚C. . Prolonged exposures to temperatures above this range have resulted in bleaching (Bayer, 1961; Harvell *et al.*, 2001; Aronson *et al.*, 2002; Lasker, 2005; Prada *et al.*, 2010). *Briareum asbestinum* exposed to 38.2˚C and plexaurid species exposed to 34.5˚-35˚C experienced mortality within

an hour (Bayer, 1961).

Stress is also often brought on by increases in sedimentation, which has been demonstrated to be a major factor attributing to octocoral regional distribution (Yoshioka and Yoshioka, 1989). Sediment in the water column reduces penetration of light, reducing photosynthetic potential. Additionally, mucus production, an energetically costly process that aids in the removal of debris from colony surfaces, was found to increase in response to sedimentation (Riegl and Branch, 1994).

Light penetration through the water column is interrelated to sedimentation, as is depth, all of which are crucially important in habitat requirements of octocorals. Depth gradients with respect to light availability are typically a reliable determinant of assemblages in the ocean (Bayer, 1961; Goldberg, 1973; Kinzie, 1973; Fabricius and Alderslade, 2001; Roberts *et al.*, 2009). Depth distributions have been found to vary roughly with phylogenetic groupings as well, where some families are fairly restricted to deeper waters e.g. Isididae (bamboo corals) and others have more representatives in shallower depths, e.g. Gorgoniidae (sea fans) (Bayer, 1961).

Largely stimulated by ecosystem-based management of fisheries, there have been significant efforts to describe octocorals' ecology in the deep sea (Roberts, 2002; Rogers, 2004; Auster, 2005; Roberts *et al.*, 2006; Scher and Martin, 2006; Stone, 2006; Wakeford *et al.*, 2006; Cairns, 2007; Maier *et al.*, 2009; Tittensor *et al.*, 2010; Davies and Guinotte, 2011; Taylor, 2011; Rogers and Sack, 2012; Rogers *et al.*, 2012; Yesson *et al.*, 2012), where they can occur

in dense 'gardens' on seamounts and other habitats and have been found as deep as 5850 m below the surface (Cairns and Bayer, 2009; Gage and Tyler 1991). These dense gardens are thought to provide habitat for commercially important juvenile fish, among other organisms (Gili and Com, 1998; Migné and Davoult, 2002; Stone, 2006).

1.1.3.4 Biological

There are a multitude of biological interactions with respect to octocorals, such as endosymbiotic associates of zooxanthellate species (Kirk *et al.*, 2005), surface microbiomes of bacterial, fungal and viral communities (Rosenberg *et al.*, 2007; Hewson *et al.*, 2011), octocoral predators (Gerhart and Coll, 1993) or various mutualistic, commensalistic and parasitic symbiotic relationships (Bayer, 1961; Gerhart, 1990; Krieger and Wing, 2002; Martin *et al.*, 2002; Buhl-Mortensen and Mortensen, 2004; Costello *et al.*, 2005; Burkepile and Hay, 2007; Greene, 2008; Braga-Henriques *et al.*, 2011). The boundaries defining predation and levels of symbiosis are complicated and more research is needed in order to accurately qualify these relationships (Martin and Schwab, 2012).

The most distinct and most researched relationship is the algal endosymbiotic *Symbiodinium* that occurs in shallow water octocorals, as well as other cnidarians, e.g. scleractinian corals. Octocorals are less flexible in hosting multiple clades of *Symbiodinium* than scleractinian corals (Baker and Romanski, 2007). However, octocorals appear to be able to switch more readily than scleractinians between mixotrophy (*Symbiodinium*-assisted living) and heterotrophy (Van Oppen *et al.*, 2005). The ancestral condition of the symbiosis is unclear

and the character has probably been gained and lost several times throughout octocoral evolution, hence the polyphyletic status of zooxanthellate and azooxanthellate, octocorals, e.g. *Euplexaura nuttingi* is the only azooxanthellate species of *Euplexaura* (Van Oppen *et al.*, 2005). Strong host specificity has developed for octocorals in the Caribbean, more specialised than scleractianians counterparts (Van Oppen *et al.*, 2005).

Only a few species are known predators of octocorals, most likely because of their prey's high content of secondary metabolites, such as terpenoids, and unsavory sclerites, which have been shown to deter predation from fish (Alstyne *et al.*, 1992; Souza *et al.*, 2007; Watling *et al.*, 2011). The commonly complex branching structure of many octocorals combined with a relatively small predator presence, provides suitable biogenic habitat for multiple species that are able to tolerate these conditions (Etnoyer and Warrenchuk, 2007; Wilson *et al.*, 2008; Reijnen *et al.*, 2011).

Many other associates have been reported to occur on or within octocoral branches such as hydroids (Puce et al. 2008), anemones (Ocaña *et al.*, 2004), small crustaceans, e.g. copepods, isopods, and amphipods (McArthur 1985; Buhl-Mortensen and Mortensen 2004; (Greene, 2008), polychaete worms (Martin et al. 2002), echinoderms (Krieger and Wing 2002), benthic ctenophores (Bayer, 1961), seahorses (Reijnen et al. 2011), and many other fish (DeLoach, 1999; Krieger and Wing, 2002; Costello *et al.*, 2005; Etnoyer and Morgan, 2005; Etnoyer and Warrenchuk, 2007).

Additionally, rich and abundant associations of bacterial, fungal and viral assemblages living

on their surfaces, in a complex community known as the holobiont, are only recently beginning to be uncovered (Rosenberg *et al.*, 2007; Toledo-Hernández *et al.*, 2008; Hewson *et al.*, 2011; Robertson *et al.*, 2016).

As stated, it is often unknown whether the nature of these associations are commensalistic, parasitic or mutualistic (Bayer, 1961; Watling *et al.*, 2011). Octocorals are associated with many sponge species, some of which are hypothesised to have mutualistic relationships (e.g. *Briareum asbestinum*) (McLean and Yoshioka, 2007). Copepods are almost always parasitic, typically forming galls in the coenchymal tissue or within the gastrovascular cavity of a polyp (Bayer, 1961; Watling *et al.*, 2011). Polychaetes are not considered parasitic, but they alter their hosts' morphology to form tunnels of interconnected polyps within which they reside (Williams and López-González, 2005; Watling *et al.*, 2011). Parasites are known to change their associations based on the environment; therefore, an understanding of parasitic relationships could be valuable in addressing impacts in an ecosystem (Lafferty, 1997). For example, the barnacle larvae of *Conopea galeatus* will only settle on the egg cases of the ovulid snail *Simnia*, which has been laid on gorgonian tissue (Crisp, 1990). Associates are a vastly understudied ecological component of the octocorals, especially in the deep sea (Watling *et al.*, 2011).

1.1.3.5 Cyphoma gibbosum

One particular associate of octocorals is the relatively well-studied flamingo tongue snail, *Cyphoma gibbosum*, which has been called both a predator and a parasite of octocorals

(Ghiselin and Wilson, 1966; Gerhart, 1986; Harvell and Suchanek, 1987; Lasker and Coffroth, 1988; Lasker *et al.*, 1988; Gerhart, 1990; Ruesink and Harvell, 1990; Rosenberg, 1991; Alstyne and Paul, 1992; Alstyne *et al.*, 1992; Nowlis, 1993; Chiappone *et al.*, 2003; Burkepile and Hay, 2007; Rypien, 2008; Rypien and Baker, 2009; Reijnen *et al.*, 2010; Schärer and Nemeth, 2010). The flamingo tongue is an ovulid snail that is primarily found living on shallow-water Caribbean octocoral hosts where it feeds, mates and lays its eggs (Chiappone *et al.*, 2003). It is one of the few associates of octocorals able to cope with the toxic biochemical artillery of its hosts.

This marine snail's mantle tissue, which covers its relatively drably coloured shell, is vividly patterned and coloured (see Chapter Five), a classic aposematic warning of its own biochemical cocktail that is derived directly from its hosts through a kleptochemical mechanism. *Cyphoma gibosum* has the ability to steal, then sequester and biotransform these toxic compounds, such as prostaglandins and terpenoids, in its mantle tissue for its own defence from marine predators (Steudler *et al.*, 1977).

The snails also practice common clumping behaviours of aposematically coloured organisms, much like the monarch butterfly and other distasteful lepidopteran species (Brower *et al.*, 1967; Rosenberg, 1991). This gregarious gathering behaviour amplifies that signal of an undesirable meal to predators and increases each individual's chance of survival while they are in the group (Alstyne *et al.*, 1992). This gathering behavior also facilitates mating in the snails and communal feeding (Nowlis, 1993). Octocoral hosts are able to regenerate tissue from low-levels of grazing by a few individuals, however population outbreaks (i.e. over 30 snails per

octocoral) of *C. gibbosum* have resulted in mass die-offs of sea fan colonies (Schärer and Nemeth, 2010). The snails are also potentially an agent of secondary dispersal in the fungal disease Aspergillosis (Nagelkerken *et al.*, 1997; Slattery, 1999; Rypien, 2008; Rypien and Baker, 2009) (see section 1.1.5.4 on Disease).

1.1.4 Coral Reefs Ecosystems

Coral reefs support 30 of the 34 phyla in the world (Birkeland, 2015). Octocorals of shallow tropical environments are an integral component of these diverse coral reef ecosystems. The relative accessibility of the shallow waters, combined with the high species richness distinctive of tropical environments has brought significant research to these communities in terms of species and environmental richness assessments, disease etiology, reproductive potentials and biochemical discoveries (Burkholder and Burkholder, 1958; Wainwright and Dillon, 1969; Kinzie, 1970; Goldberg, 1973; Opresko, 1973; Kinzie, 1974; Gerhart, 1984; Yoshioka and Yoshioka, 1989; Fabricius and Alderslade, 2001; Sánchez and Cairns, 2004; Sánchez and Wirshing, 2005; Courtney *et al.*, 2007; Prada *et al.*, 2008; Toledo-Hernández *et al.*, 2009; Devictor and Morton, 2010; Kahng *et al.*, 2011; Santavy *et al.*, 2012b; Gómez *et al.*, 2014; Lasker and Porto-Hannes, 2015; Lenz *et al.*, 2015; Serrano *et al.*, 2016).

In the Indo-Pacific, where scleractinian corals dominate reefs, the most common octocorals are the 'soft corals' mostly from the polyphyletic sub-order Alcyoniina, which have no internal skeletal axis (Fabricius and Alderslade, 2001). Octocorals of the western Atlantic are dominated by 'gorgonians', referring to octocorals which have a fused scleroproteinaceous or

calcium carbonate axis such as members from Gorgoniidae and Plexauridae (Bayer 1961; (Goldberg, 1973; Sánchez and Wirshing, 2005). In the Caribbean, octocorals occur in dense communities and long-term studies have shown them to be increasing in abundance (Lenz *et al.* 2013).

Coral reefs formed by scleractinians are in decline worldwide (Wilkinson, 2008; De'ath *et al.*, 2012; Fisher *et al.*, 2015), particularly in the Caribbean where they no longer form the dominant reef habitat (Jackson *et al.*, 2014). As scleractinan coral cover continues along its predicted path of precipitous decline worldwide (De'ath *et al.*, 2012), ecological studies in areas of low coral cover like the Caribbean, provide valuable insights into future reef ecosystems (Hughes *et al.*, 2010).

Ecosystems are highly complex in biodiverse areas such as coral reefs. Teasing apart the drivers of ecosystem dynamics (i.e. top-down or bottom-up systems) is further complicated under a revisionary dominance structure (Birkeland, 2015). Undoubtedly, there are multiple explanations for the ecosystem shifts seen in the generally accepted 'coral-seaweed-herbivore triangle' (Done, 1992; Hughes, 1994; McCook, 1999; Knowlton, 2004; Bruno *et al.*, 2009; Mumby, 2009; Norström *et al.*, 2009; Dudgeon *et al.*, 2010; Fox, 2013; Sheil and Burslem, 2013; Birkeland, 2015). There is no argument that coral reef ecosystems are experiencing fundamental shifts under current environmental conditions (De'ath *et al.*, 2012; Fisher *et al.*, 2015). However, primarily a debate exists with how ecosystem respond to these shifts and what this means for the prediction of future coral reefs (Petraitis and Dudgeon, 2004; Mumby, 2009; Dudgeon *et al.*, 2010).

Phase-shifts largely brought on by anthropogenic impacts and natural disasters, allow opportunistic organisms, such as algae, sponges and octocorals to replace the barren benthos, giving way to new dominant systems (Aronson *et al.*, 2002; Bruno *et al.*, 2009; Dudgeon *et al.*, 2010). The phase-shift hypothesis implies linearity in this shifting regime, structured around loss of biodiversity that scleractinians support (this fact is certainly not disputed) (Petraitis and Dudgeon, 2004). Largely, the phase-shift hypothesis focuses on the transition to low biodiversity reefs with macroalgae cover and argues that if stressors were relieved, the system would return to the highly biodiverse reefs characterised by scleractinian-dominance (Idjadi *et al.*, 2006; Bruno *et al.*, 2009). However, this associated biodiversity loss is largely supported through studies of fish abundance, while invertebrate biodiversity (for example, the invertebrates supported by octocoral and sponge habitats) is widely understudied (Greene, 2008).

Another hypothesis of ecosystem dynamics in reefs is the idea of alternative (or multiple) stable states, which proposes that more than one state of community stability exists (Knowlton, 1992; Knowlton, 2004; Petraitis and Dudgeon, 2004; Norström *et al.*, 2009). Disturbed ecosystems (see next section) that destabalise the natural balance of structured assemblages (e.g. over-fishing of herbivorous grazers, responsible for restricting macroalgae overgrowth on corals) are thought to settle into new alternative states of equilibrium. This is where the opposition lies between the linear trajectory implied in the phase-shift hypothesis (Petraitis and Dudgeon, 2004). Positive feedback loops are considered to encourage the stability of these states as the ecosystem restructures itself, essentially recasting the functional roles lost

from the disturbance. For example, overgrowth of algae, following the loss of herbivores, opens a new niche, which offers selective shelter to the non-herbivorous assemblage of fish and more macroinvertebrates. The selective pressures already targeting herbivore abundances are further compounded by this emergent carnivorous assemblage, which now also feeds on the herbivorous larvae (Birkeland, 2015).

Advocates of the alternative stable state hypothesis argue that the Caribbean's relatively low hard coral cover compared to other reef systems of the world is the result of an equilibrium reached prior to anthropogenic impacts (Knowlton, 1992; Bellwood *et al.*, 2004; Dudgeon *et al.*, 2010). Around 3000 years ago in the late Holocene, turbid and cool waters led to the mortality of acroporid corals and converted the ecosystem to a new stable state of dominance by octocorals, sponges and macroalage, which is still seen today (Lighty *et al.*, 1978). There are cases where Caribbean reefs have returned to an acroporid-dominant community, and these studies are used to support the phase-shift hypothesis, specifically in opposition to the alternative stable state (Idjadi *et al.*, 2006; Bruno *et al.*, 2009). If resilience of a system is in fact reliant on the functional redundancy intrinsic in its biodiversity (Bellwood *et al.*, 2004), then these cases of phase-shift reversal could potentially be underestimated given the projections of unknown biodiversity (Idjadi *et al.*, 2006; Fisher *et al.*, 2015).

It is also possible, assuming an alternative stable state had been reached, or conditions that octocorals represent one modern trajectory of surrogate reef habitat, whereby the study of octocoral communities as reef-habitat providers requires more attention (Knowlton, 2004; Petraitis and Dudgeon, 2004; Norström *et al.*, 2009; Sheil and Burslem, 2013).

Geological history has seen numerous incarnations of dominant reef-building fauna, such as stromatolites, sponges, bryozoans, crinoids, and rudist bivalves over the past 3.5 billion years (Wood, 1998). Scleractinian corals have only been in existence since the Triassic, approximately 210 mya and were not prominent until the early to mid-Cenozoic, less than 65 mya (Wood, 1998).

Ultimately, however, such determinant dismissals of an ecosystem's functioning based on over-generalisations, are ultimately missing the bigger picture (Petraitis and Dudgeon, 2004). A coral reef ecosystem is known for its biodiversity and the functional redundancy of its biodiversity, much of which remains unknown (Fisher *et al.*, 2015). Therefore, future resilience predictions are perhaps unjustly framed using such disparate pieces of that puzzle (Costello *et al.*, 2010; Sheil and Burslem, 2013). Regardless of the framing or semantics of these habitat transitions away from scleractinian-dominance, there exist measurable disturbances that threaten the biodiversity of these coral reef ecosystems (De'ath *et al.*, 2012; Birkeland, 2015).

1.1.5 Threats and disturbances

Human activity, both direct and indirect, poses the largest threat to the marine environment, most notably on coastal ecosystems of which the majority of the global population is dependent (UNEP, 2006). The primary reason for decline in coral reefs is the over-development of coastal land and the implications of associated threats of increasing population, such as over-fishing, terrestrial runoff (e.g. agricultural chemical run-off, sewage, rubbish, sedimentation, etc.), invasive species and general disturbance (Cesar *et al.*, 2003b). In

most cases, there are unknown synergistic effects from these impacts on such fragile environments, putting further strain on them (Nellemann *et al.*, 2008). In regions with lower biodiversity and reduced ecosystem complexity, such as the Caribbean, when contrasted with the Great Barrier Reef System, or the reefs of the Indo-Pacific, a comparative measure of functional groups shows higher susceptibility to impacts (Bellwood *et al.*, 2004).

Coral reefs contribute to fisheries, coastal protection, tourism and recreation, and overall biodiversity value in ecosystem services (Cesar *et al.*, 2003a). The reefs of Hawaii alone have been estimated at an overall per annum value of nearly $10 billion USD (Cesar and Pieter JH, 2004). It is necessary to understand the causes of degradation in order to implement informed and effective management plans of these ecologically and economically important ecosystems.

1.1.5.1 Ocean warming

There is a lot of evidence to suggest that an anthropogenic ocean-warming signal exists (Barnett *et al.*, 2005; Birkeland, 2015). During episodically elevated temperatures, octocorals have been recognised to undergo mass bleaching (Harvell *et al.*, 2001; Aronson *et al.*, 2002; Prada *et al.*, 2010; Parrin *et al.*, 2016) and die (Lasker, 2005). Bleaching is thought to occur when the metabolic demands of the symbiotic zooxanthellae can no longer be met, a common response in times of elevated stress, particularly high temperatures (Fabricius and Alderslade, 2001; Prada *et al.*, 2010; Netherton *et al.*, 2014); (Parrin *et al.*, 2016).

1.1.5.2 Ocean acidification

A primary factor in atmospheric and ocean warming is the greenhouse gas carbon dioxide (CO_2). Fluctuations in CO_2 also have profound effects on the ocean's chemistry (Raven *et al.*, 2005). Increasing CO_2 levels in the ocean result in decreased availability of calcium carbonate ($CaCO_3$) because it is converted to bicarbonate in a lower pH environment. This spells trouble for organisms that utilise $CaCO_3$ from the ocean in the formation of calcareous skeletons or shell-like structures, e.g. coccolithophores, foraminifera, corals, bivalves, gastropods, crustaceans, etc. (Birkeland, 2015). Once these structures are formed, they rely on high concentrations of carbonate ions (CO_3^{2-}) in the surrounding seawater, otherwise their shells or skeletons begin to dissolve. Solubility of $CaCO_3$ in seawater is positively correlated with increasing pressure and decreasing temperature. In the deep sea, these factors, combined with enriched CO_2 deep waters, form a biological boundary called the 'saturation horizon', below which $CaCO_3$ dissolves. The rise in CO_2 in the oceans has resulted in a shallowing of this horizon (Raven *et al.*, 2005).

Most octocorals have sclerites composed of calcite[3], which is less soluble than the aragonite-based skeleton of scleractinians. Additionally, some species have axes that can comprise a combination of calcite or aragonite (Bayer and Macintyre, 2001), so although they will not be the first affected, they are by no means protected from the acidification of the oceans (Guinotte *et al.*, 2006).

[3] One notable exception is the blue coral *Heliopora,* which possesses sclerites composed of aragonite (Miyazaki and Reimer, 2015).

Recent research on the Caribbean plexaurid, *Eunicea fusca*, has demonstrated the gorgonian's resistance to median levels of ocean acidification (Gómez *et al.*, 2014). They will outlast the scleractinan corals to the end of this century's projected reef degradation, but only marginally, as octocorals are still susceptible to increases in CO_2. As hard coral cover is lost, the hardier octocorals are expected to replace the remains of the habitat, and take over as the new dominant group in the shifted regime of the future (Ruzicka *et al.*, 2013). This is thought to occur specifically in shallow coastal ecosystems, where concomitant stressors from both the marine and terrestrial ecosystems collide (Ruzicka *et al.*, 2013).

1.1.5.3 Terrestrial runoff

Pollution is primarily an issue for shallow-water coastal corals close to terrestrial and riverine runoff, that brings with it many agricultural chemicals, untreated sewage (nutrients), oil, heavy-metals, silt, and a slew of other problems that negatively impact the environment (Nellemann *et al.*, 2008). Agriculture runoff is projected to increase globally along with the exponentially growing world population in coastal areas (Nellemann *et al.*, 2008). Increased terrestrial runoff is dangerous to human health, potentially spreading disease and hazardous chemicals and contributing to unnatural sediment accumulation, suffocating benthic habitats and inhibiting photosynthesis (GESAMP, 2001; Fabricius, 2005). It has been shown that some octocorals appear to be even more negatively affected by sedimentation and declining water quality than hard corals (Riegl and Branch, 1994; Fabricius, 2005).

Honduras is the highest contributor of sediment and nutrients into the Mesoamerican Barrier

Reef System, of which the Belize Barrier Reef System is part (Burke and Sugg, 2006; Paris and Chérubin, 2008). This has been suggested to be a driver behind the regime shift from coral to macroalgae-dominated lagoonal patch reefs of Belizean atolls and the Bay Islands of Honduras (e.g. Útila). A study on the impact of riverine input on scleractinian coral reefs in Los Cayos Cochinos, Honduras, found sedimentation to be a higher threat to areas of high biodiversity than competition, disease or bleaching (Shrives, 2010).

Runoff can also bring about artificial increases in phytoplankton biomass through the introduction of nutrients from sewage and fertilizers containing nitrogen and phosphorus. This phenomenon, called eutrophication, can dramatically alter food webs, resulting in large plankton and algae blooms, which consume much of the dissolved oxygen in the waters, occasionally causing large-scale mortalities of fish and other organisms. Sometimes toxic species bloom under these conditions, spreading the toxin up through the food chain to humans (GESAMP, 2001; Fabricius, 2005).

Nutrification has been shown to directly affect the symbiosis between corals and their associated zooxanthellate, leading to declines in biodiversity and large-scale phase shifts in ecosystems when combined with other factors of disturbance (McCook, 1999; Fabricius, 2005). Varieties of pollution have been shown to have a correlation with parasitism, enabling parasites to act as indicators of water quality (Möller, 1987). Experimental studies on *Gorgonia ventalina* have also demonstrated a positive correlation between nutrient concentrations and disease severity (Bruno *et al.*, 2003). Terrestrial runoff could also be a vector of coral disease, transporting and spreading the soil fungus responsible for Aspergillosis infections (Andréfouët

et al., 2002).

1.1.5.4 Disease

Abiotic stressors such as elevated temperatures, eutrophication, sedimentation, pollution, faecal contamination, solar UV radiation, poor water quality, and precipitation have been linked to disease prevalence and virulence (Smith *et al.*, 1996; Nagelkerken *et al.*, 1997; Geiser *et al.*, 1998; Shinn *et al.*, 2000; Harvell *et al.*, 2002; Bruno *et al.*, 2003; Mullen *et al.*, 2004; Harvell *et al.*, 2007; Ward *et al.*, 2007). Often these threats are synergistic; accordingly, when corals are infected with disease they are even more sensitive to stress and vice versa (Harvell *et al.*, 2002; Bruno *et al.*, 2003; Lafferty *et al.*, 2004).

Pathogens are often spread through currents, which is likely why disease outbreaks are preceded by hurricane events that have already decreased coral resilience by physically damaging colonies, fueling a destructive cycle (Andréfouët *et al.*, 2002). Coral diseases have been shown to be increasing in prevalence, and especially when combined with the myriad of impacts degrading coral reef health and biodiversity, are of great concern (Lafferty *et al.*, 2004).

Coral disease is most prevalent in the Caribbean, where it is known as a 'hotspot' of disease outbreak (Harvell *et al.*, 2007). As of 2007, with the review by the Coral Disease Working Group, there have been 20 diseases reported in the Caribbean and Indo-Pacific, infecting at least 150 species of zooxanthellate scleractinians, octocorals, and hydrocorals (Sutherland *et*

al., 2004; Harvell *et al.*, 2007). Disease reports have been published on octocorals in the Caribbean, Atlantic, and Mediterranean, and observations indicate outbreaks are also present at low levels off the coasts of Zanzibar and Kenya (Smith *et al.*, 1996; Geiser *et al.*, 1998; Harvell *et al.*, 2001; Kramer and Kramer, 2002; Harvell *et al.*, 2007).

Outbreaks have led to mass-mortalities of octocorals: *Briareum asbestinum* have been impacted by the cyanobacterium *Scytonema* sp. and sea fans *Gorgonia* spp. by the fungal pathogen *Aspergillus* (Smith *et al.*, 1996; Geiser *et al.*, 1998; Harvell *et al.*, 2001; Kramer and Kramer, 2002). Of the reported diseases of octocorals, only Aspergillosis is exclusive to octocorals, while growth anomalies, black band, and red band disease are also found in scleractinians (Harvell *et al.*, 2007).

Aspergillosis is caused by the fungal pathogen *Aspergillus* that most likely invades the marine environment via soil runoff or aeolian dust (Nagelkerken *et al.*, 1997). The flamingo tongue snail, *Cyphoma gibbosum*, has been cited as a potential secondary vector of Aspergillosis, once introduced into the environment (Rypien, 2008). The snail, *C. gibbosum,* which preferentially feeds on diseased octocoral tissue (Nagelkerken *et al.*, 1997) is thought to transport the fungal *Aspergillus* spores from host to host (Rypien and Baker, 2009).

1.1.6 Biodiversity

With the many threats that coral reefs face, their resilience to degradation has been directly related to their inherent biodiversity (Roff and Mumby, 2012). Coral reefs with high

biodiversity and well-structured communities have been shown to resist human impact more efficiently (Ferrigno *et al.*, 2016).

At the lower limit of extremes in biodiversity, inbred populations are at a greater risk of succumbing to extinction especially from environmental stressors (Bijlsma *et al.*, 2000). The fewer individuals there are in a population creates the risk of an inbreeding depression, which lowers the biological fitness of a population, increasing the chance for retention of deleterious mutations (MacArthur, 1955; Peterson *et al.*, 1998). Analogous to the diversity of an immune system, greater genetic diversity within an ecosystem is linked to its ability to recognise and respond to a range of threats (e.g. disease). This concept can be extended to the larger scope of biodiversity in general; more species translates to more genes, which provides the system with a significantly more adaptable arsenal for self-repair and survival. Currently, species extinction is 50-100 times greater than the natural rate, with dramatic rises predicted (UNEP, 2000). Tropical coral reefs have been compared to rain forests in their diversity (Reaka-Kudla, 1997). Estimates of undocumented species richness inhabiting coral reefs is between 550,000 and 1,330,000 species (Fisher *et al.*, 2015). Most of the world's diversity still yet to be described, species could be blinking out of existence without any human awareness.

Conservation efforts rely on a foundational knowledge of an area's biodiversity, placing an emphasis on first describing the resident species that form communities (Gotelli and Colwell, 2011). The Caribbean has long been considered an area for conservation concern (Jackson *et al.*, 2014) and a dominant component of its reefs are the octocorals (Sánchez *et al.*, 1997; Sánchez *et al.*, 1998; Lenz *et al.*, 2015), placing a significant emphasis for their specific study in this region.

1.1.7 Systematics

In octocoral taxonomy, the systematic classification of taxa (e.g. species), is a complicated matrix of continuous and contradicting character states. The subclass Octocorallia, belonging to the class Anthozoa, is a well-supported monophyletic clade (Bayer, 1961; France *et al.*, 1996; Wirshing *et al.*, 2005). The unanimous taxonomic agreement ends there; beneath the surface of this classification are layers of disagreement and confusion (Berntson *et al.*, 2001; Sánchez *et al.*, 2003a; Fukami *et al.*, 2004; McFadden *et al.*, 2006; Aguilar and Sanchez, 2007; Daly *et al.*, 2007; Grajales *et al.*, 2007; McFadden *et al.*, 2011).

This challenging group has led to varied attempts of systematics. Studies have used characters from morphological data (Vargas et al. 2010); nuclear, ribosomal and mitochondrial DNA sequences (Aguilar-Hurtado et al. 2012; Sánchez et al. 2003b; Sánchez et al. 2003a; McFadden et al. 2006b; Wirshing et al. 2005; McFadden et al. 2011; van der Ham et al. 2009); RNA secondary structures (Grajales et al. 2007; Aguilar, 2007 #996); and terpenoid chemicals (Gerhart 1983). Disparity of phenotypic and genotypic characters of this ecologically important group also adds to the challenge of their conservation.

1.1.7.1 Morphological systematics

The present state of knowledge of octocoral systematics has not changed much since Bayer (1961) stated, "in perhaps no other group of animals, with the possible exception of the Porifera, is classification and identification so subjective as it is in the Octocorallia." Even

Fredrick Bayer, who contributed greatly to the systematic study of octocorals (Bayer, 1956b; Bayer, 1956a; Bayer, 1961; Bayer, 1981; Bayer *et al.*, 1983; Bayer and Macintyre, 2001), said in reference to discriminating between two separately described *Plexaura* spp., "...and I gave up in despair trying to find ways in which they differ consistently..." (Bayer, 1961).

Morphological identification depends on characters such as presence or absence of an axis and its composition (most important for determining higher ordinal and sub-ordinal taxonomy), colony branching structure (an often convergent feature), individual polyp sclerite composition and configuration with other polyps, and presence or absence of sclerites and their multitude of microscopic morphological characters (most important for species-level identification) (Bayer, 1981).

The continuous character states of octocorals make delineation of species very challenging, although studies have demonstrated proper coding of characters can provide valuable phylogenetic information (Vargas et al. 2010). Sometimes morphological differences previously considered a continuous character trait of one species, subsequently shows a genetic distinctions, which corresponds to that sliding morphological scale (Prada *et al.*, 2008).

Morphology in corals does not necessarily follow a strict guideline in bauplan (i.e. bodyplan) formation and there are many degrees of hybridisation and convergent evolution (Veron, 1995; McFadden and Hutchinson, 2004; Isomura *et al.*, 2013). Genetic structure can even vary with different morphologies of the same species of *Briareum asbestinum* (Brazeau and Harvell 1994). Charles Darwin scribbled in his notebook in 1837 that, "the tree of life should

perhaps be called the coral of life" (Barrett, 1987). The complexity of coral phylogeny unearthed by genetics, and corals' tendency towards hybridisation is analogous to anastomoses (i.e. cross-connections) of some sea-fan branching patterns (Veron, 1995; McFadden and Hutchinson, 2004; Isomura *et al.*, 2013).

Systematics of higher taxa of families and genera are also problematic. In the essential octocoral key by Bayer (1981) he states, "...several of the traditional families are so ambiguous that it is impossible to break the key up by families in any defensible way." This statement holds true today, and few taxonomists in the world are still attempting to bring logic to the myriad layers of octocoral, and more broadly cnidarian, systematics (Collins, 2009; Park *et al.*, 2012; Kayal *et al.*, 2013; Van Iten *et al.*, 2014). When morphology is so unreliable and inconsistent throughout a field, molecular characters can often provide the consistent variability necessary for phylogenetics. However, within such analyses lie yet another conundrum.

1.1.7.2 Molecular systematics

Molecular phylogenetics of octocorals is just about as resolved as morphologically-defined taxa. Typical barcoding genes such as cytochrome c oxidase subunit 1 (COI or cox1), successfully used for species identification in many taxa (Hebert *et al.*, 2003a; Hebert *et al.*, 2003b; Hebert *et al.*, 2004; Smith *et al.*, 2005; Ward *et al.*, 2005; Hajibabaei *et al.*, 2006; Ward *et al.*, 2008; Rypien and Baker, 2009), do not provide enough resolution of cnidarian species because of the slow substitution rate of the mitochondrial genome in anthozoans

(Shearer *et al.*, 2002; Hebert *et al.*, 2003b).

A promising development in the search for an adequate barcode for octocorals came with the discovery of mitochondrial *mutS* gene (Pont-Kingdon et al. 1995; Pont-Kingdon et al. 1998). This gene is unique to octocorals and is thought to have originated by a horizontal gene transfer from either a bacterium or girus (giant virus) (Bilewitch and Degnan 2011). The *mtMutS* gene is known predominately in octocoral literature as *msh1* as it was thought to be homologous to the eukaryotic *MSH* nuclear gene family; phylogenetic analysis more closely relates it with the *MutS2* family of prokaryotes and plants, which calls for its current designation as *mtMutS* (Bilewitch and Degnan 2011). France and Hoover (2002) found *mtMutS* to be a useful gene in phylogenetic analysis of octocorals; however, there are still limitations in its resolution of species or morpho-species.

The lack of variability in the reportedly slow-evolving mitochondrial protein-coding genes of anthozoans (France and Hoover, 2001; Shearer *et al.*, 2002; Hellberg, 2006), pushed barcoding efforts to sequence more variable gene regions. Other mitochondrial genes, ND3, ND4L and *mtMutS* (MSH) used in analyses by France & Hoover (2001; 2002) found no differences between species *Lipidisis olapa* and *Lipidisis* sp. from locations over 3000km apart in the Pacific Ocean. The phylogenetic potential of INDELS (i.e. insertions-deletion, e.g. *igr1, igr4*), non-protein-coding regions between genes, were explored in bamboo octocorals (van der Ham *et al.*, 2009). High levels of variation exist within these intergenic regions, but when used alone they do not provide enough haplotypes for species distinction. The high degree of variability within these regions can complicate sequence alignment with ambiguities, potentially

violating positional homology and leading to inaccurate phylogenies. Some studies have used other non-coding regions called internal transcribed spacers (ITSs), to detect cryptic species and translated their predicted RNA secondary structure into phylogenetic characters (Aguilar and Sanchez, 2007; Grajales *et al.*, 2007; Sánchez and Dorado, 2008). However, ITSs are shown to possess mostly intragenomic variability and have an excessive number of INDELS (Concepcion *et al.*, 2007).

As of 2011, the most informative combination of molecular markers for octocoral species delimitation was an extended barcode of COI + *igr1* + *mtMutS* (McFadden et al. 2011). This was shown to have more success in separating morpho-species (Taylor 2011) than with any of the genes separately. However, it was still insufficient in distinguishing between species and the quest continued for the holy grail of octocoral barcoding.

In 2012, a nuclear gene, 28S[4] was added to the extended barcode in a phylogenetic analysis of 130 octocoral taxa using *mtMutS*, COI and 28S rDNA sequences (McFadden and van Ofwegen, 2012). This gene suite was used to reveal cryptic diversity in a species of Clavulariidae (McFadden and van Ofwegen, 2013). Another study using this extended barcode in shallow-water octocorals, found that only up to 70% of morpho-species were distinguished (McFadden *et al.*, 2014). More multilocus studies continue to search for a superior extended barcode (Bilewitch *et al.*, 2014; Torres-Suarez, 2014). Within sponges, another metazoan with a slow-evolving mitochondrial genome, the nuclear protein-coding gene, ALG11, has shown

[4] A part of which had been used previously in broader anthozoan phylogenetics (Chen *et al.*, 1995).

remarkable variation (Belinky *et al.*, 2012), and its suggested use for cnidarians prompted the exploration of its barcoding potential in this thesis.

1.1.7.3 Systematics of Plexauridae

Particularly understudied and unresolved are the members of the family Plexauridae, within the suborder Holaxonia (Bayer, 1961; Sánchez *et al.*, 2003a). Multiple polyphyletic groupings with mixtures of holaxonians, alcyoniinans, and a few scleraxonians and stoloniferians are found scattered throughout the quagmire of the octocoral phylogeny, in what is loosely defined as a consistent clade of primarily Holaxonia and Alcyoniina (Sánchez *et al.*, 2003b; Wirshing *et al.*, 2005; McFadden *et al.*, 2006; McFadden *et al.*, 2011). Intragenomic variants found in *Gorgonia* and *Pseudopterogorgia* suggest a higher degree of gene flow between plexaurid species than previously suspected (Sánchez and Dorado, 2008). Within Plexauridae, a large and diverse family with over 480 species in 40 genera (Williams and Cairns, 2011), the subfamily Plexaurinae (mostly shallow water) and Stenogorgiinae (mostly deep water) tend to cluster discretely (except for *Plexaurella nutans*), but not monophyletically, and in some cases are more closely related to members of the family Gorgoniidae (Wirshing *et al.*, 2005).

Gorgoniidae and Plexauridae were once classified together under the broadly used term Gorgonacea (Bayer, 1961), which comprised octocorals with proteinaceous axes to varying degrees of calcification (primarily species of the suborder Holaxonia with some scleraxonians and calcaxonians) (Sánchez *et al.*, 2003a). The division of these two families is perhaps unjustified because of the overlapping character states and unclear delineations of

morphological character states that potentially place the same taxon in either family (Grasshoff and Alderslade, 1997). Attempts to describe these groupings morphologically have largely failed (McFadden *et al.*, 2011); however, the quest continues.

1.1.8 Study area – Útila, Bay Islands, Honduras

The Honduran Bay Islands constitute the eastern most extension of the Mesoamerican Barrier Reef System (MBRS), which extends along the coasts of Mexico, Belize, Guatemala, and Honduras (see Fig. 1.3). It is the second longest barrier reef system in the world and a World Heritage Site designated by the United Nations Educational, Scientific, and Cultural Organization (Almada-Villela *et al.*, 2002; Gössling, 2003). Honduras, because of its mountainous topography, large riverine input, high annual rainfall, and intensive agriculture[5] that loosens topsoil, is the largest input of sediment into the MBRS (Burke and Sugg, 2006; Paris and Chérubin, 2008; CIA, 2012).

The Bay Islands, which lie 40-80 km off the coast, are considered of highest priority for biodiversity and conservation studies of the Mesoamerican Caribbean Reef (Kramer and Kramer, 2002). They are considered among the highest threatened areas, owing to lack of information, habitat loss, declining water quality and over-usage of resources (Kramer and Kramer, 2002). The coast of Honduras, which is subjected to heavy sedimentation, low salinities from freshwater input, and continuous turbidity, prevents much reef growth (Roberts, 2002; Rogers, 2004; Auster, 2005; Scher and Martin, 2006; Stone, 2006; Cairns, 2007; Maier *et al.*, 2009; Roberts *et al.*, 2009; Tittensor *et al.*, 2010). Away from the mainland coast there are significant reef developments on

[5] Production of bananas and coffee employs approximately 40% of the labour force

the Bay Islands, which consist of an archipelago of nearly 200 islands, more than 60 belonging to Honduras, including several larger islands such as Útila, Roatán, Cayos Cochinos, and Guanaja (Kramer and Kramer, 2002). Recognised nationally as both a terrestrial and marine conservation priority, the Bay Islands National Marine Park was created by the Honduran president in 1997 (Gössling, 2003).

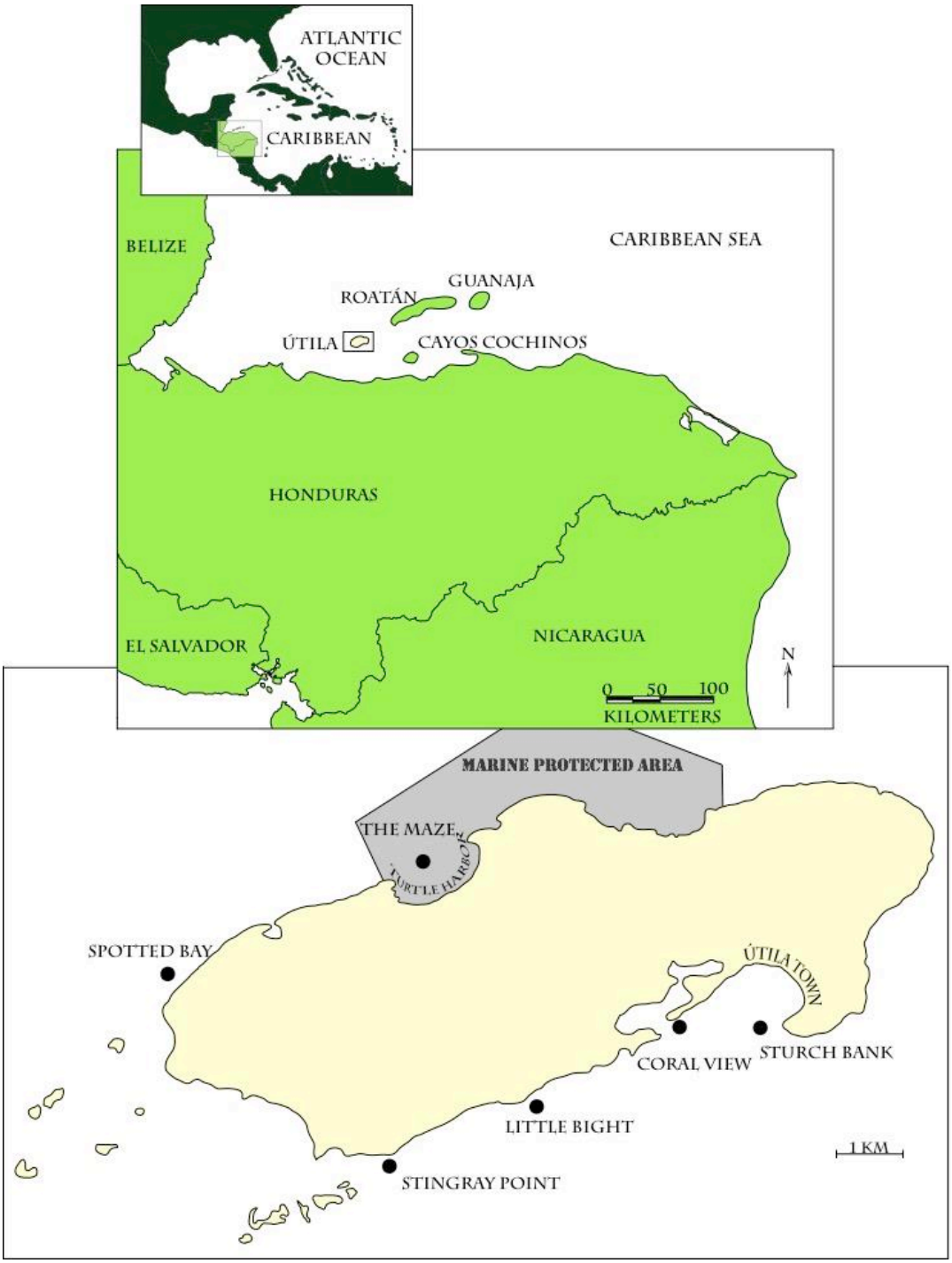


Figure 1.3 Map of wider Caribbean, with insets of Honduran coast line of Bay Islands and map of Útila with sites of data collection noted.

In the late 1990s, a series of natural disasters had catastrophic impacts on the MBRS. In 1998, Hurricane Mitch devastated much of the coastlines from the Bay Island of Guanaja northward to the Yucatán (Kramer and Kramer, 2002). This came on the coat-tails of a worldwide coral-bleaching crisis, during the first half of 1998, brought on by high sea surface temperatures from a particularly intense El Niño (Strong *et al.*, 1998; Aronson *et al.*, 2002). Additionally, during this time there were reports of multiple disease outbreaks in both scleractinians and octocorals (Smith *et al.*, 1996; Nagelkerken *et al.*, 1997; Geiser *et al.*, 1998; Goreau *et al.*, 1998; Harvell *et al.*, 2001; Kramer and Kramer, 2002). Caribbean reefs then were still recovering from Hurricane Allen's reduction of coral cover from an estimated 75% to less than 40% in 1980 (Mumby *et al.*, 2007). The 1980s also saw disease outbreaks on scleractinian corals (Aronson and Precht, 2001) and an unknown pathogen which nearly wiped-out the urchin *Diadema antillarum*, a keystone reef species that depresses macroalgal growth on reefs through grazing (Lessios *et al.*, 1983). These events spurred a concerted effort to assess and conserve Caribbean coral reefs (Almada-Villela *et al.*, 2002; Andréfouët *et al.*, 2002; Kramer and Kramer, 2002; Almada-Villela *et al.*, 2003; Burke and Sugg, 2006; Chérubin *et al.*, 2008; García-Salgado *et al.*, 2008; HRI, 2012).

Since these devastating disasters, monitoring programs of coral reef health have been developed and implemented in the Caribbean. The Synoptic Monitoring Program was developed to monitor the health of the MBRS (Almada-Villela *et al.*, 2003). This system of monitoring was used from 2004 to 2008 by García-Salgado *et al.* (2008) who described a decline in hard coral reef cover and an increase in algal cover in general. Both Cayos Cochinos and Útila support a critical level of algal coverage for which stress agents are undetermined. From 2004 to 2007, Cayos Cochinos lost half of its overall coral coverage, dropping from 24%, considered good coverage (but below optimal), to

12%, which is considered poor. Útila was measured in 2005 and 2006, where coral cover decreased from an already low 15%, which falls between poor and good in an 'alert' category, to 11%, placing it near the border of poor and critical categories (García-Salgado *et al.*, 2008). An unpublished study, looked at impacts of Hurricane Mitch and the bleaching event on the island of Roatán and found that 25% of the (observed) octocoral population (*Eunicea, Briareum, Gorgonia, Pseudopterogoriga=Antillogorgia* and *Plexaura*) had been lost, including all species of the genus *Plexaura* (Keck, 2004).

An Eco-audit for Honduras was published which ranked indicators of ecosystem health (HRI, 2011). The state of management of marine protected areas (MPAs) and sewage treatment in Honduras was considered poor because of inadequate enforcement and low cooperation from hotels and private sector assistance. Only six dive shops (11% of Bay Islands) were participating to adopt the Coral Reef Alliance (CORAL) dive standards despite better than average tax incentives for conservation and sustainable practices. The lowest rankings in ecosystem health were categories relating to international mapping collaborations of warming seas and coral bleaching. However, research, education, and awareness scores were considered fair to good.

Few studies have been conducted on the Bay Islands of Honduras, and even fewer on Útila. There are currently no known published accounts focusing on octocorals in Útila, but scleractinian studies have been conducted (Harborne *et al.*, 2001; Scaps and Saunders, 2011) and nearby octocoral distributions in Roatán, Swan Islands, Belize, and the Yucatan have been documented (Tortora and Keith, 1980; Muzik, 1982; Lasker and Coffroth, 1983; Dahlgren, 1989; Keith, 1992). General benthic surveys incorporating broad categories of octocorals have been carried out (Harborne *et*

al., 2001) but a species-level study, like that of the scleractinians, does not exist from Útila (Scaps and Saunders, 2011).

Útila, the closest of the islands to the mainland, is located approximately 35 km off the coast of Honduras in the Caribbean Sea and has been gaining in popularity with SCUBA-diving tourists. The mainland's continental shelf extends north and connects with the south coasts of the Bay Islands, in a shallow trough of gradually inclined sandy benthic habitat. These southerly shores are in direct contrast to the northern shores of the Bay Islands, which plunge rapidly very near shore to 1000s of metres into the deepest part of the Caribbean Sea, called the Cayman Trough. The north shore of Útila is also the only policy protected coast of the island, where the endangered green turtle nests on its beaches (Harborne *et al.*, 2001). Most Útila's population lives in the east harbour of the south coast, which is more sheltered from winds and the rugged surge of the open ocean (see Fig. 1.3).

1.1.9 Aims and objectives: Thesis chapter breakdown

Entirely based on the octocoral communities around the island of Útila, Honduras, a Caribbean island not previously studied for its octocoral species-level richness, this thesis consists of four data chapters, this introduction chapter, and a conclusion chapter. Most the ecological data chapters (Chapters 2-4) herein were collected from June-August of 2013. Data for Chapter Five was collected in June-August of 2014.

1.1.9.1 Chapter Two aims

The first data chapter describes the richness and abundance of the octocoral communities around the island down to 15 m. Total occurrence of species was surveyed down to 30 m. Patterns of octocoral assemblages are explored and analysed within the context of their environment. The reef depths were chosen to complement general reef surveys to coincide with volunteer collected data of Operation Wallacea. These data form the basis for which subsequent chapters are built and analysed.

1.1.9.2 Chapter Three aims

This chapter investigates the underestimation of habitat provided by octocorals and explores methods of estimating surface areas lost in traditional benthic reef transects. This research highlights the overlooked significance in abundance of octocorals as an important element of reef real estate.

1.1.9.3 Chapter Four aims

This chapter uses complementary character states of morphological and molecular characters to test the boundaries of species delimitation, using the notoriously taxonomically complex genus *Eunicea* as a model. Continuous character coding of over-lapping morphologies within *Eunicea,* were implemented, and the molecular barcoding potential of the nuclear gene ALG11 was tested in cnidarians for the first time (along with traditional markers *mtMutS* and COI).

1.1.9.4 Chapter Five aims

Signs and symptoms of the most prevalent octocoral disease Aspergillosis were noted during the collection of data for richness and abundance (Chapter Two). These data on disease presence, led to the investigation of the hypothesis that the associated snail, *Cyphoma gibbosum*, is a vector of the fungal pathogen Aspergillosis in this chapter. Populations of both adult and juvenile *C. gibbosum* were investigated for their preference in octocoral hosts based on selectivity indices. Additionally, these data combined with data collected on signs and symptoms of disease, were used to test the hypothesis that diseased hosts are preferred.

This work will form the basis for any future octocoral studies of Útila, as well as for comparative studies in Honduras and the wider Mesoamerican Barrier Reef System. Although this is a site-specific study – largely reflective of a unique assemblage of octocorals – it adds a significantly needed comparison point of biodiversity. Future research will hopefully use these connections to form a better understanding of the resilience of Caribbean reefs.

2 Chapter Two:

Species richness and abundance of octocorals around the Bay Island of Útila, Honduras

2.1 Abstract

Surveys of octocoral species were taken around the island of Útila, Honduras down to 30 m, uncovering 49 identified species (including described morpho-species) in total, the highest reported octocoral species number for any one location in the Caribbean to date. Repeated samples of species richness and abundance measurements were taken on reefs down to 15 m, which accounted for 50 'species' (i.e. not all were positively assigned a species name) categories which are used in analyses. Reefs closer to Útila town on the south shore were found to have community structures indicative of disturbance. Species richness was also found to positively correlate with distance away from Útila town. However, the highest species evenness scores were found on the south shore in an area awaiting marine protection (Stingray Point).

2.2 Introduction

The worldwide degradation of coral reefs has been occurring steadily for centuries (Pandolfi *et al.*, 2003) but within the last three decades a series of natural disasters and anthropogenic activity has significantly contributed to documented losses (Hughes *et al.*, 2003; Bellwood *et al.*, 2004; Mumby *et al.*, 2007; Jackson *et al.*, 2014). Caribbean reefs are amongst the more severely damaged and lack resilience, in part because of low functional redundancy in comparison to the rich abundance of Indo-Pacific reefs (Hughes, 1994; Gardner *et al.*, 2003; Gardner *et al.*, 2005; Mumby *et al.*, 2007; Mumby, 2009; Roff and Mumby, 2012; Newman *et al.*, 2015). In 1998, Hurricane Mitch, decreased coral cover by 20% across Central America (Aronson *et al.*, 2002; Kramer and Kramer, 2002). High growth rates of macroalgae and the loss of herbivorous grazers because of over-fishing (Jackson *et al.*, 2001; Pauly *et al.*, 2005; Mora, 2008; Roff and Mumby, 2012) and disease

outbreaks (Lessios *et al.*, 1983; Harvell *et al.*, 2001; Bodmer *et al.*, 2015) are pointed to as key factors in Caribbean reef decline.

The Mesoamerican Barrier Reef System (MBRS) in the Caribbean is the second largest of its kind in the world (after The Great Barrier Reef in Australia), extending over 1000 km from the Yucatan in Mexico to the Bay Islands of Honduras (Almada-Villela *et al.*, 2002). The reefs of the Caribbean and MBRS are uniquely characterised by their abundance of octocorals (Goldberg, 1973; Lasker and Coffroth, 1983; Sánchez *et al.*, 1997). Octocorals are often overlooked, understudied and broadly generalised in reef studies despite their ubiquity. This is likely owing to their notoriously challenging field identification (Lasker and Coffroth, 1983). However, although challenging, the study of this ecologically dominant fauna is crucial to gain a holistic picture of reef resilience.

Investigations of Caribbean octocorals have been relatively plentiful compared to octocoral literature elsewhere in the world (Kinzie, 1970; Goldberg, 1973; Jordan, 1978; Tortora and Keith, 1980; Muzik, 1982; Lasker and Coffroth, 1983; Dahlgren, 1989; Keith, 1992; Sánchez *et al.*, 1997; Sánchez *et al.*, 1998; Prada *et al.*, 2008; Sathe *et al.*, 2008; Lenz *et al.*, 2015). However, this is the first study to document the species richness and abundance of octocorals around the Honduran Bay Island of Útila and the first in over 30 years within the greater MBRS (Muzik, 1982; Lasker and Coffroth, 1983). Bayer (1961) remarked that gaps in the diversity records from Honduran reefs were 'conspicuous and vexing' and 55 years later this statement still resonates.

The reefs surrounding Útila and the Bay Islands of Honduras showcase high habitat diversity, a trait that has been correlated with higher species richness and abundance (Harborne *et al.*, 2001;

Gratwicke and Speight, 2005). Útila is considered an area of conservation interest in the ecoregion because of its sea turtle nesting grounds (*Chelonia mydas* and *Caretta caretta*), endemic lizards (*Ctenosaura bakeri*), and high reef species abundance and richness (Harborne *et al.*, 2001; Almada-Villela *et al.*, 2002; Kramer and Kramer, 2002; Almada-Villela *et al.*, 2003; García-Salgado *et al.*, 2008; Scaps and Saunders, 2011; Alegría *et al.*, 2012; HRI, 2012).

Species occurrences of octocorals from the Bay Islands of Honduras were recorded, for the first and only time, 37 years ago from collections made in 1979 on the island of Roatán (Keith, 1992). This study quantitatively investigates the variation in species richness and evenness around Útila, and relates environmental factors to observed differences. Within the island system of Útila, this study aims to measure the variability in assemblages seen within reef systems around the island.

Additionally, questions are investigated such as: Can correlations be drawn between species richness and distance from anthropogenic impact? What can be inferred from the population structure of assemblages found around the island and do any environmental variables correlate to these estimators?

2.2.1 Materials and Methods

2.2.1.1 Location

All data were collected from around reefs of Útila, a small island (45 km^2) off the northern Caribbean coast of Honduras in Central America (refer to Fig. 1.3). It is the second largest of the Bay Islands, an archipelago that demarks the eastern most extent of the Mesoamerican Barrier

Reef System (MBRS). Útila is a popular diving destination in the Caribbean and the island is home to approximately 14 dive centers, which operate mainly from the East Harbor where the town of Útila is located. The town is the main locality of the islands' population of nearly 4000 people as of 2013 (Instituto Nacional de Estadística Honduras, 2013). Diving pressure is high, especially on sites closest to town, but reef damage is mitigated by permanent mooring buoys all around the island (Canty, 2007).

The south shore of Útila is characterised by its sandy sloping benthic habitat, patchy reef systems, and sheltered bay, protected from much of the hurricane damage that the exposed shores of the north are sometimes subjected (Yoshioka and Yoshioka, 1989; Harborne *et al.*, 2001). Approximately 35 km south, lays the mainland of Honduras. High topographic relief of Honduras coupled with a large river system, persistent tropical storms, and intensive agricultural development (coffee and bananas) are often linked to coast erosion practices (Southgate and Ecuador, 1992). The riverine input off the mainland coast is substantial; Honduras contributes approximately 80% of sedimentation and nutrients to MBRS region (Burke and Sugg, 2006). Currents carry sediment plumes from the western river mouths northeast around the Bay Islands (Chérubin *et al.*, 2008). Buoyant matter plumes extend from the mainland and are concentrated on the south shores of Útila (Burke and Sugg, 2006).

The north shore is characterised by higher wind and wave energetics on the more exposed coastlines, typical of north-facing windward Caribbean shores (Kinzie, 1970). The relatively uninterrupted fringing reefs have dramatic bathymetric drop-offs to the oceanic plate (Harborne *et al.*, 2001). The Turtle Harbor Wildlife Refuge is located here, a bay characterised by its high levels of scleractinian coral cover, relatively high fish abundance and richness, and sites for turtle nesting

grounds (Harborne *et al.*, 2001).

Sturch's Bank dive site is situated adjacent to the highest population density of Útila, with anecdotal reports of reef clearance ca. 30 years ago to facilitate boat traffic. Coral View is the research station, from which this study was based, where shore diving was possible. This site is the furthest south site west of town that is still accessible by land. Little Bight is characterised by its connection to the deepest part of the south shore (about 65 m). The area near Stingray Point was noted to have high scleractinian diversity in Coral Cay Conservation rapid habitat assessments (Harborne *et al.*, 2001), and it is the furthest southwest site. Spotted Bay is a relatively sheltered site of the north shore, and is characterized by high levels of current and is often inaccessible during poor weather conditions. Here, an inner reef site was chosen because the buoy for the outer reef had been taken away by a storm. The Maze is unique for its shallow flats of gorgonian fields and vertical wall beginning around 10-12m and extending to hundreds of metres (Pinet, 1976; Harborne *et al.*, 2001).

2.2.2 Data and sample collection

Data collection was conducted using SCUBA from June-August 2013 at six reef sites (Fig. 2.1 & 2.2) along the southern and northern coasts of the island: four south shore sites: Sturch's Bank (SB), Coral View (CV), Little Bight (LB), Stingray Point (SRP); and two north shore sites: Spotted Bay (SpB) and The Maze (M) (See Fig. 2.1 for SB, CV, and LB; see Fig. 2.2 for SRP, SpB and M).

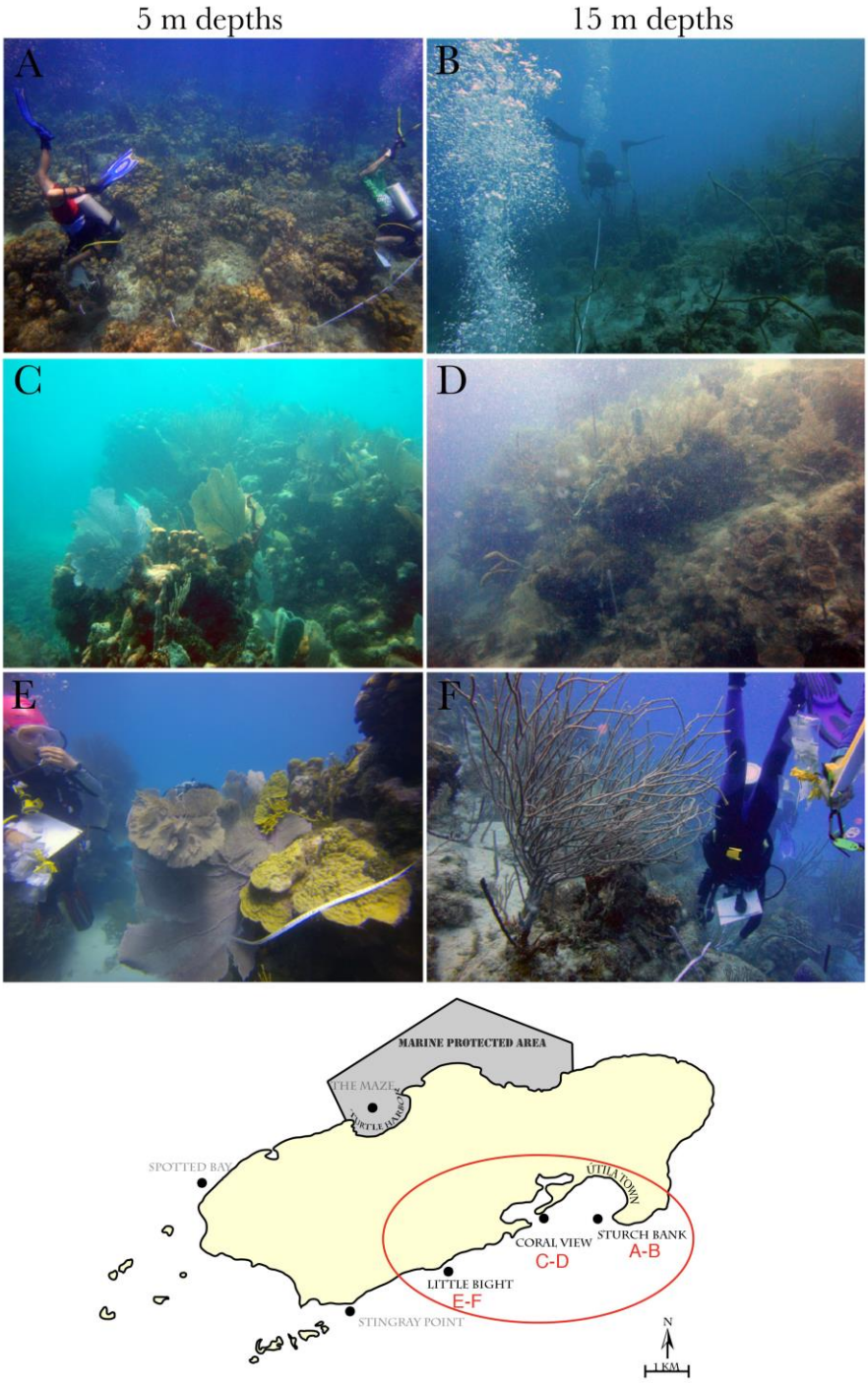


Figure 2.1 Photographs of reefs from where data were collected. A-B: Sturch's Bank at 5 m and 15 m; C-D: Coral view at 5 m and 15 m; E-F: Little Bight at 5 m and 15 m. Map of Útila below with sites encircled in red. Photo credit: J Murray.

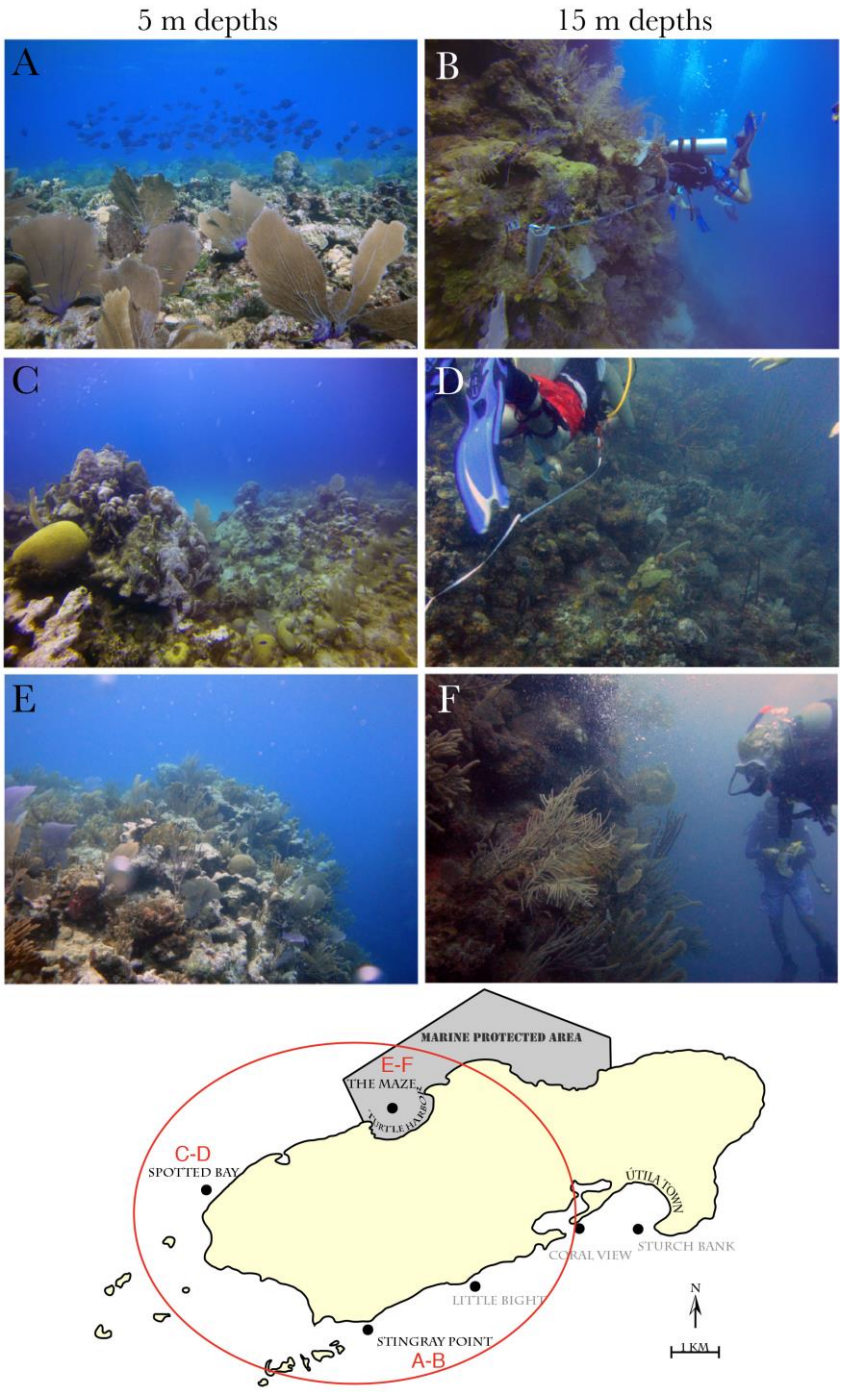


Figure 2.2 Photographs of reefs from where data were collected. A-B: Stingray Point at 5 m and 15 m; C-D: Spotted Bay at 5 m and 15 m; E-F: The Maze at 5 m and 15 m. Map of Útila below with sites encircled in red. Photo credit: J Murray.

Reef transect data were collected at 5 m and 15 m, and were used to describe species diversity as defined by the species richness, evenness and abundance. The 50 'species' reportedly recorded from these transects includes categories pertaining to unidentified species from each genera (e.g. '*Eunicea* spp.' and '*Antillogorgia* spp.'). An additional survey dive was taken down to 30 m, to look for species not seen in transects, which added four species to the recorded total identified species list of 49 species for Útila.

Data were collected within 1 m^2 quadrats, which were taken along 20 m transects for guidance (summarised in Table 2.1). Transects were laid haphazardly (true 'random' sampling was not possible because of dive site and time restrictions) away from mooring buoys, at least 5 m apart, at 5 m and 15 m depths. The weighted end of the transect tape was placed in a sand patch or dead part of reef and 20 m was reeled out along the depth gradient, carefully tucking the tape in and around the topographic complexity of the reef. The length of the transect (20 m) was chosen to incorporate the range of octocoral densities seen at each site, however, the number of quadrats sampled during each dive (limited to 50 minutes) varied between 1-20 m^2 because of variable densities observed at the sites (refer to Table 2.1). All octocorals within each square metre were counted and measured for height, width, and holdfast diameter, which characteristics were then used in other studies (see Chapter Three on surface areas). Each colony was photographed and given a field identification, its health condition was noted (relative scale from 0-4), and presence or absence of any associated fauna was recorded (see Chapter Five on *Cyphoma gibbosum*).

Table 2.1 Summary of data collected. Dive sites of Útila split into south and north shore, respectively and 5 m and 15 m depth. Quadrats were collected along 20m transects, which were limited to a single dive. Transects are equivalent to a 50 minute dive. Number of individuals refers to octocoral colonies.

Data collected	South Shore Sites								North Shore Sites				Total
	Sturge's Bank (SB)		Coral View (CV)		Little Bight (LB)		Stingray Point (SRP)		Spotted Bay (SpB)		The Maze (M)		
	5m	**15m**	**5m**	**15m**	**5m**	**15m**	**5m**	**15m**	**5m**	**15m**	**5m**	**15m**	
Transects (20m)	4	6	6	5	6	5	4	5	4	5	8	4	62
Quadrats (m^2)	31	22	79	17	26	25	22	19	4	11	20	22	298
Number of individuals	76	155	145	160	143	128	82	135	97	155	229	96	1601

If identification was not possible in the field using Sanchez and Wirshing (2005), small samples from branch tips (<5 cm in length) were taken using scissors or gardening shears and stored in Whirl-Pak bags or 5 - 10 ml sample tubes with ambient seawater until processing. Samples were transported back to a dry lab at the Coral View Dive Center (at Coral View dive site location on map, see Fig. 1.3) where samples were preserved in 95% ethanol for later morphological identification and DNA extraction. A total of 450 samples for morphological identification and molecular study were transported back to the laboratory at the University of Oxford's Department of Zoology in September 2013 and stored, after a final ethanol change, in -20°C.

Colony morphology was verified with photographs to confirm identification in correspondence with the dichotomous key by Bayer (1961). For identification, sclerites were freed from tissue by dissolving in diluted household bleach and viewed under a microscope. Calibrated photographs (within +/- 10 µm accuracy) were taken with a 5 mB Amscope USB camera (5.0 MP) used with a

Leica stereomicroscope EZ5 for branch and large spindle features, and a compound microscope, Olympus Vanox-T Research Microscope Model AHMT, for club and capstan features.

2.2.2.1 Environmental variables

Water movement and turbidity are key components in determining octocoral habitat suitability (Bayer, 1961; Kinzie, 1970), so these variables were measured with the most sophisticated technology available during field collections for each site. Relative water flow was measured at the surface with a one-metre long PVC pipe, a buoyant test tube and a stopwatch. A Secchi disk was used to gauge the transparency of the water (i.e. lower values are indicative of higher turbidity). These measurements were taken an average of 10 times per site in roughly the same location.

Additionally, distance from anthropogenic stress, measured in kilometres from the highest population density of the town of Útila, was used as an environmental and habitat factor. Sites ranged from 0.5 km to 17.5 km away from highest human population density.

2.2.2.2 Statistics

All statistical analyses were conducted using R 3.2.0 (R Core Team, 2015), with the *vegan* package (Oksanen *et al.*, 2016) and *BiodiversityR* package (Kindt, 2016) and Primer v7 (Clarke and Gorley, 2015). Species rarefaction curves, richness, and diversity indices (see equations below) were calculated as well as colony density per site and per depth. The nonparametric species richness estimator, Chao1, which factors rare species occurrences (singletons and doubletons) was calculated (Gotelli and Colwell, 2011).

Evenness scores were calculated using a modified formula implementing two diversity indices as recommended by Alatalo (1981):

Modified Hill's ratio

$$F_{2,1} = \frac{(N_2 - 1)}{(N_1 - 1)}$$

Antilogarithmic Shannon's entropy H'

$$N_1 = exp^{-\sum p_i ln\, p_i^2}$$

Reciprocal of Simpson's index

$$N_2 = \frac{1}{\sum p_i^2}$$

Count abundance data were used to model community diversity curves as proposed by Wilson (1991). The models broken-stick (MacArthur, 1957), log-normal (Preston, 1948), geometric (Motomura, 1932), Zipf (Zipf, 1949), and Zipf-Mandelbrot (Mandelbrot, 1977) were tested and those with the lowest AIC score were chosen to be the best fit.

As previously stated, the data were unevenly distributed within each transect (i.e. not all transects consisted of 20 m^2), therefore an average and standard deviation was used for each transect and those values were used as the unit of sampling for comparison between sites.

Bray-Curtis similarities (Bray and Curtis, 1957) were calculated based on the relative proportions of species (at each reef site and depth). These similarities were used to test for significant differences

in community structures in response to site, shore (north vs. south) and depth (5 m vs. 15 m) using a partial permutational multiple analysis of variance (PERMANOVA) as suggested by McArdle and Anderson (2001). Additionally, similarities were run in a cluster analyses (method=SIMPROF) to display community patterns (Clarke *et al.*, 2008), and environmental data was fitted over principal component analysis (PCA) of similarities.

2.3 Results

From surveys down to 30 m, 49 identified species were found around Útila (Table 2.2). Example photographs showcasing a small selection of the species found in this study can be found in Figure 2.3.

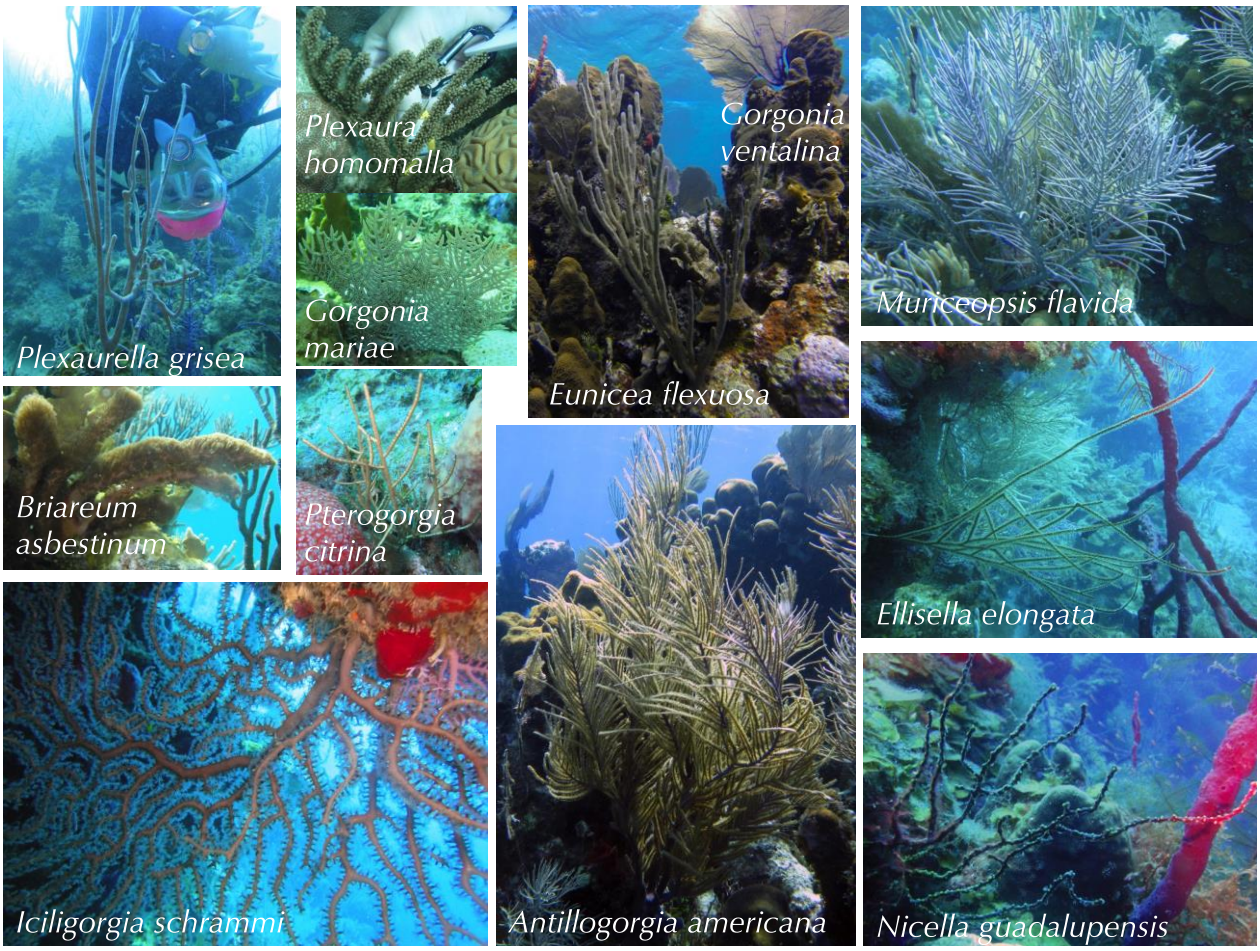


Figure 2.3 Examples of octocorals from around Útila. Photographs by VB Lovenburg and AD Rogers.

Table 2.2 A list of octocoral species from sites around the Caribbean, including the current study from for comparison. Roatán (Keith, 1992); Yucatan (Jordan, 1978; Dahlgren, 1989); Belize (Muzik, 1982; Lasker and Coffroth, 1983) , Costa Rica (Guzmán and Cortés, 1985); Bahamas, Jamaica, & FL Keys (Bayer, 1961). This list does not include species restricted to depths greater than 45 m. Begins on next page.

Octocorallia of the Caribbean

Octocoral species (under taxon classification)	Honduras			Yucatan	Belize	Costa Rica	Bahamas	Jamaica	FL Keys
	Útila	Roatan	Swan Ills						
Order Alyconacea									
Suborder Calaxonia									
Family Ellisellidae									
Ellisella elongata (Pallas, 1766)	✓	✓							
Ellisella barbadensis (Duchassaing & Michelotti, 1864)	✓	✓							
Nicella guadalupensis (Duchassaing & Michelotti, 1860)	✓								✓
Nicella schmitti Bayer, 1961	✓								
Suborder Holaxonia									
Family Gorgoniidae									
Antillogorgia acerosa (Pallas, 1766)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Antillogorgia albatrossae (Bayer, 1961)	✓								
Antillogorgia americana (Gmelin, 1791)	✓	✓	✓	✓		✓	✓	✓	✓
Antillogorgia bipinnata (Verrill, 1864)		✓			✓		✓		✓
Antillogorgia blanquillensis (Stiasny, 1941)					✓				
Antillogorgia elisabethae (Bayer, 1961)	✓			✓	✓		✓		
Antillogorgia hummelincki (Bayer, 1961)	✓	✓							
Antillogorgia hysterix (Bayer, 1961)			✓		✓		✓		
Antillogorgia kallos (Bielschowksy, 1918)	✓	✓		✓	✓				
Antillogorgia navia (Bayer, 1961)							✓	✓	
Antillogoriga rigida (Bielschowksy, 1929)	✓	✓		✓	✓	✓			✓
Gorgonia flabellum forma *occatoria,* (Milne Edwards & Haime, 1857)						✓			
Gorgonia flabellum forma *flabellum* Linnaeus, 1758				✓		✓			
Gorgonia mariae Bayer, 1961	✓	✓		✓	✓		✓		

Table 2.2 Caribbean octocoral species list continued

Octocoral species (under taxon classification)	Útila	Roatan	Swan Ills	Yucatan	Belize	Costa Rica	Bahamas	Jamaica	FL Keys
Gorgonia ventalina Linnaeus, 1758	✓	✓	✓	✓	✓	✓	✓	✓	✓
Leptogorgia cardinalis (Bayer, 1961)									✓
Leptogorgia punicea (Milne Edwards & Haime, 1857)									✓
Pterogorgia anceps (Pallas, 1766)	✓			✓	✓		✓	✓	✓
Pterogorgia citrina (Esper, 1792)	✓			✓			✓	✓	✓
Pterogorgia guadalupensis Duchassaing & Michelin, 1846				✓	✓				✓
Family Nidaliidae									
Nidalia occidentalis Gray, 1835									✓
Family Plexauridae									
Eunicea asperula (Milne Edwards & Haime, 1857)	✓								✓
Eunicea calyculata (Ellis & Solanderca, 1786) forma *coronata* Bayer, 1961	✓	✓			✓				
Eunicea calyculata (Ellis & solander, 1786) forma *calculata* Bayer, 1961	✓			✓	✓		✓		✓
Eunicea clavigera Bayer, 1961	✓			✓	✓		✓		
Eunicea flexuosa (Lamouroux, 1821)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Eunicea fusca (Duchassaing & Michelotti, 1860)	✓			✓	✓	✓			
Eunicea knighti Bayer, 1961				✓		✓			
Eunicea laciniata Duchassaing & Michelotti, 1860	✓			✓	✓	✓			✓
Eunicea laxispica (Lamarck, 1815)	✓			✓	✓		✓		
Eunicea mammosa Lamouroux, 1816	✓	✓	✓	✓	✓		✓	✓	✓
Eunicea palmeri Bayer, 1961	✓				✓				✓
Eunicea succinea forma *plantaginea* (Lamarck, 1815)	✓				✓	✓			
Eunicea succinea forma *succinea* (Pallas, 1766)	✓			✓	✓				
Eunicea tourneforti Milne Edwards & Haime, 1857 forma *atra* Verrill, 1901	✓	✓		✓	✓	✓			
Eunicea tourneforti forma *tourneforti* Milne Edwards & Haime, 1857	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 2.2 Caribbean octocoral species list continued

Octocoral species (under taxon classification)	Útila	Roatan	Swan Ills	Yucatan	Belize	Costa Rica	Bahamas	Jamaica	FL Keys
Muricea atlantica (Kükenthal, 1919)	✓			✓	✓	✓	✓	✓	✓
Muricea elongata Lamouroux, 1821				✓			✓		✓
Muricea laxa Verrill, 1864	✓			✓			✓		✓
Muricea muricata (Pallas, 1766)	✓	✓		✓	✓		✓		
Muricea pinnata Bayer, 1961	✓			✓				✓	
Muriceopsis flavida (Lamarck, 1815)	✓	✓	✓	✓	✓	✓	✓		✓
Muriceopsis petila Bayer, 1961	✓			✓	✓		✓		
Muriceopsis sulphurea (Donovan, 1825)						✓			
Plexaura homomalla (Esper, 1792) forma *kukenthali* Moser, 1921	✓	✓			✓				✓
Plexaura homomalla (Esper, 1792) forma *homomalla*	✓	✓	✓	✓	✓		✓	✓	✓
Plexaura nina Bayer & Deichmann, 1958	✓						✓		
Plexaurella dichotoma (Esper, 1791)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Plexaurella fusifera Kunze, 1916	✓					✓			✓
Plexaurella grisea Kunze, 1916	✓			✓	✓	✓			
Plexaurella nutans (Duchassaing & Michelotti, 1860)	✓			✓				✓	✓
Plexaurella pumila Verrill, 1912				✓					
Pseudoplexaura crucis Bayer, 1961	✓			✓					
Pseudoplexaura flagellosa (Houttuyn, 1772)	✓	✓		✓	✓	✓		✓	✓
Pseudoplexaura porosa (Houttuyn, 1772)	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pseudoplexaura wagenaari (Stiasny, 1941)	✓	✓	✓		✓				✓
Lytreia plana (Deichmann, 1936)					✓				
Suborder Scleraxonia									
Family Anthothelidae									
Erythropodium caribaeorum (Duchassaing & Michelotti, 1860)	✓			✓	✓	✓			

Table 2.2 Caribbean octocoral species list continued

Octocoral species (under taxon classification)	Útila	Roatan	Swan Ills	Yucatan	Belize	Costa Rica	Bahamas	Jamaica	FL Keys
Iciligorgia schrammi, Duchassaing, 1870	✓				✓		✓		✓
Family Briareidae									
Briareum asbestinum (Pallas, 1766)	✓	✓	✓	✓	✓	✓	✓		✓
Family Spongiodermatidae									
Diodogorgia nodulifera (Hargitt, 1901)									✓
Total number of species present	49	24	13	39	39	23	28	16	34

Extensive sampling, for the purposes of calculating species richness and abundance, was limited to 15 m depth and all further reports are based on these data, which had 50 'species' categories that included some unidentified morphospecies awaiting further taxonomic expertise. Raw data of species counts for each site and depth (5 m and 15 m) are given in Appendix A and relative species abundances are given for each site in Appendix B. The rarefaction curve for the entire island has reached an asymptote, demonstrating sufficient sampling (Fig. 2.4). However, when sites are broken up into their component reefs and depths for analysis, the species rarefaction curves have not yet reached a plateau, suggesting species richness was not fully characterised at each reef (Fig. 2.5).

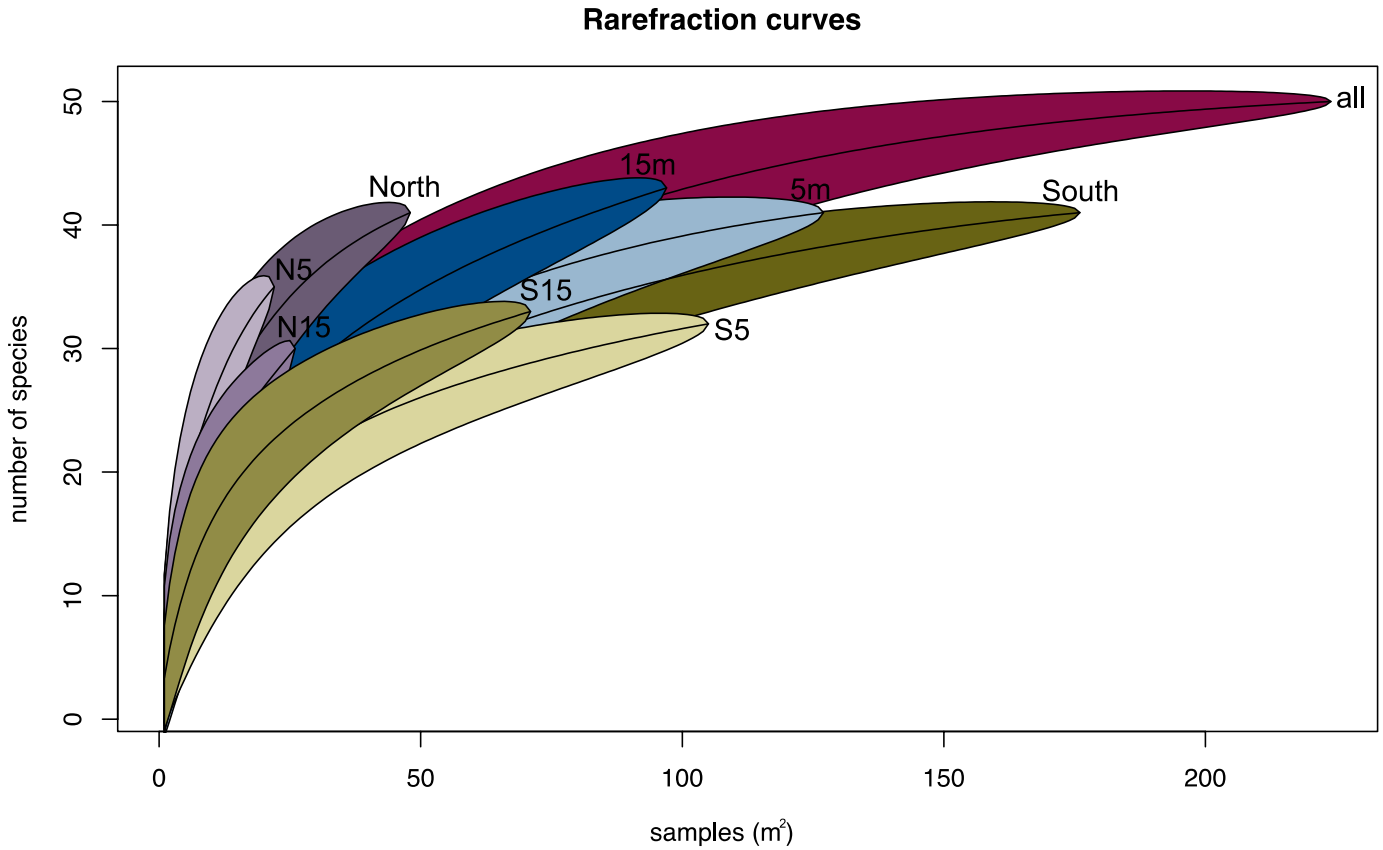


Figure 2.4 Rarefaction curves of data collected for: All sites, All sites at 5 m, All sites at 15 m, All North sites, North at 5 m, North at 15 m, All South Sites, South at 5 m, and South at 15 m. Coloured area around curves refers to standard margin of error.

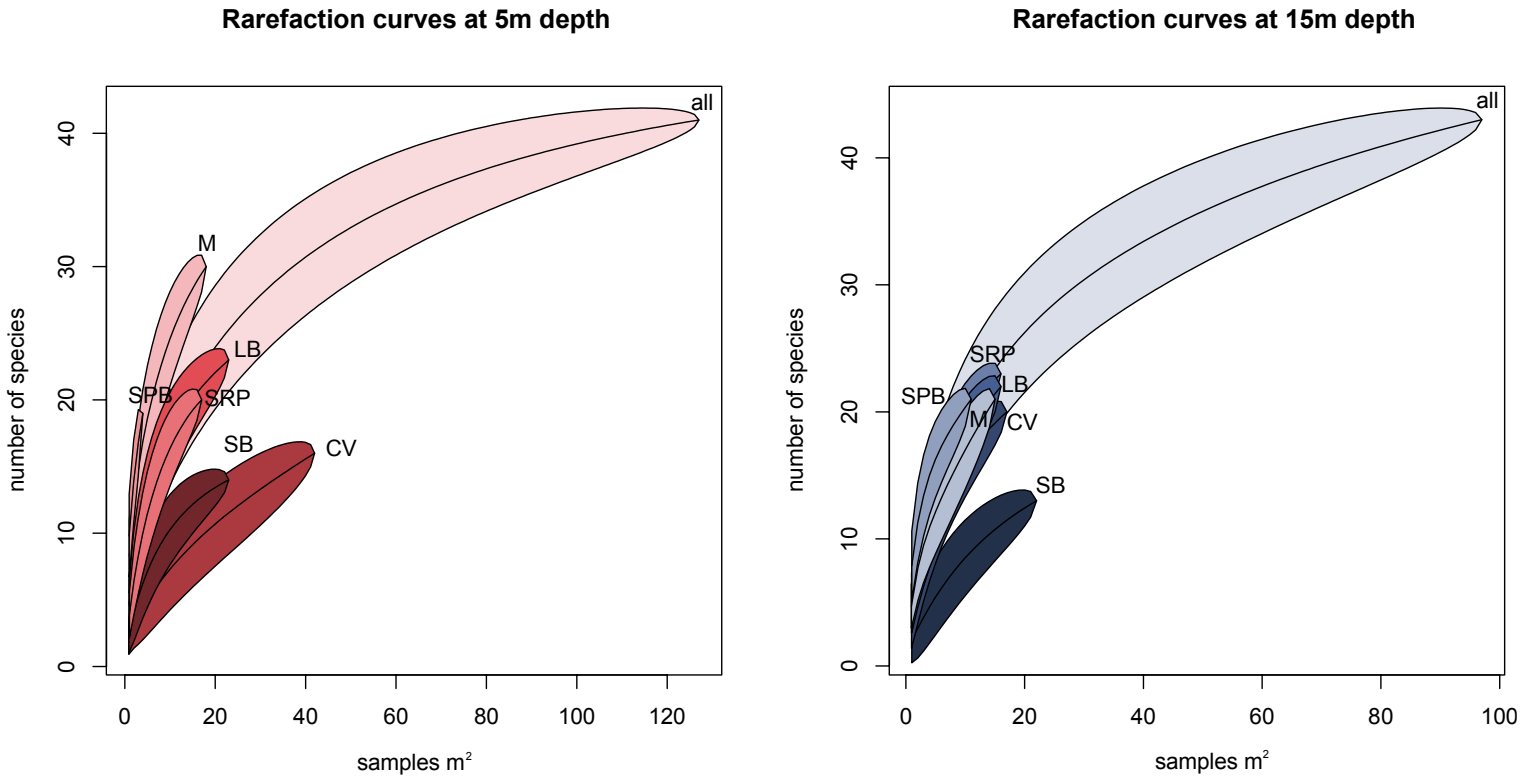


Figure 2.5 Rarefaction curves by reef site. Sites: South shore: SB= Sturch's Bank, CV= Coral View, LB= Little Bight, SRP= Stingray Point; North shore: SPB= Spotted Bay, M=The Maze. Coloured area around curves refers to standard margin of error.

The species abundance model for the entire island displayed a log-normal distribution (Fig. 2.6). The communities of the north shore were fit best with a geometric model (Preston, 1948) and a Zipf model (Zipf, 1949) fit the south shore communities best (Fig. 2.6). Approximately 80% of community structure is determined by 10 species (see Fig. 2.7).

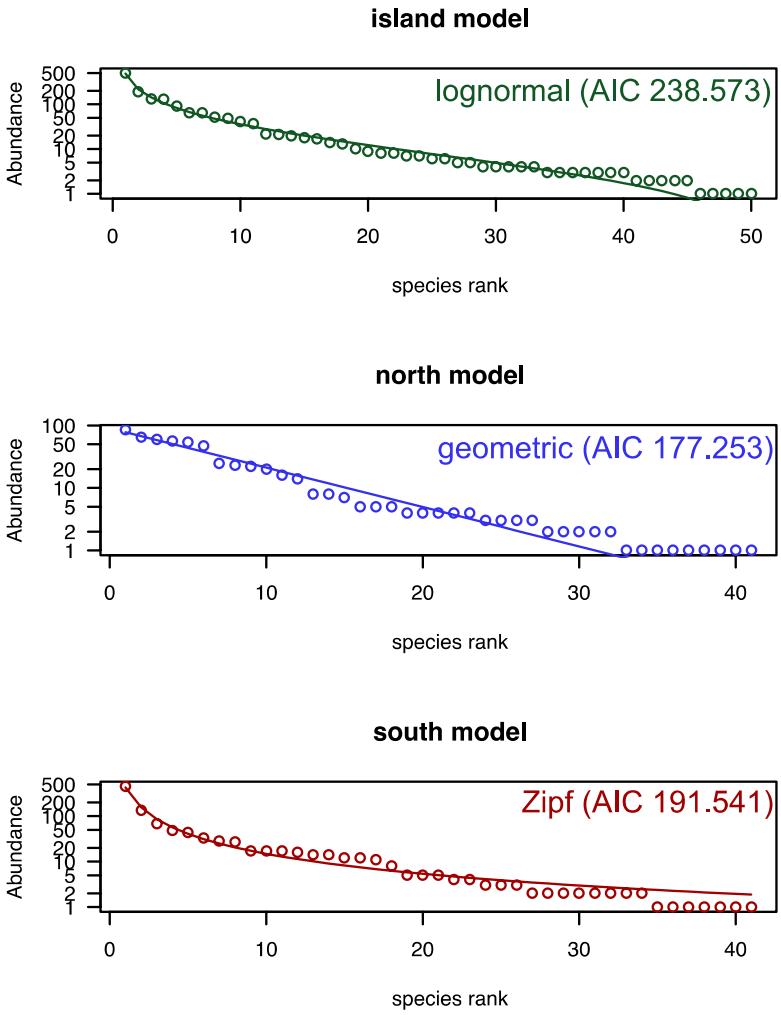


Figure 2.6 Species population models fitted to rank abundance and dominance for island and north and south communities.

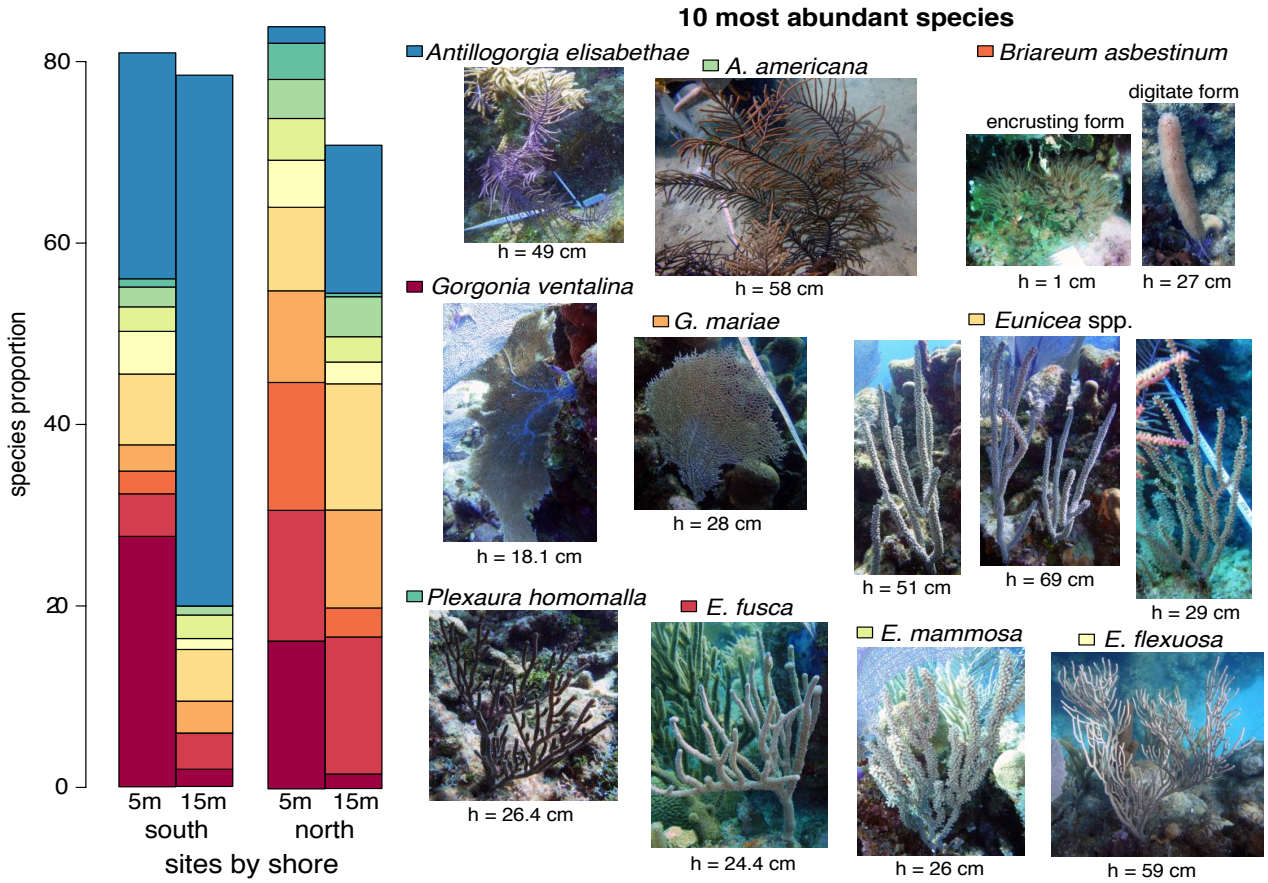


Figure 2.7 Population structure visualised by 10 most abundant species at 5 and 15 m depth of octocoral communities on the north and south shore of Útila. Photo credit: J Murray and V Lovenburg.

Partial permutational MANOVA (PERMANOVA) using Bray-Curtis similarities found the effects of shore (north or south), site, and depth to be significant on community structures of octocorals (Table 2.3). Species richness estimates and evenness averages for sites (individual reefs and grouped by island shores) and depths are visualised in Figure 2.8.

Table 2.3 PERMANOVA results testing nested differences in shore (north and south), site (SB, CV, LB, SRP, SpB, and M), and depth (5 m and 15 m) using Bray-Curtis similarities of species abundances and community structure.

PERMANOVA table of results						
Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms.
Shore	1	17169	17169	9.2112	**0.001**	999
Site(Shore)	4	24900	6225.1	3.3398	**0.001**	996
Depth(Site(Shore))	6	45089	7514.9	4.0319	**0.001**	997
Residuals	49	91330	1863.9			
Total	60	1.82E+05				

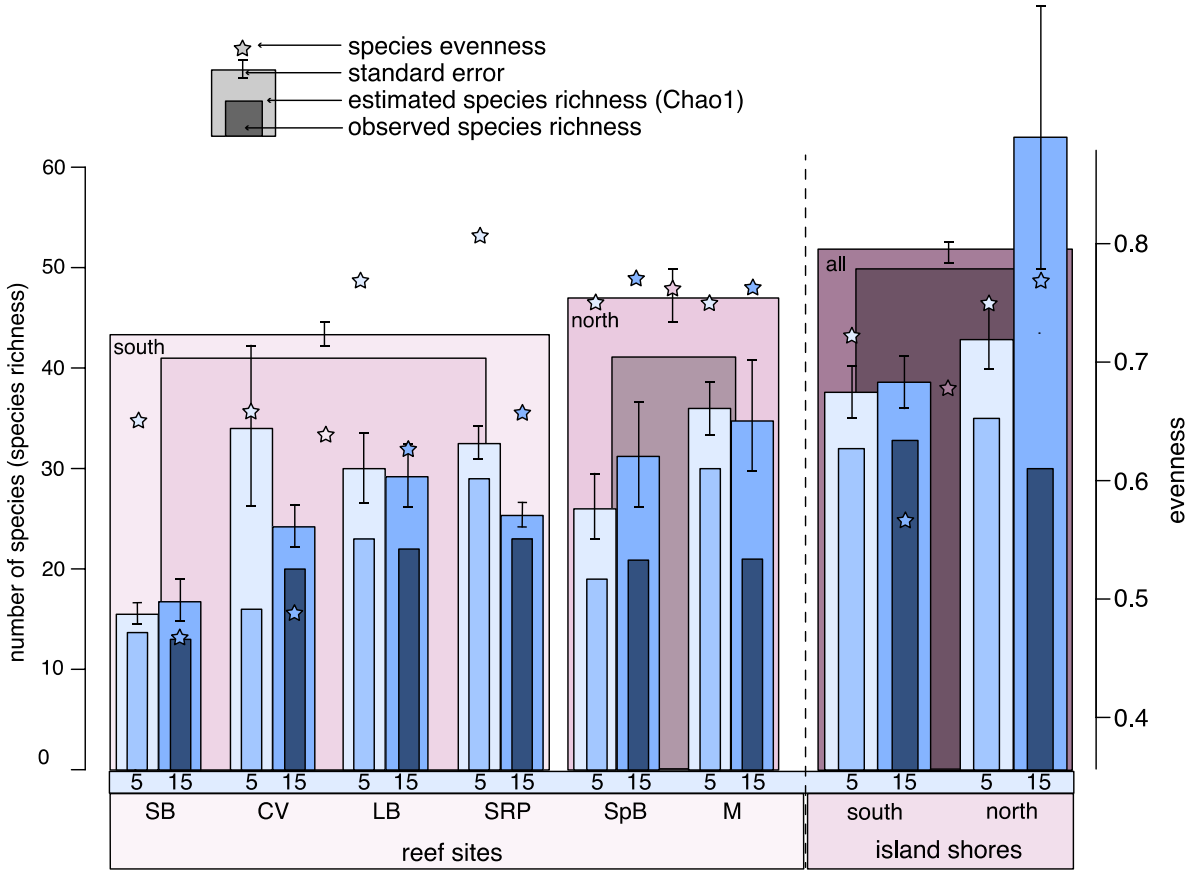


Figure 2.8 Species richness observed and estimated values for each site and depth, and averages for island side and depth. Evenness means for each richness score corresponds to right y-axis and are plotted with star symbols. Error bars indicate standard deviation.

The principal component analysis (PCA) of the Brays-Curtis similarities were fitted with environmental variables (e.g. distance from human population density and water flow) (Fig. 2.9). The first principal component (PC1) explained 39.8% of the variation and 26.2% of the variation was explained by the second principal component (PC2). PC1 was characterised by a high positive loading of distance from human density (63.7%) and PC2 was characterised by a high negative loading value of depth (- 72.8 %).

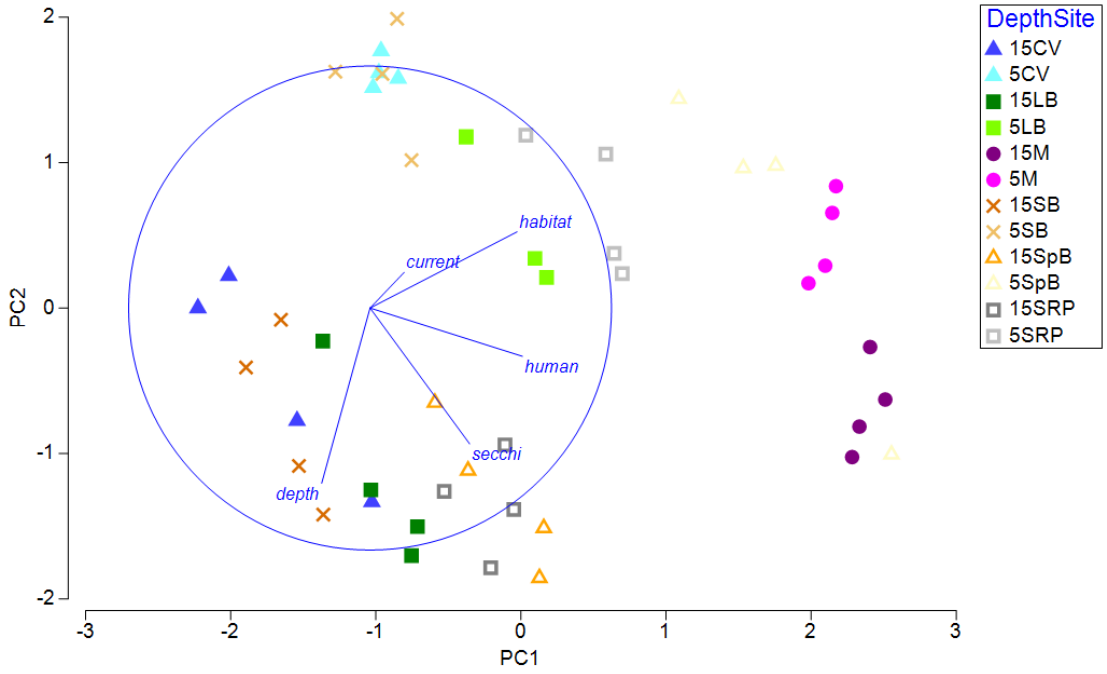


Figure 2.9 Principal component analysis of environmental variables fitted over Brays Curtis similarities of octocoral communities. Corresponding site and depth (15 = 15 m and 5 = 5 m) and colour-coded. SB= 'Sturch's Bank', CV= 'Coral View', LB= 'Little Bight', SRP= 'Stingray Point', SpB= 'Spotted Bay', M= 'The Maze'.

There was a positive correlation between species richness and evenness with distance from human population (Fig. 2.10). The furthest site from human population density (The Maze) is also within the MPA.

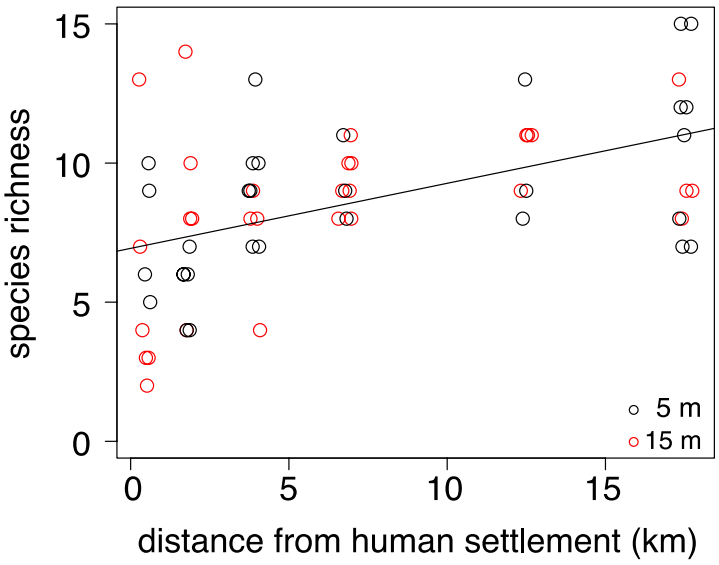


Figure 2.10 Octocoral species richness positively correlated to distance from human settlement of Útila town (ANOVA $F_{1,58}$=18.7, p<0.001). Depth was not significantly correlated within linear model (ANOVA $F_{1,58}$=0.06, p=0.80). Jittering was used on distance from human settlement to visualise overlapping data points.

2.4 Discussion

The high species richness estimated for Útila (51.7±2.2) in comparison to other studies in the area is remarkable (Jordan, 1978; Tortora and Keith, 1980; Muzik, 1982; Lasker and Coffroth, 1983; Dahlgren, 1989; Keith, 1992). Sampling effort, which is not always quantified in comparable studies of the region (Tortora and Keith, 1980; Muzik, 1982; Lasker and Coffroth, 1983; Keith, 1992), was

extensive in this study; this could have influenced the discovery of rare species and likely increased richness estimates.

The observed species richness (49) is still the highest found in the region (see Table 2.1) (Jordan, 1978; Tortora and Keith, 1980; Muzik, 1982; Lasker and Coffroth, 1983; Dahlgren, 1989; Keith, 1992). If comparable research was undertaken in areas previously studied, it might be revealed that octocoral species richness in the Caribbean is generally higher. However, a study from Yucatan reefs (north end of the MBRS) by Dahlgren (1989) identified nearly 10 times the number of colonies in surveys and only found 39 species, so this would suggest that Útila species richness is not merely a factor of intensive sampling. Unfortunately, the only studies conducted on octocorals in Honduras (Tortora and Keith, 1980; Keith, 1992) did not report sampling effort and were based on samples collected between 1979-1980.

Keith (1992) speculated that the higher richness of octocorals found on Roatán compared to Honduran islands further north (Swan Islands) (Tortora and Keith, 1980) was likely because of increased habitat diversity (e.g. multiple reef types such as patch reefs and rubble zones) and protection from storms because of distance from mainland. Útila is closer to the mainland than Roatán (35 km vs. 53 km), and the same logic could be argued for higher species richness found in Útila than Roatán. However, the distance is much less from Útila to Roatán (~17 km), than it is from Roatán to the Swan Islands (~263 km), which is approximately 187 km from the mainland of Honduras.

Species distribution patterns on Útila were comparable to other Caribbean studies of octocoral zonation on reefs (Bayer, 1961; Kinzie, 1970; Muzik, 1982; Lasker and Coffroth, 1983; Dahlgren, 1989). Sea fans, *Gorgonia ventalina*, a species typical of reefs from 5-15 m (Bayer, 1961) were predominantly found at 5 m on fore-reef zones. Species characteristic of deeper depths (e.g. 'deep-water sea fan' *Iciligorgia schrammi*) were found predominantly at 15 m, such as the 'wide mesh sea fan' *G. mariae* typically found 30-35 m (Bayer, 1961), although they have been found at shallower depths (less than 5 m) in Jamaica and Belize (Kinzie, 1970; Muzik, 1982). Like other studies from the southern MBRS there were no representatives of *G. flabellum* found here either (Tortora and Keith, 1980; Muzik, 1982; Lasker and Coffroth, 1983; Keith, 1992). There were more encrusting *Briareum asbestinum* on the north side, characteristic of an environment subjected to sustained current (see Fig. 2.7). Most notable was the general composition of species known from separate geographical locations in the Caribbean (see Table 2.2).

Although the communities of octocorals around Útila support relatively high levels of species richness for the Caribbean, community abundance structures of reefs deviated from a log-normal curve, which could be an indication of impact (Magurran, 2004) (refer to Fig. 2.6). If reef communities were completely connected around the entire island (i.e. communities are pooled) then the population structure did fit a log-normal distribution.

The communities along the south coast exhibited a Zipf abundance pattern, which reflect an unbalanced community. Such steep gradations of relative abundances are commonly found in early successional communities and populations burdened by toxins or enrichment by pollution (May, 1986; Magurran, 2004). An environmental toxicology study is recommended for the area given

these results as this is unlikely to be an early successional community. Stingray Point had the highest species richness estimates of the south shore site and the highest evenness score any of the sites. This site was suggested as an area of conservation interest by Harborne *et al.* (2001) but there is still no movement on the development of the protected area (Narozanski *et al.*, 2012). Little Bight had high species richness and evenness therefore it too should be considered for inclusion in any proposed protected area.

There are many factors that affect the distribution of octocoral colonies in the environment, e.g. current, sedimentation, topographic complexity, light attenuation (Wainwright and Dillon, 1969; Kinzie, 1970; Goldberg, 1973; Lasker and Coffroth, 1983; Dahlgren, 1989; Sánchez *et al.*, 1997). Turbidity (i.e. low values of Secchi depth) often correlates to high levels of sedimentation and low levels of light penetration. Sedimentation is known to be particularly damaging to octocorals (Yoshioka and Yoshioka, 1989). Light levels are important for healthy photosynthesis of all zooxanthellate coral organisms (Fabricius, 2005).

The characterisations of the environmental conditions were not measured with precise instrumentation, yet higher measurements of the Secchi disk were still correlated with species richness. With such high levels of estimated species richness, it would be interesting to have precise measurements of environmental conditions to accurately quantify these uniquely species-rich habitats. The causative environmental factors contributing to the different community structures would require extensive experimental design and a more complete knowledge of species responses to these variables (Austin, 2002).

Útila has experienced a boom in tourism (mostly dive-related) in the last 30 years or so, stimulating economic growth and development that has strained the natural resources available to the island, including reef health (Canty, 2007). Coastal development and diving pressures on the reefs of Útila could be a factor in this poor community structure particularly on the south shore sites (Canty, 2007).

Anthropogenic stress has been repeatedly linked to coral reef degradation (Guzmán and Jiménez, 1992; Hughes, 1994; Hughes *et al.*, 2003; Bellwood *et al.*, 2004; Mora, 2008). Distance away from human population density was correlated to richness estimates; although no causal link is confirmed. A study on coral reefs in Indonesia demonstrated that areas hosting high diversity and well-structured assemblages resist disturbances more effectively (Ferrigno *et al.*, 2016). There is unfortunately no baseline comparison for these communities to assess resistance, if any, but the implications of these findings are promising for the future of coral reefs.

Octocoral species richness has been observed to increase with depth in the Caribbean (Kinzie, 1970; Dahlgren, 1989). On the north shore of Útila there was a greater estimate of species richness at 15 m compared to 5 m, however the standard error was so large, the validity of these findings would need to be confirmed, ideally adding another depth for a gradient comparison. The south shore showed no increase in estimated species richness from 5 m to 15 m.

The estimated species richness for Coral View at 5 m and the north shore at 15 m may stand out as suspiciously high (Fig. 2.7). The preponderance of rare species was undoubtedly a factor in this estimate. However, the abundance-based coverage estimator (ACE), which is often recommended

for assemblages of numerous rare species (not just singletons or doubletons used in Chao1), projected the same richness (with a smaller standard error) (Colwell *et al.*, 2012). Another factor in these high estimates could be that colonies in these areas were more spatially aggregated and exhibited a highly patchy distribution, which is commonly found in reefs systems of octocorals (Kinzie, 1970; Dahlgren, 1989). Sample-based rarefaction, the method exercised here, usually accounts for spatial aggregation (Gotelli and Colwell, 2011). The north shore at 15 m consisted of a sloping fore-reef habitat at Spotted Bay and a nearly vertical wall at The Maze. These sites most likely did not have enough overlap in assemblages for accurate species richness estimates. Coral View at 5 m is also likely the overlap of two assemblages, however the inferred ecological separation was not abundantly apparent during sampling. Distinct populations are thought to occur within reef zones at small-scale island-like assemblages, presumably governed by precise physical suitability (Dahlgren, 1989).

An additional and not necessarily mutually exclusive hypothesis to be considered in interpreting the high richness estimates, especially for those projected for Coral View, could be is that these reefs are an example of the intermediate disturbance hypothesis on a small-scale within reef system, but also on a larger island-scale (Paine, 1966; Connell, 1978). This hypothesis predicts that species richness levels will be maximal in habitats subjected to levels of periodic intermediate disturbances (Paine, 1966; Jackson, 1977; Connell, 1978; Karlson, 1980). The intermediate disturbance hypothesis has stimulated debate over its usage and over-simplification in ecological studies (Karlson, 1980; Dial and Roughgarden, 1998; Roxburgh *et al.*, 2004; Fox, 2013; Sheil and Burslem, 2013). An assessment of this hypothesis is out of the scope of this study and there are too

many unknown components in the basic biology and dynamic interactions of octocoral species to draw conclusions.

Besides the highest estimated richness of octocorals for any Caribbean island, one of the most tangible and exceptional discoveries during this study are the large populations of *A. elisabethae.* This species is regularly harvested for its pseudopterosins, compounds with analgesic and anti-inflammatory properties (Look *et al.*, 1986; Bruckner, 2002; Lasker and Porto-Hannes, 2015). Successful management of these fisheries, using refined management models outlined by Lasker (2013), have demonstrated to be sustainable. The octocoral communities of 15 m at Sturch's Bank on Útila are nearly completely dominated by *A. elisabethae* (approximately 90%). With precautionary management strategies implemented, including a protocol of population assessment (Lasker and Porto-Hannes, 2015), there exists a potential for significant revenue for Honduras in this continued harvest.

3

Chapter Three

Underestimation of habitat provided by octocorals in the Caribbean

3.1 Abstract

Space limitation of coral reef habitat places importance on quantifying surface area available for habitat. Coral reef surveys are largely focused on defining benthic coverage suited for areas of high scleractinian cover. In the Caribbean, where an average of less than 15% of the benthic coverage is now scleractinians, new methods need to be assessed that account for more dominant habitat providers, such as the octocorals that offer habitat away from the benthic substrata. This study demonstrates that holdfast measurements of sea fans can be modelled to predict up to 67% of their available surface areas for colonies when factoring environmental conditions (e.g. depth and wave exposure). These calculations used in conjunction with benthic data could provide a more accurate estimate of historic habitat provided by these organisms. Additionally, disease signs were found to be associated with larger colonies, indicating an additional need for translating historic data that might be useful in etiological models.

3.2 Introduction

Coral reefs are being lost worldwide at dramatic rates and this loss has been most critical in the Caribbean (Gardner *et al.*, 2003; Alvarez-Filip *et al.*, 2009; Roff and Mumby, 2012). There are many factors that affect the degradation of coral reefs (e.g. pollution, sedimentation, ocean warming, and over-fishing) and their synergistic pressures create nonlinear responses (Hughes *et al.*, 2003; Mumby *et al.*, 2007). These disturbances have profound effects on diversity in an ecosystem and have dramatically changed the composition of coral reef communities (Gardner *et al.*, 2005; Mora, 2008). As living scleractinian cover is lost, there has been an observable shift from coral-

dominated to marcroalgae-dominated, or 'coral depauperate' reefs, in many areas (Bruno *et al.*, 2009; Mumby, 2009; Dudgeon *et al.*, 2010).

The consequences of this transition on environmental stability have been fervently debated in the literature (Hughes, 1994; McCook, 1999; Knowlton, 2004; Bruno *et al.*, 2009; Mumby, 2009; Dudgeon *et al.*, 2010; Fox, 2013; Sheil and Burslem, 2013). The phase-shift hypothesis, alternative stable state hypothesis, and intermediate disturbance hypothesis each attempt to describe the current condition of many coral reef systems (Done, 1992; Knowlton, 2004; Mumby, 2009). The dispute centres on the notion that the present prevailing predicament is either an emergent stable state or an ultimately unstable state. The phase shift hypothesis asserts that there is a continuous trajectory of ecosystem turnover that would revert to a coral-dominated reef if environmental pressures were mitigated (Done, 1992; Hughes, 1994; Dudgeon *et al.*, 2010). Alternative stable states and intermediate disturbance hypotheses argue that environmental feedback mechanisms strengthen the stability of coral-depauperate reefs making recovery from these alternate states nonlinear and present additional complications to conservation (Knowlton, 2004; Fox, 2013; Sheil and Burslem, 2013).

Recently it has been reported that octocorals are increasing in abundance in the Caribbean and establishing themselves as a dominant component of reefs (Sathe *et al.*, 2008; Lenz *et al.*, 2015). In a study of octocoral abundance, Lasker and Coffroth (1983) suggested that if the biomass (or another indicator of size) of the Plexauridae was measured, it would likely show them to dominate some reef communities. This observed dominance could either be an important reef stepping stone or the rise of an alternative stable state of reefs. Regardless of the nuanced status, a greater

understanding of the role these organisms play in new communities is essential for conservation monitoring of these fragile ecosystems.

3.2.1 Habitat complexity

Habitat complexity has been suggested as one of the key factors in supporting coral reef biodiversity as well as supporting coastal productivity and providing storm protection (Friedlander and Parrish, 1998; Graham and Nash, 2012). Reefs with high scleractinian coral cover and rugosity are known to support greater overall species richness and abundance of associated biota (MacArthur and MacArthur, 1961; Gratwicke and Speight, 2005; Alvarez-Filip *et al.*, 2009; Newman *et al.*, 2015). Highly heterogeneous habitats help support higher biodiversity through niche partitioning (MacArthur and MacArthur, 1961; Luckhurst and Luckhurst, 1978). For example, structural complexity and the variety of growth forms are used in measurements of fish habitat suitability (Gratwicke and Speight, 2005).

Octocorals are frequently found with various associated fauna such as crustaceans, hydroids, annelid worms, molluscs, benthic ctenophores and pygmy seahorses (Bayer, 1961; McArthur, 1985; Crisp, 1990; Gerhart, 1990; Martin *et al.*, 2002; Buhl-Mortensen and Mortensen, 2004; Puce *et al.*, 2008; Wilson *et al.*, 2008; Reijnen *et al.*, 2011). Octocorals and sponges provide essential habitat for different fish and invertebrate life stages (Greene, 2008; Wilson *et al.*, 2008). The degree of habitat complexity and presence of octocorals and sponges on reefs are inadvertently overlooked because reef surveys are typically biased to assess areas with high scleractinian densities (Almada-Villela *et al.*, 2002).

The standard reef survey methods, developed in areas of high scleractinian cover, like the Indo-Pacific (Almada-Villela *et al.*, 2002; Wilkinson, 2008), largely fail to account for the three-dimensional space that arborescent octocorals and sponges inhabit in the Caribbean. Traditional survey methods (e.g. continuous transect and point intercept transect) are suited to measure hard corals and macroalgae, which are encrusting or which grow low to the seabed (Weinberg, 1981). However, most of the surface area of octocorals and sponges are not attached to the substratum but are continuously shifting in the water column above the plane of data collection (See Fig. 3.1).

Surface area is a fundamental concept in biology that governs forces in metabolic regulation and allometric relationships in a dynamic environment (McMahon, 1973). Surface area as a function of metabolism was first purposed by Rubner (1883) for mammals and birds. Surface area calculations for scleractinian corals have been widely studied in the literature (Hoegh-Guldberg, 1988; Bythell *et al.*, 2001; Courtney *et al.*, 2007; Laforsch *et al.*, 2008). The surface area in contact with the environment is directly related to the biological functioning of that organism (i.e. autotrophic and heterotrophic feeding) and niche availability for utilisation of other reef organisms (Muscatine and Porter, 1977; Yoshioka and Yoshioka, 1989; Wilson *et al.*, 2008).

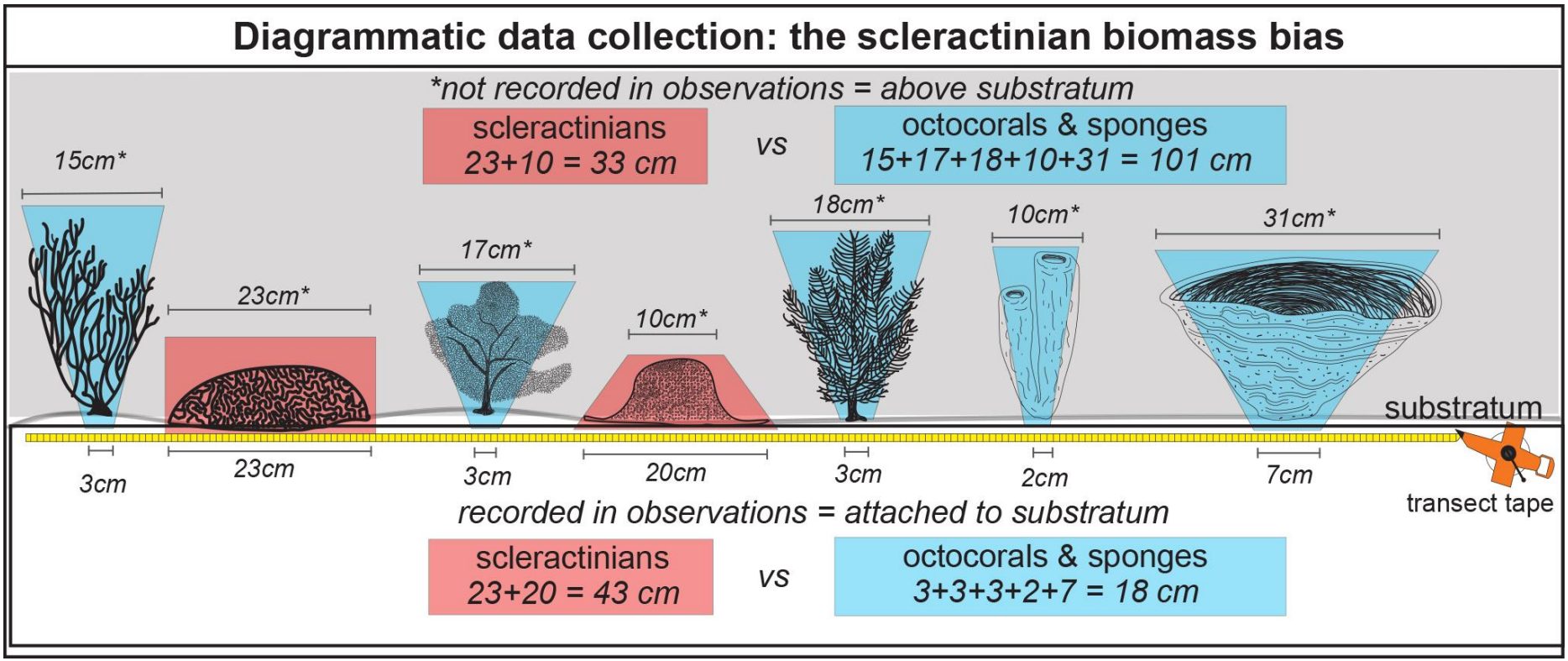


Figure 3.1 Hypothetical data collection diagram highlighting the bias in traditional transects that only record what is attached to the substratum. Octocorals and sponges are depicted and highlighted in blue; scleractinian corals are depicted and highlighted in red.

Reefs that are no longer dominated by scleractinians need to employ survey methods that appropriately quantify octocorals and sponges, in order to properly assess ecosystem services (Diaz and Rützler, 2001; McLean and Yoshioka, 2007; Santavy *et al.*, 2012a). Santavy *et al.* (2012a) explored rapid and non-invasive measurements that can be translated into regression equations to best quantify this habitat complexity for scleractinians as well as octocorals and sponges. These equations employ measurements of the colonies' height, width, and are specific to their category of growth form. Quantification of habitat availability provided by octocorals and sponges is a useful method to employ for future research and necessary for a complete assessment of reef resilience.

Long-term studies of octocoral populations in the Caribbean are limited to census data collected to measure scleractinian and macroalgae percent cover (Lenz *et al.*, 2015). In the traditionally benthic-biased transect, it may be possible to use measurements collected from an octocoral colony's holdfast – the area in contact with the benthic substratum – to estimate its corresponding surface area. Working under the idea that transects typically reflect primarily the area attached to the substrata, this study hypothesises that holdfast sizes are positively correlated with, and have the potential to predict, the surface area of colonies. Valuable reef habitats that octocorals provide is thus being underestimated and inaccurately portrayed with traditional reef transect methods. Furthermore, octocorals' surface areas and holdfast sizes are likely reflective of the health state of the colony.

3.3 Methods

The site of data collection spanned the more frequently dived southern coast (four sites) and the less frequented northern coast (two sites) of Útila. The island is uniquely positioned on the continental slope so that the southern coast's reefs connect to a sandy sloping bottom that

gradually declines in depth, while in contrast the northern coast dramatically drops to hundreds of metres depth within a kilometre off the coast and thousands of metres depth within tens of kilometres (Pinet, 1976). The island of Útila is home to approximately 4000 people, most whom reside in the Eastern harbour of the south coast, called Útila Town. The north coast is the location of a Marine Protected Area (Turtle Harbor) where sea turtles (*Chelonia mydas* and *Caretta caretta*) come to lay their eggs (Fig. 1.3) (Harborne *et al.*, 2001).

3.3.1 Data

Four south coast and two north coast sites were used in data collection at two depths (5 m and 15 m) using SCUBA diving to survey 20m line transects (see map in Fig. 1.3). Additional data collected on colonies included: species identification, degree of health (e.g. signs of the disease Aspergillosis, tissue necrosis or tumours) and any associated fauna on each colony. Two depths (5 m and 15 m) were chosen for data collection in order to get a more complete range of morphologies since many octocoral species have shallow and deep morphology forms (Bayer, 1961; Prada *et al.*, 2008). Maximum colony height, width and holdfast diameter was recorded (Fig. 3.2) for over 600 octocoral colonies that met size requirements outlined by Santavy *et al.* (2012b) for surface area regression equations (e.g. over 10 cm in height for sea fans and sea rods, and over 26 cm for sea plumes). Three morphotypes analysed by Santavy *et al.* (2012a) were used in this study: sea fan planar, sea plume and sea rod branched (see Fig. 3.3 for morphological distinctions).

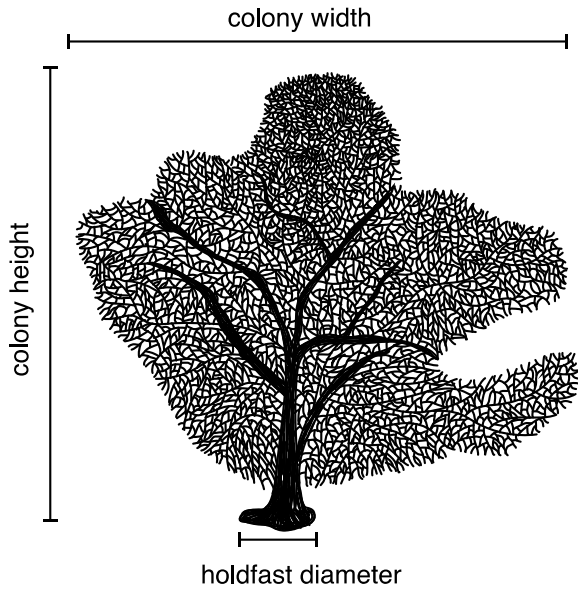


Figure 3.2 Diagrammatic example of measurements taken in the field of a sea fan (*Gorgonia* sp.).

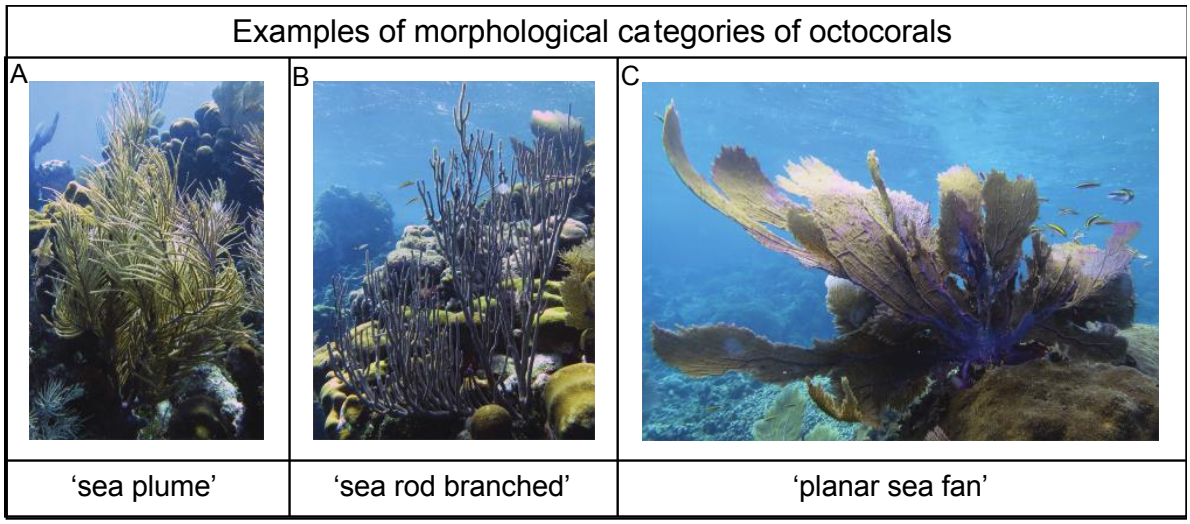


Figure 3.3 Examples of morphological categories of octocoral colonies in the field. A) *Antillogorgia americana* B) *Eunicea flexuosa* C) *Gorgonia ventalina*. Photographs by AD Rogers.

3.3.2 Statistical analyses

Size data from 168 sea fans (eq. a), 178 sea rods (eq. b) and 199 sea plumes (eq. c) were used to calculate the Santavy *et al.* (2012b) surface area regression equation and correlated with holdfast measurements.

Sea fan planar $SA = 0.68h^2 + 0.66d^2 - 3.61$ (a)

Sea rod branched $SA = 1.46d^2 + 399$ (b)

Sea plume $SA = 4.77h^2 - 2{,}990$ (c)

h = colony height

d = colony diameter or width

SA= surface area of colony

Continuous data such as colony height, colony diameter, holdfast diameter, and surface area were all log-transformed to account for heteroscedasticity. Heteroscedasticity can be problematic in regression analyses because of assumptions in modelling errors, leading to the invalidation of statistical significance (Cook and Weisberg, 1983). Log transformations of one or both variables are specifically recommended when testing allometric (i.e. body size) relationships (Quinn and Keough, 2002). Residuals were also plotted on graphs to check for heteroscedasticity and normality. Shapiro-Wilk Normality tests were performed separately on each morphological class of the log-transformed surface area and holdfast diameter to determine appropriate tests for regression analyses. Data for holdfast diameters failed to support the null hypothesis of normality for sea fans ($W = 0.98$, $p < 0.05$), sea rods ($W = 0.96$, $p < 0.001$) and sea plumes ($W = 0.98$, $p < 0.05$). Data for transformed surface areas supported the null hypothesis for sea plumes ($W = 0.99$, $p = 0.67$) but failed to support the null hypothesis for sea rods ($W = 0.81$, $p < 0.001$) and sea fans ($W = 0.98$, $p < 0.05$). Given the failure for the majority of the data to meet assumptions of normality, correlations revealed by

regression analyses were tested for statistical significance using the non-parametric Spearman Rank Correlation.

Correlations of surface area and holdfast were tested, accounting for variables of effect such as depth, site location and health of colonies measured. Differences in holdfast diameters and surface area between island shores were tested using the non-parametric Kruskal Wallis test. Correlations of colony health to surface area and holdfast dimensions were tested using the non-parametric Spearman correlation method. Statistical analyses and graphs were performed and executed in JMP (v8.0) and R (R Core Team, 2015).

3.3.2.1 Scleractinian vs. octocorals

To quantitatively illustrate the assumed under-representation of octocorals on traditional transects, benthic coverage measured in these transects of octocoral and scleractinian colonies' were compared to the colonies' surface areas. Coral colony dimensions (i.e. maximum width and height) were used to calculate mean surface areas, which were compared to mean observed presence on a transect (=centimetres measured) of scleractinian corals and octocorals. Along a 20m transect, for reference, at 5 m at Coral View, all corals (both scleractinian and octocorals) were recorded and each coral colony that met the size requirements (one dimension greater than 10cm) was measured for its maximum height and diameter in addition to the length recorded from the transect. Twenty-five colonies of scleractinians and 34 octocorals were observed for their colony dimensions and classified into a morphological category for analysis (data collection by Eric Traub). The following scleractinian coral equations from Santavy *et al.* (2012b) were used for the morphologic categories listed:

Sphere or flattened dome (e.g. *Diploria labyrinthiformis, Siderastrea siderea*)

$$SA = 0.904 \log(h)+1.165 \log(d/2)+0.610$$

Low mound or branching (e.g. *Porites astreoides, Porites porites*)

$$SA = 0.846 \log(h)+0.723 \log(d/2)+0.510 \log(h+[d/2])+0.656$$

h = maximum height

d = maximum colony diameter

Log-transformed measurements of surface areas from regression equations and lengths recorded on transects were tested for normality within coral types (i.e. scleractinian or octocoral). Data failed to reject Shapiro-Wilks test of normality (surface areas for scleractinians: W = 0.94, p = 0.15 and octocorals: W = 0.97, p = 0.39; amount recorded on transect for scleractinians: W = 0.98, p = 0.79 and octocorals: W = 0.96, p = 0.20). Therefore, differences in means for surface areas and transect measurements of coral types were tested using the parametric ANOVA analysis.

3.4 Results

All three morphological categories of octocorals had positive correlations between their holdfast diameters and colony surface areas (Fig. 3.5). Spearman rank correlation indicates a significant correlation between holdfast diameter and surface area calculated from equations (Santavy *et al.*, 2012b) for each morphology (sea fans: r_s = 0.75, p-value < 0.001; sea plumes: r_s=0.35, p < 0.001; sea rods: r_s=0.51, p < 0.001). Sea fans had the strongest trend of correlation, with a 0.52 value of R^2, meaning that 52% of the variation in holdfast diameter is explained by the colony's surface area.

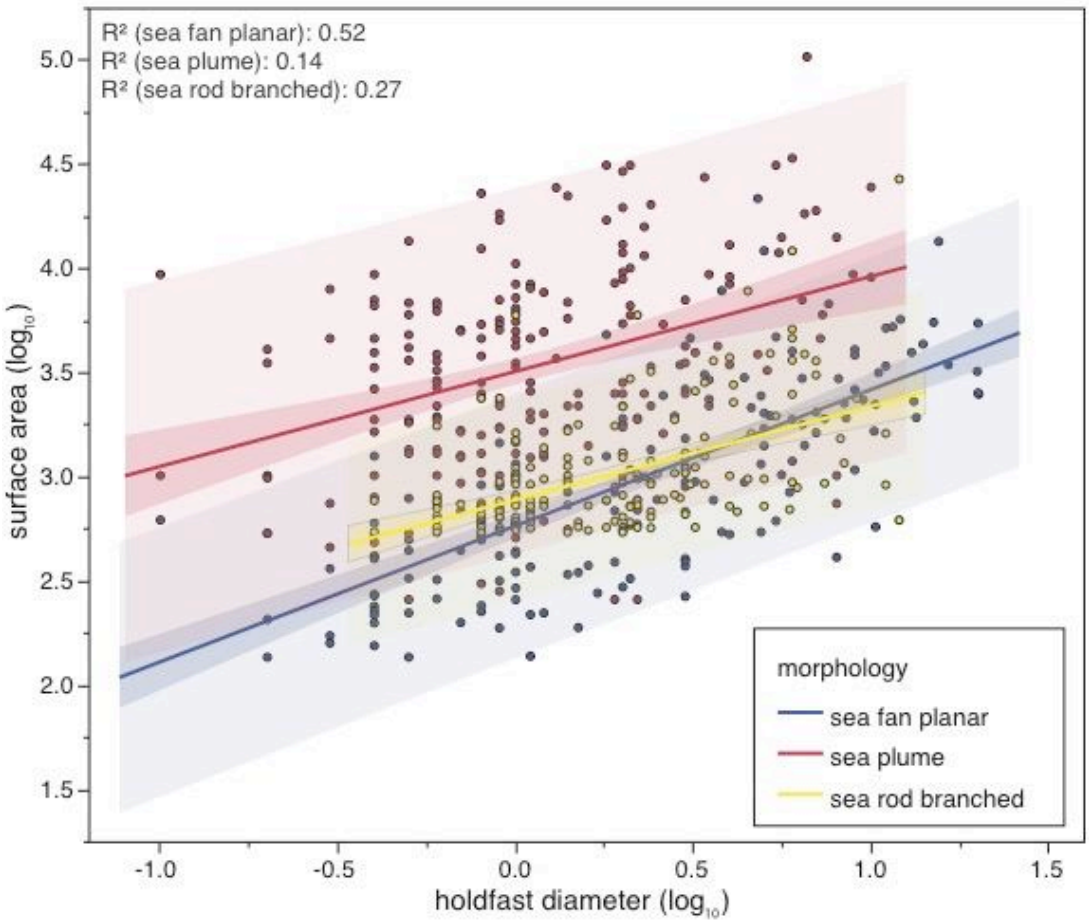


Figure 3.2 Graphical representation of the correlation of holdfast diameter and surface area calculated from morphologically dependent regression equations by Santavy *et al.* (2012b).

Holdfast diameter trends varied based on morphology types, depth, and site location. When data are parsed into site-specific groupings and depth, the correlations are strengthened (Fig. 3.6). One-way ANOVA for holdfast diameters and surface areas revealed differences in sizes of colonies and their holdfasts on the south side of the island and the north side of the island (see Fig. 3.7 for graphical visualisation). Sea fan surface area was significantly smaller on the north shore (χ^2=10.43, df=1, p=0.001) but their holdfasts were not significantly smaller (χ^2=1.59, df=1 p=0.192). Sea plume surface areas were significantly larger and had larger holdfasts on the north shore (χ^2=3.97, df=1, p=0.046; χ^2=14.90, df=1, p<0.001). Sea rod branched colonies had

similar surface areas on both shores (χ^2=1.70, df=1, p=0.19) but had larger holdfasts on the north shore (χ^2=8.43, df=1, p<0.05).

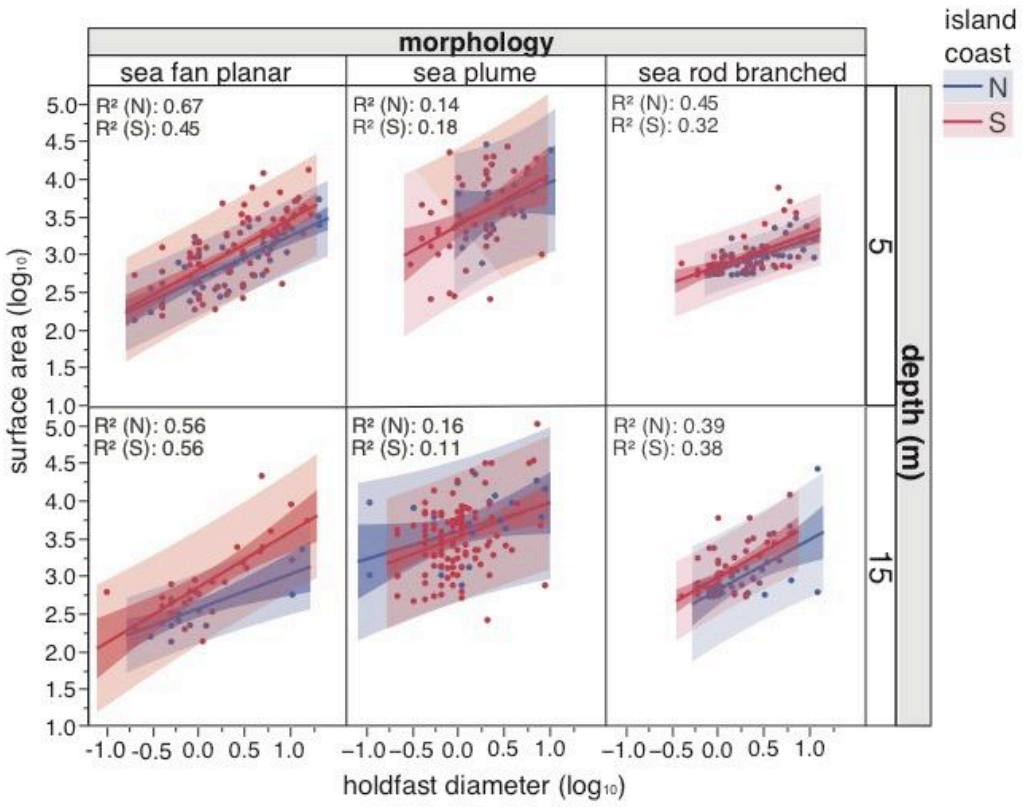


Figure 3.3 Correlations of holdfast diameter and surface area of octocoral morphologies by depth and island coast.

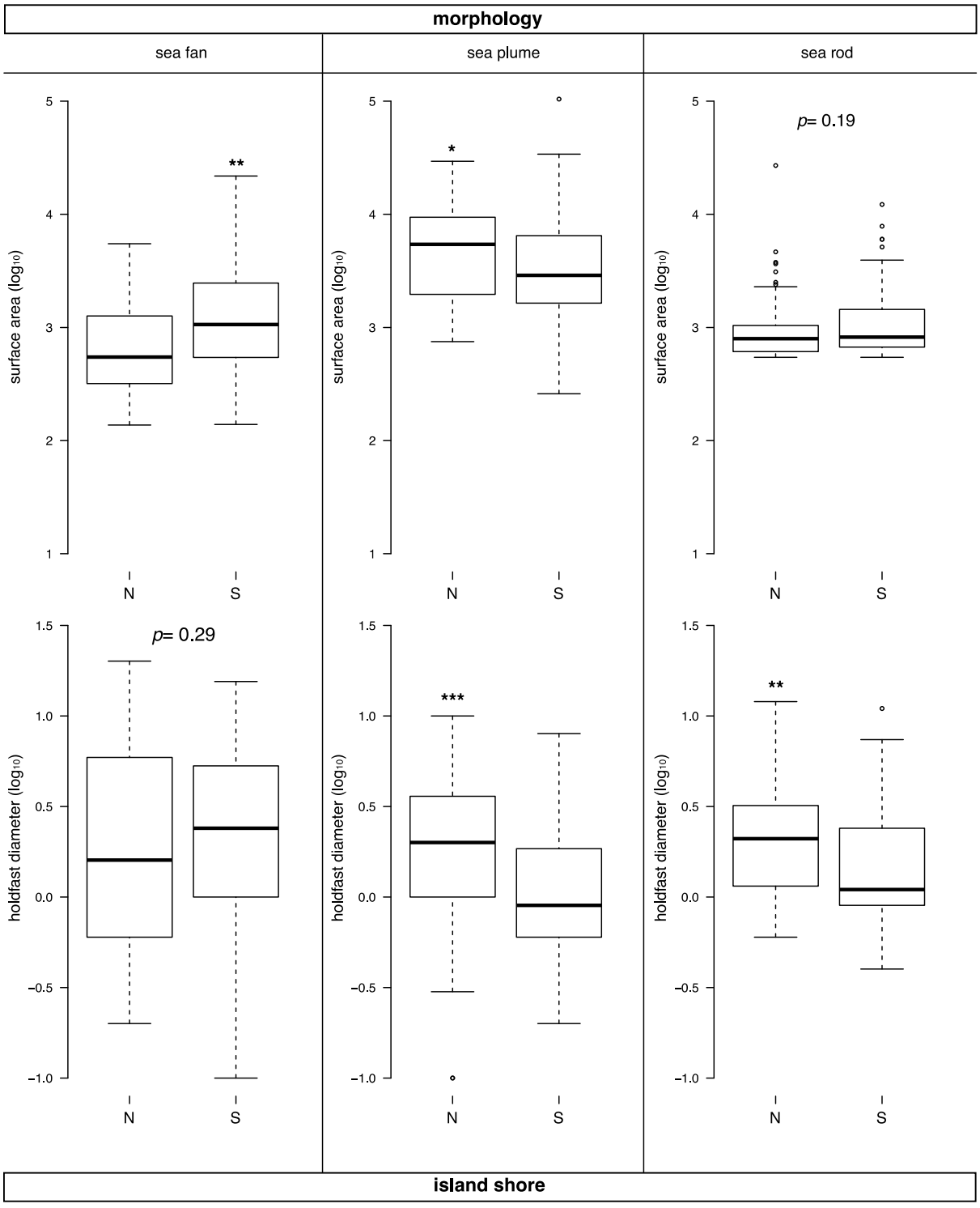


Figure 3.4 Boxplots showing morphology types (sea fan, sea plume, and sea rod) surface areas vs holdfast diameters and statistical significance from Kruskal-Wallis rank sum tests of relationship between shores. *P*-values given if not significant or coded as: * = 0.05; ** < 0.05; *** <0.001.

3.4.1.1 Octocoral vs scleractinian

Combined measurements of transect length laying over 25 scleractinian colonies accounted for 62% of the total one-dimensional measurement reflective of traditional benthic methods. Significantly more area was measured for the 25 scleractinians compared to 34 colonies of octocorals which accounted for 38% of the observed lengths ($F_{1,57}$ = 26.27, p<0.001) (see Fig. 3.8). Conversely, the surface areas of the scleractinian colonies were significantly less than surface areas in octocorals ($F_{1,57}$ = 8.56, p<0.05) (see Fig 3.8).

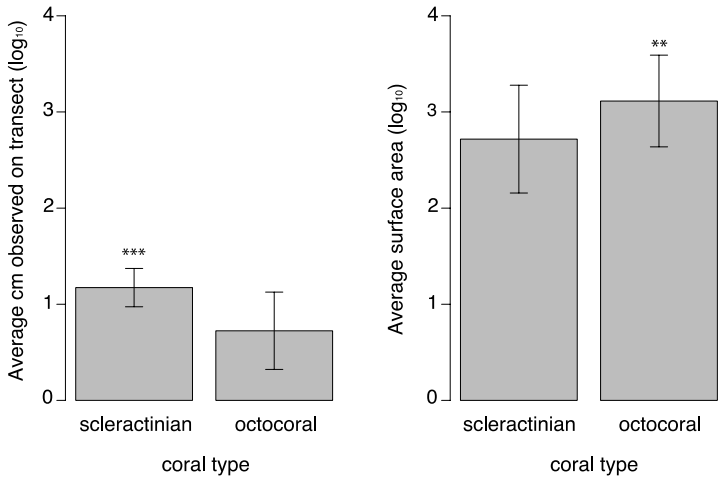


Figure 3.5 The average measurement along a continuous transect occupied by colonies of scleractinian coral vs octocorals compared to the average surface area calculated by regression equations of the same scleractinian colonies vs octocoral colonies. Error bars indicated standard error of the means. Significant differences in means are labelled with asterisks: *** indicates a *p-value* of <0.001 and ** indicates a *p-value* of <0.05.

Scleractinians' surface areas and observed transect measurements were positively correlated (r_s=0.75, p<0.001). Conversely, there was no significant correlation with transect measurements and surface area in octocorals (r_s=0.28, p=0.114).

3.4.2 Health related

Signs and symptoms of disease were found more commonly in larger colonies. In all three morphologies, signs and symptoms of disease were positively correlated with larger surface areas (sea fans: r_s=0.41, p<0.001; sea plumes: r_s=0.16, p=0.02; sea rods: r_s=0.22, p=0.003) (Fig. 3.9). There was a significant positive correlation with holdfast diameter and incidence of disease in sea plumes (r_s=0.16, p<0.05) and sea fans (r_s=0.29, p<0.001), but not in sea rods (r_s=0.14, p=0.06).

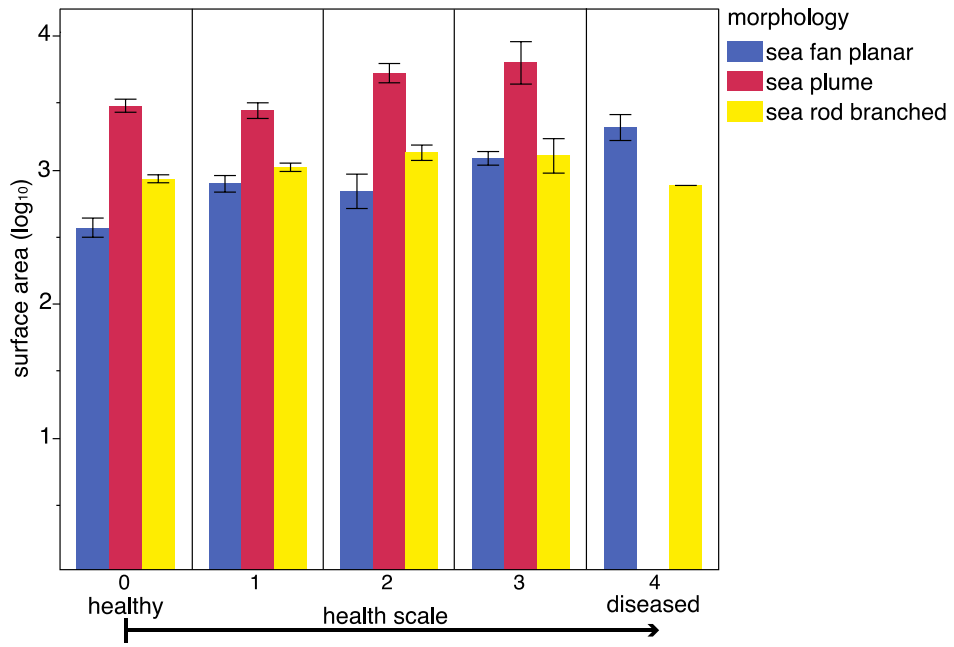


Figure 3.6 Mean surface areas and standard deviations of octocoral morphologies within relative health scale classes from healthy (0) to multiple signs of disease (4), e.g. purple tumours and tissue necrosis.

3.5 Discussion

Surface areas and their correlation to holdfast diameters were best modelled in the sea fans, especially when depth and environmental variables were separated into their respective components. The morphology category of sea fans comprises two species (i.e. *Gorgonia ventalina* and *G. mariae*), while sea plumes account for nine species (*Antillogorgia* spp. and *Muriceopsis* spp.), and sea rods represent 28 species (*Eunicea* spp., *Plexaura* spp., *Pseudoplexaura* spp., *Plexaurella* spp., and *Muricea* spp.). Differential growth rates between organisms is common amongst reef species with indeterminate growths (Sebens, 1982). Species will also often undertake various strategies to overcome space competition on a reef (Jackson, 1977). A dataset that combines multiple species of different growth rates and competitive strategies is likely to have confounding signals. This could explain why sea fans, which were only two species, fit models better than sea plumes and sea rods, which each had more species in each category.

The difference in holdfast diameters and surface area seen on the north coast versus the south coast is likely a mixture of environmental variables such as differences in wave and light exposure, and competition for space (Muscatine and Porter, 1977; Yoshioka and Yoshioka, 1989; Sánchez *et al.*, 1997). Sea fans were significantly larger on the south shore, yet their holdfasts were not significantly larger, suggesting an increase in relative surface area but no increase in holdfast size. Smaller colonies, and consequently, smaller holdfasts, are more susceptible to detachment, and increasingly vulnerable in areas of higher surge such as the north shore (Toledo-Hernández *et al.*, 2009). Another environmental factor explaining variation in the data is likely the vertical wall at 15 m located in one of the north shore locations (The Maze), which would presumably influence the

size and attachment of colonies (Kinzie, 1970). Octocorals have differential growth and settlement depending on their substrate composition and topographic relief (Yoshioka and Yoshioka, 1989).

Preliminary results reported here showcase the under-representation of octocoral habitat in common methods of benthic transects. The translations from benthic transect measurements to surface areas from historic reef data requires more research to conclusively comment. Multiple continuous transects of total benthic percentage, combined with complementary coral colony measurements from each transect, would need to be collected to rigorously test the potential of translating historically collected data.

The specific variables regulating the trade-off between surface areas and holdfasts require further investigation as well. In a shifting ecological environment, it is increasingly important to track these changes in a quantifiable way. It would be valuable and time-efficient if a single measurement could predict the available surface area of sea fans, which are predisposed to devastating diseases such as Aspergillosis, a widespread and concerning disease found in the Caribbean (Smith *et al.*, 1998; Toledo-Hernández *et al.*, 2013).

Environmental and habitat factors such as wave action and species competition were likely influencing holdfast dimensions measured from different reefs (Sánchez *et al.*, 1997; Toledo-Hernández *et al.*, 2009). Octocoral species show variation in tolerance to wave action, which can alter their settlement and growth (Yoshioka and Yoshioka, 1989). The competition for space on reefs is a well-known determinant of benthic communities, which is often variable in different habitats for all reef components (Benayahu and Loya, 1977; Jackson, 1977). In reefs where

competition between other organisms (e.g. macroalgae and sponges) was more complicated, benthic interactions could be limiting holdfast growth. Additionally, this could have influenced the accuracy of measurement by obscuring the complete holdfast dimensions from layers of subsequent overgrowth.

Octocorals add structural complexity to coral reefs and this complexity is an important constituent of the Caribbean coral reefs (Kuffner *et al.*, 2007). Their complex canopies provide a unique home for many fish and invertebrates and are especially important in areas of declining scleractinian coral cover (Kuffner *et al.*, 2007). During surveys, over 25% of the colonies were directly interacting with one or multiple other organisms (Lovenburg, pers. obs.), such as juvenile filefish, brittle stars, and shrimp.

3.5.1 Octocoral size and health

The size of holdfasts and surface areas of colonies were positively correlated with incidence and severity of disease, corroborating a study by Nagelkerken *et al.* (1997) that found larger (i.e. older) sea fans had higher incidence and severity of disease. However, smaller sea fans could be more susceptible to mortality because of disease and thus are no longer present in the community (Toledo-Hernández *et al.*, 2009). The finding that larger sea fans are more prone to disease should be considered if translating previous reef transects to surface areas of sea fans, as reefs hosting predominately larger sea fans could be more susceptible to disease outbreaks.

The results demonstrate that holdfast sizes are positivity correlated with surface areas as hypothesised. The precise translations of observed transect dimensions need further investigation. The health state of the coral colony is likely a parameter that influences the size variability seen in colonies, which could explain the imprecision of the model. Octocorals are indeed underestimated on traditional reef transect methods and it is recommended that better suited reef survey methods are adapted in the Caribbean, where these important habitat-forming octocorals dominate.

4 Chapter Four

A phylogenetic and morphometric study of the Caribbean genus *Eunicea*

4.1 Abstract

The relationships and intra- and inter-species variability within the genus *Eunicea* are tested. A new nuclear marker for Cnidaria (ALG11) is tested for its species delimitation abilities against established mitochondrial markers in the Octocorallia (*mtMutS* and COI). Collectively, all markers were used to test their barcoding efficiency of morphologically defined species. Morphologically continuous characters, reflective of the morphologic plasticity inherent in octocoral species, are analysed, and molecular agreement for species designations is tested.

4.2 Introduction

The genus *Eunicea*, an endemic and prominent Caribbean genus, has long been a source of contention in the taxonomic literature (Bayer, 1961). Relationships of *Eunicea* species are frequently unresolved with variable and inconsistent sister groupings (Wirshing *et al.*, 2005; Grajales *et al.*, 2007; Sánchez, 2009). Evidence of accelerated speciation within *Eunicea* spp. is supported by a relatively narrow geographical range, conserved gene sequences, and high species diversity within the Caribbean (Sánchez *et al.*, 2003a). Ecological separation, such as by depth, is common between reef zones for several species complexes (Prada *et al.*, 2008; Prada and Hellberg, 2013). Allopatric speciation, driven by the closing of the Panama Isthmus, is thought to be a contributor of Caribbean species evolution within the past 3.5 million years (Keigwin, 1978). Rapid speciation is often an indication of hybridisation, which has been demonstrated to occur in octocorals (McFadden and Hutchinson, 2004).

In this analysis, the most comprehensive molecular and morphological character set to date has been compiled in an attempt to resolve relationships between the most species-

rich Caribbean octocoral genus *Eunicea* and test species delimitation within the genus. An integrative approach was applied combining traditional morphological characters (continuous coding) and molecular characters (two mitochondrial genes and one nuclear gene) in order to explore the inter- and intra-specific variation in *Eunicea*. An exploration of morphological characters that contribute to molecular agreement was undertaken. Resolution of genealogies and their combined phylogenies are investigated with respect to morphological separations within *Eunicea*. The genetic and morphologic variations are compared for agreement in species delimitation. Specifically, the resolution of morphologically defined species relationship was tested with the addition of the nuclear gene ALG11. Morphological characters were analysed along with genetic clusters to determine which characters were most likely to predict molecular phylogeny.

4.3 Methods

4.3.1.1 Sample collection

All samples for analyses were collected by SCUBA diving around Útila, Honduras June-August 2013 (see Fig. 1.3). Small samples from branch tips (<5 cm in length) were taken using scissors or gardening shears and stored in Whirl-Pak bags or 5-10 mL sample tubes with ambient seawater until processing. Samples were transported back to a dry laboratory at the Coral View Dive Center (http://www.coralviewutila.com) where samples were preserved in 95% ethanol. Daily ethanol changes were made as the limited supplies permitted, with a minimum of one change within a 24-hour period per sample. A total of 40 samples were used for morphological and molecular analyses in this chapter.

4.3.1.2 DNA extraction, amplification, and sequencing

DNA was extracted from specimens using DNeasy Tissue and Blood Kit (Qiagen) as per

manufacturer's instructions. Tissue was homogenised using smooth forceps before the addition of proteinase K. To increase DNA concentration in the final step, a reduced amount of Buffer AE was added (80 µl instead of 200 µl) and for insurance, a second DNA elution was made (repeating final step) and stored in a separate -20°C freezer until amplification.

The most recent barcoding suite of genes for octocorals used COI+*igr*+*mtMutS*+28S (McFadden et al. 2014). Unfortunately, 28S failed to work reliably for these samples. A new marker gene called ALG11 was trialled. After a bioinformatics search for a superior marker was conducted in the mitochondrial and ribosomal genes of sponges, ALG11 was selected and used in a phylogenetic comparison of COI and 18S (Belinky *et al.* 2012). Belinky *et al.* (2012) suggested this marker could resolve other evolutionary relationships with slow-evolving taxa such as cnidarians. This hypothesis was tested for the species-rich genus of octocorals, *Eunicea*.

The nuclear gene ALG11 and mitochondrial genes COI and *msh1*, (Bilewitch and Degnan (2011) explain the misleading evolutionary implications of using the nomenclature '*msh1*') now called *mtMutS,* were amplified. For *mtMutS* amplification, PCR protocol was based on methods from Taylor and Rogers (2015) with previously published primers for the 5'end of *mtMutS* (ND42599F: 5'-GCCATTATGGTTAACTATTAC-3' (France and Hoover 2002); Mut-3458R: 5'-TSGAGCAAAAGCCACTCC-3' (Sánchez *et al.*, 2003a). For COI+*igr1* the region COII(5')-*igr1*-COI(5') was amplified using primers COII8068F (5'-CCATAACAGGACTAGCAGCATC-3' (McFadden *et al.*, 2004) and COIOCTR (5'-ATCATAGCATAGACCATACC-3' (France and Hoover, 2002). Primers for ALG11 developed by Belinky *et al.* (2012) were selected based on the smallest difference between annealing temperatures to best optimize PCR protocols (ALG11-D1: 5' -

TTYCAYCCNTAYTGYAAYGCNGGNGG-3'; ALG11-R2: 5' - CCRAARTGYTCRTTCCACATNGTRTG-3').

Template DNA for sequencing was obtained and 2 µl was used in PCR reactions containing 20 µl total volume with 10 µl of HotStarTaq *Plus* Master Mix (containing HotStarTaq *Plus* DNA polymerase, QIAGEN PCR Buffer, and dNTPs), 4 µl containing 0.16 µM of each primer, 2 µl of RNA-free water, and 2 µl of CoralLoad (the use of CoralLoad was confirmed to not affect sequencing).

Protocol for *mtMutS* followed a PCR protocol of one initial period of five minutes at 95°C, followed by 35 cycles of denaturing at 94°C for 60 seconds, annealing at 52°C for 60 seconds, and extending at 72°C for two minutes, with a final extension at 60°C for 30 minutes and stored at 4°C until ready for sequencing.

Protocol for amplification of COI used PCR conditions with one initial period of five minutes at 95°C, followed by 35 cycles of denaturing at 94°C for 30 seconds, annealing at 59°C for 60 seconds, and extending at 72°C for 60 seconds, with a final extension at 72°C for 10 minutes and stored at 4°C until ready for sequencing.

Protocol for ALG11 used a touchdown procedure to improve amplification: five minutes of initial conditions at 95°C, followed by 35 cycles of denaturing at 94°C for 30 seconds, annealing at 67°C for 60 seconds, and extending at 72°C for 60 seconds, followed by another 35 cycles of denaturing at 94°C for 30 seconds, annealing at 48°C for 60 seconds, and extending at 72°C for 60 seconds, with a final extension at 72°C for 10 minutes and stored at 4°C until ready for sequencing.

From 642 PCRs, 126 were successfully sequenced to produce a three gene phylogenetic dataset of *mtMutsS*+COI+ALG11. After gene amplification, Lynne Richardson in the

Department of Zoology, University of Oxford, carried out the remaining protocols.

Successfully amplified PCR reactions were cleaned using the following PEG precipitation method: 1) 60 µl addition of 20% PEG*/2.5 m NaCl to each reaction (*PEG mol wt 8000); 2) reseal and mix by vortexing; 3) incubate reactions at room temperature (or overnight at 4 °C) 4) and centrifuge PCR products at 2750 x *g* for one hour at 4°C to form a pellet; 5) discard supernatant by inverting plates onto blue roll; 6) invert plates on Whatman 3 MM paper and centrifuge for one minute at 500 *g* to remove excess supernatant; 7) wash pellet with 150 µl ice cold 70% EtOH and centrifuge at 2750 x *g* for 10 minutes and repeat step five; 8) repeat step seven; 9) re-suspend dried PCR product in 30 µl deionized water; 10) store at -20 °C.

Cleaned PCR products were then sequenced and sequencing reactions cleaned to the following protocol: 1) prepare and map a 96-well Microtitre plate; 2) dilute sequencing primers to a working stock of 10 µM with sterile deionized water; 3) dispense 4 µl dilute primer into each well; 4) make Big Dye master mix: 187.5 µl dH_2O, 187.5 µl 5 x Buffer, and 25 µl Big Dye; 5) dispense 4 µl master mix into each well; 6) add 2 µl of each PCR product to appropriate wells; 7) tap plates to ensure PCR products are in wells; 8) seal Microtitre plates and mix by vortexing, centrifuge at 500 *g* for one minute to ensure reaction mixes are at bottom of each well; 9) place in thermal cycler for the following sequencing programme: 30 cycles of 96°C for 10 seconds, 50°C for five seconds and 60°C for two minutes; 10) remove plate from thermal cycler and place in plate holder before carefully peeling back adhesive cover prior to adding 15 µl of sterile deionized water to each well using a multi-channel automatic pipette; 11) mix 7 ml of absolute EtOH and 280 µl 3M NaOAc pH 5.2; 12) dispense 52 µl of this mixture to each well, reseal and vortex; 13) incubate plate for 45 minutes at room temperature; 14) centrifuge plates for one hour at 2750 x *g* at 4 °C 15) immediately after spin cycle, remove seal and invert

plates onto blue roll and place inverted plate onto Whatman 3MM paper and spin at 500 x *g* for one minute 16) wash with 150 µl 70% EtOH and spin at 2750 x *g* for 10 minutes; 17) immediately after spin cycle remove and repeat plate inversion; 18) store at -20 °C until ready to load onto sequencer. Sequencing was then carried out via the Sanger method on an Applied Biosystems 3730xl DNA Analyzer.

4.3.1.3 Sequence alignment of Eunicea dataset

Forward and reverse chromatograms were aligned using Geneious Pro 8.1.8 (Kearse, 2012) and corrected by eye. Consensus sequences were aligned first together by gene and then to octocoral sequences retrieved from Genbank and cut to fit annotated genes, if available. Invertebrate mitochondrial protein translation was used to check nucleotide validity. COI genes were 708 nucleotide bp long and *mtMutS* genes were 750 nucleotide bp long. The sequences for COI were aligned to *E. tourneforti* (Accession: GQ342445.1 GI: 297593185), the only *Eunicea* COI sequence on Genbank. This study contributes 40 additional *Eunicea* COI sequences to Genbank, as well as one from a specimen of *Plexaurella grisea*.

In the case of ALG11, where no reference sequences were available, an entire block alignment of all chromatograms (84 in total) was checked against nuclear protein translations. ALG11 sequences were partial gene segments of 426 nucleotide bp long.

4.3.1.4 Eunicea flexuosa mtMutS dataset

One-hundred and thirty one *mtMuts* sequences from Prada *et al.* (2008) were downloaded from Genbank of 'shallow type' and 'deep type' *E. flexuosa.* Sequences from

Prada *et al.* (2008) were combined with 13 samples of *E. flexuosa* from Útila in this study in order to assess their placement amongst depth types.

Prada *et al.* (2008) describes 'shallow type' as colonies found at less than 5 m and 'deep type' as colonies typical of that found at greater than 17m. Data collections in this study were restricted to depths of 5 m and 15 m in order to complement concurrent reef surveys of the 'reef crest' and 'reef slope'. Of the twelve samples added to the *E. flexuosa* dataset, eight were collected at 5 m and four were collected at 15 m.

4.3.1.5 Outgroup selection

Outgroup selection can significantly alter model parameters if the species selected is too dissimilar to the ingroup and has been found to promote long-branch attraction (Bergsten, 2005; Mateos *et al.*, 2012). A close sister species is preferable. The species *Plexaurella grisea* was selected as an outgroup to *Eunicea* spp., which is a member of Plexauridae, but has been found to phylogenetically group with Gorgoniidae in a sister clade (Sánchez *et al.*, 2003a). A common octocoral outgroup member *Briareum asbestinum* (Berntson *et al.*, 2001), which is at best 73% genetically similar to *Eunicea* spp. (based on *mtMutS* gene used here), was initially used and found to change the model selection for the analyses in order to incorporate the range of genetic variation across all species in the phylogeny. The *Eunicea* are considered to be under an especially rapid speciation because of their low genetic divergence (often 99% genetically identical) and restrictive phylogeography (Sánchez *et al.*, 2003b). It is recommended that phylogenies with such characteristics are run with an outgroup of higher genetic similarity to avoid long-branch attraction (Mateos *et al.*, 2012). For the *E. flexuosa* dataset, *E. palmeri* was used as an outgroup based on the most differentiated pairwise distance estimates within

the genus.

4.3.1.6 Phylogenetic analyses

All genes and concatenated gene alignments were run under maximum-likelihood (ML) searches and Bayesian Inference (BI) analyses. DNA substitution model testing was determined using JModelTest 2.1.10 (Posada, 2008). A total of 88 substitution models were evaluated under Akaike Information Criterion (AIC), corrected AIC(c), and Bayesian Information Criterion (BIC). Model testing was also performed with PartitionFinder (Lanfear *et al.*, 2012). PartitionFinder, like JModelTest, utilises the program PhyML for estimating best model fits for large data sets; however, PartitionFinder analyses the entire sequence alignment, investigates the option of partitioning by codon, and has faster processing speeds than JModelTest (Lanfear *et al.*, 2012).

Bayesian Information Criterion (BIC), Akaike Information Criterion (AIC), and AICc were run for every gene separately and the resulting partition found in PartitionFinder was applied in analysis. BIC analysis was selected as it typically prefers the least partitioned dataset, while AIC and AICc models prefer more complex partitioning schemes. Bayesian Inference analyses were conducted using MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001). MrBayes was run for two million Monte Carlo Markov Chain (MCMC) generations for *Eunicea* spp. trees and 10 million MCMC generations for *E. flexuosa* trees. Concatenated genes were run together: ALG11+*mtMuts*+COI, as a mitochondrial-only concatenation, *mtMuts*+COI, and as each gene separately, producing two phylogenies and three genealogies. Burn-in was set to 10% of runs. Analyses were all run until split frequencies were below 0.01 and trace files resembled a 'hairy caterpillar' (Hall,

2011) using Tracer 1.6 (Rambaut and Drummond, 2007).

Maximum likelihood analyses were run in IQ-Tree (Berchtold *et al.*, 2000) with 10,000 bootstrap replicates in ultrafast bootstrapping (Minh *et al.*, 2013). Edge-proportional partition is the recommended analysis for multi-gene alignments (Chernomor *et al.*, 2016). Burn-in was manually set to 25% of runs. Pairwise distance values for genes were computed in MEGA 6.06 (Tamura *et al.*, 2013).

All tree files were viewed first in TreeFinder (Drummond, 2007), assigning probability bootstraps to nodes and checking outgroup rooting of tree. Trees were then exported and graphically polished in Adobe Illustrator CS6 (Illustrator 2010).

Species delimitation analyses were run using the web-based Automatic Barcoding Gap Discovery (ABGD) tool (Puillandre *et al.*, 2012). Parameters were set using a Jukes-Cantor (JC69) distance, relative gap width of X=1, distance distribution of Nb bins=20, a Pmin of 0.0001 and a Pmax of 0.1.

4.3.1.7 Morphometric dataset

Character coding for morphological phylogenies has been done in *Eunicea* with discrete coding (Sánchez, 2009) and continuous character coding (Prada *et al.*, 2008; Vargas *et al.*, 2010). Size measurements of sclerites is a key distinction for species-level identification of octocorals (Bayer, 1961). However, these traits are continuous variables and are typically avoided in character matrices (Thiele, 1993). The exclusion of these polymorphic character traits within a species may have significant influence on phylogenetic reconstruction (Wiens, 1999). Transforming qualitative continuous characters into quantitative character coding was preferred in this study to capitalise on these informative

character states (Wiens, 2001). Thiele (1993) originally proposed a model for the application of previously ignored continuous and overlapping character states in cladistics analysis. However, his formula for coding the continuous variables relied upon the *a priori* assumption of taxa and combining species data.[6] Such an assumption undermines this study since it specifically aims to address possible variation within morphologically-defined species delimitations of *Eunicea*.

Character means and standard deviations were measured (n=21) for each of the individuals in the molecular phylogeny (40 individuals of *Eunicea* and one *Plexaurella* as an outgroup) (See Table 4.1 and Fig. 4.2). Prada *et al.* (2008) used 14 continuous morphological traits for discriminate analysis and principal component analysis to differentiate *E. flexuosa* individuals. This study used 13 of the characters established by Prada *et al.* (2008) (referred to as Dataset 13), eliminating surface area as one of the characters which is more difficult to estimate in other *Eunicea* spp. with long calices (and the methods used to obtain this measurement were not detailed). Additionally, colony width was added as a measurement within a larger dataset (referred to as Dataset 19).

[6] This he addressed and acknowledged in a footnote to an anonymous reviewer who had called attention to this issue. He directed readers, intent on working with 'demonstrated monophyly' as opposed to that of 'assumed monophyly', to the works of Vrana and Wheeler (1992), and Nelson (1989)

Table 4.1 Descriptions of morphometric characters and their codes from Dataset 13 used by Prada *et al.* (2008) and Dataset 19 used by this study.

	Character	Units	Code	Dataset	
				13	19
Colony characters	**Colony height**	cm	CH	x	
	Colony width	cm	CW		
	Terminal branch length (average available for measurement)	cm	TBL	x	*
	Terminal branch number	(total no.)	TBN	x	
	Terminal branch length relative to **colony height**	(avg cm/cm)	TBL/CH		x
	Total branch number relative to **colony width**	(no./cm)	TBN/CW		x
Branch characters	**Branch thickness**	(cm)	BT	x	*
	Polyp density	(no./cm^2)	PD	x	
	Polyp density relative to **branch thickness**	PD/cm	PD/BT		x
	Calicular diameter (average of 20 calyces=the protective mounds where polyps retract into)	mm	CD	x	x
	Standard deviation of **calicular diameters**	mm	stCD		x
	Intercalicular distance (average of 20 distances between calyces)	mm	ID	x	x
	Standard deviation of **intercalicular distances**	mm	stID		x
Sclerites characters	**Club length** (surface sclerites, average of 20)	mm	CL	x	x
	Standard deviation of **club lengths**	mm	stCL		x
	Club width (surface sclerites, average of 20)	mm	CW	x	x
	Standard deviation of **club widths**	mm	stCW		x
	Spindle length (mid-layer sclerites, average of 20)	mm	SL	x	x
	Standard deviation of **spindle lengths**	mm	stSL		x
	Spindle width (mid-layer sclerites, average of 20)	mm	SW	x	x
	Standard deviation of **spindle widths**	mm	stSW		x
	Capstan length (axis-layer sclerites, average of 20)	mm	CAL	x	x
	Standard deviation of **capstan lengths**	mm	stCAL		x
	Capstan width (axis-layer sclerites, average of 20)	mm	CAW	x	x
	Standard deviation of **capstan widths**	mm	stCAW		x

* used in agreement with other characters

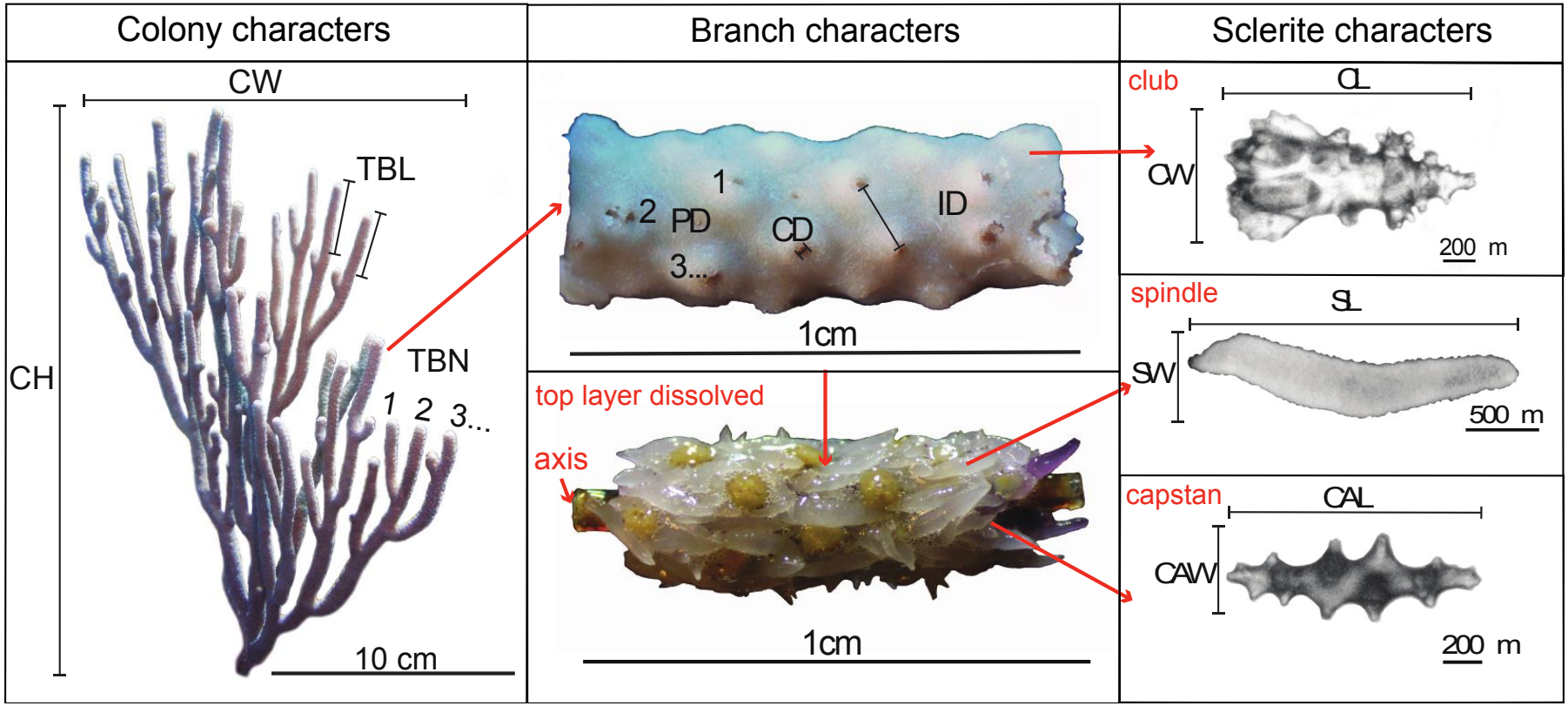


Figure 4.1 Morphometric characters collected from colony, branch and sclerites. CH=colony height, CW= colony width, PD=polyp density, CD=calicular diameter, ID=intercalicular distance, CL=club length, CW=club width, SL=spindle length, SW=spindle width, CAL=capstan length, CAW=capstan width. *Eunicea flexuosa* sample used for example. Photo credits: Lovenburg.

Sclerite characters (refer to Fig. 4.2) were measured from 20 randomly-selected sclerites for each specimen following methods by Prada *et al.* (2008). Colony characters were measured using calibrated images in ImageJ (Schneider *et al.*, 2012) and averages taken where possible (i.e. one colony had only one branch). Branch characters and sclerite characters for large spindles were taken using a Leica stereomicroscope EZ5 and small sclerite characters (e.g. club and capstans) were taken using the Olympus Vanox-T Research Microscope Model AHMT. An Amscope USB camera (5.0 MP) was used to capture all features and measurements were taken within its software (calibration to +/- 10 μm accuracy).

4.3.1.8 Data transformations on morphometrics

Characters were selected based on Prada *et al.*'s (2013) continuous morphometric analyses of *E. flexuosa*. Prada *et al.*'s (2013) dataset looked at temporal changes of depth transplants of hundreds of colonies of *E. flexuosa* to determine to what degree morphological plasticity or speciation was responsible for ecotypes. Because the dataset presented here spanned a wider number of species with fewer replicates per species, the height of the colony was less reflective of a species-specific character and more reflective of the colony's age. Dataset 19, which is better suited to a wider range of species and smaller sample size, encapsulates diagnostic morphological criteria (e.g. shape of colony and relative measurements) while still using continuous variables, employing several ratios of the characters to standardise comparisons between species (e.g. TBL/CH, TBN/CW, and PD/BT). Measurements were used in an attempt to give a morphometric description of the colony's overall form (i.e. quantifying qualitative terms like 'straggling' or 'bushy'). An important character in Bayer's (1961) dichotomous key is if the colony is either a 'candelabrum' or with 'long and straggling branches'. The ratios of the terminal branch length to height as well as the terminal branch

number to width ratio give a good approximation of these characters in continuous space. The variability within the categories of characters is also an important parameter to consider as it may represent a character in itself that mean values do not reflect. Therefore, standard deviations were also added to Dataset 19.

The measurements from Dataset 13, in the analyses by Prada *et al.* (2008), as stand-alone characters, were appropriately informative because of the large sample sizes from one species and the time series element of their study. The characters used in this study and the two datasets analysed are outlined in Table 4.1.

The data were standardised using a log(x+1) transformation for each measurement. Averages and standard deviations were calculated for use in cladistics analyses. This particular transformation was chosen in order to eliminate negative values in the matrix and was employed by Thiele (1993) in a similar way. Analyses conducted on only this transformation resulted in inflated significance of macroscopic characteristics; therefore, data were further standardized to a 0-1 range for all character categories. Prada *et al.* (2008) conducted analyses on raw and standardized (range 0-1) data. Range standardization of raw data vs log(x+1) transformations were negligible and did not change any overall outcomes; log(x+1) offered a slightly higher support for principal component scores for each dataset.

As an exploration exercise to find the best way to analyse continuous character states, Wiens' (2001) 'gap weighting' method (a modification of Thiele, (1993)) was modified to define a taxon as an individual rather than multiple individuals within one taxon. Characters with the replicated measurements (i.e. polyp and sclerite characters) were transformed to fit the model:

$$x_s = \frac{x - min}{max - min}\ x\ 1000$$

4.3.1.9 Statistical analyses

Statistical programmes used for analysing morphologic datasets include JMP (Inc., 2015) and R (R Core Team, 2015).

Cluster analyses (method=Ward) were applied to Dataset 13 used by Prada *et al.* (2008) and to Dataset 19 to visualise how accurately morphological groupings defined species designations supported by phylogenetic analyses.

Principal component analyses (PCA) were applied to both datasets (Dataset 13 and Dataset 19) to visualise trait association and test for any trends resulting from environmental variables. The PCA scores of the first three principal components were used in a two-way ANOVA, using depth and phylogenetic groups as the factors in ANOVA. Methods for morphological trait analyses were replicated for the second set of character traits (19) selected for analyses.

Testing changes in characters resulting from differences in depth within groups of species, however, required an adjustment of the model because residuals for each phylogenetic group are not normally distributed; an assumption which violates the ordinary least squares (OLS) parameters used in ANOVA. Generalised linear modelling (GLM) uses maximum likelihood to estimate population parameters in contrast to OLS for which the assumptions require a balanced linear model (Quinn and Keough, 2002).

There were not enough multiples of each species, so some of the species (e.g. *E. palmeri* and *E. tourneforti*) had to be placed together in 'groups' in order to carry out some statistical analyses. Discriminant function analyses were performed on PCA scores to determine the best morphologic predictors for phylogenetic groups revealed by genetic analyses. This was conducted in an attempt to highlight evolutionarily informative characters within *Eunicea*.

4.4 Results

Using traditional taxonomic characters (Bayer 1961; Sanchez 2009; Bayer 1982) eight morpho-species of *Eunicea* were identified from Útila, Bay Islands, Honduras from the 40 *Eunicea* individuals analysed in this study. Sequences for *mtMutS*+COI+ALG11 were obtained for 41 individuals (inclusive of the outgroup species used, *Plexaurella grisea*). The partitioning models parameterised by PartitionFinder are outlined in Table 4.2. Codon position had no effect on model selection.

Table 4.2 Best-fit partitioning models of molecular evolution for nucleotide alignments selected by PartitionFinder.

Gene(s)	PartitionFinder model selection		
	AIC	AICc	BIC
COI	K81uf	K81uf	HKY
mtMutS	TrN+G	TrN+G	HKY
ALG11	HKY+G	HKY+G	HKY
COI+*mtMutS*	TrN+I	TrN+I	HKY
COI+*mtMutS*+ALG11	HKY+G	HKY+G	HKY+G

Phylogenetic relationships within *Eunicea* are compared to previous intra-generic studies (Sánchez *et al.*, 2003b; Wirshing *et al.*, 2005; Grajales *et al.*, 2007; Prada *et al.*, 2008). These data suggest that *Eunicea flexuosa* is a complex of at least two distinct lineages, corroborating the study by Prada *et al.* (2008). Both mitochondrial (Fig. 4.3) and nuclear phylogenies (Fig. 4.4) supported two lineages of *E. flexuosa*; however, only the nuclear phylogeny with ALG11

(Fig. 4.4) resolved *E. flexuosa* as one monophyletic clade, with *E. fusca* as a sister clade (Fig. 4.5). In the concatenated alignment of *mtMutS*+COI+ALG11 (Fig. 4.5), although not well supported, the sister relationship between *E. clavigera* and *E. tourneforti* is consistent with morphological relationships found by Sanchez *et al.* (2009) and corroborates molecular relationships found by Wirshing *et al.* (2005) that were revealed using mitochondrial genes (Fig. 4.7). A relationship unique to this analysis was the indistinctness amongst *E. mammosa* and *E. succinea* samples (uncorrected *p*-distance within group = 0). This relationship had not been determined until present, as previous analyses did not include both species (Sánchez, 2009).

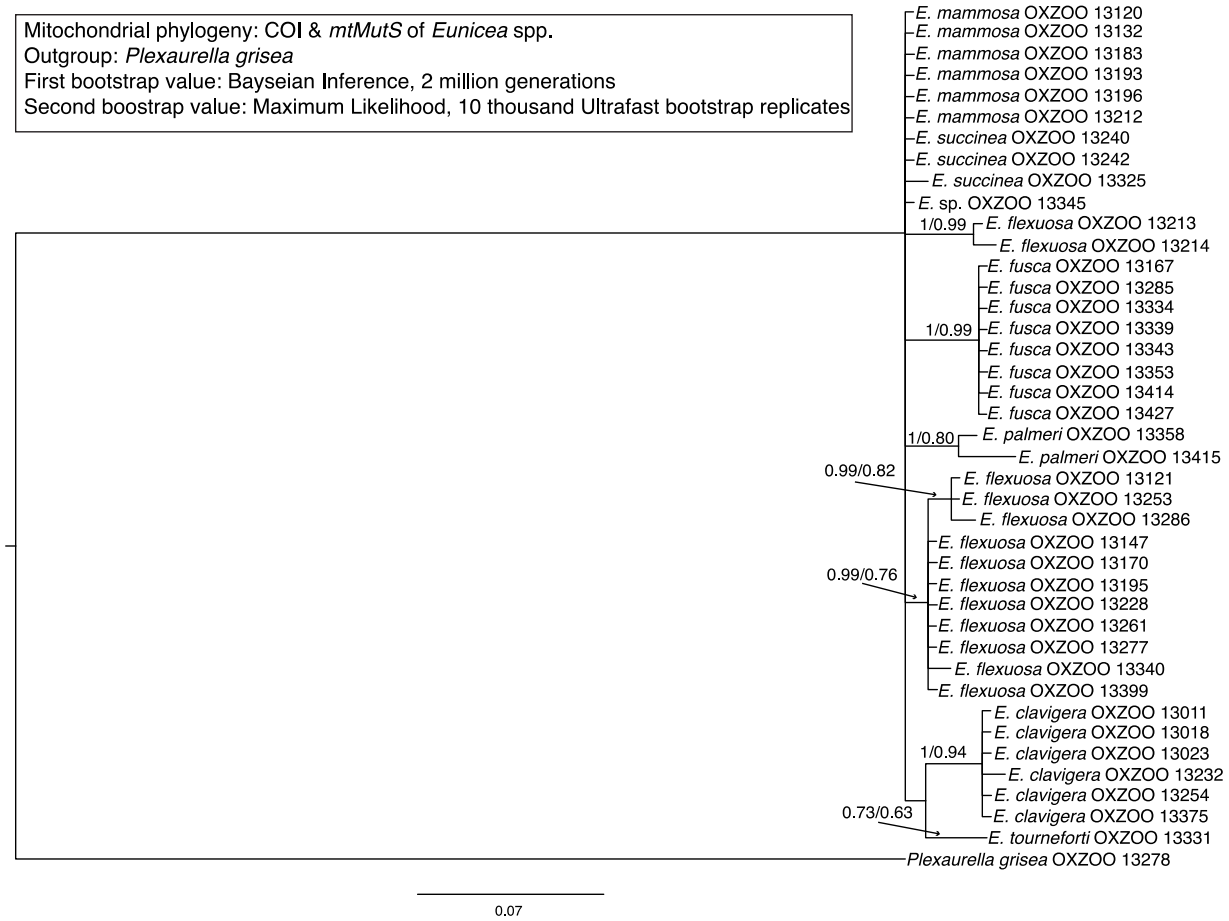


Figure 4.2 Mitochondrial phylogeny of COI+*mtMutS* Bootstrap values Maximum Likelihood and Bayesian Inference shown at nodes. Outgroup *Plexaurella grisea*. Bootstrap values Maximum Likelihood and Bayesian Inference shown at nodes. Bayseian Inference with two million generations. Maximum Likelihood with 10 thousand Ultrafast Boostrap replicates.

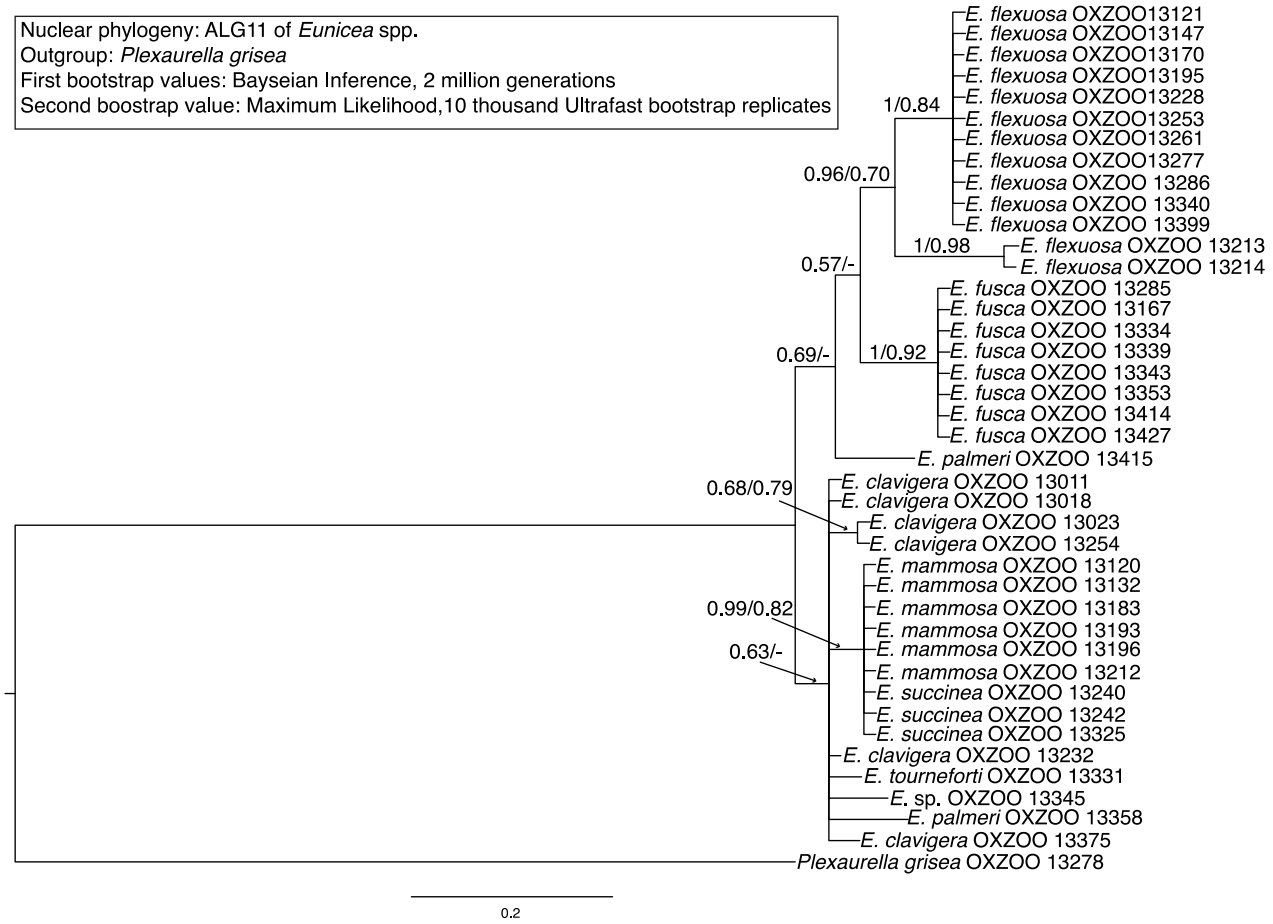


Figure 4.3 Genealogy of nuclear marker ALG11 of *Eunicea*. Bootstrap values Maximum Likelihood and Bayesian Inference shown at nodes. Outgroup *Plexaurella grisea*. Bootstrap values Maximum Likelihood and Bayesian Inference shown at nodes. Bayseian Inference with two million generations. Maximum Likelihood with 10 thousand Ultrafast Boostrap replicates.

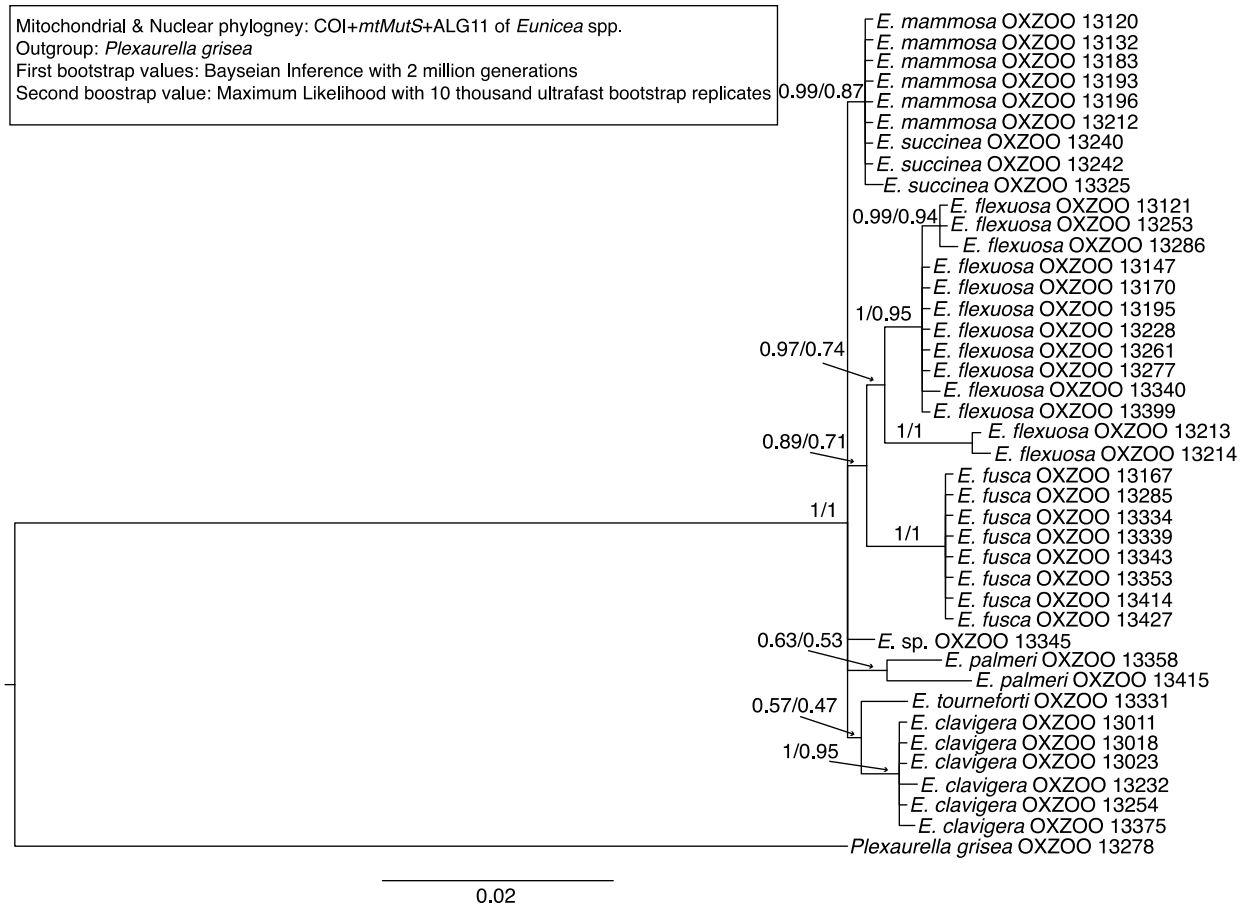


Figure 4.4 Concatenated gene alignment phylogeny of COI+*mtMutS*+ALG11 of *Eunicea* spp. Outgroup *Plexaurella grisea*. Bootstrap values Maximum Likelihood and Bayesian Inference shown at nodes. Bayesian Inference with two million generations. Maximum Likelihood with 10 thousand Ultrafast Boostrap replicates.

Congeneric genetic distances between species pairs ranged from 0-0.565% (mean= 0.323%) for COI, 0%-1.333% (mean=0.607%) for *mtMutS* and 0%-2.37% (mean=1.156%) for ALG11. Mean genetic distance percentages between and within species are compared for each gene separately (Table 4.3).

Table 4.3 Mean uncorrected pairwise *p*-distances for each gene region, within species pairs and between species pairs defined by morphological identification in the genus *Eunicea*.

Genes region	Pairwise mean distances	
	Intraspecific	Interspecific
COI	0.282	0.325
mtMutS	0.467	0.629
ALG11	0.830	1.273

Uncorrected pairwise *p*-distances of concatenated sequences found an overall average of *p-dist* = 0.007 (outgroup *Plexaurella grisea* was included) between individual concatenated sequence alignments. Between and within group (*E. clavigera*, *E. mammosa*, *E. flexuosa*, *E. fusca* and *E.* spp. = *E. tourneforti*, *E. palmeri*, & *E.* sp.) mean distances were calculated and outlined in Table 4.4. ALG11 had an overall average of *p-dist*=0.011, *mtMutS p-dist*= 0.009, and COI *p-dist*=0.003.

Table 4.4 Between and within group mean distance from 1884 nucleotide basepair comparisons over three genes (two mitochondrial: *mtMuts* and COI; one nuclear: ALG11). Numbers equal average basepair change for each nucleotide position: **6 *E. clavigera***; **9 *E. mammosa*** (6 *E. mammosa* and 3 *E. succinea* samples – 2 *E. succinea* forma *plantaginea* and 1 *E. succinea* forma *succinea*)*;* **12 *E. flexuosa***; **8 *E. fusca,*** and **4 *E.* spp.** (includes two *E. palmeri*, 1 *E. tourneforti*, and 1 *E.* sp.).

	E. clavigera	*E. mammosa*	*E. flexuosa*	*E. fusca*	*E.* spp.
E. clavigera	**0**				
E. mammosa	0.003	**0**			
E. flex	0.005	0.003	**0.002**		
E. fusca	0.005	0.004	0.005	**0**	
E. spp.	0.004	0.003	0.005	0.005	**0.004**

Unique sequences were determined in Geneious Pro 8.1.8 for each gene separately to compare the results of molecular barcoding with morphological identifications. There were seven unique sequences for COI, 13 for *mtMutS*, and 11 for ALG11. There are eight morphologically described species, however, *E. flexuosa* is now considered a complex of two species (Prada and Hellberg, 2013).

The species delimitation AGBD found support for eight species groups within *Eunicea*: Group one (n=6) was all *E. clavigera,* group two (n=10) was all *E. mammosa* and *E. succinea,* group three (n=11) was *E. flexuosa* ('shallow type', see next section), group four (n=8) was all *E. fusca,* group five (n=2) was *E. flexuosa* ('deep type', see next section), group six (n=1) was *E. tourneforti*, group seven (n=1) was one *E. palmeri*, and group eight (n=1) was the other sample of *E. palmeri.*

4.4.1.1 *Eunicea flexuosa*

Hierarchical likelihood ratio tests in JModelTest and Partition Finder agreed with Prada *et al.* (2008), preferring HKY substitution models for *mtMutS* partitioning schemes for BIC, AIC, and AICc model selections for alignments combining *mtMutS* sequences from this study and Prada *et al.* (2008). Findings support two distinct haplotype groups within *E. flexuosa* (Fig. 4.6). However, depth was not found to be a factor in the groups. Of the 12 samples collected in this study and combined in analyses with sequences from Prada *et al.* (2008), 10 samples fell out in the 'shallow' clade and two samples fell out in the 'deep' clade. Of the two samples that aligned with the 'deep' clade, one was collected at 5 m and the other was collected at 15 m. Of the 10 samples that aligned with the 'shallow' clade, seven were collected at 5 m and three were collected at 15 m.

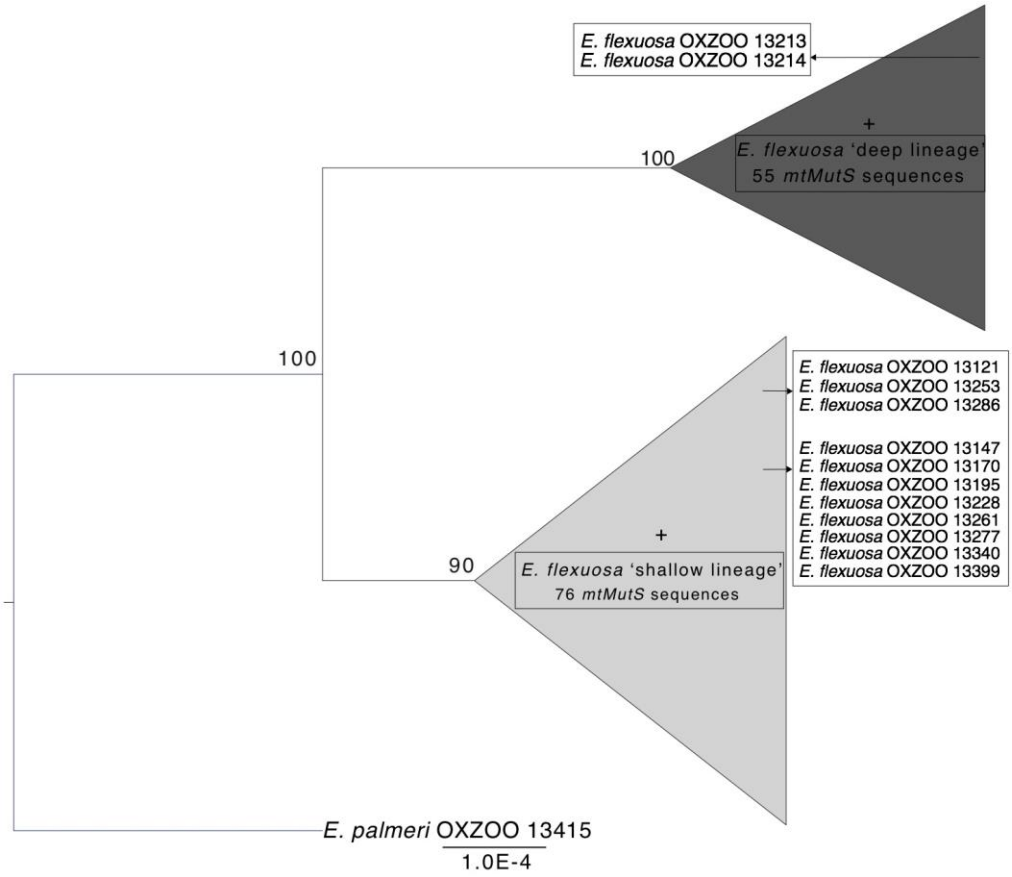


Figure 4.5 Phylogeny of 131 *mtMutS* sequences of *Eunicea flexuosa* from Prada *et al.* (2008) downloaded from Genbank combined with 13 *E. flexuosa* samples from this study. Samples from this study are listed.

4.4.1.2 Morphometrics

Using all characters and hierarchical clustering (method=ward) of four clusters there are three clusters of species *E. flexuosa* & *E. clavigera*, *E. mammosa* & *E. succinea* and another cluster with *E. fusca*, *E. tourneforti*, *E. palmeri*, and *E.* sp (Fig. 4.7). When cluster analysis was run with five potential clustering groups, the cluster of *E. tourneforti*, *E. palmeri*, and *E. fusca*, and *E.* sp. remained intact, however, *E. mammosa* split into groups according to their depths of 5 m and 15 m

(One-way ANOVA of PC1 and PC2 by depth within clusters: PC1 of *E. mammosa*: $F(1,4)=12.99$, $p=0.0227$; PC2 of *E. mammosa* $F(1,4)=20.68$, $p=0.0104$).

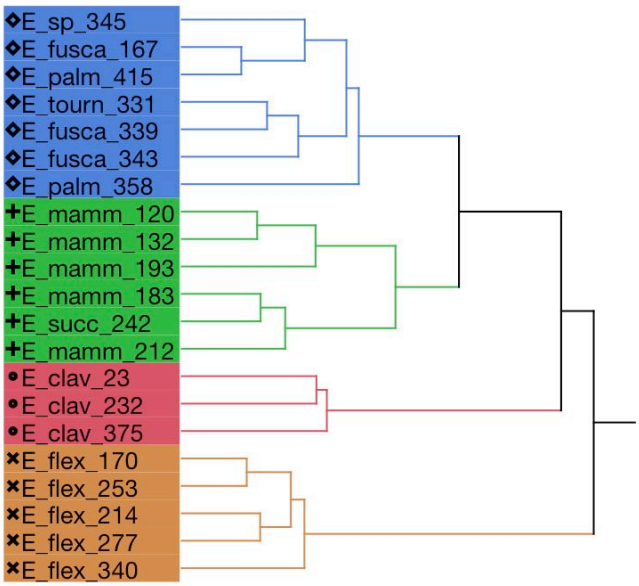


Figure 4.6 Dendrogram of octocoral species within *Eunicea* used in morphometric analysis. Hierarchical clustering (method=Ward) used 19 morphometric characters of colony, branch, and sclerite features. Four clusters are displayed: *E. flexuosa* in orange with '×' icons; *E. clavigera* in red with '°' icons; *E. mammosa* and *E. succinea* in green with '+' icons; and a mixed assemblage of *Eunicea*: *E. fusca*, *E. palmeri*, *E. tourneforti*, and *E.* sp. in blue with '◊' icons.

Height of colony was not correlated to any of the sclerite measurements but was positively correlated with the terminal branch average length [$F(1,19)=7.17$, $p=0.015$], and the terminal branch number [$F(1,19)=25.51$, $p<0.0001$).

A principal component analysis was performed on all morphological characters. The first three scores of the PCA were then used in analysis of variance tests. A two-way ANOVA was used to test full factorial effects on the spread of morphological variation.

Results using Prada *et al.* (2008) characters (Dataset 13) differed slightly from results using Dataset 19 (see Table 4.5). The first principal component (PC1) explained 32% of the variance and was characterised by high positive loadings of intercalicular distances, capstan length, and terminal branch length; and high negative loadings of polyp density and club length. PC2 explained 22% of the variance, characterised by high positive values of spindle width and spindle length and high negative loading of capstan length. PC3 explained 16% of the variance and was characterized by high positive loadings for capstan width, club length, and capstan length and high negative loadings for capstan width, calicular diameter and polyp density.

For Dataset 19, the first principal component (PC1) explained 31% of the variance and was characterized by high positive loadings for spindle width (and its standard deviation), spindle length (and its standard deviation), as well as high negative loadings of capstan length (and its standard deviation), and the standard deviation of club length. PC2 explained 29% of variance and was characterized by high positive loadings of intercalicular distance (and its standard deviation), calicular diameter, and capstan length, as well as high negative loadings of polyp density to branch thickness ratio, total branch number to width ratio, club length (and its standard deviation), and club width. PC3 explained 15% of the variance and was characterised by high positive loadings for club width, club length (and its standard deviation), capstan length (and its standard deviation) and intercalicular distance, and high negative loadings for capstan width (and its standard deviation).

Table 4.5 Explanatory factors from principal component analyses on dataset defined by Prada and Hellberg (2013) and augmented dataset used in further analyses herein. The bold is highlighting agreement between the datasets in the same PC, the italics highlights the agreement for PC1 and PC2.

Character dataset	PC1	PC1 explained by:	PC2	PC2 explained by:	PC3	PC3 explained by:
Dataset 13	32%	*ID*, **CAL**, TBL, *PD* & **CL**	22%	*SW*, *SL*, **CAL**	16%	**CW**, **CL**, CAL, CAW, CD, PD
Dataset 19	31%	*SW*, stSW, *SL*, stSL, **CAL**, stCAL, st**CL**	29%	*ID*, stID, CD, **CAL**, *PD*/BT, TBN/CW, CL, stCL, CW	15%	**CW**, **CL**, stCL, CAL, stCAL, ID, **CAW**, stCAW

Groups that were consistently supported by phylogenetic results were used to test for significance of morphological variations. Phylogenetic groupings (designated as 'phylogroups' from now on) consisted of: 1. *E. flexuosa,* 2. *E. clavigera*, 3. *E. mammosa*/*E. succinea*, 4. *E. fusca*, and 5. *E.* spp (=2 individuals of *E. palmeri*, one individual of *E. tourneforti,* and one individual of *E.* sp.).

Phylogroups were statistically different from one another in morphometric space for all three PC equations (PC1: $F(4,16)=41.5214$, $p<0.0001$; PC2: $F(4,16)=42.3803$, $p<0.0001$; $F(4,16)=12.6848$, $p<0.0001$). Depth alone was not a significant factor in affecting variation ($F(1,20)= 2.84$, $p=0.1083$). However, in a two-way ANOVA on PCA scores, depth was a significant factor affecting variation within phylogroups (Two-way ANOVA, $F(4)=4.0359$, $p=0.0297$). In a discriminant function analysis conducted on all measurements using depth as a predictor, 90.48% were correctly assigned

to depth.

For the standardised dataset of 19 characters, the significance of phylogroups and depth told the same story. Phylogroups were statistically different from one another in morphometric space for all three PC equations (ANOVA, PC1: $F(4,16)=35.1192$, $p<0.0001$; PC2: $F(4,16)=3.4176$, $p=0.0335$; $F(4,16)=3.6741$, $p=0.0263$). Depth was not a significant factor affecting mean groups (ANOVA, $F(1,19)=2.1897$, $p=0.1553$). However, in a two-way ANOVA on PCA scores, depth did significantly affect variation within phylogroups ($F(4)= 8.6687$, $p=0.0021$).

When a discriminant function analysis was conducted on all data, depth correctly predicted 95.24% of the data. There was one misclassification, which incorrectly assigned *E. flexuosa* (sample 170, LB5) to a sample from 15 m instead of 5 m.

For brevity, remaining presented analyses are only the standardised dataset using 19 characters. Within phylogroups, the variance of data was explained by depth for individuals of *E. fusca* (GLM, $\chi=10.3295$, $p=0.0013$) and *E. mammosa* (GLM, $\chi=28.3826$, $p<0.0001$). Variation within group means was not significant with depth for *E. flexuosa* (GLM, $\chi=0.7683$, $p=0.3807$), *E. clavigera* (GLM, $\chi=0.1032$, $p=0.7480$), or *E.* spp. (GLM, $\chi=1.4296$, $p=0.2318$). Reclassifying *E. flexuosa* into corresponding 'shallow' and 'deep' types based on corresponding haplotypes from the phylogenetic tree (Fig. 4.6) did not change the significance of the morphologic distinctions.

In discriminant function analysis, using step-wise variable selection, a perfect classification into designated phylogroups was obtainable using only sclerite features of: spindle width (SW) and club length (CL), and branch features of: intercalicular distance (ID) and ratio of polyp density to branch thickness (PD/BT) (refer to Fig. 4.7 for character reference). Removing PD/BT resulted in

two misclassifications.

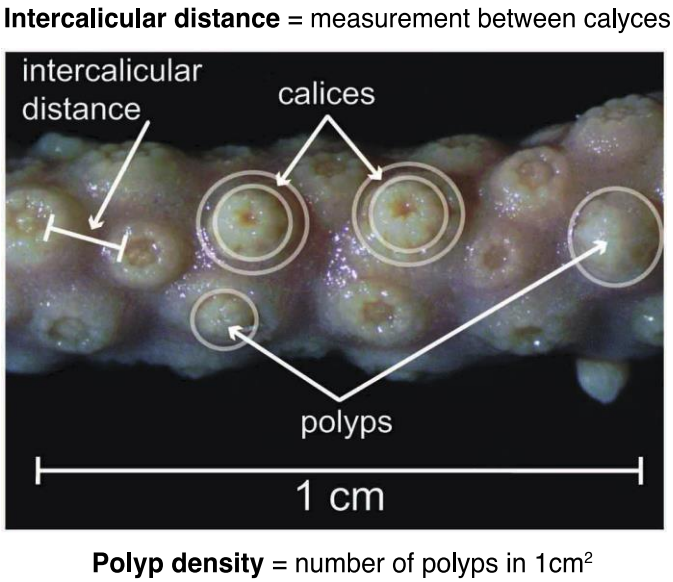


Figure 4.7 Diagrammatic detail of branch features used in discriminant function analysis. Branch features: polyp density to branch thickness ratio (PD/BT); intercalicular distance (ID).

Spindle width and inter-calice distances were statistically different between all phylogroups and were both excellent models for predicting phylogroups. GLMs comparing the two models for effective distinction between group means found spindle width to be the best predictor between groups (AICc=-26.9105, χ=57.1062, p<0.0001). Intercalicular distance (ID) was also a good overall model to predict differences between groups (AICc=-19.0129, χ=46.3568, p<0.0001) (Fig. 4.9). Polyp density to branch ratio (PD/BT) was statistically different in all groups except *E. flexuosa* (Fig. 4.9). With the exception of *E. fusca,* club lengths between groups were all statistically different from one another (Fig. 4.10).

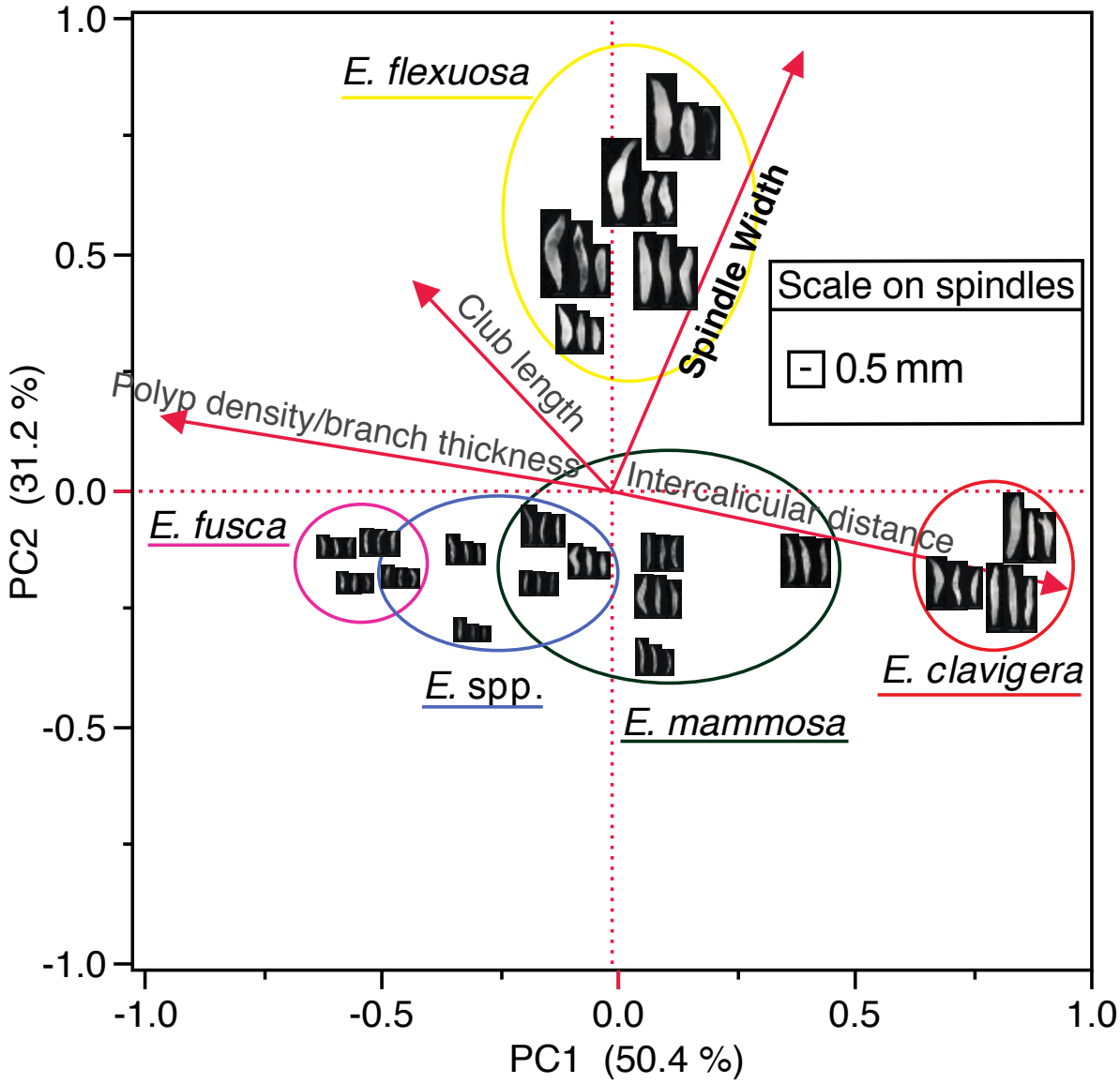


Figure 4.8 Principal component analysis of *Eunicea* using morphological characters that best predicted the five phylogenetic groupings of: *E. mammosa*+*E.succinea*, *E. flexuosa, E. fusca, E. clavigera* and *E.* spp (=*E. tourneforti*, *E. palmeri,* and *E.* sp.). Mid-axial sclerites, spindles are shown in scale to each other to demonstrate range of widths within and between groups.

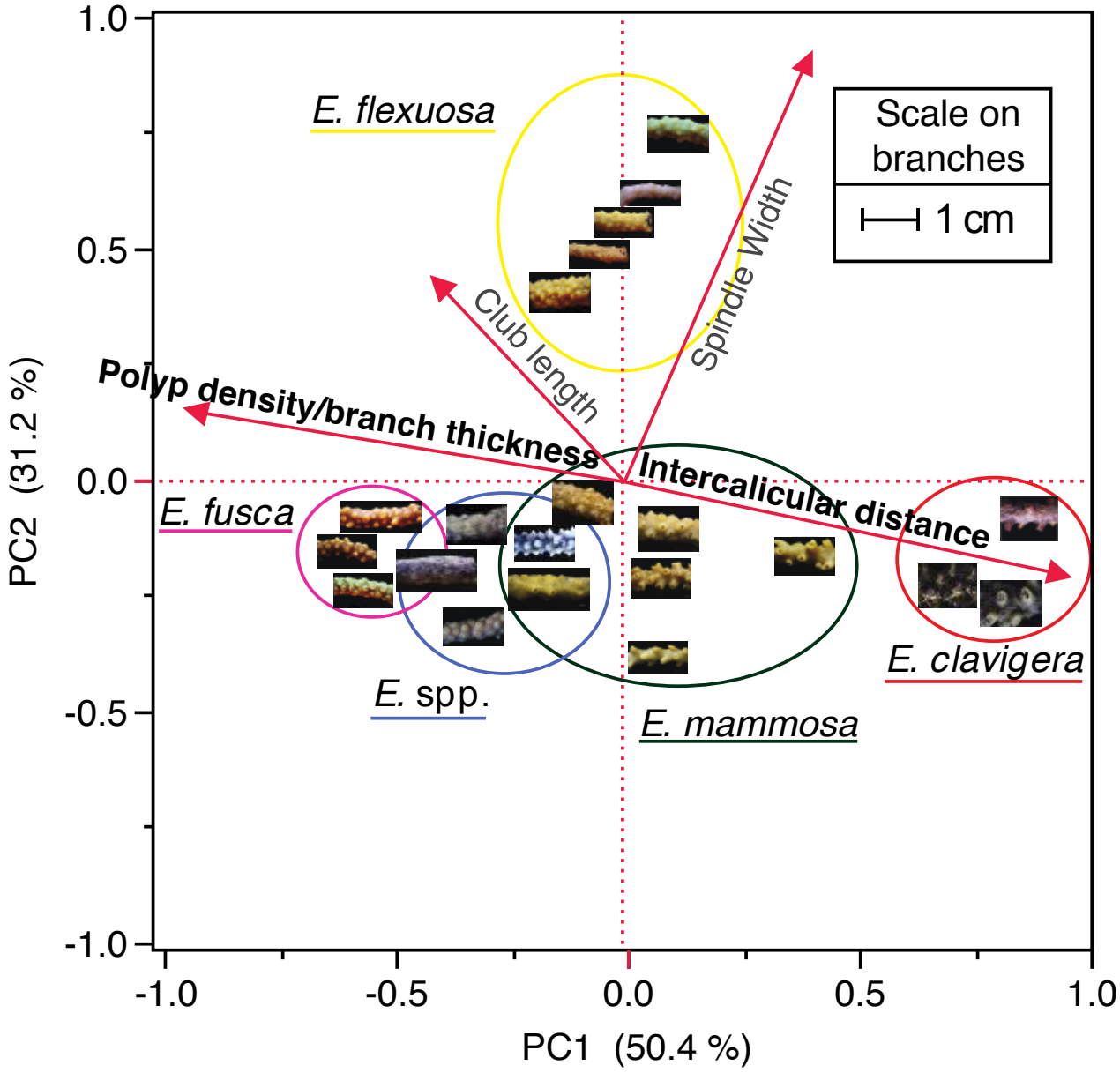


Figure 4.9 Principal component analysis of *Eunicea* using morphologic characters that best predicted the five phylogenetic groupings of: *E. mammosa*+*E.succinea*, *E. flexuosa, E. fusca, E. clavigera* and *E.* spp (=*E. tourneforti*, *E. palmeri,* and *E.* sp.). Branches are shown in scale to each other to demonstrate range of characters within and between groups.

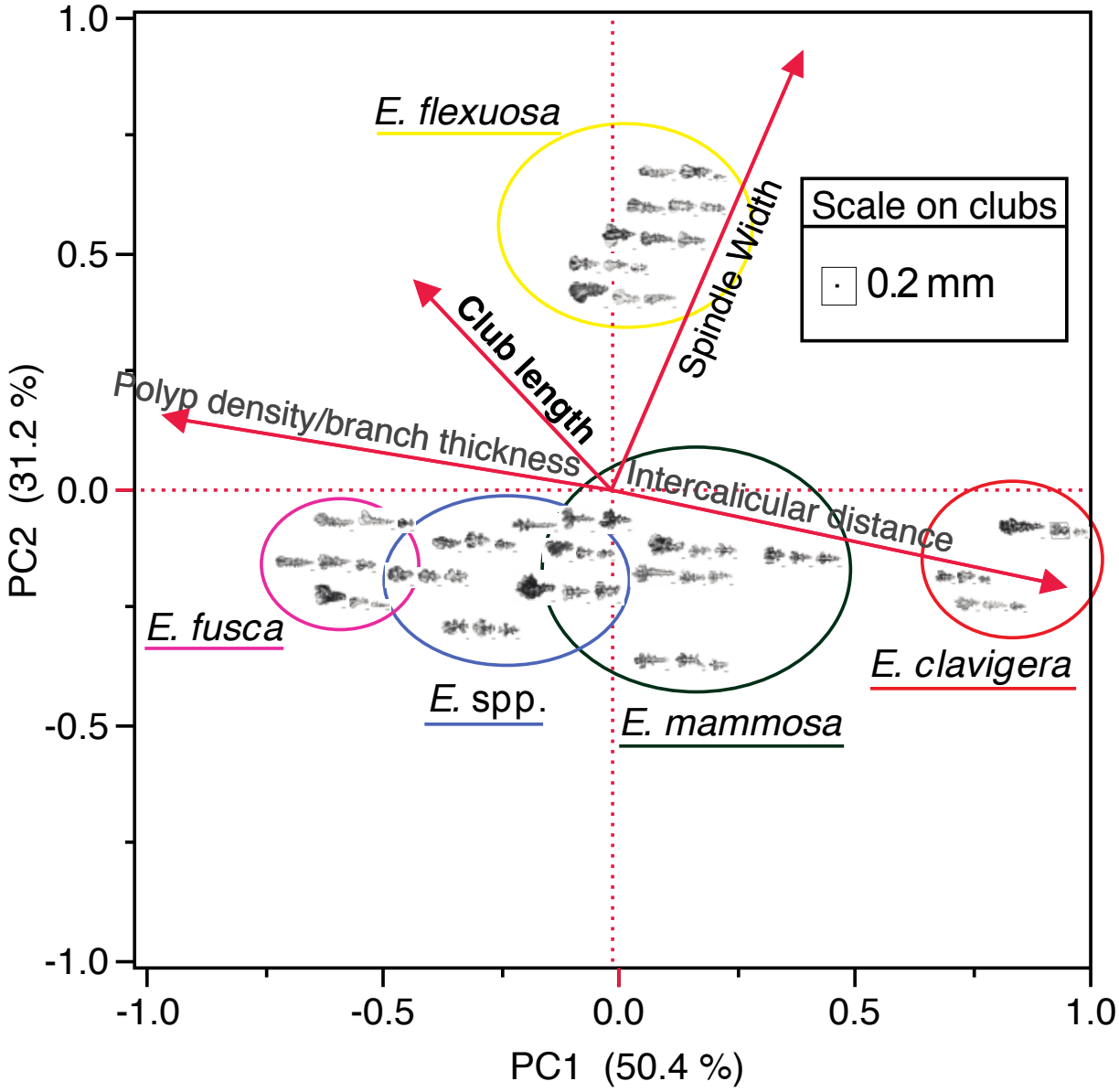


Figure 4.10 Principal component analysis of *Eunicea* using morphologic characters that best predicted the five phylogenetic groupings of: *E. mammosa*+*E.succinea*, *E. flexuosa, E. fusca, E. clavigera* and *E.* spp (=*E. tourneforti*, *E. palmeri,* and *E.* sp.). Surface layer sclerites clubs are shown in scale to each other to demonstrate range of characters within and between groups.

4.4.1.3 *Morphology of E. flexuosa*

Morphology of *E. flexuosa* samples was distinct in comparison to other species, however a morphological distinction between a 'deep' and 'shallow' type was not found in this study as it was for Prada and Hellberg (2013). The 'deep' types were typically found below 17m in the study by Prada and Hellberg (2013), however, the current study was unable to sample this deep. The depths of 5 m and 15 m did not correlate to the respective types.

4.4.1.4 Results summary

ALG11 was tested for the first time in Cnidaria and proved to be a useful nuclear marker with slightly higher variability shown than *mtMutS*. Morphology and genetics data support the union of *E. mammosa* and *E. succinea* (both forms described in Bayer, (1961) into one species. Samples of *E. flexuosa* demonstrated genetic variation similar to the group of multiple species of *Eunicea* (indicating more than one species within *E. flexuosa*), however this study did not find morphological distinctions that corresponded to those variations, nor were they related to depth.

4.5 Discussion

Molecular analyses revealed that ALG11 is a nuclear marker that has superior resolution for separating species compared to mitochondrial genes for morphologically described species of *Eunicea.* This supports the hypothesis suggested by Belinky *et al.* (2012) that ALG11 would provide phylogenetic resolution amongst cnidarians. The combined molecular dataset of ALG11+*mtMutS*+COI was a powerful predictor of morphologically-described species delineations.

Nuclear barcoding has been tested in octocorals for ribosomal genes 28S (McFadden *et al.*, 2001; Aguilar-Hurtado *et al.*, 2012; McFadden and van Ofwegen, 2012; McFadden and van Ofwegen, 2013) and 18S (Berntson *et al.*, 2001; McFadden *et al.*, 2001; Sánchez *et al.*, 2003b; Sánchez and Dorado, 2008); and internal transcribed spacer ITS2 (Aguilar and Sanchez, 2007; Grajales *et al.*, 2007; Wirshing, 2012). A similarly sized nuclear coding gene region has not been sequenced for octocorals before (18S is ~1815bp, 28S is ~765bp, compared to 426bp of ALG11 used in this study) for comparison. A thorough study of intraspecific and interspecific genetic variation for the octocoral genera *Alcyonium* and *Bellonella* was conducted by McFadden and Hutchinson (2004) and 28S had an intraspecific range of genetic variation of 0 to 0.8% and 0 to 6.4% for interspecific variation. Here ALG11 had an intraspecific variation of 0 to 1.9% (however the upper end of this range was between the two *E. flexuosa* types) and an interspecific genetic range of 0.24 and 2.37%.

A unique contribution of this study is the finding that *E. mammosa* and *E. succinea* morphological and genetic differentiation fall within the range exhibited by one species. The morphological distinction separating *E. mammosa* and *E. succinea* currently is the ratio of spindle length to width and calicular idiosyncrasies (e.g. 'mammiform'[7] or 'shelf-like') (Bayer, 1961). Both of these characters are known to fluctuate with environmental conditions (refer to group of *E. mammosa* in Figs. 4.10-4.12 which has samples of *E. succinea* and *E. mammosa* collected at 5 m and 15 m around Útila). Therefore, it is proposed here, that *E. mammosa* is a synonym of E. *succinea.* The ABGD species delimitation analysis supported this unification as well.

[7] Mammiform derives from the Latin word 'mamma' and combined with 'iform', meaning shaped like a breast.

Analyses of morphological characters strongly supported the phylogenetic clades of *E. flexuosa*, *E. mammosa+E. succinea*, and *E. clavigera*, however *E. fusca* overlapped with other *Eunicea* species in cluster analyses and PCA (Fig. 4.9 and 4.10). Notably, the union of samples from *E. fusca* and separately the 'deep' lineage of *E. flexuosa* were consistently supported in all gene trees and concatenated phylogenies. This result highlights an evolutionary element not fully captured by the morphometric data. The highly conserved molecular coding found in *E. fusca* across all genealogies and phylogenies is likely a result of their ability to reproduce asexually by fragmentation, as less genetic mixing would be present (Sánchez, 2009). *Eunicea fusca* and *E. laciniata* are the only other members of the genus known to reproduce asexually (Sánchez, 2009). All other *Eunicea* whose reproduction has been studied (i.e. *E. flexuosa*, *E. clavigera, E. succinea, E. tourneforti,* and *E. tayorna*) are gonochoristic broadcast spawners (Beiring and Lasker, 2000; Sánchez, 2009).

Analyses corroborated two distinct haplotypes within *E. flexuosa* (Prada *et al.*, 2008), however there was no morphological support for that genetic separation within these samples. This study was limited to 15 m, while the 'deep' lineage referred to by Prada *et al.* (2008) was largely composed of samples collected below 17 m, thus deeper samples from Útila would need to be analysed in order to provide suitable comparison and corroboration for the lineage separation based on morphology.

Remarkably, morphological separation was apparent between 5 m and 15 m depths for *E. mammosa+E.succinea* and *E. fusca* but not *E. clavigera* nor *E. flexuosa*, as mentioned. Given the morphological variability common in species of *Eunicea* it is likely that the two depths surveyed in this study did not adequately capture the full morphometric range of *E. clavigera* either.

Given the limitations of defining morphologically plastic species – which have been known to hybridise (McFadden and Hutchinson, 2004) and have multiple reproductive strategies (Lasker, 1984; Sánchez, 2009; Kahng *et al.*, 2011), many of which are not known – this study showed strong molecular support for morphologically defined species. In concatenated alignments of COI+*mtMutS*+ALG11, all morphologically described species grouped together with strong node support (>96% Bayesian probability), with the exception of *E. palmeri* (63% Bayesian probability). The ALG11 genealogy grouped all *E. mammosa* and *E. succinea* with strong node support (98.7% Bayesian probability). ALG11 is a useful, variable nuclear marker to complement *mtMutS* and COI for this group of octocorals. Morphologic clustering broadly agrees with phylogenetic clustering when grouped as: *E. clavigera*, *E. flexuosa*, *E. mammosa*+*E. succinea*, *E. fusca*, and *E.* spp (=*E. tourneforti*, *E. palmeri,* and *E.* sp.). This evidence is used to support the combination of *E. succinea* and *E. mammosa* into one species: *E. mammosa.* Relative to genetic differences of intra and interspecies relationships of *Eunicea,* molecular evidence indicates two species within *E. flexuosa*, however, whether they correspond to morphology or depth is not supported by this evidence. The morphological characters of spindle width, club length, intercalicular distance, and ratio of polyp density to branch thickness are the most useful predictions for phylogenetic taxa clustering.

This study has succeeded in producing a character set where morphological and molecular distinctions largely agree. However, although the gene suite predicted morphologically described species, the *Eunicea* remained as a polytomy with little hierarchical branching. Current tools for assessing species diversity within this group requires a creative action plan (Knowlton, 2000; Oppen

et al., 2001; Vollmer and Palumbi, 2002; Pigliucci, 2003; DeSalle *et al.*, 2005; Edwards, 2009; Norris and Hull, 2011). Most recently, next-generation techniques are proving useful for delimitation of species in octocorals (Pante *et al.*, 2015a; Herrera and Shank, 2016). A more complete understanding of the reproductive and dispersal capabilities of *Eunicea* will still need to be fully explored before species delimitation is completely understood (Knowlton, 2000; Vollmer and Palumbi, 2002; Pante *et al.*, 2015b). Additionally, it is recommended that research using their chemical composition as phylogenetic characters be revived (Gerhart, 1983) (see Concluding Chapter).

5 Chapter Five:

Host preference by the flamingo tongue snail *Cyphoma gibbosum*

5.1 Abstract

The host selection preferences of *Cyphoma gibbosum* were studied for the octocoral communities around reefs of Útila. Selectivity coefficients, which are the proportions of *C. gibbosum* found on octocoral hosts standardised by the available octocoral abundances, for both adult and juvenile *C. gibbosum* were calculated. Adults were found to prefer a more generalised selection of hosts from the Plexauridae family while the juveniles displayed a specialist selection of one species of Gorgoniidae (*Antillogoriga americana*). While adult *C. gibbosum* had a negative selectivity for the sea fans, *Gorgonia ventalina*, the ones that were preferred were diseased. This finding is in support of the hypothesis that members of *C. gibbosum* preferentially feed on hosts' infected with the fungal pathogen Aspergillosis.

5.2 Introduction

There are copious compounding threats to coral reefs, including overfishing and the effects of climate change (Hughes *et al.*, 2003; Hughes *et al.*, 2010; Jackson *et al.*, 2014) (Weil, 2004). Stressful environmental conditions can weaken immune system responses and cause organisms to become more susceptible to disease (Harvell *et al.*, 2002). Disease outbreaks are now a critical factor affecting coral reefs (Goreau *et al.*, 1998; Aronson and Precht, 2006; Bruno *et al.*, 2007). The past few decades have shown increased prevalence of disease in Caribbean reefs and greatly reduced coral populations (Harvell *et al.*, 2007; Lesser *et al.*, 2007; Schärer and Nemeth, 2010; Jackson *et al.*, 2014). This rise in disease outbreaks has been attributed to climate change and introduction of pathogens from the land (Lafferty *et al.*, 2004); however, because these threats are often synergistically manifested, it is difficult to assign blame to any one factor over the other.

The most widespread disease in the Caribbean, Aspergillosis, is likely transported via large Aeolian dust fluxes from Africa, and is the confirmed etiological agent in massive sea fan (*Gorgonia* spp.) die-offs (Smith *et al.*, 1996; Nagelkerken *et al.*, 1997; Geiser *et al.*, 1998; Shinn *et al.*, 2000). *Aspergillus* is a widespread genus of fungus that can be found on healthy as well as diseased sea fans (Toledo-Hernández *et al.*, 2008). *Aspergillus sydowii* specifically is identified as an opportunistic pathogen of octocorals (Barrero-Canosa *et al.*, 2012). However, more recently, experiments of inoculation with strains of *A. sydowii* have failed to infect colonies of *Gorgonia ventalina* (Toledo-Hernández *et al.*, 2013).

The *Aspergillus* fungal infection is hypothesised to be secondarily spread via the generalist octocoral predator, the flamingo tongue snail, *Cyphoma gibbosum* (Rypien and Baker, 2009) (Figure 5.1). *Cyphoma gibbosum* is one of few predators of octocorals, because it has the ability to cope with and utilise the cocktail of secondary metabolites and biochemicals from octocorals that are toxic and unpalatable to most reef predators (Chiappone *et al.*, 2003). The snails' tissues have been found to contain the same prostaglandins and sterols contained within octocoral tissues (Steudler *et al.*, 1977). Since these snails are being singled out as a potential vector of this epidemic, their patterns of host selection could offer clues to models of transmission of this deadly fungus.



Figure 5.1 An adult *Cyphoma gibbosum* snail (approximately 2.5 cm in length) feeding on octocoral colony, Eunicea flexuosa. Photo taken by Lovenburg in Útila, Honduras on Coral View reef at 5 m depth.

Ovulid snails have a purported symbiotic relationship with octocorals in the Caribbean as well as the Indo-Pacific and their concerted phylogenetic relationship has been studied in some depth (Reijnen *et al.*, 2010). The exact nature of this relationship, whether it is obligate or facultative, commensalistic, mutualistic, or parasitic, is not completely understood; however, these gastropods are often called a parasite or a specialist grazer of the octocoral. A phylogenetic study of the Ovulidae and their hosts describes the snails as obligate associates of the octocoral (Reijnen *et al.*, 2010).

Host specificity factors have been investigated several times in the flamingo tongue literature (Harvell and Suchanek, 1987; Lasker *et al.*, 1988; Ruesink and Harvell, 1990; Alstyne and Paul, 1992; Nowlis, 1993; Chiappone *et al.*, 2003; Reijnen *et al.*, 2010). It was found that *C. gibbosum* are capable of following mucous trails of conspecifics, most likely in order to locate mates (Gerhart, 1986). In the field, snails are often found aggregating on a colony, which not only increases the likelihood of finding a mate but is also an evolutionary strategy found in aposematically coloured fauna (Gerhart, 1986; Rosenberg, 1989; Rosenberg, 1991).

This seemingly gregarious behaviour is common amongst toxic individuals (e.g. butterflies) who gather together in order to increase their unpalatable signal (Sillen-Tullberg, 1988). In experimental host selection trials, *C. gibbosum* preferred to reside primarily on *Briareum asbestinum*, but females would move in order to oviposit on *Plexaurella dichotoma*, suggesting life history drivers for host choice (Nowlis, 1993). Another factor influencing host selection is the type and potency of bioactive compounds produced by the octocorals which are subsequently sequestered in the tissues of *C. gibbosum* (Gerhart, 1990). Incidentally, it has been found that *C. gibbosum* feeding activates biochemical production in *Antillogorgia* (=*Pseudopterogorgia*) *elisabethae* (Thornton and Kerr, 2002).

This study looks at the host selection preferences of adult and juvenile *C. gibbosum* in Útila for the first time. Host species data from Útila is compared with two other extensive surveys undertaken in the Caribbean by Chiappone *et al.* (2003) and Reijnen *et al.* (2010). Additionally, the preference for hosts infected with signs and symptoms of Aspergillosis is investigated.

5.3 Materials and Methods

5.3.1 Site description

Five sites around Útila, three on the south shore (Coral View, Little Bight and Stingray Point), two on the north shore (Spotted Bay and The Maze) (see map in Fig. 1.3) at two depth profiles (5 m and 15 m) for which background octocoral diversity and density had been previously assessed (see Chapter Two) were also surveyed for *Cyphoma gibbosum*. Background transects noted any adult *C. gibbosum* found present on colonies, however, juveniles were not noted during these octocoral-centric studies. Surveys searching for colonies only with *C. gibbosum* were completed across 300m^2 area at 5 m and 15 m depths (150m^2 at each depth) at each site using 50m X 1m belt transects. Every octocoral was surveyed within this area for the presence of *C. gibbosum* individuals. Colonies without snails were not recorded in the *Cyphoma* dataset to allow for as many observations of host selection as possible. Host colonies of octocorals were identified in the field or, if field identification was not possible, a small sample (<3 cm^2) was clipped from an outer branch and identified back in the laboratory (Bayer, 1961). Additionally, height of colony was recorded in the field with a metre measuring tape.

Host availability at sites was taken from previous transects (see Chapter Two) conducted for overall octocoral species diversity (richness and abundance) and health, using similar belt transect methods employed above for *C. gibbosum* surveys. Sites were selected to incorporate as wide a range in reef types as possible. Diversity transects also included sightings of adult *C. gibbosum*, however, juveniles were not searched for in the diversity dataset because of time restrictions. Any snail less

than 1 cm in shell length was considered a juvenile, as established in previous methods (Lasker *et al.*, 1988).

5.3.2 Statistical analysis

Probability of host selection was determined from diversity transects using the probability of host availability weighted against host selectivity. These probabilities were used to determine an electivity index based on utilisation of food, or in this case host type (r), in relation to their abundance or availability in the environment (p). If the hosts are selected randomly, the proportion of host selection should equate to the available proportion within the environment (Lechowicz, 1982).

Ivlev's electivity index (Ivlev, 1961) is not ideal for rare 'food' types because of the sensitivity of the equation to sampling error. For example, if there were an availability proportion of 0.3 of a food type, even if the diet selection was 1 for that food type, the index would still only be 0.54, which is smaller than if availability was 0.2 and selection was 0.8 (0.6). The Vanderploeg and Scavia's Relativized Electivity Index (Vanderploeg and Scavia, 1979) (eq. 1), which is essentially Ivlev's electivity index normalised by the availability of the food source, has been verified to be a superior index to use when there is significant variability in food source availability and is used here to look at host selection (Lechowicz, 1982). The values of Ivlev's electivity index scores host choice on a scale from -1 to 1 with a score of -1 being total rejection, 0 being no preference and 1 being preferred selection over other hosts. Prior to pooling the data in the results, depth was confirmed to not have a significant effect on data distribution.

Vanderploeg and Scavia's Relativized Electivity Index (1)

$$E_i^* = \frac{W_i - (1/n)}{W_i + (1/n)}$$

Chesson's alpha (α_i), Vanderploeg and Scavia's selectivity coefficient (W_i)

$$\alpha_i = W_i = \frac{r_i/p_i}{\sum_i r_i/p_i}$$

$$i = food$$
$$r_i = food\ in\ diet$$
$$p_i = food\ in\ environment$$

5.4 Results

The occurrence of *C. gibbosum* was relatively rare around Útila, occurring on 1.2% (19 of 1601 colonies) of the population of octocorals. However, these data were collected as peripheral notes to the dataset of octocoral abundance and diversity so it is likely many snails were overlooked in the survey, especially juveniles. As mentioned above, the *Cyphoma* dataset was not concerned with snail density but rather host selection, therefore this dataset only surveyed host colonies where snails occurred to maximise observations of host selection. Total abundances of counts are outlined in Table 5.1.

Table 5.1 Total raw counts of *Cyphoma gibbosum* snail data on octocoral host species. Data reported separately for adult and juvenile (<1 cm in length) snails.

Octocoral host species	*Cyphoma gibbosum* counts				
	adults		juveniles		all snails
	5m	15m	5m	15m	
Antillogorgia americana	9	11	38	19	77
Antillogorgia elisabethae	1	2	0	0	3
Antillogorgia kallos	0	0	1	0	1
Antillogorgia sp.	0	1	1	1	3
Briarium asbestinium	3	3	0	0	6
Eunicea clavigera	1	1	0	0	2
Eunicea flexuosa	9	3	0	0	12
Eunicea fusca	1	3	0	0	4
Eunicea sp.	0	7	0	0	7
Eunicea succinea forma *plantaginea*	1	0	0	0	1
Eunicea succinea forma *succinea*	2	2	0	0	4
Eunicea tourneforti	1	0	0	0	1
Gorgonia ventalina	19	0	1	0	20
Muricea atlantica	1	0	0	0	1
Muricea sp.	1	0	0	0	1
Muriceopsis flavida	3	0	0	5	8
Muriceopsis sp.	0	0	1	5	6
Plexaurella dichotoma	3	3	0	0	6
Plexaurella grisea	1	0	0	0	1
Pseudoplexaura porosa	4	1	0	0	5
Pseudoplexaura sp.	1	0	0	0	1
Pterogorgia anceps	1	0	0	0	1
TOTAL	62	37	42	30	171

The complete results of host selection preference as it compares to the host availability can be seen in Table 5.2. A total of 19 putative species were found to host adult *C. gibbosum* from 49 potential host species. Only five species were found to host juvenile *C. gibbosum*. Electivity indices and proportion of snails found on host species highlight host selection.

Table 5.2 Summary of octocoral host availability, proportion of adult and juvenile *Cyphoma gibbosum* found on octocoral hosts and host preference illustrated by Vanderploeg and Scavia's selectivity coefficient (E*i), given on a scale from -1 (no preference) to 1 (highest possible preference). Columns are colour-coded (relative to the category) on blue to red scale, with blue being the lowest value and red being the highest value. The bottom row reflects the number of hosts per column and total available.

Octocoral host species	Adult snails		Juvenile snails		Host proportion available
	Proportion occupying	E*I	Proportion occupying	E*I	
Antillogorgia acerosa	0.000	-1.000	0.000	-1.000	0.012
Antillogorgia albatrossae	0.000	-1.000	0.000	-1.000	0.001
Antillogorgia americana	0.202	0.585	0.792	0.951	0.029
Antillogorgia elisabethae	0.030	-0.869	0.000	-1.000	0.239
Antillogorgia hummelincki	0.000	-1.000	0.000	-1.000	0.007
Antillogorgia kallos	0.000	-1.000	0.000	-1.000	0.005
Antillogorgia rigida	0.000	-1.000	0.000	-1.000	0.003
Antillogorgia spp.	0.010	-0.325	0.042	0.696	0.011
Briareum asbestinum	0.061	-0.100	0.000	-1.000	0.041
Erythropodium caribaeorum	0.000	-1.000	0.001	0.189	0.001
Eunicea asperula	0.000	-1.000	0.000	-1.000	0.003
Eunicea calyculata forma *calyculata*	0.000	-1.000	0.000	-1.000	0.002
Eunicea calyculata forma *coronata*	0.000	-1.000	0.000	-1.000	0.007
Eunicea clavigera	0.020	-0.572	0.000	-1.000	0.041
Eunicea flexuosa	0.121	0.341	0.000	-1.000	0.033
Eunicea fusca	0.040	-0.607	0.000	-1.000	0.091
Eunicea knighti	0.000	-1.000	0.000	-1.000	0.001
Eunicea laciniata	0.000	-1.000	0.000	-1.000	0.005
Eunicea laxispica	0.000	-1.000	0.000	-1.000	0.001
Eunicea mammosa	0.000	-1.000	0.000	-1.000	0.032
Eunicea palmeri	0.000	-1.000	0.000	-1.000	0.002
Eunicea spp.	0.071	-0.427	0.000	-1.000	0.097
Eunicea succinea forma *plantaginea*	0.010	-0.517	0.000	-1.000	0.018
Eunicea succinea forma *succinea*	0.040	0.821	0.000	-1.000	0.002
Eunicea tourneforti	0.010	0.585	0.000	-1.000	0.001
Gorgonia mariae	0.000	-1.000	0.000	-1.000	0.066
Gorgonia ventalina	0.192	-0.121	0.014	-0.738	0.135
Iciligorgia schrammi	0.000	-1.000	0.000	-1.000	0.002
Muricea atlantica	0.020	0.672	0.000	-1.000	0.002
Muricea laxa	0.000	-1.000	0.000	-1.000	0.002
Muricea muricata	0.000	-1.000	0.000	-1.000	0.001
Muricea pinnata	0.000	-1.000	0.000	-1.000	0.001
Muricea waltonsmithii	0.000	-1.000	0.000	-1.000	0.001
Muriceopsis flavida	0.030	-0.335	0.069	0.504	0.034
Plexaura homomalla forma *homomalla*	0.000	-1.000	0.000	-1.000	0.012
Plexaura homomalla forma *kukenthali*	0.000	-1.000	0.000	-1.000	0.001
Plexaura spp.	0.000	-1.000	0.000	-1.000	0.005
Plexaurella dichotoma	0.061	0.532	0.000	-1.000	0.010
Plexaurella fusifera	0.000	-1.000	0.000	-1.000	0.001
Plexaurella grisea	0.010	0.312	0.000	-1.000	0.003
Plexaurella nutans	0.000	-1.000	0.000	-1.000	0.007
Plexaurella spp.	0.000	-1.000	0.000	-1.000	0.001
Pseudoplexaura crucis	0.000	-1.000	0.000	-1.000	0.003
Pseudoplexaura flagellosa	0.000	-1.000	0.000	-1.000	0.005
Pseudoplexaura porosa	0.051	0.768	0.000	-1.000	0.004
Pseudoplexaura sp.	0.010	-0.181	0.000	-1.000	0.008
Pseudoplexaura wagenaari	0.000	-1.000	0.000	-1.000	0.002
Pterogorgia anceps	0.010	0.768	0.000	-1.000	0.001
Pterogorgia citrina	0.000	-1.000	0.000	-1.000	0.006
Total species count	**19**		**5**		**49**

Nearly 80% of all juveniles were found on one species of host, the slimy sea plume, *Antillogorgia americana* (See Figure 5.2) with a selectivity index score of 0.951, indicating high preference for this host. Juveniles were only found on five species out of the 49 potential hosts surveyed. Only three of those five species were found to have a positive selectivity index. Other *Antillogorgia* spp. (for which identifications were not confirmed) had the next highest selectivity index (0.696) followed by *Muriceopsis flavida* (0.504).

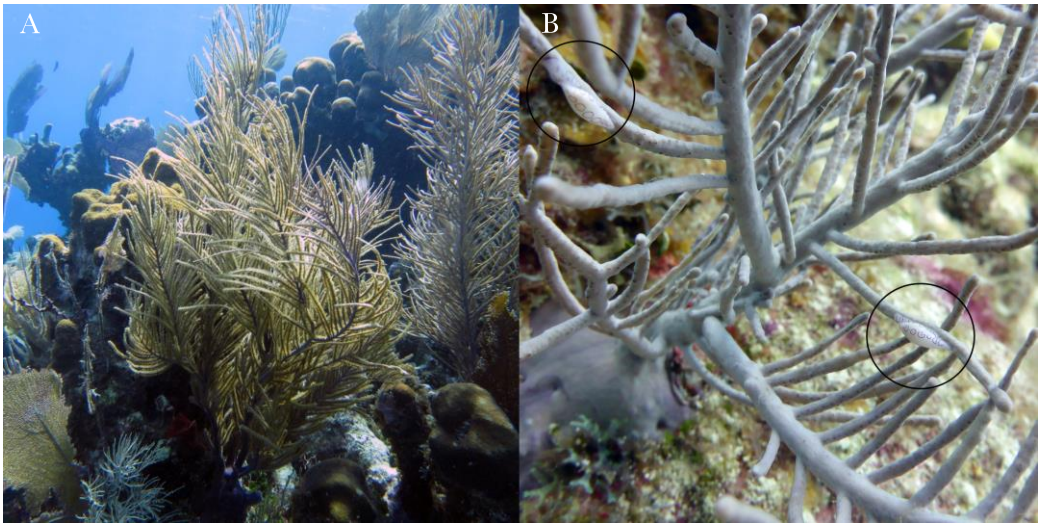


Figure 5.2 A. Octocoral colony *Antillogorgia americana*. B. Close-up of *Cyphoma gibbosum* juveniles on *A. americana* colony. Snails are circled. Photo credit: A: AD Rogers. B: VB Lovenburg.

Using Vanderploeg and Scavia's Relativized Electivity Index (Table 2), apparent host selection was confirmed by factoring the proportion of potential host species available. Adult preferences were

more generalised than juveniles; however, some preferences were evident. The highest index value found for adult *C. gibbosum* was for *Eunicea succinea* forma *succinea* (0.821), followed by *Pseudoplexaura porosa* (0.768), *Pterogorgia anceps* (0.768), *Muricea atlantica* (0.672), *Antillogorgia americana* (0.585), *Eunicea tourneforti* (0.585) and *Plexaurella dichotoma* (0.532). There was also some apparent avoidance of some species such as *Antillogorgia elisabethae* (-0.869), *Eunicea fusca* (-0.607), *Eunicea clavigera* (-0.572), and *E. succinea* forma *plantaginea* (-0.517).

This study, found 144 octocorals, hosting a total of 172 snail occupants (1609 octocorals were surveyed for host availability in a separate dataset, see Chapter Two). These data were compared to two other extensive surveys looking at host occupation patterns. Chiappone *et al.* (2003) found 73 octocorals with snails and 9,911 octocorals were surveyed for host availability. Reijnen *et al.* (2010) found 104 octocorals with snails but did not study the site for host availability. Comparisons of host selection based on these datasets are outlined in Table 5.3.

Table 5.3 Octocoral host occupancy by *Cyphoma gibbosum* from Caribbean sites in this study and studies by Chiappone *et al.* (2003) and Reijnen *et al.* (2010) (in respective order of columns). A '-' signifies the octocoral species was not found in host availability surveys, or in the case of *Erythropodium caribaeorum* from FL keys (Chiappone *et al.*, 2003), was not included in data collection. Note: *Briareum asbestinum* has two growth forms: encrusting and lobed, which could be two species (Brazeau and Harvell, 1994). Chiappone *et al.* (2003) and Reijnen *et al.* (2010) did not make any distinction between growth forms. This study only found *C. gibbosum* on lobed growth forms.

Octocoral family/species	Útila, Bay Islands, Honduras	Florida Keys	Curaçao, Netherlands Antilles
Anthothelidae			
Erythropodium caribaeorum (Duchassaing & Michelotti, 1860)	absent	-	absent
Briareidae			
Briareum asbestinum (Pallas, 1766)	present	present	present
Gorgoniidae			
Gorgonia mariae Bayer, 1961	absent	-	present
Gorgonia ventalina Linnaeus, 1758	present	present	present
Psuedopterogorgia (=*Antillogorgia*) *acerosa* (Pallas, 1766)	absent	present	present
P. americana (Gmelin, 1791)	present	present	present
P. bipinnata	-	absent	present
P. elisabethae Bayer, 1961	present	-	absent
P. kallos (Bielschowsky, 1929)	present	-	absent
P. rigida (Bielschowsky, 1929)	absent	absent	present
Pterogorgia anceps (Pallas, 1766)	present	absent	absent
P. citrina (Esper,, 1792)	absent	absent	present
P. guadalupensis Duchassaing & Michelotti, 1860	-	present	absent
Plexauridae			
Eunicea calyculata (Ellis & Sollander, 1786)	absent	absent	present
Eunicea calyculata coronata Bayer, 1961	absent	absent	absent
E. clavigera Bayer, 1961	present	-	present
E. fusca Duchassaing & Michelotti, 1860	present	-	absent
E. laciniata Duchassaing & Michelotti, 1860	absent	present	absent
E. knighti Bayer, 1961	-	-	present
E. mammosa Lamouroux, 1816	absent	present	absent
E. succinea (Pallas, 1766)	present	absent	present
E. succinea plantaginea Lamarck, 1815)	present	absent	absent
E. tourneforti Milne Edwards & Haime, 1857	present	present	present
Muricea atlantica (Kükenthal, 1919)	present	absent	absent
Muricea muricata (Pallas, 1766)	absent	present	present
M. laxa Verrill, 1864	absent	absent	absent
Muriceopsis flavida Aurivillius, 1931	present	absent	absent
Plexaura (=*Eunicea*) *flexuosa*	present	present	present
P. nina	-	-	absent
P. homomalla (Esper, 1792)	absent	present	present
Plexaurella dichotoma (Esper, 1791)	present	present	present
P. grisea Kunze, 1916	present	absent	present
P. nutans (Duchassaing & Michelotti, 1860)	absent	-	present
Pseudoplexaura flagellosa Houttuyn, 1772	absent	-	absent

Octocoral family/species	Útila, Bay Islands, Honduras	Florida Keys	Curaçao, Netherlands Antilles
P. porosa (Houttuyn, 1772)	present	present	present

The signs and symptoms of the fungal infection, Aspergillosis, are most easily identifiable in the sea fans *Gorgonia* spp., therefore the preference for *C. gibbosum* of healthy or diseased hosts was only tested for sea fans. The null hypothesis that host selection for diseased sea fans was not more than healthy sea fans was tested. A chi-squared test showed significant preference of *C. gibbosum* on diseased versus healthy sea fans (χ^2=5.09, df=1, p=0.02) (Fig. 5.3).

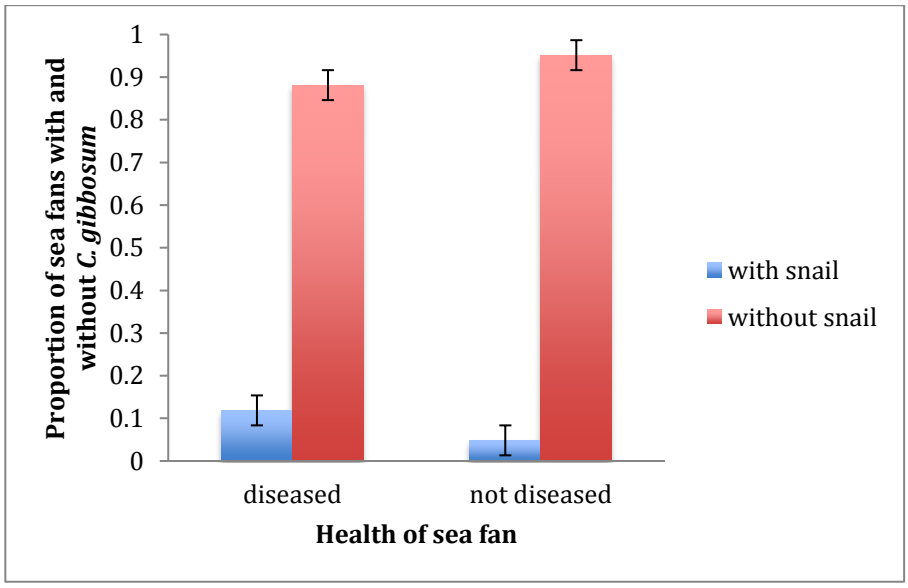


Figure 5.3 Healthy and diseased (displaying signs and symptoms fungal infection Aspergillosis) of sea fans, *Gorgonia* spp. and the proportion found with *Cyphoma gibbosum* snails with standard error bars. The numbers of snails found on diseased sea fans were statistically higher than that expected (χ^2=5.09, df=1, p=0.02).

5.5 Discussion

Overall, adult *C. gibbosum* host occupation patterns fit the traditional canon of a generalist as shown by previous studies' findings of host preference (Harvell and Suchanek, 1987; Chiappone *et al.*, 2003; Reijnen *et al.*, 2010). Despite being a generalist, there are preferred host patterns. Relative to densities in the population, adult *C. gibbosum* showed high preference (above 0.5 selectivity index score) for *Eunicea succinea* forma *plantaginea, Pseudoplexaura porosa, Pterogorgia anceps, Muricea atlantica, Antillogorgia americana, E. tourneforti* and *Plexaurella dichotoma*. Most of these species (except *Muricea atlantica* and *Antillogorgia americana*) are distinctly fleshy in nature, providing more tissue on which to graze. However, the genus *Muricea* is known for the opposite, having a high sclerite to tissue ratio (Harvell and Suchanek, 1987). Host preference for *E. flexuosa* has been found previously (Lasker *et al.*, 1988), which also contains a high sclerite to tissue ratio, suggesting that sclerite and tissue content is not the primary driver for host selection in these cases.

The typical growth form of *E. succinea, E. succinea* forma *succinea* (which is genetically identical to *E. succinea* forma *plantaginea*, refer to Chapter Four) was preferentially avoided (-0.517). The typical growth form of *E. succinea* has stout branches compared to the relatively thin branches of *E. succinea* forma *plantaginea* colonies (7mm and 3-5 mm, respectively) and has larger and denser polyps. The flamingo tongue snails are known to partially graze away polyps on species with larger skeletal armature like *Eunicea* spp. (Harvell and Suchanek, 1987). The preference for the fleshier *E. succinea* growth form is likely a selection for the denser polyps and generally higher biomass available for grazing. Notably, *E. succinea* has been found to harbor chemicals with novel anti-

cancer fighting properties (Iwamaru *et al.*, 2007), however the study did not clarify if a particular growth type was tested.

Other noteworthy absences of host occupation were on *Muriceopsis flavida* in all but Útila, where it was the second choice of host for juveniles (selectivity index of 0.504); however, it had a negative selectivity index for adults (-0.335). *Plexaura homomalla* has been found to be a preferred host species (Lasker *et al.*, 1988); however, *C. gibbosum* was not found associated with it in Útila. This finding was most likely because *P. homomalla* was not abundant around these reefs (1.2% constituent of communities).

The Florida Keys were surveyed from June through September in 2001. Curaçao was surveyed April through June 2005. This study in Útila surveyed for snails June through August 2014. It is possible the time of the year influences host preference in addition to geographic location. Predation on octocorals by the butterflyfish, *Chaetodon capistratus*, was found to correlate closely with egg production in colonies, which could also be a factor influencing grazing of *C. gibbosum*. Other factors, such as the egg laying and mating of the snails, have been suggested as influencing host selection (Nowlis, 1993).

Adult *C. gibbosum* have been noted previously to lay eggs specifically on *Plexaurella dichotma,* which likely explains the preference seen in this study (Nowlis, 1993). Snails were anecdotally observed mating on *G. ventalina* and *Eunicea* spp. most frequently and ovipositing on *Plexaurella* spp. and *Eunicea* spp. (pers. obs. Lovenburg).

The most compelling element of this study is the preference seen for *Antillogorgia americana* and the avoidance of *A. elisabethae*. The preference for *Antillogorgia americana* is unlikely because of its available tissue content, considering the similar morphologies of *A. elisabethae* (Bayer, 1961) which was actively avoided for both adults and juveniles, (-0.869 and -1.00, respectively). There were no associations documented of *C. gibbosum* with *A. elisabethae* for surveys in the Florida Keys (Chiappone *et al.*, 2003) or Curaçao (Reijnen *et al.*, 2010); and a study in Panama (Lasker and Coffroth, 1988) grouped species of *Antillogorgia* (=*Pseudopterogorgia*) together, but did remark of its preference for *A. americana* particularly.

Potentially the selection for *A. americana* over the more abundant conspecific *A. elisabethae* is owing to the octocoral's particularly potent secondary metabolites, for which composition has been shown to be highly variable between species (Gerhart, 1983; Pawlik *et al.*, 1987; Rodríguez, 1995). Secondary metabolites, bountiful in marine and terrestrial fauna, function primarily as predator deterrents (Pawlik *et al.*, 1987; Nishida, 2002; O'Neal and Pawlik, 2002). However, *C. gibbosum* is actually able to sequester and biotransform these noxious chemicals, incorporating them into their own defence systems (Cronin *et al.*, 1995; Whalen *et al.*, 2010).

Juvenile host preference was most intriguing, with 80% of all snails - and selectivity value of 0.951 - found on *A. americana*, the slimy sea plume. This could suggest a high preference of conspecifics, however adults, which have a much broader selectivity of hosts, were found aggregating just as often as juveniles (62 adult snails found on 51 colonies vs. 42 juvenile snails found on 33 colonies). Species of *Antillogorgia* belong to the family Gorgoniidae, which possess smaller skeletal armature (=sclerites) compared to Plexauridae genera (e.g. *Eunicea, Pseudoplexaura* and *Plexaurella*) that

would presumably be more manageable for a juvenile grazer (Alstyne *et al.*, 1992). However, this does not explain the particular selection of this species since *A. elisbethae* has similarly sized sclerites and was a more abundant host.

Antillogorgia americana could be a host selection based on camouflage of juvenile *C. gibbosum* (Faulkner and Ghiselin, 1983; Reijnen *et al.*, 2010; Sanchez *et al.*, 2016) (Fig. 5.4). The spot patterns on the mantle tissue resemble the extended polyps of *A. americana,* a species particularly noted for rarely retracting its polyps because of excessive mucous production (Opresko, 1973). As the young *C. gibbosum* feeds on its hosts' tissues, which contain both yellow and purple pigments (carotenoids) (Leverette *et al.*, 2008), it develops into the more flamboyant adult and is able to move to other hosts.



Figure 5.4 Juvenile *Cyphoma gibbosum* on *Antillogorgia americana.* Photo credit: VB Lovenburg.

These extended polyps, for which *A. americana* are known, could be an influencing factor, as prey are known to prefer polyps of octocorals specifically (Lasker *et al.*, 1988). Furthermore, the polyps in species of *Antillogorigia* were found to contain higher chemical content than the surrounding tissue (Harvell and Fenical, 1989). This suggests specific dietary and habitat requirements for early life stages of *C. gibbosum.* The preference of *C. gibbosum* juveniles for *A. americana* is likely linked to the octocoral's particular chemical cocktail.

As previously mentioned, the biotransformation of toxic secondary metabolites through metabolic reductive processes of certain enzymes (e.g. cytochrome P450) has been documented in the guts of *C. gibbosum* (Vrolijk and Targett, 1992; Whalen *et al.*, 2010). Direct and indirect measurements of enzyme activity (e.g. P450 and its reductase NCR) were measured in the guts of *C. gibbosum* collected from different hosts (Vrolijk and Targett, 1992). A positive correlation was found between large digestive glands and high activity levels of enzyme detoxification, suggested the size of the snail, and consequently its age, were a factor in host selection (Vrolijk and Targett, 1992). *Plexaura homomalla,* a commonly cited host preferred by *C. gibbosum*, consistently indicated high levels of metabolic processes relating to detoxification, such as large digestive glands (Vrolijk and Targett, 1992) and high expression of P450 transcripts (Whalen *et al.*, 2010). In comparison, *A. americana* diets were associated with smaller digestive glands and lower metabolic demands for detoxification (Vrolijk and Targett, 1992; Whalen *et al.*, 2010). This evidence supports the idea that younger individuals of *C. gibbosum* would preferentially feed on *A. americana.* Also, because the larvae of these snails are pelagic, and pelagic larvae commonly use chemical signals as cues for settlement (Hadfield and Paul, 2001), the chemical composition is almost certainly a factor in host selection.

The degree to which environmental factors influence this octocoral-snail relationship remain largely uncharacterised. Outbreaks of *C. gibbosum* can be incredibly damaging to their hosts (Schärer and Nemeth, 2010) and the drivers of these outbreaks is uncertain, but could be linked to predator abundances controlling *C. gibbosum* populations (Burkepile and Hay, 2007). During the time of study, Útila was not undergoing an outbreak in *C. gibbosum* populations, nor was there a high level of Aspergillosis infections. Predator abundance of *C. gibbosum,* such as the hogfish*, Lachnolaimus maximus*, pufferfishes (Tetraodontidae) and Caribbean spiny lobsters *Panuliris argus,* were not quantified in this study; however their abundances have been shown to alter populations of *C. gibbosum*, potentially altering host selection strategies and controlling disease (Burkepile and Hay, 2007). Further studies should be taken to test these links.

In the Útila ecosystem, there is a marginal avoidance of *Gorgonia* hosts (electivity index value of -0.121); however, of these cases, there was a significant preference for diseased sea fans (Fig 5.3). It has been suggested that Aspergillosis decreases anti-predator chemical defenses in octocorals, making them more attractive to predators, which consequently could amplify infection potential and disease spread (Slattery, 1999). The research of host selection reported here would be a useful tool for epidemiological models of Aspergillosis in the marine environment.

6 Concluding Chapter

6.1 Results, revelations and remarks

The last time octocorals' species richness and abundance was studied in the Mesoamerican Barrier Reef System was over 33 years ago.[8] This was also the first time species abundance and diversity were quantified for the Bay Islands of Honduras and it is the first account of species-level richness and abundance for octocorals around Útila. This body of work aimed to describe and characterise important aspects of the octocoral communities and their ecology around the island of Útila for the first time at a species-level. Set in an understudied area of the largest barrier reef system of the northern hemisphere, plagued by copious conservation challenges and with a focus on a taxonomically perplexing fauna, this was a worthwhile, albeit, formidable endeavour.

The species richness of octocoral communities in Útila is rivalled by few locations in the Caribbean (Kinzie, 1970; Muzik, 1982; Dahlgren, 1989; Keith, 1992; Sánchez *et al.*, 1997; Sánchez and Wirshing, 2005). This is particularly true considering that transects were in maximum depths of 15 m (with the exception of one collection dive to 30 m that added four species to the confirmed species list). Moreover, this observed richness is estimated to be higher still. With caveats, it is the highest recorded shallow-water octocoral species richness reported in the MBRS and from any Caribbean island to date (see section on Limitations).

The octocorals (and sponges) are considered a dominant benthic component on many Caribbean reefs (Opresko, 1973; Lasker and Coffroth, 1983; Sánchez *et al.*, 1998; Espinosa *et al.*, 2010; Lenz *et al.*, 2015; Velásquez and Sánchez, 2015); however, most surveys still fail to incorporate methods

[8] The study by Keith (1992) of octocorals from Roatán, Bay Islands of Honduras, was based on museum samples collected in 1979 and abundance was not quantified.

for accurately reflecting these abundant taxa because of a scleractinian-focused reef assessment strategy. The quantification of these reef habitat providers was here explored methodologically, revealing the degree to which under-representation can be measured and methods for translating these data to more accurately reflect their benthic dominance and substrate provisioning. This research once again highlights the need for a strategic change in monitoring projects in the Caribbean if accurate reef assessments are to be made. Incorporating a height dimension (or width if this is the largest dimension) for octocorals and sponges found in transects is recommended for accurate habitat capture in descriptive analyses of reefs.

A better understanding of reef resilience these emergent communities contribute, coupled with the challenge of characterising this biodiversity, prompted current research to focus on relationships of the species-rich genus *Eunicea.* Species relations within *Eunicea* are largely unresolved and molecular phylogenetic studies of octocorals have yet to find a suitable molecular barcode that distinguishes morphospecies (Sánchez *et al.*, 2003a; Wirshing *et al.*, 2005; McFadden *et al.*, 2014). The application of a multilocus barcode (of protein-coding regions) from established mitochondrial genes (COI and *mtMutS*) with the addition of a new nuclear marker (ALG11) was phylogenetically tested and analysed alongside continuous character coding of overlapping morphological features (Prada *et al.*, 2010; Vargas *et al.*, 2010). This work is the first cnidarian example that supports the nuclear gene ALG11 as a barcoding candidate as predicted by Belinky *et al.* (2012).

This was also the first time multiple representatives from species of the *Eunicea* were analysed together in a broader phylogeny. These results suggested that *E. mammosa, E. succinea* forma *plantaginea* and *E. succinea* forma *succinea* constitute one species, supported by all morphological

and genetic characters analysed. This is certainly interesting, considering their long-standing acceptance as distinct morphotypes (Bayer, 1961).

This is of particular interest when the results of host selectivity in the flamingo tongue snail, *Cyphoma gibbosum* are compared. Negative selectivity indices were found for associations with *E. mammosa* and *E. succinea* forma *succinea*, however, a high selectivity was found for *E. succinea* forma *plantaginea*. The reasons for this preference can only be hypothesised, however, if tested they could offer valuable clues to evolutionary drivers for the continued selection of this close association. The interspecific relationships between hosts and their closely associated fauna are known forces driving natural selection under evolutionary stable strategies (Dawkins, 2006).

Within the stable populations of *C. gibbosum* measured in Útila (i.e. there were no outbreaks), there was a negative selectivity for sea fans (*Gorgonia* spp.) over other host species. However, of the associations with this genus, *C. gibbosum* did prefer sea fans that displayed symptoms of disease. Sea fans are the most affected by the fungal pathogen Aspergillosis (Schärer and Nemeth, 2010), for which *C. gibbosum* is considered a potential secondary vector (Rypien, 2008; Rypien and Baker, 2009) and other studies have found selectivity for diseased hosts (Toledo-Hernández *et al.*, 2013). The indications of these findings suggest the fungus is somehow responsible for altering host selection preference in *C. gibbosum*, and thus increasing its own abundance in the population through this positive feedback loop. The symbiotic propagation of fungus by animals (e.g. fungus-growing ants) is a well-studied system (Chapela *et al.*, 1994). The species of *Aspergillus* fungus responsible for the disease has not been conclusive (Nagelkerken *et al.*, 1997; Slattery, 1999; Rypien, 2008; Toledo-Hernández *et al.*, 2008; Toledo-Hernández *et al.*, 2013). Co-evolutionary

patterns between the fungus and their octocoral hosts have failed to show any significance (Rypien, 2008). Additionally, co-evolutionary patterns exhibited by these ovulidae snails are not found to link with octocoral evolution either (Reijnen *et al.*, 2010). Perhaps, the co-evolutionary link missing is the comparison of evolutionary patterns of this fungus and the ovulidae. The stable systems present in Útila with clear host preferences found in juveniles could be an insight into the spread of this disease.

6.2 Conclusions

This thesis spanned a wide-ranging variety of ecologically and genetically probing investigations surrounding the octocorals of Útila, hence its primary title of 'Omnifarious Octocoral Observations'. The octocorals around Útila represent the highest estimates of species richness for a Caribbean island. The communities, although hosting a rich abundance of species, still displayed signatures associated with impacted assemblages and authorities are encouraged to move forward with hitherto delayed plans of marine protection. These characteristic assemblages provide a framework to explore the mechanisms of resilience within these emergent habitats.

The results from Chapter Three confirmed the correlation of surface areas (i.e. available habitat) and holdfast dimensions, providing a framework for the potential translation of historic reef transects in a time-series analyses of octocoral abundance around the Caribbean. The abundant octocoral habitat compared to scleractinian provided habitat on Caribbean reefs was further highlighted. This work emphasised the importance of adding a height dimension to reef surveys in the Caribbean to more accurately capture this three-dimensional habitat above the benthos. The

molecular work successfully sequenced a nuclear marker novel to cnidarian phylogenies. This work added significantly to the genetic diversity available for testing relationships within *Eunicea* and opened new avenues for species delimitation for octocorals.

Finally, this was also the first study of host preference of *C. gibbosum* conducted in the Mesoamerican Barrier Reef System. This filled in a large geographic gap to the host selection puzzle, which could be valuable in characterising potential transmission pathways of the deadly fungus *Aspergillus*. Additionally, octocoral host preference had not been previously studied for juvenile *C. gibbosum,* and contrary to the generalist strategy found in adults, juvenile *C. gibbosum* are found to be highly host-specific (i.e. *Antillogorgia americana*). Implications of which are potentially significantly valuable in describing the etiology of Aspergillosis.

6.3 Limitations

There were several issues implicit in the nature of this thesis, which sought to describe the diversity of a phylogenetically challenging group and its ecology. Although there was little comparison to type specimens or input from established taxonomic experts in this group, rigorous and repeated identifications with molecular confirmation satisfied the designation of a species or morphospecies to the best of any expert's ability in this field. This is particularly true, as type specimens of *Eunicea* are largely collected from the northern Caribbean (e.g. Bahamas, Bermuda, and Florida) and geographical inconsistencies are commonplace within octocorals (Bayer, 1961). Ideally, a complete molecular dataset would complement all morphological identifications; however, funding and time restrictions did not allow for such luxuries.

Repeated surveys at depths greater than 18m were not possible for reasons related to health insurance, which limited some of the genetic studies in this thesis that would have benefitted from a greater depth margin, e.g. *Eunicea flexuosa* 'deep' lineages were typically collected below 17m in Prada *et al.* (2008).

Signs and symptoms of the disease Aspergillosis were not confirmed histologically. Samples were taken for the purposes of this investigation; however, funding and time did not permit the confirmation of fungal spores in the population. The disease is characterised by distinctive tumours and purpling, however, it would have been preferable as a complete study of the disease (conforming to Koch's postulates) to confirm this infection (Harvell *et al.*, 2007).

6.4 Future research suggestions

First and foremost, any and all gaps in the knowledge of biodiversity is the paramount suggestion for future research. The complete species richness of octocorals has yet to be fully uncovered for Útila's reefs. There are many deep-sea and mesophotic species of octocorals in the Caribbean and these assemblages are likely to host high species richness as well. The morphological variability associated with depth is known for many octocoral species (Bayer, 1961; Sánchez, 2009; Prada and Hellberg, 2013). An intriguing hypothesis, yet to be tested for octocorals, is the potential for deep reefs to serve as refugia for species with broad depth ranges. Deeper populations in the mesophotic (~30m and below), which are insulated from the severity of habitat disturbances in shallow-waters

(e.g. hurricane damage, warming), are hypothesised to seed shallow populations with genetic diversity (Bongaerts *et al.*, 2010).

Útila's reefs were cited to host higher scleractinian species richness and abundance than the neighbouring Bay Island of Roatán. Octocorals here in Útila were also found to have higher species richness in comparison. Future studies linking this observed richness should be investigated. Potentially, an evolutionary stable strategy has been reached within these habitats of well-structured communities of scleractinians, octocorals and sponges that supports the intermediate disturbance hypothesis (Roxburgh *et al.*, 2004).

In the face of global climate change, studies of emerging dominant benthic component require focused research attention. On many Caribbean reefs, even more overlooked than octocorals, are the sponges (Porifera) (McLean and Yoshioka, 2007), for which the communities need much attention (Bell, 2008). Like octocorals, sponge abundance is also increasing on monitored Caribbean reefs (Pawlik *et al.*, 2013; McMurray *et al.*, 2015). The species richness of sponges is remarkably high in some cases, especially in contrast to scleractinians and octocorals (Diaz and Rützler, 2001). Species richness of a Belizean reef was found to support 47 scleractinians, 35 octocorals, and over 300 estimated species of sponges (Diaz and Rützler, 2001).

Both octocorals and sponges support untold amounts of invertebrate species richness and abundance (Greene, 2008). Sponges are also considered highly important in food web dynamics, water purification, reef regeneration and many beneficial relationships with corals (Wulff, 2001). Their study is perhaps daunting with the multitude of growth forms and difficulty in defining an

individual, let alone a species; however, an entire world of scientific discoveries – crucial in confronting the crisis facing coral reefs – await those up to the challenge (Wulff, 2001; Bell, 2008).

Sponges and octocorals have both been prolific sources of secondary metabolites (see Biochemical section in Chapter One) (reviewed in(Bhakuni and Rawat, 2005; Paul *et al.*, 2011). The compositions and levels of these bioactive chemicals in organisms can vary by species and geographic location (Rodríguez, 1995) and more studies cataloguing this biochemical diversity are always warranted, especially for diverse communities like Útila. The abundant populations uncovered here of *Antillogorgia elisabethae* would benefit from a focused biochemical study. These ecologically and economically important species are regularly harvested for anti-inflammatory diterpenes for medical and commercial purposes (Look *et al.*, 1986; Lasker, 2013). Therefore, biochemical study of these populations is highly recommended and funding should be more readily obtained when presented in the aforementioned contexts.

Populations of *A. elisabethae* in Útila would make excellent candidates for studies on their microbial communities (Robertson *et al.*, 2016). Diverse microbial, fungal and viral communities thrive on the surface of corals and are hugely important in disease regulation and other physiological processes still slowly being unravelled (Woodley *et al.*, 2003; Ellner *et al.*, 2007; Harvell *et al.*, 2007; Hewson *et al.*, 2011). These microorganisms are potentially the source of many of the potent biochemical compounds found in octocorals (Paul *et al.*, 2011) and the communities on *A. elisabethae* have been shown to be highly variable (Robertson *et al.*, 2016) and

diverse (Correa *et al.*, 2013). More focus in general is needed on the coral holobiont[9], which reacts to fight off ill effects from diseases, sedimentation and ocean warming (Harvell *et al.*, 2007). This is especially crucial when disease is increasing in prevalence in the oceans and two thirds of the reports come from the Caribbean (Green and Bruckner, 2000; Lafferty *et al.*, 2004; Harvell *et al.*, 2007). Studies of and relating to disease are of dire importance and should refer to the well-researched and planned management strategies of the Coral Disease Working Group (www.gefcoral.org) (Harvell *et al.*, 2007).

To end on a more (perhaps subjectively) uplifting note, the discovery of an unknown – and to current knowledge undocumented – parasitic copepod living within the mantle tissue of several individuals of *C. gibbosum* (Fig. 6.1). The copepod was most similar to the genus *Ismaila,* known endoparasites of opisthobranch gastropods (Anton and Schrödl, 2013). Only one specimen of the copepod was seen in its entirety – when it crawled out from within a dying snail under the microscope – but the distinct green appendages are quite noticeable once recognised.

[9] The relationship of the coral animal, the endosymbiotic algae, and microbial communities.

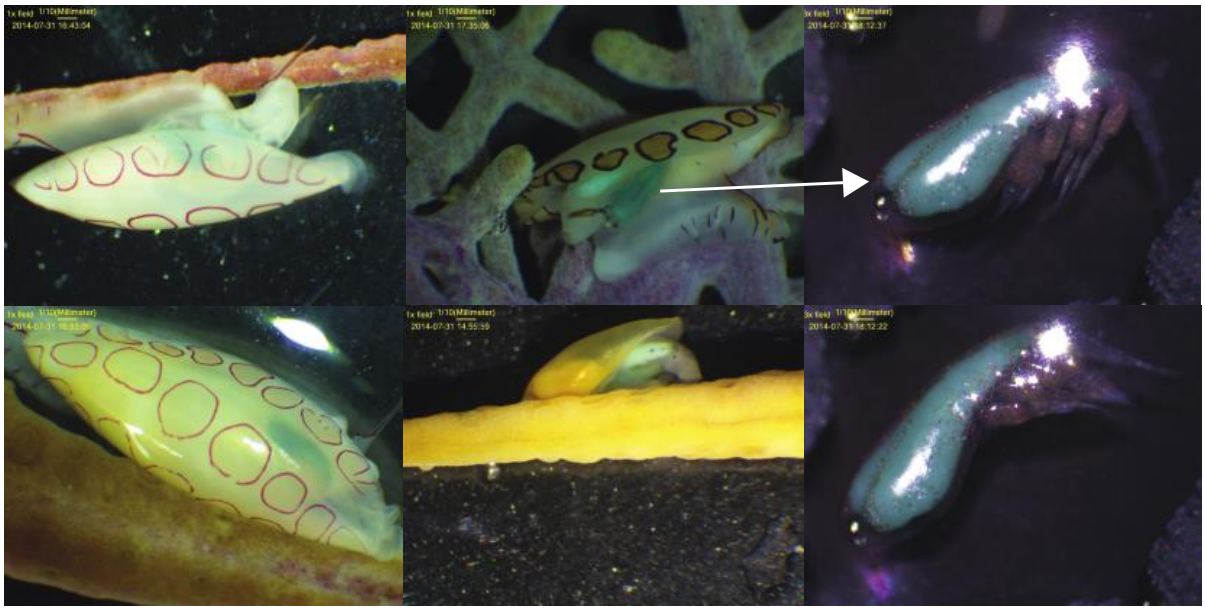


Figure 6.1 Several examples of a presumed endoparasitic copepod in juvenile ovulid *Cyphoma gibbosum* snails collected in Útila. Photo credit: VB Lovenburg.

7 References

ABREU, P.A., D.V. WILKE, A.J. ARAUJO, J.D.B. MARINHO-FILHO, E.G. FERREIRA, C.M.R. RIBEIRO, L.S. PINHEIRO, J.W. AMORIM, A.L. VALVERDE, R.A. EPIFANIO, L.V. COSTA-LOTUFO & P.C. JIMENEZ 2015. Perezone, from the gorgonian Pseudopterogorgia rigida, induces oxidative stress in human leukemia cells. *Revista Brasileira de Farmacognosia,* 25**,** 634-640.

AGUILAR, C. & J.A. SANCHEZ 2007. Phylogenetic hypotheses of gorgoniid octocorals according to ITS2 and their predicted RNA secondary structures. *Molecular Phylogenetics and Evolution,* 43**,** 774-86.

AGUILAR-HURTADO, C., M. NONAKA & J.D. REIMER 2012. The Melithaeidae (Cnidaria: Octocorallia) of the Ryukyu Archipelago: molecular and morphological examinations. *Molecular Phylogenetics and Evolution,* 64**,** 56-65.

ALATALO, R.V. 1981. Problems in the measurement of evenness in ecology. *Oikos,* 37**,** 199-204.

ALDERSLADE, P. & C.S. MCFADDEN 2011. A new sclerite-free genus and species of Clavulariidae (Coelenterata: Octocorallia). *Zootaxa,* 3104**,** 64-68.

ALEGRÍA, A., C. BOUCHON, S. BOX, I. DRYSDALE, D. FENNER, M. MCFIELD, A. NAROZANSKI, P. PORTILLO, B. STENECK, AGRRA, H.R. INITIATIVE & R. CHECK 2012. Honduras. *In:* JACKSON, J., DONOVAN, M., CRAMER, K. & LAM, V. (eds.) *Status and trends of Caribbean coral reefs: 1970-2012.*

ALMADA-VILLELA, P., M. MCFIELD, P. KRAMER, P.R. KRAMER & E. ARIAS-GONZALEZ 2002. Status of coral reefs of Mesoamerica - Mexico, Belize, Guatemala, Honduras, Nicaragua, and El Salvador. *In:* WILKINSON, C. R. (ed.) *Status of coral reefs of the world: 2002. GCRMN Report.* Townsville: Australian Institute of Marine Science.

ALMADA-VILLELA, P.C., P.F. SALE, G. GOLD-BOUCHOT & B. KJERFVE 2003. Conservation and sustainable use of the Mesoamerican Barrier Reef Systems Project (MBRS). *Manual of methods for the MBRS synoptic monitoring program.* Belize City, Belize.

ALSTYNE, K.L.V. & V.J. PAUL 1992. Chemical and structural defenses in the sea fan *Gorgonia ventalina*: effects against generalist and specialist predators. *Coral Reefs,* 11**,** 155-159.

ALSTYNE, K.L.V., C.R. WYLIE, V.J. PAUL & K. MEYER 1992. Antipredator defenses in tropical pacific soft corals (Coelenterata: Alcyonacea). I. Sclerites as defenses against generalist carnivorous fishes. *Biological Bulletin,* 182**,** 231-240.

ALVAREZ-FILIP, L., N.K. DULVY, J.A. GILL, I.M. COTE & A.R. WATKINSON 2009. Flattening of Caribbean coral reefs: region-wide declines in architectural complexity. *Proc Biol Sci,* 276**,** 3019-25.

ANDRAS, J.P., K.L. RYPIEN & C.D. HARVELL 2013. Range-wide population genetic structure of the Caribbean sea fan coral, Gorgonia ventalina. *Mol Ecol,* 22**,** 56-73.

ANDRÉFOUËT, S., P.J. MUMBY, M. MCFIELD, C. HU & F.E. MULLER-KARGER 2002. Revisiting coral reef connectivity. *Coral Reefs,* 21**,** 43-48.

ANTON, R.F. & M. SCHRÖDL 2013. The gastropod-crustacean connection: towards the phylogeny and evolution of the parasitic copepod family Splanchnotrophidae. *Zoological Journal of the Linnean Society,* 167**,** 501-530.

APPELTANS, W., S.T. AHYONG, G. ANDERSON, M.V. ANGEL, T. ARTOIS, N. BAILLY, R. BAMBER, A. BARBER, I. BARTSCH, A. BERTA, M. BLAZEWICZ-PASZKOWYCZ, P. BOCK, G. BOXSHALL, C.B. BOYKO, S.N. BRANDAO, R.A. BRAY, N.L. BRUCE, S.D. CAIRNS, T.Y. CHAN, L. CHENG, A.G. COLLINS, T. CRIBB, M. CURINI-GALLETTI, F. DAHDOUH-GUEBAS, P.J. DAVIE, M.N. DAWSON, O. DE CLERCK, W. DECOCK, S. DE GRAVE, N.J. DE VOOGD, D.P. DOMNING, C.C. EMIG, C. ERSEUS, W. ESCHMEYER, K. FAUCHALD, D.G. FAUTIN, S.W. FEIST, C.H. FRANSEN, H. FURUYA, O. GARCIA-ALVAREZ, S. GERKEN, D. GIBSON, A. GITTENBERGER, S. GOFAS, L. GOMEZ-DAGLIO, D.P. GORDON, M.D. GUIRY, F. HERNANDEZ, B.W. HOEKSEMA, R.R. HOPCROFT, D. JAUME, P. KIRK, N. KOEDAM, S. KOENEMANN, J.B. KOLB, R.M. KRISTENSEN, A. KROH, G. LAMBERT, D.B. LAZARUS, R. LEMAITRE, M. LONGSHAW, J. LOWRY, E. MACPHERSON, L.P. MADIN, C. MAH, G. MAPSTONE, P.A. MCLAUGHLIN, J. MEES, K. MELAND, C.G. MESSING, C.E. MILLS, T.N. MOLODTSOVA, R. MOOI, B. NEUHAUS,

P.K. NG, C. NIELSEN, J. NORENBURG, D.M. OPRESKO, M. OSAWA, G. PAULAY, W. PERRIN, J.F. PILGER, G.C. POORE, P. PUGH, G.B. READ, J.D. REIMER, M. RIUS, R.M. ROCHA, J.I. SAIZ-SALINAS, V. SCARABINO, B. SCHIERWATER, A. SCHMIDT-RHAESA, K.E. SCHNABEL, M. SCHOTTE, P. SCHUCHERT, E. SCHWABE, H. SEGERS, C. SELF-SULLIVAN, N. SHENKAR, V. SIEGEL, et al. 2012. The magnitude of global marine species diversity. *Curr Biol,* 22**,** 2189-202.

ARONSON, R. & W. PRECHT 2001. White-band disease and the changing face of Caribbean coral reefs. *Hydrobiologia,* 460**,** 25-38.

ARONSON, R., W. PRECHT, M. TOSCANO & K. KOLTES 2002. The 1998 bleaching event and its aftermath on a coral reef in Belize. *Marine Biology,* 141**,** 435-447.

ARONSON, R.B. & W.F. PRECHT 2006. Conservation, precaution, and Caribbean reefs. *Coral Reefs,* 25**,** 441-450.

AUSTER, P.J. (ed.) 2005. *Are deep-water corals important habitats for fishes?*: Springer-Verlag Berline Heidelberg.

AUSTIN, M. 2002. Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. *Ecological Modelling,* 157**,** 101-118.

BAKER, A.C. & A.M. ROMANSKI 2007. Multiple symbiotic partnerships are common in scleractinian corals, but not octocorals: Comment on Goulet (2006). *Marine Ecology Progress Series,* 335**,** 237-242.

BARNETT, T.P., D.W. PIERCE, K.M. ACHUTARAO, P.J. GLECKLER, B.D. SANTER, J.M. GREGORY & W.M. WASHINGTON 2005. Penetration of human-induced warming into the world's oceans. *Science,* 309**,** 284-7.

BARRERO-CANOSA, J., L.F. DUEÑAS & J.A. SÁNCHEZ. 2012. Isolation of potential fungal pathogens in gorgonian corals at the Tropical Eastern Pacific. *Coral Reefs* [Online].

BAYER, F., M. GRASSHOFF & J. VERSEVELDT 1983. Illustrated trilingual glossary of morphological and anatomical terms applied to Octocorallia. Leiden: EJ Brill.

BAYER, F.M. 1956a. Descriptions and redescriptions of the Hawaiian octocorals collected by the U.S. fish commission steamer "Albatross" (2. Gorgonacea: Scleraxonia). *Pacific Science,* X**,** 67-95.

BAYER, F.M. 1956b. Octocorallia. *In:* MOORE, R. C. (ed.) *Treatise on Invertebrates Paleontology.*

BAYER, F.M. 1961. The shallow-water Octocorallia of the West Indian region. *Studies of the Fauna Curacao and Other Caribbean Islands,* 55.

BAYER, F.M. 1981. Key to the genera of Octocorallia exclusive of Pennatulacea (Coelenterata: Anthozoa), with diagnoses of new taxa. *Proceedings of the Biological Society of Washington,* 94**,** 902-947.

BAYER, F.M. & I.G. MACINTYRE 2001. The mineral component of the axis and holdfast of some gorgonacean octocorals (Coelenterata: Anthozoa), with special reference to the family Gorgoniidae. *Proceedings of the Biological Society of Washington,* 114**,** 309-345.

BEHRMANN-GODEL, J. & A. BRINKER 2015. Biology and Ecology of Perch Parasites. 193-229.

BEIRING, E.A. & H.R. LASKER 2000. Egg production by colonies of a gorgonian coral. *Marine Ecology Progress Series,* 196**,** 169-177.

BELINKY, F., A. SZITENBERG, I. GOLDFARB, T. FELDSTEIN, G. WORHEIDE, M. ILAN & D. HUCHON 2012. ALG11--a new variable DNA marker for sponge phylogeny: comparison of phylogenetic performances with the 18S rDNA and the COI gene. *Molecular Phylogenetics and Evolution,* 63**,** 702-13.

BELL, J.J. 2008. The functional roles of marine sponges. *Estuarine, Coastal and Shelf Science,* 79**,** 341-353.

BELLWOOD, D.R., T.P. HUGHES, C. FOLKE & M. NYSTRÔM 2004. Confronting the coral reef crisis. *Nature,* 429**,** 827-833.

BENAYAHU, Y. & Y. LOYA 1977. Space partitioning by stony corals, soft corals, and benthic algae on the coral reefs of the northern Gulf of Eilat (Red Sea). *Helgoländer Wissenschaftliche Meeresuntersuchungen,* 30**,** 362-382.

BERCHTOLD, S., C. BÖHM, H. JAGADISH, H.-P. KRIEGEL & J. SANDER 2000. Independent quantization: an index compression technique for high-dimensional data spaces. *16th International Conference on Data Engineering, 2000. Proceedings.* San Diego, CA: IEEE.

BERGSTEN, J. 2005. A review of long-branch attraction. *Cladistics,* 21**,** 163-193.

BERNTSON, E.A., F.M. BAYER, A.G. MCARTHUR & S.C. FRANCE 2001. Phylogenetic relationships within the Octocorallia (Cnidaria: Anthozoa) based on nuclear 18S rRNA sequences. *Marine Biology,* 138**,** 235-246.

BHAKUNI, D. & D. RAWAT 2005. *Bioactive marine natural products,* New York, USA and New Delhi, India, Springer with Anamaya Publishers.

BIJLSMA, R., J. BUNDGAARD & A.C. BOEREMA 2000. Does inbreeding affect the extinction risk of small populations?: predictions from Drosophila. *J. Evol. Biol.,* 13**,** 502-514.

BILEWITCH, J.P. & S.M. DEGNAN 2011. A unique horizontal gene transfer event has provided the octocoral mitochondrial genome with an active mismatch repair gene that has potential for an unusual self-contained function. *BMC Evol Biol,* 11**,** 228.

BILEWITCH, J.P., M. EKINS, J. HOOPER & S.M. DEGNAN 2014. Molecular and morphological systematics of the Ellisellidae (Coelenterata: Octocorallia): parallel evolution in a globally distributed family of octocorals. *Mol Phylogenet Evol,* 73**,** 106-18.

BIRKELAND, C. 2015. Coral reefs in the Anthropocene. *In:* BIRKELAND, C. (ed.). Dordrecht Heidelberg New York London: Springer.

BODMER, M.D.V., A.D. ROGERS, M.R. SPEIGHT, N. LUBBOCK & D.A. EXTON 2015. Using an isolated population boom to explore barriers to recovery in the keystone Caribbean coral reef herbivore Diadema antillarum. *Coral Reefs,* 34**,** 1011-1021.

BONGAERTS, P., T. RIDGWAY, E.M. SAMPAYO & O. HOEGH-GULDBERG 2010. Assessing the 'deep reef refugia' hypothesis: focus on Caribbean reefs. *Coral Reefs,* 29**,** 309-327.

BRAGA-HENRIQUES, A., M. CARREIRO-SILVA, F. TEMPERA, F.M. PORTEIRO, K. JAKOBSEN, J. JAKOBSEN, M. ALBUQUERQUE & R. SERRÃO SANTOS 2011. Carrying behavior in the deep-sea crab Paromola cuvieri (Northeast Atlantic). *Marine Biodiversity,* 42**,** 37-46.

BRAY, J.R. & J. CURTIS 1957. An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs,* 27**,** 325-349.

BRAZEAU, D.A. & C.D. HARVELL 1994. Genetic structure of local populations and divergence between growth forms in a clonal invertebrate, the Caribbean octocoral *Briareum asbestinum*. *Marine Biology,* 119**,** 53-60.

BRIDGE, D., C.W. CUNNINGHAM, R. DESALLE & L.W. BUSS 1995. Class-level relationships in the Phylum Cnidaria: Molecular and morphological evidence. *Mol Biol Evol,* 12**,** 679-689.

BROWER, L.P., J.V.Z. BROWER & J.M. CORVINO 1967. Plant poisons in a terrestrial food chain. *Proceedings of the National Academy of Sciences of the United States of America,* 57**,** 893–898.

BRUCKNER, A.W. 2002. Life-Saving Products from Coral Reefs. *Issues in Science and Technology,* 18**,** 39-44.

BRUNO, J.F., L.E. PETES, C. DREW HARVELL & A. HETTINGER 2003. Nutrient enrichment can increase the severity of coral diseases. *Ecology Letters,* 6**,** 1056-1061.

BRUNO, J.F., E.R. SELIG, K.S. CASEY, C.A. PAGE, B.A. WILLIS, C.D. HARVELL, H. SWEATMAN & A.M. MELENDY 2007. Thermal stress and coral cover as drivers of coral disease outbreaks. *PloS Biology, 5***,** 1220-1227.

BRUNO, J.F., H. SWEATMAN, W.F. PRECHT, E.R. SELIG & V.G. SCHUTTE 2009. Assessing evidence of phase shifts from coral to macroalgal dominance on coral reefs. *Ecology,* 90**,** 1478-1484.

BRUSCA, R.C., W. MOORE & S.M. SHUSTER 2016. *Invertebrates*, Sunderland: Sinauer Associates.

BUHL-MORTENSEN, L. & P.B. MORTENSEN 2004. Crustaceans associated with the deep-water gorgonian corals *Paragorgia arborea* (L., 1758) and *Primnoa resedaeformis* (Gunn., 1763). *Journal of Natural History,* 38**,** 1233-1247.

BURKE, L. & Z. SUGG 2006. Hydrologic modeling of watersheds discharging adjacent to the Mesoamerican Reef. On the water-shed analysis for the Mesoamerican Reef. WRI/ICRAN.

BURKEPILE, D.E. & M.E. HAY 2007. Predator release of the gastropod Cyphoma gibbosum increases predation on gorgonian corals. *Oecologia,* 154**,** 167-73.

BURKHOLDER, P.R. & L.M. BURKHOLDER 1958. Antimicrobial activity of horny corals. *Science,* 127**,** 1174-1175.

BYTHELL, J.C., P. PAN & J. LEE. 2001. Three-dimensional morphometric measurements of reef corals using underwater photogrammetry techniques. *Coral Reefs,* 20**,** 193-199.

CAIRNS, S.D. 2007. Deep-water corals: an overview with special reference to diversity and distribution of deep-water scleractinian corals. *Bulletin of Marine Science,* 81**,** 311-322.

CAIRNS, S.D. & F.M. BAYER 2009. A generic revision and phylogenetic analysis of the Primnoidae. *Smithsonian Contributions to Zoology,* 629**,** 1-73.

CANTY, S.W. 2007. *Positive and negative impacts of dive tourism: the case study of Útila, Honduras.* Lunds Universitet.

CARMELY, S., Y. KASHMAN, Y. LOYA & Y. BENAYAHU 1980. New prostaglandin (PGF) derivatives from the soft coral *Lobophyton depressum Tetrahedron Letters,* 21**,** 875-878.

CESAR, H., L. BURKE & L. PET-SOEDE 2003a. The Economics of Worldwide Coral Reef Degradation. Cesar Environmental Economics Consulting, Arnhem, and WWF-Netherlands, Zeist, The Netherlands.

CESAR, H., L. BURKE & L. PET-SOEDE 2003b. The economics of worldwide coral reef degradation. Cesar Environmental Economics Consulting (CEEC).

CESAR, H.S. & V.B. PIETER JH 2004. Economic valuation of the coral reefs of Hawai'i. *Pacific Science,* 58**,** 231-242.

CHANGYUN, W., L. HAIYAN, S. CHANGLUN, W. YANAN, L. LIANG & G. HUASHI 2008. Chemical defensive substances of soft corals and gorgonians. *Acta Ecologica Sinica,* 28**,** 2320-2328.

CHAPELA, E., S. REHNER, T. SCHULTZ & U. MUELLER 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science,* 266**,** 1691-1694.

CHEN, C.A., D.M. ODORICO, M.T. LOHUIS, J.E.N. VERON & D.J. MILLER 1995. Systematic relationships within the Anthozoa (Cnidaria: Anthozoa) using the 5'-end of the 28S rDNA. *Moelcular Phylogenetics and Evolution,* 4**,** 175-183.

CHEN, J.Y., P. OLIVERI, F. GAO, S.Q. DORNBOS, C.W. LI, D.J. BOTTJER & E.H. DAVIDSON 2002. Precambrian Animal Life: Probable Developmental and Adult Cnidarian Forms from Southwest China. *Developmental Biology,* 248**,** 182-196.

CHERNOMOR, O., A. VON HAESELER & B.Q. MINH 2016. Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices. *Syst Biol.*

CHÉRUBIN, L.M., C.P. KUCHINKE & C.B. PARIS 2008. Ocean circulation and terrestrial runoff dynamics in the Mesoamerican region from spectral optimization of SeaWiFS data and a high resolution simulation. *Coral Reefs,* 27**,** 503-519.

CHIAPPONE, M., H. DIENES, D.W. SWANSON & S.L. MILLER 2003. Density and gorgonian host-occupation pattern by flamingo tongue snails (*Cyphoma gibbosum*) in the Florida Keys. *Caribbean Journal of Science,* 39**,** 116-127.

CIA. 2012. *Central America and Caribbean: Honduras* [Online]. Washington, DC: Central Intelligence Agency. Available: https://www.cia.gov/library/publications/the-world-factbook/index.html.

CIERESZKO, L.S., M.A. JOHNSON, R.W. SCHMIDT & C.B. KOONS 1968. Chemistry of coelenterates - VI. Occurence of gorgosterol, A C30 sterol, in coelenterates and their zooxanthellae. *Comparative Biochemistry and Physiology,* 24**,** 899-904.

CINEL, B., M. ROBERGE, H. BEHRISCH, L.V. OFWEGEN, C.B. CASTRO & R.J. ANDERSEN 2000a. Antimitotic diterpenes from *Erythropodium caribaeorum* teset pharmacophore models for microtubule stabilization. *Organic Letters,* 2**,** 257-260.

CINEL, B., M. ROBERGE, H. BEHRISCH, L.V. OFWEGEN, C.B. CASTRO & R.J. ANDERSEN 2000b. Antimitotic diterpenes from *Erythropodium caribaeorum* test pharmacophore models for microtubule stabilization. *Organic Letters,* 2**,** 257-260.

CLARKE, K. & R. GORLEY 2015. PRIMER v7: User Manual/Tutorial. . *PRIMER-E.* Plymouth.

CLARKE, K., P. SOMERFIELD & R. GORLEY 2008. Testing null hypotheses in exploratory community analyses: similarity profiles and biota-environmental linkage. *Journal of Experimental Marine Biology and Ecology,* 366**,** 56-69.

COLL, J.C. 1992. The chemistry and chemical ecology of octocorals (Coelenterata, Anthozoa, Octocorallia). *Chemical Reviews,* 92**,** 613-631.

COLLINS, A.G. 2009. Recent insights into cnidarian phylogeny. *Smithsonian Contributions to the Marine Sciences,* 38**,** 139-149.

COLWELL, R.K., A. CHAO, N.J. GOTELLI, S.Y. LIN, C.X. MAO, R.L. CHAZDON & J.T. LONGINO 2012. Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. *Journal of Plant Ecology,* 5**,** 3-21.

CONCEPCION, G.T., M.W. CREPEAU, D. WAGNER, S.E. KAHNG & R.J. TOONEN 2007. An alternative to ITS, a hypervariable, single-copy nuclear intron in corals, and its use in detecting cryptic species within the octocoral genus Carijoa. *Coral Reefs,* 27**,** 323-336.

CONNELL, J.H. 1978. Diversity in tropical rain forests and coral reefs. *Science,* 199**,** 1302-1310.

COOK, R. & S. WEISBERG 1983. Diagnostics for heteroscedasticity in regression. *Biometrika,* 70**,** 1-10.

CORREA, H., B. HALTLI, C. DUQUE & R. KERR 2013. Bacterial communities of the gorgonian octocoral Pseudopterogorgia elisabethae. *Microb Ecol,* 66**,** 972-85.

COSTANZA, R., R. DE GROOT, P. SUTTON, S. VAN DER PLOEG, S.J. ANDERSON, I. KUBISZEWSKI, S. FARBER & R.K. TURNER 2014. Changes in the global value of ecosystem services. *Global Environmental Change,* 26**,** 152-158.

COSTELLO, M.J., M. COLL, R. DANOVARO, P. HALPIN, H. OJAVEER & P. MILOSLAVICH 2010. A census of marine biodiversity knowledge, resources, and future challenges. *Plos One,* 5**,** 15.

COSTELLO, M.J., M. MCCREA, A. FREIWALD, T. LUNDALV, L. JONSSON, B.J. BETT, J.C.E.V. WEERING, H.D. HAAS, J.M. ROBERTS & D. ALLEN (eds.) 2005. *Role of cold-water*

Lophelia pertusa coral reefs as fish habitat in the NE Atlantic, Berlin Heidelberg: Springer-Verlag.

COURTNEY, L.A., W.S. FISHER, S. RAIMONDO, L.M. OLIVER & W.P. DAVIS 2007. Estimating 3-dimensional colony surface area of field corals. *Journal of Experimental Marine Biology and Ecology,* 351**,** 234-242.

CRISP, D.J. 1990. Gregariousness and systematic affinity in some North Carolinian barnacles. *Bulletin of Marine Science,* 47**,** 516-525.

CRONIN, G., M. HAY, W. FENICAL & N. LINDQUIST 1995. Distribution, density, and sequestration of host chemical defenses by the specialist nudibranch *Tritonia hamnerorum* found at high densities on the sea fan *Gorgonia ventalina*. *Marine Ecology Progress Series,* 119**,** 177-189.

DAHAN, M. & Y. BENAYAHU 1997. Clonal propagation by the azooxanthellate octocoral *Dendronephthya hemprichi*. *Coral Reefs,* 16**,** 5-12.

DAHLGREN, E.J. 1989. Gorgonian community structure and reef zonation patterns on Yucatan coral reefs. *Bulletin of Marine Science,* 45**,** 678-696.

DALY, M., M.R. BRUGLER, P. CARTWRIGHT, A.G. COLLINS, M.N. DAWSON, D.G. FAUTIN, S.C. FRANCE, C.S. MCFADDEN, D.M. OPRESKO, E. RODRIGUEZ, S.L. ROMANO & J.L. STAKE 2007. The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. *Zootaxa***,** 127-182.

DAVIES, A.J. & J.M. GUINOTTE. 2011. Global habitat suitability for framework-forming cold-water corals. *Plos One* [Online], 6.

DAWKINS, R. 2006. *The Selfish Gene,* Oxford, Oxford University Press.

DE'ATH, G., K.E. FABRICIUS, H. SWEATMAN & M. PUOTINEN 2012. The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proc Natl Acad Sci U S A,* 109**,** 17995-9.

DELOACH, N. 1999. *Reef fish behavior,* Jacksonville, FL, New World Publications, Inc.

DEMIRJIAN, D.C., F. MORÍS-VARAS & C.S. CASSIDY 2001. Enzymes from extremophiles. *Current Opinion in Chemical Biology,* 5**,** 144-151.

DESALLE, R., M.G. EGAN & M. SIDDALL 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philos Trans R Soc Lond B Biol Sci,* 360**,** 1905-16.

DEVICTOR, S.T. & S.L. MORTON 2010. Identification guide to the shallow water (0-200 m) octocorals of the South Atlantic Bight. *Zootaxa,* 2599**,** 1-62.

DIAL, R. & J. ROUGHGARDEN 1998. Theory of marine communities: the intermediate disturbance hypothesis. *Ecology,* 79**,** 1412-1424.

DIAZ, M. & K. RÜTZLER 2001. Sponges: an essential component of Caribbean coral reefs. *Bulletin of Marine Science,* 69**,** 535-546.

DONE, T. 1992. Phase shifts in coral reef communities and their ecological significance. *Hydrobiologia,* 247**,** 121-132.

DRUMMOND, A. 2007. FigTree v1.3.1.

DUDGEON, S.R., R.B. ARONSON, J.F. BRUNO & W.F. PRECHT 2010. Phase shifts and stable states on coral reefs. *Marine Ecology Progress Series,* 413**,** 201-216.

EDWARDS, S.V. 2009. Is a new and general theory of molecular systematics emerging? *Evolution,* 63**,** 1-19.

ELLNER, S.P., L.E. JONES, L.D. MYDLARZ & C.D. HARVELL 2007. Within-host disease ecology in the sea fan Gorgonia ventalina: modeling the spatial immunodynamics of a coral-pathogen interaction. *Am Nat,* 170**,** E143-61.

EPIFANIO, R.N.D.A., L.F. MAIA, J.R. PAWLIK & W. FENICAL 2007. Antipredatory secosterols from the octocoral *Pseudopterogorgia americana*. *Marine Ecology Progress Series,* 329**,** 307-310.

ESPINOSA, Y., L. HERNÁNDEZ-FERNÁNDEZ & A. CAPOTE 2010. Structure and injuries of octocoral communities (Octocorallia: Alyconacea) of ecological reserve Siboney-Juticí, Santiago de Cuba, Cuba. *International Journal of Tropical Biology,* 58**,** 1211-1221.

ETNOYER, P. & L. MORGAN 2005. Habitat-forming deep-sea corals in the Northeast Pacific Ocean. *In:* FREIWALD, A. & ROBERTS, J. M. (eds.) *Cold-water Corals and Ecosystems.* Berlin Heidelberg: Springer-Verlag.

ETNOYER, P. & J. WARRENCHUK 2007. A catshark nursery in a deep gorgonian field in the Mississippi Canyon, Gulf of Mexico. *Bulletin of Marine Science,* 81**,** 553-559.

FABRICIUS, K. & P. ALDERSLADE 2001. *Soft corals and sea fans: a comprehensive guide to the tropical shallow water genera of the central-west Pacific Ocean and the Indian Ocean and the Red Sea,* Melbourne, New Litho.

FABRICIUS, K. & G. DE'ATH 2008. Photosynthetic symbionts and energy supply determine octocoral biodiversity in coral reefs. *Ecology,* 89**,** 3163-3173.

FABRICIUS, K. & D. KLUMPP 1995. Widespread mixotrophy in reef-inhabiting soft corals: the influence of depth, and colony expansion and contraction on photosynthesis. *Marine Ecology Progress Series,* 125**,** 195-204.

FABRICIUS, K.E. 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Marine Pollution Bulletin,* 50**,** 125-46.

FABRICIUS, K.E. & D. MCCORRY 2006. Changes in octocoral communities and benthic cover along a water quality gradient in the reefs of Hong Kong. *Mar Pollut Bull,* 52**,** 22-33.

FAULKNER, D. & M. GHISELIN 1983. Chemical defense and evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. *Marine Ecology Progress Series,* 13**,** 295-301.

FERRIGNO, F., C.N. BIANCHI, R. LASAGNA, C. MORRI, G.F. RUSSO & R. SANDULLI 2016. Corals in high diversity reefs resist human impact. *Ecological Indicators,* 70**,** 106-113.

FISHER, R., R.A. O'LEARY, S. LOW-CHOY, K. MENGERSEN, N. KNOWLTON, R.E. BRAINARD & M.J. CALEY 2015. Species richness on coral reefs and the pursuit of convergent global estimates. *Curr Biol,* 25**,** 500-5.

FITZSIMMONS-SOSA, K., P. HALLOCK, J. WHEATON, K. HACKETT & M. CALLAHAN 2004. Annuals cycles of gonadal development of six common gorgonians from Biscayne National Park, Florida, USA. *Caribbean Journal of Science,* 40**,** 144-150.

FOX, J.W. 2013. The intermediate disturbance hypothesis should be abandoned. *Trends Ecol Evol,* 28**,** 86-92.

FRANCE, S.C. & L.L. HOOVER 2001. Analysis of variation in mitochondrial DNA sequences (ND3, ND4L, MSH) among Octocorallia (=Alcyonaria) (Cnidaria: Anthozoa). *Bulletin of the Biological Society of Washington,* 10**,** 110-118.

FRANCE, S.C. & L.L. HOOVER 2002. DNA sequences of the mitochondrial COI gene have low levels of divergence among deep-sea octocorals (Cnidaria : Anthozoa). *Hydrobiologia,* 471**,** 149-155.

FRANCE, S.C., P.E. ROSEL, J.E. AGENBROAD, L.S. MULLINEAUX & T.D. KOCHER 1996. DNA sequence variation of mitochondrial large-subunit rRNA provides support for a two-subclass organization of the Anthozoa (Cnidaria). *Molecular Marine Biology and Biotechnology 5***,** 15-28.

FREUDENTHAL, H.D. 1962. *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a Zooxanthella: taxonomy, life cycle, and morphology. *Journal of Protozoology,* 9**,** 45-52.

FRIEDLANDER, A.M. & J.D. PARRISH 1998. Habitat characteristics affecting fish assemblages on a Hawaiian coral reef. *Journal of Experimental Marine Biology and Ecology,* 224**,** 1-30.

FUKAMI, H., A.F. BUDD, G. PAULAY, A. SOLÉ-CAVA, C.A. CHEN, K. IWAO & N. KNOWLTON 2004. Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature,* 427**,** 832-835.

GARCÍA-SALGADO, M., G. NAVA-MARTÍNEZ, M. VASQUEZ, N. JACOBS, I. MAJIL, A. MOLINA-RAMÍREZ, B. YAÑEZ-RIVERA, A. CUBAS, J. DOMINGUEZ-CALDERON, W. HADAAD, M. MALDONADO & O. TORRES. Declining trend on the Mesoamerican Reef System marine protected areas. Proceedings of the 11th International Coral Reef Symposium 7-11 July 2008 2008 Ft. Lauderdale, Florida.

GARDNER, T.A., I.M. COTE, J.A. GILL, A. GRANT & A.R. WATKINSON 2003. Long-term region-wide declines in Caribbean corals. *Science,* 301**,** 958-60.

GARDNER, T.A., I.M. CÔTÉ, J.A. GILL, A. GRANT & A.R. WATKINSON 2005. Hurricanes and Caribbean coral reefs: impacts, recovery, paterns, and role in long-term decline. *Ecology,* 86**,** 174-184.

GEISER, D.M., J.W. TAYLOR, K.B. RITCHIE & G.W. SMITH 1998. Cause of sea fan death in the West Indies. *Nature,* 394**,** 137-138.

GERHART, D. & J. COLL 1993. Pukalide, a widely distributed octocoral diterpenoid, induces vomiting in fish. *Journal of Chemical Ecology,* 19**,** 2697-2704.

GERHART, D.J. 1983. The chemical systematics of colonial marine animals: an estimated phylogeny of the order Gorgonacea based on terpenoid characters. *Biological Bulletin,* 164**,** 71-81.

GERHART, D.J. 1984. Prostaglandin A2: an agent of chemical defense in the Caribbean gorgonian *Plexaura homomalla*. *Marine Ecology - Progress Series,* 19**,** 181-187.

GERHART, D.J. 1986. Gregariousness in the gorgonian-eating gastropod *Cyphoma gibbosum*: tests of several possible causes *Marine Ecology Progress Series,* 31**,** 255-263.

GERHART, D.J. 1990. Fouling and gastropod predation: consequences of grazing for a tropical octocoral. *Marine Ecology Progress Series,* 62**,** 103-108.

GESAMP 2001. Protecting the oceans from land-based activities. Land-based sources and activities affecting the quality and uses of the marine, coastal and assocaited freshwater environment. . Nairobi, Kenya: United Nations Environment Program.

GHISELIN, M.T. & B.R. WILSON 1966. On the anatomy, natural history, and reproduction of *Cyphoma*, a marine prosobranch gastropod. *Bulletin of Marine Science,* 16**,** 132-144.

GILI, J.M. & R. COM 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. *TREE,* 13**,** 316-321.

GOLDBERG, W.M. 1973. The ecology of the coral-octocoral communities off the Southeast Florida coast: geomorphology, species composition, and zonation. *Bulletin of Marine Science,* 23**,** 465-488.

GÓMEZ, C.E., V.J. PAUL, R. RITSON-WILLIAMS, N. MUEHLLEHNER, C. LANGDON & J.A. SÁNCHEZ 2014. Responses of the tropical gorgonian coral Eunicea fusca to ocean acidification conditions. *Coral Reefs,* 34**,** 451-460.

GOREAU, T.J., J. CERVINO, M. GOREAU, R. HAYES, M. HAYES, L. RICHARDSON, G. SMITH, K. DEMEYER, I. NAGELKERKEN, J. GARZON-FERRERA, D. GIL, G. GARRISON, E.H.

WILLIAMS, L. BUNKLEY-WILLIAMS, C. QUIROLO, K. PATTERSON, J.W. PORTER & K. PORTER 1998. Rapid spread of diseases in Caribbean coral reefs. *Revista de Biologia Tropical,* 46**,** 157-171.

GÖSSLING, S. 2003. The political ecology of marine protected areas: the case of the Bay Islands. *In:* GÖSSLING, S. (ed.) *Tourism and development in tropical islands: political ecology perspectives.* Cheltenham, UK and Northampton, USA: Edward Elgar Publishing.

GOTELLI, N. & R. COLWELL 2011. Estimating species richness. *In:* MAGURRAN, A. & MCGILL, B. (eds.) *Frontiers in Measuring Biodiversity.* New York: Oxford University Press.

GOTELLI, N.J. 1988. Determinants of recruitment, juvenile growth, and spatial distribution of a shallow-water gorgonian. *Ecology,* 69**,** 157-166.

GRAHAM, N.A.J. & K.L. NASH 2012. The importance of structural complexity in coral reef ecosystems. *Coral Reefs,* 32**,** 315-326.

GRAJALES, A., C. AGUILAR & J.A. SANCHEZ 2007. Phylogenetic reconstruction using secondary structures of Internal Transcribed Spacer 2 (ITS2, rDNA): finding the molecular and morphological gap in Caribbean gorgonian corals. *BMC Evol Biol,* 7**,** 90.

GRASSHOFF, M. & P. ALDERSLADE 1997. Gorgoniidae of Indo-Pacific reefs with descritpion of two new genera (COelenterata: Octocorallia). . *Senckenbergiana Biologica,* 77**,** 23-35.

GRATWICKE, B. & M. SPEIGHT 2005. The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. *Journal of Fish Biology,* 66**,** 650-667.

GREEN, E. & A. BRUCKNER 2000. The significance of coral disease epizootiology for coral reef conservation. *Biological Conservation,* 96**,** 347-361.

GREENE, A.K. 2008. *Invertebrate endofauna associated with sponge and octocoral epifauna at Gray's Reef National Marine Sanctuary off the coast of Georgia.* The Graduate School of the College of Charleston.

GROSS, H. & G.M. KÖNIG 2006. Terpenoids from Marine Organisms: Unique Structures and their Pharmacological Potential. *Phytochemistry Reviews,* 5**,** 115-141.

GUERRIERO, A., M. D'AMBROSIO & F. PIETRA 1988. 124. Leiopathic acid, a novel optically active hydroxydocosapentaenoic acid, and related compounds, from the black coral *Leiopathes* sp. of Saint Paul Island (S. Indian Ocean). *Helvetica Chimica Acta,* 71**,** 1094-1100.

GUINOTTE, J.M., J. ORR, S. CAIRNS, A. FREIWALD, L. MORGAN & R. GEORGE 2006. Will human-induced changes in seawater chemistry alter the distribution of deep-sea scleractinian corals? *Frontiers in Ecology and the Environment,* 4**,** 141-146.

GUZMÁN, H.M. & J. CORTÉS 1985. Organismos de arrecifes coralinos de Costa Rica. IV. Descripcion y distribucion geografica de octocoralarios (Cnidaria: Anthozoa) de la Costa Caribe. *Brenesia,* 24**,** 125-173.

GUZMÁN, H.M. & C.E. JIMÉNEZ 1992. Contamination of coral reefs by heavy metals along the Caribbean coast of Central America. *Marine Pollution Bulletin,* 24**,** 554-561.

HADFIELD, M.G. & V.J. PAUL 2001. Natural chemical cues for settlement and metamorphosiss of marine invertebrate larave. *In:* MCCLINTOCK, J. & BAKER, B. (eds.) *Marine chemical ecology.* Boca Raton, FL: CRC Press.

HAJIBABAEI, M., D.H. JANZEN, J.M. BURNS, W. HALLWACHS & P.D. HEBERT 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proc Natl Acad Sci U S A,* 103**,** 968-71.

HALL, B.G. 2011. *Phylogenetic Trees Made Easy: A How-To Manual,* Bellingham Research Institute, Sinauer Associates, Inc.

HARBORNE, A., S. EVANS, D. AFZAL, M. ANDREWS & P. RAINES 2001. Summary of Coral Cay Conservation's habitat mapping data from Útila, Honduras. Centre for Advanced Spatial Analysis, University College London.

HARVELL, C. & W. FENICAL 1989. Chemical and structural defense in Caribbean gorgonians (*Pseudopterogorgia* spp.): intracolony localization of defense. *Limnology and Oceanography,* 34**,** 382-389.

HARVELL, C.D., C.E. MITCHELL, J.R. WARD, S. ALTIZER, A.P. DOBSON, R.S. OSTFELD & M.D. SAMUEL 2002. Climate warming and disease risks for terrestrial and marine biota. *Science,* 296**,** 2158-62.

HARVELL, C.D. & T.H. SUCHANEK 1987. Partial predation on tropical gorgonians by *Cyphoma gibbosum* (Gastropoda). *Marine Ecology Progress Series,* 38**,** 37-44.

HARVELL, D., E. JORDAN-DAHLGREN, S. MERKEL, E. ROSENBERG, L. RAYMUNDO, G. SMITH, E. WEIL & B. WILLIS 2007. Coral disease, environmental drivers, and the balance between coral and microbial associates. *Oceanography,* 20**,** 172-195.

HARVELL, D., K. KIM, C. QUIROLO, J. WEIR & G. SMITH 2001. Coral bleaching and disease: contributors to 1998 mass mortality in *Briareum asbestinum* (Octocorallia, Gorgonacea). *Hydrobiologia,* 460**,** 97-104.

HEBERT, P.D., A. CYWINSKA, S.L. BALL & J.R. DEWAARD 2003a. Biological identifications through DNA barcodes. *Proc Biol Sci,* 270**,** 313-21.

HEBERT, P.D., S. RATNASINGHAM & J.R. DEWAARD 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc Biol Sci,* 270 Suppl 1**,** S96-9.

HEBERT, P.D.N., M.Y. STOECKLE, T.S. ZEMLAK & C.M. FRANCIS 2004. Identification of birds through DNA barcodes. *Plos One,* 2**,** 1657-1663.

HELLBERG, M.E. 2006. No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evolutionary Biology,* 6.

HERRERA, S. & T.M. SHANK 2016. RAD sequencing enables unprecedented phylogenetic resolution and objective species delimitation in recalcitrant divergent taxa. *Mol Phylogenet Evol,* 100**,** 70-9.

HEWSON, I., J.M. BROWN, C.A. BURGE, C.S. COUCH, B.A. LABARRE, M.E. MOUCHKA, M. NAITO & C.D. HARVELL 2011. Description of viral assemblages associated with the Gorgonia ventalina holobiont. *Coral Reefs,* 31**,** 487-491.

HOEGH-GULDBERG, O. 1988. A method for determining the surface area of corals. *Coral Reefs,* 7**,** 113-116.

HRI 2011. Eco-audit results for Honduras.

HRI. 2012. *Healthy Reefs for Healthy People* [Online]. Available: http://www.healthyreefs.org/cms/.

HUELSENBECK, J.P. & F. RONQUIST 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics Applications Note,* 17**,** 754-755.

HUGHES, T.P. 1994. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. *Science,* 265**,** 1547-1551.

HUGHES, T.P., A.H. BAIRD, D.R. BELLWOOD, M. CARD, S.R. CONNOLLY, C. FOLKE, R. GROSBERG, O. HOEGH-GULDBERG, J.B. JACKSON, J. KLEYPAS, J.M. LOUGH, P. MARSHALL, M. NYSTROM, S.R. PALUMBI, J.M. PANDOLFI, B. ROSEN & J. ROUGHGARDEN 2003. Climate change, human impacts, and the resilience of coral reefs. *Science,* 301**,** 929-33.

HUGHES, T.P., N.A. GRAHAM, J.B. JACKSON, P.J. MUMBY & R.S. STENECK 2010. Rising to the challenge of sustaining coral reef resilience. *Trends in Ecology & Evolution,* 25**,** 633-42.

IDJADI, J.A., S.C. LEE, J.F. BRUNO, W.F. PRECHT, L. ALLEN-REQUA & P.J. EDMUNDS 2006. Rapid phase-shift reversal on a Jamaican coral reef. *Coral Reefs,* 25**,** 209-211.

IGUCHI, K., M. IWASHIMA & K. WTANABE 1995. Clavulocatones, new marine prostanoids with a γ-lactonic moiety in the α-side-chain from the Okinawan soft coral, *Clavularia viridis*. *Journal of Natural Products,* 58**,** 790-793.

IMBS, A., S.S. DAUTOV & N. LATYSHEV 1989. DEGREE OF MATURITY OF THE GONADS AND THE EICOSANOIDS OF THE SOFT CORAL GERSEMIA-RUBIFORMIS. *JOURNAL OF EVOLUTIONARY BIOCHEMISTRY AND PHYSIOLOGY,* 25**,** 307-311.

IMBS, A.B. 2011. Prostaglandins and oxylipins of corals. *Russian Journal of Marine Biology,* 37**,** 325-334.

INC., S.I. 2015. JMP, Version 12. Cary, NC.

INSTITUTO NACIONAL DE ESTADÍSTICA HONDURAS 2013. Honduras: Bay Islands (Islas de la Bahía).

ISOMURA, N., K. IWAO & H. FUKAMI 2013. Possible natural hybridization of two morphologically distinct species of Acropora (Cnidaria, Scleractinia) in the Pacific: fertilization and larval survival rates. *PLoS One,* 8**,** e56701.

IVLEV, V. 1961. *Experimental ecology of the feeding of fishes,* New Haven, Connecticut, Yale University Press.

IWAMARU, A., E. IWADO, S. KONDO, R.A. NEWMAN, B. VERA, A.D. RODRIGUEZ & Y. KONDO 2007. Eupalmerin acetate, a novel anticancer agent from Caribbean gorgonian octocorals, induces apoptosis in malignant glioma cells via the c-Jun NH2-terminal kinase pathway. *Molecular Cancer Therapeutics,* 6**,** 184-92.

JACKSON, J. 1977. Competition on marine hard substrata: adaptive significance of solitary and colonial strategies. *The American Naturalist,* 111**,** 743-767.

JACKSON, J., M. DONOVAN, K. CRAMER & V. LAM (eds.) 2014. *Status and trends of Caribbean coral reefs: 1970-2012,* Gland, Switzerland: Global Coral Reef Monitoring Network, IUCN.

JACKSON, J.B.C., M.X. KIRBY, W.H. BERGER, K.A. BJORNDAL, L.W. BOTSFORD, B.J. BOURQUE, R.H. BRADBURY, R. COOKE, J. ERLANDSON, J.A. ESTES, T.P. HUGHES, S. KIDWELL, C.B. LANGE, H.S. LENIHAN, J.M. PANDOLFI, C.H. PETERSON, R.S. STENECK, M.J. TEGNER & R.R. WARNER 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science,* 293**,** 629-638.

JENG, M.S., H.D. HUANG, C.F. DAI, Y.C. HSIAO & Y. BENAYAHU 2011. Sclerite calcification and reef-building in the fleshy octocoral genus Sinularia (Octocorallia: Alcyonacea). *Coral Reefs,* 30**,** 925-933.

JORDAN, E. An analysis of a gorgonian community in a reef calcareous platform on the Caribbean coast of Mexico. Third International Coral Reef Symposium, 1978 Miami Florida.

KAHNG, S.E., Y. BENAYAHU & H.R. LASKER 2011. Sexual reproduction in octocorals. *Marine Ecology Progress Series,* 443**,** 265-283.

KARLSON, R.H. 1980. Alternative competitive strategies in a periodically disturbed habitat. *Bulletin of Marine Science,* 30**,** 894-900.

KAYAL, E., B. ROURE, H. PHILIPPE, A.G. COLLINS & D.V. LAVROV 2013. Cnidarian phylogenetic relationships as revealed by mitogenomics. *BMC Evolutionary Biology,* 13**,** 1-18.

KEARSE, M., MOIR, R., WILSON, A., STONES-HAVAS, S., CHEUNG, M., STURROCK, S., BUXTON, S., COOPER, A., MARKOWITZ, S., DURAN, C., THIERER, T., ASHTON, B., MENTJIES, P., DRUMMOND, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics,* 28**,** 1647-1649.

KECK, J.R. 2004. *Changes in coral populations on the Northwest coast of Roatán, Honduras, subsequent to the 1998 coral bleaching event and Hurricane Mitch.* Master's.

KEIGWIN, L.D.J. 1978. Pliocene closing of hte Isthmus of Panama, based on biostratigraphic evidence from nearby Pacific Ocean and Caribbean Sea cores. *Geology,* 6**,** 630-634.

KEITH, D.E. 1992. Shallow-water gorgonians (Ocotocorallia) of Roatan, Honduras. *Bulletin of Marine Science,* 50**,** 212-226.

KIM, J.W. & T.L. PEEPLES 2006. Screening extremophiles for bioconversion potentials. *Biotechnology Progress,* 22**,** 1720-1724.

KINDT, R. 2016. BiodiversityR: package for community ecology and suitability.

KINZIE, R.A. 1970. *The ecology of the gorgonians (Cnidaria, Octocorallia) of Discovery Bay, Jamaica.* Ph.D., Yale University.

KINZIE, R.A. 1973. The zonation of West Indian gorgonians. *Bulletin of Marine Science,* 23**,** 93-155.

KINZIE, R.A.I. 1974. *Plexaura homomalla*: the biology and ecology of a harvestable marine resource. *In:* BAYER, F. M., WEINHEIMER, A. J. & VOSS, G. L. (eds.) *Prostaglandins from Plexaura homomalla: Ecology, Utilization and Conservation of a Major Medical Marine Resource.* Coral Gables, Florida: University of Miami Press.

KIRK, N.L., J.R. WARD & M.A. COFFROTH 2005. Stable *Symbiodinium* composition in the sea fan *Gorgonia ventalina* during temperature and disease stress. *Biol. Bull.,* 209**,** 227-234.

KNOWLTON, N. 1992. Thresholds and multiple stable states in coral reef community dynamics. *American Zoologist,* 32**,** 674-682.

KNOWLTON, N. 2000. Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia,* 420**,** 73-90.

KNOWLTON, N. 2004. Multiple "stable" states and the conservation of marine ecosystems. *Progress in Oceanography,* 60**,** 387-396.

KRAMER, P.A. & P.R. KRAMER 2002. Ecoregional conservation planning for the Mesoamerican Caribbean Reef (MACR). *In:* MCFIELD, M. (ed.). WWF-ORE.

KRIEGER, K.J. & B.L. WING 2002. Megafauna associations with deepwater corals (Primnoa spp.) in the Gulf of Alaska. *Hydrobiologia,* 471**,** 83-90.

KUFFNER, I.B., J.C. BROCK, R. GROBER-DUNSMORE, V.E. BONITO, T.D. HICKEY & C.W. WRIGHT 2007. Relationships Between Reef Fish Communities and Remotely Sensed Rugosity Measurements in Biscayne National Park, Florida, USA. *Environmental Biology of Fishes,* 78**,** 71-82.

LAFFERTY, K.D. 1997. Envrionmental parasitology: what can parasites tell us about human impacts on the environment. *Parasitology Today,* 13**,** 251-255.

LAFFERTY, K.D., J.W. PORTER & S.E. FORD 2004. Are Diseases Increasing in the Ocean? *Annual Review of Ecology, Evolution, and Systematics,* 35**,** 31-54.

LAFORSCH, C., E. CHRISTOPH, C. GLASER, M. NAUMANN, C. WILD & W. NIGGL 2008. A precise and non-destructive method to calculate the surface area in living scleractinian corals using X-ray computed tomography and 3D modeling. *Coral Reefs,* 27**,** 811-820.

LANFEAR, R., B. CALCOTT, S.Y.W. HO & S. GUINDON 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution,* 29**,** 1695-1701.

LASKER, C.G.-R.A.H.R. 2004. Reproductive biology, devlopment, and planula behavior in the Caribbean gorgonian *Pseudopterogorgia elisabethae*. *Invertebrate Biology,* 123**,** 54-67.

LASKER, H. 2005. Gorgonian mortality during a thermal even in the bahamas. *Bulletin of Marine Science,* 76**,** 155-162.

LASKER, H.R. 1983. Vegetative reproduction in the octocoral *Briareum asbestinum*. *Journal of Experimental Marine Biology and Ecology,* 72**,** 157-169.

LASKER, H.R. 1984. Asexual reproduction, fragmentation, and skeletal morphology of a plexaurid gorgonian. *Marine Ecology Progress Series,* 19**,** 261-268.

LASKER, H.R. 2013. Recruitment and resilience of a harvested Caribbean octocoral. *PLoS One,* 8**,** e74587.

LASKER, H.R. & M.A. COFFROTH 1983. Octocoral distribution at Carrie Bow Cay, Belize. *Marine Ecology Progress Series,* 13**,** 21-28.

LASKER, H.R. & M.A. COFFROTH 1988. Temporal and spatial variability among grazers: variability in the distribution of the gastropod *Cyphoma gibbosum* on octocorals. *Marine Ecology Progress Series,* 43**,** 285-295.

LASKER, H.R., M.A. COFFROTH & L.M. FITZGERALD 1988. Foraging Patterns of *Cyphoma gibbosum* on Octocorals: the roles of host choice and feeding preference. *Biological Bulletin,* 174**,** 254-266.

LASKER, H.R. & I. PORTO-HANNES 2015. Population structure among octocoral adults and recruits identifies scale dependent patterns of population isolation in The Bahamas. *PeerJ,* 3**,** e1019.

LECHOWICZ, M.J. 1982. The sampling characteristics of electivity indices. *Oecologia,* 52**,** 22-30.

LEENHARDT, P., V. STELZENMÜLLER, N. PASCAL, W.N. PROBST, A. AUBANEL, T. BAMBRIDGE, M. CHARLES, E. CLUA, F. FÉRAL, B. QUINQUIS, B. SALVAT & J. CLAUDET 2017. Exploring social-ecological dynamics of a coral reef resource system using participatory modeling and empirical data. *Marine Policy,* 78**,** 90-97.

LENZ, E.A., L. BRAMANTI, H.R. LASKER & P.J. EDMUNDS 2015. Long-term variation of octocoral populations in St. John, US Virgin Islands. *Coral Reefs.*

LESSER, M.P., J.C. BYTHELL, R.D. GATES, R.W. JOHNSTONE & O. HOEGH-GULDBERG 2007. Are infectious diseases really killing corals? Alternative interpretations of the experimental and ecological data. *Journal of Experimental Marine Biology and Ecology,* 346**,** 36-44.

LESSIOS, H., D. ROBERTSON & J. CUBIT 1983. Spread of *Diadema* mass mortality through the Caribbean. *Science,* 226**,** 335-337.

LEVERETTE, C.L., M. WARREN, M.A. SMITH & G.W. SMITH 2008. Determination of carotenoid as the purple pigment in Gorgonia ventalina sclerites using Raman microscopy. *Spectrochimica Acta Part A,* 69**,** 1058-61.

LIGHTY, R., I. MACINTYRE & R. STUCKENRATH 1978. Submerged early Holocene barrier reef south-east Florida shelf. *Nature,* 276**,** 59-60.

LINDEL, T., P.R. JENSEN, W. FENICAL, B.H. LONG, A.M. CASAZZA, J. CARBONI & C.R. FAIRCHILD 1997. Eleutherobin, a new cytotoxin that mimics paclitaxel (taxol) by stabilizing microtubules. *J. Am. Chem. Soc.,* 119**,** 8744-8745.

LOOK, S.A., W. FENICAL, G.K. MATSUMOTO & J.CLARDY 1986. The pseudopterosins: a new class of anti-inflammatory and analgesic diterpene pentosides from the marine sea whip *Pseudopterogorgia elisabethae* (Octocorallia). *J. Org. Chem.,* 51**,** 5140-5145.

LUCKHURST, B. & K. LUCKHURST 1978. Analysis of the influence of substrate variables on coral reef fish communities. *Marine Biology,* 49**,** 317-323.

MACARTHUR, R. 1955. Fluctuations of animal populations and a measure of community stability. *Ecology,* 36**,** 533-536.

MACARTHUR, R.H. 1957. On the relative abundance of bird species. *Proceedings of the National Academy of Sciences,* 43**,** 293-295.

MACARTHUR, R.H. & J.W. MACARTHUR 1961. On bird species diversity. *Ecology,* 42**,** 594-598.

MAGURRAN, A.E. 2004. *Measuring biological diversity,* USA, UK, and Australia, Blackwell Publishing.

MAIER, C., J. HEGEMAN, M.G. WEINBAUER & J.P. GATTUSO 2009. Calcification of the cold-water coral *Lophelia pertusa* under ambient and reduced pH. *Biogeosciences,* 6**,** 1671-1680.

MANDELBROT, B.B. 1977. *Fractals*, Wiley Online Library.

MARTIN, B.D. & E. SCHWAB 2012. Current Usage of Symbiosis and Associated Terminology. *International Journal of Biology,* 5.

MARTIN, D., J. NÚÑEZ, R. RIERA & J. GIL 2002. On the associations between Haplosyllis (Polychaeta, Syllidae) and gorgonians (Cnidaria, Octocorallaria), with the description of a new species. *Biological Journal of the Linnean Society,* 77**,** 455-477.

MATEOS, M., L.A. HURTADO, C.A. SANTAMARIA, V. LEIGNEL & D. GUINOT 2012. Molecular systematics of the deep-sea hydrothermal vent endemic Brachyuran family Bythograeidae: a comparison of three Bayesian species tree methods. *PLoS One,* 7**,** e32066.

MAY, R.M. 1986. The search for patterns in the balance of nature: advances and retreats. *Ecology,* 67**,** 1115-1126.

MCARDLE, B.H. & M.J. ANDERSON 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology,* 82**,** 290-297.

MCARTHUR, A.G. 1985. Cnidarians and copepods: a success story. *Transactions of the American Microscopial Society,* 104**,** 313-320.

MCCOOK, L.J. 1999. Macroalgae, nutrients and phase shifts on coral reefs: scientific issues and management consequences for the Great Barrier Reef. *Coral Reefs,* 18**,** 357-367.

MCFADDEN, C.S., Y. BENAYAHU, E. PANTE, J.N. THOMA, P.A. NEVAREZ & S.C. FRANCE 2011. Limitations of mitochondrial gene barcoding in Octocorallia. *Molecular Ecology Resources,* 11**,** 19-31.

MCFADDEN, C.S., A.S. BROWN, C. BRAYTON, C.B. HUNT & L.P. VAN OFWEGEN 2014. Application of DNA barcoding in biodiversity studies of shallow-water octocorals: molecular proxies agree with morphological estimates of species richness in Palau. *Coral Reefs.*

MCFADDEN, C.S., R. DONAHUE, B.K. HADLAND & R. WESTON 2001. A molecular phylogenetic analysis of reproductive trait evolution in the soft coral genus *Alcyonium*. *Evolution, 55***,** 54-67.

MCFADDEN, C.S., S.C. FRANCE, J.A. SANCHEZ & P. ALDERSLADE 2006. A molecular phylogenetic analysis of the Octocorallia (Cnidaria : Anthozoa) based on mitochondrial protein-coding sequences. *Molecular Phylogenetics and Evolution,* 41**,** 513-527.

MCFADDEN, C.S. & M.B. HUTCHINSON 2004. Molecular evidence for the hybrid origin of species in the soft coral genus *Alcyonium* (Cnidaria : Anthozoa : Octocorallia). *Molecular Ecology,* 13**,** 1495-1505.

MCFADDEN, C.S., L.D. TULLIS, M.B. HUTCHINSON, K. WINNER & J.A. SOHM 2004. Variation in coding (NADH dehydrogenase subunits 2, 3, and 6) and noncoding intergenic spacer regions of the mitochondrial genome in Octocorallia (Cnidaria : Anthozoa). *Marine Biotechnology,* 6**,** 516-526.

MCFADDEN, C.S. & L.P. VAN OFWEGEN 2012. Stoloniferous octocorals (Anthozoa, Octocorallia) from South Africa, with descriptions of a new family of Alcyonacea, a new genus of Clavulariidae, and a new species of Cornularia (Cornulariidae). *Invertebrate Systematics,* 26**,** 331.

MCFADDEN, C.S. & L.P. VAN OFWEGEN 2013. A second, cryptic species of the soft coral genus Incrustatus (Anthozoa: Octocorallia: Clavulariidae) from Tierra del Fuego, Argentina, revealed by DNA barcoding. *Helgoland Marine Research,* 67**,** 137-147.

MCGREGOR, T. & S. WILLS 2016. Natural assests: surfing a wave of economic growth.

MCLEAN, E. & P. YOSHIOKA 2007. Associations and interactions between gorgonians and sponges. *In:* CUSTÓDIO, M., LÔBO-HAJDU, G., HAJDU, E. & MURICY, G. (eds.) *Porifera Research: Biodiversity, Innovation and Sustainability.* Museu Nacional de Rio de Janeiro, Brasil.

MCMAHON, T. 1973. Size and shape in biology: elastic criteria impose limits on biological proportions, and consequently on metabolic rates. *Science,* 179**,** 1201-1204.

MCMURRAY, S.E., C.M. FINELLI & J.R. PAWLIK 2015. Population dynamics of giant barrel sponges on Florida coral reefs. *Journal of Experimental Marine Biology and Ecology,* 473**,** 73-80.

MIGNÉ, A. & D. DAVOULT 2002. Experimental nutrition in the soft coral *Alcyonium digitatum* (Cnidaria: Octocorallia): removal rate of phytoplankton and zooplankton. *Cahiers de Biologie Marine,* 43**,** 9-16.

MINH, B.Q., M.A. NGUYEN & A. VON HAESELER 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution,* 30**,** 1188-95.

MIYAZAKI, Y. & J.D. REIMER 2015. A new genus and species of octocoral with aragonite calcium-carbonate skeleton (Octocorallia, Helioporacea) from Okinawa, Japan. *Zookeys***,** 1-23.

MÖLLER, H. 1987. Pollution and parasitism in the aquatic environment. *International Journal of Parasitology,* 17**,** 353-361.

MONCADA, S., S.H. FERREIRA & J.R. VANE 1975. Inhibition of prostaglandin biosynthesis as the mechanism of analgesia of aspirin-like drugs in the dog knee joint. *European Journal of Pharmacology,* 31**,** 250-260.

MORA, C. 2008. A clear human footprint in the coral reefs of the Caribbean. *Proc Biol Sci,* 275**,** 767-73.

MOTOMURA, I. 1932. On the statistical treatment of communities. *Zool. Mag,* 44**,** 379-383.

MULLEN, K.M., E.C. PETERS & C.D. HARVELL 2004. Coral resistance to disease. *In:* ROSENBERG, E. & LOYA, Y. (eds.) *Coral Health and Disease.* Springer-Verlag.

MUMBY, P.J. 2009. Phase shifts and the stability of macroalgal communities on Caribbean coral reefs. *Coral Reefs,* 28**,** 761-773.

MUMBY, P.J., A. HASTINGS & H.J. EDWARDS 2007. Thresholds and the resilience of Caribbean coral reefs. *Nature,* 450**,** 98-101.

MUSCATINE, L. & J.W. PORTER 1977. Reef corals: mualistic symbioses adapted to nutrient-poor environments. *Bioscience,* 27**,** 454-460.

MUZIK, K. 1982. Octocorallia (Cniaria) from Carrie Bow Cay, Belize. *In:* RÜTZLER, K. & MACINTYRE, I. G. (eds.) *The Atlantic Barrier Reef Ecosystem at Carrie Bow Cay, Belize, I: Structure and communities.* Washington, D.C.: Smithsonian Institution Press.

MYDLARZ, L.D., R.S. JACOBS, J. BOEHNLEIN & R.G. KERR 2003. Pseudopterosin Biosynthesis in Symbiodinium sp., the Dinoflagellate Symbiont of Pseudopterogorgia elisabethae. *Chemistry & Biology,* 10**,** 1051-1056.

NAGELKERKEN, I., K. BUCHAN, G.W. SMITH, K. BONAIR, P. BUSH, J. GARZÓN-FERREIRA, L. BOTERO, P. GAYLE, C.D. HARVELL, C. HEBERER, K. KIM, C. PETROVIC, L. PORS & P.

YOSHIOKA 1997. Widespread disease in Caribbean sea fans: II. Patterns of infection and tissue loss. *Marine Ecology Progress Series,* 160**,** 255-263.

NAROZANSKI, A., S. BOX & G. STOYLE. Developing management tools to enchance efficiency of marine protected area in Honduras. Proceedings of the 65th Gulf and Caribbean Fisheries Institute, 2012 Santa Marta, Colombia.

NELLEMANN, C., S. HAIN & J. ALDER (eds.) 2008. *In Dead Water - Merging of climate change with pollution, over-harvest, and infestation in the world's fishing grounds,* Norway: GRID-Arendal.

NELSON, G. 1989. Cladistics and evoutionary models. *Cladistics,* 5**,** 275-289.

NETHERTON, S.E., D.M. SCHEER, P.R. MORRISON, A.P. PARRIN & N.W. BLACKSTONE 2014. Physiological correlates of symbiont migration during bleaching of two octocoral species. *J Exp Biol,* 217**,** 1469-77.

NEWMAN, S.P., E.H. MEESTERS, C.S. DRYDEN, S.M. WILLIAMS, C. SANCHEZ, P.J. MUMBY & N.V. POLUNIN 2015. Reef flattening effects on total richness and species responses in the Caribbean. *J Anim Ecol,* 84**,** 1678-89.

NISHIDA, R. 2002. Sequestration of defensive subtances from plants by Lepidoptera. *Annual Review Entomology,* 47**,** 57-92.

NORRIS, R.D. & P.M. HULL. 2011. The temporal dimension of marine speciation. *Evolutionary Ecology* [Online].

NORSTRÖM, A.V., M. NYSTRÖM, J. LOKRANTZ & C. FOLKE 2009. Alternative states on coral reefs: beyond coral–macroalgal phase shifts. *Marine Ecology Progress Series,* 376**,** 295-306.

NOWLIS, J.P. 1993. Mate and oviposition-influence host preferences in the coral-feeding snail *Cyphoma gibbosum. Ecology,* 74**,** 1959-1969.

O'NEAL, W. & J. PAWLIK 2002. A reappraisal of the chemical and physical defenses of Caribbean gorgonian corals against predatory fishes. *Marine Ecology Progress Series,* 240**,** 117-126.

OCAÑA, O., J.C.D. HARTOG & L.P.V. OFWEGEN 2004. Ring sea anemones, an overview (Cnidaria, Anthozoa, Actiniaria). *Graellsia,* 60**,** 143-154.

OKSANEN, J., F.G. BLANCHET, M. FRIENDLY, R. KINDT, P. LEGENDRE, D. MCGLINN, P.R. MINCHIN, R. O'HARA, G.L. SIMPSON, P. SOLYMOS, M. HENRY, H. STEVENS, E. SZOECS & H. WAGNER 2016. vegan: Community Ecology Package.

OPPEN, M.J.V., B.J. MCDONALD, B. WILLIS & D.J. MILLER 2001. The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence? *Molecular Biology and Evolution,* 18**,** 1315-1329.

OPRESKO, D.M. 1973. Abundance and distribution of shallow-water gorgonians in the area of Miami, Florida. *Bulletin of Marine Science,* 23**,** 535-558.

PAINE, R. 1966. Food web complexity and species diversity. *The American Naturalist,* 100**,** 65-75.

PAKES, M.J. & R.M. WOOLLACOTT 2008. Reproduction of the gorgonian Plexaura flexuosa in Bermuda. *Journal of Experimental Marine Biology and Ecology,* 357**,** 121-127.

PANDOLFI, J.M., R.H. BRADBURY, E. SALA, T.P. HUGHES, K.A. BJORNDAL, R.G. COOKE, D. MCARDLE, L. MCCLENACHAN, M.J. NEWMAN, G. PAREDES, R.R. WARNER & J.B. JACKSON 2003. Global trajectories of the long-term decline of coral reef ecosystems. *Science,* 301**,** 955-957.

PANTE, E., J. ABDELKRIM, A. VIRICEL, D. GEY, S.C. FRANCE, M.C. BOISSELIER & S. SAMADI 2015a. Use of RAD sequencing for delimiting species. *Heredity (Edinb),* 114**,** 450-9.

PANTE, E., N. PUILLANDRE, A. VIRICEL, S. ARNAUD-HAOND, D. AURELLE, M. CASTELIN, A. CHENUIL, C. DESTOMBE, D. FORCIOLI, M. VALERO, F. VIARD & S. SAMADI 2015b. Species are hypotheses: avoid connectivity assessments based on pillars of sand. *Mol Ecol,* 24**,** 525-44.

PARIS, C.B. & L.M. CHÉRUBIN 2008. River-reef connectivity in the Meso-American Region. *Coral Reefs,* 27**,** 773-781.

PARK, E., D.-S. HWANG, J.-S. LEE, J.-I. SONG, T.-K. SEO & Y.-J. WON 2012. Estimation of divergence times in cnidarian evolution based on mitochondrial protein-coding genes and the fossil record. *Molecular Phylogenetics and Evolution,* 62**,** 329-345.

PARRIN, A.P., T.L. GOULET, M.A. YAEGER, L.S. BROSS, C.S. MCFADDEN & N.W. BLACKSTONE 2016. Symbiodinium migration mitigates bleaching in three octocoral species. *Journal of Experimental Marine Biology and Ecology,* 474**,** 73-80.

PAUL, V.J., R. RITSON-WILLIAMS & K. SHARP 2011. Marine chemical ecology in benthic environments. *Nat Prod Rep,* 28**,** 345-87.

PAULY, D., R. WATSON & J. ALDER 2005. Global trends in world fisheries: impacts on marine ecosystems and food security. *Philos Trans R Soc Lond B Biol Sci,* 360**,** 5-12.

PAWLIK, J.R. 1992. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanography and Marine Biology Annual Reviews,* 30**,** 273-335.

PAWLIK, J.R. 1993. Marine invertebrate chemical defenses. *Chemical Review,* 93**,** 1911-1922.

PAWLIK, J.R. 2012. Antipredatory Defensive Roles of Natural Products from Marine Invertebrates. 677-710.

PAWLIK, J.R., M.T. BURCH & W. FENICAL 1987. Patterns of chemical defenses among Caribbean gorgonian corals: a prliminary survey. *Journal of Experimental Marine Biology and Ecology,* 108**,** 55-66.

PAWLIK, J.R., T.L. LOH, S.E. MCMURRAY & C.M. FINELLI 2013. Sponge communities on Caribbean coral reefs are structured by factors that are top-down, not bottom-up. *PLoS One,* 8**,** e62573.

PETERSON, G., C.R. ALLEN & C. HOLLING 1998. Ecological resilience, biodiversity, and scale. *Ecosystems,* 1**,** 6-18.

PETRAITIS, P.S. & S.R. DUDGEON 2004. Detection of alternative stable states in marine communities. *Journal of Experimental Marine Biology and Ecology,* 300**,** 343-371.

PIGLIUCCI, M. 2003. Species as family resemblance concepts: the (dis-)solution of the species problem? *Bioessays,* 25**,** 596-602.

PIKE, J.E. 1974. The prostaglandins: their history, development and potential. *In:* BAYER, F. M., WEINHEIMER, A. J. & VOSS, G. L. (eds.) *Prostaglandins from Plexaura homomalla: Ecology, Utilization and Conservation of a Major Medical Marine Resource: A Symposium.* Coral Gables, Florida: University of Miami Press.

PINET, P.R. 1976. Morphology off northern Honduras, northwestern Caribbean Sea. *Deep-Sea Research,* 23**,** 839-847.

POSADA, D. 2008. jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution,* 25**,** 1253-1256.

PRADA, C. & M.E. HELLBERG 2013. Long prereproductive selection and divergence by depth in a Caribbean candelabrum coral. *PNAS,* 110**,** 3961-3966.

PRADA, C., N.V. SCHIZAS & P.M. YOSHIOKA 2008. Phenotypic plasticity or speciation? A case from a clonal marine organism. *BMC Evolutionary Biology,* 8**,** 1-19.

PRADA, C., E. WEIL & P.M. YOSHIOKA 2010. Octocoral bleaching during unusual thermal stress. *Coral Reefs,* 29**,** 41-45.

PRESTON, F. 1948. The commonness, and rarity, of species. *Ecology,* 29**,** 254-283.

PUCE, S., C.G. DI CAMILLO & G. BAVESTRELLO 2008. Hydroids symbiotic with octocorals from the Sulawesi Sea, Indonesia. *Journal of the Marine Biological Association of the United Kingdom,* 88**,** 1643.

PUILLANDRE, N., A. LAMBERT, S. BROUILLET & G. ACHAZ 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol Ecol,* 21**,** 1864-77.

QUINN, G.P. & M.J. KEOUGH 2002. *Experimental Design and Data Analysis for Biologists,* Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo, Cambridge University Press.

R CORE TEAM 2015. R: a language and environment for statistical computing. *R Foundation for Statistical Computing.* Vienna, Austria.

RAMBAUT, A. & A.J. DRUMMOND 2007. Tracer v1.6.

RAVEN, J.K., H. CALDEIRA, O. ELDERFIELD, P. HOEGH-GULDBERG, U.L.U. RIEBESELL, J. SHEPHERD, C.T. AND & A. WATSON 2005. Ocean acidification due to increasing atmospheric carbon dioxide. *London, Royal Society*.

REAKA-KUDLA, M.L. 1997. The global diversity of coral reefs: a comparison with rain forests. *In:* REAKA-KUDLA, M. L., WILSON, D. E. & WILSON, E. O. (eds.) *Biodiversity II: Understanding and Protecting Our Biological Resources.* Washington, D.C.: Joseph Henry Press.

REIJNEN, B.T., B.W. HOEKSEMA & E. GITTENBERGER 2010. Host specificity and phylogenetic relationships among Atlantic Ovulidae (Mollusca: Gastropoda). *Contributions to Zoology,* 79**,** 69-78.

REIJNEN, B.T., S.E. VAN DER MEIJ & L.P. VAN OFWEGEN 2011. Fish, fans and hydroids: host species of pygmy seahorses. *Zookeys***,** 1-26.

RIEGL, B. & G.M. BRANCH 1994. Effects of sediment on the energy budgets of four scleractinian (Bourne 1900) and five alcyonacean (Lamouroux 1816) corals. *Journal of Experimental Marine Biology and Ecology,* 186**,** 259-275.

ROBERTS, C.M. 2002. Deep impact: the rising toll of fishing in the deep sea. *TRENDs in Ecology & Evolution,* 17**,** 242-245.

ROBERTS, J.M., A. WHEELER, A. FREIWALD & S. CAIRNS 2009. *Cold-Water Corals: The Biology and Geology of Deep-Sea Coral Habitats,* USA, Cambridge University Press, New York.

ROBERTS, J.M., A.J. WHEELER & A. FREIWALD 2006. Reefs of the deep: the biology and geology of cold-water coral ecosystems. *Science,* 312**,** 543-7.

ROBERTSON, V., B. HALTLI, E.P. MCCAULEY, D.P. OVERY & R.G. KERR 2016. Highly Variable Bacterial Communities Associated with the Octocoral Antillogorgia elisabethae. *Microorganisms,* 4.

ROCHA, J., L. PEIXE, N.C. GOMES & R. CALADO 2011. Cnidarians as a source of new marine bioactive compounds--an overview of the last decade and future steps for bioprospecting. *Mar Drugs,* 9**,** 1860-86.

RODRÍGUEZ, A.D. 1995. The natural products chemistry of West Indian gorgonian octocorals. *Tetrahedron,* 51**,** 4571-4618.

RODRÍGUEZ, A.D. & C. RAMÎREZ 1994. Further butenolides from the Caribbean octocoral *Pterogorgia citrina*. *Journal of Natural Products,* 57**,** 339-347.

RODRÍGUEZ, A.D., J.-G. SHI & S.D. HUANG 1999. Highly oxygenated pseydopterane and cembranolide dipterpenes from the Caribbean sea feather *Pseudopterogorgia bipinnata*. *Journal of Natural Products,* 62**,** 1228-1237.

ROFF, G. & P.J. MUMBY 2012. Global disparity in the resilience of coral reefs. *Trends Ecol Evol,* 27**,** 404-13.

ROGERS, A.D. 2004. The biology, ecology and vulnerability of deep-water coral reefs. Cambridge: British Antarctic Survey.

ROGERS, A.D. & K. SACK 2012. Not seeing the wood for the trees. *Global Policy,* 3**,** 515-516.

ROGERS, A.D., C. YESSON & P. GRAVESTOCK 2012. A biophysical, economic and legal profile of South Georgia and the South Sanwich Islands. PEW Environmental Group.

ROSENBERG, E., O. KOREN, L. RESHEF, R. EFRONY & I. ZILBER-ROSENBERG 2007. The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol,* 5**,** 355-62.

ROSENBERG, G. 1989. Aposematism evolves by individual selection: evidence from marine gastropods with pelagic larvae. *Evolution,* 43**,** 1811-1813.

ROSENBERG, G. 1991. Aposematism and synergistic selection in marine gastropods. *Evolution,* 45**,** 451-454.

ROUSSIS, V., Z. WU & W. FENICAL 1990. New antiinflammatory pseydopterosins from the marine octocoral *Pseudopterogorgia elisabethae*. *Journal of Organic Chemistry,* 55**,** 4916-4922.

ROXBURGH, S.H., K. SHEA & J.B. WILSON 2004. The intermediate disturbance hypothesis: patch dynamics and mechanisms of species coexistence. *Ecology,* 85**,** 359-371.

RUBNER, M. 1883. Ueber den Einfluss der Körpergrosse auf Stoff- und Kraft- wechsel. *Zeitschrift fur Biologie,* 19**,** 536-562.

RUEDA, A., E. ZUBÍA, M.J. ORTEGA & J. SALVÁ 2001. Structure and cytotoxicity of new polyhydroxylated sterols from the Caribbean gorgonian *Plexaurella grisea*. *Steroids,* 66**,** 897-904.

RUESINK, J. & C.D. HARVELL 1990. Specialist predation on the Caribbean gorgonian *Plexaurella* spp. by *Cyphoma signatum* (Gastropoda). *Marine Ecology Progress Series,* 65**,** 265-272.

RUZICKA, R.R., M.A. COLELLA, J.W. PORTER, J.M. MORRISON, J.A. KIDNEY, V. BRINKHUIS, K.S. LUNZ, K.A. MACAULAY, L.A. BARTLETT, M.K. MEYERS & J. COLEE 2013. Temporal changes in benthic assemblages on Florida Keys reefs 11 years after the 1997/1998 El Niño. *Marine Ecology Progress Series,* 489**,** 125-141.

RYPIEN, K.L. 2008. *The origins and spread of Aspergillus sydowii, an opportunistic pathogen of Caribbean gorgonian corals.* Doctor of Philosophy, Cornell University.

RYPIEN, K.L. & D.M. BAKER 2009. Isotopic labeling and antifungal resistance as tracers of gut passage of the sea fan pathogen Aspergillus sydowii. *Diseases of Aquatic Organisms,* 86**,** 1-7.

SÁNCHEZ, J.A. 2009. Systematics of the candelabrum gorgonian corals (*Eunicea* Lamouroux; Plexauridae; Octocorallia; Cnidaria). *Zoological Journal of the Linnean Society,* 157**,** 237-263.

SÁNCHEZ, J.A. & S.D. CAIRNS 2004. An unusual new gorgonian coral (Anthozoa: Octocorallia) from the Aleutian Islands, Alaska. *Zoologische Mededelingen,* 78**,** 1-10.

SÁNCHEZ, J.A., J.M. DÍAZ & S. ZEA 1997. Gorgonian communities in two contrasting environments on oceanic atolls of the Southwestern Caribbean. *Bulletin of Marine Science,* 61**,** 453-465.

SÁNCHEZ, J.A. & D. DORADO. Intragenomic ITS2 variation in Caribbean seafans. Proceedings of the 11th International Coral Reef Symposium, 7-11 July 2008 2008 Ft. Lauderdale, Florida.

SANCHEZ, J.A., A.P. FUENTES-PARDO, I. NI ALMHAIN, N.E. ARDILA-ESPITIA, J. CANTERA-KINTZ & M. FORERO-SHELTON 2016. The masquerade game: marine mimicry adaptation between egg-cowries and octocorals. *PeerJ,* 4**,** e2051.

SÁNCHEZ, J.A., H.R. LASKER & D.J. TAYLOR 2003a. Phylogenetic analyses among octocorals (Cnidaria): mitochondrial and nuclear DNA sequences (lsu-rRNA, 16S and ssu-rRNA, 18S) support two convergent clades of branching gorgonians. *Molecular Phylogenetics and Evolution,* 29**,** 31-42.

SÁNCHEZ, J.A., C.S. MCFADDEN, S.C. FRANCE & H.R. LASKER 2003b. Molecular phylogenetic analyses of shallow-water Caribbean octocorals. *Marine Biology,* 142**,** 975-987.

SÁNCHEZ, J.A. & H.H. WIRSHING 2005. A field key to the identification of tropical Western Atlantic zooxanthellate octocorals (Octocorallia: Cnidaria). *Caribbean Journal of Science,* 41**,** 508-522.

SÁNCHEZ, J.A., S. ZEA & J.M. DÍAZ 1998. Patterns of octocoral and black coral distribution in the oceanic barrier reef-complex of Providencia Island, Southwestern Caribbean. *Caribbean Journal of Science,* 34**,** 250-264.

SANTAVY, D., W. FISHER, J. CAMPBELL & R. QUARLES 2012a. Field manual for coral reef assessments. Gulf Breeze, FL.: U.S. Environmental Protection Agency, Office of Research and Development.

SANTAVY, D.L., L.A. COURTNEY, W.S. FISHER, R.L. QUARLES & S.J. JORDAN 2012b. Estimating surface area of sponges and gorgonians as indicators of habitat availability on Caribbean coral reefs. *Hydrobiologia,* 707**,** 1-16.

SATHE, M., D. GILLIAM, R. DODGE & L. FISHER. Patterns in southeast Florida coral reef community composition. Proceedings of the 11th International Coral Reef Symposium, 2008 Ft. Lauderdale, Florida.

SCAPS, P. & J. SAUNDERS 2011. Shallow Water Stony Corals (Scleractinia, Milleporidae, and Stylasteridae) from Útila and Cayos Cochinos, Honduras. *ISRN Zoology,* 2011**,** 1-9.

SCHÄRER, M.T. & M.I. NEMETH 2010. Mass mortality of gorgonians due to a Cyphoma gibbosum (Linnaeus) population outbreak at Mona Island, Puerto Rico. *Coral Reefs,* 29**,** 533-533.

SCHER, H.D. & E.E. MARTIN 2006. Timing and climatic consequences of the opening of Drake Passage. *Science,* 312**,** 428-430.

SCHNEIDER, C.A., W.S. RASBAND & K.W. ELICEIRI 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods,* 9**,** 671-675.

SEBENS, K.P. 1982. Competition for space: growth rate, reproductive output, and escape in size. *The American Naturalist,* 120**,** 189-197.

SERRANO, X.M., I.B. BAUMS, T.B. SMITH, R.J. JONES, T.L. SHEARER & A.C. BAKER 2016. Long distance dispersal and vertical gene flow in the Caribbean brooding coral Porites astreoides. *Sci Rep,* 6**,** 21619.

SHEARER, T.L., M.J.H.V. OPPEN, S.L. ROMANO & G. WÖRHEIDE 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria) *Molecular Ecology,* 11**,** 2475-2487.

SHEIL, D. & D.F. BURSLEM 2013. Defining and defending Connell's intermediate disturbance hypothesis: a response to Fox. *Trends Ecol Evol,* 28**,** 571-2.

SHINN, E.A., G.W. SMITH, J.M. PROSPERO, P. BETZER, M.L. HAYES, V. GARRISON & R.T. BARBER 2000. African dust and the demise of Caribbean Coral Reefs. *Geophysical Research Letters,* 27**,** 3029.

SHRIVES, J.P. 2010. *Investigating riverine impact upon the coral reefs of Los Cayos Cochinos, Honduras.* Doctor of Philosophy, University of Oxford.

SILLEN-TULLBERG, B. 1988. Evolution of gregariousness in aposematic butterfly larvae: a phylogenetic analysis. *Evolution,* 42**,** 293-305.

SIMPSON, A. 2009. *Reproduction in Octocorals (Subclass: Octocorallia): a review of published literature* [Online]. Available: http://www.ucs.louisiana.edu/~scf4101/Bambooweb/.

SLATTERY, M. 1999. Fungal pathogenesis of the sea fan *Gorgonia ventalina*: direct and indirect consequences. *Chemoecology,* 9**,** 97-104.

SMITH, C.R., F.C. DE LEO, A.F. BERNARDINO, A.K. SWEETMAN & P.M. ARBIZU 2008. Abyssal food limitation, ecosystem structure and climate change. *Trends Ecol Evol,* 23**,** 518-28.

SMITH, G., C. HARVELL & K. KIM 1998. Response of sea fans to infection with *Aspergillus* sp. (Fungi). *Revista de Biologia Tropical,* 46**,** 205-208.

SMITH, G.W., L.D. IVES, I.A. NAGELKERKEN & K.B. RITCHIE 1996. Caribbean sea-fan mortalities. *Nature Immunology,* 383**,** 487.

SMITH, M.A., B.L. FISHER & P.D. HEBERT 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. *Philos Trans R Soc Lond B Biol Sci,* 360**,** 1825-34.

SOUTHGATE, D. & M. ECUADOR 1992. Promoting resource degradation in Latin America: tropical deforestation, soil erosion, and coastal ecosystem disturbance in Ecuador. *Economic Development and Cultural Change,* 40**,** 787-807.

SOUZA, J.R.B., H.A. RODRIGUES, B.M. NEVES & C.D. PEREZ 2007. First report of bristleworm predator of the reef octocoral Carijoa riisei. *Coral Reefs,* 26**,** 1033-1033.

STEUDLER, P.A., F.J. SCHMITZ & L.S. CIERESZKO 1977. Chemistry of coelenterates. Sterol composition of some predator-prey pairs on coral reefs. *Comparative Biochemistry and Physiology Part B: Comparaitve Biochemistry,* 56B**,** 385-392.

STONE, R.P. 2006. Coral habitat in the Aleutian Islands of Alaska: depth distribution, fine-scale species associations, and fisheries interactions. *Coral Reefs,* 25**,** 229-238.

STRONG, E., T.J. GOREAU & R.L. HAYES 1998. Ocean hotspots and coral reef bleaching. January-July 1998. *Reef Encounter,* 24**,** 20-22.

SUTHERLAND, K.P., J.W. PORTER & C. TORRES 2004. Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. *Marine Ecology Progress Series,* 266**,** 273-302.

TAMURA, K., G. STECHER, D. PETERSON, A. FILIPSKI & S. KUMAR 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution,* 30**,** 2725-9.

TAYLOR, M.L. 2011. *Distribution and diversity of octocorals from longline by-catch around South Georgia, UK.* Doctor of Philosophy, Imperial College London.

TAYLOR, M.L. & A.D. ROGERS 2015. Evolutionary dynamics of a common sub-Antarctic octocoral family. *Mol Phylogenet Evol,* 84**,** 185-204.

TEEB 2010. The economics and ecosystems and biodiversity: mainstreaming the economics of nature: a synthesis of the approach, conclusions and recommendations of TEEB.

THIELE, K. 1993. The holy grail of the perfect character: the cladistic treatment of morphometric data. *Cladistics,* 9**,** 275-304.

THORNTON, R.S. & R.G. KERR 2002. Induction of pseudopterosin biosynthesis in the gorgonian *Pseudopterogorgia elisabethae*. *Journal of Chemical Ecology,* 28**,** 2083-2090.

TITTENSOR, D.P., A.R. BACO, J.M. HALL-SPENCER, J.C. ORR & A.D. ROGERS 2010. Seamounts as refugia from ocean acidification for cold-water stony corals. *Marine Ecology,* 31**,** 212-225.

TOLEDO-HERNÁNDEZ, C., V. GULIS, C.P. RUIZ-DIAZ, A.M. SABAT & P. BAYMAN 2013. When aspergillosis hits the fan: Disease transmission and fungal biomass in diseased versus healthy sea fans (Gorgonia ventalina). *Fungal Ecology,* 6**,** 161-167.

TOLEDO-HERNÁNDEZ, C., P. YOSHIOKA, P. BAYMAN & A.M. SABAT 2009. Impact of disease and detachment on growth and survivorship of sea fans Gorgonia ventalina. *Marine Ecology Progress Series,* 393**,** 47-54.

TOLEDO-HERNÁNDEZ, C., A. ZULUAGA-MONTERO, A. BONES-GONZÁLEZ, J.A. RODRÍGUEZ, A.M. SABAT & P. BAYMAN 2008. Fungi in healthy and diseased sea fans (Gorgonia ventalina): is Aspergillus sydowii always the pathogen? *Coral Reefs,* 27**,** 707-714.

TOMONO, Y., H. HIROTA, Y. IMAHARA & N. FUSETANI 1999. Four new steroids from two octocorals. *Journal of Natural Products,* 62**,** 1538-1541.

TORRES-SUAREZ, O.L. 2014. Gorgonia mariae and Antillogorgia bipinnata populations inferred from compensatory base change analysis of the internal transcribed spacer 2. *Mol Phylogenet Evol,* 79**,** 240-8.

TORTORA, L. & D. KEITH 1980. Octocorallia of the Swan Islands, Honduras. *Caribbean Journal of Science,* 15**,** 87-93.

UNEP 2000. Sustaining life on Earth: How the Convention on Bioloigcal Diversity promotes nature and human well-being. *In:* DIVERSITY, S. O. T. C. O. B. (ed.).

UNEP 2006. Marine and coastal ecosystems and human well-being: a synthesis report based on the findings of the Millennium Ecosystem Assessment. Nairobi, Kenya: UNEP.

UNEP-WCMC 2015. Experimental Biodiversity Accounting as a component of the System of Environmental-Economic Accounting Experimental Ecosystem Accounting (SEEA-

EEA). *Supporting document to the Advancing the SEEA Experimental Ecosystem Accounting project.* United Nations.

VAN DER HAM, J.L., M.R. BRUGLER & S.C. FRANCE 2009. Exploring the utility of an indel-rich, mitochondrial intergenic region as a molecular barcode for bamboo corals (Octocorallia: Isididae). *Mar Genomics,* 2**,** 183-92.

VAN ITEN, H., A.C. MARQUES, J.D.M. LEME, M.L.A.F. PACHECO, M.G. SIMÕES & A. SMITH 2014. Origin and early diversification of the phylum Cnidaria Verrill: major developments in the analysis of the taxon's Proterozoic-Cambrian history. *Palaeontology,* 57**,** 677-690.

VAN OPPEN, M.J., J.C. MIEOG, C.A. SANCHEZ & K.E. FABRICIUS 2005. Diversity of algal endosymbionts (zooxanthellae) in octocorals: the roles of geography and host relationships. *Mol Ecol,* 14**,** 2403-17.

VANDERPLOEG, H. & D. SCAVIA 1979. Two electivity indices for feeding with special reference to zooplankton grazing. *Journal of Fisheries Research Board Canada,* 36**,** 362-365.

VARGAS, S., O. BREEDY & H.M. GUZMAN 2010. The phylogeny of Pacifigorgia (Coelenterata, Octocorallia, Gorgoniidae): a case study of the use of continuous characters in the systematics of the Octocorallia. *Zoosystema,* 32**,** 5-18.

VELÁSQUEZ, J. & J.A. SÁNCHEZ 2015. Octocoral species assembly and coexistence in Caribbean coral reefs. *PLoS One,* 10**,** 1-16.

VERON, J.E.N. 1995. *Corals in space and time. The biogeography and evolution of the Scleractinia,* Sydney, Australia, University of New South Wales Press.

VOLLMER, S.V. & S.R. PALUMBI 2002. Hybridization and the evolution of reef coral diversity. *Science,* 296**,** 2023-2025.

VRANA, P. & W. WHEELER 1992. Individual organisms as terminal entities: laying the species problem to rest. *Cladistics,* 8**,** 67-72.

VROLIJK, N. & N. TARGETT 1992. Biotransformation enzymes in *Cyphoma gibbosum* (Gastropoda: Ovulidae): implications for detoxification of gorgonian allelochemicals. *Marine Ecology Progress Series,* 88**,** 237-246.

WAINWRIGHT, S.A. & J.R. DILLON 1969. On the orientation of sea fans (genus *Gonrgonia*). *Biological Bulletin,* 136**,** 130-139.

WAKEFORD, R.C., D.J. AGNEW, N. LIPPENS, J. MOIR-CLARK & A. PAYNE 2006. Developing recommendations to mitigate the impact of longlining on coldwater coral around South Georgia. *A report for the Government of South Georgia and the South Sandwich Islands.* Marine Resources Assessment Group.

WARD, J.R., K. KIM & C.D. HARVELL 2007. Temperature affects coral disease resistance and pathogen growth. *Marine Ecology Progress Series,* 329**,** 115-121.

WARD, R.D., F.O. COSTA, B.H. HOLMES & D. STEINKE 2008. DNA barcoding of shared fish species from the North Atlantic and Australasia: minimal divergence for most taxa, but Zeus faber and Lepidopus caudatus each probably constitute two species. *Aquatic Biology,* 3**,** 71-78.

WARD, R.D., T.S. ZEMLAK, B.H. INNES, P.R. LAST & P.D. HEBERT 2005. DNA barcoding Australia's fish species. *Philos Trans R Soc Lond B Biol Sci,* 360**,** 1847-57.

WATLING, L., S.C. FRANCE, E. PANTE & A. SIMPSON 2011. Biology of Deep-Water Octocorals. *In:* LESSER, M. (ed.) *Advances in Marine Biology.*

WEI, X., A.D. RODRÍGUEZ, P. BARAN, R.G. RAPTIS, J.A. SÁNCHEZ, E. ORTEGA-BARRIA & J. GONZÁLEZ 2004. Antiplasmodial cembradiene diterpenoids from a Southwestern Caribbean gorgonian octocoral of the genus Eunicea. *Tetrahedron,* 60**,** 11813-11819.

WEIL, E. 2004. Coral reef diseases in the wider Caribbean. *In:* ROSENBERG, E. & LOYA, Y. (eds.) *Coral health and disease.* Berlin: Springer.

WEINBERG, S. 1981. A comparison of coral reef survey methods. *Bijdragen tot de Dierkunde,* 51**,** 199-218.

WEINHEIMER, A.J. 1974. The discovery of 15-epi PGA_2 in *Plexaura homomalla In:* BAYER, F. M., WEINHEIMER, A. J. & VOSS, G. L. (eds.) *Prostaglandins from Plexaura homomalla: Ecology, Utilization and Conservation of a Major Medical Marine Resource.* Coral Gables, Florida: University of Miami Press.

WEINHEIMER, A.J. & R.L. SPRAGGINS 1969. Chemistry of coelenterates. XV: The occurence of two new prostaglandin derivatives (15-epi-PGA2 and its acetate, methyle ester) in the gorgonian *Plexaura homomalla*. *Tetrahedron Letters,* 59**,** 5185-5188.

WHALEN, K.E., V.R. STARCZAK, D.R. NELSON, J.V. GOLDSTONE & M.E. HAHN 2010. Cytochrome P450 diversity and induction by gorgonian allelochemicals in the marine gastropod Cyphoma gibbosum. *BMC Ecology,* 10**,** 24.

WIENS, J.J. 1999. Polymorphism in systematics and comparative biology. *Annual Review of Ecology, Evolution, and Systematics,* 30**,** 327-362.

WIENS, J.J. 2001. Character analysis in morphological phylogenetics: problems and solutions. *Systematic Biology,* 50**,** 689-699.

WILKINSON, C. 2008. Status of coral reefs of the world. Townsville, Australia: Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre.

WILLIAMS, G.C. & S.D. CAIRNS. 2011. *Systematic list of octocoral genera* [Online]. Available: http://researcharchive.calacademy.org/research/izg/OCTOCLASS.htm.

WILLIAMS, G.C. & P.J. LÓPEZ-GONZÁLEZ 2005. A new genus and species of gorgonian octocoral (Anthozoa: Plexauridae) from Antarctic waters. *Proceedings of the California Academy of Sciences,* 56**,** 379-390.

WILSON, J.B. 1991. Methods for fitting dominance/diversity curves. *Journal of Vegetation Science,* 2**,** 35-46.

WILSON, S.K., S.C. BURGESS, A.J. CHEAL, M. EMSLIE, R. FISHER, I. MILLER, N.V. POLUNIN & H.P. SWEATMAN 2008. Habitat utilization by coral reef fish: implications for specialists vs. generalists in a changing environment. *J Anim Ecol,* 77**,** 220-8.

WIRSHING, H.H. 2012. *Molecular ecology and evolution of reef corals and their algal symbionts.* University of Miami.

WIRSHING, H.H., C.G. MESSING, C.J. DOUADY, J. REED, M.J. STANHOPE & M.S. SHIVJI 2005. Molecular evidence for multiple lineages in the gorgonian family Plexauridae (Anthozoa: Octocorallia). *Marine Biology,* 147**,** 497-508.

WON, J.H., B.J. RHO & J.I. SONG 2001. A phylogenetic study of the Anthozoa (phylum Cnidaria) based on morphological and molecular characters. *Coral Reefs,* 20**,** 39-50.

WOOD, R. 1998. The ecological evolution of reefs. *Annual Review of Ecology and Systematics,* 29**,** 179-206.

WOODLEY, C., A. BRUCKNER, S. GALLOWAY, S. MCLAUGHLIN, C. DOWNS, J. FAUTH, E. SHOTTS & K. LIDIE 2003. Coral Disease and Health: A National Research Plan. Silver Spring, MD: National Oceanic and Atmospheric Administration.

WULFF, J. 2001. Assessing and monitoring coral reef sponges: why and how? *Bulletin of Marine Science,* 69**,** 831-846.

YESSON, C., M.L. TAYLOR, D.P. TITTENSOR, A.J. DAVIES, J. GUINOTTE, A. BACO, J. BLACK, J.M. HALL-SPENCER & A.D. ROGERS 2012. Global habitat suitability of cold-water octocorals. *Journal of Biogeography,* 39**,** 1278-1292.

YOSHIOKA, P.M. & B.B. YOSHIOKA 1989. Effects of wave energy, topographic relief and sediment transport on the distribution of shallow-water gorgonians of Puerto Rico. *Coral Reefs,* 8**,** 145-152.

ZHENG, J.J., C.L. SHAO, M. CHEN, L.S. GAN, Y.C. FANG, X.H. WANG & C.Y. WANG 2014. Ochracenoids A and B, guaiazulene-based analogues from gorgonian Anthogorgia ochracea collected from the South China Sea. *Mar Drugs,* 12**,** 1569-79.

ZIPF, G.K. 1949. *Human Behaviour and the Principle of Least Effort,* New York: Hafner, Addison-Wesley.

8 Appendix A

Raw data of octocoral species abundances of Útila by site and depths. Begins on next page.

Species	South Shore Sites								North Shore Sites				Sites Combined				Total
	Sturge's Bank (SB)		Coral View (CV)		Little Bight (LB)		Stingray Point (SRP)		Spotted Bay (SpB)		The Maze (M)		South Shore Sites		North Shore Sites		
	5m	15m	5m	15m	5m	15m	5m	15m	5m	15m	5m	15m	5m	15m	5m	15m	
Antillogorgia acerosa	3	0	1	1	3	0	2	2	0	6	2	0	9	3	2	6	20
Antillogorgia albatrossae	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	2
Antillogorgia americana	0	1	1	1	4	2	5	2	1	10	13	1	10	6	14	11	41
Antillogorgia elisabethae	36	132	63	104	10	47	2	55	2	39	4	2	111	338	6	41	496
Antillogorgia hummelincki	2	1	0	6	1	0	2	0	0	0	1	0	5	7	1	0	13
Antillogorgia kallos	0	1	0	0	5	0	0	2	0	0	0	0	5	3	0	0	8
Antillogorgia rigida	0	0	0	0	0	0	0	0	0	1	1	2	0	0	1	3	4
Antillogorgia sp.	0	2	1	5	1	3	0	2	2	0	0	1	2	12	2	1	17
Briareum asbestinum	9	0	0	0	2	0	0	0	31	7	15	1	11	0	46	8	65
Erythropodium sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1
Eunicea asperula	0	0	0	1	0	1	0	1	0	0	0	1	0	3	0	1	4
Eunicea calyculata forma *calyculata*	0	0	0	0	0	0	2	0	0	1	0	0	2	0	0	1	3
Eunicea calyculata forma *coronata*	1	0	1	2	0	0	0	1	0	0	3	1	2	3	3	1	9
Eunicea clavigera	4	4	3	7	1	24	2	3	1	5	0	10	10	38	1	15	64
Eunicea flexuosa	1	1	7	2	7	2	6	2	5	1	12	5	21	7	17	6	51
Eunicea fusca	0	1	0	2	18	4	3	16	12	25	35	13	21	23	47	38	129
Eunicea laciniata	0	0	0	1	0	1	0	0	0	3	0	1	0	2	0	4	6
Eunicea laxispica	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1
Eunicea mammosa	0	1	1	3	3	5	8	6	4	5	11	2	12	15	15	7	49
Eunicea palmeri	0	0	0	0	0	0	0	0	0	0	3	1	0	0	3	1	4
Eunicea sp.	1	3	2	4	25	11	7	15	15	17	15	18	35	33	30	35	133
Eunicea succinea forma *plantaginea*	2	0	1	0	7	4	0	3	1	0	3	1	10	7	4	1	22
Eunicea succinea forma *typical*	0	0	0	0	0	1	1	0	0	0	1	0	1	1	1	0	3
Eunicea tourneforti	0	0	0	0	1	0	0	0	0	0	2	0	1	0	2	0	3

Species	South Shore Sites								North Shore Sites				Sites Combined				Total
	Sturge's Bank (SB)		Coral View (CV)		Little Bight (LB)		Stingray Point (SRP)		Spotted Bay (SpB)		The Maze (M)		South Shore Sites		North Shore Sites		
	5m	15m	5m	15m	5m	15m	5m	15m	5m	15m	5m	15m	5m	15m	5m	15m	
Gorgonia mariae	3	0	3	8	3	10	4	2	3	16	30	11	13	20	33	27	93
Gorgonia ventalina	6	0	53	1	36	2	28	8	10	3	43	1	123	11	53	4	191
Iciligorgia schrammi	0	0	0	0	0	3	0	0	0	0	0	0	0	3	0	0	3
Muricea atlantica	0	0	1	0	1	0	0	0	0	0	1	0	2	0	1	0	3
Muricea laxa	0	0	0	0	0	0	1	1	1	1	0	0	1	1	1	1	4
Muricea muricata	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1
Muricea pinnata	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1
Muricea waltonsmithii	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2	0	2
Muriceopsis flavida	0	0	5	0	4	1	1	6	1	0	4	15	10	7	5	15	37
Muriceopsis petila	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	2
Muriceopsis sp.	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	7	7
Plexaura homomalla	2	0	0	0	1	0	1	0	2	0	11	1	4	0	13	1	18
Plexaura homomalla forma *kukenthali*	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	2
Plexaura sp.	0	0	0	0	4	0	0	1	0	0	1	0	4	1	1	0	6
Plexaurella dichotoma	5	2	0	4	0	2	2	2	0	3	1	0	7	10	1	3	21
Plexaurella fusifera	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	0	2
Plexaurella grisea	0	0	0	1	0	1	0	0	0	1	2	0	0	2	2	1	5
Plexaurella nutans	0	4	0	4	3	1	1	1	0	0	0	0	4	10	0	0	14
Plexaurella sp.	0	2	0	0	1	0	0	0	0	0	0	0	1	2	0	0	3
Pseudoplexaura crucis	0	0	1	0	0	0	0	0	0	0	3	0	1	0	3	0	4
Pseudoplexaura flagellosa	0	0	1	0	0	1	0	2	3	0	0	0	1	3	3	0	7
Pseudoplexaura porosa	0	0	0	0	0	0	0	0	0	0	5	0	0	0	5	0	5
Pseudoplexaura sp.	0	0	0	0	2	0	3	0	1	2	2	0	5	0	3	2	10
Pseudoplexaura wagenaari	0	0	0	0	0	0	0	1	0	0	2	0	0	1	2	0	3
Pterogorgia anceps	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1
Pterogorgia citrina	0	0	0	0	0	0	0	0	0	7	1	0	0	0	1	7	8
TOTAL	**76**	**155**	**145**	**160**	**143**	**128**	**82**	**135**	**97**	**155**	**229**	**96**	**446**	**578**	**326**	**251**	**1601**

9 Appendix B

Octocoral species relative abundances (%) of Útila. Colour coding of abundances based upon relative scale within each column from low (blue) to high (red). Begins on next page.

Species	South Shore Sites								North Shore Sites				South Shore Sites		North Shore Sites		Total
	Sturge's Bank (SB)		Coral View (CV)		Little Bight (LB)		Stingray Point (SRP)		Spotted Bay (SpB)		The Maze (M)						
	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	5 m	15 m	
Antillogorgia elisabethae	47.4	85.2	43.5	65.0	6.99	36.8	2.44	40.8	2.06	25.2	1.75	2.08	24.9	58.5	1.84	16.3	30.98
Gorgonia ventalina	7.89	0.00	36.6	0.63	25.2	1.56	34.6	5.93	10.3	1.94	18.8	1.04	27.6	1.90	16.3	1.59	11.93
Eunicea sp.	1.32	1.94	1.38	2.50	17.5	8.59	8.54	11.1	15.5	11	6.55	18.8	7.85	5.71	9.20	14	8.31
Eunicea clavigera	5.26	2.58	2.07	4.38	0.70	18.8	2.44	2.22	1.03	3.23	0.00	10.4	2.24	6.57	0.31	5.98	4.00
Eunicea fusca	0.00	0.65	0.00	1.25	12.6	3.13	3.66	11.9	12.4	16.1	15.3	13.6	4.71	3.98	14.4	15.1	8.06
Gorgonia mariae	3.95	0.00	2.07	5.00	2.10	7.81	4.88	1.48	3.09	10.3	13.1	11.5	2.91	3.46	10.2	10.8	5.81
Eunicea flexuosa	1.32	0.65	4.83	1.25	4.90	1.56	7.32	1.48	5.15	0.65	5.24	5.21	4.71	1.21	5.21	2.39	3.19
Eunicea mammosa	0.00	0.65	0.69	1.88	2.10	3.91	9.76	4.44	4.12	3.23	4.80	2.08	2.69	2.60	4.60	2.79	3.06
Muriceopsis flavida	0.00	0.00	3.45	0.00	2.80	0.78	1.22	4.44	1.03	0.00	1.75	15.6	2.24	1.21	1.53	5.98	2.31
Eunicea succinea forma	2.63	0.00	0.69	0.00	4.90	3.13	0.00	2.22	1.03	0.00	1.31	1.04	2.24	1.21	1.23	0.40	1.37

Species	South Shore Sites								North Shore Sites				South Shore		North Shore		Total
plantaginea																	
Plexaurella dichotoma	6.58	1.29	0.00	2.50	0.00	1.56	2.44	1.48	0.00	1.94	0.44	0.00	1.57	1.73	0.31	1.20	1.31
Antillogorgia americana	0.00	0.65	0.69	0.63	2.80	1.56	6.10	1.48	1.03	6.45	5.68	1.04	2.24	1.04	4.29	4.38	2.56
Antillogorgia sp.	0.00	1.29	0.69	3.13	0.70	2.34	0.00	1.48	2.06	0.00	0.00	1.04	0.45	2.08	0.61	0.40	1.06
Plexaurella nutans	0.00	2.58	0.00	2.50	2.10	0.78	1.22	0.74	0.00	0.00	0.00	0.00	0.90	1.73	0.00	0.00	0.87
Antillogorgia acerosa	3.95	0.00	0.69	0.63	2.10	0.00	2.44	1.48	0.00	3.87	0.87	0.00	2.02	0.52	0.61	2.39	1.25
Antillogorgia hummelincki	2.63	0.65	0.00	3.75	0.70	0.00	2.44	0.00	0.00	0.00	0.44	0.00	1.12	1.21	0.31	0.00	0.81
Briareum asbestinum	11.9	0.00	0.00	0.00	1.40	0.00	0.00	0.00	32	4.52	6.55	1.04	2.47	0.00	14.1	3.19	4.06
Antillogorgia kallos	0.00	0.65	0.00	0.00	3.50	0.00	0.00	1.48	0.00	0.00	0.00	0.00	1.12	0.52	0.00	0.00	0.50
Pseudoplexaura sp.	0.00	0.00	0.00	0.00	1.40	0.00	3.66	0.00	1.03	1.29	0.87	0.00	1.12	0.00	0.92	0.80	0.62
Eunicea calyculata forma *coronata*	1.32	0.00	0.69	1.25	0.00	0.00	0.00	0.74	0.00	0.00	1.31	1.04	0.45	0.52	0.92	0.40	0.56
Plexaura sp.	0.00	0.00	0.00	0.00	2.80	0.00	0.00	0.74	0.00	0.00	0.44	0.00	0.90	0.17	0.31	0.00	0.37
Plexaura homomalla	2.63	0.00	0.00	0.00	0.70	0.00	1.22	0.00	2.06	0.00	4.80	1.04	0.90	0.00	3.99	0.40	1.12
Pseudoplexaura flagellosa	0.00	0.00	0.69	0.00	0.00	0.78	0.00	1.48	3.09	0.00	0.00	0.00	0.22	0.52	0.92	0.00	0.44

Species	South Shore Sites								North Shore Sites				South Shore		North Shore		Total
Eunicea asperula	0.00	0.00	0.00	0.63	0.00	0.78	0.00	0.74	0.00	0.00	0.00	1.04	0.00	0.52	0.00	0.40	0.25
Iciligorgia schrammi	0.00	0.00	0.00	0.00	0.00	2.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.52	0.00	0.00	0.19
Plexaurella sp.	0.00	1.29	0.00	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.35	0.00	0.00	0.19
Eunicea laciniata	0.00	0.00	0.00	0.63	0.00	0.78	0.00	0.00	0.00	1.94	0.00	1.04	0.00	0.35	0.00	1.59	0.37
Plexaurella grisea	0.00	0.00	0.00	0.63	0.00	0.78	0.00	0.00	0.00	0.65	0.87	0.00	0.00	0.35	0.61	0.40	0.31
Muricea laxa	0.00	0.00	0.00	0.00	0.00	0.00	1.22	0.74	1.03	0.65	0.00	0.00	0.22	0.17	0.31	0.40	0.25
Eunicea calyculata forma *calyculata*	0.00	0.00	0.00	0.00	0.00	0.00	2.44	0.00	0.00	0.65	0.00	0.00	0.45	0.00	0.00	0.40	0.19
Eunicea succinea forma *typical*	0.00	0.00	0.00	0.00	0.00	0.78	1.22	0.00	0.00	0.00	0.44	0.00	0.22	0.17	0.31	0.00	0.19
Muricea atlantica	0.00	0.00	0.69	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.44	0.00	0.45	0.00	0.31	0.00	0.19
Antillogorgia albatrossae	1.32	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.17	0.00	0.00	0.12
Plexaurella fusifera	0.00	0.00	0.00	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	0.00	0.00	0.12
Pseudoplexaura crucis	0.00	0.00	0.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.31	0.00	0.22	0.00	0.92	0.00	0.25
Eunicea tourneforti	0.00	0.00	0.00	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.87	0.00	0.22	0.00	0.61	0.00	0.19

Species	South Shore Sites								North Shore Sites				South Shore		North Shore		Total
Pseudoplexaura wagenaari	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.74	0.00	0.00	0.87	0.00	0.00	0.17	0.61	0.00	0.19
Muriceopsis petila	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.74	0.00	0.00	0.00	1.04	0.00	0.17	0.00	0.40	0.12
Erythropodium sp.	0.00	0.00	0.00	0.00	0.00	0.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.06
Muricea muricata	0.00	0.00	0.00	0.00	0.00	0.00	1.22	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.06
Muricea pinnata	0.00	0.00	0.00	0.00	0.00	0.78	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.06
Pterogorgia citrina	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.52	0.44	0.00	0.00	0.00	0.31	2.79	0.50
Muriceopsis sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.29	0.00	0.00	0.00	2.79	0.44
Pseudoplexaura porosa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.18	0.00	0.00	0.00	1.53	0.00	0.31
Antillogorgia rigida	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.44	2.08	0.00	0.00	0.31	1.20	0.25
Eunicea palmeri	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.31	1.04	0.00	0.00	0.92	0.40	0.25
Muricea waltonsmithii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.03	0.00	0.44	0.00	0.00	0.00	0.61	0.00	0.12
Plexaura homomalla forma *kukenthali*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.44	0.00	0.00	0.00	0.31	0.40	0.12
Eunicea laxispica	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.40	0.06
Pterogorgia anceps	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.03	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.06