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Exploring Mesophotic Depth Refuges on Coral Reefs

A Thesis Submitted in Fulfilment of the Degree of Doctor of Philosophy



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Abstract

Tropical coral reefs extend deeper than many people are aware. Though we may be familiar with zooxanthellate coral just below the surface, the deepest recorded are further than 150m down. This thesis focusses on mesophotic coral ecosystems (MCEs), beginning at 30m depth and extending to the deepest photosynthetic coral holobionts. Such understudied reefs require attention not just for their novelty. As the pressures which threaten shallow reefs attenuate with depth, MCEs may represent a refuge from anthropogenic stressors such as ocean warming. This DPhil occurred during the third global coral bleaching event, and considers whether the mesophotic zone could provide a refuge for shallow coral species into the future.

Unfortunately, directly testing for deep coral refuges would require the destruction of shallow reefs and patient observation of any recovery. To side-step this process I consider a number of barriers to refuge dynamics. If MCEs are to act as a refuge for shallow species both depth zones must contain similar species pools. In chapter two I undertake a systematic review and meta-analysis to synthesise existing ecological surveys. I find that after half a century of research the mesophotic literature is still largely descriptive, likely because of the difficulties associated with accessing MCEs. I quantify the proportion of shallow species also observed deeper than 30m. The degree of community overlap varies by geographic region and with taxonomic groups, yet in some cases a substantial proportion of the shallow species pool can be found on MCEs as currently defined.

Chapter three is based on primary research on the MCEs of Utila, Honduras. I consider a biological approach for recognising MCEs, as a global 30m depth limit is unlikely to capture variation in community zonation dependent on local conditions. I show that a change from shallow-specialist to depth-generalist taxa is largely coincident with current depth-based definitions, but accommodates site-specific variation in scleractinian communities. Locations with deeper maximum depth ranges for shallow species may be more likely to provide refuge from negative impacts. In addition, the recognition of mesophotic reefs as a biological assemblage rather than a depth zone will aid the design of experimental manipulations and allow logical comparisons between locations.

Site-specific variation in species depth ranges, and the ability to survive both on MCEs and shallow reefs, are likely determined by the physiology of the coral holobiont. In chapter four I quantify metabolic change in the depth-generalist coral *Agaricia lamarcki* from two locations around Utila. Depth ranges are known to differ at these two sites. I find site-specific physiological profiles, with heterotrophy increasing with depth and a reduction in photosynthetic capacity at the site with the smallest depth range. I also find evidence to suggest greater niche conservation with depth at the site with the largest depth range.

The same colonies assessed in chapter four undergo experimental manipulation in chapter five. Fragments were transplanted to a shallow or mesophotic station at a third site. Following a coral bleaching event on Utila a survival and bleaching analysis was conducted.

Fragments located shallow were almost twice as likely to die, and were more likely bleached, than those at mesophotic depths. Site and depth of collection did not influence the chances of survival suggesting any reduction in bleaching thresholds for mesophotic colonies were unimportant in this case. It is important to note that depth can offer protection from other agents of mortality, such as water movement and coastal development, and this experimental design does not answer what drives differences in survival.

Together these findings show that there is potential for MCEs to protect species also present on shallow reefs in the face of disturbance. Further, individuals collected from MCEs may be capable of surviving on shallow reefs when artificially moved. Substantial variation in depth ranges and metabolism, at least for Scleractinia, ensures that the likelihood of a deep reef refuge existing will always be site-specific. These findings will be best supported by further work correlating the patterns observed with local abiotic measurements, considering the fecundity of mesophotic reefs, and studying shallow water settlement and survival of mesophotic propagules in the face of different competitive regimes.

Declaration of Authenticity

The enclosed work has not been submitted totally, or in part, for consideration for another degree. When chapters have already been published in peer reviewed journals, the DOI is displayed beneath the chapter title. The underlying data can be found with the open access publication. For chapters not yet published elsewhere the data will be uploaded as supporting files with the digital version of this thesis to the Oxford university Research Archive (ORA).

Modern science is collaborative in nature, as such, an agreed authors' contributions section is included at the end of each piece of work. The introduction and synthesis section of the thesis were written by Jack Laverick and reviewed by Professor Alex Rogers. All authors have consented to this document being submitted.

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Chapter One

General Introduction: Mesophotic Coral Ecosystems and Deep Reef Refuges

Jack H. Laverick

1.1 Mesophotic Coral Ecosystems:

Marine biology as a field has always faced challenges of access. Utilising newly developed SCUBA and submersible technologies in the 20th century began to open marine environments to detailed study by scientists. SCUBA allowed taxonomists to document new taxa otherwise missed in complex 3D habitats and to adopt experimental approaches to subtidal ecology. Despite this, SCUBA studies are largely limited to depths shallower than 30m to remain within recreational dive regulations. Submersible studies and the use of remotely-operated vehicles (ROVs) meanwhile allowed the study of deep water ecosystems thousands of metres beneath the surface (Roberts and Wheeler, 2008). Submersibles, however, are expensive to hire and operate, historically lacked dexterity, and may miss small taxa. The large ROVs necessary for taking biological samples face operational difficulties in shallow waters (e.g. overheating of cables). Typically, submersible and ROV studies have focussed on ecosystems below the ranges of zooxanthellate corals (for some exceptions see Fricke and Meischner, 1985; Fricke and Knauer, 1986). As a result a 2008 review (Menza, Kendall and Hile, 2008) reported 90% of all publications mentioning coral fail to report data deeper than 30m. Recent research has begun to show that photosynthetic hard corals regularly occur far deeper than 30m. The current depth record stands at 165m at Johnston Atoll (Maragos and Jokiel, 1986). This is not a globally unusual case with further depth records set for the Bahamas, Red Sea, and Great Barrier Reef past depths of 100m (Reed, 1985; Schlichter, Fricke and Weber, 1986; Englebert *et al.*, 2014), as reviewed by Kahng et. al (2010).

In 2008 a workshop was hosted by NOOA to bring together researchers interested in studying the hard to access 'middlingly deep' coral reefs; found below recreational dive

limits. They coined the term Mesophotic Coral Ecosystems (MCEs), to help coordinate researchers and define shared research interests (Puglise *et al.*, 2009). MCEs are tropical reef ecosystems containing photosynthetic hard corals, defined as starting at 30 - 40m deep and ending with the last photosynthetic hard corals. In practice, the term mesophotic has been applied to studies deeper than the 30m of recreational diving (Laverick *et al.*, 2018), and has started to be used for Temperate Mesophotic Ecosystems as well (TMEs).

MCEs significantly increase global reef area (Harris *et al.*, 2012). As hard corals typically rely on photosynthesis to satisfy their calorific demand, and light energy decreases exponentially with increasing depth (Fricke and Knauer, 1986), we would expect MCEs to be subject to a differing ecological paradigm to shallow reefs. Research on MCEs, plugging the knowledge gap reported by Menza et. al (2008), has become increasingly common as a result of advances in diving technology and autonomous exploration (Pyle, 1998). The work described in this thesis has been facilitated by training in the use of mixed gas closed circuit rebreathers for diving.

Despite increasing research effort, MCEs are still poorly defined. An absolute upper depth limit of 30m fails to capture the variability of environmental conditions responsible for changing rates of reef transition between sites. The 30m limit is linked to limits of SCUBA diving rather than biology. A number of site descriptions discuss changing richness with depth and identify clusters of deep and shallow species (Bridge *et al.*, 2012; Muir *et al.*, 2015), yet very few attempt to define reef structure in terms of indicator taxa (Bejarano, Appeldoorn and Nemeth, 2014). Following the second international MCE workshop in 2014 the need to address such issues was acknowledged (Loya *et al.*, 2016).

1.2 The Deep Reef Refugia Hypothesis:

In spite of issues of definition, the recent MCE-focussed research was inspired by a conservation angle. In 1996 Peter Glynn suggested "Possible refuge habitats that would be protected from rapid temperature rise might be found at moderate depths, in upwelling centres, on oceanic banks or island shores exposed to vigorous circulation, and at some high latitude sites" (Glynn, 1996). From this the Deep Reef Refugia Hypothesis (DRRH) was formalised, positing deeper reefs may be protected from climate pressures threatening shallow reefs. Discussions of deep reef refuges have followed mesophotic research from the beginning. As initially the mesophotic was treated as a depth zone, it was easy to consider whether shallow species exist in mesophotic depth refuges, i.e. existing at depths, suitably large (below 30m), to avoid shallow water disturbances (Bongaerts *et al.*, 2010; Laverick *et al.*, 2018). MCEs are thought to potentially store mature individuals of a taxon, which could act as a source of recruits for a future recovery in the shallows. In the same way MCEs may also provide refuge for shallow reefs from non-climatic pressures, such as physical disturbance and coastal development (Bridge, Hughes, *et al.*, 2013; Kavousi and Keppel, 2017). This way of thinking can, however, run into difficulties when considering the shallows and mesophotic as different communities (Laverick *et al.*, 2016; Laverick, Andradi-Brown and Rogers, 2017). MCEs are not synonymous with deep reef refuges; with 'deep' reef refuges reported in shallow waters (Smith *et al.*, 2014), and distinct communities detected beneath 30 m (Semmler, Hoot and Reaka, 2016; Laverick, Andradi-Brown and Rogers, 2017; Baldwin, Tornabene and Robertson, 2018; Rocha *et al.*, 2018)

Testing whether mesophotic reefs can provide refuge directly is difficult, it would require damaging multiple shallow reefs and waiting for potentially lengthy regeneration

times. There are a number of assumptions, however, which must be true in order for recovery dynamics to occur on a given reef. This thesis aims to test some of these assumptions, outlined below, to validate the logical argument.

1.2.1 There is an overlap in hard coral communities between MCEs and Shallow reefs

In order for MCEs to provide refuge for shallow taxa, species present on shallow reefs must also be found on MCEs. In the eventuality there is no community overlap the recolonization of shallow reefs may still occur, but by a different species pool formerly excluded by competitive influences. Clearly future decisions regarding a new definition for MCEs, not based on a static depth limit, will have implications for this assumption. It may be that MCEs become defined by their own unique pool of species. The likelihood of refuge dynamics occurring is then a matter of how rapidly with increasing depth does the transition in reef structure occur. A deep transition will afford shallower reef species with the protection of depth. Such a definition may then allow the realisation of protection for MCEs in their own right, this is currently a rarity (NPA, 2009).

A number of mesophotic site descriptions now exist. It seems there is a degree of community overlap. In general, shallow reef communities occur shallower than 30m, a specialist mesophotic community beneath 60m (lower mesophotic) and a transitional zone between 30 m and 60 m (upper mesophotic) (Kahng, Copus and Wagner, 2014). The vertical zonation of coral reef communities has been detected in fish (Bejarano, Appeldoorn and Nemeth, 2014; Rosa *et al.*, 2015; Baldwin, Tornabene and Robertson, 2018; Rocha *et al.*, 2018), macroalgae (Brasileiro *et al.*, 2015), and Brachyuran crabs (Hurley *et al.*, 2015), as well as hard corals (Rooney *et al.*, 2010). The amount of variability between sites and a

consistent method for detecting changes in community structure are yet to be considered explicitly.

For some of the locations with longer histories of MCE research, modelling studies are beginning to reveal the forces driving the occurrence of species on MCEs and their structure. Costa et. al (2015) generated an accurate predictive model of occurrence for three hard coral genera with depth ranges entering MCEs. Depth, mean euphotic depth, mean euphotic depth variability, distance from shoreline, sea surface temperature, and sea surface temperature variability were the six strongest predictors of hard coral occurrence offshore of Maui, Hawai'i. Exceptional water clarity and abiotic stability favoured the formation of deep hard coral communities. This highlights the potential for differing rates of vertical zonation with increasing depth between sites. This suggests deep reef refuges will require case-specific assessment based on the reef and pressures of interest.

1.2.2 MCEs are reproductively active and not sink populations supported by shallow reefs

It may be the case, if mesophotic depths receive less potential energy, that MCEs are sink populations. There may be enough energy to support metabolism but not reproduction. Broken fragments from the shallows which roll down the reef slope, or juveniles settling in sub-optimal habitat, may allow MCEs to exist on long time scales. In this instance, MCEs would not be able to provide a source of recruits to aid recovery of an impacted shallow reef. Also, as spawning times for hard corals are determined by solar insolation, depth may disrupt synchronicity between shallow and deep populations (Van Woesik, Lacharmoise and Köksal, 2006; Shlesinger *et al.*, 2018). It must be determined whether MCEs are

reproductively active, and if they are, whether increased depth impacts fecundity and phenology.

Directly observing and quantifying coral spawning down a shallow to mesophotic depth gradient is logistically demanding. The synchronicity of spawning across species on the Great Barrier Reef (Babcock *et al.*, 1986) limits the opportunities in a given year for researchers to collect data. Opportunities in the Caribbean are species specific. The brief spawning windows also necessitate either large numbers of reliable traps and cameras or many coordinated observers. Initial observations confirmed MCEs may be reproductively active. An ROV dive in the Gulf of Mexico recorded broadcast corals spawning in the mesophotic zone in synchrony with individuals of the same species in the shallows (Vize, 2006). In order to generate larger datasets, studies of MCE reproduction turned to genetics and estimation of vertical connectivity of populations.

For the broadcast spawning species *Montastraea cavernosa,* significant genetic structuring was detected with increasing depth (Brazeau, Lesser and Slattery, 2013; Serrano *et al.*, 2014; Goodbody-Gringley *et al.*, 2015). This was in spite of a high degree of genetic connectivity within depth bands across the Caribbean (Goodbody-Gringley, Woollacott and Giribet, 2012). This implies reproduction at depth. A greater chance of migration from shallow populations to depth was detected (Serrano *et al.*, 2014). As this could result from greater fecundity in the shallows or higher coral cover it is still a possibility that deep propagules could settle shallower following shallow reef mortality.

The closely related species *Orbicella faveolata* has also been observed spawning at mesophotic depths (Daniel M Holstein *et al.*, 2015). Detailed histological analyses revealed, in spite of reduced polyp density at depth, greater coral cover and a higher average

fecundity per polyp for mesophotic colonies. Together, this meant increased reproductive output with increasing depth. A modelling exercise considering currents, sink rates, and settlement success, suggested mesophotic populations of *O. faveolata* are largely self-seeded (Daniel M. Holstein *et al.*, 2015). In spite of this, in even the most pessimistic scenarios considering reduced survivorship and fertilisation, there may be a local subsidy of up to 10% from mesophotic populations to the shallows.

For comparison, *Porites astreoides* is a depth generalist brooding species. Similar histological analyses reported no change in fecundity with increasing depth (Holstein, Smith and Paris, 2016). Holstein et al. (2015) conclude the DRRH may apply to populations of *P. astreoides* as a function of coral cover. The same modelling exercise suggests the DRRH is more likely for this brooding coral than *O. faveolata*, as a result of a more resilient larval network in the shallows which connects to MCEs over multiple generations. It appears MCEs are reproductively active, but the likelihood of these propagules settling in the shallows and allowing damaged reefs to recover is still only considered theoretical.

1.2.3 Recruits from MCEs are physiologically capable of surviving on shallow reefs

Physiology studies of MCE hard corals have largely considered the balancing of energy budgets. The low light levels at mesophotic depths provides a challenge for hard corals as photosynthetic organisms. Three options are available in order to offset the reduction in available light energy. Either hard corals could increase photosynthetic efficiency, reduce basal metabolic rate or increase the proportion of heterotrophic subsidy. A constituently lower metabolic rate and increased capture of light energy may be adaptations which

increase the susceptibility of MCE hard corals to shallow pressures therefore hindering refuge dynamics.

Multiple hard coral adaptations have been reported, presumed to increase photosynthetic efficiency. These include extensive symbiont shuffling with depth (Bongaerts, Carmichael, *et al.*, 2015), an increased flattening of branches and growth form with enlarged polyps (Graus and MacIntyre, 1982; Nir *et al.*, 2011; Muir *et al.*, 2015), and a highly organised skeletal structure in comparison to shallow species (Kahng *et al.*, 2012). Lower respiration rates have been reported (Kahng *et al.*, 2010) and gracile growth forms at depth may represent a metabolic trade-off between the need to increase surface area for light capture and the energy requirements of calcification. Other species instead appear to rely on heterotrophic subsidy rather than increasing photosynthetic efficiency (Crandall *et al.*, 2016) or compliment photosynthetic adaptations in this way (Lesser *et al.*, 2010).

It is important to remember that the mesophotic physiology studies conducted so far have been on mature colonies, and not on coral larvae. As such, whether the offspring of a mesophotic colony can survive in the shallows is unknown. The implicit assumption of research to date, is that if the adult could survive certain conditions, so too could the recruit.

1.2.4 MCEs are impacted less by pressures threatening shallow coral reefs

The assumption that increased depth protects from shallow water disturbance is core to the deep reef refugia hypothesis. Light levels, and typically water temperatures, decline with increasing depth which may lead to lower levels of coral bleaching (Bridge, Hughes, *et al.*,

2013). Similarly the physical effect of water movement declines with increasing depth (Lentz *et al.*, 2017). Therefore, deeper corals may avoid physical damage during storms**.** Deeper reefs are also often further from shore than shallow reefs, when a reef system is spread along a slope. It is conceivable mesophotic reefs further from shore would be comparatively protected from the effects of sedimentation, coastal development, and fishing (Bridge, Hughes, *et al.*, 2013). Naturally different stresses and disturbances have differing frequencies and intensities between sites. The Red Sea for instance is known for its high water temperatures, however it also has a local pool of species capable of tolerating these levels (Osman *et al.*, 2018). In Palau, shifting thermoclines can transport thermal stress from the shallows onto MCEs (Schramek *et al.*, 2018). Quite how the depth-stress relationship varies between sites will depend on the intrinsic properties of reef taxa, as well as the magnitude of any disturbance event.

Though the logic of reduced stress with depth is enticing, and damage has been limited to (or is worse on) shallow reefs during disturbance in some cases (Sinniger, Morita and Harii, 2012; Frade *et al.*, 2018), reduced mortality with depth is still simply assumed for most sites. MCEs may still be vulnerable because of increased sensitivity. Hard corals from more stable environments may be less tolerant of changes in ambient conditions as a result of reduced phenotypic plasticity (Oliver and Palumbi, 2011). Bleaching, the expulsion of endosymbionts by the host, has been observed on MCEs (Nir *et al.*, 2014) and in some instances the thermal susceptibility of mesophotic hard corals has been shown to be higher than shallow species (Smith *et al.*, 2015). In addition to bleaching pressures, disease (Smith *et al.*, 2010), and storm damage (White *et al.*, 2013) has been observed. The fragile nature of mesophotic hard corals leaves them particularly susceptible to physical disturbance (Bongaerts, Muir, Englebert, Bridge, *et al.*, 2013), while the typically large plating growth

forms at mesophotic depths are more vulnerable to the negative effects of sedimentation (Rogers, 1990).

There will always be cases of damage recorded from specific sites. The challenge will be determining whether on average MCEs are more stable than neighbouring shallow reefs. A simple proxy for this may be to determine the relative growth rates and ages of live hard coral cover down the depth gradient. Unfortunately, colony coring is not an option for fragile growth forms and stable isotope techniques may require the removal of whole coral colonies. Observations of species surviving at depth despite extirpation in the shallows lends support to the DRRH in some cases (Sinniger, Morita and Harii, 2012; Smith *et al.*, 2014). Especially as corals known to occur on MCEs have exhibited reductions in fecundity lasting for at least three years following thermal stress shallower than 10 m (Levitan *et al.*, 2014), stress potentially avoided at depth.

1.2.5 Meta-populations

Analogies to the DRRH can be found in other areas, from the spill over effect expected from marine no-take zones for fisheries (da Silva *et al.*, 2015), to thermal refugia permitting forest expansion after the last ice age (Anderson *et al.*, 2006). Perhaps the most important lessons to be learnt for scientists interested in the DRRH, from broader ecology, come from studies of meta-population dynamics (Hanski and Simberloff, 1997). Key parameters and assumptions of meta-population models map directly onto the above discussion of the DRRH.

Firstly these models often consider a single taxon (Akcakaya, Mills and Doncaster, 2007), rather than applying to entire ecosystems. Such formulations acknowledge the difficulties posed by shifting community structure across gradients (1.2.1). It is highly likely that whether deep reef refuges exist is a species-specific question, with our examples to date focussing on single species (Smith *et al.*, 2014; Sinniger *et al.*, 2017), or at best apply to groups of species sharing similar physiologies (Laverick, Andradi-Brown and Rogers, 2017).

Meta-population models drop the assumption from early demographic and population models of a panmictic population, and instead consider populations which are structured in space (Shallow – Mesophotic depth gradient), that breed locally (1.2.2), which are able to effect the population dynamics of each other through the exchange of individuals (1.2.2, 1.2.3, population connectivity), and that the drivers of population suppression or boosts are also structured spatially (1.2.4). Though the field is not yet at the point where we can take full advantage of these models, as we are lacking crucial parameter estimates, it is important to bear in mind some of their findings. For example, the relationship between the connectivity of patches and extinction risk can be non-linear (Molofsky and Ferdy, 2005). Under low connectivity individuals do not migrate fast enough to rescue dying patches, while high levels of connectivity can cause patches to homogenise, for the populations to cycle in phase, and consequently increase extinction risk. In addition, with increasing assemblage complexity the viability of meta-populations may reduce, as competitive interactions and community reorganisations prevent any rescue effects (Bull *et al.*, 2007). Such findings may be pertinent to phase-shifts observed across depth gradients on coral reefs (Lesser and Slattery, 2011). Understanding how these studies relate to reefs and the DRRH will require not only studies of reef to reef connectivity, but also the population dynamics of connected reefs over time.

1.3 Study site - Utila:

Chapters three, four, and five of this thesis involved fieldwork and primary research. From 2014 through 2016 we visited Utila, one of the Honduran Bay Islands on the southern boundary of the Mesoamerican Barrier Reef in the Caribbean. The Island's approximate population of 8,000 people is centred around Sandy Bay on the south shore (Korda, Hills and Gray, 2008). The shallow south shore reefs create a spur-and-groove system, which slopes to a sandy bottom at approximately 30-45m depth. Here the reefs change to a patch system. The slope continues to descend gradually to 70m depth towards mainland Honduras. The coral view research station is located on the south shore of the island and provided the centre for our dive and research operation. Boat trips were also available to the north shore of the island permitting us to study five dive sites in total, spread around the island (Figure 1.1). The north shore reefs are primarily steep walls descending deeper than 100m, with an extensive shallow back-reef environment.

Chapter three is based on video data collected from all five dive sites down to the bottom of each reef. Exploratory dives on closed circuit rebreathers were conducted to a maximum of 100m to check for developed reef systems before selecting sampling profiles. Chapter four uses coral fragments collected from the maze (TMA) on the north shore and little bight (LB) on the south shore (Figure 1.1). These two sites were selected as typical of the two shorelines. Material was collected to the maximum and minimum depths of occurrence for the species of interest, following the ecological survey work. The experiment reported in chapter five used fragments from the same colonies sampled in chapter four, but was conducted at the coral view research centre (CV).

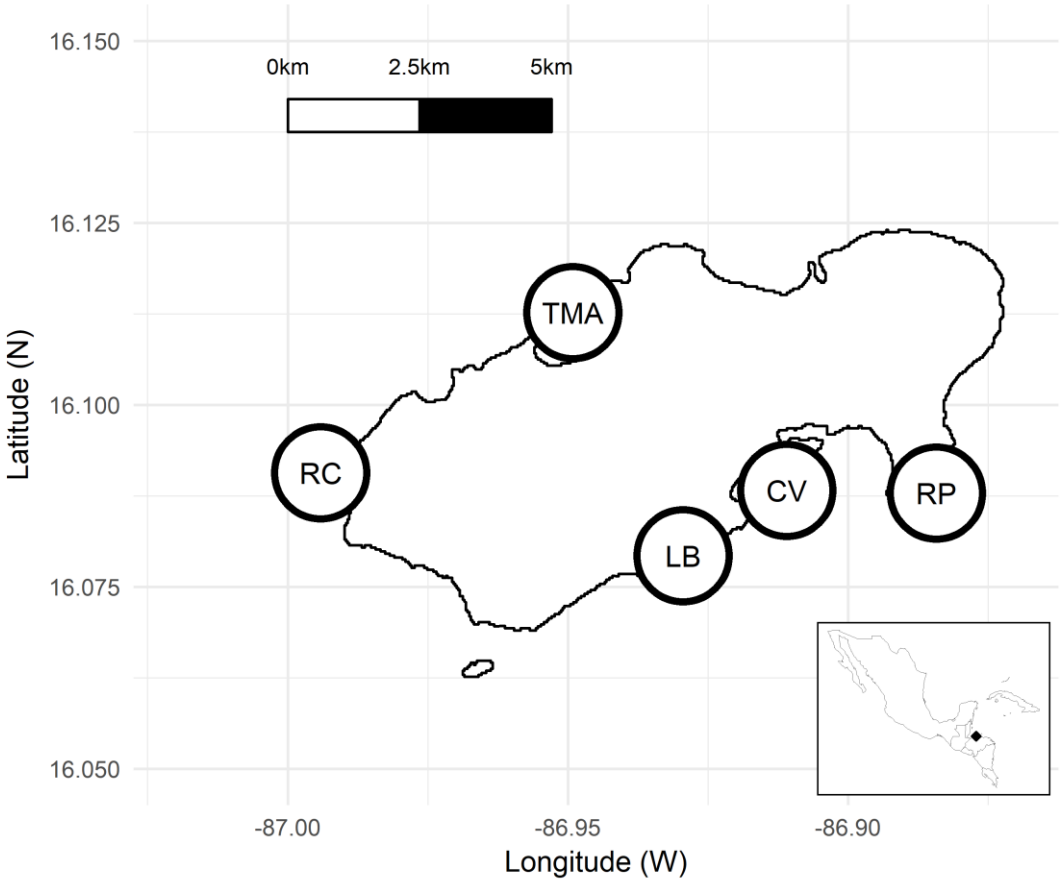


Figure 1.1 Dive site locations around the island of Utila, Honduras, Caribbean

Sites are listed with GPS co-ordinates and abbreviations starting left in WGS84 format: Raggedy Cay (RC: N 16.09065964, W -86.9941015), The Maze (TMA: N 16.11266214, W -86.94911793), Little Bight (LB: N 16.07926302, W -86.92942222), Coral View (CV: N 16.08823274,W -86.91094506), Rocky Point (RP: N 16.08784039, W -86.88423403). The inset shows the position of Utila in the Caribbean. The base map was sourced from GADM database of Global Administrative Areas under a CC BY licence with permission.

1.4 Thesis outline:

Chapter two details the protocol and findings of a systematic review and meta-analysis. I asked 'to what extent do mesophotic coral ecosystems and shallow reefs share species of conservation concern?'. A broad search strategy, retrieving ~ 170,000 bibliographic entries, ensured we collected research before and after the coining of the

term “mesophotic”. We screened and synthesised the captured ecological surveys to create a consensus measure, as well as capturing the effects of geographic region and study taxon on the estimate. If MCEs are to act as a refuge for shallow species both depth zones must contain similar species pools.

Currently, mesophotic reefs are defined as beginning at 30-40m depth (Puglise *et al.*, 2009). As little guidance exists on recognising MCEs in the absence of depth limits researchers have largely used 30m as an arbitrary upper boundary. This is reflected in chapter two. Chapter three takes a biological approach to identifying MCEs. A global 30m depth limit is unlikely to capture variation in community zonation dependent on local conditions. We use a species level dataset of scleractinian abundances from five locations around Utila to identify mesophotic and shallow communities. We then map these assemblages onto transect locations to reveal the variable depths of community transition. Locations with deeper maximum depth ranges for shallow species may be more likely to provide refuge from negative impacts. In addition, the recognition of mesophotic reefs as a biological assemblage, rather than a depth zone, will aid the design of experimental manipulations and permit logical comparisons between locations.

A key finding from chapter three was mesophotic reefs seem to be characterised by depth-generalist Scleractinia, and an absence of shallow species. This raises the question, how do depth-generalist corals satisfy their energy budgets under differing light regimes? And, could physiological adaptation or acclimation prevent survival under high-light levels? Site-specific variation in species depth ranges, and the ability to survive both on MCEs and shallow reefs, are likely determined by the physiology of the coral holobiont. In chapter four we quantify metabolic change in the depth-generalist coral *Agaricia lamarcki,* from two

locations known to have differing depth ranges. We use PAM fluorometry to investigate variation in photosynthetic parameters and mass spectrometry to help understand how the relative importance of photosynthesis and heterotrophy may vary with depth.

Chapter five details a transplant experiment using material from the same colonies assessed in chapter four. Fragments were transplanted away from their original depth to a shallow or mesophotic station at a third site, with controls. This was intended as a long term physiology experiment, however, following a coral bleaching event on Utila a survival and bleaching analysis was conducted. This opportunistic study allows us to experimentally assess the level of protection offered by depth, and to disaggregate this from any past exposure to a particular environment. It is important to note that depth can offer protection from other agents of mortality, such as water movement and coastal development, and this experimental design does not answer what drives differences in survival. This chapter addresses the root claim of the deep reef refugia hypothesis, that corals at deeper depths experience lower levels of mortality than those on shallow reefs, while controlling for the effects of acclimation and differences in susceptibilities.

Chapter Two

To What Extent do Mesophotic Coral Ecosystems and Shallow Reefs Share Species of Conservation Interest? A Systematic Review

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2.1 Abstract:

Globally, shallow-water coral reef biodiversity is at risk from a variety of threats, some of which may attenuate with depth. Mesophotic coral ecosystems (MCEs), occurring from 30-40m and deeper in tropical locations, have been subject to a surge of research this century. Though a number of valuable narrative reviews exist, a systematic quantitative synthesis of published MCE studies is lacking.

We conducted a systematic review to collate mesophotic research, including studies from the 20th century to the present. We highlight current biases in research effort, regarding locations and subject matter, and suggest where more attention may be particularly valuable. Following a notable number of studies considering the potential for mesophotic reefs to act as refuges, it is important to know how comprehensive these sources of recruits and organisms capable of moving to shallow water reefs may be.

We search seven sources of bibliographic data with two search strings, as well as personal libraries. Articles were included if they contained species presence data from both shallower and deeper than 30m depth on tropical coral reefs. Studies were critically appraised based on the number of species identified and balanced sampling effort with depth. Maximum and minimum depths per species were extracted from each study, along with study region and taxon.

We quantified the degree of community overlap between shallow tropical reefs (<30m) and reefs surveyed at the same locations below 30m. Proportions of shallow species, across all studied taxa, observed deeper than 30m were used to generate log odds ratios and passed to a mixed-effects model. Study location and taxon were included as

effect modifiers. Funnel plots, regression tests, fail safe numbers, and analysis of a high validity subgroup contributed to sensitivity analyses and tests of bias.

Across all studies synthesised we found two thirds of shallow species were present on mesophotic reefs. Further analysis by taxon and broad locations show that this pattern is influenced geographically and taxonomically. Community overlap was estimated as low as 26% and as high as 97% for some cases.

There is clear support for the hypothesis that protecting mesophotic reefs will also help to conserve shallow water species. At the same time, it is important to note that this study does not address mesophotic-specialist communities, or the ecological forces which would permit refuge dynamics. As we limit our analysis to species only present above 30m it is also possible diversity found exclusively deeper than 30m warrants protection in its own right. Further research into relatively ignored taxa and geographic regions will help improve the design of protected areas in future.

2.2 Introduction:

Mesophotic Coral Ecosystems (MCEs) occur between 30 and 40m at their shallowest in tropical and sub-tropical regions (Puglise *et al.*, 2009) and can be found deeper than 150m (Kahng *et al.*, 2010). MCEs are considered understudied in comparison to shallow reefs (Menza, Kendall and Hile, 2008), likely because of difficulties of access (Pyle, 1998), yet when accounted for they may greatly increase global reef area (Harris *et al.*, 2012). MCEs may be protected from commonly cited threats in comparison to shallow reefs (Glynn, 1996; Bridge, Hoey, *et al.*, 2013; Lindfield *et al.*, 2015; Semmler, Hoot and Reaka, 2016), and additionally may provide recruits to aid shallow reef recovery (Daniel M Holstein *et al.*, 2015; Daniel M. Holstein *et al.*, 2015). The amount of research published on mesophotic reefs is increasing (Turner *et al.*, 2017) as technology advances and research groups grow (Baker, Puglise and Harris, 2016b).

Research efforts are attempting to increase awareness regarding MCEs, while calling for their conservation (Bridge, Hughes, *et al.*, 2013). Protecting MCEs is often justified by the hypothesised existence of deep reef refuges (Baker, Puglise and Harris, 2016b) as shallow reefs continue to suffer damage (Hughes *et al.*, 2017). In areas where there is a substantial degree of community overlap between shallow reefs and MCEs, species may persist locally on MCEs despite extirpation in the shallows (Sinniger, Morita and Harii, 2012; Sinniger *et al.*, 2017). If these deep populations are reproductively active (Bongaerts *et al.*, 2017) then shallow reefs may be able to recover through vertical connectivity (Daniel M. Holstein *et al.*, 2015; Hammerman *et al.*, 2017).

Some studies have found that MCEs support high levels of endemism (Kosaki *et al.*, 2017), offering alternative justification for protecting MCEs as they may harbour distinct

geographical communities. Similarly, some researchers now argue MCEs could be identified by their own depth-specific ecological communities (Loya *et al.*, 2016; Laverick, Andradi-Brown and Rogers, 2017). These studies bring into question the general degree of community overlap between MCEs and shallow reefs (Semmler, Hoot and Reaka, 2016) as currently defined by a 30-40m depth limit based on SCUBA regulations. Understanding how many shallow reef species may be protected on reefs at depths greater than 30m will aid the future decisions of conservation managers (NPA, 2009).

Narrative reviews of the MCE literature have been conducted a number of times (Lesser, Slattery and Leichter, 2009; Bongaerts *et al.*, 2010; Kahng *et al.*, 2010; Olson and Kellogg, 2010; Slattery *et al.*, 2011; Kahng, Copus and Wagner, 2014; Baker, Puglise and Harris, 2016b), highlighting older studies and changes of key terms used to describe MCEs historically (Puglise *et al.*, 2009; Laverick *et al.*, 2016). Narrative reviews, however, may be subject to subconscious biases in article selection (Schmidt and Gotzsche, 2005). It is also difficult to ensure older studies, without keywords relating to depth ranges which fit in the modern framework, are re-discovered. Recently the bibliographies of a number of key reviews, publications from an online MCE database (www.mesophotic.org), and broader databases were systematically collated to show the state of the field (Turner *et al.*, 2017). However, existing reviews have stopped short of quantitatively synthesising the results of past studies.

As shallow reefs globally are increasingly threatened (Hughes *et al.*, 2017), we conduct a meta-analysis quantifying the extent of community overlap between shallow and mesophotic reefs across all taxa. We determine the proportion of shallow species which are present on MCEs, while highlighting the variation which can be ascribed to the study

location and focus taxon. If species present below 30m are protected from impact, then the higher the proportion of overlap the more effectively the conservation and management of MCEs will protect shallow water species. We collate the research field (Laverick *et al.*, 2016), depositing a bibliography of all identified MCE research online (www.mesophotic.org) to ensure wide and easy accessibility for future studies.

2.2.1 Stakeholder engagement-

Invited discussion with our advisory committee generated our research question (Laverick *et al.*, 2016), which aims to quantify shallow water taxa present on MCEs that may contribute to refuge dynamics. The need for this information, and the raising of awareness in management circles, was expressed in a recent United Nations Environment Programme report (Baker, Puglise and Harris, 2016b). The stakeholders on our advisory committee are researchers active in the mesophotic filed, from a geographical spread of universities and research institutions. Committee members are listed as authors following further involvement in generating the screening criteria, assisting with full-text retrieval, and commenting on this manuscript.

2.2.2 Objective of the review-

Primary question:

To what extent do mesophotic coral ecosystems and shallow reefs share species of conservation interest?

This can be broken into the following structure:

Subject (Population)	Exposure	Comparator	Outcome
Tropical and subtropical coral reefs (an ecosystem of conservation concern)	Reefs deeper than 30m	Reefs shallower than 30m	Species presence or absence

Secondary questions consider effect modifiers:

Does the extent of community overlap vary by region or between broad taxonomic groups?

2.3 Methods:

A protocol paper for this systematic review details the search strings used, databases searched, and the screening process amongst other information (Laverick *et al.*, 2016). Here, we reiterate our methods while detailing deviations from the protocol. The final sets of screening criteria used are reported along with the statistical analysis selected as suitable after the retrieval of studies. We report our review following the reporting standards for systematic evidence syntheses in environmental research (ROSES) (N. Haddaway *et al.*, 2017).

2.3.1 Deviations from the protocol-

JSTOR (all years) was not searched as a database, despite being detailed in our protocol. This was because an information request was declined as a result of the high volume of enquiries. Journal articles were still retrieved from JSTOR through a Google Scholar search.

Articles were categorised by type as part of a supporting narrative synthesis. We generated a word cloud based on term frequencies from included titles and abstracts, in

consultation with the advisory committee. The five, objectively derived, tags were: ecology, disturbance, trophic interactions, symbionts, and reproduction. Tags were assigned manually while screening on outcome at abstract level. Our full text screening for the primary review question, and quantitative synthesis, use studies tagged as ecology.

Method of data collection was not extracted, despite being identified in the protocol paper (Laverick *et al.*, 2016), as many articles used a mixture of different survey methods for shallow and deep sampling. AIC are not compared between models fitted to the full dataset and thea high validity subgroup as the metric is affected by sample size.

2.3.2 Search strategy-

We use two search strings to balance capturing relevant literature against articles captured after name changes in the field over time, as detailed in our protocol (Laverick *et al.*, 2016). "Mesophotic" from 01/01/2010 to 31/12/2016, and "Mesophotic OR "Deep reef" OR ((Submersibl* OR Submarine* OR "Deep water" OR Trimix) AND (biolog* OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*))" from the start of the source to 31/12/2009. The scoping exercise and search string development are reported in the protocol paper. The final search strings were successful in retrieving 87% of our test library.

We conducted our literature search in ISI Web of Science, Science Direct, Proquest, AGRICOLA and Scopus in English, with a full list of databases presented in our protocol (Laverick *et al.*, 2016). We downloaded all bibliographic data from mesophotic.org at the end of 2016 and used Google Scholar for a complimentary search, capable of capturing grey literature (Haddaway *et al.*, 2015). The Google Scholar search was conducted using web-scraping software in R (Wickham and RStudio, 2016). A detailed methodology can be found in supplementary 2.1, based on work by Haddaway et al. (N. R. Haddaway *et al.*, 2017),

retrieving up to the first 1000 entries. Following our literature search the personal libraries (Private collection of manuscripts) of authors and advisory committee members were checked for missed publications. Additional publications were also provided by the reviewers of this manuscript. Literature searches were conducted in the period from 19/04/2015 to 28/02/2017.

2.3.3 Article screening and study inclusion criteria-

Before screening based on exclusion criteria occurred in the software EPPI Reviewer 4 (Thomas, Brunton and Graziosi, 2010), duplicate entries resulting from the use of multiple databases were removed. We set EPPI Reviewer to automatically mark as duplicates full bibliographic entries with 95% similarity. This level of similarity generally corresponded to the identical entries with differing capitalisation, or entries with one database field left blank in comparison to the master entry. Other possible duplicates were checked manually. When retrieving full-text articles for detailed screening not all were readily available. The lead author was contacted for any manuscripts the screening team could not find available online. A final list of full texts not retrieved after two attempts at contact with the authors was circulated to the advisory committee. If full texts were not present in personal libraries after these checks the articles were excluded. The screening was conducted by authors who had no publications in the list screened, preventing bias in the inclusion of data.

The original screening and validity appraisal criteria were reviewed to reconcile disagreements during Kappa analysis, at each level of screening, between the two reviewers. 1000 articles were coded by both reviewers at title and abstract level, 50 full texts were coded by both reviewers. Any differently assigned articles were discussed. The final criteria were as follows:

Title and Abstract criteria:

1. Relevant subject: Extant Tropical and Subtropical coral reefs (exclude Mediterranean and palaeobiological studies)

2. Relevant exposure: Sampling at depths greater than 30m.

3. Relevant outcome: Ecological data such as richness, biodiversity, species lists and abundance. (Other study types were tallied for discussion of the broader field, but omitted from the primary review question).

Full text criteria:

1. Relevant subject: Mention of reefs containing photosynthetic stony (scleractinian) corals (exclude non-biology studies i.e. geology).

2. Relevant study design: Observational or experimental

3. Relevant exposure: Sampling at depths greater than 30m.

4. Relevant comparator: Ecological data from shallow reefs above 30m.

5. Relevant outcome: Any of the following data are reported: taxon richness, taxon abundance, taxon biomass and biodiversity indices along with a measure of variability and number of replicates.

2.3.4 Critical appraisal-

External validity considers the generalisability, and the fairness of comparison between different studies. Internal validity considers the risk of bias within a specific study. A number of methods exist to critically assess these concepts, but most are developed with randomised control trials in mind, or at least observational studies with a level of

randomisation in allocation and blinding rarely found outside of the medical sciences (Bilotta, Milner and Boyd, 2014).

We have no reason to believe the observation of a species depth range from one article is any more important than that from another (Laverick *et al.*, 2016). By including geographical area and taxon as effect modifiers, we have controlled for the primary confounding sources of variability when comparing studies. We therefore consider the spatial scales of different studies are the primary remaining threat to external validity. A key based on a whole ocean region is likely to find a greater depth range for a given species than a study at a specific location, because the key is likely based on data from a broader range of abiotic conditions.

With respect to internal validity, our primary concern with mesophotic research is that sampling effort may decline with increasing depth. This can lead to an underestimate of the number of species present deeper than 30m as rarer species are found in shallow water, but are missed at depth. Similarly, studies reporting only a small number of species may provide a less reliable estimate of community level overlap. This is because the importance of a single species depth range is elevated, when community overlap is calculated as a proportion. Our critical appraisal is therefore based on the following criteria:

Validity appraisal criteria:

1. More than 10 species are identified within a taxon

2. The study is not a regional taxonomic key

3. Even sampling effort is reported across depths

Studies passing all these criteria were included in a high validity sub-group for sensitivity analysis. We choose to limit our high validity group to studies with more than ten species to avoid undue influence of single species over community level overlap values.

2.3.5 Data extraction-

Maximum and minimum depths for each species reported in an article were extracted manually. A subset of 12 articles were checked for consistency of data extraction by a second reviewer. Discussion of these extractions produced the following clarifications. Depth ranges for adults and juveniles of a species were combined into a single depth range. For the purposes of defining multiple studies within an article, locations were considered different when across multiple countries, when provided. Australia and the USA were exceptions because of their size, here we took into account the seas around Australia, and the islands/coastlines sampled of the USA. The review was conducted by authors who had no publications in the list screened at full text, preventing bias in the inclusion of data. Data were restricted to species occurring on tropical and sub-tropical reefs when multiple habitats were studied in a single article, such as mangroves and reefs. If these data were not available, the corresponding author was contacted. Contact was attempted twice before asking members of the advisory committee to try a third time with any researchers they knew personally. Failure to respond to these contact attempts led to exclusion of the data set from the analysis (Supplementary 2.2). A list of all articles contributing data to the final meta-analysis can be found in supplementary 2.2.

2.3.6 Potential effect modifiers/ reasons for heterogeneity-

The location and broad taxonomic group were extracted from articles as effect modifiers, following consultation with the advisory committee (Laverick *et al.*, 2016), as different regions may have different transition depths and taxa may respond differently to depth.

2.3.7 Data synthesis-

After reviewing the retrieved articles, a meta-analysis for the primary review question was deemed appropriate. The list of observed depth ranges was limited to species observed shallower than 30m. Of these species, the proportion also present deeper than 30m was calculated, grouped by article, site, and taxon. Log odds ratios were calculated in R (R-Core-Team, 2013) in the package metafoR (Viechtbauer, 2010) and passed to a mixed-effects model. A maximum-likelihood estimator with Knapp & Hartung adjustments (Knapp and Hartung, 2003) was used with location and taxon as effect modifiers. Likelihood-ratio tests assessed the statistical significance of model elements and determined whether to retain an interaction term.

Our sensitivity analysis and tests for bias follow our protocol (Laverick *et al.*, 2016), and were as follows. Funnel plots were used to identify potential outliers (Sterne and Egger, 2001), and a regression test for asymmetry assessed the chance of publication bias affecting the dataset (Sterne and Egger, 2001). As a sensitivity analysis, Rosenthal's fail-safe number (Rosenthal, 1979) was calculated for the model to determine how many studies, averaging to null results, are required to lose statistical significance. To check sensitivity further, the analysis was repeated on a subset of high validity studies and the effects compared to a full model.

The broader research field was summarised using the bibliographic data from papers retained at full text level. Articles by year, categorisation, geographical area, and the mesophotic depth limit used were counted and plotted.

2.4 Results:

2.4.1 Review descriptive statistics-

Our methods retrieved almost 170,000 articles, with 3,011 screened at full text (Figure 2.1). Kappa analysis was used for consistency checking and refining of the inclusion criteria by two reviewers for 1,000/122,305 at title and abstract level and 50/311 at full text level. A list of all ecology articles screened at full text level, with decisions, can be found in supplementary 2.3. The following summary and figures of the systematic review, as opposed to the meta-analysis, are created from the data extracted from all articles; not excluded on outcome at the abstract level and verified as mesophotic at full text level. This brief discussion provides context of the wider field of mesophotic research, before we address the primary review question with a meta-analysis. The bibliographic data for these 461 articles were deposited with mesophotic.org.

There was a notable acceleration in the number of articles per year as we entered the 21st century (Figure 2.2a). Publication levels were relatively stable during the 20th century with only one year yielding more than 10 papers. The earliest article we identified was from 1960.

After more than half a century of research, the mesophotic field is still largely descriptive and focussed on pattern. This is supported by the majority of studies being

assigned an ecology tag during our screening process (Figure 2.2b). The next most common tag was assigned six times less frequently and indicates articles considering disturbance or damage on mesophotic reefs. Other common MCE study types refer to trophic interactions, symbioses, and reproduction.

This exercise was used as an opportunity to see how researchers define mesophotic reefs. 27% of articles include a depth limit in their introductions. The overwhelming majority of papers providing a definition state MCEs start at 30m depth (89%), however, some papers exist claiming the upper bound is 20m or even as deep as 60m.

Mesophotic reefs in the western Atlantic, Caribbean, and Gulf of Mexico, are the focus of most published studies, followed by those on archipelagos in the Pacific (Figure 2.2d). Australian reefs represent another hot spot of activity, but are nonetheless not as well studied. The Red Sea has provided a relatively large number of articles for its area, while the Indian Ocean has been comparatively understudied.

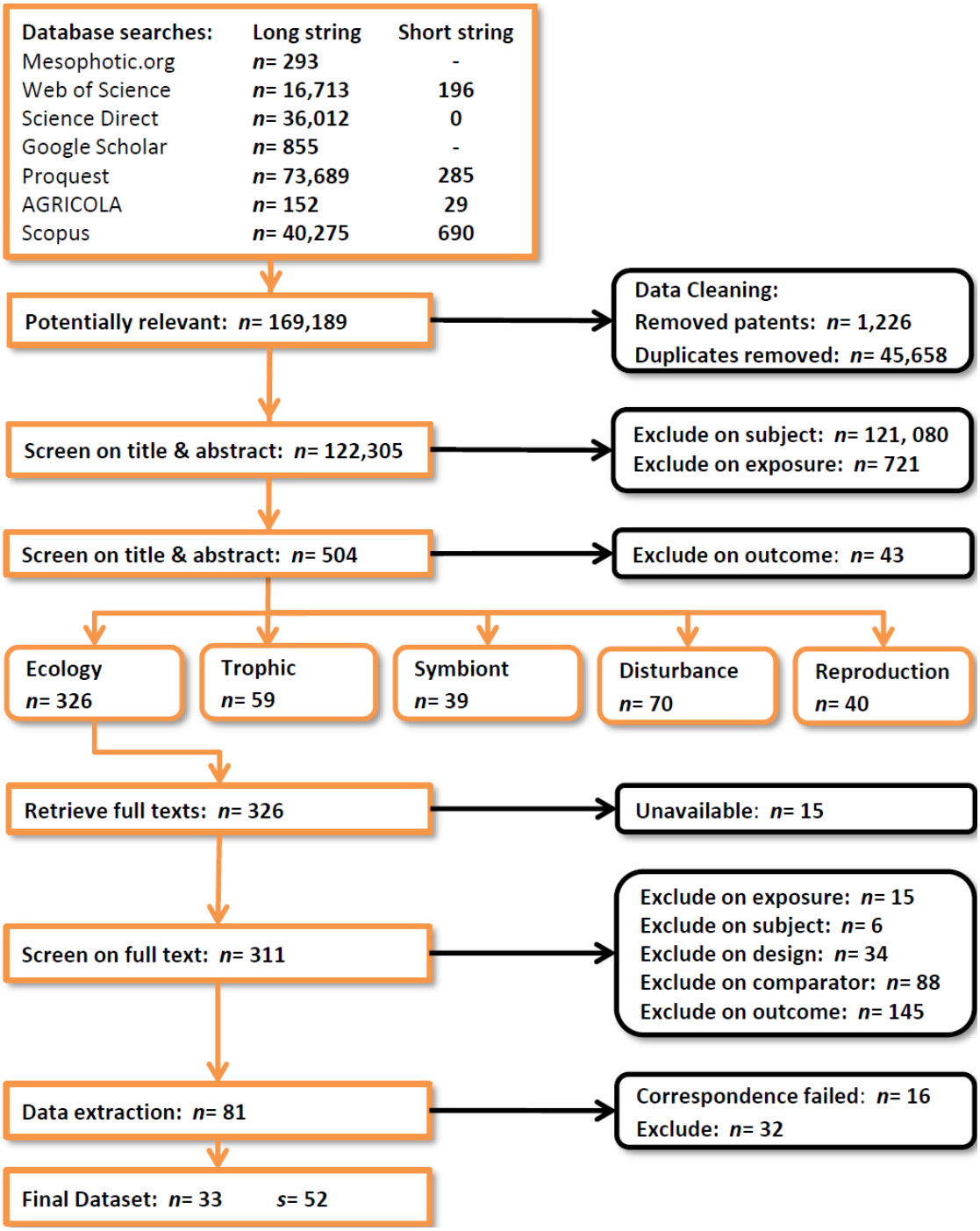


Figure 2.1 Screening report

The number of articles (n) *assigned to each screening criteria is reported. Black boxes are articles removed from consideration. Orange boxes contain articles included for the next level of screening. Note that a single article could be excluded for multiple reasons i.e. not reporting data shallower than 30m AND not containing the right type of data. Short and long string refer to the two search strings defined in our protocol paper. Our five tags were assigned to articles while screening by outcome at abstract level. At data extraction multiple studies* (s)*, data from combinations of taxa and locations, were reported in some articles*

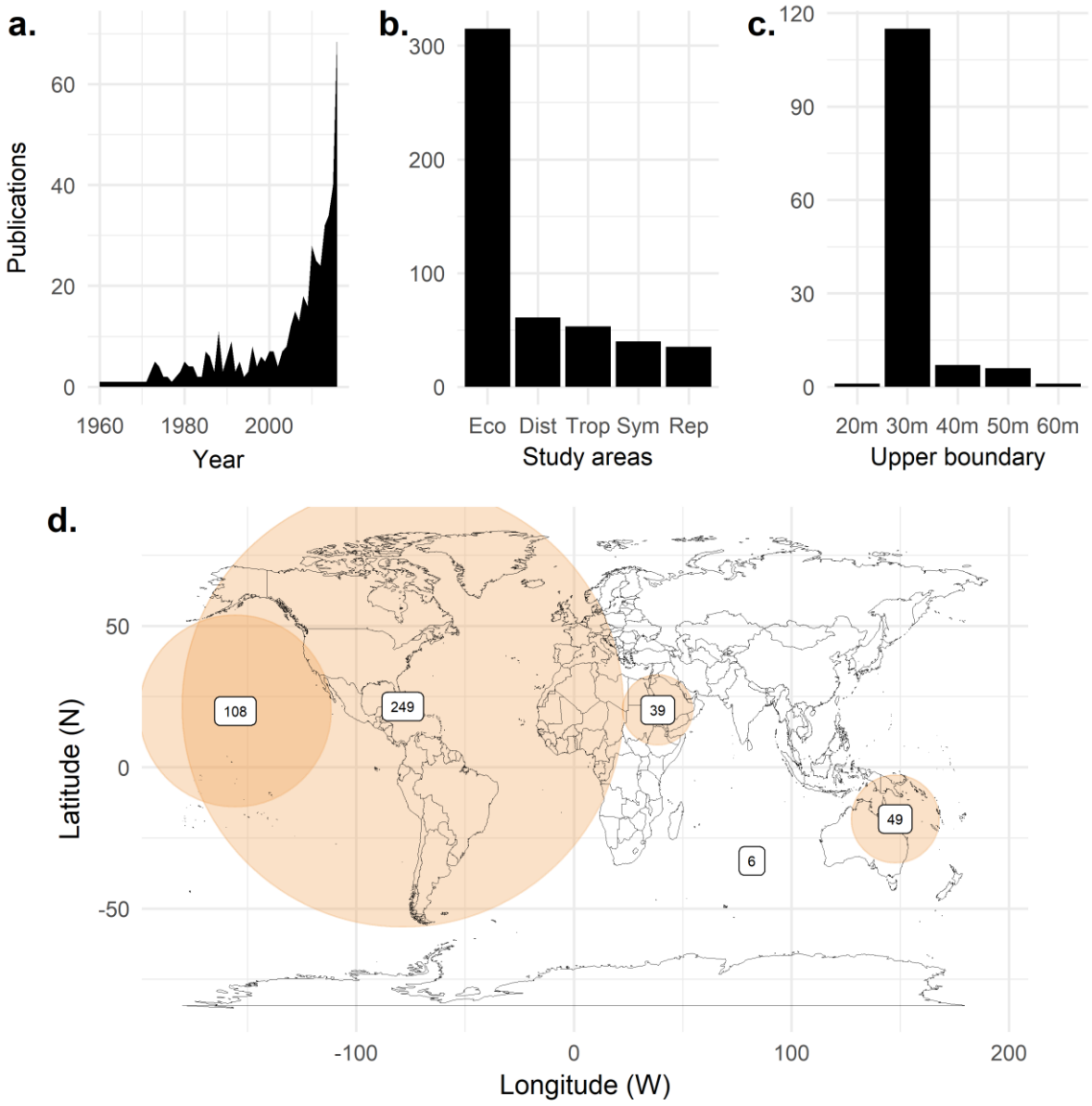


Figure 2.2 Literature base summary

A graphical summary of mesophotic research since 1960. a. the number of articles published in a given year. b. the number of articles identified as considering ecology, disturbance, trophic interactions, symbionts, and reproduction. Article categories were chosen from a word cloud generated from abstracts, then assigned manually. c. the number of articles reporting an upper depth limit for mesophotic reefs. If a range was reported the shallowest value was extracted. d. The geographical distribution of research effort on MCEs. The size of the orange circles indicates the number of articles in the region, also displayed in text labels.

2.4.2 Data-synthesis-

Across the whole dataset, the median proportion of shallow species also present below 30m is 0.67. The meta-analysis was performed on articles assigned as Eco at abstract level screening, and subsequently passed full-text level screening, as well as two further publications provided as titles at the review stage. Of these 81 + 2 articles (Figure 2.1), data were successfully extracted from 35, yielding 52 studies (combinations of broad taxon and locations within articles) after contacting corresponding authors (Supplementary 2.2). The consistency of data extraction was checked by a second reviewer for 12/35 articles included. Ecology papers we could not source a full text for are listed in (Supplementary 2.4). Both location and taxon were included as effect modifiers to aid biological interpretation and reduce the variance assigned to between study heterogeneity (I^2 of 10.1% reallocated). An interaction term was not included as intended in our protocol. This decision was made as studies were not evenly distributed across all factor-level combinations (Supplementary 2.5)) and a likelihood-ratio test failed to distinguish the two models (χ^2 = 10.8, P = 0.14). A Q-test detected significant between-study heterogeneity (QE = 182.2, df = 27, P < 0.0001). An I^2 of 72.3% can be interpreted as “substantial” under Cochrane review guidelines (Higgins and Green, 2008), but does increase with study number (Rücker *et al.*, 2008) and may be less important for an ecological synthesis. The R^2 of our model is 75.9%. A forest plot of effect size contributions from individual studies can be found in Supplementary 2.6.

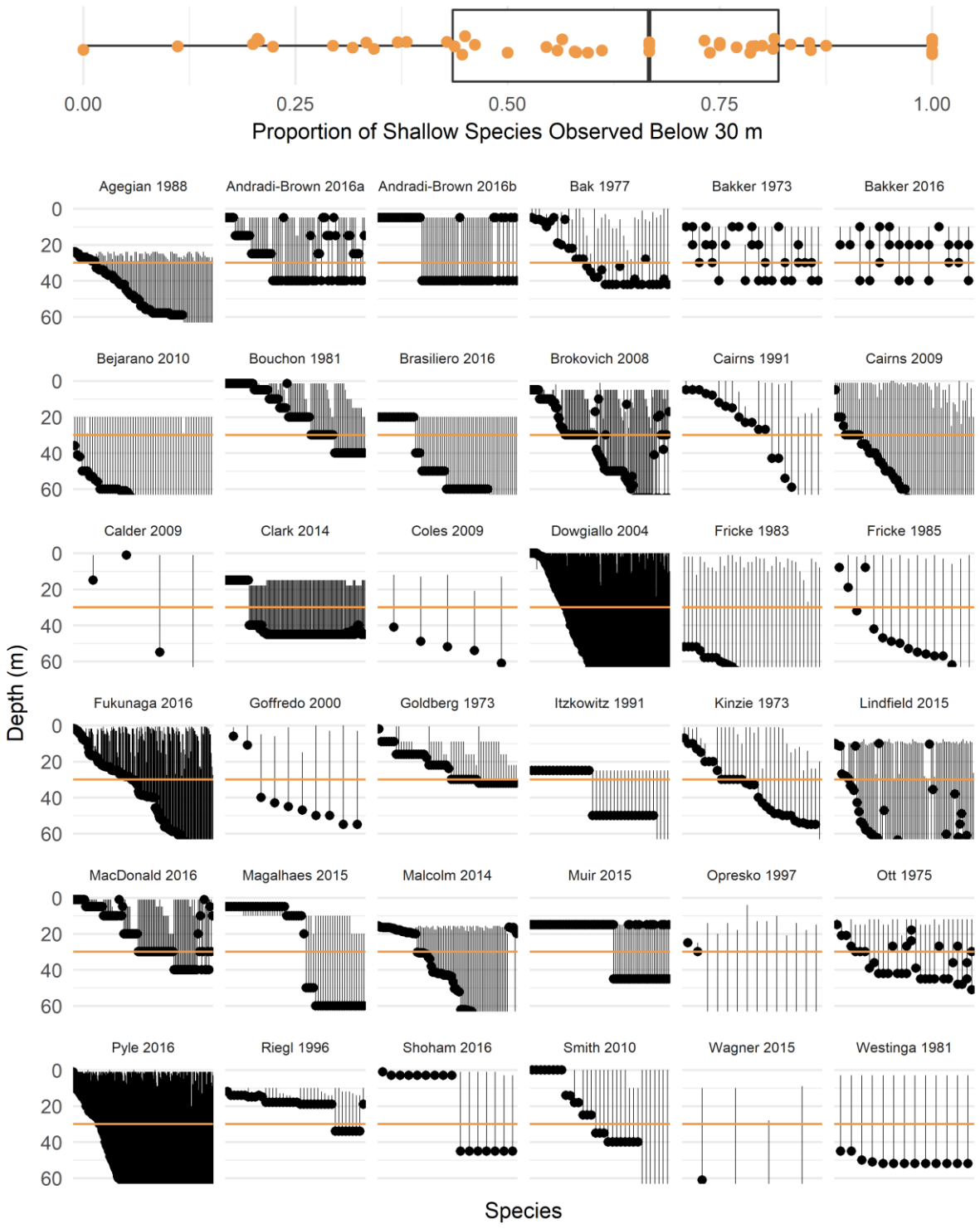


Figure 2.3 Shallow reef species depth ranges by publication

A boxplot shows the distribution of overlap estimates from all articles contributing data to the meta-analysis. Beneath, each panel illustrates the distribution of species depth ranges within the article. The scale ends at 60m for clarity. An orange line indicates the 30m mesophotic boundary. 36 'articles' are shown as two time periods were sampled in Bakker 2016 and two additional articles were provided at review.

Figure 2.4 visualises the proportion of shallow species present deeper than 30m, predicted from the model, for each modifier level. The central Pacific has the highest community overlap between shallow and mesophotic reefs at 72% (95% C.I. 53-85%). Studies off the cost of Brazil report the lowest at 30% (7-72%) when ignoring areas represented by a single study. For taxa, models of algal groups all returned predictions of over 75% overlap. 86% (37-98%) of shallow-water Antipatharia were found on mesophotic reefs, while octocorals were predicted to have the lowest overlap of all factor levels at 38% (15-68%). The amount of studies retrieved for a given factor level does influence the width of the confidence interval. Scleractinia with 18 studies has a prediction ranging by 29%, whereas Octocorallia with three studies ranges by 53%. Data underlying the figure can be found in supplementary 2.7.

As part of the sensitivity analysis 22 studies were excluded from the high validity group, following our validity appraisal criteria. The analysis was then re-run to quantify the influence of these studies over our synthesis (Figure 2.4). These studies are indicated in the comments column of supplementary 2.2. The dataset-wide estimate of community overlap dropped by 10 percentage points to 0.57. No change in broad pattern is noted, only a slight increase in the range of predictions, and a slight decrease in estimated overlap. We would generally expect a high validity sub-group analysis to narrow the prediction intervals. Our results suggest that the overriding influence may be the reduction in sample size, and that studies outside of the high validity group are largely similar to those retained. This is despite low validity studies operating at larger spatial scales and with unbalanced sampling effort with depth. A regression test provides no evidence for publication bias in the dataset (t = 0.5382, df = 33, P = 0.594), also visualised with a funnel plot (Supplementary 2.8). This is further supported by a Rosenthal's fail safe number (Rosenthal, 1979) of 1612 studies, it is

highly unlikely this many mesophotic surveys remain unpublished given the number we retrieved.

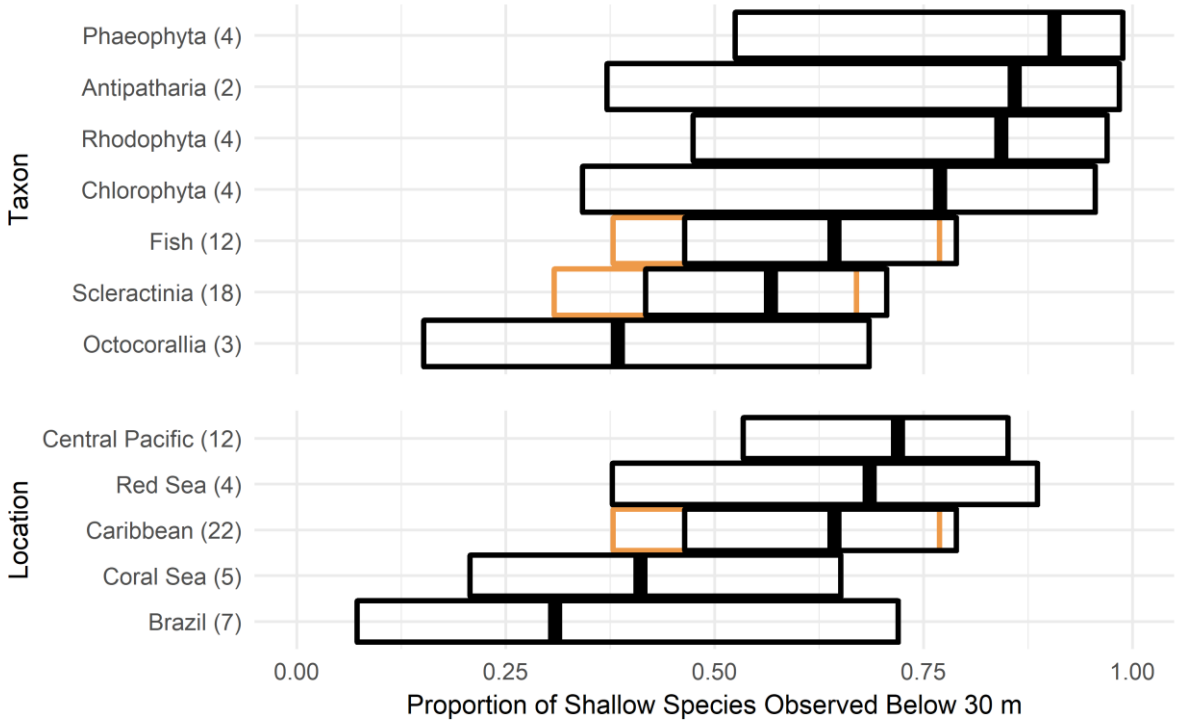


Figure 2.4 Effect of modifiers on community overlap

Crossbars show back transformed model predictions of community overlap by taxon and location. The thick central bar represents the prediction, the thinner bars on either extreme are 95% confidence intervals. The bars are asymmetrical because of the scaling of the effect sizes before back transformation. The number of studies contributing to the full model for a given level are in brackets. Orange crossbars are repeat predictions for high validity subgroups; only presented for three factor levels because of a lack of high validity data for other levels.

2.5 Discussion:

2.5.1 Quantitative results-

Two thirds of shallow-water species can be found on MCEs (Figure 2.3). This suggests MCEs should potentially be considered as a significant conservation target when protecting shallow reefs. This observation is in broad agreement with expert opinion regarding the

Caribbean; suggesting over 40% similarity for shallow and mesophotic benthic taxa and approximately 60% for fish (Semmler, Hoot and Reaka, 2016). Semmler et. al (2016) use Jaccard similarity and will therefore estimate lower similarity than this study. Jaccard similarity returns a lower score for reefs with mesophotic-specialist taxa while in this study community overlap was calculated excluding species not observed on shallow reefs.

As individual studies would lead us to expect, community overlap varies by region (Figure 2.4, Supplementary 2.7). The highest proportion of shallow species present on mesophotic reefs was predicted for the central Pacific at 72% (95% C.I. 53-85%). Brazilian reefs and the Coral Sea with the Great Barrier Reef both harbour less than half of shallow reef taxa on MCEs at 31% (7-72%) and 41% (20-65%) respectively. A number of drivers may explain these patterns but there are two likely candidates.

Reefs located in biodiversity hot spots, such as the Coral Triangle and Red Sea (Veron, 2000), may experience greater competition and subsequent specialisation (Knowlton and Jackson, 1994). This may lead to narrower depth ranges and so less community overlap in comparison to species-poor regions such as the Caribbean and Hawai'i. Additionally, the boundaries between mesophotic communities and shallow reef communities may vary between sites (Laverick, Andradi-Brown and Rogers, 2017). This is likely underpinned by abiotic factors interacting with species physiology. The current use of an arbitrary 30m depth limit, derived from recreational SCUBA limitations, may be limiting our ability to accurately report on ecological pattern. If the transition depth occurs deeper, a larger portion of shallow water species will be present below 30m. The deviation in community transition depth from 30m between sites may explain some of the variability in our estimates of community overlap. The maximum depth limits of photosynthetic corals

are known to vary by region (Baker *et al.*, 2016) and with light (Kahng *et al.*, 2010) and loosely correlate with our findings.

Community overlap can be broken down further by predicting the effect of different taxon levels (Figure 2.4, Supplementary 2.7). The two taxonomic groups with the largest number of studies contributing to the synthesis, fish and Scleractinia, are estimated as having 64% (46-79%) and 57% (42-71%) of shallow taxa on MCEs respectively. These values are relatively close to our synthesis-wide estimate. Other taxonomic groups have relatively large confidence intervals, but all three algal taxa return high community overlap of over 75%. This may be surprising for autotrophic taxa, but it appears a 30m boundary is within the physiological envelope of most algal species identified. The high estimate of 86% (37-98%) for Antipatharia, though unintuitive for a taxon traditionally considered 'deep' (Cairns, 2007), likely results from limiting the analysis to species observed shallower than 30m. For the most part, if a black coral is observed shallower than 30m it is likely a deep species encroaching on shallow reefs. Alternatively, a small number of species may have evolved towards shallow-water emergence. The smaller number of species contributing to estimates for taxa other than fish and Scleractinia highlights the need for taxonomic diversity in future mesophotic studies. It is also important to maintain shallow sampling for contemporary comparisons to allow for site to site variation.

Our finding of over half of shallow taxa being recorded on MCEs is not in direct contention with research suggesting mesophotic-specific communities on MCEs (Bejarano, Appeldoorn and Nemeth, 2014; Muir *et al.*, 2015; Semmler, Hoot and Reaka, 2016; Shoham and Benayahu, 2016; Laverick, Andradi-Brown and Rogers, 2017). Firstly, we deliberately exclude taxa only observed on mesophotic reefs from our analysis. This is as MCEs may

extend below 150m (Kahng *et al.*, 2010) while the majority of the studies synthesised here do not sample as deep. Any estimate of the proportion of mesophotic taxa not on shallow reefs would likely be misleading because of an absence of evidence (Supplementary 2.9). Additionally, the lower limit of MCEs is acknowledged to be variable (Kahng, Copus and Wagner, 2014; Loya *et al.*, 2016). Further analyses considering overlap at the lowest limit of MCEs would therefore be inappropriate because of a similar lack of sampling. Our primary research question considers the potential for shallow water species to exploit deep reef refuges.

Secondly, we consider MCEs in a narrow sense as a 30m depth limit. As depth increases the ratio of shallow taxa to mesophotic-specialists declines (Supplementary 2.9) (Semmler, Hoot and Reaka, 2016). When sampling is evenly distributed across the whole depth range of an MCE it is possible to detect depth-specific community structure (Laverick, Andradi-Brown and Rogers, 2017) and high levels of geographically endemic biodiversity (Kosaki *et al.*, 2017); though this likely varies between locations (Laverick, Andradi-Brown and Rogers, 2017). It is important to note consensus in the field is to recognise an upper mesophotic zone, in which shallow species are gradually lost with depth, and a lower mesophotic zone containing specialist communities (Bongaerts *et al.*, 2010; Loya *et al.*, 2016), rather than rigidly following the 30m boundary. Any conclusions regarding shallow taxon occurrence deeper than 30m is therefore likely driven by pattern in the upper mesophotic. We are reticent to perpetuate the definition of these zones by arbitrary depth limits because of the potential for between site variation (Laverick, Andradi-Brown and Rogers, 2017).

2.5.2 Qualitative analysis-

At the outset of this project www.mesophotic.org represented the largest publicly available compilation of mesophotic literature, with the metadata of 293 articles available. This study succeeded in boosting this number to at least 461, considerably increasing the visibility of research. Absent from this list of papers are any published in 2017 or later. The new database offered the opportunity to categorise these studies, describing the field as a whole.

Past reviews have noted the geographical bias of mesophotic research (Kahng *et al.*, 2010; Baker *et al.*, 2016; Turner *et al.*, 2017). This is to some extent unavoidable because of the locations of research groups in developed countries near accessible reefs (i.e., Hawai'i, US Virgin Islands, and Israel among others). We confirm the bias towards studies in the central Pacific and western Atlantic region. A better global understanding of MCEs requires increased research attention in the Coral Triangle and the wider Indian Ocean to the west. Currently MCE research attention focuses on reefs with lower coral species richness (Veron, 2000). Though this may be convenient for a number of reasons, it may mean ecological differences between shallow and mesophotic reefs resulting from greater competition and niche diversification are missed.

In addition to geographic biases, the mesophotic field lacks diversity in terms of study types. As expected for a field which has only recently taken off (Figure 2.2a), a large proportion of the studies focus on ecological description. This has already been noted across existing research and narrative reviews (Turner *et al.*, 2017) but we lend support to the observation with a larger literature base. This pattern has been shifting over time (Turner *et*

al., 2017), but there is certainly room for an increase in experimental, modelling, and longitudinal studies considering processes instead of pattern.

A final interesting observation is that the term "mesophotic" has been defined differently across published work. Though the overwhelming majority of papers define mesophotic reefs as starting at 30m, the mesophotic research strategy more loosely states they can start from 30-40m (Puglise *et al.*, 2009). This 30m limit is rooted in recreational dive limits, rather than biological boundaries which can vary (Laverick, Andradi-Brown and Rogers, 2017). Not surprisingly the next most common definition is for mesophotic communities starting at 40m. More interestingly, six papers within the last 10 years define MCEs from 50m with another one at 20m and one at 60m. As the number of papers on mesophotic reefs increases it is important to remain consistent in the application of terminology, or specify explicitly how these boundaries may be recognised to vary.

2.5.3 Reasons for heterogeneity-

Our analysis returns an I^2 of 72.3%, under Cochrane review guidelines this can be interpreted as 'substantial' unexplained heterogeneity (Higgins and Green, 2008). However, Cochrane review guidelines were developed by the health sector, and so largely with the synthesis of controlled clinical trials in mind. In ecological studies higher degrees of residual variation are often expected, as a result of the messiness of the 'real' world and confounding variables arising outside of an experimental framework.

As already mentioned, if the 30m boundary of shallow and mesophotic systems is not shared across all studies the estimates of community overlap could be impacted. A community transition at 20m would result in few shallow species deeper than 30, similarly in areas of high light penetration mesophotic communities may begin deeper (Kahng, Copus

and Wagner, 2014), inflating the proportion of shallow water species occurring deeper than 30m. If this is the cause of our high between study heterogeneity, then this study may suggest the 30m depth boundary can be misleading. Our analysis could be improved by including abiotic data believed to affect the rates of community transition for each study, such as light attenuation coefficients and topography (Costa *et al.*, 2015). These data are not available for the synthesised studies, and new research should ensure environmental data is collected alongside ecological surveys.

2.5.4 Review limitations-

We hoped to incorporate abundance into a meta-analysis of communities (Warwick and Clarke, 1993; Savage, Field and Warwick, 2001; Laverick *et al.*, 2016), yet this approach would sharply reduce the amount of included studies because of differing measures of abundance, as well as preventing the incorporation of sessile and motile taxa into one analysis. Adopting a presence-absence approach does, however, miss the importance of population density. Our analysis could have been influenced by population over-spill where a few out-lying individuals from an otherwise shallow species were observed deeper than 30m (Bongaerts, under review). The refuge dynamics which may occur on MCEs must be underpinned by reproductively active source populations, rather than the sinks represented by outlier observations as hypothesised in deep-sea ecosystems (Rex *et al.*, 2005). Though such outliers are unlikely to represent source populations for a deep reef refuge, they may none-the-less protect genetic diversity (Sinniger *et al.*, 2017).

Additionally, our interpretation of the impacts of effect modifiers should be taken with caution. Confidence intervals are wide because of a lack of studies across most

taxonomic groups, only fish and Scleractinia predictions are based on more than five studies (Supplementary 2.7).

2.6 Conclusion:

2.6.1 Information for policy/management-

It is clear that a notable proportion of shallow reef taxa are also present on MCEs, though this varies significantly by taxon and location. The findings of this systematic review validate conservation decisions supporting the protection of MCEs (NPA, 2009) as potential "lifeboats" for shallow reefs (Baker, Puglise and Harris, 2016b) based on community overlap, though further research into refuge dynamics specifically is required. It is important to note distinct mesophotic biodiversity may deserve protection in its own right, something our analysis is unable to inform.

2.6.2 Implications for research-

The degree of overlap varies by region and taxon, however, so too does research effort and the information available to us. We recommend that in the years to come researchers conduct survey work of lesser studied, but ecologically important, taxa and also attempt to visit MCEs as yet unstudied. This extra knowledge will aid conservation prioritisation efforts and allow us to identify deeper coral reef regions currently unknown to conservation managers.

2.7 Acknowledgements:

We would like to thank all of the authors who supplied us with PDF copies of their manuscripts and underlying data when requested. We also would like to thank our editor and anonymous reviewers, who greatly helped improve the transparency of this manuscript.

2.8 Authors contributions:

In addition to the contributions to the project detailed in our protocol paper, JHL conducted the literature search. JHL and SP carried out the screening of all articles, extracted, and formatted the data. JHL and SP retrieved full texts and underlying data with help from the advisory committee (DAAB, DAE, PB, TCLB, MPL, RLP, MS and DW). JHL performed the statistical analysis and wrote the initial draft. ADR supervised the project and edited the manuscript. The advisory committee and SP also commented on the manuscript.

2.9 Supplementary information:

Supplementary 2.1 Google scholar web-scraping

The following outlines the process used to extract search results from Google Scholar. The lack of a 'download results' feature on Google Scholar results pages makes the generation of a workflow necessary. This was pieced together from a number of online help files, papers, and conversations, referred to when available. We are grateful to the multiple sources of help.

Storing Search Results

The steps used to download the search results were outlined by Haddaway et al. (N. R. Haddaway *et al.*, 2017) https://environmentalevidencejournal.biomedcentral.com/articles/10.1186/s13750-016-0079-2.

Data Scraping

Unfortunately, the import.io method described for the extraction of data from HTML files on a local server is no longer functional (import.io, Pers. comm). Instead we used the package rvest (Wickham and RStudio, 2016) in the programming language R (R-Core-Team, 2013).

Below is the code used. It is worth noting a lower number of hits were returned than expected from the scraped pages. This results from inconsistencies in the format Google Scholar presents data. We were also unable to retrieve abstracts as not all search results contained them. The inclusion of only a handful of abstracts caused difficulties in constructing a data frame.

First a list of locally stored files to be scraped needed to be created. With potentially a hundred pages per search this is time-consuming manually. The below allows the automatic generation of files names, provided they follow a pattern.

```
rm(list=ls())
setwd("_____")

library(rvest)

#### list of URLs ####

files <- c(0:197)
#number of saved webpages.

files <- lapply(0:197, function(files){paste0("scholar_", files, ".htm")})
#take the unique IDs and turn them into file names.

files <- lapply(files, read_html)
#apply read_html to all of the file names in the list.
```

Open one of the files in a web browser with 'selector gadget' https://cran.r-project.org/web/packages/rvest/vignettes/selectorgadget.html present on the favourites bar. This tool was used to identify the location on the page of information we are interested in. A video tutorial is available here http://selectorgadget.com/.

Next the data from a single file were extracted. We use the text strings returned by selector gadget as the argument in `html_nodes()`. This checks the extraction is working correctly, but also generates the first entry in the data frame for the coming for loop to bind to.

```
start <- read_html('scholar_0.htm')

Bib <- data.frame( Titles = start %>%  html_nodes(".gs_rt a") %>% html_tex
t(),
                   Authors = start %>%  html_nodes(".gs_a") %>% html_text
())
```

Unfortunately, a lack of structure in Google Scholar outputs prevented the extraction of authors, journals, and dates separately. These were extracted as one and were later cleaned

in excel. Next `for` was used to repeat the extraction through all locally stored htm files and to bind the results to a data frame.

```
for (i in files)
#Loop through all the files.


{

Bib_temp <- data.frame( Titles = i %>%  html_nodes(".gs_rt a") %>% html_te
xt(),
                   Authors = i %>%  html_nodes(".gs_a") %>% html_text())

Bib <- rbind(Bib, Bib_temp)
#bind new data to Bib
}


write.csv(Bib, 'Google_Scholar.csv')
```

Data Cleaning

The .csv file was then opened in excel to manipulate the data into a RIS format for upload to EPPI Reviewer 4.

1) Select the authors column, then data > text to columns > delimited > comma.
2) Set new column headings - Title - Author(1-n) - Source - Date
3) Review each author column to ensure no double-barreled surnames have been split, correct instance where the have.
4) To make sure Source and Date data is in the correct column, insert empty cells as required for rows with less than the maximum number of authors.
5) use =concatenate(cell,",",cell,",") to combine all the author columns. You should now have 4 columns
6) use replace ,, with , until no more replacements are made. This removes the excess commas from the end of short author lists.

Import to Endnote

These data were then ready to be converted into the format of the reference management software selected. Here endnote was used to manage references and convert them into RIS format for upload to EPPI Reviewer 4. To import the .csv file into Endnote the guide from UTS library was used at http://www.lib.uts.edu.au/question/5951/how-can-i-transfer-references-endnote-excel-spreadsheet

The bullet points are repeated below as a direct quotation, in case the URL breaks in future:

1) We will be using line 1 of the spreadsheet for the field names used in all the references (author, title, etc), and column A of the spreadsheet for their reference types. So you will need to insert a new, empty column A; and possibly a new, empty line 1.
2) Type the words Reference Type into cell A1. Leave the rest of column A empty for the moment. Field names (see step 3) should be added into line 1 starting with cell B1. The order of the field names along line 1 doesn't matter, so long as each field name corresponds to the data in its column (eg the field name Author should be above the column that contains all the author names).
3) You must take the field names from EndNote as follows:

- In EndNote, click on Edit (on a Mac, click on EndNote X5), Preferences, Reference Types, Modify Reference Types
- Use the drop down menu to select different reference types. For each reference type, the field names you need to use are in bold, in the column headed by the word "Generic". So for example, for journal articles, your column of journal names should be headed in line 1 by the field name "Secondary Title".

4) The rest of the spreadsheet should contain all the references, starting in line 2, one reference per line, so that the columns below each of the field names contain the correct data for that field (authors, titles, page numbers, etc).

5) The order of the references doesn't matter, and it's OK to leave empty cells here and there; but don't leave whole columns empty.

6) Authors and editors should be surname, first name (or initials). If there are multiple authors or editors, type // between them, eg Smith, Jenny//Jones, K//Brown, VM

7) Then type all the appropriate reference types into column A.

8) If you have any double quotes (") anywhere (eg in some of your titles), change them all to single quotes (')

9) Save this Excel file with a new name.

10) Then click on File, Save As...

11) Change Save as type to Text (Tab delimited), call the file any name you like, and save it somewhere convenient (eg desktop). Say OK and Yes to any notices that pop up.

12) Close the Excel file, this time saying No to saving changes.

13) Open up the saved text file, and put the cursor in top left corner. Hit , move the cursor back to top left corner, and type *Generic

14) Click on Edit, Replace. In the Find what box, type a double quote ". Leave the Replace with box empty. Then click on Replace All. (If we don't get rid of the double quotes around the multiple authors at this point they end up in our EndNote references and are much more trouble to get rid of. This is why we changed double quotes to single quotes in step 8).

15) Close the text file, saving changes.

16) Open EndNote, open a reference library, click on File, Import. Choose File - choose your saved text file. Import Option - choose Tab Delimited. Click Import. The first time you do this, try it on a new, empty reference library just to test the process out! Once you're sure it works OK, repeat with the same text file into your real reference library.
17) References should now be in EndNote correctly. Once you've checked them, if they're OK you can delete the saved text file, and any of the Excel files that you don't want to keep.

Upload to EPPI Reviewer 4

The entire library of hits in RIS format can be exported, from the reference management software of your choosing, for upload to EPPI Reviewer 4. The library should be split into chunks of around 3,000 hits to ensure a complete upload. This can be done manually, but the program 'GSplit3' was used for greater ease. A split was specified in the text RIS formatted file 'BEFORE the 3,000th occurrence of TY-'. The footer of each chunk file was checked to ensure no meta-data from GSplit3 was appended. Each resulting chunk file was uploaded to EPPI Reviewer 4.

Supplementary 2.2 Data extraction report

All studies identified during full text screening as containing data of interest are listed below. The comments column provides explanations for publications which did not contribute data for the final meta-analysis, studies which were assigned to the low validity group, as well as whether the authors were contacted.

Short Title	Title	Contacted	Comment
Included			
(Agegian and Abbott, 1985)	Deep water macroalgal communities: a comparison between Penguin Bank, Hawaii, and Johnston Atoll		Low validity - fewer than 11 species for Phaeophyta
(D. A. Andradi-Brown, Gress, *et al.*, 2016) -a	Reef fish community biomass and trophic structure changes across shallow to upper- Mesophotic reefs in the mesoamerican barrier reef, Caribbean		
(D. A. Andradi-Brown, Macaya-Solis, *et al.*, 2016) -b	Assessing caribbean shallow and mesophotic reef fish communities using Baited-Remote Underwater Video (BRUV) and diver-operated video (DOV) survey techniques		
(Bak, 1977)	LONG-TERM CHANGE IN CORAL COMMUNITIES ALONG DEPTH GRADIENTS OVER LEEWARD REEFS IN THE NETHERLANDS-ANTILLES		
(De Bakker *et al.*, 2016)	Long-term shifts in coral communities on shallow to deep reef slopes of Curaçao and Bonaire: are there any winners?		
(Bejarano, Nemeth and Appeldoorn, 2011)	Use of mixed-gas rebreathers to access fish assemblages in Mesophotic Coral Ecosystems (MCE) off La Parguera shelf-Edge, Puerto Rico		Low validity – Uneven sampling
(Bouchon, 1981)	Quantitative Study of the Scleractinian Coral Communities of a Fringing Reef of Reunion Island (Indian Ocean)		Provided at review
(Brasileiro *et al.*, 2015)	Macroalgal composition and community structure of the largest rhodolith beds in the world		
(Brokovich *et al.*, 2008)	Descending to the twilight-zone: changes in coral reef fish assemblages along a depth gradient down to 65 m		
(Cairns, 1991)	A REVISION OF THE AHERMATYPIC SCLERACTINIA OF THE GALAPAGOS AND COCOS ISLANDS		Low validity – Uneven sampling
(Cairns, Jaap and Lang, 2009)	Scleractinia (Cnidaria) of the Gulf of Mexico		Low validity – regional key

Short Title	Title	Contacted	Comment
(Calder and Cairns, 2009)	Hydroid diversity and species composition along a gradient from shallow waters to deep sea around Bermuda		Passed to Calder 1998, Low validity - fewer than 11 species, Uneven sampling
(Clark *et al.*, 2014)	Fish and Benthic Communities of the Flower Garden Banks National Marine Sanctuary: Science to Support Sanctuary Management		
(Coles *et al.*, 2009)	Spatial distribution of deepwater seagrass in the inter-reef lagoon of the Great Barrier Reef World Heritage Area		Low validity - fewer than 11 species
(Dowgiallow, 2004)	Patterns in diversity and distribution of benthic molluscs along a depth gradient in the Bahamas		
(Fricke and Schuhmacher, 1983)	THE DEPTH LIMITS OF RED SEA STONY CORALS AN ECO PHYSIOLOGICAL PROBLEM A DEEP DIVING SURVEY BY SUBMERSIBLE		Low validity – Uneven sampling
(Fricke and Meischner, 1985)	Depth limits of Bermudan scleractinian corals: a submersible survey		Low validity – Uneven sampling
(Fukunaga *et al.*, 2016)	Structure of mesophotic reef fish assemblages in the Northwestern Hawaiian Islands		Low validity – Uneven sampling
(Goffredo and Chadwick-Furman, 2000)	Abundance and distribution of mushroom corals (Scleractinia : Fungiidae) on a coral reef at Eilat, northern Red Sea		Low validity - fewer than 11 species
(Goldberg, 1973)	ECOLOGY OF CORAL-OCTOCORAL COMMUNITIES OFF SOUTHEAST FLORIDA COAST - GEOMORPHOLOGY, SPECIES COMPOSITION, AND ZONATION		
(Itzkowitz *et al.*, 1991)	A RECONNAISSANCE OF THE DEEPER JAMAICAN CORAL REEF FISH COMMUNITIES		
(Kinzie, 1973)	The zonation of west indian gorgonians		Provided at review Low validity – Uneven sampling
(Lindfield *et al.*, 2015)	Mesophotic depths as refuge areas for fishery-targeted species on coral reefs		
(MacDonald, Bridge and Jones, 2016)	Depth, bay position and habitat structure as determinants of coral reef fish distributions: Are deep reefs a potential refuge?		

Short Title	Title	Contacted	Comment
(Magalhães *et al.*, 2015)	Changes in benthic communities along a 0-60 m depth gradient in the remote St. Peter and St. Paul Archipelago (Mid-Atlantic Ridge, Brazil)		Low validity - fewer than 11 species for Phaeophyta, Chlorophyta, Cnidaria
(Malcolm, Jordan and Smith, 2011)	Testing a depth-based Habitat Classification System against reef fish assemblage patterns in a subtropical marine park		
(Muir *et al.*, 2015)	Diverse staghorn coral fauna on the mesophotic reefs of north-east Australia		Low validity – Uneven sampling
(Opresko and Sánchez, 2005)	Caribbean shallow-water black corals (Cnidaria: Anthozoa: Antipatharia)		Low validity – regional key
(Ott, 1975)	QUANTITATIVE ANALYSIS OF COMMUNITY PATTERN AND STRUCTURE ON A CORAL REEF BANK IN BARBADOS, WEST INDIES		
(Pyle *et al.*, 2016)	A comprehensive investigation of mesophotic coral ecosystems in the Hawaiian Archipelago		Low validity – Uneven sampling
(Riegl and Riegl, 1996)	Studies on coral community structure and damage as a basis for zoning marine reserves		
(Shoham and Benayahu, 2016)	Higher species richness of octocorals in the upper mesophotic zone in Eilat (Gulf of Aqaba) compared to shallower reef zones		
(Smith *et al.*, 2010)	Benthic structure and cryptic mortality in a Caribbean mesophotic coral reef bank system, the Hind Bank Marine Conservation District, US Virgin Islands		Passed to 2017 monitoring data
(Wagner, 2015)	A taxonomic survey of the shallow-water (<150 m) black corals (Cnidaria: Antipatharia) of the Hawaiian Islands		Low validity - fewer than 11 species, Uneven sampling
(Westinga and Hoetjes, 1981)	The Intrasponge Fauna of Spheciospongia vesparia (Porifera, Demospongiae) at Curacao and Bonaire		Low validity – Uneven sampling
Excluded			
Amado-Filho (2007)	Structure of rhodolith beds from 4 to 55 meters deep along the southern coast of Espírito Santo State, Brazil	Y	Communication failed
Amado-Filho (2012)	Occurrence and distribution of rhodolith beds on the Fernando de Noronha Archipelago of Brazil	Y	Communication failed

Short Title	Title	Contacted	Comment
Andradi-Brown (2016)	Identifying zooplankton community changes between shallow and upper-mesophotic reefs on the Mesoamerican Barrier Reef, Caribbean	N	Not to Species level
Avery (1998)	Bahamian reef communities: Composition, recruitment and change (10 meters to 250 meters)	N	Not to Species level
Bak (2005)	Coral reef crisis in deep and shallow reefs: 30 years of constancy and change in reefs of Curacao and Bonaire	Y	Passed to Bak (1977)
Baldwin (2015)	A new, mesophotic Coryphopterus goby (Teleostei, Gobiidae) from the southern Caribbean, with comments on relationships and depth distributions within the genus	N	Not a community level
Barrios-Suárez (2002)	Distribution of anemones (Anthozoa: Actiniaria and Corallimorpharia) in the area of Santa Marta, Colombian Caribbean	Y	Communication failed
Bouchon-Navaro (2005)	Biogeographic patterns of coastal fish assemblages in the West Indies	Y	Communication failed
Cheney (1974)	Deep-water benthic algae of the Florida Middle Ground	Y	Communication failed
Cordeiro (2015)	Mesophotic coral ecosystems occur offshore and north of the Amazon River	Y	Communication failed
Etnoyer (2010)	Rapid assessment of octocoral diversity and habitat on Saba Bank, Netherlands Antilles	Y	Communication failed
Eyal (2015)	Euphyllia paradivisa, a successful mesophotic coral in the northern Gulf of Eilat/Aqaba, Red Sea	N	Duplicate data
Feitoza (2005)	Ecology and Zoogeography of Deep-Reef Fishes in Northeastern Brazil	Y	Survey below 35m
Francini (2014)	Reef communities of the Saint Peter and Saint Paul's Archipelago across an euphotic-mesophotic depth gradient (0-90 m)	N	Thesis captured as scientific paper
Fraser (2008)	Reef morphology and invertebrate distribution at continental shelf edge reefs in the South Atlantic Bight	N	Not a community level
Garcia-Sais (2010)	Reef habitats and associated sessile-benthic and fish assemblages across a euphotic-mesophotic depth gradient in Isla Desecheo, Puerto Rico	Y	Communication failed
Harris (2004)	Submerged coral reefs in the Gulf of Carpentaria, Australia	N	Topography not community focussed

Short Title	Title	Contacted	Comment
Harvey (2007)	Bait attraction affects the performance of remote underwater video stations in assessment of demersal fish community structure	Y	Communication failed
Hurley (2016)	An assessment of shallow and mesophotic reef brachyuran crab assemblages on the south shore of O'ahu, Hawai'i	Y	Communication failed
Luck (2013)	Polyphyly and hidden species among Hawai'i's dominant mesophotic coral genera, Leptoseris and Pavona (Scleractinia: Agariciidae)	N	does not consider shallow communities
Madin (2016)	The Coral Trait Database, a curated database of trait information for coral species from the global oceans	N	Repeats already captured data
Meirelles (2015)	Baseline Assessment of Mesophotic Reefs of the Vitoria-Trindade Seamount Chain Based on Water Quality, Microbial Diversity, Benthic Cover and Fish Biomass Data	Y	Communication failed
Newman (1996)	Variation in reef associated assemblages of the Lutjanidae and Lethrinidae at different distances offshore in the central Great Barrier Reef	N	Not community level
Parrish (2004)	Habitat and reef-fish assemblages of banks in the Northwestern Hawaiian Islands	Y	Depth range of 24-40m considered too narrow
Pereira-Filho (2011)	Reef fish and benthic assemblages of the Trindade and Martin Vaz Island group, southwestern Atlantic	Y	Communication failed
Przeslawski (2015)	Implications of Sponge Biodiversity Patterns for the Management of a Marine Reserve in Northern Australia	N	Sledges used, no depths
Reed (1997)	Biodiversity and distribution of deep and shallow water sponges in the Bahamas	Y	Communication failed
Reed (2011)	Final Cruise Report: Florida Shelf-Edge Expedition (FLoSEE) Deepwater Horizon Oil Spill Response: Survey of Deepwater and Mesophotic Reef Ecosystems in the Eastern Gulf of Mexico and Southeastern Florida. R/V Seward Johnson and Johnson-Sea-Link Submersible. July 9-August 9, 2010	N	Not to Species level
Robertson (2016)	The fishes of Cayo Arcas (Campeche bank, Gulf of Mexico): An updated checklist	Y	Depth ranges unavailable
Rooney (2010)	Mesophotic coral ecosystems in the Hawaiian Archipelago	N	Not to Species level

Short Title	Title	Contacted	Comment
Sammarco (2012)	Geographic expansion of hermatypic and ahermatypic corals in the Gulf of Mexico, and implications for dispersal and recruitment	Y	Communication failed
Sánchez (1998)	Patterns of octocoral and black coral distribution in the oceanic barrier reef-complex of Providencia island, southwestern Caribbean	Y	Communication failed
Sarano (1988)	Morphology and ecology of the deep fore reef slope at Osprey Reef (Coral Sea)	N	No contact information
Semmler (2016)	Are mesophotic coral ecosystems distinct communities and can they serve as refugia for shallow reefs?	Y	Significant element of expert opinion and undue influence due to study size
Sheppard (1980)	Coral cover, zonation and diversity on reef slopes of Chagos Atolls, and population structures of the major species	Y	Deep data lost
Smith (1999)	Short term variability in larval fish assemblages of the Sydney shelf: Tracers of hydrographic variability	Y	Not to Species level
Smith (2008)	Assessing coral reef health across onshore to offshore stress gradients in the US Virgin Islands	N	Not to Species level
Spalding (2012)	Ecology of mesophotic macroalgae and Halimeda kanaloana meadows in the Main Hawaiian Islands	N	Thesis captured as scientific paper
Stanley (2000)	Variation in the density and species composition of fishes associated with three petroleum platforms using dual beam hydroacoustics	N	Pelagic species
Starr (2007)	Spatial dynamics of the Nassau grouper Epinephelus striatus in a Caribbean atoll	N	Not community level
Vermeij (2003)	Species-Specific Population Structure of Closely Related Coral Morphospecies Along a Depth Gradient (5-60 m) Over a Caribbean Reef Slope	N	Not community level
Wagner (2011)	Azooxanthellate? Most Hawaiian black corals contain Symbiodinium	Y	Author suggests data was superseded by Wagner (2015)
Wagner (2012)	The Biology and Ecology of Hawaiian Black Corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia)	Y	Thesis captured as scientific paper
Wagner (2015)	The spatial distribution of shallow-water (< 150 m) black corals (Cnidaria: Antipatharia) in the Hawaiian Archipelago	Y	Author suggests data was superseded by Wagner (2015)

Short Title	Title	Contacted	Comment
Waheed (2015)	Scleractinian corals (Fungiidae, Agariciidae and Euphylliidae) of Pulau Layang-Layang, Spratly Islands, with a note on Pavona maldivensis (Gardiner, 1905)	Y	Data unavailable and author failed to respond
Weaver (2006)	Deep reef fish surveys by submersible on Alderdice, McGrail, and Sonnier Banks in the northwestern Gulf of Mexico	N	Depth data unavailable
Whitfield (2014)	Native fish community structure and Indo-Pacific lionfish Pterois volitans densities along a depth-temperature gradient in Onslow Bay, North Carolina, USA	Y	Communication failed
Zuschin (2005)	Diversity patterns of bivalves in a coral dominated shallow-water bay in the northern Red Sea - High species richness on a local scale	Y	Could not remove non reef species

Supplementary 2.3 Full text report

All studies with full texts retrieved and identified during the screening process as ecological are listed. Code details the screening decision for the entry based on full text screening

Short Title	Title	Code
Abbey (2013)	Deglacial mesophotic reef demise on the Great Barrier Reef	EXCLUDE on outcome, on comparator
Affendi (2011)	Coral ecology of a unique continental island of Pulau Perak, Kedah, Malaysia/Affendi Yang Amri	EXCLUDE on outcome, on comparator
Agegian (1988)	Deep water macroalgal communities: a comparison between Penguin Bank, Hawaii, and Johnston Atoll	INCLUDE on full text
Aguilar-Perera (2016)	Lionfish invaded the mesophotic coral ecosystem of the Parque Nacional Arrecife Alacranes, Southern Gulf of Mexico	EXCLUDE on outcome
Allen (2004)	The recent species of the genera Limatula and Limea (Bivalvia, Limacea) present in the Atlantic, with particular reference to those in deep water	EXCLUDE on subject
Amado (2012)	Occurrence and distribution of rhodolith beds on the Fernando de Noronha Archipelago of Brazil	INCLUDE on full text
Amado-Filho (2007)	Structure of rhodolith beds from 4 to 55 meters deep along the southern coast of Espírito Santo State, Brazil	INCLUDE on full text
Amado-Filho (2016)	Mesophotic ecosystems of the unique South Atlantic atoll are composed by rhodolith beds and scattered consolidated reefs	EXCLUDE on outcome
Amado-Filho (2016)	The mesophotic zone of the only South Atlantic Atoll is dominated by rhodolith beds	EXCLUDE on outcome
Andradi-Brown (2016)a	Identifying zooplankton community changes between shallow and upper-mesophotic reefs on the Mesoamerican Barrier Reef, Caribbean	INCLUDE on full text
Andradi-Brown (2016)b	Assessing caribbean shallow and mesophotic reef fish communities using Baited-Remote Underwater Video (BRUV) and diver-operated video (DOV) survey techniques	INCLUDE on full text
Andradi-Brown (2016)	Reef fish community biomass and trophic structure changes across shallow to upper- Mesophotic reefs in the mesoamerican barrier reef, Caribbean	INCLUDE on full text
Anonymous (2011)	Oceanographic data collected during the Bonaire 2008: Exploring Coral Reef Sustainability with New Technologies (bonaire2008) on Fetch1 AUV and Gavia AUV's in Netherlands, Antilles from January 6, 2008 - January 29, 2008 (NODC Accession 0072312)	EXCLUDE on outcome

Short Title	Title	Code
Anonymous (2011)	Thriving 'Middle Light' Reefs Found in Puerto Rico	EXCLUDE on study design, on outcome
Appeldoorn (2016)	Mesophotic coral ecosystems under anthropogenic stress: a case study at Ponce, Puerto Rico	EXCLUDE on comparator
Armstrong (2006)	Characterizing the deep insular shelf coral reef habitat of the Hind Bank marine conservation district (US Virgin Islands) using the Seabed autonomous underwater vehicle	EXCLUDE on comparator
Armstrong (2006)	Remote sensing of deep hermatypic coral reefs in Puerto Rico and the US Virgin Islands using the seabed autonomous underwater vehicle	EXCLUDE on comparator
Armstrong (2007)	Deep zooxanthellate coral reefs of the Puerto Rico: US Virgin Islands insular platform	EXCLUDE on outcome
Armstrong (2009)	Monitoring coral reefs in optically-deep waters	EXCLUDE on outcome
Armstrong (2010)	Large-scale mapping and characterization of deep reef habitats in the US Caribbean	EXCLUDE on comparator
Armstrong (2012)	Mesophotic coral Reefs of the Puerto Rico Shelf	EXCLUDE on comparator
Avery (1998)	Bahamian reef communities: Composition, recruitment and change (10 meters to 250 meters)	INCLUDE on full text
Bahia (2012)	Non-geniculate coralline red algae of mesophotic rhodolith beds from southeastern and northeastern Brazil	EXCLUDE on outcome
Bailey-Brock (1999)	Ecology and biodiversity of coral reef polychaetes of Guam and Saipan, Mariana islands	EXCLUDE not mesophotic
Bak (1980)	Constancy and change in coral reef habitats along depth gradients at Curacao	EXCLUDE on outcome
Bak (1995)	LONG-TERM CHANGE IN CORAL COMMUNITIES ALONG DEPTH GRADIENTS OVER LEEWARD REEFS IN THE NETHERLANDS-ANTILLES	INCLUDE on full text
Bak (2005)	Coral reef crisis in deep and shallow reefs: 30 years of constancy and change in reefs of Curacao and Bonaire	INCLUDE on full text
Baker (2016)	Mesophotic coral ecosystems ? A lifeboat for coral reefs?	EXCLUDE on study design
Bakker (2016)	Long-term shifts in coral communities on shallow to deep reef slopes of Curaçao and Bonaire: are there any winners?	INCLUDE on full text
Baldwin (2015)	A new, mesophotic Coryphopterus goby (Teleostei, Gobiidae) from the southern Caribbean, with comments on relationships and depth distributions within the genus	INCLUDE on full text
Ballantine (1982)	HALIMEDA-HUMMII SP-NOV HALIMEDA-CRYPTICA V ACERIFOLIA VAR NOV (CAULERPALES, CHLOROPHYTA), AND ADDITIONAL RECORDS OF HALIMEDA SPECIES FROM PUERTO-RICO	EXCLUDE on outcome

Short Title	Title	Code
Ballantine (1988)	THE LIFE-HISTORY AND DEVELOPMENT OF HYPOGLOSSUM-RHIZOPHORUM (DELESSERIACEAE, RHODOPHYTA) IN CULTURE, A NEW DEEP-WATER SPECIES FROM THE CARIBBEAN	EXCLUDE on outcome, on comparator
Ballantine (2011)	ALGAL COMPOSITION AND DYNAMICS IN MESOPHOTIC CORAL REEFS, SOUTHWEST PUERTO RICO	EXCLUDE on outcome, on comparator
Bare (2010)	Mesophotic communities of the insular shelf at Tutuila, American Samoa	EXCLUDE on outcome, on comparator
Barrios-Suárez (2002)	Distribution of anemones (Anthozoa: Actiniaria and Corallimorpharia) in the area of Santa Marta, Colombian Caribbean	INCLUDE on full text
Beaman (2012)	Habitats and Benthos at Hydrographers Passage, Great Barrier Reef, Australia	EXCLUDE on outcome
Beaman (2016)	Spatial patterns in the distribution of benthic assemblages across a large depth gradient in the Coral Sea, Australia	EXCLUDE on comparator
Beaver (2002)	Fishery productivity and trophodynamics of platform artificial reefs in the northwestern Gulf of Mexico	EXCLUDE on subject
Bejarano (2010)	Use of mixed-gas rebreathers to access fish assemblages in Mesophotic Coral Ecosystems (MCE) off La Parguera shelf-Edge, Puerto Rico	INCLUDE on full text
Bejarano (2014)	Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico	EXCLUDE on comparator
Blair (1985)	STUDIES OF DEEP-WATER CARIBBEAN SPECIES OF HALIMEDA UDOTEACEAE OBSERVATIONS ON DEPTH DISTRIBUTION AND MORPHOLOGY	EXCLUDE on study design, on outcome
Blair (1988)	The deep-water species of Halimeda Lamouroux (Halimedaceae, Chlorophyta) from San Salvador Island, Bahamas: species composition, distribution and depth records	EXCLUDE on comparator
Blyth-Skyrme (2013)	Mesophotic coral ecosystems- potential candidates as essential fish habitat and habitat areas of particular concern	EXCLUDE on outcome, on comparator
Bongaerts (2010)	Assessing the deep reef refugia' hypothesis: focus on Caribbean reefs	EXCLUDE on study design
Bongaerts (2011)	Mesophotic coral ecosystems on the walls of Coral Sea atolls	EXCLUDE on outcome, on comparator
Bongaerts (2013)	Sharing the slope: depth partitioning of agariciid corals and associated Symbiodinium across shallow and mesophotic habitats (2-60 m) on a Caribbean reef	EXCLUDE on outcome
Bongaerts (2015)	Deep down on a Caribbean reef: lower mesophotic depths harbor a specialized coral-endosymbiont community	EXCLUDE on comparator

Short Title	Title	Code
Bongaerts (2015)	Prevalent endosymbiont zonation shapes the depth distributions of scleractinian coral species	EXCLUDE on outcome
Bouchon-Navaro (2005)	Biogeographic patterns of coastal fish assemblages in the West Indies	INCLUDE on full text
Brasileiro (2016)	Macroalgal composition and community structure of the largest rhodolith beds in the world	INCLUDE on full text
Breedy (2013)	A NEW SPECIES OF THE GENUS EUGORGIA (CNIDARIA: OCTOCORALLIA: GORGONIIDAE) FROM MESOPHOTIC REEFS IN THE EASTERN PACIFIC	EXCLUDE on outcome
Breedy (2013)	Two new species of gorgonian octocorals from the Tropical Eastern Pacific biogeographic region (Cnidaria, Anthozoa, Gorgoniidae)	EXCLUDE on outcome
Breedy (2016)	A new Muricea species (Cnidaria, Anthozoa, Octocorallia) from the eastern tropical Pacific	EXCLUDE on outcome, on comparator
Bridge (2011)	Habitats and sessile benthic megafaunal communities in the mesophotic zone of the Great Barrier Reef World Heritage Area, Australia	EXCLUDE on subject
Bridge (2011)	Topography, substratum and benthic macrofaunal relationships on a tropical mesophotic shelf margin, central Great Barrier Reef, Australia	EXCLUDE on outcome, on comparator
Bridge (2011)	Variability in mesophotic coral reef communities along the Great Barrier Reef, Australia	EXCLUDE on comparator
Bridge (2012)	Abundance and diversity of anemonefishes and their host sea anemones at two mesophotic sites on the Great Barrier Reef, Australia	EXCLUDE on outcome
Bridge (2012)	Diversity of Scleractinia and Octocorallia in the mesophotic zone of the Great Barrier Reef, Australia	EXCLUDE on comparator
Bridge (2013)	Mesophotic Coral Reef Ecosystems in the Great Barrier Reef World Heritage Area	EXCLUDE on subject
Bridge (2016)	Ecological and morphological traits predict depth-generalist fishes on coral reefs	EXCLUDE on comparator
Bright (1977)	Chapter 6 Reconnaissance of Reefs and Fishing Banks of the Texas Continental Shelf	EXCLUDE on comparator
Brokovich (2008)	Descending to the twilight-zone: changes in coral reef fish assemblages along a depth gradient down to 65 m	INCLUDE on full text
Bryan (2006)	Preliminary Comparisons Between Reef Fish Assemblages on Vessel-Reefs and Natural Substrate in Depths of 70-95 Meters	EXCLUDE on subject, on comparator
Bryan (2013)	Characterization of the mesophotic reef fish community in south Florida, USA	EXCLUDE on comparator
Cairns (1979)	STUDIES ON THE FAUNA OF CURACAO AND OTHER CARIBBEAN ISLANDS 57. THE DEEP WATER SCLERACTINIA OF THE CARIBBEAN SEA AND ADJACENT WATERS	EXCLUDE on outcome

Short Title	Title	Code
Cairns (1988)	NEW RECORDS OF STYLASTERIDAE CNIDARIA HYDROZOA FROM WESTERN AUSTRALIA INCLUDING THE DESCRIPTION OF TWO NEW SPECIES	EXCLUDE on study design, on outcome
Cairns (1991)	A REVISION OF THE AHERMATYPIC SCLERACTINIA OF THE GALAPAGOS AND COCOS ISLANDS	INCLUDE on full text
Cairns (1991)	NEW RECORDS OF STYLASTERIDAE (HYDROZOA, HYDROIDA) FROM THE GALAPAGOS AND COCOS ISLANDS	EXCLUDE on outcome
Cairns (2001)	Studies on western Atlantic Octocorallia (Coelenterata: Anthozoa). Part 1: The genus Chrysogorgia Duchassaing and Michelotti, 1864	EXCLUDE not mesophotic
Cairns (2006)	Studies on western Atlantic Octocorallia (Coelenterata: Anthozoa). Part 6: The genera Primnoella Gray, 1858; Thouarella Gray, 1870; Dasystenella Versluys, 1906	EXCLUDE on study design, on outcome
Cairns (2009)	Scleractinia (Cnidaria) of the Gulf of Mexico	INCLUDE on full text
Calder (1998)	Hydroid diversity and species composition along a gradient from shallow waters to deep sea around Bermuda	INCLUDE on full text
Calder (2000)	Assemblages of hydroids (Cnidaria) from three seamounts near Bermuda in the western North Atlantic	EXCLUDE on comparator
Cerrano (2009)	Gold coral (Savalia savaglia) and gorgonian forests enhance benthic biodiversity and ecosystem functioning in the mesophotic zone	EXCLUDE on subject
Chabanet (1997)	Relationships between coral reef substrata and fish	EXCLUDE not mesophotic
Chan (2009)	Generalist dinoflagellate endosymbionts and host genotype diversity detected from mesophotic (67-100 m depths) coral Leptoseris	EXCLUDE on outcome, on comparator
Chaytor (2015)	Demersal Fish Assemblages on Seamounts and Other Rugged Features in Deep Waters of the Greater and Lesser Antilles	EXCLUDE not mesophotic, on comparator
Cheney (1973)	A systematic study of the Florida species of Eucheuma; preliminary results	EXCLUDE on outcome
Cheney (1974)	Deep-water benthic algae of the Florida Middle Ground	INCLUDE on full text
Clark (2014)	Fish and Benthic Communities of the Flower Garden Banks National Marine Sanctuary: Science to Support Sanctuary Management	INCLUDE on full text
Coles (2009)	Spatial distribution of deepwater seagrass in the inter-reef lagoon of the Great Barrier Reef World Heritage Area	INCLUDE on full text
Colin (1974)	Observation and collection of deep-reef fishes off the coasts of Jamaica and British Honduras (Belize)	EXCLUDE on comparator
Colin (1976)	Observations of deep-reef fishes in the Tongue-of-the-Ocean, Bahamas	EXCLUDE on comparator

Short Title	Title	Code
Colin (1986)	Geology and biological zonation of the reef slope, 50-360 m depth at Enewetak Atoll, Marshall Islands	EXCLUDE on comparator
Cordeiro (2015)	Mesophotic coral ecosystems occur offshore and north of the Amazon River	INCLUDE on full text
Corgosinho (2013)	Archeolourinia shermani, a new genus and species of Louriniidae (Copepoda: Harpacticoida) from a Caribbean mesophotic zone	EXCLUDE on study design, on outcome
Corgosinho (2016)	A new species of Atergopedia (Copepoda: Harpacticoida: Novocriniidae) from a Caribbean mesophotic reef	EXCLUDE on outcome
Cortes (1981)	The coral reef at Cahuita, Costa Rica, a reef under stress	EXCLUDE not mesophotic
Cortes (1990)	Coral reef decline in Golfo Dulce, Costa Rica, eastern Pacific: Anthropogenic and natural disturbances	EXCLUDE not mesophotic
Cortés (2003)	Coral reefs of the Americas: An introduction to Latin American Coral Reefs	EXCLUDE on outcome
Cortes (2008)	Life to 450 m depth at Isla del Coco, Costa Rica	EXCLUDE on outcome, on comparator
Culter (2006)	Pulley reef: a deep photosynthetic coral reef on the West Florida Shelf, USA	EXCLUDE on outcome, on comparator
Dai (1992)	Distribution of substrates and macrobenthos at depths between 35 and 120 M in Southern Taiwan	EXCLUDE on outcome, on comparator
Dai (1993)	Patterns of coral distribution and benthic space partitioning on the fringing reefs of southern Taiwan	EXCLUDE not mesophotic
de Oliveira (2016)	Mesophotic ecosystems: coral and fish assemblages in a tropical marginal reef (northeastern Brazil)	EXCLUDE on comparator
de Voogd (2003)	Amphimedon denhartogi spec. nov. (Porifera: Haplosclerida) from deep reef habitats in Indonesia	EXCLUDE on study design, on outcome, on comparator
Denis (2015)	Extension of the known distribution and depth range of the scleractinian coral Psammocora stellata: first record from a Taiwanese mesophotic reef	EXCLUDE on outcome, on comparator
Dennis (1988)	Reef fish assemblages on hard banks in the northwestern Gulf of Mexico	EXCLUDE on outcome
Dennis (2004)	New records of marine fishes from the Puerto Rican Plateau	EXCLUDE on outcome
Diaz (1996)	Morphology and marine habitats of two southwestern Caribbean atolls: Albuquerque and Courtown	EXCLUDE on outcome

Short Title	Title	Code
Dixon (2014)	Microhabitat use and photoacclimation in the clownfish sea anemone Entacmaea quadricolor	EXCLUDE on outcome
Dowgiallo (2004)	Patterns in diversity and distribution of benthic molluscs along a depth gradient in the Bahamas	INCLUDE on full text
Dullo (1990)	THE FORALGAL CRUST FACIES OF THE DEEPER FORE REEFS IN THE RED-SEA - A DEEP DIVING SURVEY BY SUBMERSIBLE	EXCLUDE on outcome
Edmunds (1990)	Reefs of the northeastern Caribbean. I. Scleractinian populations	EXCLUDE not mesophotic
Eiseman (1981)	DUDRESNAYA-PATULA SP-NOV, AN UNUSUAL DEEP-WATER RED ALGA FROM FLORIDA	EXCLUDE on study design, on outcome
Eiseman (1982)	NEW RECORDS AND RANGE EXTENSIONS OF DEEP-WATER ALGAE FROM EAST FLOWER GARDEN BANK, NORTHWESTERN GULF OF MEXICO	EXCLUDE not mesophotic
Englebert (2014)	Deepest zooxanthellate corals of the Great Barrier Reef and Coral Sea	EXCLUDE on outcome, on comparator
Etnoyer (2010)	Rapid assessment of octocoral diversity and habitat on Saba Bank, Netherlands Antilles	INCLUDE on full text
Etnoyer (2016)	Decline in condition of gorgonian octocorals on mesophotic reefs in the northern Gulf of Mexico: before and after the Deepwater Horizon oil spill	EXCLUDE on outcome, on comparator
Eyal (2015)	Euphyllia paradivisa, a successful mesophotic coral in the northern Gulf of Eilat/Aqaba, Red Sea	INCLUDE on full text
Feitoza (2005)	Ecology and Zoogeography of Deep-Reef Fishes in Northeastern Brazil	INCLUDE on full text
Fowler (2012)	Evidence of sustained populations of a small reef fish on artificial structures. Does depth affect production on artificial reefs?	EXCLUDE on outcome, on comparator
Francini (2014)	Reef communities of the Saint Peter and Saint Paul's Archipelago across an euphotic-mesophotic depth gradient (0-90 m)	INCLUDE on full text
Fraser (2008)	Reef morphology and invertebrate distribution at continental shelf edge reefs in the South Atlantic Bight	INCLUDE on full text
Frederic (2013)	Locally extinct coral species Seriatopora hystrix found at upper mesophotic depths in Okinawa	EXCLUDE on outcome, on comparator
Fricke (1983)	THE DEPTH LIMITS OF RED SEA STONY CORALS AN ECO PHYSIOLOGICAL PROBLEM A DEEP DIVING SURVEY BY SUBMERSIBLE	INCLUDE on full text
Fricke (1985)	Depth limits of Bermudan scleractinian corals: a submersible survey	INCLUDE on full text
Fricke (1986)	Diversity and spatial pattern of coral communities in the Red Sea upper twilight zone	EXCLUDE on comparator

Short Title	Title	Code
Fukunaga (2016)	Structure of mesophotic reef fish assemblages in the Northwestern Hawaiian Islands	INCLUDE on full text
Garcia-Sais (2010)	Reef habitats and associated sessile-benthic and fish assemblages across a euphotic-mesophotic depth gradient in Isla Desecheo, Puerto Rico	INCLUDE on full text
García-Sais (2010)	Mesophotic benthic habitats and associated marine communities at Abrir La Sierra, Puerto Rico	EXCLUDE on comparator
García-Sais (2012)	Fishery independent survey of commercially exploited fish and shellfish populations from mesophotic reefs within the Puertorican EEZ	EXCLUDE on comparator
Garth (1973)	New taxa of brachyuran crabs from deep water off western Peru and Costa Rica	EXCLUDE on study design, on outcome
Gavio (2005)	Platoma chrysymenioides sp. nov. (Schizymeniaceae), and Sebdenia integra sp. nov. (Sebdeniaceae), two new red algal species from the Northwestern Gulf of Mexico, with a phylogenetic assessment of the Cryptonemiales complex (Rhodophyta)	EXCLUDE on study design, on outcome
Gilmartin (1960)	THE ECOLOGICAL DISTRIBUTION OF THE DEEP-WATER ALGAE OF ENIWETOK ATOLL	EXCLUDE on outcome
Ginsburg (2012)	Spatial extent of potential habitats of the Mesophotic Coral Ecosystem (MCE, 20-80 m) in the tropical North Atlantic (TNA)	EXCLUDE on study design
Gleason (2010)	Landscape video mosaic from a mesophotic coral reef	EXCLUDE on outcome
Gledhill (2001)	Reef fish assemblages on Gulf of Mexico shelf-edge banks	EXCLUDE on outcome
Glynn (1976)	Some physical and biological determinants of coral community structure in the eastern Pacific	EXCLUDE not mesophotic
Goffredo (2000)	Abundance and distribution of mushroom corals (Scleractinia : Fungiidae) on a coral reef at Eilat, northern Red Sea	INCLUDE on full text
Goldberg (1973)	ECOLOGY OF CORAL-OCTOCORAL COMMUNITIES OFF SOUTHEAST FLORIDA COAST - GEOMORPHOLOGY, SPECIES COMPOSITION, AND ZONATION	INCLUDE on full text
Greenfield (1981)	THE BLENNIOID FISHES OF BELIZE AND HONDURAS CENTRAL AMERICA WITH COMMENTS ON THEIR SYSTEMATICS ECOLOGY AND DISTRIBUTION BLENNIIDAE CHAENOPSIDAE LABRISOMIDAE TRIPTERYGIIDAE	EXCLUDE not mesophotic
Grigg (2006)	Depth limit for reef building corals in the Au'au Channel, S.E. Hawaii	EXCLUDE on outcome
Grill (2001)	Modern shallow- to deep-water bivalve death assemblages in the Red Sea - ecology and biogeography	EXCLUDE on subject
Guerra-Garcia (2015)	New genus and new species of Caprellidae (Crustacea: Peracarida: Amphipoda) from the mesophotic coral ecosystems of Puerto Rico and St. Croix, Caribbean Sea	EXCLUDE on study design, on outcome, on comparator

Short Title	Title	Code
Gutner-Hoch (2011)	Genotypic diversity and distribution of Ostreobium quekettii within scleractinian corals	EXCLUDE on outcome
Harris (2004)	Submerged coral reefs in the Gulf of Carpentaria, Australia	INCLUDE on full text
Harris (2013)	Submerged banks in the Great Barrier Reef, Australia, greatly increase available coral reef habitat	EXCLUDE on study design
Harvey (2007)	Bait attraction affects the performance of remote underwater video stations in assessment of demersal fish community structure	INCLUDE on full text
Hernandez-Agreda (2016)	The microbial signature provides insight into the mechanistic basis of coral success across reef habitats	EXCLUDE on outcome
Hillis-Colinvaux (1986)	Halimeda growth and diversity on the deep fore-reef of Enewetak Atoll	EXCLUDE on outcome
Ho (2004)	New records of 31 species of brachyuran crabs from the joint Taiwan-France expeditions, "Taiwan 2000" and "Taiwan 2001", off deep waters in Taiwan	EXCLUDE on study design
Hoeksema (2016)	High coral cover at lower mesophotic depths: a dense Agaricia community at the leeward side of Curaçao, Dutch Caribbean	EXCLUDE on outcome, on comparator
Honey-Escandón (2008)	Echinoderms (Echinodermata) from the Mexican Pacific	EXCLUDE on outcome
Hurley (2016)	An assessment of shallow and mesophotic reef brachyuran crab assemblages on the south shore of O'ahu, Hawai'i	INCLUDE on full text
Irving (1995)	Near-shore bathymetry and reef biotopes of Henderson Island, Pitcairn Group	EXCLUDE on outcome
Iryu (1995)	Distribution of marine organisms and its geological significance in the modern reef complex of the Ryukyu Islands	EXCLUDE on outcome
Itzkowitz (1991)	A RECONNAISSANCE OF THE DEEPER JAMAICAN CORAL REEF FISH COMMUNITIES	INCLUDE on full text
Jankowski (2015)	Depth gradients in diversity, distribution and habitat specialisation in coral reef fishes: implications for the depth-refuge hypothesis	EXCLUDE not mesophotic
Jarrett (2005)	Strange bedfellows-a deep-water hermatypic coral reef superimposed on a drowned barrier island; southern Pulley Ridge, SW Florida platform margin	EXCLUDE on comparator
Jordan (2016)	Characterizing mesophotic reef fish communities at five South Texas relic coral-algal banks	EXCLUDE on outcome
Kahng (2006)	Ecology and ecological impact of an alien octocoral, Carijoa riisei, in Hawai'i	EXCLUDE on comparator
Kahng (2006)	The Deepest, Zooxanthellate Scleractinian Corals in the World?	EXCLUDE on outcome

Short Title	Title	Code
Kahng (2007)	Vertical zonation of megabenthic taxa on a deep photosynthetic reef (50-140 m) in the Auau Channel, Hawaii	EXCLUDE on comparator
Kahng (2010)	Community ecology of mesophotic coral reef ecosystems	EXCLUDE on study design
Kahng (2014)	Recent advances in the ecology of mesophotic coral ecosystems (MCEs)	EXCLUDE on study design, on outcome
Kane (2014)	High levels of mesophotic reef fish endemism in the Northwestern Hawaiian Islands	EXCLUDE on comparator
Kobara (2009)	Regional analysis of seafloor characteristics at reef fish spawning aggregation sites in the Caribbean	EXCLUDE on outcome
Kosaki (1991)	New records of fishes from Johnston Atoll, with notes on biogeography	EXCLUDE on study design, on outcome
Kosaki (2016)	100% endemism in mesophotic reef fish assemblages at Kure Atoll, Hawaiian Islands	EXCLUDE on outcome, on comparator
Kramer (1994)	New deep-water fish records from the Great Barrier Reef, Australia	EXCLUDE on outcome
Krumm (1996)	Endolithic bivalves and host corals from the Upper Oligocene Lares Formation of Puerto Rico	EXCLUDE on subject
Lam (2008)	Ahermatypic corals (scleractinia : Dendrophylliidae, Oculinidae and Rhizangiidae) recorded from submarine caves in Hong Kong	EXCLUDE on outcome, on comparator
Lane (2016)	Mesophotic mushroom coral records at Brunei Darussalam support westward extension of the coral triangle to the South China sea waters of Northwest Borneo	EXCLUDE on outcome
Lang (1974)	Biological zonation at the base of a reef	EXCLUDE on outcome
Lehnert (1996)	North Jamaican deep fore-reef sponges	EXCLUDE on study design, on outcome, on comparator
Leichter (2008)	Deep water macroalgal communities adjacent to the Florida Keys reef tract	EXCLUDE on comparator
Lesser (2009)	Ecology of mesophotic coral reefs	EXCLUDE on study design, on outcome
Lesser (2010)	Ocean Exploration: Consequences of lionfish invasion to stability of Mesophotic Coral Reefs	EXCLUDE on outcome
Lesser (2011)	Phase shift to algal dominated communities at mesophotic depths associated with lionfish (Pterois volitans) invasion on a Bahamian coral reef	EXCLUDE on comparator

Short Title	Title	Code
Lewis (1966)	A preliminary description of some marine benthic communities from Barbados, West Indies	EXCLUDE on outcome, on comparator
Lewis (2006)	Biology and Ecology of the Hydrocoral Millepora on Coral Reefs	EXCLUDE on study design
Liddell (2000)	Temporal change in hard substrate communities 10-250 m, the Bahamas	EXCLUDE on outcome
Liddell (2009)	Mexican coral reefs	EXCLUDE on study design
Lindfield (2014)	Depth Refuge and the Impacts of SCUBA Spearfishing on Coral Reef Fishes	EXCLUDE not mesophotic
Lindfield (2014)	Silent fish surveys: bubble-free diving highlights inaccuracies associated with SCUBA-based surveys in heavily fished areas	EXCLUDE not mesophotic
Lindfield (2015)	Mesophotic depths as refuge areas for fishery-targeted species on coral reefs	INCLUDE on full text
Linklater (2016)	High coral cover on a mesophotic, subtropical island platform at the limits of coral reef growth	EXCLUDE on outcome, on comparator
Linklater (2016)	Past and present coral distribution at the latitudinal limit of reef development, southwest Pacific Ocean	EXCLUDE on outcome
Littler (1991)	Deep-water rhodolith distribution, productivity, and growth history at sites of formation and subsequent degradation	EXCLUDE on outcome, on comparator
Littler (1997)	A unique coral reef formation discovered on the Great Astrolabe Reef, Fiji	EXCLUDE not mesophotic
Locke (2007)	Newly documented species of Madracis (Scleractinia : Pocilloporidae) from the Caribbean	EXCLUDE on study design, on outcome
Locke (2013)	Biogeography, biodiversity and connectivity of Bermuda's coral reefs	EXCLUDE on study design
Locke (2013)	Scleractinia, Octocorallia and Antipatharia of Bermuda's reefs and deep-water coral communities: a taxonomic perspective including new records	EXCLUDE on outcome
Locker (2010)	Geomorphology of mesophotic coral ecosystems: current perspectives on morphology, distribution, and mapping strategies	EXCLUDE on study design, on outcome
Logan (2013)	The Reefs of the Turks and Caicos Islands	EXCLUDE on outcome
Luck (2013)	Polyphyly and hidden species among Hawai'i's dominant mesophotic coral genera, Leptoseris and Pavona (Scleractinia: Agariciidae)	INCLUDE on full text
MacDonald (2016)	Depth, bay position and habitat structure as determinants of coral reef fish distributions: Are deep reefs a potential refuge?	INCLUDE on full text

Short Title	Title	Code
Madin (2016)	The Coral Trait Database, a curated database of trait information for coral species from the global oceans	INCLUDE on full text
Magalhaes (2015)	Changes in benthic communities along a 0-60 m depth gradient in the remote St. Peter and St. Paul Archipelago (Mid-Atlantic Ridge, Brazil)	INCLUDE on full text
Malcolm (2014)	Testing a depth-based Habitat Classification System against reef fish assemblage patterns in a subtropical marine park	INCLUDE on full text
Maragos (1986)	Reef corals of Johnston Atoll: one of the world's most isolated reefs	EXCLUDE on outcome
Matsuda (2011)	Rhodoliths from deep fore-reef to shelf areas around Okinawa-jima, Ryukyu Islands, Japan	EXCLUDE on outcome
Meirelles (2015)	Baseline Assessment of Mesophotic Reefs of the Vitoria-Trindade Seamount Chain Based on Water Quality, Microbial Diversity, Benthic Cover and Fish Biomass Data	INCLUDE on full text
Mergner (1984)	The ecological research on coral reefs of the Red Sea	EXCLUDE on study design, on outcome
Morais (2016)	Lush underwater forests in mesophotic reefs of the Gulf of Guinea	EXCLUDE on outcome
Muir (2015)	Diverse staghorn coral fauna on the mesophotic reefs of north-east Australia	INCLUDE on full text
Newman (1996)	Variation in reef associated assemblages of the Lutjanidae and Lethrinidae at different distances offshore in the central Great Barrier Reef	INCLUDE on full text
Ng (1998)	Lamoha hystrix, a new species of deep-water porter crab (Crustacea : Decapoda : Brachyura : Homolidae) from the central Pacific	EXCLUDE on outcome
Noren (2016)	Can Twilight Reefs Usher In A New Dawn For Depauperate Shallow Coral Reefs?	EXCLUDE on outcome
Nugues (2006)	Differential competitive abilities between Caribbean coral species and a brown alga: A year of experiments and a long-term perspective	EXCLUDE not mesophotic
Nugues (2008)	Long-term dynamics of the brown macroalga Lobophora variegata on deep reefs in Curacao	EXCLUDE on outcome
Ohara (2013)	First record of a mesophotic Pachyseris foliosa reef from Japan	EXCLUDE on outcome
Olavo (2011)	Shelf-edge reefs as priority areas for conservation of reef fish diversity in the tropical Atlantic	EXCLUDE on comparator
Opresko (2005)	Caribbean shallow-water black corals (Cnidaria: Anthozoa: Antipatharia)	INCLUDE on full text
Ott (1975)	QUANTITATIVE ANALYSIS OF COMMUNITY PATTERN AND STRUCTURE ON A CORAL REEF BANK IN BARBADOS, WEST INDIES	INCLUDE on full text
Paddack (2005)	Herbivorous coral reef fishes in a changing ecosystem	EXCLUDE on outcome

Short Title	Title	Code
Pagan (2010)	Use of small ROV systems to survey mesophotic ecosystems	EXCLUDE on outcome
Papastamatiou (2015)	Movements and foraging of predators associated with mesophotic coral reefs and their potential for linking ecological habitats	EXCLUDE on outcome
Parrish (2004)	Habitat and reef-fish assemblages of banks in the Northwestern Hawaiian Islands	INCLUDE on full text
Paulay (2003)	The non-scleractinian Anthozoa (Cnidaria) of the Mariana Islands	EXCLUDE on outcome
Pearson (2015)	Distinct cross-shelf gradient in mesophotic reef fish assemblages in subtropical eastern Australia	EXCLUDE on comparator
Pereira-Filho (2011)	Reef fish and benthic assemblages of the Trindade and Martin Vaz Island group, southwestern Atlantic	INCLUDE on full text
Pereira-Filho (2012)	Extensive Rhodolith Beds Cover the Summits of Southwestern Atlantic Ocean Seamounts	EXCLUDE on outcome
Pešic (2012)	A new species of Pontarachna (Acari, Hydrachnidia, Pontarachnidae) from a mesophotic coral ecosystem off Vieques Island, Puerto Rico, Caribbean Sea	EXCLUDE on outcome, on comparator
Pesic (2014)	A new species of Litarachna (Acari, Hydrachnidia, Pontarachnidae) from a Caribbean mesophotic coral ecosystem	EXCLUDE on outcome
Petrescu (2012)	New genus and new species of Cumacea (Crustacea: Peracarida) from the mesophotic coral ecosystem of SW Puerto Rico, Caribbean Sea	EXCLUDE on outcome, on comparator
Petrescu (2013)	Two new species of the genus Cumella (Crustacea: Cumacea: Nannastacidae) associated with mesophotic reefs of Puerto Rico and St. Croix, Caribbean Sea	EXCLUDE on study design, on outcome
Petrescu (2014)	New species of Cumella (Crustacea: Cumacea: Nannastacidae) from mesophotic habitats of Mona Island, Puerto Rico, Caribbean Sea	EXCLUDE on outcome, on comparator
Petrescu (2014)	Three new Nannastacidae (Crustacea: Cumacea) species from a Caribbean mesophotic ecosystem	EXCLUDE on outcome
Petrescu (2016)	A new species of the genus Campylaspis (Crustacea: Cumacea) from the mesophotic reefs of St. John, US Virgin Islands	EXCLUDE on outcome
Petrescu (2016)	New species and new records of Cumacea (Crustacea: Peracarida: Cumacea) from mesophotic reefs of Puerto Rico and US Virgin Islands, Caribbean Sea	EXCLUDE on outcome
Pickard (2013)	Characterization of Shark Movements on a Mesophotic Caribbean Coral Reef and Temporal Association with Fish Spawning Aggregations	EXCLUDE on outcome
Pickard (2016)	Comparative Use of a Caribbean Mesophotic Coral Ecosystem and Association with Fish Spawning Aggregations by Three Species of Shark	EXCLUDE on outcome
Pinheiro (2014)	Reef oases in a seamount chain in the southwestern Atlantic	EXCLUDE on outcome

Short Title	Title	Code
Pinheiro (2016)	Upper and lower mesophotic coral reef fish communities evaluated by underwater visual censuses in two Caribbean locations	EXCLUDE on comparator
Pochon (2015)	Depth specialization in mesophotic corals (Leptoseris spp.) and associated algal symbionts in Hawai'i	EXCLUDE on outcome, on comparator
Przeslawski (2015)	Implications of Sponge Biodiversity Patterns for the Management of a Marine Reserve in Northern Australia	INCLUDE on full text
Pyle (2000)	Assessing undiscovered fish biodiversity on deep coral reefs using advanced self-contained diving technology	EXCLUDE on outcome
Pyle (2008)	Five new species of the damselfish genus Chromis (Perciformes : Labroidei : Pomacentridae) from deep coral reefs in the tropical western Pacific	EXCLUDE on study design, on outcome, on comparator
Pyle (2016)	A comprehensive investigation of mesophotic coral ecosystems in the Hawaiian Archipelago	INCLUDE on full text
Pyle (2016)	Tosanoides obama, a new basslet (Perciformes, Percoidei, Serranidae) from deep coral reefs in the Northwestern Hawaiian Islands.	EXCLUDE on outcome
Randall (1973)	Distribution of corals after Acanthaster panci (L.) infestation at Thnguisson Point, Guam	EXCLUDE not mesophotic
Randall (2015)	A New Mesophotic Branching Coral Species of Psammocora from the Mariana Islands Archipelago (Cnidaria: Scleractinia: Psammocoridae)	EXCLUDE on outcome
Reed (1997)	Biodiversity and distribution of deep and shallow water sponges in the Bahamas	INCLUDE on full text
Reed (2011)	Final Cruise Report: Florida Shelf-Edge Expedition (FLoSEE) Deepwater Horizon Oil Spill Response: Survey of Deepwater and Mesophotic Reef Ecosystems in the Eastern Gulf of Mexico and Southeastern Florida. R/V Seward Johnson and Johnson-Sea-Link Submersible. July 9-August 9, 2010	INCLUDE on full text
Reimer (2014)	Zoanthid (cnidaria: anthozoa: hexacorallia: zoantharia) species of coral reefs in Palau	EXCLUDE not mesophotic
Richmond (2014)	Classifying benthic biotopes on sub-tropical continental shelf reefs: how useful are abiotic surrogates?	EXCLUDE on comparator
Riegl (1996)	Studies on coral community structure and damage as a basis for zoning marine reserves	INCLUDE on full text
Rivero (2010)	Ecological aspects of sponges in mesophotic coral ecosystems	EXCLUDE on comparator
Rivero-Calle (2009)	Biological and physical characteristics of a mesophotic coral reef: Black Jack reef, Vieques, Puerto Rico	EXCLUDE on outcome, on comparator
Roberts (1996)	Patterns in subtidal marine assemblages associated with a deep-water sewage outfall	EXCLUDE on comparator
Robertson (2016)	The fishes of Cayo Arcas (Campeche bank, Gulf of Mexico): An updated checklist	INCLUDE on full text

Short Title	Title	Code
Rodriguez (2013)	Deep Reef Fishes off La Parguera Insular Slope, Puerto Rico, and their Connectivity with Shallow Reefs	EXCLUDE on comparator
Rogers (2008)	Ecology of coral reefs in the US Virgin Islands	EXCLUDE on study design
Rooney (2010)	Mesophotic coral ecosystems in the Hawaiian Archipelago	INCLUDE on full text
Rosa (2016)	Mesophotic reef fish assemblages of the remote St. Peter and St. Paul's Archipelago, Mid-Atlantic Ridge, Brazil	EXCLUDE on comparator
Rosenberg (2008)	Honing in on Deep-water Corals: Ground-truthing AUV Data in the Straits of Florida	EXCLUDE on outcome
Rutzler (2014)	Diversity of sponges (Porifera) from cryptic habitats on the Belize barrier reef near Carrie Bow Cay	EXCLUDE on outcome
Sammarco (2012)	Geographic expansion of hermatypic and ahermatypic corals in the Gulf of Mexico, and implications for dispersal and recruitment	INCLUDE on full text
Sánchez (1998)	Patterns of octocoral and black coral distribution in the oceanic barrier reef-complex of Providencia island, southwestern Caribbean	INCLUDE on full text
Sarano (1988)	Morphology and ecology of the deep fore reef slope at Osprey Reef (Coral Sea)	INCLUDE on full text
Schizas (2010)	Preliminary Findings On The Copepod Fauna Associated With The Caribbean Mesophotic Coral Ecosystem Of Puerto Rico	EXCLUDE on outcome, on comparator
Schizas (2015)	A new species of Longipedia Claus, 1863 (Copepoda: Harpacticoida: Longipediidae) from Caribbean mesophotic reefs with remarks on the phylogenetic affinities of Polyarthra	EXCLUDE on outcome
Sedberry (2006)	Spawning locations for Atlantic reef fishes off the Southeastern U.S	EXCLUDE on outcome
Semmler (2016)	Are mesophotic coral ecosystems distinct communities and can they serve as refugia for shallow reefs?	INCLUDE on full text
Senna (2014)	A new species of Boca Lowry & Stoddart, 1997 (Amphipoda: Lysianassoidea: Aristiidae) from a mesophotic coral ecosystem off Puerto Rico, Caribbean Sea	EXCLUDE on outcome
Sheppard (1980)	Coral cover, zonation and diversity on reef slopes of Chagos Atolls, and population structures of the major species	INCLUDE on full text
Sheppard (1982)	Coral populations on reef slopes and their major controls	EXCLUDE on study design
Sherman (2010)	Geomorphology and benthic cover of mesophotic coral ecosystems of the upper insular slope of southwest Puerto Rico	EXCLUDE on comparator
Sherman (2013)	Exploring the mesophotic zone: Diving operations and scientific highlights of three research cruises across Puerto Rico and US Virgin Islands	EXCLUDE on outcome, on comparator

Short Title	Title	Code
Shoham (2016)	Higher species richness of octocorals in the upper mesophotic zone in Eilat (Gulf of Aqaba) compared to shallower reef zones	INCLUDE on full text
Simmons (2014)	Distribution and diversity of coral habitat, fishes, and associated fisheries in US waters of the Gulf of Mexico	EXCLUDE on outcome
Simon (2016)	Mesophotic fishes of the Abrolhos Shelf, the largest reef ecosystem in the South Atlantic	EXCLUDE on comparator
Slattery (2011)	Connectivity and stability of mesophotic coral	EXCLUDE on study design, on outcome
Slattery (2014)	Allelopathy in the tropical alga Lobophora variegata (Phaeophyceae): mechanistic basis for a phase shift on mesophotic coral reefs?	EXCLUDE on comparator
Slattery (2015)	Trophic ecology of sponges from shallow to mesophotic depths (3 to 150 m): Comment on Pawlik et al.(2015)	EXCLUDE on study design, on outcome
Sluka (2001)	Herbivorous fish assemblages and herbivory pressure on Laamu Atoll, Republic of Maldives	EXCLUDE not mesophotic
Smith (1999)	Short term variability in larval fish assemblages of the Sydney shelf: Tracers of hydrographic variability	INCLUDE on full text
Smith (2008)	Assessing coral reef health across onshore to offshore stress gradients in the US Virgin Islands	INCLUDE on full text
Smith (2010)	Benthic structure and cryptic mortality in a Caribbean mesophotic coral reef bank system, the Hind Bank Marine Conservation District, US Virgin Islands	INCLUDE on full text
Smith (2016)	Potential structuring forces on a shelf edge upper mesophotic coral ecosystem in the US Virgin Islands	EXCLUDE on outcome
Spalding (2012)	Ecology of mesophotic macroalgae and Halimeda kanaloana meadows in the Main Hawaiian Islands	INCLUDE on full text
Spalding (2013)	BIODIVERSITY OF MESOPHOTIC MACROALGAE IN THE PAPAHANAUMOKUAKEA MARINE NATIONAL MONUMENT, NORTHWESTERN HAWAIIAN ISLANDS	EXCLUDE on subject
Spalding (2016)	New Ulvaceae (Ulvophyceae, Chlorophyta) from mesophotic ecosystems across the Hawaiian Archipelago	EXCLUDE on study design, on outcome
Stanley (2000)	Variation in the density and species composition of fishes associated with three petroleum platforms using dual beam hydroacoustics	INCLUDE on full text
Starr (2007)	Spatial dynamics of the Nassau grouper Epinephelus striatus in a Caribbean atoll	INCLUDE on full text
Starr (2012)	Deepwater fish assemblages at Isla del Coco National Park and Las Gemelas Seamount, Costa Rica	EXCLUDE on comparator
Stevens (2005)	Local-scale mapping of benthic habitats to assess representation in a marine protected area	EXCLUDE on outcome

Short Title	Title	Code
Thomas (2007)	New species and host associations of commensal leucothoid amphipods from coral reefs in Florida and Belize (Crustacea:Amphipoda)	EXCLUDE on outcome, on comparator
Tornabene (2016)	Repeated invasions into the twilight zone: evolutionary origins of a novel assemblage of fishes from deep Caribbean reefs	EXCLUDE on outcome, on comparator
Tornabene (2016)	Varicus lacerta, a new species of goby (Teleostei, Gobiidae, Gobiosomatini, Nes subgroup) from a mesophotic reef in the southern Caribbean	EXCLUDE on outcome, on comparator
TSUDA (2015)	Marine Benthic Algae from Seamounts along the Mariana Islands, Western Pacific1	EXCLUDE on outcome
Tuohy (2015)	In situ Tagging of Nassau Grouper Epinephelus striatus Using Closed-Circuit Rebreathers at a Spawning Aggregation in Puerto Rico	EXCLUDE on outcome, on comparator
Van Der Meij (2015)	A mesophotic record of the gall crab Opecarcinus hypostegus from a Curaçaoan reef	EXCLUDE on outcome
van Soest (2014)	Deep-water sponges (Porifera) from Bonaire and Klein Curacao, Southern Caribbean	EXCLUDE on outcome, on comparator
Veazey (2016)	The implementation of rare events logistic regression to predict the distribution of mesophotic hard corals across the main Hawaiian Islands	EXCLUDE on outcome, on comparator
Venn (2009)	Deep zooxanthellate corals at the high latitude Bermuda Seamount	EXCLUDE on outcome
Vermeij (2002)	How are coral populations structured by light? Marine light regimes and the distribution of Madracis	EXCLUDE on outcome
Vermeij (2003)	Species-Specific Population Structure of Closely Related Coral Morphospecies Along a Depth Gradient (5-60 m) Over a Caribbean Reef Slope	INCLUDE on full text
Vora (1990)	Submerged reef systems on the central western continental shelf of India	EXCLUDE on outcome, on comparator
Wagner (2010)	Using morphometrics, in situ observations and genetic characters to distinguish among commercially valuable Hawaiian black coral species; a redescription of Antipathes grandis Verrill, 1928 (Antipatharia: Antipathidae)	EXCLUDE on outcome
Wagner (2011)	Azooxanthellate? Most Hawaiian black corals contain Symbiodinium	INCLUDE on full text
Wagner (2011)	New Records of Commercially Valuable Black Corals (Cnidaria: Antipatharia) from the Northwestern Hawaiian Islands at Mesophotic Depths	EXCLUDE on outcome, on comparator

Short Title	Title	Code
Wagner (2012)	The Biology and Ecology of Hawaiian Black Corals (Cnidaria: Anthozoa: Hexacorallia: Antipatharia)	INCLUDE on full text
Wagner (2013)	Mesophotic surveys of the Northwestern Hawaiian Islands with new records of black coral species	EXCLUDE on outcome, on comparator
Wagner (2014)	Mesophotic surveys of the flora and fauna at Johnston Atoll, Central Pacific Ocean	EXCLUDE on comparator
Wagner (2015)	A taxonomic survey of the shallow-water (<150 m) black corals (Cnidaria: Antipatharia) of the Hawaiian Islands	INCLUDE on full text
Wagner (2015)	The spatial distribution of shallow-water (< 150 m) black corals (Cnidaria: Antipatharia) in the Hawaiian Archipelago	INCLUDE on full text
Waheed (2013)	A tale of two winds: species richness patterns of reef corals around the Semporna peninsula, Malaysia	EXCLUDE on outcome
Waheed (2015)	Scleractinian corals (Fungiidae, Agariciidae and Euphylliidae) of Pulau Layang-Layang, Spratly Islands, with a note on Pavona maldivensis (Gardiner, 1905)	INCLUDE on full text
Weaver (2006)	Deep reef fish surveys by submersible on Alderdice, McGrail, and Sonnier Banks in the northwestern Gulf of Mexico	INCLUDE on full text
Weaver (2006)	Deepwater reef fishes and multibeam bathymetry of the Tortugas south Ecological Reserve, Florida Keys National Marine Sanctuary, Florida	EXCLUDE on comparator
Webster (2008)	From corals to canyons: The Great Barrier Reef Margin	EXCLUDE on outcome
Weinstein	Deep reef sedimentology: Impact on the structural sustainability of mesophotic coral reefs in the US Virgin Islands	EXCLUDE on outcome, on comparator
Westinga (1981)	The Intrasponge Fauna of Spheciospongia vesparia (Porifera, Demospongiae) at Curacao and Bonaire	INCLUDE on full text
White (2013)	Typhoon damage on a shallow mesophotic reef in Okinawa, Japan	EXCLUDE on outcome
Whitfield (2014)	Native fish community structure and Indo-Pacific lionfish Pterois volitans densities along a depth-temperature gradient in Onslow Bay, North Carolina, USA	INCLUDE on full text
Wind (2015)	A comparison of extinction risk in shallow versus deep water reefs	EXCLUDE on study design
Woesik (2015)	Reef-coral refugia in a rapidly changing ocean	EXCLUDE on study design, on outcome
Zuschin (2005)	Diversity patterns of bivalves in a coral dominated shallow-water bay in the northern Red Sea - High species richness on a local scale	INCLUDE on full text

Supplementary 2.4 Unavailable full texts report

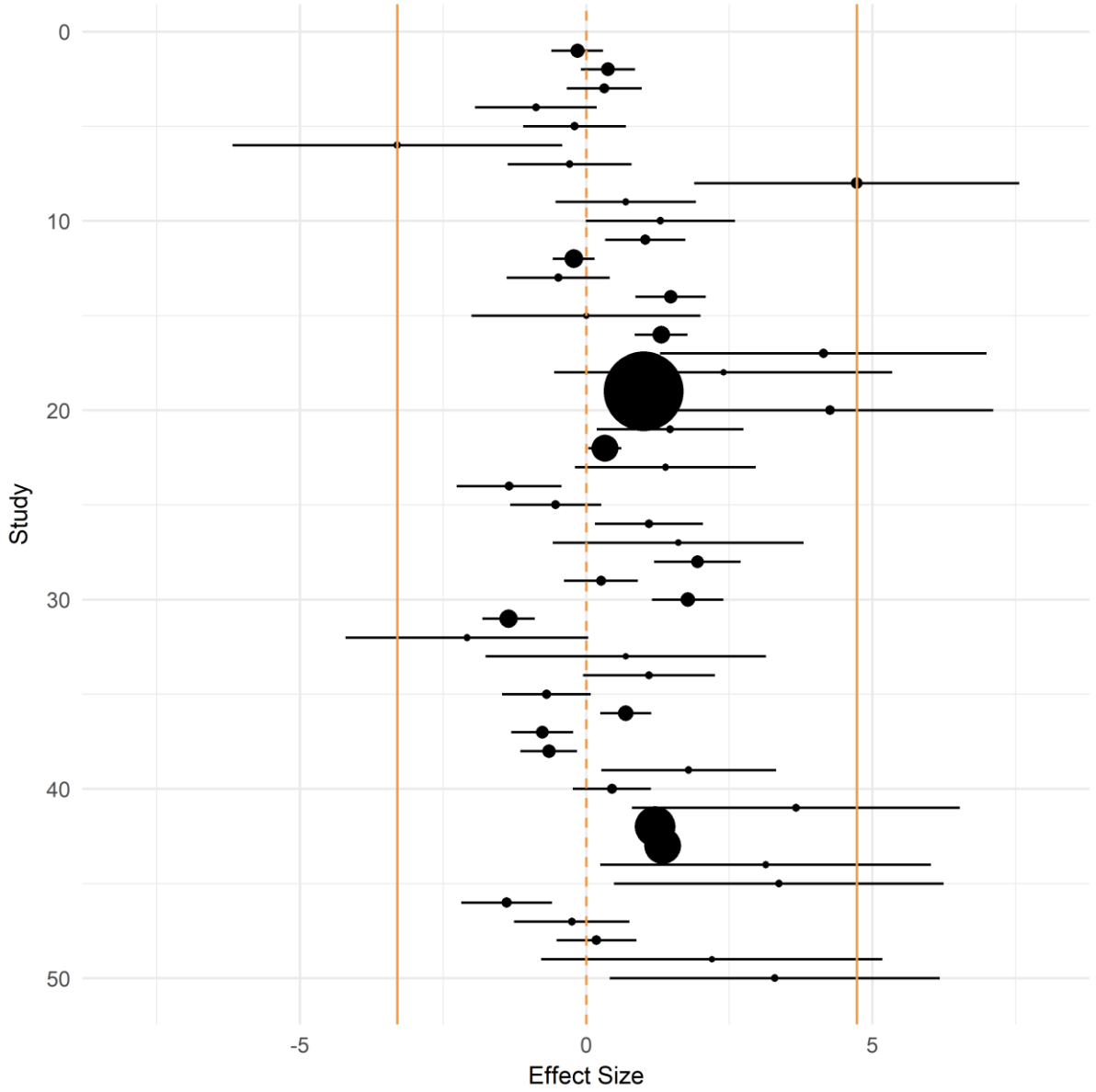
List of articles screened at abstract level and assigned an eco tag, which we could not retrieve a full text for.

Short Title	Title
Ghiold (1990)	BLEACHING AND RECOVERY OF DEEP-WATER REEF-DWELLING INVERTEBRATES IN THE CAYMAN ISLANDS BRITISH WEST INDIES
Grasshoff (2000)	The gorgonians of the Sinai coast and the Strait of Gubal, Red Sea (Coelenterata, Octocorallia)
Noble (1986)	HALIMEDA-MAGNIDISCA (CAULERPALES, CHLOROPHYTA), A NEW SPECIES FROM THE GREAT-BARRIER-REEF, AUSTRALIA
Ortiz (1999)	The Recent marine Mollusca of Puerto Rico
Yang (1980)	Community Structure and Species Diverstiy of Reef Corals at Nan-Wan Bay, Taiwan
Nuttall (2013)	Antipatharian diversity and habitat suitability mapping in the mesophotic zone of the Northwestern Gulf of Mexico
Ruiz (2006)	Deep-water octocorals off eastern Venezuela
Ballantine (2009)	Notes on the benthic marine algae of Puerto Rico. IX. Additions to the flora including reports of three species for the first time in the Atlantic
Glynn (1990)	Coral Mortality and Disturbances to Coral Reefs in the Tropical Eastern Pacific
Glynn (2003)	Reef-building coral communities of Easter Island (Rapa Nui), Chile
Moshchenko (1998)	Distribution of the hydroid Millepora platyphylla on the reefs of southern Vietnam
Newman (2001)	Spatial and temporal variation in assemblages of Lutjanidae, Lethrinidae and associated fish species among mid-continental shelf reefs in the central great barrier reef
Sammarco (2016)	The positive relationship between relief and species richness in mesophotic communities on offshore banks, including geographic patterns
Searles (1987)	OBSERVATIONS ON THE DEEP-WATER FLORA OF BERMUDA
Womersley (2004)	Additions to the Marine Algal Flora of southern Australia

Supplementary 2.5 Distribution of studies across factor levels

The number of studies included in the full meta-analysis of 50 studies is reported for each combination of taxon and location. The total number of studies for a given factor level is in brackets after the level name. Many combinations are not represented, prompting the decision not to include an interaction term in the statistical analysis.

	Brazil (7)	Caribbean (22)	Central Pacific (12)	Indian Ocean (1)	Coral Sea (5)	Red Sea (4)	South Africa (1)
Angiosperm (1)	0	0	0	0	1	0	0
Antipatharia (2)	0	1	1	0	0	0	0
Chlorophyta (4)	2	0	2	0	0	0	0
Fish (12)	0	5	4	0	2	1	0
Hydroid (1)	0	1	0	0	0	0	0
Mollusca (1)	0	1	0	0	0	0	0
Octocorallia (2)	0	2	0	0	0	1	0
Phaeophyta (4)	2	0	2	0	0	0	0
Porifera (1)	1	0	0	0	0	0	0
Rhodophyta (4)	2	0	2	0	0	0	0
Scleractinia (18)	0	11	1	1	2	2	1
Sponge infauna (1)	0	1	0	0	0	0	0



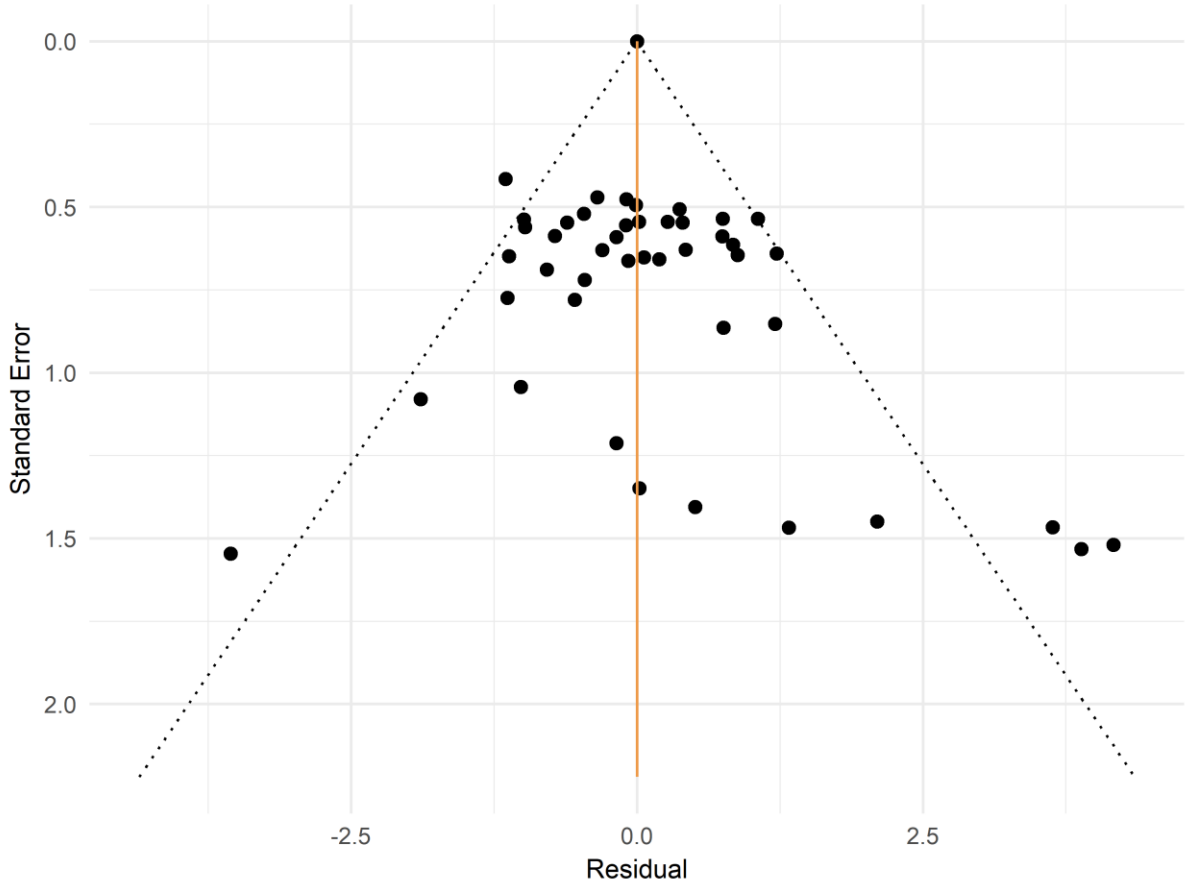
Supplementary 2.6 Forest plot

The effect sizes for 50 included studies are displayed. Dots indicate the effect size estimate and are scaled to the number of species observed within each study. The black lines extend from the lower to upper 95% confidence limit of each study estimate. Orange lines are for reference to help interpretation. The dashed line at 0 indicates the effect size of studies finding 50% community overlap. The solid lines indicate the effect size of the largest study reporting 0% overlap (negative effect size) and 100% (positive effect size). Note that due to the scaling of effect sizes the confidence intervals become asymmetrical when back transformed to proportions (Figure 2.4).

Supplementary 2.7 Factor level effects

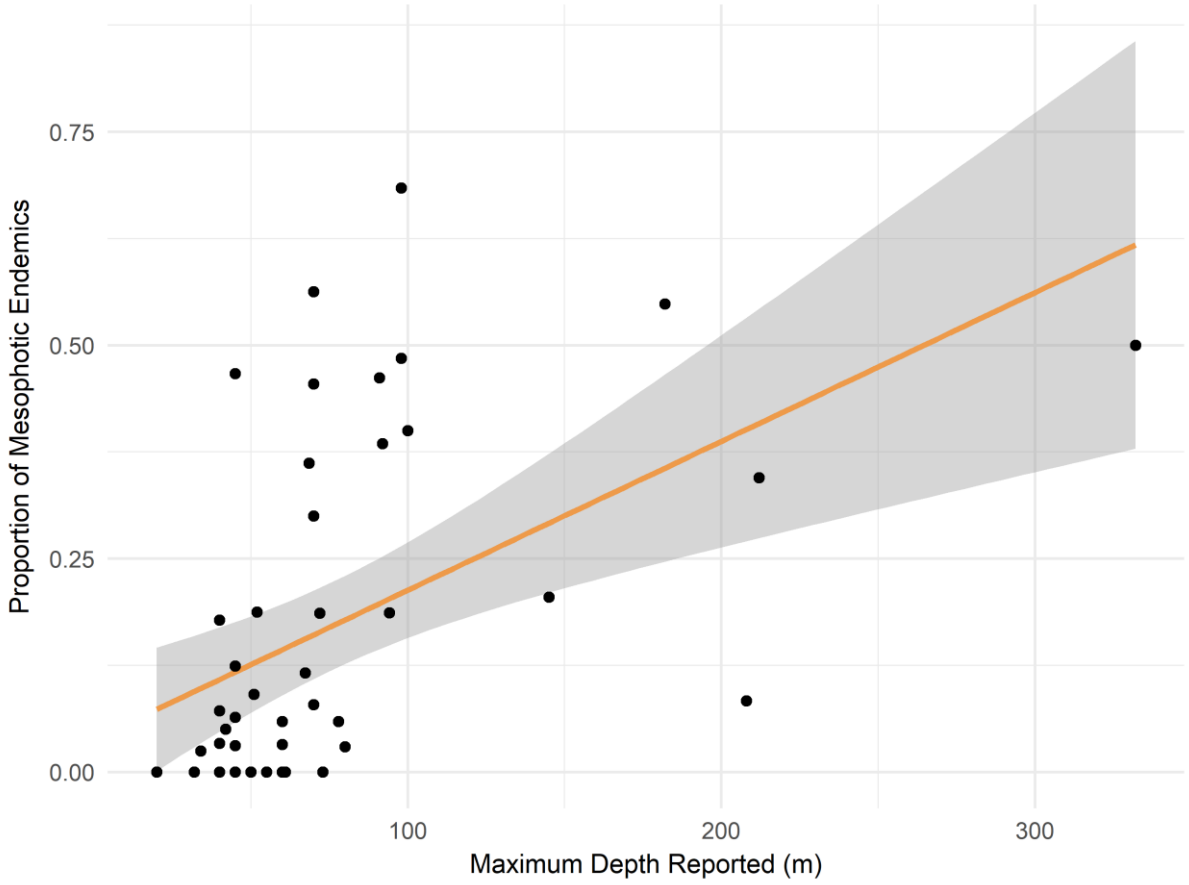
Predictions of community overlap between shallow and mesophotic reefs by taxon and location, as visualised in Figure 2.4. Values are reported as per computer output and in descending order of estimate. The number of studies for each factor level contributing to the model is provided. Reference level indicates which factor level was held constant as the factor of interest was changed. The use of different reference levels should not affect the analysis in the absence of an interaction term in the model.

Factor Effects	Studies	Estimate	CI Lower Bound	CI Upper Bound	Reference Level
Location					
Central Pacific	12	0.7194247	0.534483024	0.8513297	Fish
Red Sea	4	0.6852092	0.377676908	0.8864571	Fish
Caribbean	22	0.6431862	0.464331211	0.7894076	Fish
Coral Sea	5	0.4113170	0.207502203	0.6509007	Fish
Brazil	7	0.3091140	0.072371112	0.7195631	Fish
Indian Ocean	1	0.2832185	0.052005551	0.7399855	Fish
South Africa	1	0.2556105	0.038277893	0.7476340	Fish
Taxon					
Angiosperm	1	0.9659617	0.225985463	0.9996376	Caribbean
Sponge infauna	1	0.9642857	0.256304806	0.9995275	Caribbean
Porifera	1	0.9235859	0.383969094	0.9957515	Caribbean
Phaeophyta	4	0.9064932	0.524904966	0.9883807	Caribbean
Antipatharia	2	0.8588630	0.371228229	0.9843068	Caribbean
Rhodophyta	4	0.8429059	0.474622522	0.9695757	Caribbean
Chlorophyta	4	0.7697910	0.342322043	0.9555204	Caribbean
Mollusca	1	0.7319149	0.339283583	0.9355478	Caribbean
Fish	12	0.6431862	0.464331211	0.7894076	Caribbean
Scleractinia	18	0.5675435	0.417488401	0.7061511	Caribbean
Hydroid	1	0.5000000	0.037195620	0.9628044	Caribbean
Octocorallia	3	0.3844480	0.152213956	0.6848000	Caribbean



Supplementary 2.8 Funnel plot

Funnel plot for the full meta-analysis. Dotted lines indicate the 95% confidence limit for expected deviation of study estimates from the model. Studies falling outside the triangle are potential outliers. With 50 studies we would expect fewer than 3. A lack of asymmetry to the plot provides no evidence of publication bias within the collected dataset.



Supplementary 2.9 Proportion of mesophotic-specialists reported with maximum study depth

The proportion of all species reported, found exclusively deeper than 30m, increases with the maximum depth of a study. An absence of deep sampling efforts does not allow us to claim there are low levels of MCE specific biodiversity. As such we limit the discussion in this paper to the shallow taxa occurring on MCEs, not of the total change in community between the two depth zones. In order for a fair comparison to be made between shallow and mesophotic biodiversity we must more strictly define the lower limit of MCEs. Data was limited to studies reporting a maximum depth <500m for legibility.

Supplementary 2.10 Scoping exercise

A scoping exercise was undertaken as part of protocol development in February 2015, reported in the protocol paper. Search strings were trialled and refined in Web of Science initially, then Scopus as outlined below. When search strings were tested the number of hits were recorded and the first 200 titles ordered by relevance (or all results, whichever was smaller) were reviewed. The results were then searched for the titles of test library papers. If a paper failed to be found the authors and date of the paper were used as a second search. If the paper was still not found it was recorded as a failed retrieval. The number of accessible papers retrieved was then reported for the search string. When comparing search strings a higher retrieval rate was considered more valuable than a smaller number of hits. When a paper is referred to specifically the number correlates with the label in the test library.

The scoping exercise is reported in the following format:

Search element ID) Search term – Hits,
Number of returned articles from test library.
comments.

Web of science – 33/36 test library entries present.

SUBJECT:

1) *"Mesophotic Coral Ecosystem" – 21,*
3/33 from test library.
New species records, very few results.

2) *Mesophotic – 131,*
9/33 from test library.
Additional relevant titles. Lots of genetic studies.

****1) OR 2) = 2). Reject "Mesophotic Coral Ecosystem"****

3) *"Deep reef" – 180*
5/33 from test library
Additional relevant titles. Struggling to pick up submersible studies so far.

**** 2) OR 3) returns 300 with 12/33 from test library. Retain "Deep reef"****

4) *Submersibl* OR submarine* - 62,767,*
7/33 from test library. 5 new
Unwieldy number of results, given return rate.

5) *((Submersibl* OR submarine*) AND (biolog* OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) -10,346*
7/33 from test library
Significant improvement but further refinement worth attempting.

6) *((Submersibl* OR submarine*) AND Tropical AND (biolog* OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) -171*

1/33 from test library

Lack of sensitivity, Tropical deemed an inappropriate addition.

****2) OR 3) OR 5) returns 11,016 with 19/33 from the test library, retain submarine study capture terms****

****Same search in Scopus returns 32,742 with 18/33 but 4 new. Both searches combined retrieve 23, Scoping continues in Scopus****

7) 2) OR 3) OR 5) OR "Deep water" – 107,665,
Unwieldy number of results

8) *2) OR 3) OR 5) OR "Deep water" (qualified as 5)) – 67,25*

23/33 from test library

5 new, deep water term is useful

** **No new results when search string ran in Web of Science -27, 514, 28/33 retrieved from combined search. Retain Deep water term.****

****last 5 missing test papers are reviewed to identify potentially useful terms in the abstract****

9) *Modify qualifying terms to include 'Hermatyp*' targets missing paper (6) – 27,514,*

Fails to capture targeted paper in Web of Science.

Fails to capture targeted paper in Scopus.

**** Reject Hermatyp* qualifier ****

10) Modify submersible* to submer* targets missing paper (7) with submerged in the abstract – *56,164*

Fails to capture targeted paper in Web of Science.

Target doesn't appear available in Scopus.

****Reject Submersibl* modification ****

The remaining 3 missing papers present in the databases were entered without abstracts. A google search retrieved the abstracts. Useful terms were not identified

Two other papers were not present in either database and represent grey literature. These papers may be captured in other depositories.

Circulated search string:

(Mesophotic) OR ("Deep reef") OR ((Submersibl OR submarine*) AND (biolog* OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) OR (("Deep water") AND (biolog* OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*))*

Deemed appropriate capturing 84% of accessible test library from test sources.

An advisory committee was arranged and presented with the above search string. An additional 5 papers were added to the test library, published before the term mesophotic was coined. The Capture rate for these new papers was 100%. As well as more test papers the following search terms were suggested and trialled in Scopus.

11) ((Trimix) AND (biolog OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) – 23*

some new relevant results

****Accept term Trimix with filter****

12) (("Technical diving") AND (biolog OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) – 7*

Irrelevant hits

13) (("mixed gas diving") AND (biolog OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) – 4*

Irrelevant hits

14) ((rebreather) AND (biolog OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) - 21*

no new relevant terms

15) ((CCR OR "Closed Circuit Rebreather") AND (biolog OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) - 728*

large number of medical studies, remove CCR

16) (("Closed Circuit Rebreather") AND (biolog OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) - 5*

none new

After advisory committee feedback incorporate 11) and finalise search string as

(Mesophotic) OR ("Deep reef") OR ((Submersibl OR submarine*) AND (biolog* OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) OR (("Deep water") AND (biolog* OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*)) OR ((Trimix) AND (biolog* OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*))*

This can be more succinctly expressed as:

Mesophotic OR "Deep reef" OR ((Submersibl OR submarine* OR "Deep water" OR Trimix) AND (biolog* OR Reef* OR Cora* OR Spong* OR Alga* OR Fish*))*

Capturing 87% of accessible updated test library from test sources

Supplementary 2.11 Test library

A list of all papers used to determine the effectiveness of trialled search strings from our protocol paper. Papers were chosen from personal libraries of the initial authors, and then built upon by the advisory committee. Papers were deliberately chosen to include articles labelled as mesophotic and more difficult papers published before the terms "mesophotic" or "twilight zone" enter the literature.

Fish papers

Brokovich, E. et al., 2008. Descending to the twilight-zone: changes in coral reef fish assemblages along a depth gradient down to 65 m. *Marine Ecology Progress Series*, 371, pp.253–262.

Bejarano, I., Appeldoorn, R.S. & Nemeth, M., 2014. Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico. *Coral Reefs*.

Garcia-Sais, J.R., 2010. Reef habitats and associated sessile-benthic and fish assemblages across a euphotic–mesophotic depth gradient in Isla Desecheo, Puerto Rico. *Coral Reefs*, 29(2), pp.277–288.

Fitzpatrick, B.M. et al., 2012. Habitat Specialization in Tropical Continental Shelf Demersal Fish Assemblages *PLoS ONE*, 7(6), p.e39634.

Strasburg, D.W., Jones, E.C. & Iversen, R.T.B., 1968. Use of a Small Submarine for Biological and Oceanographic Research. *J. Cons. perm. int. Explor. Mer*, 31(3), pp.410–426.

Colin, P.L., 1974. Observation and collection of deep-reef fishes off the coasts of Jamaica and British Honduras (Belize). *Marine Biology*, 24(1), pp.29–38.

Thresher, R.E. & Colin, P.L., 1986. Trophic structure, diversity and abundance of fishes of the deep reef (30-300m) at Enewetak, Marshall Islands. *Bulletin of Marine Science*, 38(1), pp.253–272.

Parker, F.O. & Ross, S.W., 1986. Observing reef fishes from submersibles off North Carolina. *Northeast Gulf Science*, 8(1), pp.31–49.

Bryan, D.R. et al., 2013. Characterization of the mesophotic reef fish community in south Florida, USA. *Journal of Applied Ichthyology*, 29(1), pp.108–117.

Parrish, F.A. & Boland, R.C., 2004. Habitat and reef-fish assemblages of banks in the Northwestern Hawaiian Islands. *Marine Biology*, 144(6), pp.1065–1073.

Pereira-Filho, G.H. et al., 2011. Reef fish and benthic assemblages of the Trindade and Martin Vaz Island group, Southwestern Atlantic. *Brazilian Journal of Oceanography*, 59(3), pp.201–212.

Feitoza, B.M., Rosa, R.S. & Rocha, L.A., 2005. Ecology and Zoogeography of Deep-Reef Fishes in Northeastern Brazil. *Bulletin of Marine Science*, 76(3), pp.725–742.

Lesser, M.P. & Slattery, M., 2011. Phase shift to algal dominated communities at mesophotic depths associated with lionfish (Pterois volitans) invasion on a Bahamian coral reef. *Biological Invasions*, 13(8), pp.1855–1868.

Frédou, T. & Ferreira, B.P., 2005. Bathymetric trends of northeastern Brazilian snappers (Pisces, Lutjanidae): implications for the reef fishery dynamic. *Brazilian Archives of Biology and Technology*, 48(5), pp.787–800.

Shinn, E.A. & Wicklund, R.I., 1989. Artificial Reef Observations from a Manned Submersible off Southeast Florida. *Bulletin of Marine Science*, 44(2), pp.1041–1050.

Ralston, S., Gooding, R.M. & Ludwig, G.M., 1986. An ecological survey and comparison of bottom fish resource assessments(submersible versus handline fishing) at Johnston Atoll. *Fishery Bulletin*, 84(1), pp.141–155.

Algal papers

Ballantine, D. L., H. Ruiz, et al. (2011). "ALGAL COMPOSITION AND DYNAMICS IN MESOPHOTIC CORAL REEFS, SOUTHWEST PUERTO RICO." Journal of Phycology 47: 15-15.

Amado-Filho, G. M., G. H. Pereira-Filho, et al. (2012). "Occurrence and distribution of rhodolith beds on the Fernando de Noronha Archipelago of Brazil." Aquatic Botany 101: 41-45.

Nilda E. Aponte, David L. Ballantine. (2001). Depth distribution of algal species on the deep insular fore reef at Lee Stocking Island, Bahamas. Deep Sea Research Part I: Oceanographic Research Papers. Volume 48, Issue 10, Pages 2185–2194

Hillis-Colinvaux, L., 1986. Deep water populations of Halimeda in the economy of an atoll.Bulletin of Marine Science, 38(1), pp.155–169.

Liddell, W.D. & Ohlhorst, S.L., 1988. Hard substrata community patterns, 1-120 M, North Jamaica. Palaios, pp.413–423.

Leichter, J.J., Stokes, M.D. & Genovese, S.J., 2008. Deep water macroalgal communities adjacent to the Florida Keys reef tract. Marine Ecology Progress Series, 356, pp.123–138.

Wagner, D., Kosaki, R. K., Spalding, H. L., Whitton, R. K., Pyle, R. L., Sherwood, A. R., … Calcinai, B. (2014). Mesophotic surveys of the flora and fauna at Johnston Atoll, Central Pacific Ocean. *Marine Biodiversity Records*, *7*, 1–10. doi:10.1017/S1755267214000785

Coral Papers

Bridge, T. C. L., Fabricius, K. E., Bongaerts, P., Wallace, C. C., Muir, P. R., Done, T. J., & Webster, J. M. (2012). Diversity of Scleractinia and Octocorallia in the mesophotic zone of the Great Barrier Reef, Australia. *Coral Reefs*, *31*(1), 179–189. doi:10.1007/s00338-011-0828-1

Fricke, H., & Meischner, D. (1985). Depth limits of Bermudan scleractinian corals – a submersible study *Marine Biology*, *187*(88), 175–187.

Fricke, H.W., Schuhmacher, H. , 1983, The depth limits of Red Sea stony corals: an ecophysiological problem (a deep diving survey by submersible) (Sinai coast). Marine Ecology, Volume 4, Issue 2, 1983, Pages 163-194

Kühlmann D (1983) Composition and ecology of deep-water coral associations. Helgol Mar Res 36:183–204

Sheppard CRC (1980) Coral cover, zonation and diversity on reef slopes of Chagos Atolls, and population structures of the major species. Mar Ecol Prog Ser 2:193–205

Sherman C, Nemeth M, Ruı´z H, Bejarano I, Appeldoorn R, Paga´n F, Scha¨ rer M, Weil E: Geomorphology and benthic cover of mesophotic coral ecosystems of the upper insular slope of southwest Puerto Rico. Coral Reefs 2010, 29:347-360.

Uncaptured papers

During the Scoping exercise, we failed to capture several papers. These were studied more closely to discover why.

Fish

(1) Luckhurst, B.E. & Luckhurst, K., 1977. Recruitment patterns of coral reef fishes on the fringing reef of Curaçao, Netherlands Antilles. *Canadian Journal of Zoology*, 55(4), pp.681–689.

Absent in Scopus. Abstract absent in Web of Science.

(2) Weaver, D.C., Dennis, G.D., III & Sulak, K.J., 2001. *Northeastern Gulf of Mexico Coastal and Marine Ecosystem Program: Community structure and Trophic Ecology of Demersal Fishes on the Pinnacles Reef Tract*, New Orleans, LA: US Department of the Interior, Geological Survey, USGS BSR-2001-0008 and Minerals Management Service Gulf of Mexico OCS Region.

Absent in both test databases.

(3) Wenner, C.A., 1983. Species associations and day-night variability of trawl-caught fishes from the inshore sponge-coral habitat, South Atlantic bight. *Fishery Bulletin*, 80(3), pp.537–552.

Abstract absent in Web of Science. Abstract unhelpful in Scopus.

Algae

(4) Agegian CR, Abbott IA (1985) Deep water macroalgal communities: a comparison between Penguin Bank (Hawaii) and Johnston Atoll. Proceedings of the 5th international coral reef symposium 5, pp 47–50

Absent in both test databases.

(5) Liddell, W.D. & Avery, W.E., 2000. Temporal change in hard substrate communities 10-250 m, the Bahamas. In Proceedings of the 9th International Coral Reef Symposium, Bali, Indonesia 23-27 October 2000. pp. 1053–1058.

Absent in Scopus. Abstract absent Web of Science.

Coral

(6) Bright TJ, Kraemer GP, Minnery GA, Viada ST (1984) Hermatypes of the Flower Garden Banks, northwestern Gulf of Mexico: a comparison to other western Atlantic reefs. Bull Mar Sci 34:461–476

Abstract did not contain helpful terms.

(7) C. Bouchon (1981) Quantitative study of the Scleractinian coral communities of a fringing reef of Reunion Island (Indian Ocean) *Mar. Ecol. Prog. Ser.* **4:** 273-288

Absent in Scopus. Abstract absent in Web of Science.

Additional test papers from the advisory committee

Bak RPM, Nieuwland G, Meesters EH (2005) Coral reef crisis in deep and shallow reefs: 30 years of constancy and change in reefs of Curacao and Bonaire. Coral Reefs 24:475-479

Grigg RW (2006) Depth limit for reef building corals in the Au'au Channel, S.E. Hawaii. Coral Reefs 25:77-84

Pyle RL (2000) Assessing undiscovered fish biodiversity on deep coral reefs using advanced self-contained diving technology. Mar Tech Soc J 34:82–91

Fricke, H., Knauer, B.,1986. Diversity and spatial pattern of coral communities in the Red Sea upper twilight zone. Oecologia 71, 29-37

Kahng, S.E., Kelley, C.D., 2007. Vertical zonation of megabenthic taxa on a deep photosyntheic reef (50-140 m) in the Aú au Channel, Hawaii. Coral Reefs 26, 679-687

Supplementary 2.12 Full database list

From our protocol paper, an expanded database list detailing all sub resources the review intended to search. In addition to these resources are the specialist resources, search engines, and personal libraries referred to in the protocol.

- ISI Web of Knowledge platform http://isiknowledge.com
    - Web of Science (1970-present)
    - BIOSIS Citation Index (1969–2008)
    - Current Contents Connect (1998- present)
    - CABI: CAB Abstracts (1973- present)
    - MEDLINE (1950- present)
    - Zoological Record (1864- present)
- Science Direct - http://www.sciencedirect.com (all years)
- JSTOR - http://www.jstor.org (all years)
- Scopus - http://www.scopus.com
- AGRICOLA - http://agricola.nal.usda.gov (all years)
- Proquest - http://search.proquest.com (all years)
    - ABI/INFORM Global
    - ABI/INFORM Trade & Industry (1971 - current)
    - American Periodicals (1740 - 1940)
    - Applied Social Sciences Index and Abstracts (ASSIA) (1987 - current)
    - Aqualine (1960 - current)
    - ARTbibliographies Modern (ABM) (1974 - current)
    - ASFA: Aquatic Sciences and Fisheries Abstracts (1971 - current)

- Aquatic Science & Fisheries Abstracts (ASFA) 1: Biological Sciences & Living Resources (1971 - current)
- Aquatic Science & Fisheries Abstracts (ASFA) 2: Ocean Technology, Policy & Non-Living Resources (1971 - current)
- Aquatic Science & Fisheries Abstracts (ASFA) 3: Aquatic Pollution & Environmental Quality (1990 - current)
- Aquatic Science & Fisheries Abstracts (ASFA) Aquaculture Abstracts (1984 - current)
- Aquatic Science & Fisheries Abstracts (ASFA) Marine Biotechnology Abstracts (1989 - current)
- Oceanic Abstracts (1981 - current)
- ProQuest Deep Indexing: Aquatic Sciences

o Biological Sciences (1946 - current)

- Algology Mycology and Protozoology Abstracts (Microbiology C) (1982 - current)
- Animal Behavior Abstracts (1982 - current)
- Aquatic Science & Fisheries Abstracts (ASFA) 1: Biological Sciences & Living Resources (1971 - current)
- Aquatic Science & Fisheries Abstracts (ASFA) Aquaculture Abstracts (1984 - current)
- Aquatic Science & Fisheries Abstracts (ASFA) Marine Biotechnology Abstracts (1989 – current)
- Bacteriology Abstracts (Microbiology B) (1982 - current)
- Biochemistry Abstracts 1 (1981 - 1993)

- Biochemistry Abstracts 3 (1982 - 1993)
- Biotechnology Research Abstracts
- Calcium & Calcified Tissue Abstracts (1982 - current)
- Chemoreception Abstracts (1982 - current)
- COS Conference Papers Index (1982 - current)
- Ecology Abstracts (1982 - current)
- Endocrinology Abstracts (1985 - 1986)
- Entomology Abstracts (1981 - current)
- Genetics Abstracts (1982 - current)
- Health & Safety Science Abstracts (1981 - current)
- Human Genome Abstracts (1982 - 1995)
- Immunology Abstracts (1982 - current)
- Industrial and Applied Microbiology Abstracts (Microbiology A) (1982 - current)
- MEDLINE® (1946 - current)
- Neurosciences Abstracts (1982 - current)
- Nucleic Acids Abstracts (1982 - current)
- Oncogenes and Growth Factors Abstracts (1989 - current)
- Plant Science (1994 - current)
- ProQuest Deep Indexing: Biological Sciences
- Toxicology Abstracts (1981 - current)
- TOXLINE (1999 - current)
- Virology and AIDS Abstracts (1982 - current)

- British Humanities Index (BHI) (1962 - current)

- British Periodicals (1681 - 1939)
- Design and Applied Arts Index (DAAI) (1973 - current)
- ebrary® e-books
- EconLit (1969 - current)
- Environmental Impact Statements (1985 - current)
- Environmental Sciences and Pollution Management (1967 - current)
    - Aquatic Science & Fisheries Abstracts (ASFA) 3: Aquatic Pollution & Environmental Quality (1990 - current)
    - Bacteriology Abstracts (Microbiology B) (1982 - current)
    - COS Conference Papers Index (1982 - current)
    - Ecology Abstracts (1982 - current)
    - Environment Abstracts
    - Environmental Engineering Abstracts (1990 - current)
    - Environmental Impact Statements: Digests (1985 - current)
    - Health & Safety Science Abstracts (1981 - current)
    - Industrial and Applied Microbiology Abstracts (Microbiology A) (1982 - current)
    - Pollution Abstracts (1981 - current)
    - ProQuest Deep Indexing: Environmental Sciences
    - Risk Abstracts (1990 - current)
    - Sustainability Science Abstracts (1995 - current)
    - Toxicology Abstracts (1981 - current)
    - TOXLINE (1999 - current)
    - Water Resources Abstracts (1967 - current)

- ERIC (1966 - current)
- Ethnic NewsWatch
- International Bibliography of the Social Sciences (IBSS) (1951 - current)

- Library and Information Science Abstracts (LISA) (1969 - current)
- Linguistics and Language Behavior Abstracts (LLBA) (1973 - current)
- MLA International Bibliography (1926 - current)
- National Criminal Justice Reference Service (NCJRS) Abstracts Database (1975 - current)
- PAIS International (1972 - current)
- Periodicals Archive Online
- Periodicals Index Online
- Physical Education Index (1970 - current)
- PILOTS: Published International Literature On Traumatic Stress (1871 - current)
- PRISMA (Publicaciones y Revistas Sociales y Humanísticas) (1966 - current)

- ProQuest Dissertations & Theses: UK & Ireland
- ProQuest Dissertations & Theses Global
- ProQuest Historical Annual Reports
- ProQuest Historical Newspapers: Chicago Defender (1909 - 1975)
- ProQuest Historical Newspapers: The Guardian and The Observer (1791 - 2003)

- ProQuest Historical Newspapers: The Irish Times and The Weekly Irish Times (1859 - 2012)
- ProQuest Historical Newspapers: New York Amsterdam News (1922 - 1993)
- ProQuest Historical Newspapers: The New York Times with Index (1851 - 2011)
- ProQuest Historical Newspapers: Pittsburgh Courier (1911 - 2002)
- ProQuest Historical Newspapers: The Scotsman (1817 - 1950)
- ProQuest Historical Newspapers: The Times of India (1838 - 2005)
- ProQuest Historical Newspapers: The Washington Post (1877 - 1998)
- Social Services Abstracts (1979 - current)
- Sociological Abstracts (1952 - current)
- Technology Research Database (1962 - current)
- The Vogue Archive
- Worldwide Political Science Abstracts (1975 - current)

Chapter Three

Using Light-Dependent Scleractinia to Define the Upper Boundary of Mesophotic Coral Ecosystems on the Reefs of Utila, Honduras

DOI: 10.1371/journal.pone.0183075

Jack H. Laverick, Dominic A. Andradi-Brown, Alex D. Rogers

3.1 Abstract:

Shallow water zooxanthellate coral reefs grade into ecologically distinct mesophotic coral ecosystems (MCEs) deeper in the euphotic zone. MCEs are widely considered to start at an absolute depth limit of 30m deep, possibly failing to recognise that these are distinct ecological communities that may shift shallower or deeper depending on local environmental conditions. This study aimed to explore whether MCEs represent distinct biological communities, the upper boundary of which can be defined and whether the depth at which they occur may vary above or below 30m. Mixed gas diving and closed-circuit rebreathers were used to quantitatively survey benthic communities across shallow to mesophotic reef gradients around the island of Utila, Honduras. Depths of up to 85m were sampled, covering the vertical range of the zooxanthellate corals around Utila. We investigate vertical reef zonation using a variety of ecological metrics to identify community shifts with depth, and the appropriateness of different metrics to define the upper MCE boundary. Patterns observed in scleractinian community composition varied between ordination analyses and approaches utilising biodiversity indices. Indices and richness approaches revealed vertical community transition was a gradation. Ordination approaches suggest the possibility of recognising two scleractinian assemblages. We could detect a mesophotic and shallow community while illustrating that belief in a static depth limit is biologically unjustified. The switch between these two communities occurred across bathymetric gradients as small as 10m and as large as 50m in depth. The difference between communities appears to be a loss of shallow specialists and increase in depth-generalist taxa. Therefore, it may be possible to define MCEs by a loss of shallow specialist species. To support a biological definition of mesophotic reefs, we advocate this analytical framework

should be applied around the Caribbean and extended into other ocean basins where MCEs are present.

3.2 Introduction:

Mesophotic coral ecosystems (MCEs) represent a distinct environment from shallow-water tropical coral reefs; primarily in terms of lower light levels and increased depth. In 2008 MCEs were defined as starting at 30-40m and extending to the depth of occurrence of the last zooxanthellate scleractinian (Puglise *et al.*, 2009), this definition has been widely adopted (Hinderstein *et al.*, 2010; Loya *et al.*, 2016). However, the use of an absolute 30m depth limit by many MCE researchers (Bongaerts *et al.*, 2010; Kahng *et al.*, 2010; Lesser *et al.*, 2010; Rooney *et al.*, 2010; Bridge *et al.*, 2013; Wagner *et al.*, 2014; Pinheiro *et al.*, 2015; Baker, Puglise and Harris, 2016; Andradi-Brown *et al.*, 2016) potentially misses site-specific patterns in environmental variables; which may alter the character of a coral reef and so change the depths at which MCEs occur. There has been limited effort to identify whether a reef community can be identified as an MCE in the absence of depth information (Semmler, Hoot and Reaka, 2016). This is despite the recognition that the lower extents of MCEs vary by region, based on environmental conditions (Englebert *et al.*, 2014).

Previous work has found evidence of changes in photosynthetic coral reef community composition at a variety of depths (Rooney *et al.*, 2010; Bridge *et al.*, 2011; Hurley *et al.*, 2015; Magalhães *et al.*, 2015), as well as observations of unusual or unexpected reef communities at particularly shallow or deep locations (Muir and Wallace, 2015; Muir *et al.*, 2015). This has led to calls to rethink the static definition of MCEs (Laverick *et al.*, 2016; Loya *et al.*, 2016). In some cases, the differences in detected transitions in

ecological communities across the depth gradient may result from different study taxa (Semmler, Hoot and Reaka, 2016), in others because of factors specific to the study sites such as the availability of hard substrate and topography (Bridge *et al.*, 2011). A third source of variation in transitions, previously little considered in MCE literature, may lie in differences in analyses employed to detect changes in reef communities.

The use of a static upper depth limit for MCEs by some appears to be at odds with these observations, and the methods for defining ecosystems in other areas of ecology. Different forest ecosystems may be defined by predominant tree species while deserts are defined in terms of rainfall (Noy-Meir, 1973). Within marine ecology, community structure in the intertidal zone is driven by tidal water movements, exposure to wave action, and biological competition either for space or through predation (Paine, 1974; Sousa, 1979; Burrows, Harvey and Robb, 2008). In the UK, the littorina, balanoid and laminarian zones are easily spotted at low tide without the need to measure heights above tide marks (Stephenson and Stephenson, 1949). The Different shallow reef zones, such as the flat and crest, have been recognised to differ in terms of ecology while often occurring at similar water depths (M. A. Huston, 1985). Such definitions recognise variability in prevailing conditions at a given location, often using community data as a surrogate for integrated environmental parameters. This sort of approach allows for the design of natural experiments (Diamond, 1983) where we can compare the effects of varying conditions on a particular biological assemblage.

More specific to MCEs, the Deep Reef Refugia Hypothesis (DRRH) suggests MCEs may provide a source of recruits to repopulate shallows reefs in the event of impacts on the latter (Glynn, 1996; Bridge, Hughes, *et al.*, 2013). For the DRRH to be correct, MCEs must

have a degree of community overlap with the shallows. If conditions vary between sites, and determine the rate of species turnover with depth, this will affect the size of any species refuges on deeper reefs. Faster rates of transition with depth from a shallow community through to a mesophotic community would reduce the vertical space occupied by shallow reef species. This reduction in depth range would reduce the likelihood that shallow taxa exist at depths, suitably large, to afford protection from near-surface environmental or anthropogenic stressors. In addition, MCEs face many threats in their own right, and clearer identification of what composes a MCE regardless of depth is crucial to inform conservation decision-making (Andradi-Brown *et al.*, 2016). To try and address these problems, it is necessary to consider a method of defining MCEs which accommodates the variable nature of biology. There are, however, several different approaches which may be employed (Table 3.1); this list is not exhaustive and there are multiple ways to analyse data sources which may improve their utility. It is valid to argue for using a combination of data types.

Here, firstly, we report on a study of zooxanthellate scleractinian coral communities from Utila, Honduras ranging from shallow reefs down to, what we consider to be, the maximum depth for this grouping at this location. Secondly, we use this dataset as a case study in detecting signals in community composition for defining the vertical zonation of the reefs, between different statistical methods. Finally, this study suggests a method of identifying MCEs and the zonation within them that may be broadly applicable regardless of geographic context.

Table 3.1 Different potential bases for a definition of MCEs

Information Base	Benefits	Weaknesses
Absolute Depth limits (Bongaerts *et al.*, 2010)	Easily implemented and consistent.	Fails to capture between site variability in environmental conditions. Remains biologically unjustified.
Light levels (Fricke and Meischner, 1985)	Accurately captures the proposed driving force of zooxanthellate coral reef structure. Mesophotic contains 'photic', which means 'relating to light'. It could be argued light levels should be integrated into any definition. Other ecological zones in marine science are defined based on light attenuation (eg. euphotic zone).	Reef structure may lag behind changes in environmental conditions by years. Light levels and penetration profiles vary daily. An average signal requires a long observation period to be representative.
Indicator species (Bejarano, Appeldoorn and Nemeth, 2014)	Integrates a number of environmental signals over long time scales. Does not require extensive taxonomic knowledge.	Indicator taxa may not be shared between sites. What does a MCE indicator taxon truly reflect?
Biodiversity measures (e.g. species richness or evenness)	Integrates a number of environmental signals over long time scales. Metrics are taxon identity-independent so may be applied globally	Misses taxon turn over.
Community composition (Bridge *et al.*, 2012)	Integrates a number of environmental signals over long time scales. Incorporates species turnover. Allows the detection of different types of MCE.	Data intensive. Requires detailed taxonomic knowledge. Requires standardisation of analyses and agreement on prioritisation of rare or common taxa.
Growth forms (Bare *et al.*, 2010)	Integrates a number of environmental signals over long time scales. Easily recognized by the non-specialists. May be applicable globally. Incorporates turnover of forms.	Different regions have different pools of growth forms. Certain taxa are phenotypically plastic and vary in growth form.

3.3 Methods:

3.3.1 Study site-

Utila is one of the Honduran Bay Islands, on the southern boundary of the Mesoamerican Barrier Reef in the Caribbean. Five sampling sites were chosen for their spread around the island (Figure 1.1). Further detail on the study site can be found in chapter 1.

3.3.2 Data Collection-

50m video transects were collected by divers using mixed-gas closed-circuit rebreathers (Hollis Prism 2, Hollis, San Leandro, California, USA) in the Summer of 2015. Research permits were granted by the Instituto de Conservación Forestal, Honduras (Permit number: ICF-261-16), through Operation Wallacea. A Veho K2 action camera (Veho, Southampton, UK) in a 100m depth-rated housing was aimed down at the sea floor 20 cm from the bottom with a dive torch for illumination. Sample depths were 5, 15, 25, 40, 55, 70 and 85m, with four replicate transects collected at each depth following the respective depth contour at each site. Two transects were collected reef-on-left, and two reef-on-right starting 10m from the GPS location (Figure 1.1) with 10m between adjacent transects. The Utila south shore sites, Little Bight (LB) and Coral View (CV), reach a maximum depth of approximately 45m. The south-easterly site, Rocky Point (RP), levelled at approximately 63 m depth. The north shore sites, Raggedy Cay (RC) and The Maze (TMA), are located on a reef wall punctuated with shelves extending past 100m depth. These sites were sampled to 55m and 85m depth respectively as exploratory dives found no scleractinian cover at greater depths.

3.3.3 Data Analysis-

Videos were analysed following scleractinian visual identification resources (Atlantic Gulf Reef Rapid Assessment (AGRRA) Program, 2006; Humann and Deloach, 2013; CARMABI Caribbean research and management of biodiversity, 2015d, 2015b, 2015a, 2015c; Sheppard, 2015). The bottom cover under the transect tape was identified every 25cm. If the bottom cover beneath the tape was not a scleractinian coral, or *Millepora*, hereafter included with Scleractinia for brevity, then the area 10 cm either side of the tape was inspected. The nearest coral to the tape in this area was reported in preference of the benthic cover type under the transect, if present. This modification to the standard point intercept approach maximised the information collected for a study primarily concerned with scleractinian community composition, while also reducing the task-loading of rebreather divers by avoiding the need to use quadrats. This method does, however, produce an over-estimate in scleractinian percentage cover, especially when in low abundance. Identification was to species level when possible.

Percentage cover of broad benthic categories; Scleractinia, soft coral, macroalgae, coralline crustose algae, sponge, and sand, were calculated to detect the changing benthic composition with depth at all sites. The identified scleractinian data formed a species by transect matrix of counts. Transects recording no Scleractinia were removed to allow the generation of an ordination. Counts were used instead of percentages to prevent elevating the importance of rare species in the analysis. A lone observation of a species on a transect will appear more distinct to an ordination if recorded as comprising 100% of the community as opposed to an abundance count of 1.

Non-metric Multi-Dimensional Scaling (NMDS) (Minchin, 1987) was used to generate

an ordination with the packages Vegan and MASS (Venables and Ripley, 2002; Jari *et al.*, 2009) in the programming language R (R-Core-Team, 2013). All results are reported as per the software output. All transects were plotted based on Bray-Curtis dissimilarities generated with scleractinian species data to visualise patterns between sites and depths.

To detect potential boundaries between shallow and mesophotic scleractinian assemblages, multiple Principal Coordinate Analyses (PCoA) were conducted using different levels of taxonomic resolution: species, genus and family. While NMDS provides a robust start point for visualisation, PCoA was used to allow further analyses conducted in Euclidean space. The data were Hellinger transformed to allow for the use of a method based in Euclidean space, standardising observations of each species by its total abundance across all transects (Legendre and Gallagher, 2001). Conceptually, species are plotted as points in as many dimensions as there were transects. Closely plotted species have more similar patterns of occurrence. The Euclidean distances between points can be considered as measures of dissimilarity. Points were assigned to an optimal number of groups, indicating distinct species assemblages. Communities observed on transects were statistically compared to these assemblages to see whether depth and site segregate communities spatially. The depth profiles of taxa were plotted against groups to illustrate the allocation of generalist and specialist taxa with respect to depth.

K-means clustering (Hartigan and Wong, 1979) was used to generate clusters to overlay on the PCoA. The optimal number of clusters was determined by trialling multiple potential clustering solutions and selecting the number of clusters which maximised the Calinski criterion (Calinski and Harabasz, 1974). Clusters returned were tested statistically using a multi-response permutation procedure with 999 iterations (Biondini, Mielke and

Beryy, 1988; Jari *et al.*, 2009). Dufrene-Legendre indicator species analyses (Dufrene and Legendre, 1997) were conducted using the R package labdsv (Roberts, 2016). The analysis was performed on the transect data to determine which depths at which sites revealed patterns of co-occurrence in these clusters. Maximum indicator values occur when all Scleractinia observed on a transect have been previously assigned to a single cluster, which we interpret as an assemblage. The depth profiles of taxa were then compared to cluster occupancy to determine whether the structuring of reef communities is driven by depth-generalists or depth-specialists.

To compare other methods of detecting reef zonation, the family Agariciidae was analysed in isolation as a potential indicator taxon. Species and genera richness were recorded across all sites and Shannon's diversity indices were calculated for depths, trend lines were fitted. The choice was limited to considering the use of a linear, logarithmic, exponential or second order polynomial fit to prevent over-fitting. The equation which maximised R^2 was selected. This equation was differentiated and the result plotted to reveal rates of change with increasing depth.

3.4 Results:

Patterns of benthic cover with depth appear dependent on site (Figure 3.1). At Coral View increasing depth led to a decrease in scleractinian cover and macro-algae. The same applied at The Maze with the addition of a distinct lag between the reduction of algae behind the reduction in scleractinians and an increase in octocoral cover with increasing depth. At Little Bight live benthic cover remained constant in all categories except for a belt of increased

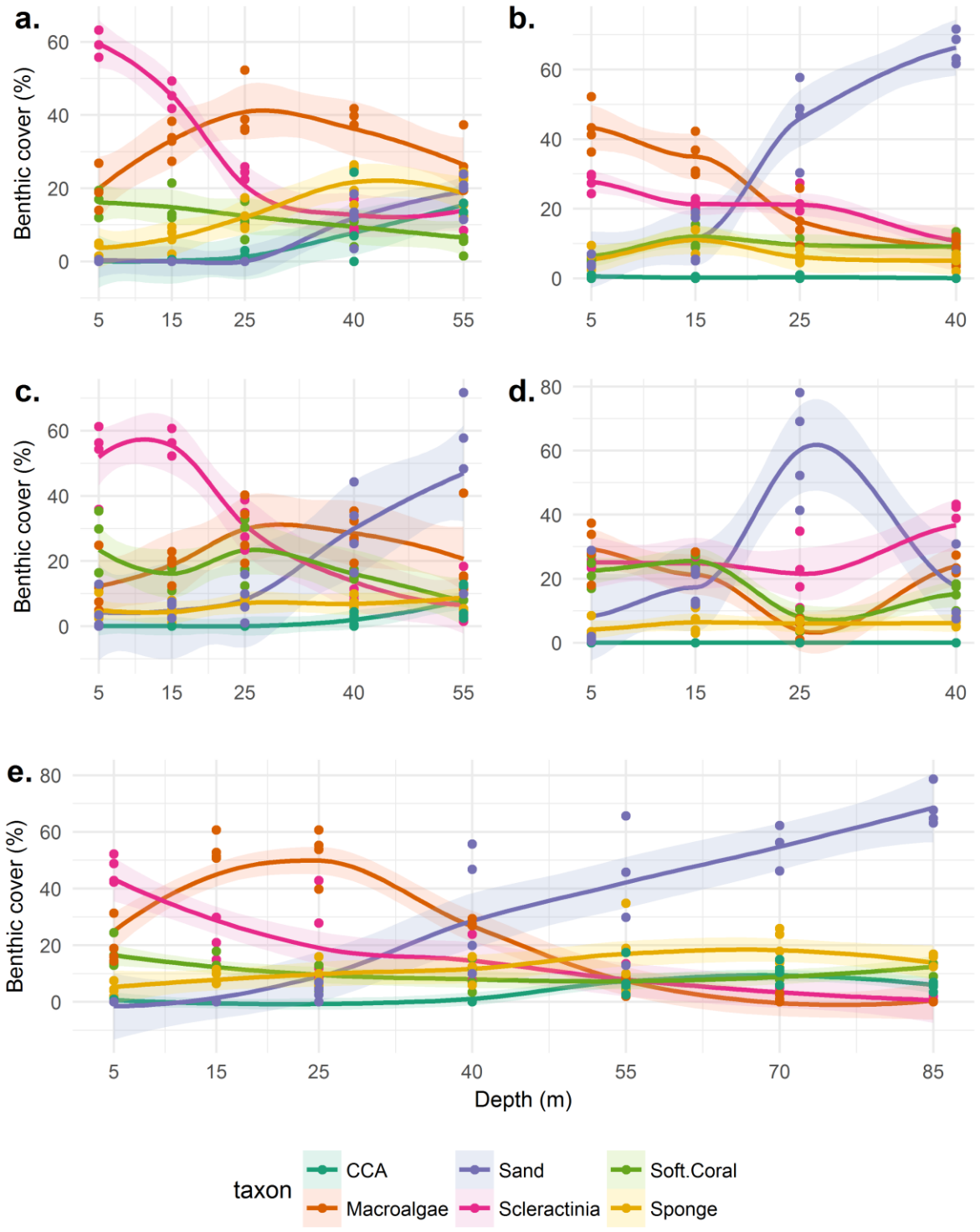


Figure 3.1 Benthic percentage cover by category and depth at each sampling site

Each site was sampled with four replicate transects at each depth except 5m at RC which had three. Trends are plotted with a loess line and their 95% confidence intervals. CCA= Coralline Crustose Algae. Panels represent different sites: (a) RC, (b) CV, (c) RP, (d) LB, (e) TMA.

sand cover at 25m which breaks the reef structure. Raggedy Cay and Rocky Point had persistently high macro-algal cover while octocorals and scleractinians both decreased with depth. All sites showed an increase in sand with increasing depth indicating a reef structure which becomes increasingly fragmented.

In all, 41 scleractinian species were documented (Supplementary 3.1). A non-metric fit to a Shepard plot (Supplementary 3.3) returned an $R^2 = 0.976$, and the stress of the NMDS plot=0.155 2 s.f. suggests an informative amount of variation is presented in figure 3.2. Variation along the first NMDS axis appears to correlate with increasing depth, with depth bands appearing clustered (Figure 3.2). The increased spread of points at the deepest sampling points is possibly an artefact of low counts. There are hints of varying rates of community change with depth between sites. For example, the 15m Rocky Point transects group with 5m transects for other sites, and three of the 25m Rocky Point transects appear to sit within a cluster of 15m transects from other sites. There appears to be no evidence for a clear two clusters marking shallow and mesophotic species.

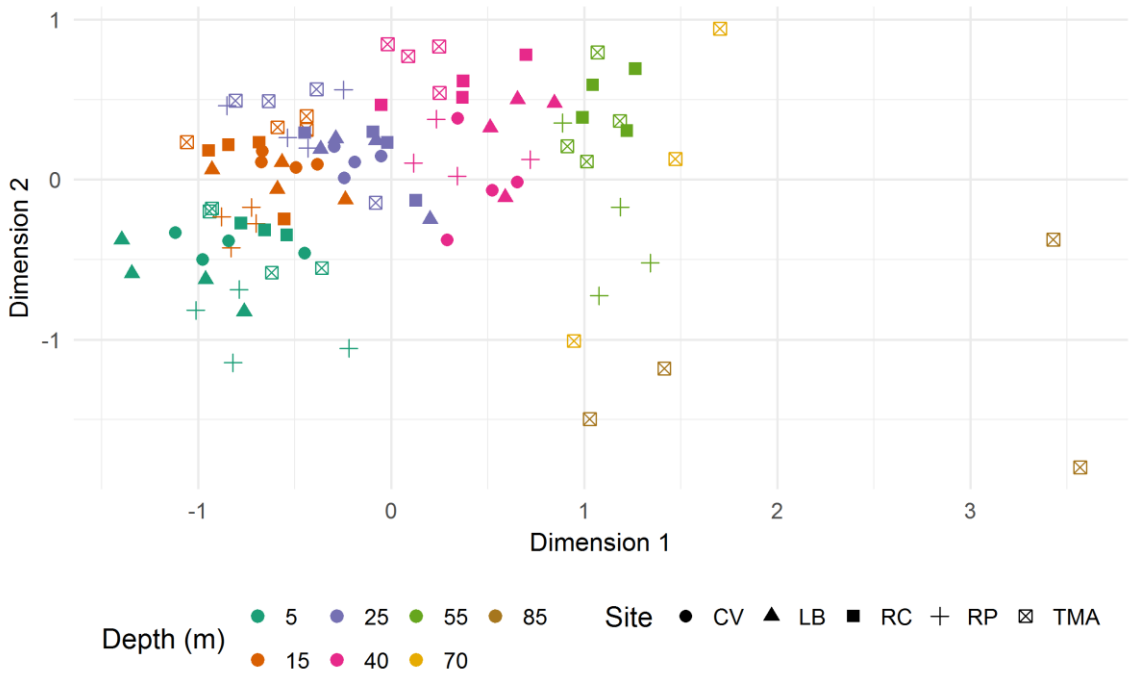


Figure 3.2 Transects discriminated by observed Scleractinia communities

NMDS plot of transects based on Bray-Curtis dissimilarities from Scleractinia species counts. Symbols denote site and colours depths.

To further explore the structure of scleractinian communities PCoAs were generated. For species- and genera-level analyses the Calinski criterion (CC) increased monotonically, with the highest value supporting two clusters of taxa with values of 6.6 and 5.0, respectively (Supplementary 3.4a,b). A multi-response permutation procedure testing for statistical significance of clusters returned *P*= 0.006 and 0.042 for two clusters at species and genus level, respectively. Family-level analyses revealed an erratic relationship between cluster number and CC presenting two peaks (Supplementary 3.4c). Seven clusters maximised the Calinski criterion (CC= 5.4, *P*= 0.881). Dufrene-Legendre indicator species analyses determined which transects were representative of defined assemblages of taxa. Transects significantly associated with clusters are displayed in Supplementary 3.2 along

with *P*-values. Figure 3.3 presents the data visually, picking out the locations where the species assemblages occur.

Figure 3.3a indicates that the assemblages detected segregate by depth, when data are analysed at the species level. There are significant shallow and deep assemblages. In the middle exists a transitional zone (white) equally ascribed to either assemblage. Note this is not a statistically supported group in its own right. In hashed colours are transects containing a single assemblage when $P > 0.05$. More specifically there appears to be a differing rate of transition with increasing depth between sites. Our results suggest the south shore sites Coral View and Little Bight show distinct deep assemblages at 40m. It is not until 55m the north shore sites Raggedy Cay and The Maze show similar significant deep assemblages. In contrast, Rocky Point shows a statistically significant shallow grouping at 15m, deeper than at other sites, and doesn't consistently form patches of reef with the deep assemblage. The lack of significance of the signal at The Maze at 70 m and 85 m may be an artefact of low cover reducing power of the analysis.

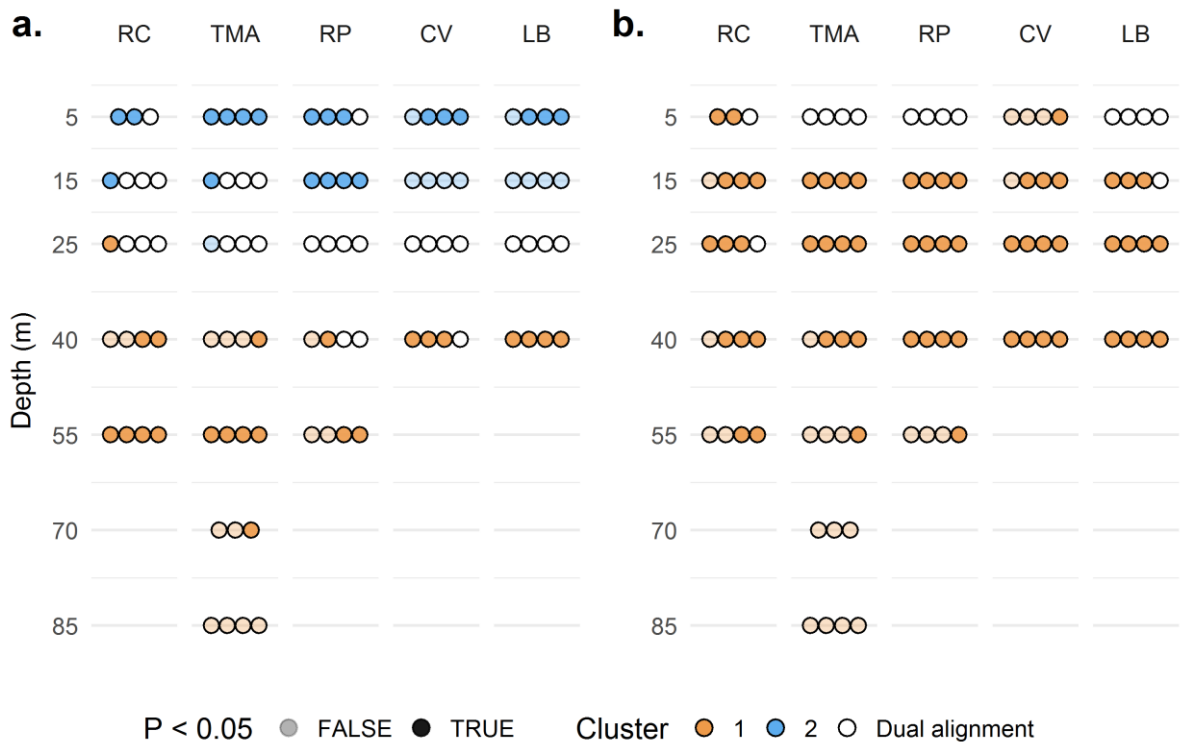


Figure 3.3 A map of assemblages at transect locations

Circles have been coloured based on cluster assignment of a transect at the indicated site and depth, with four transects (circles) per depth at each site. The number of the cluster relates to the analyses explicitly presented in supplementary 3.2. Dual alignment was assigned to any transect for which R provided an indicator value for more than one cluster. There are 4 transects visualised per depth at a site. One 70m transect at TMA was removed as it captured no Scleractinia. The video file for one 5m transect at RC was corrupted. (a) Analysis based on species level ID. (b) Analysis based on genus level ID.

The genus-level analysis was able to detect two statistically significant clusters, yet when mapped onto locations (Figure 3.3b) the only signal visible is a single assemblage with a broad depth range. This assemblage had a large spread across all sites, losing statistical significance past 55m depth. 5m appeared to represent a transitional zone. The family-level analysis maps were uninformative, possibly resulting from the large number of clusters lacking statistical significance.

Figure 3.4 reveals the two different species assemblages determined by the above analysis across all sites. It appears the shallow assemblage is characterised by shallow-water

specialists such as *Acropora cervicornis* and members of the genus *Orbicella*. The deep-water assemblage consists primarily of depth-generalist species. *Madracis pharensis* is the only species in the deep cluster not detected shallower than 40m. *Agaricia grahamae, Agaricia undata, Madracis formosa* and *Madracis senaria* are the next deepest, with ranges starting at 25m. The transition zone depicted in Figure 3.3a was characterised by a loss of shallow-water specialists and increasing dominance in depth-generalists. This suggestion is supported by Figure 3.3b only mapping a single assemblage with a broad depth range, only giving way to a transitional signal at 5m. This is what would be expected if the statistically significant deep cluster reflected all depth generalists, a signal which becomes diluted at 5m by the appearance of shallow specialists. A similar plot of depth distributions for genus-level analysis by cluster can be found in the supplementary material (Supplementary 3.5). The general pattern described with a shallow-specialist and depth-generalist community appears to hold true for genus level distributions (Supplementary 3.5). Exceptions are the genus *Agaricia* aligning with the shallow assemblage and *Undaria* and *Colpophyllia* with the depth-generalist assemblage. The lack of statistical significance and a large number of mono-family clusters removes the ability to discuss patterns in the family-level analysis.

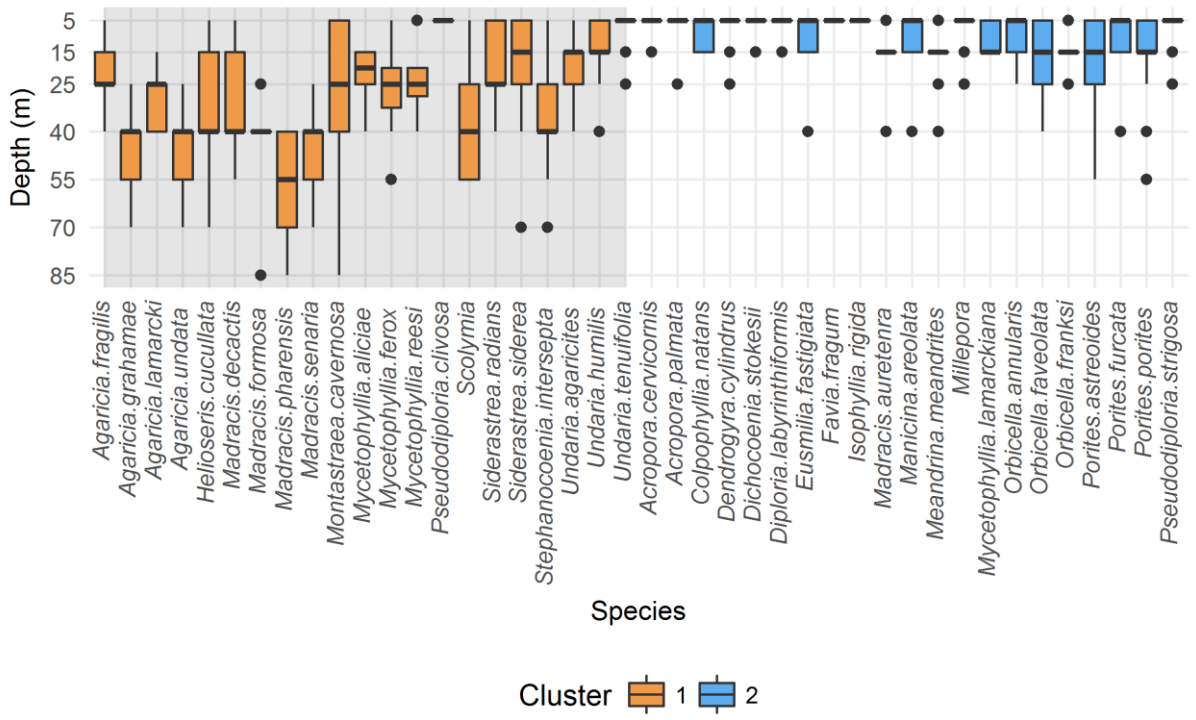


Figure 3.4 Dataset average depth ranges of Scleractinia species

Box plots of species depth ranges generated from pooled data of all sites. Box plots are coloured internally to reveal the assemblage they belong to. All box plots on the darker background belong to Cluster 1, the lighter background denotes cluster 2. Lines extend 1.5x the interquartile range or to the last observation. Points are outliers beyond this limit.

Following these results, *a posteriori* analysis of agariciid corals as a putative indicator taxon was performed. The Agariciidae exhibit a large depth range as a family and were relatively common across all depths. The methodology followed was the same as applied to the species-genus-family analyses. Five clusters appeared optimal, CC= 3.1, *P*= 0.566, but again there were two peaks. As *Helioseris cucullata* and *Agaricia fragilis* formed mono-specific clusters these were removed and the analysis re-run. This was in case a large number of clusters hid a signal, as may have been the case with the family- level analysis. Three clusters were found with CC= 4 at a single peak and *P*= 0.067. Defrene-Legendre indicator species analysis failed to return any transect aligning with a single cluster of

statistical significance. The three clusters were *Undaria tenuifolia* and *Undaria humilis*, *Agaricia lamarcki* and *Undaria agaricites*, *Agaricia grahamae* and *Agaricia undata*.

To explore whether the patterns in Figure 3.3 reflect a loss of shallow specialists and the maintenance of a depth generalist community, rather than the gain of depth specialists, a non-parametric Mann-Whitney U test was performed. The depth ranges of species in both clusters were compared. Outliers were defined as the few observations falling further than 1.5 times the interquartile range from the median depth value of the species; these were excluded from the analysis. The results (W= 68, P < 0.0001, with 21 species in each group) show there is a significant difference. The mean depth range of the depth generalist cluster was 35.2m ±20.4m while for shallow specialists, it was 8.3m ± 13.4m.

Figure 3.5 compares the trends detected using different metrics at differing levels of taxonomic resolution. Figure 3.5a presents scleractinian species richness within assemblages plotted with depth. Total species richness of scleractinians declined steadily with depth yet the pattern does not hold true for each assemblage. The shallow assemblage showed a rapid decline with depth while the 'deep' assemblage exhibits a low humped relationship. Shannon's diversity drops at an increasing rate with depth, with no obvious feature coinciding with changes in community detected by the ordination approach.

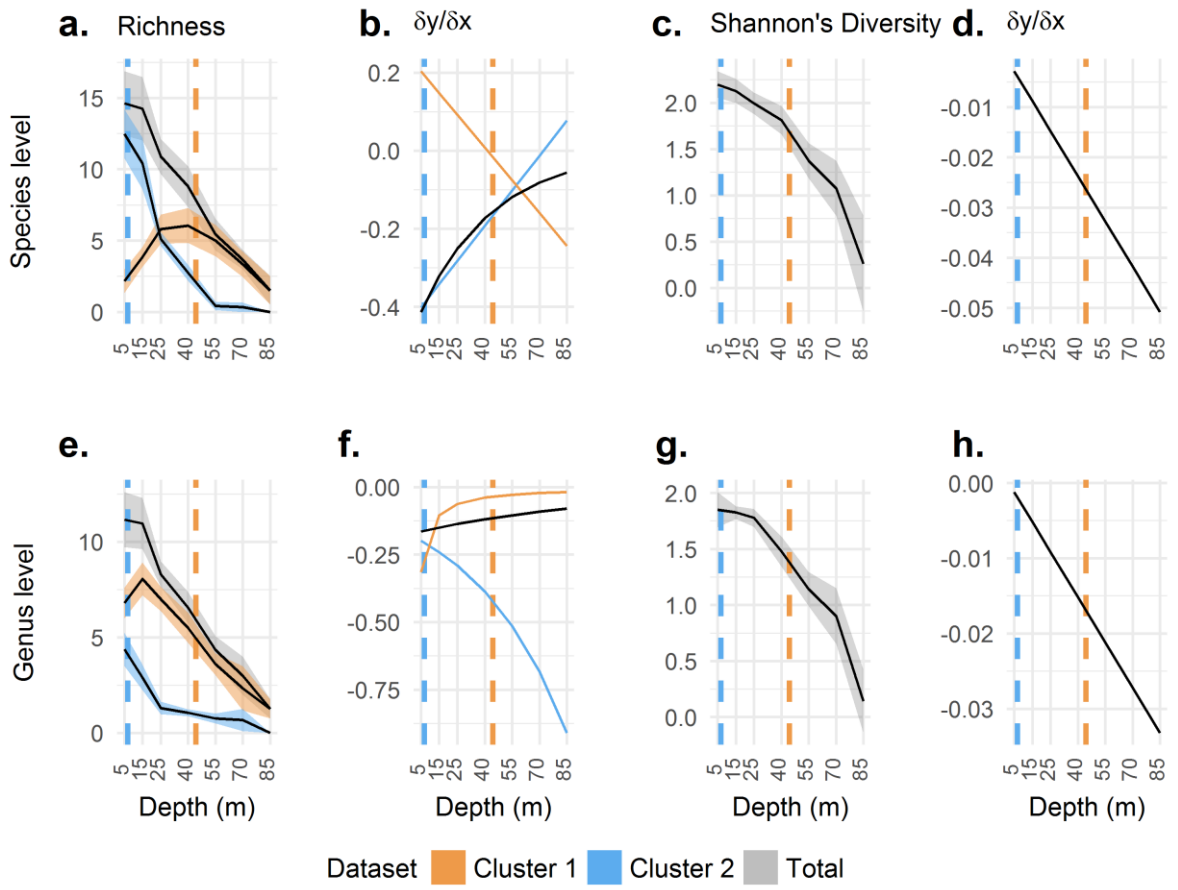


Figure 3.5 Biodiversity metrics and their relationships with depth

Comparison of different metrics for detecting changes in reef structure. Data are pooled from all sites. Shaded areas are one standard error from the mean (the denominator of the equation was the number of sites sampled at a given depth). The colour coding of the shading represents assemblages consistent with previous plots. Dashed lines are the dataset average boundaries for the deepest transects statistically significantly aligning as shallow and the shallowest transects similarly aligning as deep. Shannon's diversity, richness, and their respective rates of change are plotted for comparison between the four columns. The top row plots use species level data, the second row uses genus level data. Note other metrics show no obvious features to demark a switch in community composition.

An exponential trend line for the total richness data was appropriate with an R^2 of 0.6575 at species level and 0.7408 at genus level. The differentials were $dy/dx=-0.46823e^{-0.025x}$ for species and $dy/dx=-0.17172e^{-0.009x}$ for genus. The estimated rate of change in richness revealed a faster loss of taxa in the shallows than at depth; this relationship was more marked for species than genera.

For comparison, rates of change in the assemblages defined by *K*-means clustering were calculated from trend lines ($Y = -0.0028X^2 + 0.2323X + 1.1877$ and $Y = 9.5235e^{-0.019X}$), with R^2 of 0.354 and 0.5659 for cluster one at species and genus level respectively. Differentials were $dy/dx=-0.0056X +0.2323$ and $dy/dx=-0.1809465e^{-0.019X}$. For cluster two as above, the trend lines ($Y = 0.003X^2 - 0.4323X + 14.998$ and $Y = -1.576\ln(X) + 6.8803$) had R^2 of 0.7405 and 0.5763 yielding $dy/dx=0.006X -0.4323$ and $dy/dx=-1.576/X$ at species and genus level.

A second order polynomial was fitted to the Shannon's diversity data with an R^2 of 0.67 at species level and 0.7152 at genus level, $dy/dx=-0.0006X + 0.0002$ for species and $dy/dx=-0.0004X + 0.0008$ for genus. The rate of change in Shannon's biodiversity became increasingly negative with depth. Interestingly the patterns in all metrics represented in Figure 3.5 are largely preserved when taxonomic resolution is reduced to the level of the genus, despite the difficulties of mapping Figure 3.3b.

3.5 Discussion:

The analysis presented here generates a representation of vertical changes in scleractinian community assemblage, only informed by patterns of co-occurrence and abundance, yet able to recognise distinct MCE and shallow communities regardless of depth. For the species-level PCoA analysis the depths of occurrence for the two assemblages and transition region loosely coincide with currently held boundaries (Bongaerts *et al.*, 2010). However, on transects for Rocky Point and Raggedy Cay, communities identified as shallow or MCE were on one occasion each separated by 10m of water vertically, demonstrating remarkable changes in community structure with small changes in depth (Figure 3.4). For other patches

of reef, the same difference in community was only detected over 50m vertically at Rocky Point and The Maze (Figure 3.3a). This approach allows the reef community to inform on the type of reef viewed, as opposed to following a set depth limit. These observations highlight the importance of formalising a mobile definition for MCEs capable of incorporating unusual reefs (Muir and Wallace, 2015).

The changes in scleractinian community structure indicate a loss of shallow specialists and rising dominance of depth-generalists with increasing depth. We see no evidence here for a MCE specialist assemblage based on a definition of typically occurring below 30m, as we record only one species range beginning deeper than this limit. Depth-generalists may be excluded by competitive influences in the shallows, either mediated by photosynthesis and sensitivity to light (Costa *et al.*, 2015), or resistance to water movement (Dollar, 1982). This is supported by the depth ranges of species assigned to each assemblage (Figure 3.4), the lack of new species in the species list of our study to 85m in comparison to a study surveying to only 18m a few years prior (Scaps and Saunders, 2011) and the differing relationships between the two identified assemblages with respect to species richness and depth (Figure 3.5a), as well as more general patterns across taxa studied elsewhere (Semmler, Hoot and Reaka, 2016). This finding is in line with established ecological theory regarding the trade off between resource-availability, species richness, and stress-tolerance (Yang *et al.*, 2015). Shallow specialists dominate under high resource (light) availability, with a monotonic decrease in richness as resources decline (Figure 3.5a), while mesophotic taxa capable of tolerating a varied light environment demonstrate a humped richness relationship along the depth gradient.

Unfortunately, this study is unable to provide the environmental data required to fully explain the patterns in community observed. Our study recorded 41 species of scleractinians in comparison to 46 in an earlier study to a maximum depth of 18 m (Scaps and Saunders, 2011). Scaps & Saunders reported two *Scolymia* species; in this study *Scolymia* was identified to genus level. All other species present in the earlier study but not reported here were classified as 'uncommon' or 'rare'. It is possible the lack of these uncommon species is a result of differing methodology. Scaps & Saunders employed roving diver surveys to identify as many species as possible, while we used transects to standardise survey areas for quantitative analysis.

Patterns in benthic coverage with depth were variable between sites, likely affected by site-specific histories of anthropogenic impact, prevailing environmental conditions, and topography. Though there are no data to support these expectations at Utila, such factors may mask generalised patterns associated with increasing depth (Bare *et al.*, 2010; Andradi-Brown *et al.*, 2016). Sponge cover remained largely constant with depth at all sites except Raggedy Cay where it increased with depth (Figure 3.1). All sites share an increasingly fragmented reef structure with increasing depth, represented by increasing sand cover (Figure 3.1). Despite the potential for systematic over-estimates of scleractinian cover, the patterns across the depth gradient presented here agrees with an earlier transect-based survey in 2014 to a maximum depth of 40m (Andradi-Brown, Gress, *et al.*, 2016), though our estimates of scleractinian cover are substantially higher. This was likely caused by preferential analysis of scleractinians if they appeared adjacent to the transect. Though mature scleractinian reef structure ended at 55m for Utila, and scleractinians were not seen below 85m (all colonies 70 – 85m were small encrusting patches), other Caribbean reefs have dense *Agaricia* beds at 85m (Hoeksema, Bongaerts and Baldwin, 2016) and Scleractinia

recorded as deep as 107m (Busby, Bright and Pruna, 1966). Our observations while conducting surveys around Utila suggest some MCEs may have suffered high scleractinian mortality at their deepest extents. Mature agariciid reefs may have once existed to at least 70m deep and have since become increasingly shallow (Supplementary 3.6).

We need to improve our understanding of mesophotic species to accurately predict future reef change. The shallow bias of coral biology (Menza, Kendall and Hile, 2008) may have missed a major feature of community structure in coral reefs. Our results suggest, for Utila, Scleractinia should be broadly classed as shallow specialists and depth generalists. This pattern was recently revealed to apply more generally across taxa as well (Semmler, Hoot and Reaka, 2016). Past physiological data also hints at a further subdivision within the depth-generalists of those utilising light energy (phyto-generalists) such as *Montastraea cavernosa*, able to promiscuously switch symbionts (Lesser *et al.*, 2010; Bongaerts, Carmichael, *et al.*, 2015), and species such as *Agaricia lamarcki* (trophic-generalists) possibly able to rely on heterotrophic subsidy to offset light levels (Crandall *et al.*, 2016). Teasing apart reef community structure and future change will require an understanding of the differing responses of these three groups of coral; shallow, phyto-generalists, and trophic-generalists, to a variety of pressures, and the competitive influences between them. Coral reefs may not be committed to a gloomy future if it is possible that the relative abundance of these three groups of coral will shuffle. To assess this, and move towards explaining the ecological structure of coral reefs across their whole vertical range, more physiological work is required. Determining how a broad suite of taxa satisfy their calorific demands, their relationship with zooxanthellae, growth rates and competitive abilities will lead to explanations of ecology rather than documentation of patterns. This could not only improve our understanding of coral reefs from an ecological view, but also their conservation.

Understanding the drivers of coral distribution may help improve the choices of species targeted for nursery schemes by matching physiology to future conditions. It may also help us in identifying which species are particularly in need of future protection because of limited plasticity.

From the perspective of the DRRH, our results raise two questions of interest. Firstly, how quickly does the decline in shallow assemblage richness occur with depth at a location? Answering this will reveal whether depth can afford protection for shallow species (Semmler, Hoot and Reaka, 2016) . When comparing between sites, sites where the decline in shallow assemblage richness and the potential competitive influences with depth occurs more slowly are more likely to satisfy the requirements of the DRRH. Secondly, the abundance of the depth-generalist assemblage peaks after the rapid decline of the shallow assemblage, potentially implying competitive interactions. Understanding whether the generalist species can survive in the shallows in the absence of competition with shallow specialists will reveal whether the DRRH could work, but may result in a phase shift on the reef from one scleractinian assemblage to another.

We chose to focus on scleractinians when considering possible definitions of MCEs for several reasons, though we accept patterns in other taxa may vary and are of interest. The advantages with benthic organisms such as scleractinians include that they are static, attract other associated fauna, and they may offer indications of change under varying environments. There is a lag time between changes in environmental conditions and changing reef structure dictated by rates of recruitment, growth and death of corals in now unfavourable habitats. Rather than a disadvantage we believe this offers opportunity for the detection of natural experiments (Diamond, 1983). When the environment changes, the

time window before the community shifts in response provides an opportunity to monitor the effects of unfavourable conditions on these reefs, revealing potential future trends. These opportunities may be missed when definitions are based on mobile taxa.

Despite this, a major challenge of a definition based on scleractinian distribution is the required analysis of species-level community structure. Not every researcher interested in MCEs necessarily has the time or knowledge to conduct a similar study simply to detect which patches of reef are MCEs. Unfortunately, as demonstrated here (Figure 3.3), reducing the level of taxonomic accuracy used only reduces the ability of the analysis to statistically detect patterns in reef community structure. This is therefore not an option for making the analysis tractable for non-coral researchers. We attempted to select an indicator taxon, the family Agariciidae, to reduce the number of species a non-specialist would need to be able to identify. This failed to resolve any transects as belonging to a distinct assemblage as the three clusters resolved overlap in their distribution. The member species of this family are also similar in their morphology adding difficulty for the non-specialist.

Before wider use, this method would need to be applied more broadly through the Caribbean for validation, and the different pool of species present in the Indo-Pacific poses a problem for direct comparison. It may be that patterns in community structure remain the same in the Indo-Pacific despite taxonomic composition changes between the two regions because of biogeography. However, the degree of taxonomic resolution possible in field studies in the Indo-Pacific is generally lower than in Caribbean studies, possibly limiting the power of analysis in the former. We would encourage other researchers to apply this method to their own data to see if the patterns hold true. Other methods for detecting vertical reef structure also have flaws (Table 3.1) and utility must be balanced with accuracy.

To this end, Figure 3.5 shows how other biodiversity metrics capture the patterns of the ordination analysis. Reducing taxonomic resolution was explored to improve the accessibility of a new definition. Analysis at the level of the genus may be subject to lower error and requires a less detailed knowledge of the Scleractinia. In the Indo-Pacific with greater diversity in scleractinian genera, genus-level identification may be sufficient to detect patterns.

Richness and rates of change as opposed to biodiversity and ordination techniques were explored as these do not require abundance data. This allows quicker sampling protocols such as roving diver surveys rather than quantitative transects. Species richness has been widely used as it is less labour intensive and does not require a strictly quantitative sampling methodology provided effort is standardised. We show here, however, that it is possible to miss structure if species identities, and so community turnover, are not taken into account (Figure 3.5). There is no evidence in Figure 3.5 for peaks in richness or biodiversity, of the total community, which may be expected under an intermediate disturbance hypothesis (Wilkinson, 1999) or indicative of zones of faunal transition. Biodiversity indices, therefore, may similarly fail to detect changes in community structure while requiring the same data to be collected as for the ordination-based approach.

Rates of change in richness and Shannon's diversity failed to show any obvious features coinciding with community boundaries detected by ordination approaches. Rates of change fail to equal 0 or peak at any boundary other than for species cluster 1 with the mesophotic boundary. This relationship, however, is based on the worst fitting model of change and requires the ordination approach to identify the cluster. Interestingly, however, these approaches revealed a similar relationship with depth when taxonomic resolution was

reduced. It may be possible to recreate the groupings returned here by *K*-means clustering by identifying two groups of scleractinia with differing depth ranges using Figure 3.4 with the statistical analysis of depth ranges for support. This would allow for a method approximating the ordination without the need for abundance data.

3.5.1 Conclusions-

It is clear that traditional biodiversity measures and absolute depth limits are insufficient for defining vertical biological community zonation across shallow to mesophotic reef gradients. We therefore present a methodology for detecting this zonation and reveal changes in the rate of transition with depth between reefs located geographically within 200m of each other. We accept further discussion is needed to improve on the amount of data required to accurately define these ecosystems for those without taxonomic expertise. It may be possible to generate workable definitions without the need for abundance data if rapid assessment is required; however, doing so removes the ability to detect transitional zones. Despite this, if the main questions of interest are 'how deep do shallow specialists descend?' to answer questions regarding the DRRH, or 'where do MCEs start' for zone-specific work, this can be achieved by ranking species by depth range to identify the lower depth boundary of the shallow cluster. Other researchers should also investigate other potential definitions to inform debate.

We found evidence of formerly extensive MCEs at depths of 70m on the north shore of Utila, and that reefs with similar species compositions are now found at approximately 55m depth. These MCEs appear to be populated by depth generalists which may rely either on heterotrophic subsidy or the manipulation of photosynthetic adaptations to accommodate

their depth range. The transition between reef types is characterised by the loss of shallow-specialists and subsequent increase depth-generalist dominance. Further work on the physiology of Scleractinia on MCEs will help to determine the mechanisms behind such ecological patterns.

3.6 Acknowledgements:

The Authors would like to thank the Royal Geographical Society with IBG (Ralph-Brown Award), the Zoological Society of London (Erasmus Darwin Barlow Expeditions fund), the Oxford University Expeditions Council, the University of Oxford John-Fell fund, the Natural Environment Research Council, Merton College Oxford and Operation Wallacea for funding. They also thank Gradient Scientific and Technical Diving and the Thinking Deep expedition team 2015 for dive support. A final thanks to the staff of Operation Wallacea at the Coral View research station and the Bay Islands Conservation Association for logistical support. DAAB was funded by a Fisheries Society of the British Isles (FSBI) PhD Studentship. We wish to thank two anonymous reviewers who provided useful advice that improved this manuscript.

3.7 Authors contributions:

JHL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing

DAAB: funding acquisition, Investigation, Writing – review & editing

ADR: Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing

3.8 Supplementary information:

Supplementary 3.1 Study Scleractinia species list

All Identified Scleractinia around the island of Utila from 5 m to 85 m depth during the survey period. The taxonomic hierarchy here defines the species, genus and family level analyses conducted as part of the study. Millepora was included as a group in all analyses as a common hermatype, Scolymia spp. was included as a single taxon in the species level analysis. The total number of species recorded was 41 across 22 genera and 8 families.

Family	Genus	Species	Family	Genus	Species
Acroporidae	Acropora	cervicornis	Faviidae	Montastraea	cavernosa
		palmata		Orbicella	annularis
Agariciidae	Agaricia	fragilis			faveolata
		grahamae			franksi
		lamarcki	Meandrinidae	Dendrogyra	cylindrus
		undata		Dichocoenia	stokesii
	Helioseris	cucullata		Eusmilia	fastigiata
	Undaria	agaricites		Meandrina	meandrites
		humilis	Mussidae	Scolymia	Spp.
		Tenuifolia		Isophyllia	rigida
Astrocoeniidae	Madracis	auretenra		Mycetophyllia	aliciae
		decactis			ferox
		Formosa			lamarckiana
		pharensis			reesi
		senaria	Poritidae	Porites	astreoides
	Stephanocoenia	intersepta			furcata
Faviidae	Colpophyllia	natans			porites
	Diploria	labyrinthiformis	Siderastreidae	Siderastrea	radians
	Favia	fragum			siderea
	Manicina	areolata			

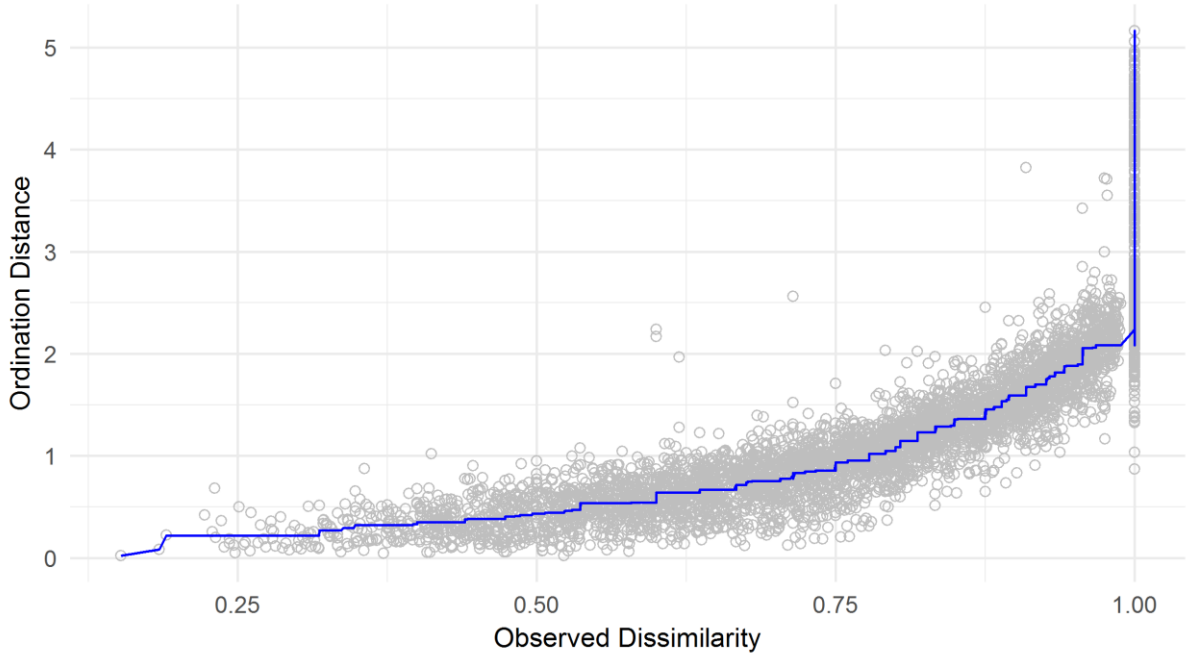
Supplementary 3.2 Locations significantly associated with scleractinian assemblages

Transect IDs are listed with the P values returned from Defrene-Lengendre indicator species analysis performed on three levels of taxonomic resolution. Only P values <0.05 are reported. Underlined numbers are the cluster identity the transect aligns with.

Transect	Species	Genus	Family	Transect	Species	Genus	Family
CV.5m.1		1 0.005		RC.55m.1	1 0.006		4 0.047
CV.5m.2	2 0.025			RC.55m.2	1 0.005	1 0.044	
CV.5m.3	2 0.032			RC.55m.3	1 0.005		
CV.5m.4	2 0.002			RC.55m.4	1 0.004	1 0.031	
CV.15m.2		1 0.029		RP.5m.1	2 0.001		
CV.15m.3		1 0.015		RP.5m.2	2 0.001		
CV.15m.4		1 0.001		RP.5m.4	2 0.003		
CV.25m.1		1 0.007		RP.15m.1	2 0.003	1 0.008	
CV.25m.2		1 0.007		RP.15m.2	2 0.002	1 0.012	
CV.25m.3		1 0.001		RP.15m.3	2 0.014	1 0.003	
CV.25m.4		1 0.002		RP.15m.4	2 0.007	1 0.034	
CV.40m.1	1 0.047	1 0.005		RP.25m.1		1 0.001	
CV.40m.2	1 0.023	1 0.013		RP.25m.2		1 0.001	
CV.40m.3		1 0.027		RP.25m.3		1 0.006	
CV.40m.4	1 0.021	1 0.032		RP.25m.4		1 0.001	
LB.5m.1	2 0.018			RP.40m.1		1 0.006	
LB.5m.3	2 0.014			RP.40m.2		1 0.001	3 0.023
LB.5m.4	2 0.016			RP.40m.3	1 0.001	1 0.001	
LB.15m.2		1 0.01		RP.40m.4		1 0.006	
LB.15m.3		1 0.025		RP.55m.1	1 0.001	1 0.003	
LB.15m.4		1 0.003		RP.55m.2	1 0.016		
LB.25m.1		1 0.005		RP.55m.3			3 0.05

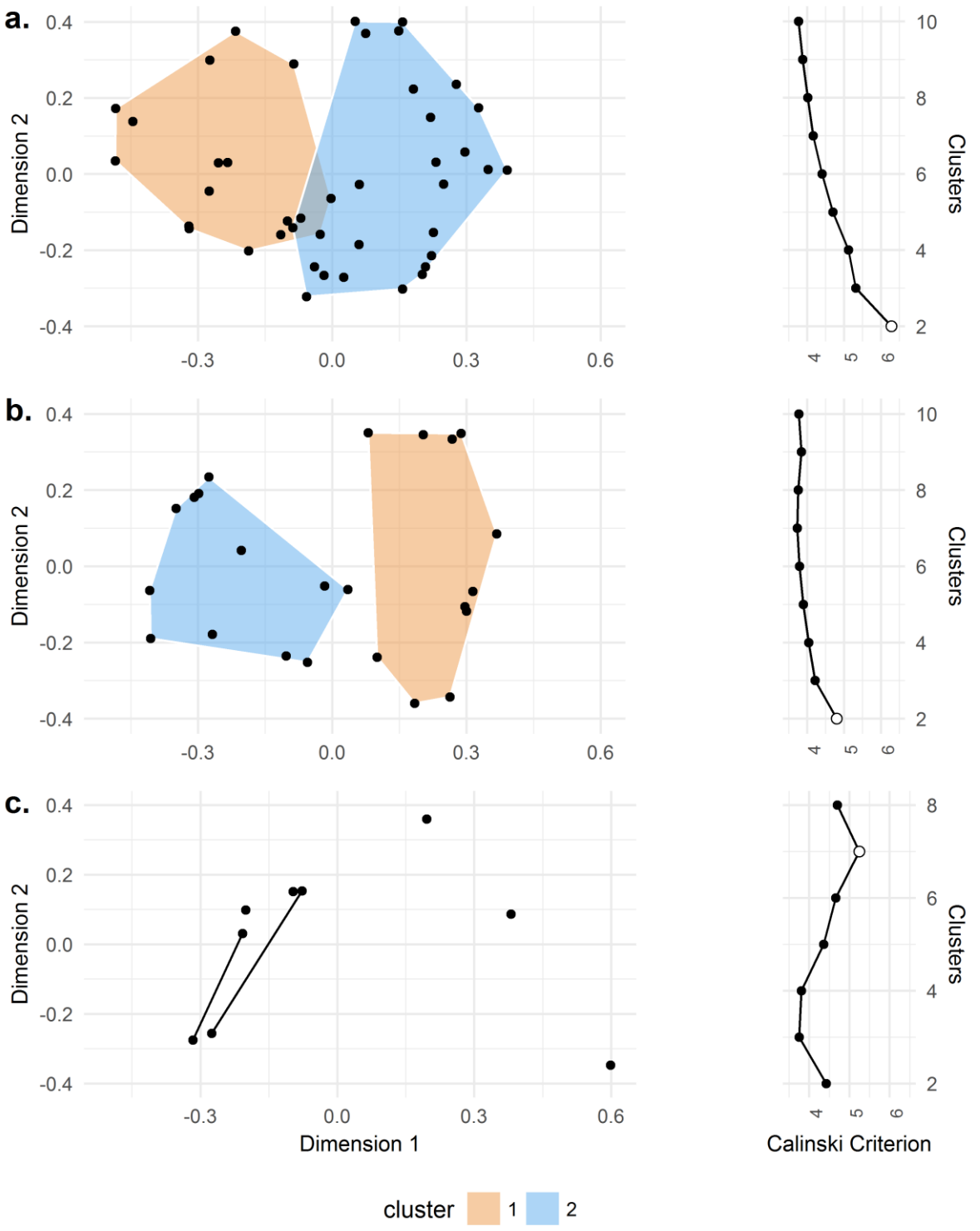
Supplementary 3.2 Continued

Transect	Species	Genus	Family	Transect	Species	Genus	Family
LB.25m.2		1 0.002		TMA.5m.1	2 0.001		
LB.25m.3		1 0.006		TMA.5m.2	2 0.002		
LB.25m.4		1 0.001	4 0.035	TMA.5m.3	2 0.03		
LB.40m.1	1 0.002	1 0.016	3 0.035	TMA.5m.4	2 0.001		
LB.40m.2	1 0.001	1 0.003		TMA.15m.1		1 0.002	
LB.40m.3	1 0.001	1 0.012		TMA.15m.2	2 0.049	1 0.013	
LB.40m.4	1 0.002	1 0.003		TMA.15m.3		1 0.005	
RC.5m.1		1 0.012		TMA.15m.4		1 0.003	
RC.5m.2	2 0.023			TMA.25m.1		1 0.001	
RC.5m.4	2 0.004	1 0.032		TMA.25m.2		1 0.007	
RC.15m.1		1 0.013		TMA.25m.3		1 0.033	
RC.15m.2	2 0.029		2 0.041	TMA.25m.4		1 0.012	
RC.15m.3		1 0.001		TMA.40m.1	1 0.004	1 0.001	
RC.15m.4		1 0.004		TMA.40m.3		1 0.042	
RC.25m.2		1 0.001		TMA.40m.4		1 0.033	
RC.25m.3	1 0.015	1 0.001		TMA.55m.1	1 0.004	1 0.044	
RC.25m.4		1 0.003		TMA.55m.2	1 0.013		
RC.40m.1	1 0.044	1 0.004	4 0.038	TMA.55m.3	1 0.015		
RC.40m.2		1 0.001	4 0.026	TMA.55m.4	1 0.025		
RC.40m.3		1 0.037	4 0.013	TMA.70m.2	1 0.009		
RC.40m.4	1 0.01		4 0.038				



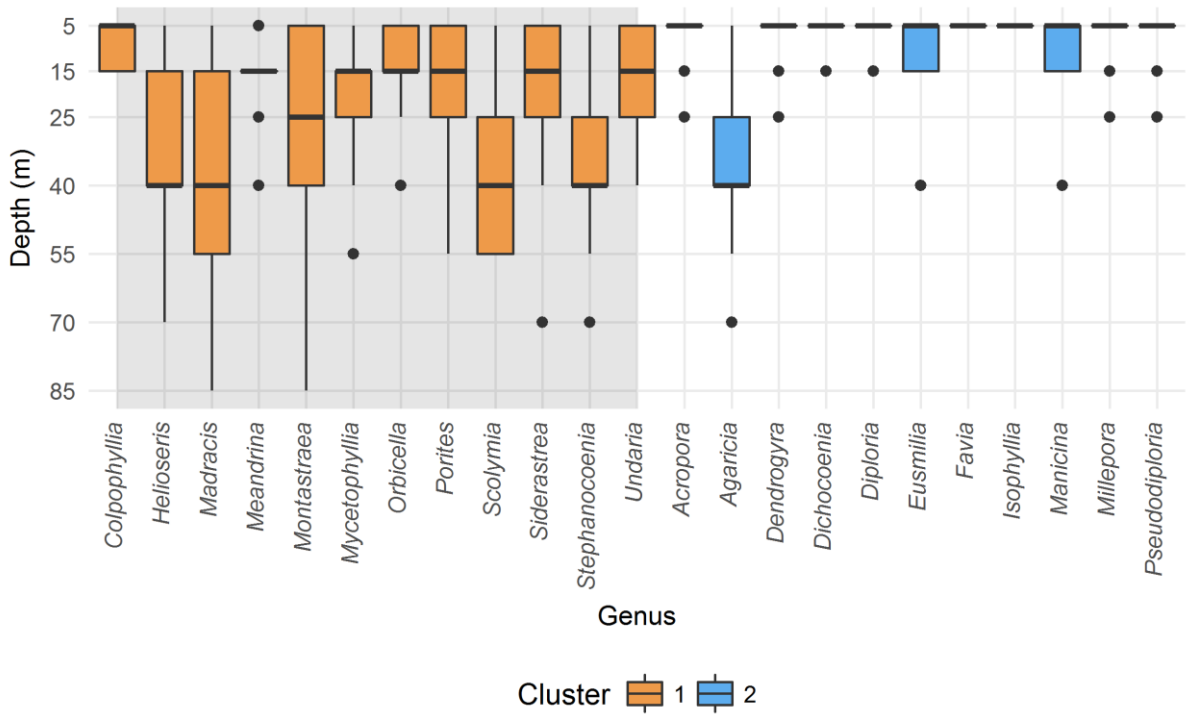
Supplementary 3.3 Shepard plot for Figure 3.2

Showing the distance between points in the ordination of Figure 3.2 and the correlation with observed dissimilarity between points. The ordination appears to faithfully reflect the dissimilarity between transects with a non-metric fit $R^2 = 0.976$.



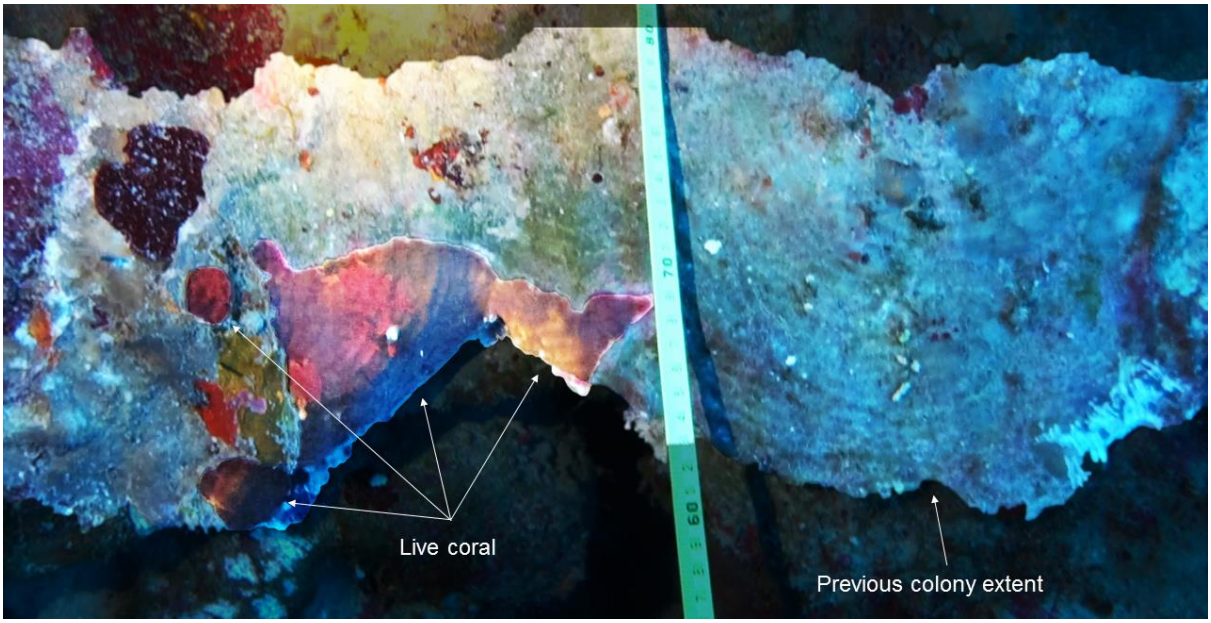
Supplementary 3.4 Scleractinia assemblage detection

The left pane shows a K means clustered Principal Co-ordinate analysis. Polygons enclose points within a cluster. The right pane shows the Calinski criterion for a different proposed number of clusters to be fitted to the data. The largest value was selected as the best choice of number of clusters, denoted by a hollow point. (a.) Analysis based on species level ID. (b.) Analysis based on genus level ID. (c.) Analysis based on family level ID.



Supplementary 3.5 Dataset average depth ranges of Scleractinia genera

Box plots of genera depth ranges with data pooled across sites, coloured by cluster identity. All box plots on the darker background belong to Cluster 1, the lighter background denotes cluster 2. Lines extend 1.5x the interquartile range or to the last observation. Points are outliers beyond this limit.



Supplementary 3.6 Evidence of past mature Agaricia colonies at 70 m depth at TMA

Live and inferred dead portions are highlighted. Old colony extent is evident from preserved corallite patterns in the substrate. The bare skeleton has not been heavily fouled.

Chapter Four

Depth Alone is an Inappropriate Proxy for Physiological Change in the Mesophotic Coral *Agaricia lamarcki*.

Jack H. Laverick, Tamara K. Green, Heidi L. Burdett, Jason Newton, Alex D. Rogers

4.1 Abstract:

The physiology of mesophotic Scleractinia is expected to vary with depth in response to environmental change. Previous research has documented trends in heterotrophy and photosynthesis with depth, but has not addressed between-site variation for a single species. Environmental differences between sites at a local scale and heterogeneous microhabitats, because of irradiance and food availability, are likely important factors when explaining the occurrence and physiology of Scleractinia. Here, 108 colonies of *Agaricia lamarcki* were sampled from two locations off the coast of Utila, Honduras, distributed evenly down the observed 50m depth range of the species. We found that depth alone was not sufficient to fully explain physiological variation. PAM fluorometry and stable isotope analyses revealed that trends in photochemical and heterotrophic activity with depth varied markedly between sites. Our isotope analyses do not support an obligate link between photosynthetic activity and heterotrophic subsidy with increasing depth. We found that *A. lamarcki* colonies at the bottom of the species depth range can be physiologically similar to those nearer the surface. As a potential explanation, we hypothesise that sites with high topographical complexity, and therefore varied microhabitats, may provide more physiological niches distributed across a larger depth range. Varied microhabitats with depth may reduce the dominance of depth as a physiological determinant. Thus, *A. lamarcki* may be able to 'avoid' changes in environment with depth, by instead existing in a subset of favourable niches.

4.2 Introduction:

Mesophotic coral ecosystems (MCEs) are zooxanthellate coral reefs widely considered to occur from between 30 - 40m to at least 150m depth (Puglise *et al.*, 2009; Bongaerts *et al.*, 2010; Kahng, Copus and Wagner, 2014; Baker, Puglise and Harris, 2016b). Deeper reefs are typically darker, colder and further offshore (Bridge, Hughes, *et al.*, 2013). Recently, MCEs in the Caribbean have been recognised as their own distinct biological assemblage, characterised by the absence of shallow-specialist taxa and the presence of depth-generalists (Semmler, Hoot and Reaka, 2016; Laverick, Andradi-Brown and Rogers, 2017). The upper and lower boundaries of MCEs may therefore be considered variable, with distributions likely underpinned by physiological responses to the environment.

Photosynthetic scleractinian corals derive a significant portion of their energy from sunlight (Hatcher, 1988). However, as depth increases, photosynthetically active radiation (PAR) declines (Sathyendranath and Platt, 1988). The depth-generalist profile typical of mesophotic Scleractinia in the Caribbean (Semmler, Hoot and Reaka, 2016; Laverick, Andradi-Brown and Rogers, 2017) therefore poses a significant physiological challenge. Photosynthetic corals may increase their photosynthetic efficiency to accommodate changing light profiles (Anthony and Hoegh-Guldberg, 2003; Hennige *et al.*, 2008). This can be achieved through symbiont switching (Bongaerts, Carmichael, *et al.*, 2015), changing growth form (Graus and MacIntyre, 1982), or even by modifying the reflective properties of the coral skeleton (Enríquez *et al.*, 2017) with differences noted between shallow and mesophotic taxa (Kahng *et al.*, 2012). Additionally, scleractinian corals sit on a spectrum of mixotrophy, with variable rates of heterotrophic feeding (Palardy, Grottoli and Matthews, 2005). Heterotrophic subsidy may be used as a strategy to survive coral bleaching events,

when the energy contribution from photosynthesis declines (Grottoli *et al.*, 2014). Heterotrophic subsidy, therefore, has also been recognised as a possible mechanism permitting the depth-generalist distribution of mesophotic hard corals (Alamaru *et al.*, 2009; Lesser *et al.*, 2010; Crandall *et al.*, 2016).

A third conceivable physiological adaptation to low light levels is a reduced metabolic rate, and so energy requirement (Davies, 1980). Though mass specific respiration rates at rest (basal metabolic rates) appear remarkably consistent across biology (Suarez, Darveau and Childress, 2004; Makarieva *et al.*, 2008), energy could be saved by reduced investment in reproduction (Feldman, Shlesinger and Loya, 2017; Shlesinger *et al.*, 2018) or growth.

Pulse Amplitude Modulated (PAM) fluorometry is an established method for studying photochemistry (Schreiber, 2004) and can be used to calculate a variety of metrics, such as photosynthetic efficiency and capacity, light-related stress, and other features (Jassby and Platt, 1976; Juneau, Green and Harrison, 2005). Further, the ratios of heavy to light nitrogen isotopes in coral tissue can provide a measure of heterotrophic feeding (Peterson and Fry, 1987), providing environmental differences are accounted for (Heikoop *et al.*, 2000; Baker *et al.*, 2010). Discrimination between carbon isotopes is partly dependent on photosynthetic activity in the absence of feeding (Alamaru *et al.*, 2009); the translocation of carbon from the zooxanthellae symbionts to the coral host may be affected by depth and produce an isotopic signature (Muscatine, Porter and Kaplan, 1989). However, lipid content may also affect bulk $\delta^{13}C$ measurements (Alamaru *et al.*, 2009).

Stable isotope analyses and PAM fluorometry of a number of mesophotic Scleractinia, including *Agaricia lamarcki* (Crandall *et al.*, 2016) and *Montastraea cavernosa*

(Lesser *et al.*, 2010, 2014; Crandall *et al.*, 2016) in the Caribbean, and *Favia favus* (Alamaru *et al.*, 2009) and *Stylophora pistillata* (Alamaru *et al.*, 2009; Einbinder *et al.*, 2009; Brokovich *et al.*, 2010; Nir *et al.*, 2011; Cohen and Dubinsky, 2015) in the Red Sea, have revealed between-species variation in the changing rate of heterotrophy and photosynthetic efficiency with depth. However, there has been little effort to investigate intra-species variation between sites with different abiotic conditions, such as light levels and slope.

The relationship between these factors and the cellular physiology of corals across depth gradients has also yet to be examined. The intracellular ratio between the secondary metabolites dimethylsulphoxide and dimethylsulphoniopropionate (DMSO:DMSP) has been previously used as an early indicator for cellular oxidative 'stress' in the cordgrass *Spartina alterniflora* (Husband and Kiene, 2007; McFarlin and Alber, 2013) because of their role in cellular antioxidant cascades (Sunda *et al.*, 2002). Since corals (and associated symbionts) harbour significant quantities of DMSP (Raina *et al.*, 2013; Burdett *et al.*, 2014), it may be hypothesised that the DMSO:DMSP ratio is also a useful oxidative stress indicator for these organisms.

Here, we consider the physiology of the mesophotic depth-generalist scleractinian *A. lamarcki,* one of the dominant mesophotic taxa at our Caribbean study sites. *A. lamarcki* is a brooding species, which has shown limited symbiont switching around the mesophotic-shallow reef boundary (Bongaerts, Carmichael, *et al.*, 2015). We sampled two sites down a continuous depth gradient to assess the consistency of physiological patterns with depth. We use techniques which have already been used to assess physiological change across the shallow- mesophotic depth gradient (Alamaru *et al.*, 2009; Einbinder *et al.*, 2009; Lesser *et*

al., 2010; Crandall *et al.*, 2016); PAM fluorometry, oxygen flux, stable isotope analyses, and also investigate trends in intracellular DMSP:DMSO ratios.

4.3 Methods:

4.3.1 Research site-

Utila is one of the Honduran Bay Islands on the southern end of the meso-American barrier reef. A quantitative benthic description exists to a maximum depth of 85m (Laverick, Andradi-Brown and Rogers, 2017). Of the five sites described (Figure 1.1), two are considered here: 'The Maze' on the north shore (TMA) and 'Little Bight' on the south shore (LB). Generally, south shore reefs are slopes ending in sand at ~45m depth. In contrast, north shore reefs are typically walls extending deeper than 100m. Though we do not have light data, the sites are known to have differing maximum depth ranges for *A. lamarcki* and the rate of change in the scleractinian community with depth (i.e. from a shallow to a mesophotic assemblage) is known to be faster at TMA than LB (Laverick, Andradi-Brown and Rogers, 2017).

4.3.2 Collection-

Coral fragments were collected (Permit number: ICF-261-16) by SCUBA divers using mixed gas closed circuit rebreathers during July 2015. During collection dives, *A. lamarcki* colonies were identified as plating and encrusting agariciid colonies with white, star-shaped polyps (Humann and Deloach, 2013). Species identity was verified by the alternation of long and short septo-costae following examination under a microscope in the field (Veron, Stafford-Smith, M.G. Turak and DeVantier, 2016).

Sampled depths were from 10m to 45m at the site LB and 16m to 60m at TMA. These depths reflect the shallowest and deepest observed colonies of *A. lamarcki* at each site. We are confident the whole depth range of *A. lamarcki* was sampled for the following reasons. The lower limit of LB coincides with a sandy plain, and concurrent ecological studies at TMA, which reported no Scleractinia deeper than 85m with maximum dive depths of 100m (Laverick, Andradi-Brown and Rogers, 2017), did not report deeper incidences of *A. lamarcki* than sampled here. Additionally, roaming divers reported no *A. lamarcki* deeper than 60m. Sampled coral colonies were >40cm in diameter, to minimise damage to newly-recruited colonies, and 5m+ from their nearest sampled neighbour to minimise the sampling of clones. All colonies were sampled as they were found, so long as they satisfied these selection criteria, with up to 12 colonies per 10m vertical depth band. Half the samples were collected by swimming with the reef on the divers' left side from the dive site mooring buoy, half with the reef on the right side.

To sample a suitable colony, a thumb sized fragment was excised using a chisel from the plate margin. This was placed in a labelled zip-lock bag and stowed in a PVC tube that was opaque to light. Fragments were kept in the dark prior to analysis to mitigate light associated stress during the divers' ascent. Once stowed, the fractured margin of the colony was lined with pre-mixed Milliput modelling putty to prevent infection or fouling (Downs, 2011). The samples were returned to a temperature-controlled field lab and placed within an opaque plastic aquarium filled with water from the fore-reef. The aquaria were heated to 28°C (= ambient in situ temperature), aerated, and covered in four layers of plastic tarp to allow dark acclimation of fragments. Samples were acclimated in the dark for 12 hours prior to analysis for photosynthetic characteristics and dissolved oxygen consumption.

Water and sediment samples were collected at both sites at 5, 15, 25, 40, and also at 55 and 70m at TMA. These samples provide an environmental isotope signature for context when interpreting the trends in coral values (Heikoop *et al.*, 2000; Baker *et al.*, 2010). Sediment was collected in sediment traps deployed on the reef for 4 weeks before the contents were drained and desiccated. Water was collected by SCUBA divers and poisoned in the lab with 10µl of mercuric chloride solution per 12ml of water, and stored without headspace in exetainer vials (Labco Ltd).

4.3.3 Oxygen incubations-

Coral fragments were removed from their aquarium, in the dark, and isolated in plastic chambers with fresh sea water from the fore-reef. The chambers were left for an hour, deemed a suitable time for generating a detectable signal after pilot tests. The lab was kept in darkness during the incubations. Water samples were taken at the beginning and end of the incubation. The change in dissolved oxygen (DO_2) content was quantified using a Fibox oxygen optode sensor spot system (PreSens Precision Sensing GmbH). The probe was held steady until the reading plateaued before recording. The change in dissolved oxygen during the incubation (ΔDO_2) was standardised to 10g of coral tissue, measured when later removed from the fragment (details below), and to the hour.

4.3.4 PAM fluorometry-

Immediately after the oxygen incubation, Rapid Light Curves (RLCs) were conducted on the submerged, polyp-bearing side of the coral fragment using PAR levels of 2, 3, 5, 8, 12, 19, 37, 64, 110 µmol m^{-2} s^{-1} set on a Diving-PAM (Heinz Walz GmbH). The fibre optic was positioned 5 mm away from the coral surface using the Surface Holder attachment during all RLCs. PAR levels for the RLCs were chosen based on a balance between avoiding rapid

light saturation of mesophotic fragments, whilst still achieving a detectable response from shallow fragments.

For RLCs a steady state is not reached during each light step (Ralph and Gademann, 2005), unlike traditional light curves. Therefore, results from RLCs yield information on the actual, rather than optimal, photosynthetic state as suggested by traditional light curves (Ralph and Gademann, 2005). Additionally, comparing RLCs from different species or under different environmental conditions should be conducted with care, as the irradiance absorption of a photosynthetic organism may change, affecting electron transport rates (Saroussi and Beer, 2007). Comparisons between sites and/or depths may therefore be affected by changes in the coral's irradiance absorption, such as changes in coral optics (Chalker, Dunlap and Oliver, 1983; Anthony and Hoegh-Guldberg, 2003), and have been taken into account when interpreting the PAM fluorometry results. Our results represent an integrated photosynthetic and bio-optical response, providing relative comparisons of the same species between sites. Variations are likely to have arisen in response to a varied environmental regime, thereby enabling comparison between sites, albeit without the capacity to identify if any observed changes are as a result of photosynthetic or bio-optical characteristics.

4.3.5 Stable isotope analysis-

All stable isotope samples were prepared as described below before shipping to the UK for analysis at the NERC Life Sciences Mass Spectrometry Facility in East Kilbride. In the field, following RLCs, coral fragments were patted dry and their mass recorded. Surface coral tissue was removed using a Waterpik filled with sea water (Johannes and Wiebe, 1970). The mass of the air-dried skeleton was later recorded to allow the mass of coral tissue to be

determined (= original mass – mass of bare skeleton). Air-dried skeleton was ground into a powder using a pestle and mortar and sealed in micro-centrifuge tubes. Coral slurry was left to settle and then pipetted into micro-centrifuge tubes. The tubes were centrifuged at 14,000rpm for 60 seconds and the supernatant removed. This was repeated three times, topping with more slurry between spins to maximise material recovery. The resulting material was left standing for 10 hours, in an aluminium tray under a sheet of glass in direct sunlight, to allow desiccation before storing at -20°C. Upon return to the UK these samples, and sediments, were further dried at 50°C overnight. We were unable to separate symbiont and host tissue in the field, we therefore interpret our results at the level of the holobiont following other similar studies (Crandall *et al.*, 2016). When host and symbiont have been analysed independently, the results tend to show a shift in mean values between the two fractions, but similar relationships with increasing depth (Alamaru *et al.*, 2009; Einbinder *et al.*, 2009; Lesser *et al.*, 2010).

Sediments and Tissue samples ($\delta^{15}N$, $\delta^{13}C$):

Samples were weighed (0.7mg for organic tissues, 5mg for sediment) into tin capsules and loaded into an Elementar (Hanau, Germany) Pyrocube elemental analyser (EA) run in NC mode. Samples were combusted and gases purified such that N_2 (for $\delta^{15}N$) and CO_2 (for $\delta^{13}C$) were admitted consecutively into a Thermo (Bremen, Germany) Delta XP isotope ratio mass spectrometer (IRMS). The protocol loosely follows simultaneous nitrogen, carbon, and sulphur analysis (Fourel *et al.*, 2014) with the following deviations: we did not run for sulphur and so did not use a SO_2 trap; oxidation and reduction reactors were cooler at 950°C and 600°C respectively; the oxidation reactor was centrally filled with CuO as a catalyst, succeeded by a plug of silver wool filtering Cl species. Three standards were used to correct

for linearity and drift of a range of $\delta^{15}N$ and $\delta^{13}C$ (Werner and Brand, 2001; Newton, 2010): a gelatine solution (GEL), a ^{13}C-enriched alanine/gelatine solution (ALAGEL), and a ^{15}N-enriched glycine/gelatine solution (GLYGEL). All standard solutions were dispensed into tin capsules and oven dried at 70°C prior to analysis. C and N abundance, and an independent evaluation of isotope ratio, was provided by four USGS40 standards (Qi *et al.*, 2003). Measurement error of all four reference materials can be found in the supplementary information (Supplementary 4.1).

Dissolved Inorganic Carbon (DIC, $\delta^{13}C$):

Two drops of 103% Phosphoric acid were added to exetainers (Labco Ltd), which were then flushed with helium. 1ml of each water sample was added to the exetainer via a syringe through the septum. The phosphoric acid liberated gaseous CO_2 from the sample into the headspace of the exetainer. Standards were treated differently as these were solid sodium bicarbonate and calcium carbonate powders (Waldron *et al.*, 2014). Standards were loaded into dry exetainers with 1ml of dilute phosphoric acid, ensuring the H_3PO_4 concentration was the same as for the samples. For both standards and samples, the headspace CO_2 was dried in a Gas Bench (Thermo, Bremen, Germany) and the $\delta^{13}C$ measured on a Thermo (Bremen, Germany) Delta V IRMS (Torres, Mix and Rugh, 2005; Yang and Jiang, 2012).

Skeletal carbonates (δ13C):

$\delta^{13}C$ of skeletal carbonates were analysed on an 'Analytical Precision' sampler/mass spectrometer (de Groot, 2008). 1mg samples of each powdered coral skeleton were sealed in vacutainers and flushed with helium. Phosphoric acid was injected through the septum in excess by the autosampler. The reaction was left at 70°C for 20 minutes to liberate CO_2 into the headspace. The resulting gas was analysed by the instrument's IRMS.

4.3.6 Dimethylated sulphur analyses-

Approximately 1mg of centrifuged tissue was diluted to 5ml volume with MilliQ 18Ω water with 1ml 10M NaOH and stored in 20ml chromatography vials (Fisher Scientific) sealed with Pharma-Fix septa (Fisher Scientific), to hydrolyse DMSP into DMS. Samples were stored in the dark and transported back to the University of St Andrews for analysis. The sample headspace was analysed by direct injection using an SRI-8610C gas chromatograph (GC) (SRI Instruments UK) fitted with a 15m 5.0U MXT-1 capillary column (N_2 carrier gas @ 8psi, 45°C), and a sulphur-specific flame photometric detector (air pressure: 2psi, H_2 pressure: 27 psi, 150°C).

Samples were then analysed for DMSO concentration, using the reductase enzyme method (Hatton, AD *et al.*, 1994). Samples were purged of DMS with N_2 following addition of Tris-buffer and neutralisation to pH 7.0. Where samples could not be analysed within 24hrs of preparation, they were frozen (-20°C) until analysis. 2 ml of flavin mononucleotide solution was added to each vial and irradiated with 3 x 60 W bulbs for one hour to catalyse the reaction of DMSO to DMS, following Hatton et al (1994). Samples were left for 12 hours to allow DMS equilibration in the vial headspace, before direct-injection GC analysis, as described above. All sample concentrations were quantified from DMSP standard calibration curves (DMSP standard from Research Plus Inc.). The limit of detection for both DMSP and DMSO samples was 1µg S per 100µl headspace injection; standard and sample precision was within 1%.

4.3.7 Statistical analyses-

All statistical analyses and data manipulation were conducted in the programming language R (R-Core-Team, 2013). The minimum saturating irradiance (RLC[Ek]) and initial

photosynthetic rate (RLC[alpha]) were calculated for each fragment by fitting rapid light curve (RLC) data to the equations of Jassby & Platt (1976) in the package Phytotools (Silsbe and Malkin, 2015). Maximum Relative Electron Transport Rate (RLC[rETRmax]) was calculated as RLC[Ek]*RLC[alpha]. As the δ13C skeletal value attains an equilibrium with the environment (McConnaughey *et al.*, 1997), and the difference to tissue δ13C represents a metabolic effect, we calculate a δ13C differential as δ13C tissue – δ13C skeleton. We rely on sea water δ13C DIC as an additional control for potential between site variation in δ13C sources, as it provides the basis for coral carbonate production (Allison *et al.*, 2014).

Linearity, normality, heteroskedasticity and influential outliers were assessed using residual plots. Statistical tests were not used to assess these as the large number of data points caused spurious rejection of assumptions with high *P*-values associated with only small deviations. Tests are robust to the assumption of homogeneity of variance across groups because of balanced sample sizes between sites. Data remained untransformed to ensure fair comparisons between tests.

In turn, RLC[Ek], RLC[alpha], RLC[rETRmax], change in dissolved oxygen during incubation (ΔDO_2), tissue molar C:N, DMSO:DMSP, $\delta^{13}C$ tissue differential, tissue $\delta^{15}N$, sediment $\delta^{15}N$, and sea water $\delta^{13}C$ DIC were all fitted as the dependent variable of a linear model with depth as the independent variable, site as a grouping factor, and an interaction term. RLC[rETRmax], ΔDO_2, tissue molar C:N, $\delta^{13}C$ tissue differential, tissue $\delta^{15}N$ were also fitted in the same way against DMSO:DMSP. For the $\delta^{13}C$ tissue differential, tissue molar C:N was included as a control variable to account for possible fluctuations in lipid content. These models show the physiological profile of *A. lamarcki* with depth at Utila, in terms of photosynthesis, heterotrophy, respiration, and oxidative stress:

$$Y \sim (Depth \; \boldsymbol{or} \; DMSO{:}DMSP) \; + \; Site \; + \; interaction$$

Additional models included:

$$Tissue \; \delta 15N \sim rETRmax \; + \; Site \; + \; rETRmax{:}Site$$

$$\delta 13C \; differential \sim Tissue \; \delta 15N \; + Tissue \; molar \; C{:}N \; + \; Site \; + \; Tissue \; \delta 15N{:}Site$$

Statistically significant model elements were detected with heteroskedastically constant variance using 'Anova(white.adjust = HC3)' (Long and Ervin, 2000).

4.4 Results:

The changes in physiology recorded for *A. lamarcki* with increasing depth differ markedly between TMA and LB. A summary of ANCOVA results and model parameters are presented in tables 4.1 and 4.2 respectively. Plots of environmental controls can be found in the supplementary material (Supplementary 4.2 and 4.3), as well as Residual plots (Supplementary 4.4). Residual plots revealed no serious deviations from model assumptions. For analyses considering the $\delta^{13}C$ tissue differential, molar C:N was included as an additional parameter to control for lipid content. Molar C:N did not vary with depth (F = 2.40 P = 0.12) or tissue $\delta^{15}N$ (F = 8.94 P = 0.54).

During our sampling we did not encounter any intermediate morphologies. Though morphological variation and change in growth form down depth gradients has been documented in some species of Scleractinia (Dustan, 1975; Amaral, 1994; Nir *et al.*, 2011; Goodbody-Gringley and Waletich, 2018), we observed only modest plasticity in growth form. At the extremes of *A. lamarcki's* depth range, within a given site, there was a

ANCOVA Summaries	Sample size		Effect of X		Effect of Site on Means		Effect of Site on Slope		X variable
	n : LB	*n : TMA*	*F*	*P*	*F*	*P*	*F*	*P*	
RLC[Ek]	50	58	17.0856	**0.00007251***	0.0135	0.90756	5.7756	**0.01802***	Depth
RLC[alpha]	50	58	43.4559	**1.835E-09***	0.3176	0.5743	2.1614	0.1445	Depth
RLC[rETRmax]	50	58	8.464	**0.00443***	0.0199	0.888178	8.8711	**0.003606***	Depth
DMSP:DMSO	44	44	1.7308	0.19158	4.2328	**0.04248***	0.0505	0.82277	Depth
Incubation δDO_2	47	41	0.0064	0.9366	0.1543	0.6955	2.7145	0.103	Depth
Tissue molar C:N	48	54	0.0108	0.9176	0.0133	0.9083	2.5248	0.1153	Depth
$\delta^{13}C$ differential	48	54	5.5309	**0.02068***	0.4084	0.52427	3.2029	0.0766	Depth
Tissue $\delta^{15}N$	48	54	19.378	**0.00002711***	25.591	**0.000001944***	14.128	**0.0002889***	Depth
Sediment $\delta^{15}N$	14	24	8.1582	**0.00726***	2.5446	0.11992	0.2265	0.6372	Depth
DIC sea water	4	18	10.9894	**0.003851***	1.5271	0.23243	1.6271	0.218326	Depth
Tissue $\delta^{15}N$	48	54	1.786	0.1845	17.2591	**0.00006928***	0.0972	0.7559	RLC[rETRmax]
$\delta^{13}C$ differential	48	54	0.3741	0.542172	4.9821	**0.027888***	8.5822	**0.004223***	Tissue $\delta^{15}N$
RLC[rETRmax]	44	44	0.0232	0.8794	0.3086	0.5799	1.2962	0.2579	DMSO:DMSP
Incubation δDO_2	43	33	0.0924	0.762	2.2564	0.1371	0.372	0.5437	DMSO:DMSP
Tissue molar C:N	44	44	4.1628	**0.04429***	0.1721	0.67921	0.0833	0.77361	DMSO:DMSP
$\delta^{13}C$ differential	44	44	1.367	0.24545	3.9114	0.05105	1.0363	0.31145	DMSO:DMSP
Tissue $\delta^{15}N$	44	44	0.7734	0.3815	18.2509	**4.839E-05***	0.5592	0.4566	DMSO:DMSP

tendency toward smaller encrusting colonies. Plating forms were most common between ~20-55m depth.

Table 4.1 ANCOVA results

*Values are reported as they appeared in computer outputs. P values < 0.05 are in bold and followed by an *. LB = Site Little Bight. TMA = Site The Maze*

Table 4.2 Model parameters

(Overleaf) Values are reported as they appeared in computer outputs. References to the residual plots in supplementary information for each model are under column 'Residuals'. 'Interpretation' is a brief explanation of what the model represents. LB = Site Little Bight. TMA = Site The Maze.

Model summaries	Little Bight		The Maze				X variable	Interpretation
	Intercept	*Slope*	*Intercept*	*Slope*	*Adjusted R^2*	*Residuals*		
RLC[Ek]	206.227352	-2.692050	143.694792	-0.879897	0.1705	N	Depth	Minimum saturating irradiance
RLC[alpha]	0.492716055	0.001374574	0.449357211	0.002833639	0.228	M	Depth	Initial photosynthetic rate
RLC[rETRmax]	100.220204	-1.204346	67.201354	-0.175732	0.1412	A	Depth	Photosynthetic capacity (relative electro transport rate)
DMSP:DMSO	0.75419628	-0.002717019	0.816521507	-0.001934149	0.01403	Q	Depth	Inferred oxidative stress
Incubation δDO_2	-17.9271223	0.3868667	-64.6352683	-0.7049168	0.0003409	D	Depth	Net photosynthesis during dark trials
Tissue molar C:N	13.6679714	0.0446042	8.9962262	-0.0825296	0.006482	E	Depth	Holobiont condition
$\delta^{13}C$ differential	-15.88822	-0.01171	-13.94745	-0.08405	0.1926	C	Depth	Long term photosynthetic signal
Tissue $\delta^{15}N$	1.63560878	0.03637871	2.29028497	-0.00204339	0.2585	B	Depth	Inferred trophic level
Sediment $\delta^{15}N$	2.86968	0.7132	2.40521	0.69883	0.2104	K	Depth	Environmental control
DIC sea water	0.88	8.29E-19	0.6542	0.005055	0.4287	L	Depth	Environmental control
Tissue $d^{15}N$	3.000773	-0.004688	2.9671088	-0.007505	0.1366	O	RLC[rETRmax]	Investigating site differences
$\delta^{13}C$ differential	-14.37608	-0.97474	-20.32498	1.10782	0.1584	P	Tissue δ15N	Investigating site differences
RLC[rETRmax]	53.35772	16.19293	66.32352	-7.45428	0.01279	F	DMSO:DMSP	Photosynthetic capacity (relative electro transport rate)
Incubation δDO_2	-6.487366	-3.052744	-13.446896	30.751496	0.007288	I	DMSO:DMSP	Net photosynthesis during dark trials
Tissue molar C:N	17.385736	-3.462559	18.745543	-4.712544	3.32E-05	J	DMSO:DMSP	Holobiont condition
$\delta^{13}C$ differential	-13.212548	-2.262587	-15.850777	-4.685545	0.05017	H	DMSO:DMSP	Long term photosynthetic signal
Tissue $\delta^{15}N$	2.7326381	-0.1110724	2.5391774	-0.5638523	0.0002416	G	DMSO:DMSP	Inferred trophic level

4.4.1 Physiological variation with depth-

Three coral measures significantly varied with depth: δ15N as a signal of heterotrophy ($F = 19.38$ $P < 0.001$), δ13C differential as a signal of long term photosynthetic activity ($F = 5.53$ $P = 0.02$), and RLC[rETRmax] as an instantaneous measure of potential photosynthetic capacity ($F = 8.46$ $P = 0.004$). ΔDO2, molar tissue C:N, and DMSO:DMSP did not significantly vary with depth (Table 4.1). Mean values of tissue δ15N differed between sites for a given depth ($F = 25.59$ $P < 0.001$) – the mean at LB was 0.53‰ higher. Mean DMSO:DMSP values were 0.08 higher at TMA than LB ($F = 4.23$ $P = 0.04$). Differing slopes with depth were detected for RLC[rETRmax] and tissue δ15N (Figure 4.1) between sites ($F = 8.87$ $P = 0.004$, $F = 14.13$ $P < 0.001$,(Table 4.1). We found potential photosynthetic capacity (RLC[rETRmax]) significantly declined with depth (Table 4.1), however, this appears to only be true at LB (Table 4.2, Figure 4.1). To further understand how photosynthetic profiles vary with depth, RLC[Ek] and RLC[alpha] were tested independently against depth, as RLC[rETRmax] is a composite of these two quantities. While RLC[alpha] significantly increased with depth, RLC[Ek] significantly decreased. Only RLC[Ek] returned a significant interaction term (Table 4.1), suggesting the observed differences in photosynthetic capacity with depth between the two sites were caused by differing RLC[Ek] values, i.e. the minimum saturating irradiance.

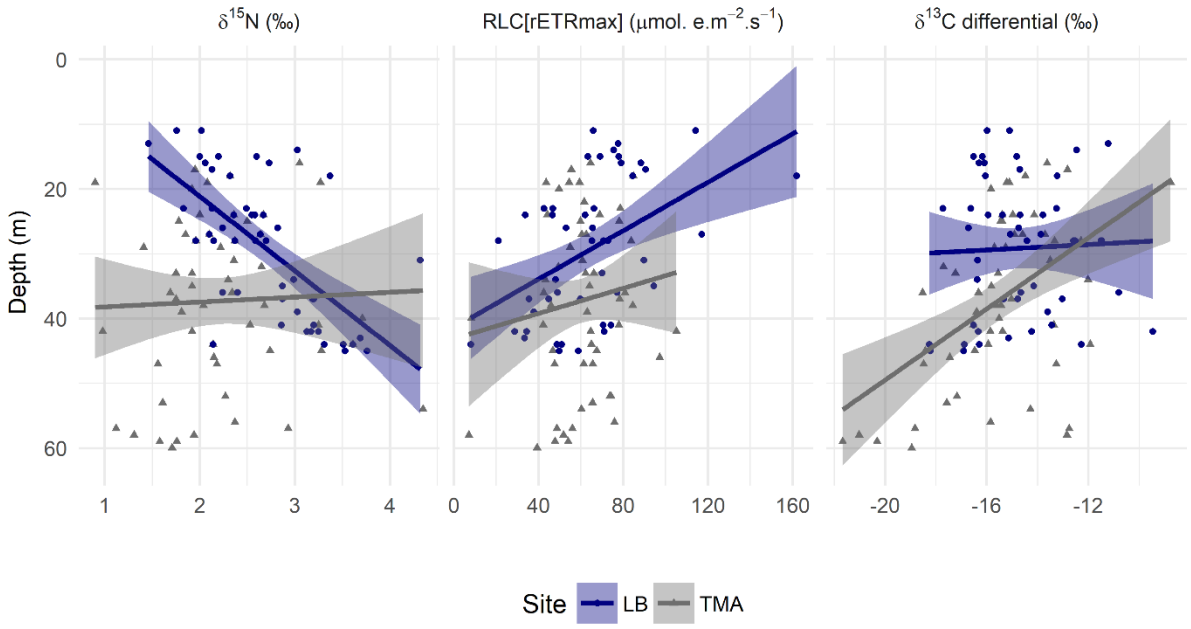


Figure 4.1 Principle physiological relationships of Agaricia lamarcki with depth across two sites

Linear models of bulk tissue $\delta^{15}N$, RLC[rETRmax], and the $\delta^{13}C$ differential against depth. Shaded areas are the 0.95 confidence interval. Statistical assessment and model parameters can be found in Tables 4.1 and 4.2. LB = Site Little Bight, TMA = Site The Maze.

4.4.2 Environmental controls-

δ15N of sediment samples at LB and TMA (Supplementary 4.2) were collected as environmental baselines for comparison to changes in tissue values which may be affected by local enrichment (Heikoop *et al.*, 2000; Baker *et al.*, 2010). Both the environmental signal and tissue showed a significant relationship with depth (Table 4.1). The mean δ15N in sediment at LB was 0.36‰ higher than TMA, this was not statistically significant (Table 4.1). It is not believed that environmental patterns drive the relationship in the tissue since the gradients in tissue δ15N are in opposing directions, whereas the environmental signal is consistently positive (Table 4.2). δ13C DIC of seawater (Supplementary 4.3) was collected and analysed as an environmental comparison to coral δ13C. Though DIC exhibits a statistically significant relationship with depth, this was in the opposite direction to the coral

holobiont δ13C measurement, and the effect size was at least an order of magnitude lower at each site (Table 4.2). LB had a mean δ13C 0.05‰ higher than TMA, again this was not significant (Table 4.1). These results suggest the trends we see between sites are due to physiological variation, and not differing environmental baselines between sites

4.4.3 Relationships between photosynthesis (RLC[rETRmax] and δ13C), inferred heterotrophy (δ15N), and stress (DMSO:DMSP)-

We detect two statistically supported relationships between DMSO:DMSP and other physiological variables. Firstly a reduction in tissue molar C:N with increasing DMSO:DMSP ($F = 4.16$ $P = 0.04$). Secondly an effect of site on mean levels of tissue bulk $\delta^{15}N$ ($F = 18.25$ $P < 0.001$), with higher $\delta^{15}N$ values for a given DMSO:DMSP at LB than TMA (Table 4.1). With $P = 0.051$, a notable effect of DMSO:DMSP on mean levels of the $\delta^{13}C$ tissue differential is identified, with higher $\delta^{13}C$ values at TMA than LB (Table 4.1).

A significant site and depth interaction for RLC[rETRmax] and for tissue $\delta^{15}N$ (Table 4.1, Figure 4.1) could be driven by two factors. Either, certain physiological relationships within the coral holobiont were not constant, or unmeasured sources of variation were confounded differently with depth at the two sites. To aid interpretation, tissue $\delta^{15}N$ was plotted against RLC[rETRmax] (Figure 4.2) and statistically assessed, determining whether the physiological relationships remained constant between sites. Site affected mean values at the two sites, but only to the degree expected by the sediment control (Supplementary 4.2). There is no statistically supported relationship between RLC[rETRmax] and tissue $\delta^{15}N$ ($F = 1.79$ $P = 0.18$, (Table 4.1), nor a significant interaction.

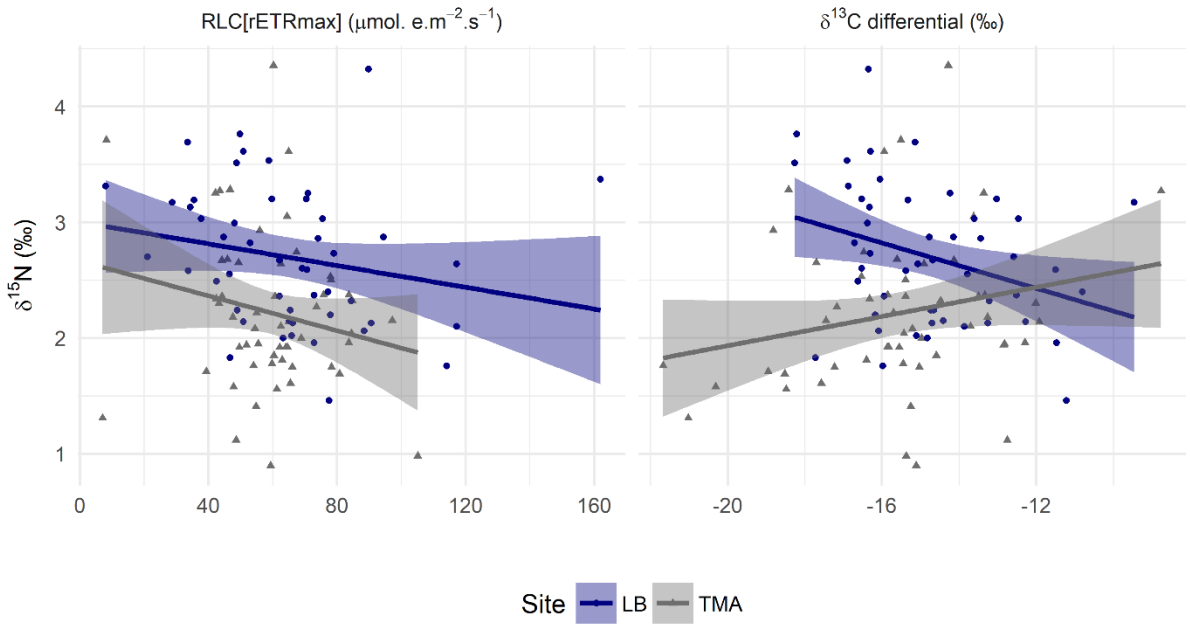


Figure 4.2 Principle physiological relationships of Agaricia lamarcki with DMSO:DMSP across two sites

Linear model of $\delta^{15}N$ by RLC[rETRmax]. Linear model of $\delta^{13}C$ differential by bulk tissue $\delta^{15}N$. Shaded areas are the 0.95 confidence interval. Statistical assessment and model parameters can be found in Tables 4.1 and 4.2. *LB = Site Little Bight, TMA = Site The Maze.*

As there was, unexpectedly, no relationship between RLC[rETRmax] and tissue $\delta^{15}N$, we further explored the $\delta^{13}C$ differential result. When controlling for variability in molar C:N, the difference in slope between LB and TMA $\delta^{13}C$ with depth (Figure 4.1) was no longer statistically significant, $P = 0.08$ (Table 4.1). A relationship might be expected between photosynthetic parameters and the degree of heterotrophy, based on previous work (Alamaru *et al.*, 2009; Lesser *et al.*, 2010; Crandall *et al.*, 2016). We therefore plot the $\delta^{13}C$ differential against tissue $\delta^{15}N$ (Figure 4.1) and statistically assessed the relationships. Whilst there was no overall relationship between the two variables, a significant interaction term (Table 4.1) revealed opposing gradients at the two sites and differing group means (Table 4.2).

4.4.4 Variability of physiological measures between sites-

Despite differences in physiology with depth between LB and TMA, the probability distributions of parameter values are broadly comparable (Figure 4.3). If the physiological parameters in figure 4.3 were linearly correlated with depth, we would expect the probability distributions to reflect the sampling effort with depth. The distributions at both sites return a modal value in close agreement and are more tightly grouped around this value than expected with sampling effort, despite sampling different depth ranges at the two sites. The exception is a shift in DMSO:DMSP between the two sites, consistent with the differences in mean levels detected by linear models (Table 4.1). The probability distribution for TMA is consistently narrower than for LB, despite TMA being sampled over a larger depth range which we would expect to necessitate greater physiological variation.

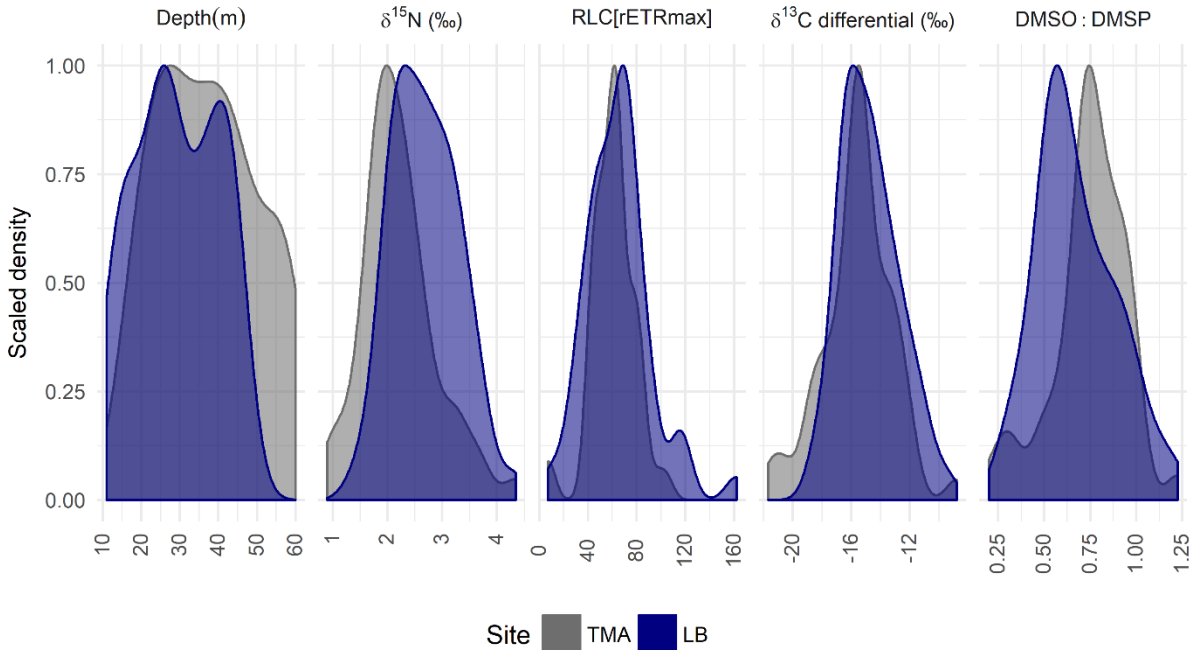


Figure 4.3 Probability distributions of select parameter values

The height of the curve indicates the relative probability of a particular parameter value. Each curve is scaled so 1 reflects the modal value within a site, the area under each curve sums to a probability of 1. The distributions from left to right show the sampled colonies with depth, values of bulk tissue $\delta^{15}N$, RLC[rETRmax], $\delta^{13}C$ differential, and DMSO:DMSP. If physiological parameters were correlated linearly with depth, we would expect distributions to be similar to those shown for sample collections depths. LB = Site Little Bight, TMA = Site The Maze.

4.5 Discussion:

4.5.1 Agaricia lamarcki expresses site-specific physiological profiles with depth-

This study aimed to assess the constancy of physiological patterns with depth between sites in the depth-generalist mesophotic coral, *Agaricia lamarcki*. We found clear site-specific trends, both in terms of PAM fluorometry and stable isotope analyses. At LB, *A. lamarcki* exhibited a reduction in potential photosynthetic capacity and an increase in heterotrophic feeding with depth (Figure 4.1). These patterns were absent at TMA, despite a wider vertical depth range. These observations highlight the variability of scleractinian physiology, and the importance of taking into consideration local/regional scale variation when attempting to generalise biological response. Unfortunately as little abiotic data was collected, and we surveyed only two sites, we are unable to test the potential mechanisms driving these physiological inconsistencies. We have, however, shown that the same species of coral will not necessarily behave in the same way down a depth gradient in different locations. Depth alone may therefore be an inappropriate proxy for physiological change through the mesophotic zone.

4.5.2 The deepest colonies of **A. lamarcki** *can be comparable to those more shallow-*

In addition to the trends in tissue $\delta^{15}N$ and RLC[rETRmax], there were no detected trends in respiration (as inferred from ΔDO_2), and no trend in the molar C:N ratio (Table 4.1). The lack of trend in respiration rate and molar C:N is of interest when taking into account the significant reduction in RLC[alpha] with depth (Table 4.2), which underpins a reduction in potential photosynthetic capacity (Figure 4.1). The change in RLC[alpha] clearly indicates that deeper *A lamarcki* colonies are acclimated to lower light levels; they do not exhibit lower fat stores or rates of energy consumption at the light levels used in this study. This

leads us to believe that the potential adaptation of deeper colonies may not prevent connectivity between shallow and mesophotic reefs, and may permit a deep-water refuge for *A. lamarcki* at this location. This is supported by the lack of an observed effect of depth on the cellular oxidative stress indicator DMSO:DMSP, and by no impact of collection site on the survival of the same colonies sampled here during a transplant experiment (Laverick and Rogers, 2017). ΔDO_2 in the dark, however, approximates basal metabolic rate which is expected to be largely constant (Suarez, Darveau and Childress, 2004; Makarieva *et al.*, 2008). In situ measurements of net-photosynthesis year round are necessary to better understand the importance of respiration in balancing energy budgets with increasing depth.

It is worth noting that coral growth rates can vary with increasing depth (Baker and Weber, 1975), and that this in turn can leave isotopic signals (Patzold, 1984). With respect to skeletal carbon fractionation, growth signals can also correlate with light exposure (Shimamura *et al.*, 2008). It is likely photosynthesis which translates light exposure into a growth rate in these studies. As these three variables are closely linked, we do not believe potential changes in growth rates with depth affect our interpretation of the stale isotope data for determining photosynthetic activity. For δ15N, zooxanthellae growth rate does not affect nitrogen isotope fractionation (Muscatine and Kaplan, 1994).

The observed negative relationship between C:N and DMSO:DMSP supports the role of tissue C:N as an indicator of holobiont health (Szmant and Gassman, 1990) and further supports the use of DMSO:DMSP as an indicator of cellular stress (Husband and Kiene, 2007; McFarlin and Alber, 2013). Between-site differences in mean DMSO:DMSP supports the

hypothesis that spatial variation in environmental conditions is impacting the local-scale physiology of *A. lamarcki*.

4.5.3 Physiological profiles are coincident with differences in ecological patterns-

Our findings also reveal a connection between physiological parameters and ecological patterns. If we interpret the difference between mean tissue δ15N at the two sites (Figure 4.1, Table 4.2) as resulting from differing environmental baselines (Supplementary 4.2, Table 4.2), then there was no difference in the mean value of any physiological parameter between the two sites, except DMSO:DMSP. This observation is despite the larger depth range at TMA. Significant interaction terms for tissue δ15N and RLC[rETRmax] show that it is the rates of change with depth which vary, such that the same physiological limits are reached for these parameters. In fact, the minimum saturating irradiance (RLC[Ek]) reduces at a rate three times faster at LB than TMA with depth, while RLC[alpaha] increases at more than twice the rate with depth at TMA (0.28% m-1) than LB (0.13% m-1), although this relationship was too noisy to return a statistically significant interaction term. Both quantities are considered key photoadaptations with increasing depth in coral (Chalker, Dunlap and Oliver, 1983). Although we are able to detect variation in the rates of photoadaptation with depth, the use of RLCs prevents us from distinguishing between photosynthetic characteristics and bio-optical properties as the causative factor in these observations. These findings do suggest that depth ranges and physiological change were related. If site-specific environmental conditions are the root cause (Anthony and Hoegh-Guldberg, 2003), observations of mesophotic taxa at ‘unusual’ (Muir and Wallace, 2015; Laverick, Andradi-Brown and Rogers, 2017) depths may be explained, supporting calls for a

biologically-informed, rather than depth-lineated, definition of mesophotic reefs (Laverick *et al.*, 2016; Loya *et al.*, 2016; Semmler, Hoot and Reaka, 2016).

4.5.4 **Agaricia lamarcki *appears to be a mixotroph* -**

Previous studies have attempted to interpret physiological data and claim particular species are primarily heterotrophic or photoautotrophic (Lesser *et al.*, 2010; Crandall *et al.*, 2016). Specifically *A. lamarcki* has been previously identified as a heterotrophic coral (Crandall *et al.*, 2016). Our high degree of replication within sites, in conjunction with a cross-site comparison, provides robust evidence for notable mixotrophy in *A. lamarcki*. Our trends in bulk tissue δ15N and δ13C at LB indicate heterotrophy (Figure 4.1), in agreement with previously published research (Crandall *et al.*, 2016). The trends detected at TMA, however, are more similar to those reported by others for *Montastraea cavernosa* (Lesser *et al.*, 2010; Crandall *et al.*, 2016). Though we do not have the compound-specific stable isotope analysis of sterols used by Crandall et al. (2010), we do detect a decrease in δ13C without a commensurate increase in δ15N. This suggests a primarily photosynthetic strategy at TMA. Further, the statistically significant trends in RLC[Ek] and RLC[alpha] with depth indicate photoadaptation (due to changes in photosynthetic characteristics and / or bio-optical properties) is occurring. As RLC[alpha] increases at twice the rate with depth at TMA, and over a larger depth gradient, it may be that photoadaptation is sufficient to maintain an autotrophic strategy at this location, but not at LB. This difference in strategy between the two sites is interesting, as we observed no differences by site in the relationship between photosynthetic capacity and tissue δ15N (Figure 4.2). Site differences in the change in environmental conditions by depth may therefore have been responsible for the apparent switch in hetero/autotrophic strategy with depth between the two sites.

4.5.5 Exploiting available microhabitat may explain physiological constancy at The Maze, a hypothesis-

In comparison to LB, very few physiological changes with depth were observed at TMA. This was despite colonies being collected across a 44m depth range and comparable modal parameter values between both sites (Figure 4.3). One potential explanation is that the topography of TMA is more complex than the gentle slope of LB, affording more light-equivalent microhabitats for colonies to exploit (Brakel, 1979). Photosynthetic capacity in Scleractinia has been shown to correlate with the light environment of microhabitats (Anthony and Hoegh-Guldberg, 2003; Bessell-Browne *et al.*, 2017). Given the rate of light attenuation with depth, we may expect the relative difference in light intensity between microhabitats (e.g. illuminated vs shaded) to be greater at shallower depths than deeper, and we do not expect mesophotic reefs to be exposed to a higher light intensity than shallower reefs. Given a random distribution of coral colonies across these microhabitats and in situ acclimation, we would expect similar heteroscedasticity in photosynthetic capacity. Our residual plots revealed no notable deviation from the assumption of homoscedasticity in photosynthetic capacity with depth (Supplementary 4.4A).

Similarly microhabitats with low flow rates, and therefore food availability, have been shown to impact the growth of *Agaricia tenuifolia* in shallow waters down a depth gradient (Sebens *et al.*, 2003). Low flow rates in the mesophotic favour ciliary mucus feeders such as *A. lamarcki* in general (Sebens and Johnson, 1991). Varying flow rates between microhabitats at TMA may permit a more constant heterotrophic contribution to the energy budget with depth (Figure 4.1).

Further, figure 4.3 show the probability distributions of the parameters with the greatest between site differences, as well as the depth distribution of sampled colonies for a null comparison. In all cases, the colonies at TMA have a tighter distribution around a modal value, despite being sampled over a larger depth range than at LB. Figure 4.3 shows a tighter distribution at TMA in terms of DMSO:DMSP, suggesting lower inter-colony variability in oxidative stress, and so potentially irradiance. Differing site means of DMSO:DMSP with depth may also indicate maintained higher irradiance levels at TMA (Table 4.1), corroborated by lower rates of change in RLC[Ek] at TMA with depth . This suggests *A. lamarcki* is better able to exist in a sub-set of preferred microhabitats at TMA. Though our low number of sites and lack of abiotic data prevents us from exploring the causes of these different physiological profiles with depth, the evidence discussed above leads us to hypothesise that topography modulating the local light field could be an important factor in mesophotic ecology (Lesser, Slattery and Mobley, 2018).

Consideration of environmental conditions is already being used to predict the occurrence of mesophotic taxa in the Hawai'ian archipelago (Costa *et al.*, 2015). Environmental data (e.g. temperature, irradiance, sedimentation, turbidity) and holobiont genetic information (e.g. symbiont clade, gene regulation) may have provided a mechanism to explain the differences we observed. Because of an absence of environmental measures, we are unable to explain the cause of documented pattern. We do, however, make some suggestions for further research. At TMA, appropriate microhabitats appear to extend deeper than at LB, increasing the vertical range of *A. lamarcki* and mitigating physiological response to depth, at this location. This leads us to hypothesise that sites with greater topographical complexity are more likely to act as depth refuges, though this will require explicit testing across other species. Such sites begin to break the correlation of

environmental conditions with depth (Brakel, 1979), allowing suitable microhabitats to exist below surface pressures (Bridge, Hughes, *et al.*, 2013). We have found that site specific conditions may influence physiology to a greater degree than depth for *A. lamarcki*. Future physiology studies should try to record the light environment that colonies are located in, preferably with temporal variation, and relate this to substrate slope and shading.

4.6 Acknowledgements:

The Authors would like to thank the Royal Geographical Society (Ralph-Brown Award), the Zoological Society of London (EDB Expeditions fund), the Oxford University Expeditions Council, Oxford University Press (John-Fell fund), the Natural Environment Research Council (NE/L002612/1; EK258-13/15), Merton College Oxford and Operation Wallacea for funding. TG is supported by a 7th Century University scholarship. Fieldwork was completed when HB was in receipt of a Marine Alliance for Science and Technology for Scotland (MASTS) Research Fellowship. DMSO analyses were supported by a MASTS Small Grant awarded to TG. MASTS is funded by the Scottish Funding Council (grant reference HR09011) and contributing institutions. We thank the Natural Environment Research Council (NERC) Field Spectroscopy Facility for loan of the Diving-PAM instrument.

The authors also thank Grace C. Young for dive time, especially at TMA, Andrea P. Izaguirre-Luque from BICA for help with sample preparation in the field and 'coral-flossing', and Dominic Andradi-Brown for sediment aliquots. The staff of Operation Wallacea at the Coral View research station, the Bay Islands Conservation Association, and the Natural Environment Research Council Life Sciences Mass Spectrometry Facility provided invaluable

logistical and technical support. A final thank you to the reviewers who helped improve this manuscript.

4.7 Authors contributions:

JHL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing

TKG: Conceptualization, Investigation, Writing – review & editing

HLB: Formal analysis, Supervision, Writing – review & editing

JN: Funding acquisition, Supervision, Writing – review & editing

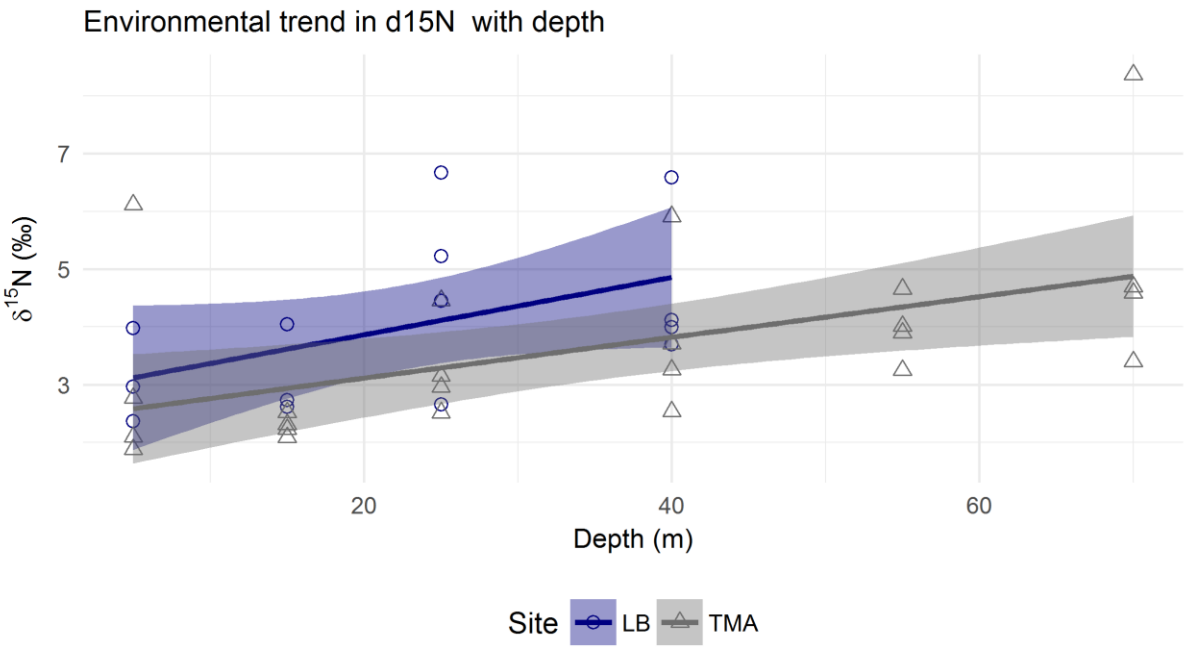
ADR: Conceptualization, Funding acquisition, Supervision, Writing – review & editing

4.8 Supplementary information:

Supplementary 4.1 Measurement error for stable isotope analyses.

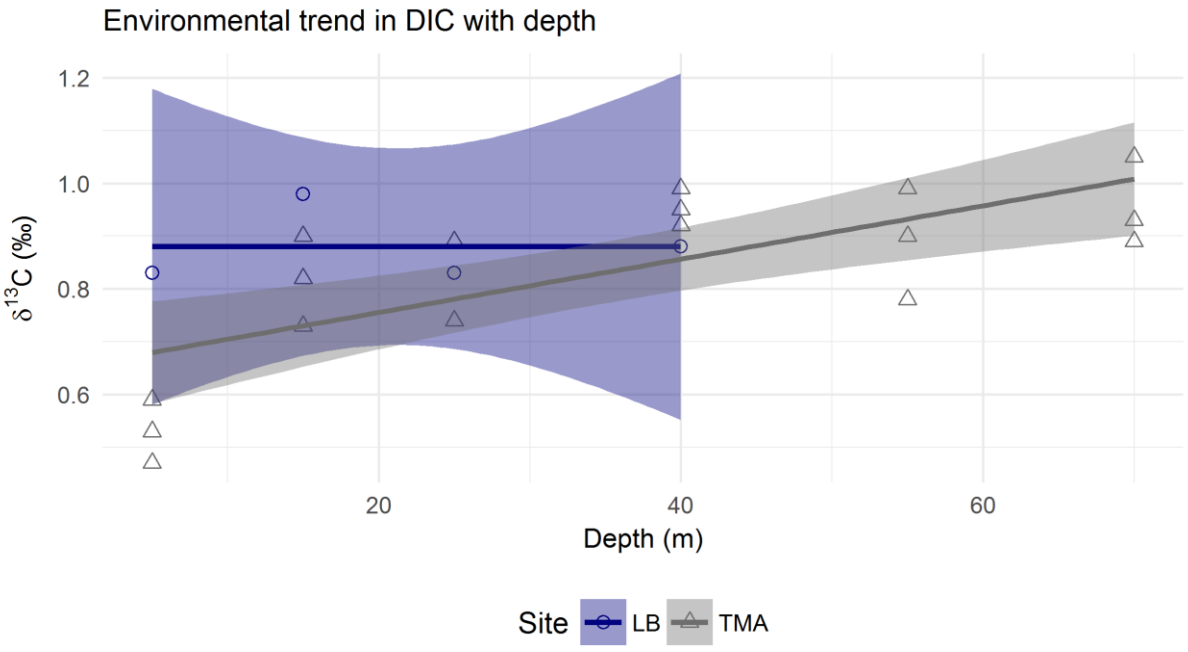
The standard deviations of the measurement distribution of four different standards, by isotope, used during stable isotope analyses of coral tissue and sediment.

	Tissue Analysis		**Sediment Analysis**	
Standard Deviations	*δ15N*	*δ 13C*	*δ 15N*	*δ 13C*
GEL	0.18	0.03	0.13	0.05
ALAGEL	0.11	0.04	0.15	0.09
GLYGEL	0.12	0.03	0.12	0.04
USGS40	0.15	0.07	0.08	0.13



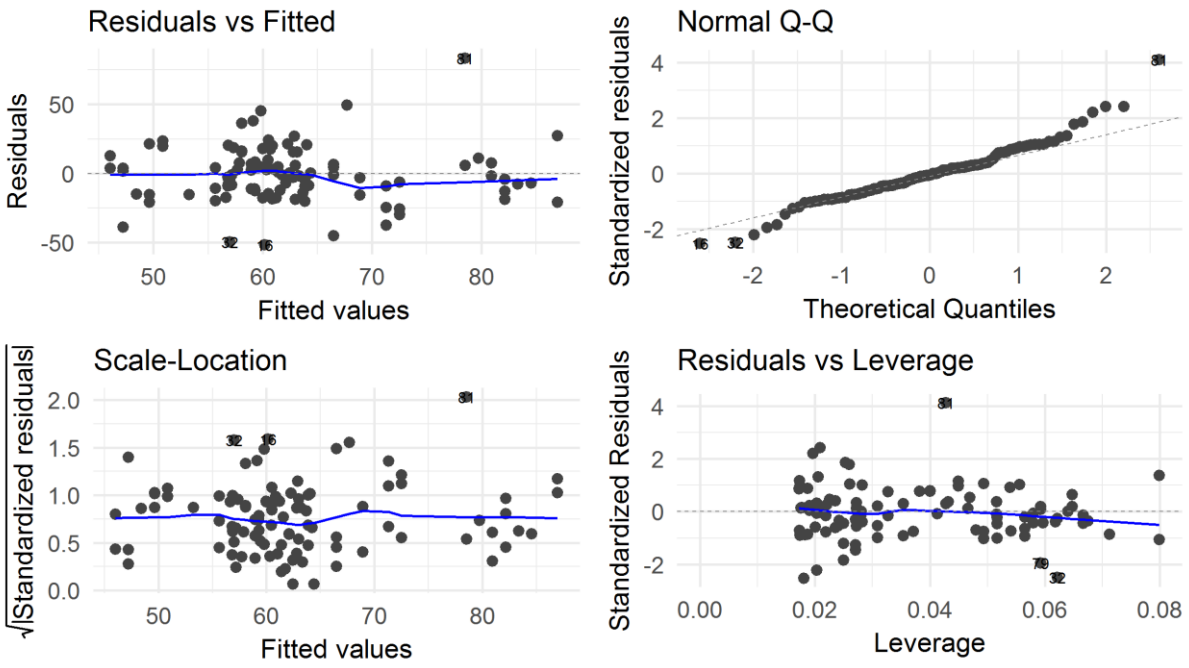
Supplementary 4.2 δ15N of Sediment at LB and TMA with depth

Shaded areas are the 0.95 confidence interval. Statistical results and model parameters can be found in Tables 4.1 and 4.2.



Supplementary 4.3 DIC of sea water at LB and TMA with depth

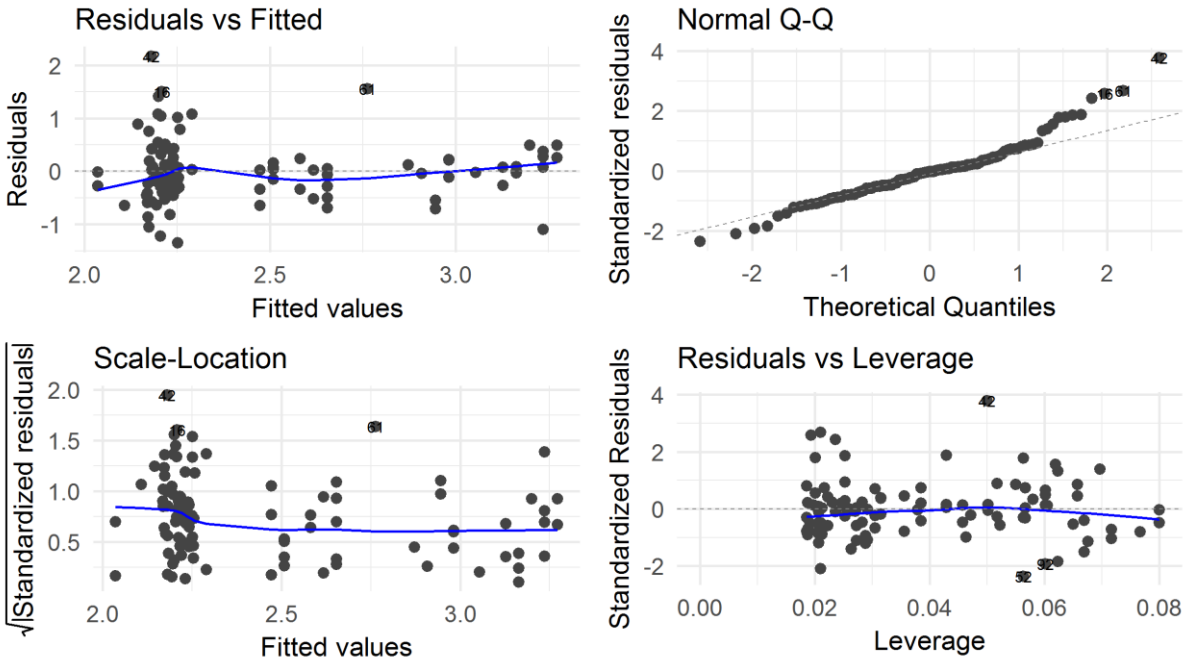
Shaded areas are the 0.95 confidence interval. Statistical results and model parameters can be found in Tables 4.1 and 4.2.



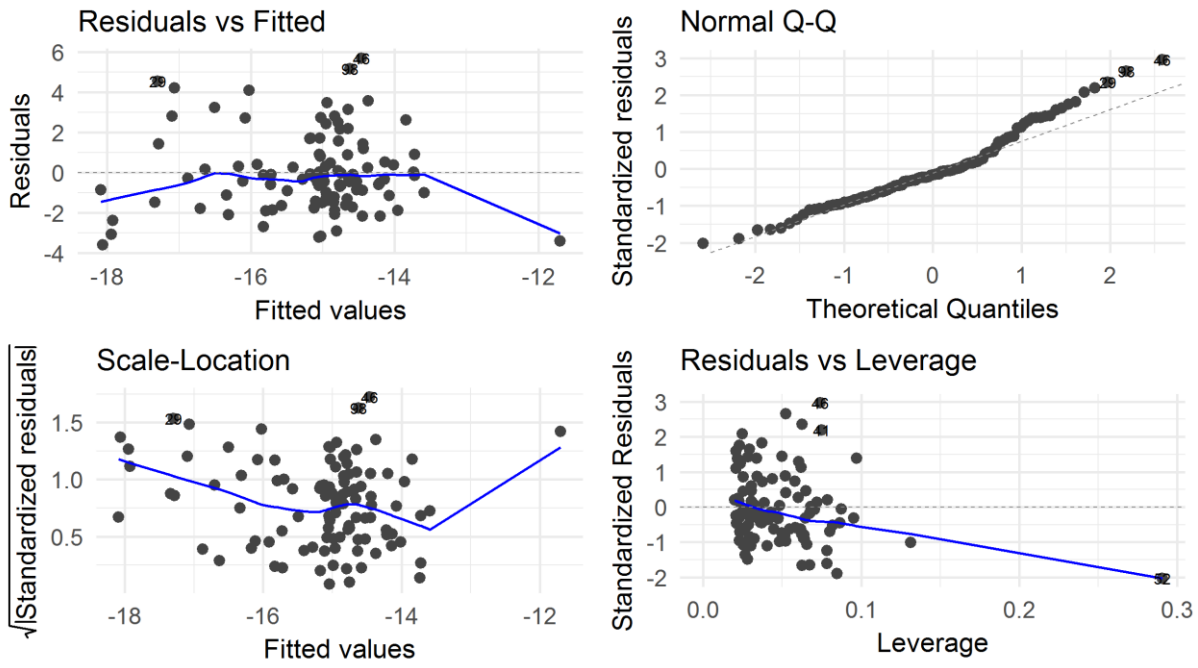
Supplementary 4.4 Residual plots considering the assumptions of fitted models

A: RLC[rETRmax] by Depth

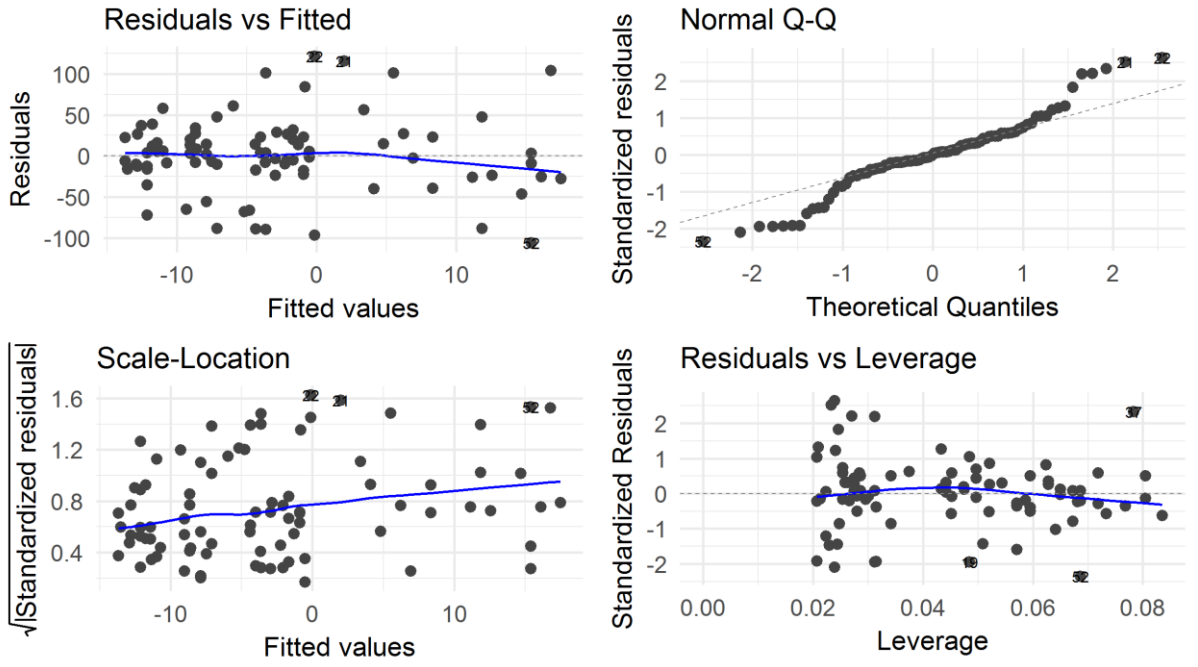
From top left, clockwise, plots reveal deviations from: linearity, normality, outliers, and heteroskedasticity.



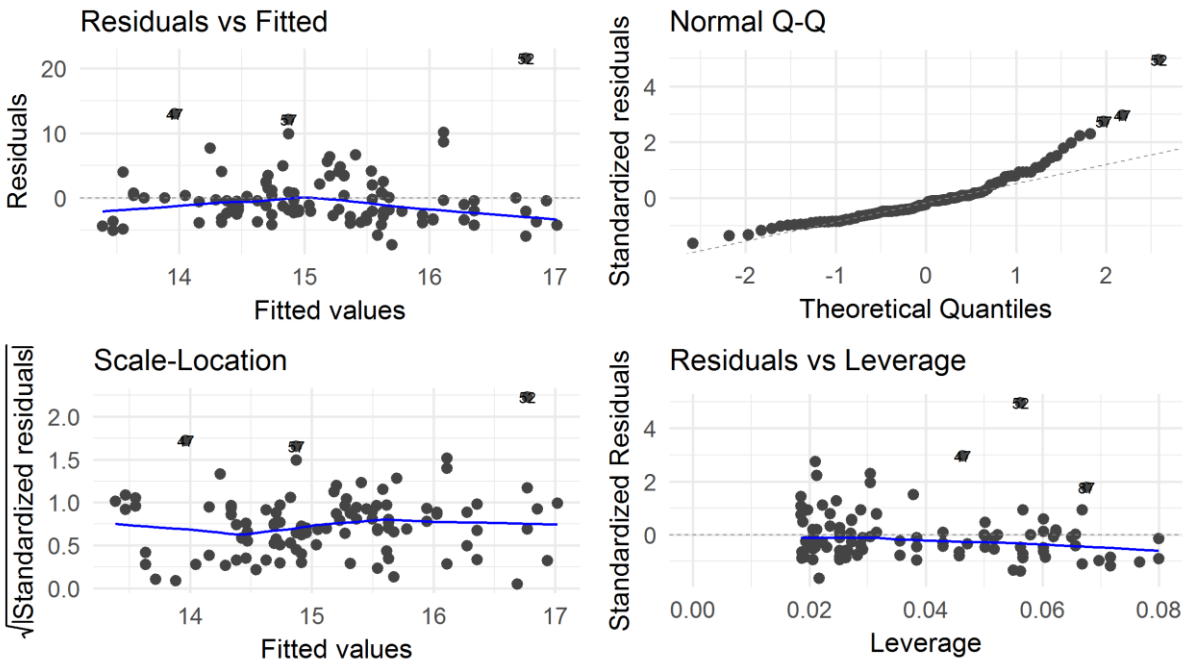
B: Tissue δ15N by Depth



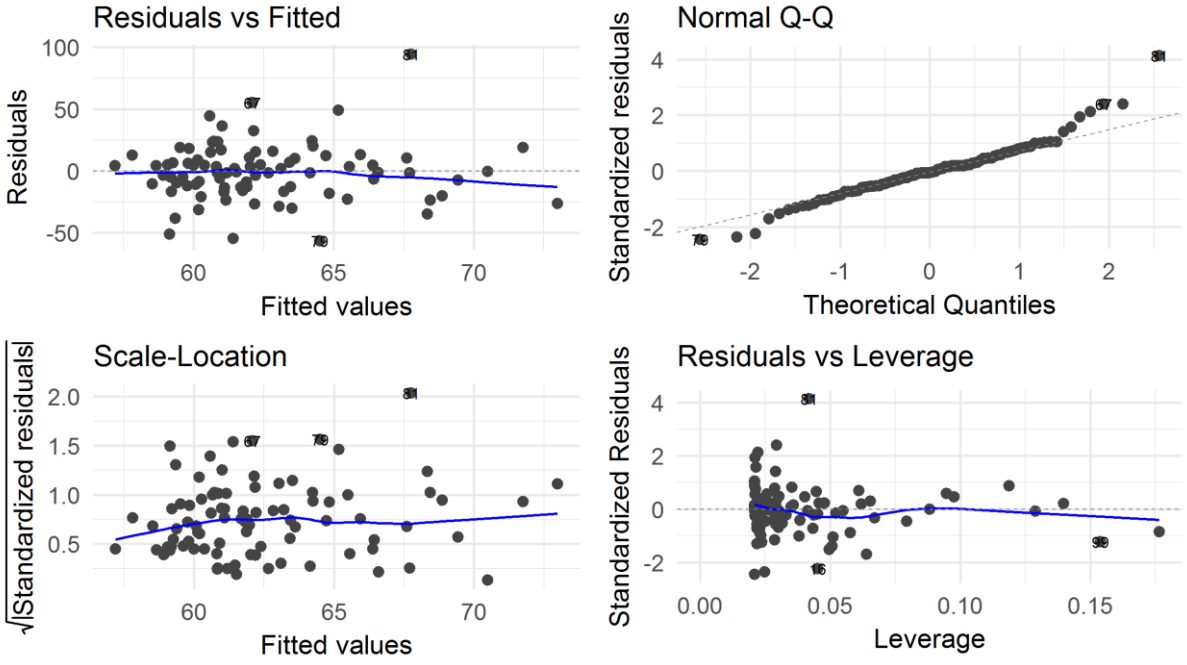
C: δ13C differential by Depth



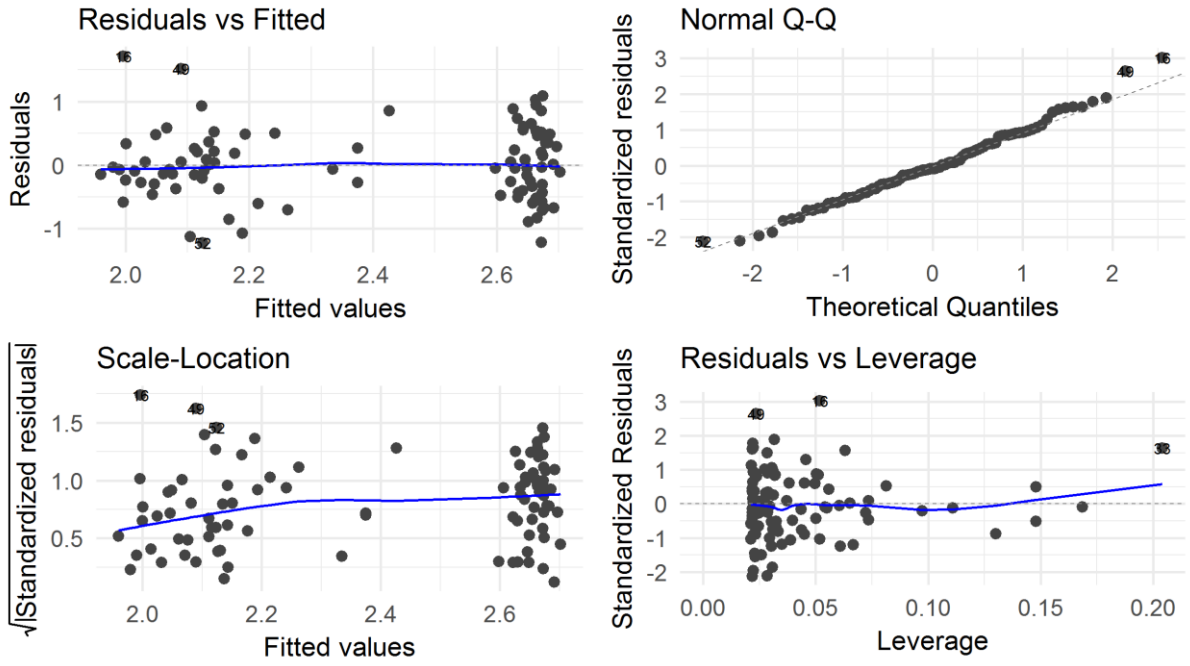
D: Incubation δDO2 by Depth



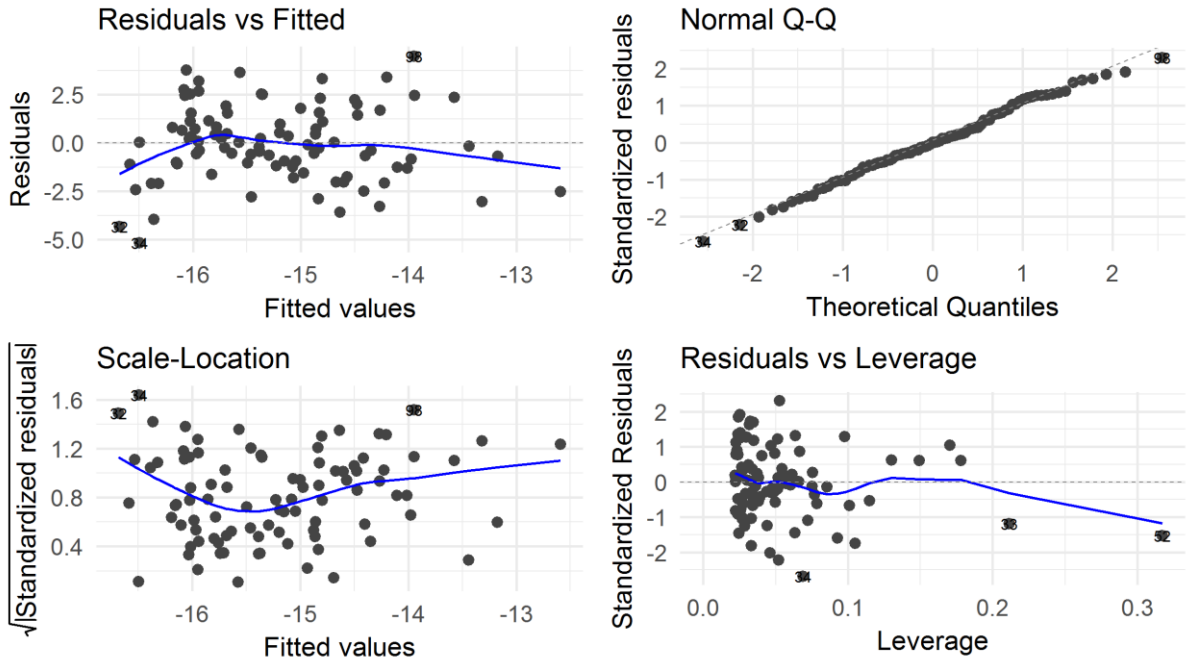
E: Tissue molar C:N by Depth



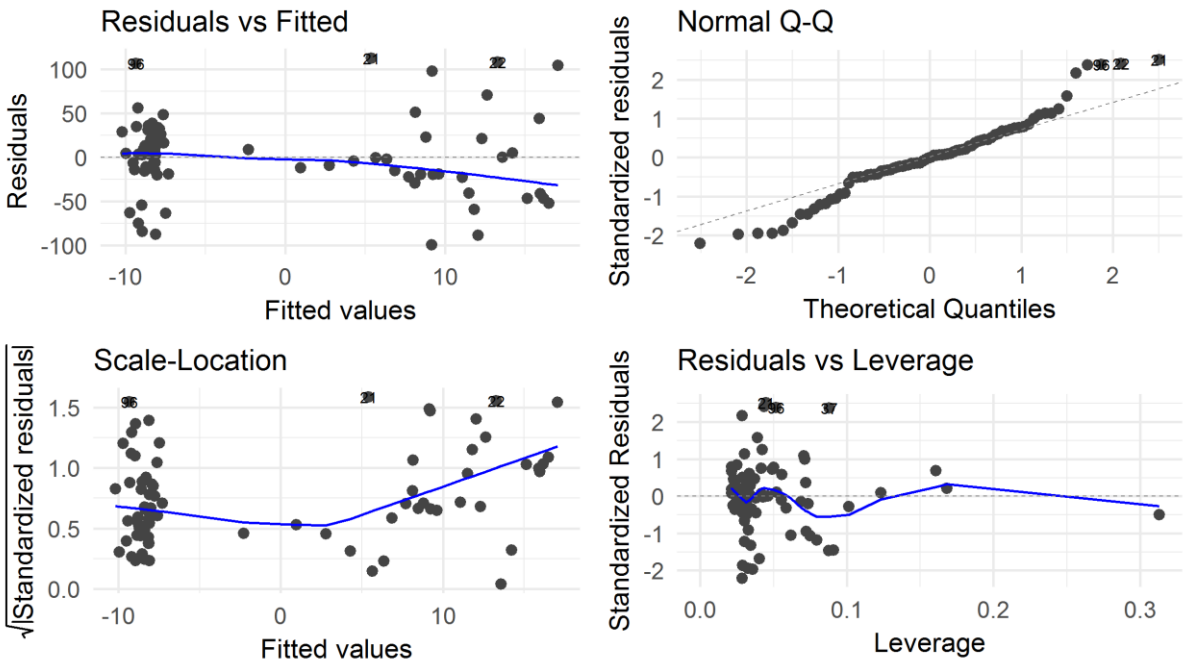
F: RLC[rETRmax] by DMSO:DMSP



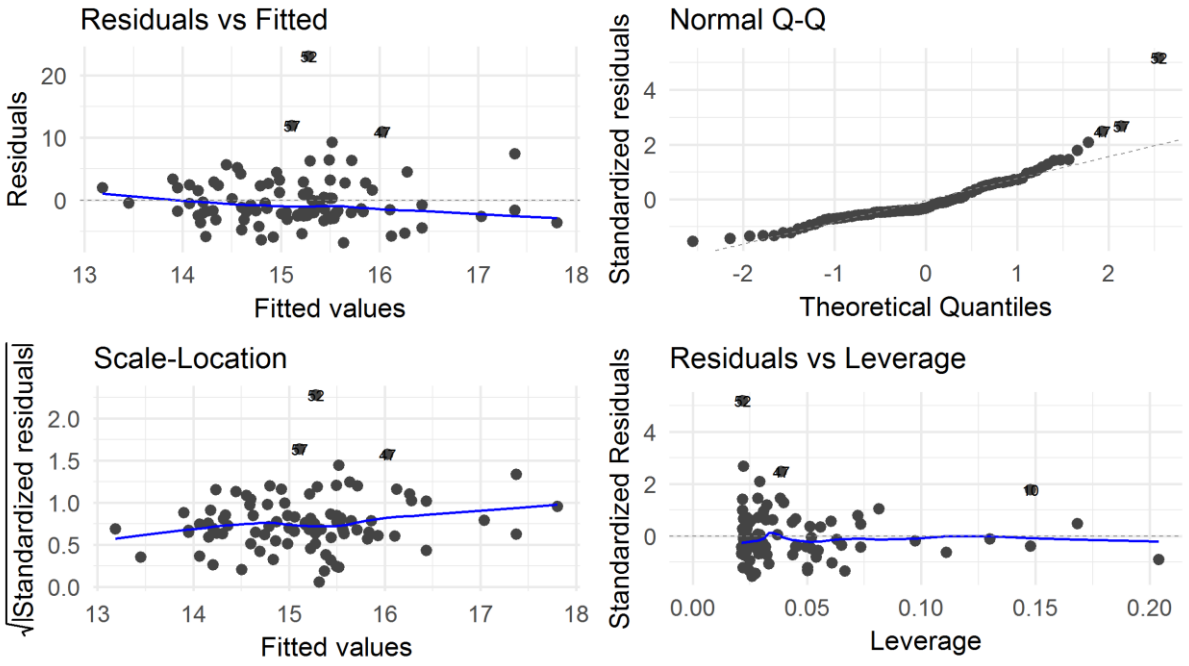
G: Tissue δ15N by DMSO:DMSP



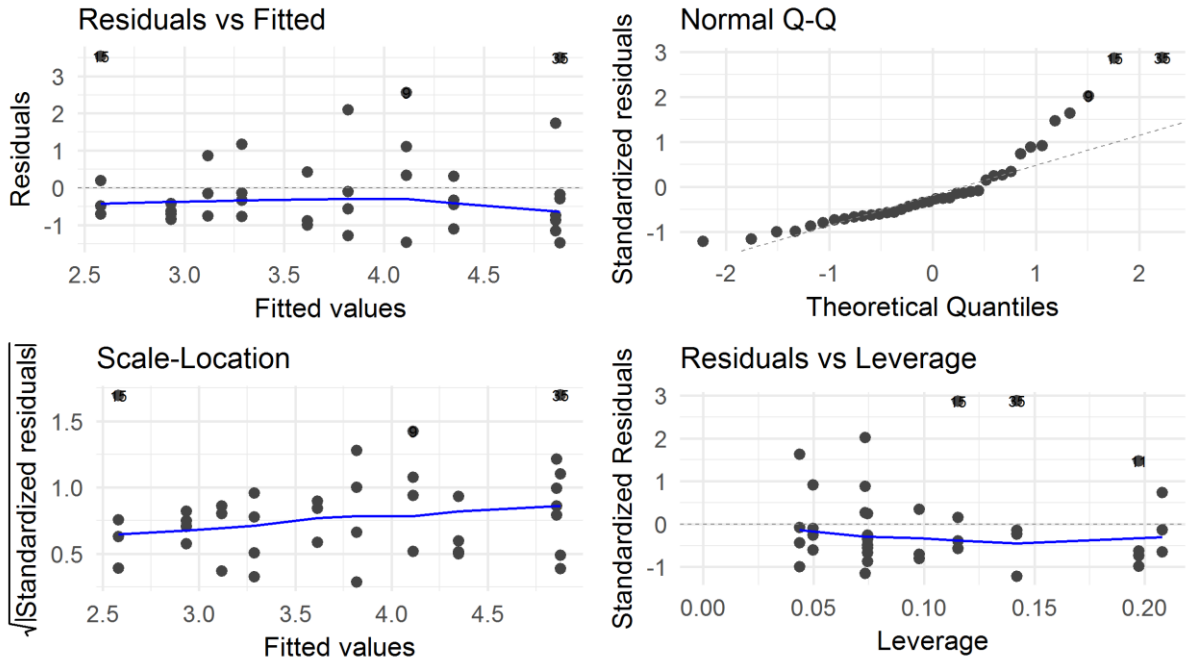
H: δ13C differential by DMSO:DMSP



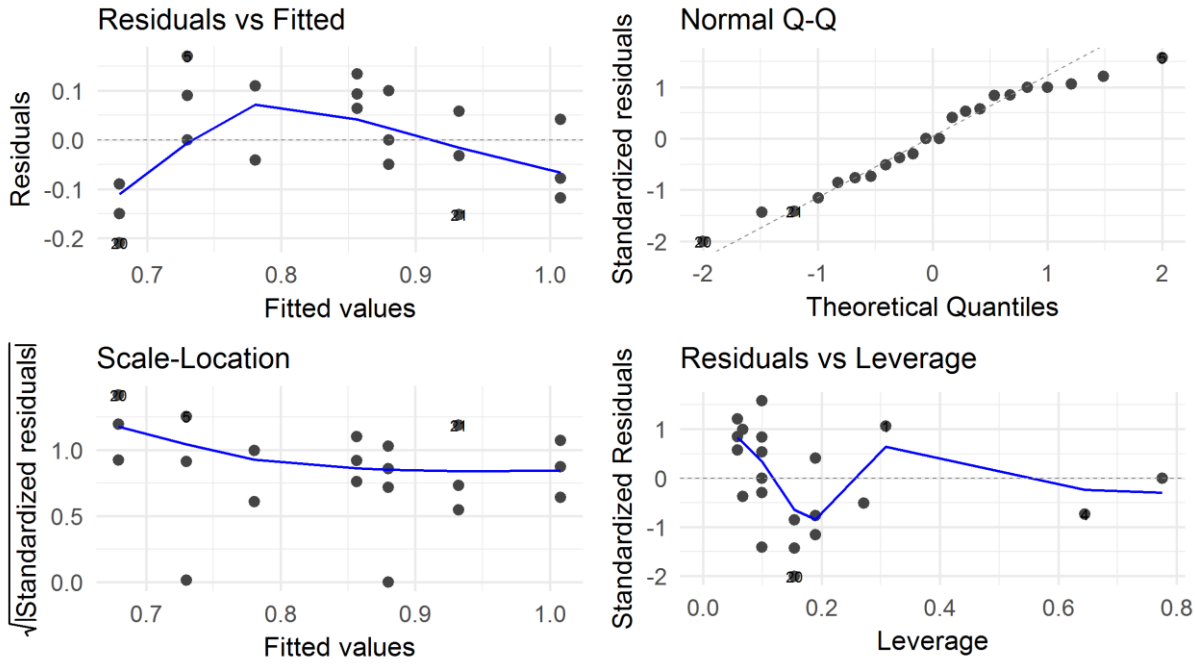
I: Incubation δDO2 by DMSO:DMSP



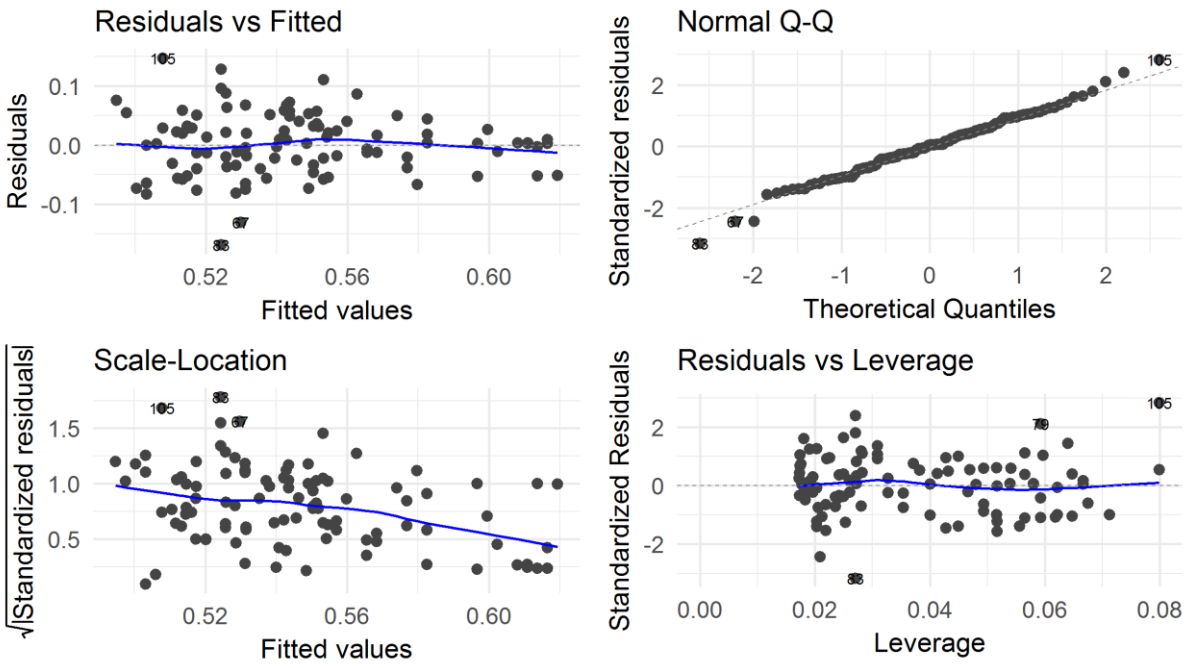
J: Tissue molar C:N by DMSO:DMSP



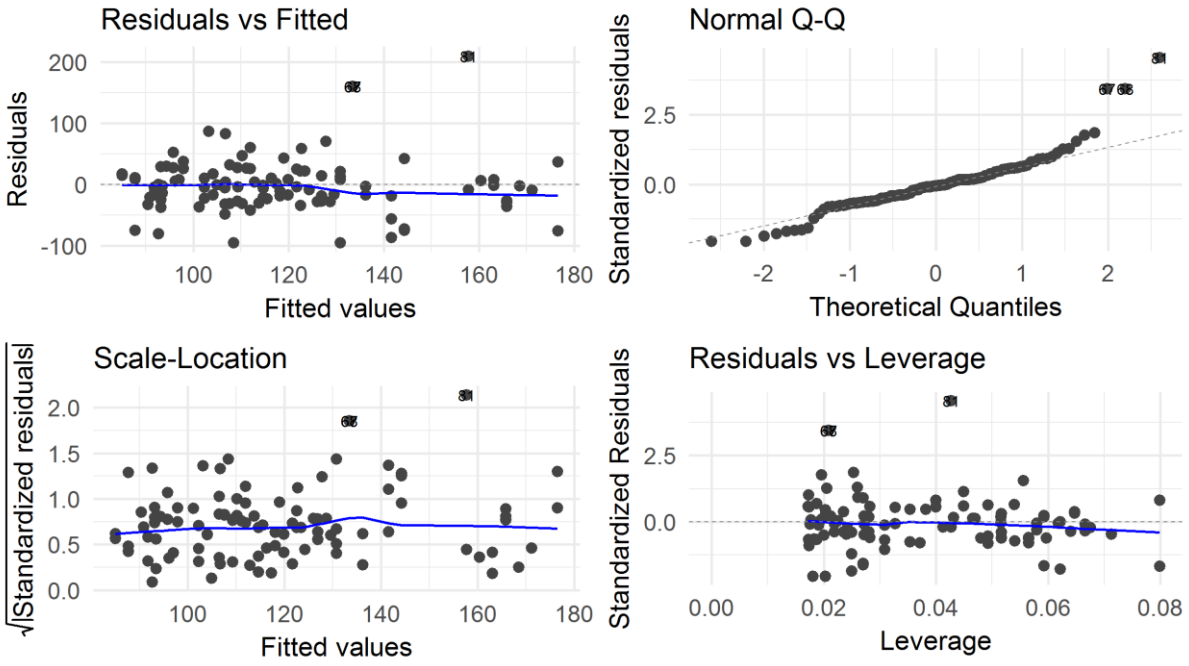
K: Sediment δ15N by Depth



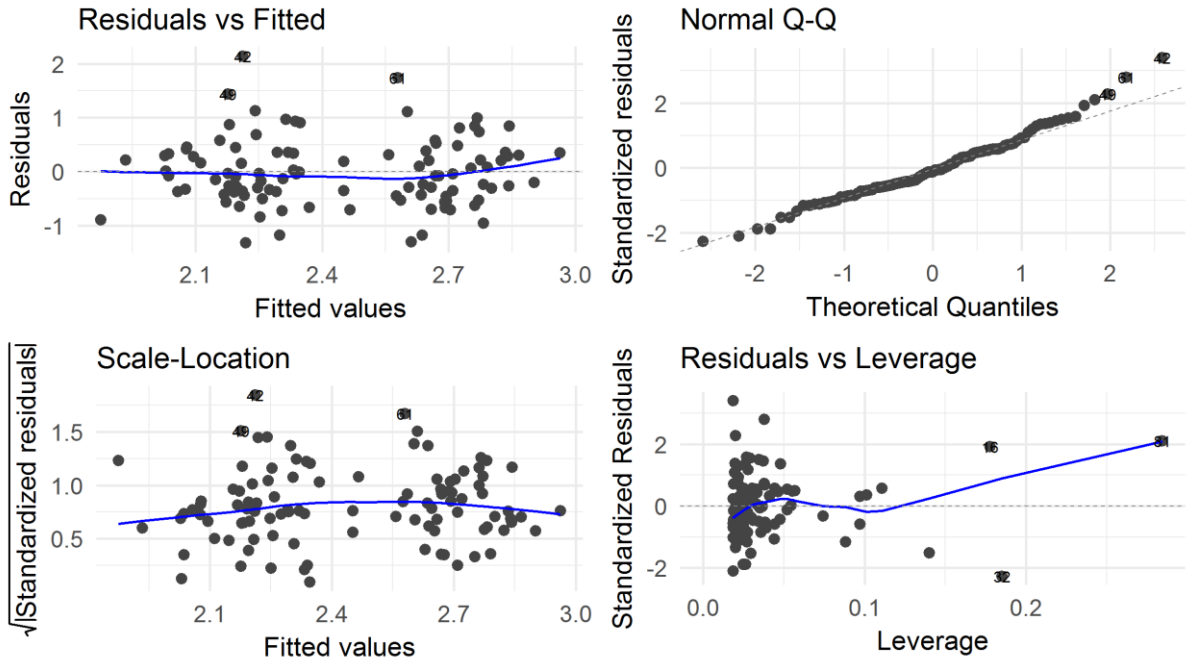
L: DIC of sea water by Depth



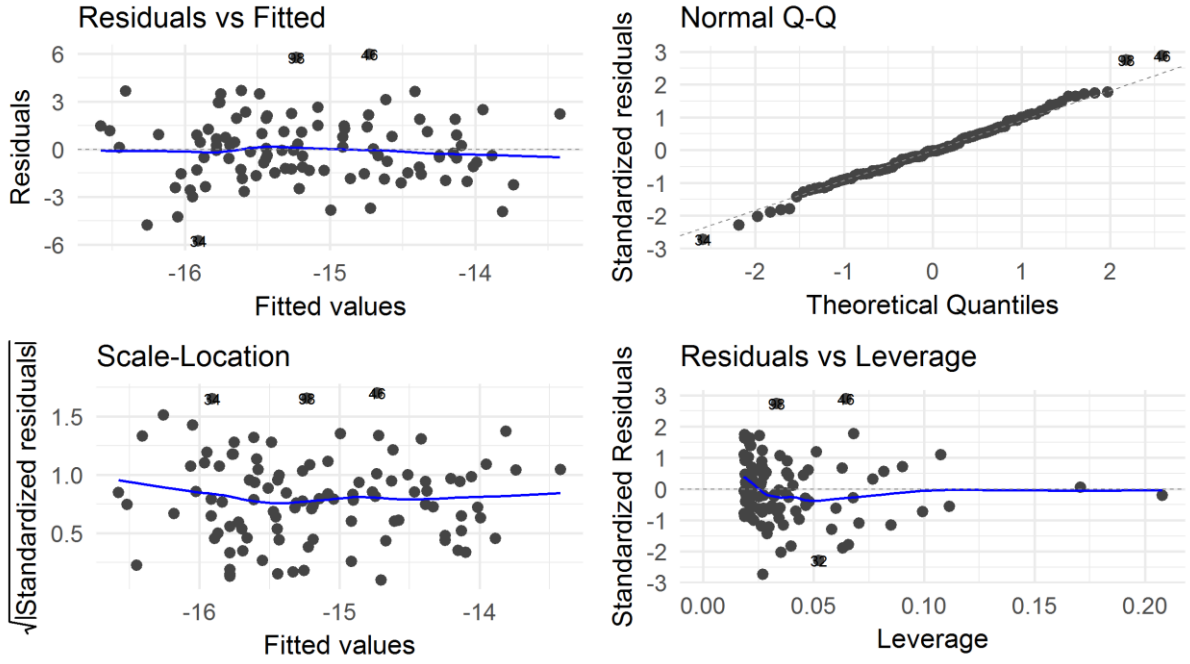
M: RLC[Alpha] by Depth



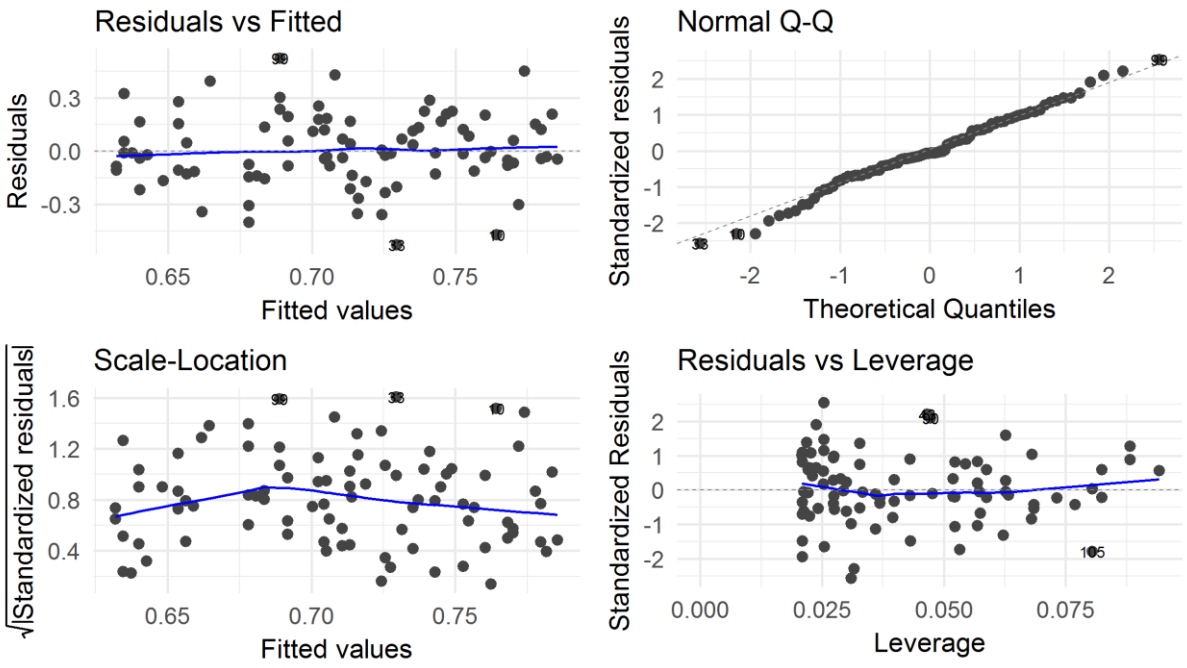
N: RLC[Ek] by Depth



O: Tissue δ15N by RLC[rETRmax]



P: Tissue δ15N by δ13C differential



Q: DMSO:DMSP by Depth

Chapter Five

Experimental Evidence for Reduced Mortality of Agaricia lamarcki on a Mesophotic Reef

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Jack H. Laverick, Alex D. Rogers

5.1 Abstract:

Mesophotic Coral Ecosystems (MCEs) may act as a refuge for impacted shallow reefs as some of the stressors affecting tropical reefs attenuate with depth. A less impacted population at depth could provide recruits to recolonise shallow reefs. Recently, disturbance has been reported on several mesophotic reefs including storm damage, biological invasions, and coral bleaching; calling into question the extent of deep-reef refuges. We report on a reciprocal transplant experiment between shallow and mesophotic reefs in the Caribbean, which occurred during a period of coral bleaching. 102 fragments of *Agaricia lamarcki* were collected down a continuous depth gradient at two sites to a maximum depth of 60m. Fragments were transplanted to either a shallow or mesophotic station at a third site, with controls. This allowed the disaggregation of the effect of the disturbance experienced during the observation period, and any potential acclimation resulting from the historical location of a fragment. Mortality and bleaching were quantitatively assessed. We found the relocation depth of a coral fragment had the strongest effect on both survival and the degree of bleaching recorded. The site a fragment was collected from, and the original collection depth, failed to explain mortality or bleaching with statistical significance. This experiment provides support for the assumption that mesophotic corals may be protected in comparison to shallow reefs, in spite of the potential effects of differing susceptibilities to stress.

5.2 Introduction:

Mesophotic coral ecosystems (MCEs) are tropical and sub-tropical reefs containing depth-generalist photosynthetic hard corals, while lacking shallow-specialist taxa (Semmler, Hoot and Reaka, 2016; Laverick, Andradi-Brown and Rogers, 2017). Broadly MCEs are thought to occur between a shallow depth limit of 30 - 40m and end at depths where light levels are too low to support photosynthetic scleractinian holobionts (Puglise *et al.*, 2009; Bongaerts *et al.*, 2010; Kahng, Copus and Wagner, 2014; Baker, Puglise and Harris, 2016b).

It has been suggested MCEs may provide refuge for species otherwise impacted on shallow reefs (Glynn, 1996). Specifically in the context of climate change this is formally referred to as the deep reef refugia hypothesis (Bongaerts *et al.*, 2010; Bridge, Hughes, *et al.*, 2013). Such suggestions are underpinned by the assumption that stressors and disturbance on shallow reefs attenuate with depth, leading to lower mortality on MCEs. Instances of corals avoiding disturbance at greater depths have been observed (Muir *et al.*, 2017). In 2013 a population of *Seriatopora hystrix* was reported at mesophotic depths, despite extirpation in the shallows after bleaching events in 1998 and 2001 (Sinniger, Morita and Harii, 2012). Similarly, the hydrocoral *Millepora intricata* recovered in Panama from the 1982 and 1997 bleaching events by exploiting a depth refuge (Smith *et al.*, 2014). The protection offered by depth has also been detected in other taxa, including fish avoiding over-exploitation rather than climate-related impacts (Lindfield *et al.*, 2015; Asher, Williams and Harvey, 2017; Kadison *et al.*, 2017).

Despite a number of supportive case studies, MCEs are still under threat from a variety of factors (D. Andradi-Brown *et al.*, 2016). Storm damage (Bongaerts, Muir, Englebert, T. C. L. Bridge, *et al.*, 2013; White *et al.*, 2013), invasive species (Lesser and

Slattery, 2011; Slattery and Lesser, 2014), and seasonal bleaching (Nir *et al.*, 2014) have been reported on mesophotic reefs. This combined with greater susceptibility to thermal (Smith *et al.*, 2016) and light-related (Gleason and Wellington, 1995) stress for deeper corals, challenges the suggestion MCEs may provide refuge to organisms present on shallow reefs. What the field lacks in a broad sense, are experimental validations.

In 2015 a long-term, factorial, reciprocal transplant experiment was established on the island of Utila, Honduras, to explore potential physiological change over time. Fragments of *Agaricia lamarcki* were collected from two mesophotic and two shallow reefs, and transplanted to a third location at different depths. Informal weekly inspections of the transplant stations showed fragments were apparently healthy. After a month had elapsed, colonies of *Agaricia lamarcki* on the reef began to bleach at shallow and mesophotic depths (Figure 5.1). This coincided with NOAA's Coral Watch programme reporting the first Degree Heating Week (DHW) of the year for Utila (Supplementary 5.1b)(NOAA-Coral-Reef-Watch, 2013). These events provided us as an opportunity to experimentally test:

Is a coral less likely to die if located at greater depths?

This is a core assumption for deep reef refuges. The experimental approach allowed us to control for the effects of acclimation and so differing susceptibilities. Transplant experiments involving changing the depths of corals have a long history (Fricke and Knauer, 1986; Rosenfeld *et al.*, 2003; Cohen and Dubinsky, 2015; Eyal *et al.*, 2015; Einbinder *et al.*, 2016), but this is the first time we are aware of a mesophotic transplant experiment coinciding with a bleaching event.



Figure 5.1 Bleaching of* Agaricia lamarcki *at mesophotic depths

During the 2015 field season, large colonies of A. lamarcki *were noticed to be partially bleached at depths of up to 35m. Image credit – Ally McDowell.*

5.3 Methods:

5.3.1 Research site-

The transplant experiment was conducted at the Coral View research centre (CV) on the island of Utila, Honduras. Though the experiment occurred at the research centre, coral material was sourced from The Maze (TMA) on the north shore, and Little Bight (LB) to the south (Figure 1.1). The research centre reef follows a spur and grove formation in the shallows, with hard substrata declining with depth until a patch reef system is reached on a silt plain at ~ 40m.

5.3.2 Experimental setup-

Fragments of *Agaricia lamarcki* were collected by a technical dive team using mixed gas closed circuit rebreathers (Hollis Prism 2, Hollis, San Leandro, California, USA) during July 2015 (Permit number: ICF-261-16). Material was collected evenly across the depth gradient with 48 fragments between 10m and 45m depth at LB and 54 fragments between 16m and 60m at TMA (Figure 5.2a); the upper and lower bounds of the depth range of *A. lamarcki* at these sites. Utilising an experimental approach instead of island-wide in-situ assessments greatly improved the feasibility of the study. The time requirement for collecting data was reduced, permitting the research team to spend more time surveying additional sites for concurrent ecological projects (Laverick, Andradi-Brown and Rogers, 2017). The design also allowed us to control for depth:site specific susceptibilities to stress during the survey period (Smith *et al.*, 2016).

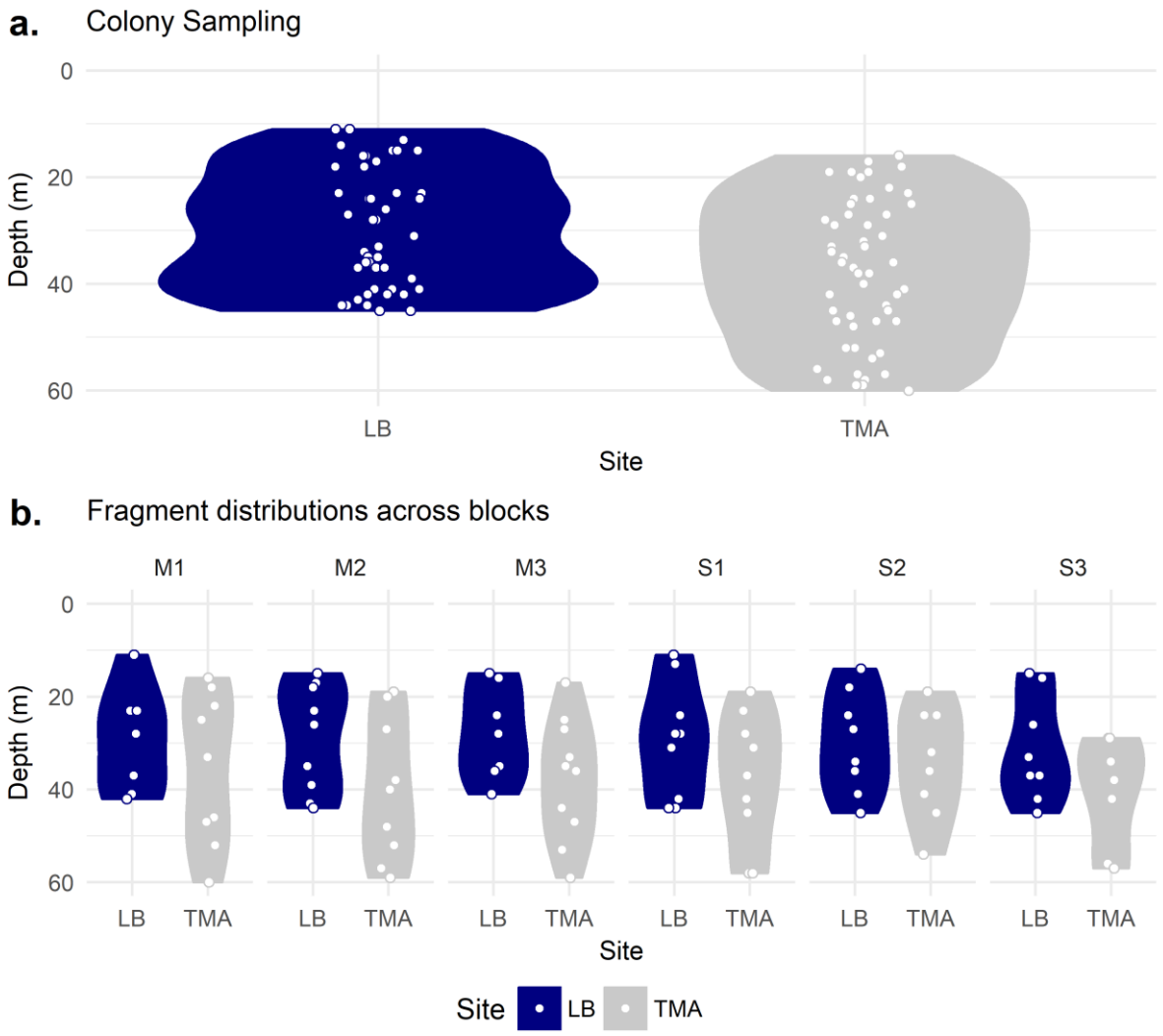


Figure 5.2 Sampling and transplant distributions of fragments

Violins represent the probability distribution of fragments occurring for a given depth based on sampling. The total coloured area sums to 1. a. Fragments were collected with even intensity from the maximum to the minimum depths of occurrence of A. lamarcki *at TMA and LB. b. Fragments were then attached to 1 of 3 mesophotic (M) or shallow (S) blocks. 48 fragments were collected and transplanted from LB and 54 from TMA. See n in Table 5.2 for the number of fragments used in statistical analyses as attrition occurred over the course of the study.*

Transplant racks were made from concrete blocks and sections of PVC tubing glued side-by-side with a hot glue gun (Figure 5.). Three racks were placed at a shallow depth (12m) and three at a mesophotic depth (37m). These depths represent the extreme ends of the depth range of *A. lamarcki* at CV (Laverick, Andradi-Brown and Rogers, 2017). Fragments were attached to transplant racks in the afternoon of their day of collection. This was done in-situ

using the same brand of pre-mixed Milliput modelling putty. Two Fragments from unique colonies per 10m depth bin, per site, were placed on each rack to ensure an even spread of material. Depth bins started from the surface and fragments were distributed in this way until the material from a site was completely used, as such a 50-60m depth bin existed for TMA but not at LB, these bins were not used in statistical analysis as it was possible to include depth as a continuous variable (Figure 5.2b.). This ensured fragments originally shallow or mesophotic were transplanted back to similar depths, therefore providing control fragments which can disaggregate the effects of acclimation and treatment. NOAA's Coral Reef Watch remote-sensing data (2013) were used to contextualise the experiment. Though note there is no guarantee satellite data that are appropriate for shallow reef monitoring will capture the stress experienced by mesophotic reefs. At the point of transplant 0 cumulative DHWs were reported for the preceding three months in the waters of Utila (Supplementary 5.1a.).



Figure 5.3 Experimental rack

Two coral fragments from each 10m depth window were placed randomly on one of three blocks, at either mesophotic or shallow depths. Each block had two racks, one for each collection site. Image credit – Ally McDowell.

5.3.3 Fragments assessments-

Formal monitoring of transplanted fragments occurred from six weeks after their placement, when bleaching was observed on the reef, and continued weekly until the end of the 2015 field season in September. Transplants were not assessed again in the following year as human interference destroyed the experimental set up. All six transplant racks were photographed in-situ with a 20 level #13 Danes-Picta greyscale card in shot. Fragment mortality was scored from photos for use in survival analyses. A fragment was considered dead when fouled. The reference card was used for quantitative bleaching assessments in Matlab (2017) using the colour correction method and script of Winters et al. (2009). The colour intensities of the red, green, and blue channels were recorded for 10 digital quadrats

of 25 pixels on each coral fragment at each time point. Digital quadrats were placed evenly across a fragment's surface to within a quadrat width of the fragment boundary, and to avoid the diagnostic white polyp mouths of *A. lamarcki*, which may affect bleaching assessments.

5.3.4 Statistical analyses-

All statistical analyses and data manipulation were conducted in the programming language R (R-Core-Team, 2013). Survival analyses were performed using the package 'survival' (Therneau and Lumley, 2017). Non-parametric log-rank tests (Mantel, 1966) were used to determine the effect of transplant depth and site on fragment survival in two univariate analyses. The effect of collection depth, as a continuous variable, on survival was determined by a Cox Proportional Hazard analysis (Cox and Oakes, 1984). The assumption of proportional hazards was assessed by regressing Schoenfeld residuals against time. Statistically significant differences in survival were visualised by Kaplan-Meier survival curves.

Quantitative bleaching assessments were performed with mixed effect linear models, using the maximum likelihood method, with the package nlme (Pinheiro *et al.*, 2017). The colour intensities in the red, green, and blue colour channels across time were response variables. Colour intensity was averaged across the 10 quadrats from each fragment at each time point as technical replicates before analysis. The general form of the model equation follows with fixed effects of treatment, site and collection depth grouped by site, then random effects of week (time since transplant) nested within ID shown in brackets permitting varying intercepts and slopes.

$$\text{Colour} \sim \text{Treatment} + \text{Site} / \text{Collection Depth} + (\sim 1 + \text{Week}|\text{ID})$$

Collection depth was nested within Site to account for differing depth ranges of *A. lamarcki* between the two sites. Fragment ID was included as a random effect to respect the chronology between specific points in the analysis, rather than fitting lines through the dataset as a whole. Grouping by site allowed us to control for the differences between the fragments collected from two sites and their different number of samples, avoiding pseudo-replication. Linearity, normality and heteroscedasticity were assessed using residual plots. Statistically significant model elements were detected with a likelihood ratio test between a model containing all elements and one omitting the variable of interest.

5.4 Results:

5.4.1 Bleaching analyses-

Transplant to a mesophotic rack appears protective against bleaching. Results of linear mixed effect models (LMEs) explaining variation in coral colour intensity are reported in Table 5.1 and depicted graphically in Figure 5.4. The residual plots for each model can be found in Supplementary 5.2. Figure 5.4a shows the effect of transplant depth on mean colour intensity in three channels across the whole observation window. Each fragment at each time point was assessed with 10 digital quadrats, which were averaged for single data points in the analysis. Row b. shows the effect of collection site, and collection depth nested within collection site to account for differing depth ranges, over the observation window. Fragments with greater intensity in the green and blue channels, and weaker in the red channel, are more severely bleached.

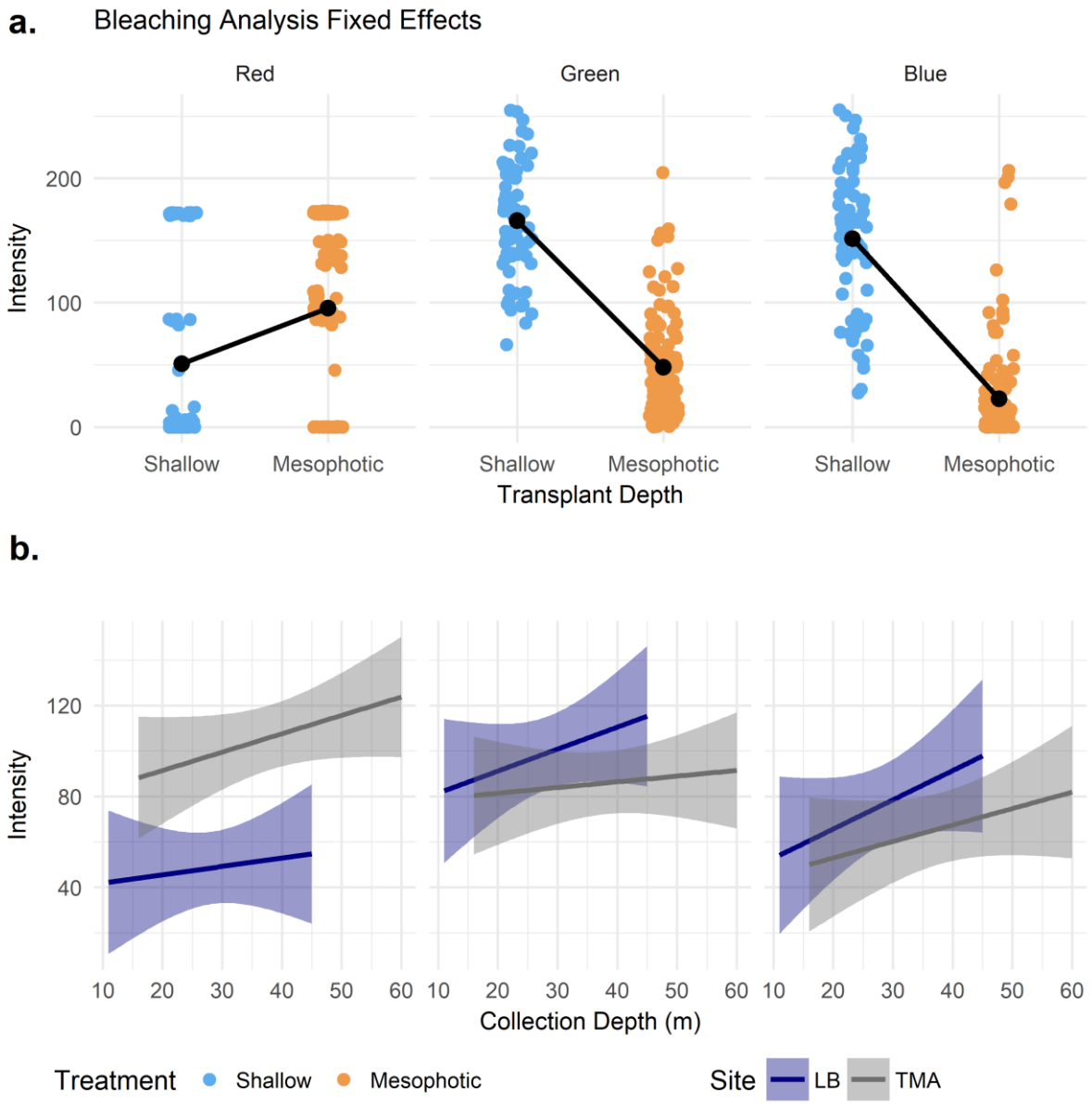


Figure 5.4 Quantitative bleaching assessments of transplanted fragments

Row a. shows the effect of transplant depth on mean colour intensity in three channels across the whole observation window. Each fragment at each time point was assessed with 10 digital quadrats, which were averaged for single data points in the analysis. Row b. shows the effect of collection site, and collection depth nested within collection site to account for differing depth ranges, over the observation window. The shaded areas represent the 95% confidence interval of colour intensity. Fragments with greater intensity in the green and blue channels, and weaker in the red channel, are more severely bleached.

Table 5.1 Bleaching analysis results

*Likelihood ratio tests of significance for fixed effects in three mixed effects linear models, each fitted to explain variation in one of three colour channels used for quantitative bleaching analysis. All 59 fragments surviving until week 8 were used. Values are reported as they appeared in computer outputs. P values < 0.05 are in bold and followed by an *.*

Bleaching Analysis	Model element	Effect		Colour
		χ^2	*P*	
Model 1	Transplant depth	8.579909	**0.0034 ***	Red
	Site	6.848727	**0.0326 ***	Red
	Collection depth	1.921921	0.3825	Red
Model 2	Transplant depth	50.43146	**<0.0001 ***	Green
	Site	0.9742721	0.6144	Green
	Collection depth	2.092902	0.3512	Green
Model 3	Transplant depth	26.13712	**<0.0001 ***	Blue
	Site	0.687022	0.7093	Blue
	Collection depth	3.721327	0.1556	Blue

5.4.2 Survival analyses-

Only transplant depth had a significant effect on the chance of a coral fragment surviving the observation period. The effect of transplant depth is visualised in Figure 5.5, while the results of univariate survival analyses are reported in Table 5.2. A coral fragment transplanted to a shallow rack had a 19 percentage point higher probability of mortality by week 8 in comparison to those on mesophotic racks. Almost one quarter of fragments transplanted to mesophotic racks died during observation, while for shallow fragments this number was closer to a half. Collection depth, and therefore any legacy effects from past acclimation to specific depths, had no significant effect on survival. Multivariate analyses to consider interaction effects were not performed as only a single variable of interest returned statistically significant results.

Table 5.2 Survival analysis results

*Values are reported as they appeared in computer outputs. P values < 0.05 are in bold and followed by an *. For transplant depth, n1= Shallow, n2= Mesophotic. For Site n1 = Little Bight n2 = The Maze. More fragments were collected at TMA as a constant density of sampling was maintained with depth, and TMA had a larger depth range for* A. lamarcki. *Collection depth was analysed as a continuous variable using a Cox Proportional Hazard model, the assumption of proportional hazards was satisfied with* χ^2*= 0.435 and P= 0.51.*

Survival Analysis	**Sample size**		**Effect**	
	n 1:	*n2:*	*Z* or χ^2	*P*
Transplant depth	42	48	5.1	**0.0235***
Site	39	51	1.2	0.273
Collection Depth	90		1.163	0.245

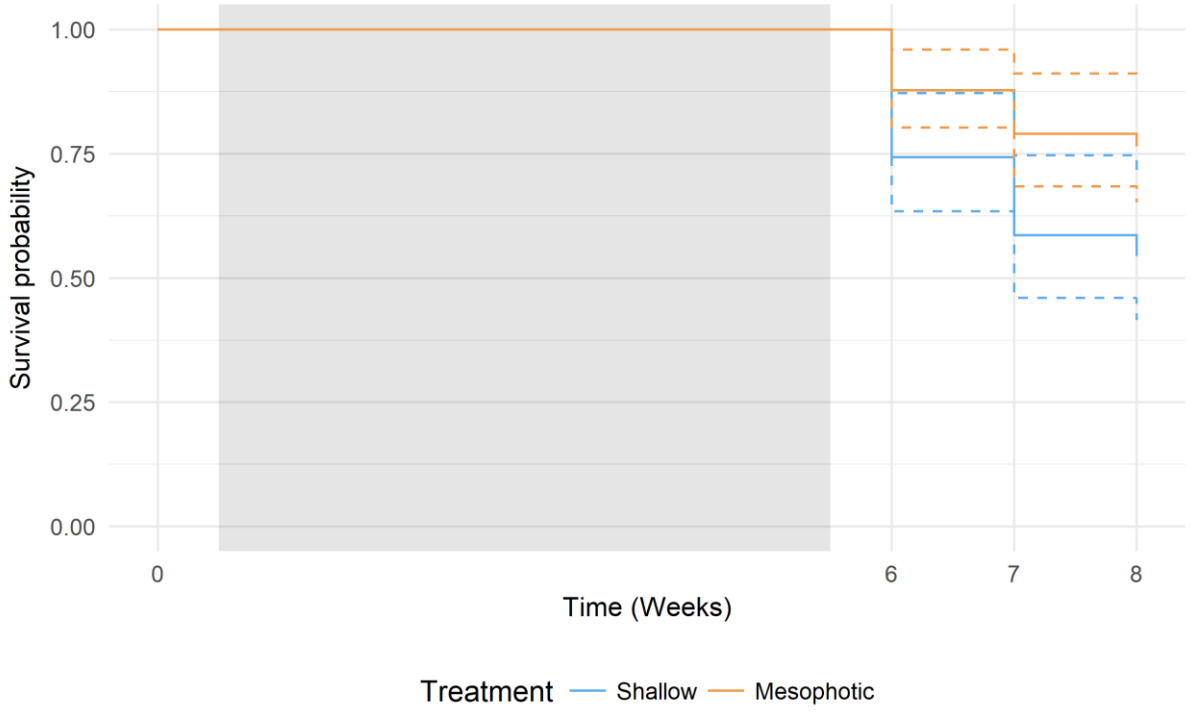


Figure 5.5 Survival curves of coral fragments by transplant depth

Survival curves of transplanted fragments from placement date with formal observations at six, seven and eight weeks later. Mortality from time of transplant to week six was not formally assessed, and is represented by the grey shading, but was sufficiently low to go unnoticed. Dashed lines represent the 95% confidence interval.

5.5 Discussion:

5.5.1 Transplanted **Agaricia lamarcki** *fragments were more likely to survive, and less likely to be bleached, at mesophotic depths-*

During the three weeks of observation after bleaching commenced, fragments on shallow transplant racks were twice as likely to be completely fouled than those at mesophotic depths (Figure 5.5). Similarly, quantitative monitoring of bleaching over the three weeks revealed high levels of coral bleaching across shallow transplant racks, with little bleaching observed at mesophotic depths (Table 5.1). The bleaching levels displayed in figure 5.4 represent completely white fragments in the shallows and paled fragments at depth. Our results corroborate reports of corals surviving at depth during bleaching events in past observational studies (Sinniger, Morita and Harii, 2012; Smith *et al.*, 2014), in spite of reported greater thermal susceptibilities in mesophotic corals elsewhere (Smith *et al.*, 2016).

It is important to note that coral bleaching and mortality can be induced by more than thermal disturbances. Coral bleaching has been reported as a response to: high light levels (Bellworthy and Fine, 2017), disease (Brown, 1997), ocean acidification (Anthony *et al.*, 2008), and chemical pollution (Danovaro *et al.*, 2008), while mortality could also result from direct physical disturbance (S.Rogers, Gilnack and Fitz III, 1983). Though Coral Reef Watch reported 2-3 DHW for Utila by our last observation (Supplementary 5.1d), indicating thermal stress in the shallows, we cannot claim to know this as the driver when multiple stresses may interact and in the absence of detailed environmental data. For this study the actual cause of disturbance is not important. A disturbance of some kind did occur and mesophotic reefs were protected for a time.

As well as relating directly to whether natural deep reef refuges can exist, our results have management implications. Current projects involving the rearing of corals outside of an aquarium setting may benefit by moving their operations deeper in times of significant heat, or other, stress. Floating nurseries, designed to accommodate water movement, may be particularly well suited to exploiting the protection of depth. These nurseries may need to be moved shallower once thermal stress abates in the shallows, to avoid delayed thermal stress at mesophotic depths (Smith *et al.*, 2016).

5.5.2 Where a fragment came from is less important than where a fragment is during stress-

Our results failed to return a statistically significant effect on survival of collection site or depth (Table 5.2). This is despite research showing these two populations of coral have differing physiological profiles (Laverick, under review) and any additional stress caused by transplantation to different depths. Unfortunately, we lack detailed in situ environmental data to help characterise the sites and transplant location. Coral Reef Watch data do show that no thermal stress had accumulated in the shallows until after the transplant experiment had occurred (Supplementary 5.1a). Further, during the course of fragment collection dive computers reported water temperatures differing by 2˚C across the depth range of *A. lamarcki*, leading us to believe that there is likely local acclimation to low light and different temperatures within sites. Even if fragments had experienced similar environments shallow and deep at their site of collection, mortality was still greater once on shallow transplant racks. It appears, in this instance, the stress and disturbance a coral fragment is subject to, is the overriding control on survival, as opposed to any past acclimation.

Interestingly our results for bleaching assessments differ slightly. Collection depth again failed to be statistically significant, as did site in green and blue channels, but significant in red. The mean effect of site in the red channel was comparable to the effect of transplant location (40-50, Figure 5.4) . It may be that the base level of pigmentation at TMA was more red than at LB, as TMA is a steep wall while LB a gentle slope. The effect of collection depth in the red channel remained the same for both sites and mortality appeared independent of collection depth.

5.5.3 Limitations-

Unfortunately, the field season ended after three weeks of observation. We are uncertain whether the trends observed will have continued as the bleaching event continued, or whether the mesophotic racks eventually bleached and died after a delay. Coral reef watch reported one additional DHW in the three months after our observation period (Supplementary 5.1e), however, we cannot know how remote sensing data will translate to stress on mesophotic reefs as heating at these depths can be delayed (Smith *et al.*, 2016). Regardless of this possibility, at a minimum we know that mesophotic corals remained healthier for longer than their shallow counterparts during our observations of a period of shallow water stress.

Though we successfully detected an effect on survival and bleaching of transplant depth, we cannot with certainty claim the bleaching is the causative agent of mortality. Additional pressures are confounded with depth such as: water movement, light levels, proximity to coastal development, and proximity to anthropogenic activity (Bridge, Hughes, *et al.*, 2013). We have been able to show that depth reduces mortality for *A. lamarcki* on Utila, but because of a lack of environmental data we are unable to explain the mechanisms

behind shallow reef mortality with certainty. In addition, reduced mortality at depth is necessary but not sufficient for the realisation of a deep reef refuge (Bongaerts *et al.*, 2010). There is a requirement of reproductive connectivity, not yet assessed on Utila, but found elsewhere (Hammerman *et al.*, 2017). We stress the distinction between refuge from general disturbance, and refugium specifically referring to climate related effects (Kavousi and Keppel, 2017).

5.6 Conclusions:

This experiment reveals that colonies of *Agaricia lamarcki* at deeper depths have a greater chance of survival in the face of shallow water stress on Utila. Further, despite reports of increased thermal sensitivity with increasing depth (Smith *et al.*, 2016), this particular Caribbean coral was less likely to bleach on mesophotic racks. The assumption for deep reef refuges, that depth affords protection, seems validated. However, meta-population dynamics will only occur if the surviving colonies at depth are reproductively active, and capable of settling in the shallows (Gleason and Wellington, 1995; Bongaerts *et al.*, 2017). Though gaps remain in understanding whether a natural deep reef refuge exists on Utila for *A. lamarcki*, protection at depth could be exploited by nursery and assisted settlement projects.

5.7 Acknowledgements:

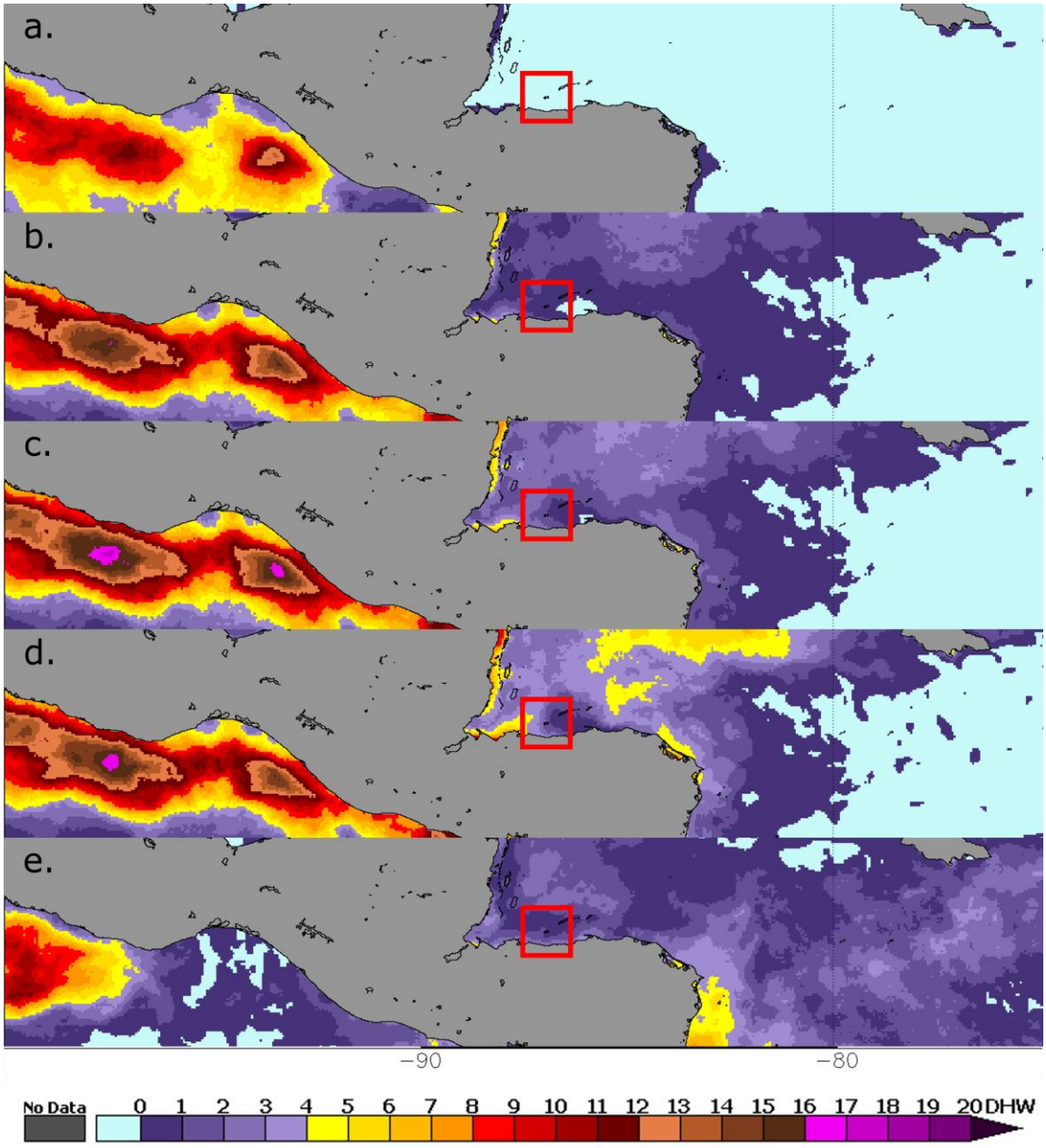
The Authors would like to thank the Royal Geographical Society (Ralph-Brown Award), the Zoological Society of London (EDB Expeditions fund), the Oxford University Expeditions Council, Oxford University (John-Fell fund), the Natural Environment Research Council (NE/L002612/1), Merton College Oxford and Operation Wallacea for funding. The authors also thank Grace C. Young and the Thinking Deep team for dive time. A final thanks to the staff of Operation Wallacea at the Coral View research station.

5.8 Authors contributions:

JHL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing

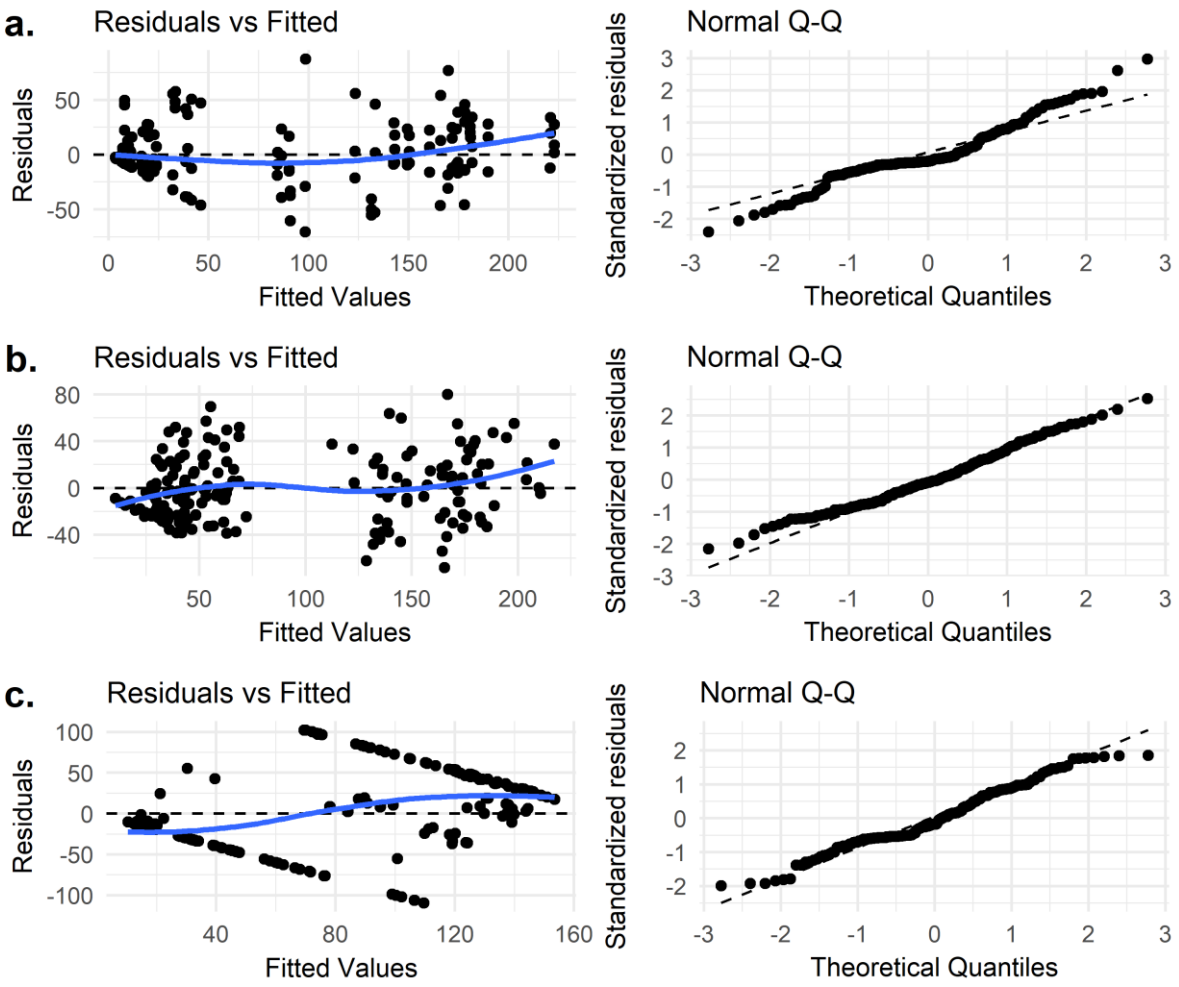
ADR: Funding acquisition, Supervision, Writing – review & editing

5.9 Supplementary information:



Supplementary 5.1 Degree heating weeks (DHW) for three months preceding transplant date

NOAA Coral Reef Watch 5km resolution maps of cumulative degree heating weeks over 3 month windows in the Caribbean. Images have been cropped and panelled to centre on Utila (red box). a. At the date of first transplant (July 2015) no heat stress had accumulated around Utila. b. One week before observation one (September), 1 DHW. c. At observation one and bleaching onset, 2 DHW accumulated. d. By observation three and the end of the study 2-3 DHW. e. Over the 3 months directly after the final observation (December 2015), 1 additional DHW accumulated.



Supplementary 5.2 *RESIDUAL PLOTS FOR BLEACHING ANALYSIS*

Residual plots for bleaching analyses in three colour channels a. Blue b. Green c. Red. Residual vs fitted plots (left) reveal deviations from linearity and heteroscedasticity. Normality is assessed by quantile plots (right).

Chapter Six

Diving Deeper

Jack H. Laverick

6.1 Synthesis:

This thesis has looked at several barriers to the existence of deep reef refuges. I assessed the degree of community overlap between shallow and mesophotic reefs, both globally with a meta-analysis (Chapter two), and in greater detail for the island of Utila, Honduras (Chapter three). Both Chapter two and three lend support to the notion that shallow taxa can be found deeper than 30m, and so may avoid shallow water disturbance. The Honduran case study further highlights the variable nature of community overlap in relation to the current 30m depth limit used to identify MCEs. I also looked into the physiology of a depth-generalist coral on the reefs of Utila to understand how these straddling species survive across the depth zones (Chapter four). Interestingly physiological profiles are site specific, but may result in similar individuals existing at either end of the species depth range. This observation highlights that the presence of individuals capable of surviving a stress event at depth will be site specific. Finally, I experimentally demonstrate this species has higher survivorship on deeper reefs in the face of disturbance, despite any variation in physiological benchmarks (Chapter five). This is a direct test of the core assumption of the DRRH, that deeper individuals are comparatively protected (Glynn, 1996; Bridge, Hughes, *et al.*, 2013). What I notably missing from this thesis is discussion of propagule exchange between depths.

In combination these chapters show some, but not all, shallow water species may be found on MCEs. Physiological profiles with depth can vary from site to site within a species and do not influence the pattern of increased survivorship with depth. This research suggests there is a chance of depth refuges existing on the reefs of Utila, though this will be site and species specific.

Depth-generalist species are more likely to survive at deeper depths in the face of disturbance than shallow-specialists as disturbance may attenuate with depth (Bridge, Hughes, *et al.*, 2013). We must remember, however, that susceptibilities may also differ down a depth gradient (Neal *et al.*, 2014; Smith *et al.*, 2015). In addition to the effect of a species depth range, the transition from a shallow to a mesophotic community can occur at different depths at different sites, sometimes by as much as 50m (Chapter three). When the transition between communities occurs particularly deep, there is a greater chance of shallow-specialist species existing at depths sufficiently large to avoid disturbance. Another caveat however, is the changes in abiotic factors which permit a deeper transition may also influence the degree of disturbance experienced at a given depth. An example of this may be when greater light penetration and a deeper upper mixed layer of water permits shallow corals to increase their lower depth bound. These factors may also increase the degree of thermal and light stress a coral is subject to for a given depth, increasing the chance of bleaching (Jones *et al.*, 1998). Despite these caveats, our initial studies suggest depth refuges are likely to exist for species with generalist depth ranges and at sites with the deepest community transitions. It is also becoming clear that MCEs are distinct ecosystems and may warrant protection in their own right.

6.2 Conflating deep reef refuges and mesophotic coral ecosystems?:

Initially, the term mesophotic was coined to bring together researchers with an interest in studying a difficult to access system. Though the definition published states a range that MCEs can begin from (30-40m), there is little to no guidance on how to apply this range (Puglise *et al.*, 2009). Consequently, most researchers use 30m as a static depth limit

(Laverick *et al.*, 2018) and the mesophotic became a depth zone (Figure 6.1a). Discussions of deep reef refuges have followed mesophotic research from the beginning. As the mesophotic was just a depth zone, language started to blur with talk of shallow species existing in mesophotic depth refuges, i.e. existing at depths, suitably large (below 30m), to avoid shallow water disturbances (Bongaerts *et al.*, 2010; Laverick *et al.*, 2018).

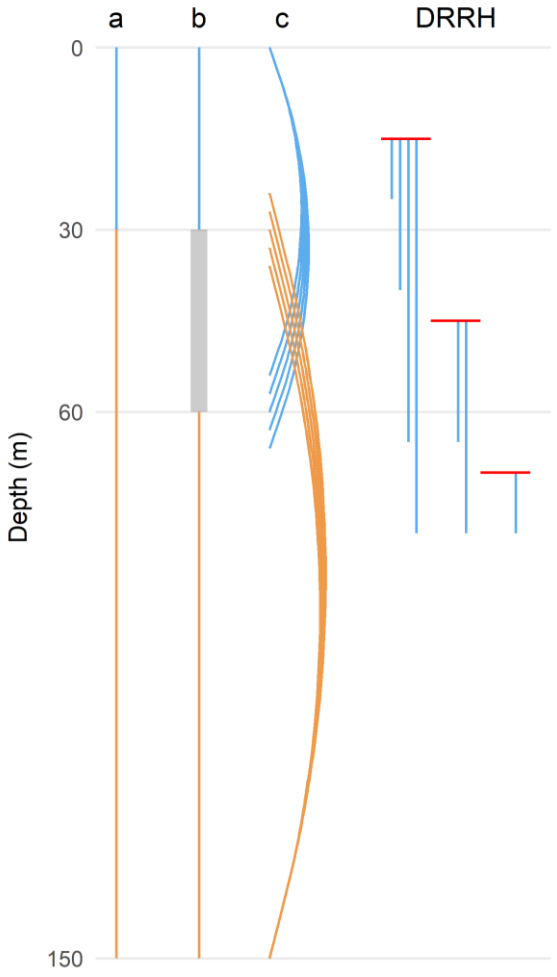


Figure 6.1 Schematic representation of the deep reef refuge hypothesis and evolving use of 'mesophotic'

Blue represents shallow zones/species depths. Orange represents mesophotic. The grey box in (b) is the area of community overlap. Curved lines in (c) represent distributions instead of a depth zones. The red lines (DRRH) indicate successively deeper impacts.

(a) Mesophotic, difficult to access reef zone (Puglise et al., 2009)

(b) Upper and Lower Mesophotic, Upper defined by community overlap, lower has unique mesophotic taxa. The '60m rule' (Kahng, Copus and Wagner, 2014)

(c) Shallow and mesophotic are two distinct community assemblages with site-specific depth distributions. (Laverick, Andradi-Brown and Rogers, 2017)

Shortly after 'mesophotic' entered the literature, a distinction was made between the upper and lower mesophotic (Kahng, Copus and Wagner, 2014). The upper mesophotic represented an area of overlap, containing some shallow species, while the lower mesophotic contained taxa with specialist adaptations to the low light environment (Figure 6.1b). The upper mesophotic became the area of interest for the DRRH.

In this thesis, and in other publications, the term mesophotic has been moved forward again to now refer to a distinct community (Figure 6.1c) found deeper than shallow reef taxa (Semmler, Hoot and Reaka, 2016; Laverick, Andradi-Brown and Rogers, 2017; Baldwin, Tornabene and Robertson, 2018; Rocha *et al.*, 2018). These communities may overlap at the limits of their depth ranges, but crucially, these depth limits are site-specific.

One important consequence of this advance, is that by definition mesophotic communities cannot protect shallow communities during a period of disturbance. There has been a tendency to reduce mesophotic reefs and research to the DRRH and the protection of shallow reefs (Baker, Puglise and Harris, 2016). We should instead get back to the original formulation of the DRRH which is concerned with shallow reef taxa existing at depths, suitably larger, to avoid shallow water disturbance (Glynn, 1996). Mesophotic communities may then be recognised as needing protection in their own right (D. Andradi-Brown *et al.*, 2016; Laverick *et al.*, 2016, 2018; Rocha *et al.*, 2018). Part of this will require researchers to identify the boundaries between, and depth distributions of, both shallow and mesophotic communities in sites of interest (Laverick, Andradi-Brown and Rogers, 2017; Laverick *et al.*, 2018).

The DRRH can then also be assessed logically as the mismatch between the depth ranges of the taxa of interest, and the depth of the impact/stressor (Figure 6.1DRRH). As the depth range of an organism increases, so too does the probability that individuals will survive at depths below a given disturbance event. Some of our current examples of deep reef refuges have happened over relatively small depth ranges (Smith *et al.*, 2014), as the combination of stress and susceptibilities limited extirpation to the very shallowest portions of the reef (<11m).

6.3 Future research:

Three broad areas of research naturally follow on from the contents of this thesis. Firstly, it is important to advance the field from documenting pattern and towards understanding the processes that shape mesophotic reefs. This can be achieved by more experimental studies, revealing the causal relationships of existing observations. Similarly uniting biological and abiotic measurements during longitudinal studies will help contextualise new findings in the field. It has been unfortunate that light data has been missing from each of the field studies in this thesis. Secondly, an important component regarding deep reef refuge dynamics, missing from this thesis, is the fecundity of mesophotic reefs and how their propagules may settle and survive on shallow reefs. Even if mesophotic individuals survive disturbance, and could survive on shallow reefs, unless they are reproductively active the life cycle won't complete at shallower depths. The third broad theme considers established ecological concepts and how they may relate to mesophotic reefs. In the absence of longitudinal studies we have just a snapshot of tropical reef ecology. In the event that a shallow reef is cleared by disturbance, questions regarding competition and succession become pertinent.

6.3.1 Experiments-

Experiments focussing on mesophotic systems are becoming increasingly common. These are largely aquaria based trials, or translocation experiments in the field. Slattery and Lesser (2011) provide an illustration of how an experimental approach may help unravel ecological process. Bahamian MCEs underwent a phase-shift to an algae-dominated system, with sponge and coral mortality following invasion by lionfish (*Pterois volitans*) (Lesser and Slattery, 2011). A shift in fish community was detected, likely due to predation by lionfish, while levels of nitrate, light, storm disturbance, and bleaching, did not appear to change

coincidently with the phase-shift. This study was shortly followed by aquaria trials exposing coral and sponge to the dominant algae from the genus *Lobophora* (Slattery and Lesser, 2014). Stable isotope and PAM fluorometry revealed there were no physiological limits excluding *Lobophora* from mesophotic reefs before the phase-shift. It appeared *Lobophora* was able to exploit allelopathy to outcompete corals and sponges already occupying space. This was only able to occur after ecological release from grazing pressure caused by lionfish predation. Only by utilising an experimental approach were Lesser and Slattery able to determine how the arrival of lionfish had caused the shift in mesophotic benthic communities.

Additional experimental work on mesophotic reefs has yielded multiple findings. Sponges facultatively express chemical defence against higher grazing pressures on shallow reefs (Slattery *et al.*, 2015). High grazing dominates bioerosion on shallow reefs while sponges primarily erode mesophotic reefs (Weinstein, Smith and Klaus, 2014). Agariciid coral growth rates may be comparable across at least a 60m depth gradient in the Caribbean (Bongaerts, Frade, *et al.*, 2015). Seasonally bleached corals may actually be better able to satisfy their calorie demand (Nir *et al.*, 2014). Adaptive photosynthetic characteristics may improve survival at mesophotic depths (Einbinder *et al.*, 2016; Eyal *et al.*, 2016; Smith *et al.*, 2017) and could even prevent survival on shallow reefs (Eyal-Shaham *et al.*, 2016).

A revealing strand of research, to further our understanding of the Honduran study system, would be quantifying the light limits tolerable to different coral species identified in chapter three in an aquaria setting. This information could be combined with year-long light

profiles with depth at the survey sites to see whether light drives the variations in community transition.

6.3.2 Completing the life cycle-

As already reviewed during the introduction of this thesis, the fecundity of mesophotic reefs is a crucial area of research needed to understand deep reef refuges. Unless mesophotic individuals are reproductively active, and the offspring survive in shallow water until reproductively active themselves, observations of reduced mortality and community overlap at depth mean little for future shallow reef survival. This thesis initially intended to explore this question, yet we did not recover enough coral tissue to perform population genetics as well as stable isotope analyses (Chapter four). As part of the Thinking Deep expedition to Utila in 2015 coral spawning was observed in the shallows. Unfortunately risk assessments prevented us diving at night on mesophotic reefs, causing us to rely on spawn traps. The design failed to retain spawn on all reefs, leaving us unable to comment on reproduction at mesophotic depths. In spite of this, other studies are beginning to show that MCEs may be reproductively active (Vize, 2006; Daniel M Holstein *et al.*, 2015; Holstein, Smith and Paris, 2016). Though observations of spawning may miss important detail, such as phenological mismatch across depth zones, reduced fecundity, and reduced gamete size with increasing depth (Shlesinger *et al.*, 2018).

In addition to the production of offspring on mesophotic reefs, equally important are studies considering shallow water settlement and survival of mesophotic propagules. Genetic differentiation over depths as small as 10m indicate little exchange of recruits between depth bands in the Caribbean (Serrano *et al.*, 2014). Further research quantifying whether planulae from different depths exhibit a bias in the depths they swim too when

settling on reefs, or whether the survival of planulae and recruits is maximised by assortative settlement (Gleason and Wellington, 1995; Babcock and Mundy, 1996), will help close the life cycle across depths. Though these studies would first require us to successfully catch and rear spawn, they would finish what this thesis started.

6.3.3 Broader ecological theory-

If we are considering mesophotic depth refuges for species found on shallow tropical reefs it becomes logical to consider the forces driving current community structure and ecological succession. With our current snapshot of coral reef communities we do not know whether the shallow-mesophotic transition is caused by biotic processes such as competition, or by community level responses to abiotic factors such as physical disturbance and light. These are directly relevant to deep reef refuges. If abiotic processes dominate, mesophotic taxa are unlikely to migrate to the shallows as space becomes available after disturbance. If competition primarily causes the distinction between the two communities then shallow reefs may be replenished by mesophotic taxa. This would require that mesophotic taxa are able to effectively compete for empty space against other benthic functional groups, such as fleshy macroalgae.

Past studies considering succession on coral reefs often failed to breakdown their results by species (Woodley *et al.*, 1981; Grigg, 1983; Done, 1992; Doropoulos *et al.*, 2017). Generally however, the apparent pattern follows rapid colonisation by macroalgae, before a transition to red algae and eventually coral recruits. Specifically for corals it may be expected that differing species are able to outcompete each other. This raises the possibility that once corals settle and grow on the reef there may be multiple seral stages reflecting different coral communities. In the absence of a species breakdown for coral reef succession

could it be that depth-generalist species found on mesophotic reefs settle first, before being outcompeted by corals of more robust growth forms? These opportunistic species with lower calcification demands due to higher surface area to volume ratios (Dustan, 1975; Kahng, 2013) may then be able to persist on patches of 'poor-quality' mesophotic reef after being driven to low densities in the shallows until the next bout of disturbance.

Hard coral species typically found on Caribbean mesophotic reefs have a rather stable rate of growth with depth in comparison to shallow species (Hubbard and Scaturo, 1985; M. Huston, 1985). Though mesophotic individuals grow more slowly than shallow corals (Dullo, 2005), this may be offset by the propensity for some mesophotic taxa to grow as encrusting patches when newly settled or in shallow waters (Helmuth and Sebens, 1993). Such fast spreading patches may help consolidate a damaged reef matrix before more robust massive growth form coral colonies outcompete over longer time scales.

6.4 Out of sight out of mind:

Just as we start to research MCEs in earnest, asking whether they provide deep reef refuges, it is clear these ecosystems are increasingly coming under threat (D. Andradi-Brown *et al.*, 2016). Storm damage (Bongaerts, Muir, Englebert, T. C. L. Bridge, *et al.*, 2013; White *et al.*, 2013), invasive species (Lesser and Slattery, 2011; Slattery and Lesser, 2014), seasonal bleaching (Nir *et al.*, 2014), and damaging exploitation (D. Andradi-Brown *et al.*, 2016) are all known to occur on MCEs and can cause long-term changes in community structure (De Bakker *et al.*, 2016). While the shallow reef research community has proven itself capable of galvanising conservation efforts, one of the greatest challenges facing mesophotic reefs is their relative obscurity. It is increasingly clear that in order to generate momentum the

public and policy makers must be made to care. This is difficult when most people have never heard of, let alone seen, a mesophotic reef.

Researchers are waking up to this, with high-quality and high profile public engagement appearing from a number of avenues. Mesophotic reefs were mentioned during the 2016 Attenborough Great Barrier Reef documentary. The release of 'Mesophotic Coral Ecosystems: a Lifeboat for coral reefs?' (Baker, Puglise and Harris, 2016b), a UNEP report considering MCEs and the deep reef refugia hypothesis, was publicised by the Huffington post and IFLScience.com amongst others. The 2016 Nekton mission (*Mission I: XL Catlin Deep Ocean Survey, NW Atlantic*, 2016), which sampled MCEs around Bermuda, found international exposure through interviews with Sky News, the BBC, and New Scientist. Cal-academy are also worth a mention for their prolific string of short science communication videos, and their 2016 Twilight zone exhibit displaying fish species brought to the surface in purpose-built miniature recompression chambers (*Twilight Zone: Deep Reefs Revealed*, 2016). These science communication efforts will help bring stunning imagery of reefs, far deeper than most people will ever go, to the public. We can then hope the technical research carried out by scientists may lead to protection for MCEs, and incidentally benefit shallow reefs under threat.

Appendix

Additional work completed during the course of this DPhil:

1) Andradi-Brown D, **Laverick J**, Bejarano I, Bridge T, Colin PL, Eyal G, Jones R, Kahng S, Reed J, Smith T, Spalding H, Weil E, Wood E (2016) *'Threats to mesophotic coral ecosystems and management options'*. In: Baker EK, Puglise KA, Harris PT (eds) Mesophotic coral ecosystems – a life boat for coral reefs? The United Nations Environment Programme and GRID-Arendal, Nairobi and Arendal.

2) Chaired by Gideon Henderson (2017). *'Future ocean resources: Metal-rich minerals and genetics – evidence pack'*. The Royal Society

3) Andradi-Brown, D. A., Gress, E., **Laverick, J. H.,** Monfared, M. A. A., Rogers, D. A., Exton, D. A. (2017). *'Wariness of reef fish to passive diver presence with varying dive gear type across a shallow to mesophotic depth gradient'*. Marine Biological Association of the United Kingdom

4) Project lead Henry Green (2018). *'Foresight Future of the Sea: A report from the Government Chief Scientific Adviser'*. Government Office for Science

5) Turner, J. A., Andradi-Brown, D. A., Gori, A., Bongaerts, P., Burdett H. L., Ferrier-Pagès C., Voolstra C.R., Bridge T., Costantini F., Gress E., **Laverick J. H.**, Loya Y., Goodbody-Gringley G., Rossi S., Taylor M. L., Viladrich N., Voss J., Weinstein D. K., Williams J., Woodall L. C., Eyal G. (2019). '*Twenty-Four Key Questions for Mesophotic Ecosystem Research and Conservation'*. In: Mesophotic Coral Reefs of the World. Springer

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