FISH ECOLOGY OF MESOPHOTIC CORAL ECOSYSTEMS

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It is a curious situation that the sea, from which life first arose should now be threatened by the activities of one form of that life. But the sea, though changed in a sinister way, will continue to exist; the threat is rather to life itself.

Rachel Carson, The Sea Around Us

As we know, there are known knowns; there are things we know we know.
We also know there are known unknowns; that is to say we know there are some things we do not know.
But there are also unknown unknowns – the ones we don't know we don't know.

Donald Rumsfeld

When we actually come to examine the question, we find that we do not know where shallow-water faunas end and the deep begin, or whether they gradually merge into one another.

J. Stanley Gardiner & C. Forster Cooper,
The Percy Sladen Trust Expedition to the Indian Ocean in 1905

DECLARATION

I declare that the work presented here is my own, performed under the supervision of Prof. Alex D Rogers and Dr. Dan A Exton. All data chapters have been prepared as scientific journal articles, and are published, in review, or will shortly be submitted. While these articles include co-authors, I have designed each study, conducted the fieldwork (with the exception of Chapter 6 where I gathered the data from a variety of sources), performed the statistical analysis and written each data chapter. Any data used in analysis that I did not collect myself has been acknowledged within the relevant chapter. Co-author involvement has been limited to assisting with field data collection or video analysis, and providing comments on draft manuscripts when being submitted for review. I have clearly indicated and referenced where I have quoted and discussed others' work in the document. In addition I can confirm this thesis has not been submitted for any other qualification at any institution.

I hereby confirm this thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Mesophotic coral ecosystems (MCEs; reefs 30-150 m depth) are largely unstudied. This thesis uses the shallow reef to MCE depth gradient around Utila, Honduras, to address research questions in three themes: (i) understanding fish community ecological processes on MCEs; (ii) evaluating effective survey techniques for MCE fish research; and (iii) exploring the role of MCEs in the western Atlantic lionfish invasion. Around Utila, herbivorous reef fish declined with increasing depth, but remained present on MCEs, suggesting a possible role in structuring mesophotic benthic communities. To test this I artificially excluded fish from areas of the reef with controls for changing light levels. The results indicate strong effects of light availability on MCE hard coral, macroalgal and sponge coverage, while little detectable effects of fish exposure. Fish surveys play a crucial role in informing reef management, yet few studies consider how biases in survey techniques varies across depth gradients. I explored differences between baited-remote underwater video (BRUV) and diver-operated video (DOV) finding BRUVs consistently recorded more species regardless of depth, but that DOV is likely better for surveys of herbivores. I also assessed fish responses to divers using open-circuit SCUBA or closed-circuit rebreathers (CCR) and, while both recorded similar fish abundances, CCR divers were able to approach fish more closely. In addition, I conducted a meta-analysis identifying widespread invasion of western Atlantic MCEs by Indo-Pacific lionfish, with similar relative abundance distributions across the depth gradient to native range sites. Around Utila, MCE lionfish occurred at greater densities than on shallow reefs, with MCE individuals larger and more mature than their shallow counterparts. This suggests deeper lionfish populations may represent an extension of ontogenetic migrations, and act as a disproportionately large source of new lionfish recruits. Overall, this thesis provides insights applicable to the western Atlantic region more generally and highlights the need for MCEs to be considered by reef managers.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIC	Akaike information criterion
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BICA	Bay Islands Conservation Association
BRUV	Baited Remote Underwater Video
CAP	Constrained analysis of principal coordinates
CCR	Closed-circuit rebreather
DDRH	Deep reef refugia hypothesis
DOV	Diver operated video
DSMB	Delayed surface marker buoy
FID	Flight initiation distance
GLM	Generalized linear model
ICF	Instituto de Conservación Forestal
LED	Light emitting diode
MAD	Minimum approach distance
MANOVA	Multivariate analysis of variance
MBRS	Mesoamerican Barrier Reef System
MCE	Mesophotic coral ecosystem
MPA	Marine protected area
NMDS	Non-metric multidimensional scaling
OC	Open-circuit
PERMANOVA	Permutational multivariate analysis of variance
SCUBA	Self contained underwater breathing apparatus
SVS	Stereo-video system
THSD	Tukey's honest significant difference

LIST OF ACCEPTED PUBLICATIONS FROM THIS THESIS

1. **Andradi-Brown DA**, Gress E, Wright G, Exton DA, Rogers AD (2016) Reef Fish Community Biomass and Trophic Structure Changes Across Shallow to Upper-Mesophotic Reefs in the Mesoamerican Barrier Reef, Caribbean. *PLoS ONE 11(6): e0156641*

2. **Andradi-Brown DA**, Macaya-Solis C, Exton DA, Gress E, Wright, G, Rogers AD (2016) Assessing Caribbean shallow and mesophotic reef fish communities using baited-remote underwater video (BRUV) and diver-operated video (DOV) survey techniques. *PLoS ONE 11(12):e0168235*

3. **Andradi-Brown DA**, Gress E, Laverick JH, Monfared MAA, Rogers AD, Exton DA. (2017) Wariness of reef fish to passive diver presence with varying dive gear type across a coral reef depth gradient. *Journal of the Marine Biological Association of the United Kingdom doi: 10.1017/S0025315417001278*

4. **Andradi-Brown DA**, Vermeij MJA, Slattery M, Lesser M, Bejarano I, Appeldoorn R, Goodbody-Gringley G, Chequer AD, Pitt JM, Eddy C, Smith SR, Brokovich E, Pinheiro HT, Jessup ME, Shepherd B, Rocha LA, Curtis-Quick J, Eyal G, Noyes TJ, Rogers AD, Exton DA (2017) Large-scale invasion of western Atlantic mesophotic reefs by lionfish potentially undermines culling-based management. *Biological Invasions 19:939-954*

5. **Andradi-Brown DA**, Grey R, Hendrix A, Hitchner D, Hunt C, Gress E, Madej K, Parry R, Régnier-McKellar C, Jones O, Arteaga M, Izaguirre A, Rogers AD, Exton DA (2017) Depth-dependent effects of culling – do mesophotic lionfish populations undermine current management? *Royal Society Open Science 4:170027*

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1 INTRODUCTION

1.1 Introduction

Mesophotic coral ecosystems (MCEs) are light-dependent communities found in tropical and sub-tropical waters from 30-40 m depth down to the limit of light-dependent corals (normally 150 - 200 m) (Hinderstein et al. 2010), sometimes referred to as the "Twlight Zone" because of their low light conditions (Fricke and Knauer 1986). Commonly they are sub-divided into the upper-MCEs (30–60 m) and lower-MCEs (>60 m), and support rich benthic communities of corals, sponges and algae (Figure 1.1) (Hinderstein et al. 2010; Loya et al. 2016). The upper boundary was originally defined anthropocentrically based on the limits of recreational open-circuit SCUBA diving (Kahng et al. 2010), but studies are increasingly recognising a shift in species composition in the 30–40 m depth range from a shallow to deeper reef community (Hinderstein et al. 2010). The variable nature of the lower boundary reflects the natural variation in ecological and physical parameters such as light penetration between different geographical locations (Smith et al. 2010), which restricts the maximum depth of zooxanthellate corals (Bongaerts et al. 2010). Armstrong & Singh (2012) observe that on the mesophotic reefs of Puerto Rico, while temperature and light availability are important for MCE communities to establish, often it was the channelling of sediment at depth that separated highly developed MCEs from poorly established ones at very close proximity. MCE presence and absence can often be predicted based on levels of sediment and availability of types of substrata (Beaman et al. 2012; 2016).

Approximately three quarters of shallow coral reefs (<30 m) are threatened by both global and local stressors (Burke et al. 2011). Yet the natural depth distributions of many shallow reef species extend onto MCEs (Bongaerts et al. 2010; Kahng et al. 2010). Despite this, most research on tropical reefs has been limited to those shallower than 20 m (Menza et al. 2008). However, recent work suggests including these deeper zooxanthellate reefs in surveys can nearly double total reef area in some regions (Harris et al. 2013). The diversity and ecological role of these deeper reefs is poorly understood, but they are likely to be faced by many similar threats as their shallow counterparts.

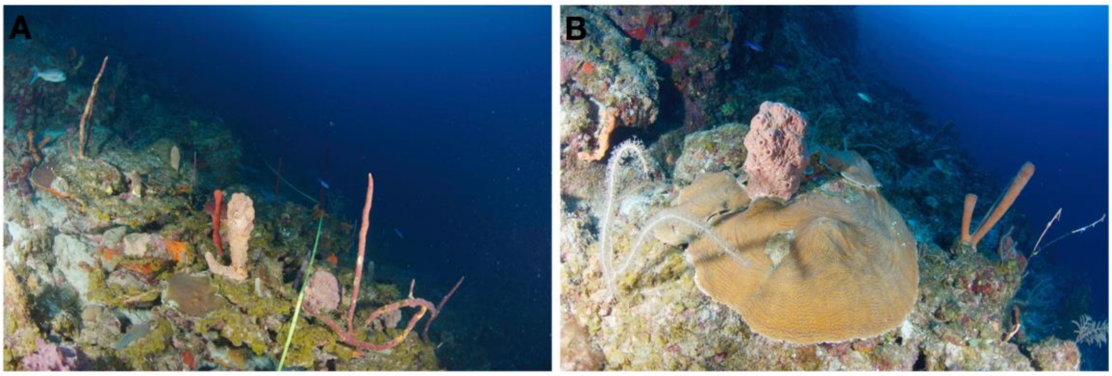

Figure 1.1 MCEs at 55 m off Utila, Honduras. Photos: Ally McDowell

MCEs are often connected to adjacent marine systems, most commonly existing as extensions of shallow-reef communities down the depth gradient (Slattery et al. 2011). The few studies conducted suggest patterns of vertical connectivity between shallow reefs and MCEs are species and location specific (Slattery et al. 2011; Kahng et al. 2014). Approximately 25 % of shallow Caribbean coral species have been recorded on upper-MCEs (Bongaerts et al. 2010; Bridge et al. 2013)**,** yet within species depth partitioning has been observed in several examples from the Caribbean (Bongaerts et al. 2013a) and Hawaii (Luck et al. 2013)**.** The importance of understanding connectivity has been highlighted by recent calls to integrate MCEs into ecosystem-scale reef resilience management practices and conservation plans (Bridge et al. 2013).

Existing reef studies are highly depth biased because of the safety and logistical challenges of working at depth (Pyle 1998; 2000). Menza et al. (2008) conducted a literature search for articles on corals, and randomly selected papers and examined the depth distributions reported. When classifying articles reporting coral health in 5 m depth intervals across the depth gradient, their results show 87 % of coral health research has been restricted to <30 m depth. More recently, there has been renewed interest in MCE research, as technical diving has become more popular and established within diver training programs (Mitchell and Doolette 2013), making it easier for researchers to access MCEs. This has led to large increases in the number of MCE publications, from <5 in 2008 to >30 in 2015 (Loya et al. 2016).

1.1.1 Early MCE fish work

Until the 1960s species diversity was thought to decline rapidly with depth (Vinogradova 1962), but following the advent of deep-sea benthic biology far greater species richness began to be recognised in deeper locations (Grassle 1991). Early mesophotic work, dependent on remote sampling, adopted many of the techniques used by deep-sea biologists (Pyle 1998). Wenner (1983) used trawls on shallow reefs and MCEs to survey fish populations, finding that locations with the greatest coral and sponge abundance had the greatest fish diversity. Goreau & Graham (1967) used dredges to map benthic changes down depth gradients but, while this work did yield samples, it was highly destructive to reef habitats.

Submersibles were used for the first in situ MCE surveys of reef fish (e.g. Strasburg et al. 1968; Colin 1974; Parker and Ross 1986), and remained in regular use into the 1990s because of their ability to extend depth and time limits associated with open-circuit SCUBA (Pyle and Chave 1994; Pyle 1998). However, because of the high costs associated with operating submersibles, their use on MCEs has been limited. Pyle (1998) found that of 133 marine ecology studies published using submersible-collected data, fewer than 5 % were on habitats less than 150 m. Of these limited studies looking at MCEs, few looked at fish communities. For example, Strasburg et al. (1968) investigated tuna populations in Hawaii, while Colin (1974) used a submersible in Jamaica and Belize to generate the first fish abundance data down the shallow to mesophotic depth gradient. In the following decades most MCE submersible work was focused around a few locations, such as; Hawaii (e.g. Brock and Chamberlain 1968; Randall et al. 1985; Ralston et al. 1986; Moffitt et al. 1989; Pyle and Chave 1994), Jamaica (e.g. Lang 1974; Itzkowitz et al. 1991), the Bahamas (e.g. Colin 1976), Florida and the Carolinas (e.g. Wenner 1983; Parker and Ross 1986; Shinn and Wicklund 1989) and Enewetak Atoll, Marshall Islands (e.g. Colin et al. 1986; Thresher and Colin 1986; Hillis-Colinvaux 1986). These early submersible-based MCE studies greatly expanded the known range for many reef fish (Ralston et al. 1986; Quattrini et al. 2004), as well as discovering that many shallow reef fish species exist down to much greater depths than expected, for example at Johnson Atoll (Brock and Chamberlain 1968; Randall et al. 1985), the Hawaiian Islands (Strasburg et al. 1968) and the Cook Islands and Papua New Guinea (Pyle 1998).

While these early studies showed it was possible to survey fish communities from submersibles, they were far from ideal. Thresher & Colin (1986) state: ‘It was clearly impossible to accurately assess (and in many cases even see) the small and/or cryptic fishes, such as gobies, blennies and small pomacentrids, that are well-documented to abound on coral reefs. Conclusions drawn from the present study, therefore, must relate principally to the larger species present on the reef.’ Strasburg et al. (1968) in Hawaii reported observing new fish species that were unable to be officially documented as sample collection was impossible. Sighting unknown species in the 60-150 m depth range was common, with 33 % of fish species observed in Jamaica and Belize (Colin 1974) and 40 % of those at Enewetak Atoll, Marshall Islands (Thresher and Colin 1986) appearing to be undescribed species but unable to be confirmed.

1.1.2 Modern MCE fish surveys

The use of in-water diving technologies revolutionised MCE research (Pyle 1998; 2000). Open-circuit SCUBA diving had previously been used for a handful of early deep reef studies such as Goreau & Wells (1967) to study mesophotic corals down to 96 m, and in association with a diver lock-out submarine by Starck & Colin (1978) to collect new fish species at 100 m. The development and adoption of rebreathers in the 1990s, however, provided a shift in the way survey work was undertaken (Pyle 1998). In one MCE study using both rebreathers and open-circuit SCUBA, the open-circuit divers consumed 17 times as much gas and required 70 % more decompression time, greatly increasing survey costs (Parrish and Pyle 2002). Rebreathers also removed the survey location restrictions associated with submersibles, allowing expansion of the first MCE work into South-East Asia (Pyle 2000).

Pyle (2000) used rebreathers to describe over 50 new species from MCEs, mostly within the Labridae, Gobiidae and Serranidae families, with discovery rates of 4.3 new species/hour in Rarotonga, Cook Islands, 5.0/hour in Papua New Guinea and 6.9/hour in Palau. Throughout these surveys the daily discovery rate did not decline, suggesting that the cumulative new species discoveries were not approaching the total number of species present (Pyle 2000). Despite nine previous species lists published for Johnston Atoll, Hawaii, based on line collecting, shallow dives and submersibles (see Kosaki et al. 1991), Wagner et al. (2014) found 22 new fish species when surveying MCEs using rebreathers, highlighting once again the need for in-water survey work. New MCE-associated fish species have now been documented across wide geographical ranges such as Indonesia (Allen and Erdmann 2009), Papua New Guinea (Earle and Pyle 1997), Solomon Islands and Loyalty Islands (Randall and Pyle 2001), Caroline Islands, Fiji and Vanuatu (Pyle 1998) and the Bahamas (Sparks and Gruber 2012).

More recent work has started using rebreathers for MCE fish community structure assessments (e.g. Brokovich et al. 2008; Garcia-Sais 2010). Closed-circuit rebreathers (CCR) recycle the divers’ breathing gas, making them ‘bubble free’ (Sieber and Pyle 2010).

In contrast, semi-closed rebreathers recycle gas less efficiently and so release some bubbles, though less than by open-circuit SCUBA. This lack of visual and audible stimuli from bubbles means CCRs likely improve fish counts in areas with active dive spear fisheries, as fish are known to detect the sounds of divers (Radford et al. 2005). Work by Cole et al. (2007) found no differences in fish assemblage structure when measured by semi-closed rebreathers compared to open-circuit SCUBA in temperate New Zealand reef communities, while Lindfield et al. (2014a) identified major differences between CCR and open-circuit SCUBA on shallow reefs in Guam. These differences in Guam appeared to be caused by fish behavioural responses, as in no-take marine protected areas (MPAs) there was no difference between techniques, but in fished areas 48 % more species and 260 % greater fish abundance were recorded by CCR than open-circuit. Lindfield et al. (2014a) suggest that the differing exposure to levels of spearfishing by open-circuit SCUBA is likely conditioning the fish to flee from divers at greater distances in the fished areas. In MCE research the increased use of CCR has been driven by a technological need for larger breathing gas supplies compared to open-circuit. However, this suggests that fish surveys conducted by CCR on MCEs are unlikely to be comparable to fish data collected on shallow reefs where the typical dive survey technique remains open-circuit SCUBA.

Despite improved availability and efficiency of rebreathers they have only had limited (though growing) uptake for MCE research because of their high initial entry costs (Sieber and Pyle 2010; Mitchell and Doolette 2013). For example, while completing this thesis and undergoing CCR training in 2015 we calculated the total costs for rebreather purchase and training an experienced open-circuit diver to a suitable level to conduct CCR research to 100 m was approximately USD 20,000. Therefore, many MCE fish studies use baited remote underwater video (BRUV) cameras. BRUVs have the advantage of avoiding dive safety management issues, and the increased availability and low price of high-definition action cameras makes them attractive. These camera systems, often containing stereo-video systems to allow fish lengths to be measured (e.g. Lindfield et al. 2016), can be dropped from boats with multiple systems deployed simultaneously. As the BRUV system remains static on the benthos for a set time period they are akin to a fish point count on the reef and provide a low cost easy way to conduct surveys. Several comparisons have been made of differences between fish communities recorded on BRUVs and other transect techniques on shallow reefs (see Watson et al. 2005; 2010; Goetze et al. 2015), though it is not clear whether these standardisations are valid for MCEs. For example, fish communities are known to react to the presence of SCUBA divers, though the effects are often species specific (Watson and Harvey 2007). In areas with high levels of recreational diving fish are also known to habituate to diver presence (Titus et al. 2015), so it is likely that behavioural differences in fish will not remain consistent across the depth gradient.

1.1.3 Fish distributions on MCEs

Fish distributions and ecology on MCEs are still poorly understood. Generally fish species richness and abundance declines with depth from shallow reefs to MCEs (Kahng et al. 2010; 2014). This broad trend masks finer patterns, for example in the Red Sea Brokovich et al. (2008) found species richness declined from the shallow reef to MCEs, but with a small increase at 30 m; the boundary between the shallow and upper-MCE (30-60 m), caused by an overlap of deep- and shallow-specialist and depth-generalist fish species. A study in Puerto Rico found a small fish species richness peak at 25 m (Garcia-Sais 2010), though another study, also from Puerto Rico, found constant species richness across this boundary (Bejarano et al. 2014). Other early submersible studies found a species richness peak slightly deeper, at the transition between the upper- and lower-MCE, so trends are far from clear and it is likely they are modified by local environmental conditions.

Many species found in the upper-mesophotic zone (30–60 m) are depth extensions of species documented on shallow reefs above. While comprehensive analysis of fish depth patterns across shallow reefs to MCEs is lacking, reviews of corals in the Caribbean suggests over three-quarters of shallow coral species extend onto MCEs (Bongaerts et al. 2010; Bridge et al. 2013). Bejarano et al. (2014) surveyed MCEs down to 70 m and found approximately three-quarters of fish species on Puerto Rican MCEs were species that could be found on shallower reefs in the region. Many of these shallow species did not occur past 60 m, although eleven species found on shallow reefs increased in abundance with depth. In the Marshall Islands, Thresher & Colin (1986) reported a shallow fore-reef fish community that extended from the surface to approximately 75–90 m after which many shallow species were lost. In the Red Sea the shallower fish species were found to drop off rapidly from 30 m into the mesophotic zone (Brokovich et al. 2008). Much of this variation in the depth at which shallow species are lost is likely to be explained by local and regional variations in species and environmental conditions, however, the general trend of the upper-mesophotic zone harbouring shallow fish species appears widespread.

At a site level, the presence of corals and other benthic taxa is critical for MCE fish communities providing the structural complexity and associates that many fish require. For example Garcia-Sais (2010) reported large grouper abundance peaked at 25 m in Puerto Rico, but only in association with *Montastrea spp.*, the dominant scleractinian coral at those depths. Another Puerto Rican study reported that the grouper *Mycteroperca phenax* was also associated with maximum structural complexity reefs at 70–100 m depth (Gilmore and Jones 1992). In the Red Sea changes in MCE fish community composition is highly correlated with reductions in branching coral abundance (Brokovich et al. 2008). Boland and Parrish (2005) studied the association between mesophotic reef fish and the black coral *Antipathes dichotoma* in Hawaii, finding 24 fish species using this coral for refuge, including four that did this to evade divers, and two species (*Dascyllus albisella* and *Centropyge potteri*) that were resident in specific colonies. In some cases, MCE fish form obligate associations with benthic species. For example, MCE anemone fish distributions in the Red Sea (Brolund et al. 2004) and the Great Barrier Reef (Bridge et al. 2012) appear to be driven by the distribution of their host anemones rather than any depth limit for the fish themselves. These examples illustrate the importance of associations between benthic and fish species on MCEs, though they have been poorly studied to date.

While biotic benthic components are important for some MCE fish species, it is not clear how many require specific benthic species to associate with, or whether it is the structural complexity they provide that is most important. Early Caribbean MCE studies found the distribution of structural complexity on reef walls was correlated with the abundance of many benthic-associated MCE fish species, including both small (e.g. gobies) and large-bodied species (e.g. basses) (Colin 1974; 1976). More recent work has suggested small carnivorous reef fish are constrained to relatively small areas of high relief in upper-MCEs restricting their population size, while the herbivorous reef fish population are less constrained (Parrish and Boland 2004). Locations at mesophotic depths without developed MCEs but with artificial structures have also been identified as harbouring diverse fish communities (Fowler and Booth 2012). A study in Florida compared low structural complexity natural areas to artificial reefs at mesophotic depths (Bryan et al. 2013). They reported the artificial reefs had similar fish assemblages to those found on high relief MCEs. Most interestingly, however, was that the majority of fish found on low structural complexity ‘natural’ MCEs were actually associated with small artificial structures such as old tyres. Abiotic or artificial complexity on reef slopes and walls are likely to be increasingly important in structuring fish populations at lower mesophotic depths (60–150 m) as the density of corals rapidly declines (Thresher and Colin 1986; Bongaerts et al. 2010).

1.1.4 Depth-dependent reef fish life histories

Many fish species incorporate depth into their life histories, migrating up or down depth gradients as they mature or for spawning (Macpherson and Duarte 1991). Strasburg et al. (1968) identified juvenile *Sargocentron punctatissimum* below 100 m depth in Hawaii, a key component in tuna and marlin diets, while groupers in Florida (*Mycteroperca phenax* and *Mycteroperca microlepis*) have been recorded migrating temporarily to MCEs to form spawning aggregations (Gilmore and Jones 1992). Though in the case of groupers, it is not clear if it is the MCE or the continental shelf edge that is attracting them. In the Caribbean, key herbivorous fish species such as striped parrotfish (*Scarus iserti*) and bottom-feeding predators such as French grunt (*Haemulon flavolineatum*) and yellowtail snapper (*Ocyurus chrysurus*) migrate to deeper habitats as they move through life stages (Cocheret de la Morinière et al. 2002). Similarly, some grouper species (*Epinephelus costae* and *Epinephelus marginatus*) show increasing size with depth on rocky reefs in the Mediterranean (La Mesa et al. 2006). Larger, older fish are likely to contribute a disproportionate number of recruits to a fished stock as a result of higher fecundity (Hixon et al. 2014). Traits such as maternal size and age have also been suggested to correlate with offspring survival, growth and size (Berkeley et al. 2004), though there is still mixed evidence on this issue (Marshall et al. 2010). In reef populations of orange-fin anemonefish (*Amphiprion chrysopterus*), Beldade et al. (2012) found larger females contributed more to local recruitment than smaller females, but that the increased contribution could not be fully explained by increased fecundity with size. Deep areas harbouring larger individuals may therefore have an enhanced role as a source of recruits and so in shallow reef fisheries resilience (McClanahan 2000).

Patterns in reef fish species life histories with depth are far from consistent. In the Gulf of Aqaba, Red Sea, juvenile zebra angelfish (*Genicanthus caudovittatus*) abundance peaks at 60–65 m where 80 % of juveniles could be found, compared to the adults peak abundance at

30 m (Brokovich et al. 2007). Some shallow coral reef fish populations may therefore have a potentially obligate juvenile MCE life stage. As 13 other surveyed reef fish in the area were found to occupy the same or shallower bathymetric distributions to adults, and the increase in zebra angelfish juvenile abundance appears to correlate with a decline in piscivorous fish, Brokovich et al. (2007) suggest these deep reefs may be offering a refuge from predation.

1.1.5 MCE fish feeding guilds

Different fish species and trophic groups respond to depth differently, though common patterns in changes down the shallow to MCE gradient are increasingly being recognised (Kahng et al. 2014). Generally, reef fish communities shift from being dominated by herbivores at shallow depths to being dominated by carnivores (mainly zooplanktivores) at mesophotic depths (Kahng et al. 2010). This trend has been observed in the Caribbean (2001; Garcia-Sais 2010; Bejarano et al. 2014), Brazil (Feitoza et al. 2005; Pereira-Filho et al. 2011), the Marshall Islands (Thresher and Colin 1986) and the Red Sea (Brokovich et al. 2008; Brokovich et al. 2010a). Kahng et al. (2010) summarises that planktivores make up 22 % of fish species recorded at mesophotic depths in the Caribbean. When considering relative abundance at specific sites the importance of planktivores quickly becomes clear; they make up 52 % of all fish observed on MCEs in Puerto Rico, with the next largest group mobile-invertebrate feeders at 20 % (Bejarano et al. 2014). In the Gulf of Mexico it was reported the subfamily Anthiinae (small planktivores in the family Serranidae) were hugely abundant on MCEs and are considered keystone species transferring energy from the water column where they feed onto the reef (Weaver et al. 2001). Garcia-Sais (2010) also identified several fish species as indicators of Caribbean MCEs based on increased abundance below 30 m. These are mostly carnivorous and include: pygmy angelfish (*Centropyge argi*), an omnivore, sunshine fish (*Chromis insolata*) a planktivore, the sargassum triggerfish (*Xanthichthys ringens*) and longsnout butterflyfish (*Chaetodon* *aculeatus*), both carnivores, yellow-cheek wrasse (*Halichoeres cyanocephalus*), an obligate cleaner, and the green-blotch parrotfish (*Sparisoma atomarium*), a herbivore.

Carnivorous fish at mesophotic depths often exhibit a range of depth specific adaptations and dietary patterns. For example, many shallow reef planktivores use vision for feeding, and so exhibit diurnal feeding patterns (Rickel and Genin 2005). The marginate dascyllus (*Dascyllus marginatus*) in the Red Sea shows similar foraging duration at 5 m and 40 m depth because of plastic adaptive ability in their visual systems to lower light (Brokovich et al. 2010b). Trianni & Tenorio (2012) conducted gut analysis showing that the spot-cheek emperor (*Lethrinus rubrioperculatus*) feeding on mesophotic reefs in Micronesia is primarily a piscivore, but this varied by season with crustaceans and molluscs making up important dietary components. This contrasts with work in New Caledonia which found that the same species primarily feeds on crustaceans (Kulbicki et al. 2005), suggesting that larger MCE carnivorous fish are likely to be generalist feeders. Focusing on the hugely important mesophotic zooplanktivorous subfamily Anthiinae (Weaver et al. 2001), Cummings et al. (2010) analysed the dietary preferences and trophic interactions of reef red-barred anthias (*Pseudanthias rubrizonatus*) collected between 80 and 160 m in western Australia. Gut content analysis showed that red-barred anthias have an opportunistic feeding strategy, mainly feeding on larval fishes, crustaceans (mysids and isopods) and gastropods (heteropods), undergoing ontogenetic dietary shifts. Other feeding interactions include cleaner wrasse (*Bodianus pulchellus*) and cleaner gobies (*Elacatinus phthirophagus*), both recorded below 60 m in the tropical southwestern Atlantic, with the goby cleaning groupers and the wrasse cleaning groupers and jacks (Sazima et al. 2010).

With a few exceptions (see: Pinheiro et al. 2016), the majority of studies of fish trophic guilds across the depth gradient have used abundance-weighted community data. However, it is increasingly recognised that, when considering ecological functions carried out by reef fish, biomass of different trophic groups gives more accurate assessments than abundance of fish (MacNeil and Connolly 2015). Therefore, it is important to relate the existing abundance-based studies of fish trophic changes across depth gradients to biomass-based studies to identify whether previous reported patterns still hold.

1.1.6 Importance of reef fish in structuring benthic communities

Reef fish are known to play a critical role in structuring the benthic community on shallow reefs (Lewis 1986; Hughes et al. 2007). This often occurs through direct predation and herbivory of benthic organisms affecting competition dynamics for space, either allowing other benthic organisms to increase in size or providing space for new recruitment. For example, overfishing of spongivores has been found to favour a shift towards a sponge community dominated by quick-growing species with few chemical defences against predation (Loh and Pawlik 2014). Many fish families, including Chaetodontidae, Labridae, Balistidae and Scaridae, contain corallivorous fish which directly feed on coral (Cole et al. 2008). Predation effects on benthic communities can also occur through trophic cascades. This is often observed in conjunction with human impacts. For example, following increased large grouper and snapper fishing in Belize, smaller grouper mesopredators increased over 850 % in abundance (Mumby et al. 2012). These smaller grouper species (*Epinephelus guttatus, Cephalopholis fulvus and Cephalopholis cruentatus*) feed on juvenile damselfish, and so are believed to be behind the decline in adult *Stegastes planifrons*, an algal feeder. Territorial algal-farming damselfish species in particular can have impacts on reef algal diversity (Russ 1987; Klumpp et al. 1987), and increase algal coverage to the detriment of corals (Sammarco and Williams 1982). Other ways in which reef fish can structure benthic habitats can be more direct, for example the sand tilefish (*Malacanthus plumieri*), at mesophotic depths on rhodolith beds, has been observed to transport rhodoliths in their mouths to form new mounds outside the edge of the main bed (Amado-Filho et al. 2012).

Herbivorous reef fish in particular have an important role in preventing algal dominance on shallow reefs. Strong relationships exist between grazing and algal cover on reefs (Fox and Bellwood 2007), with the loss of reef herbivores being associated with phase-shifts to algal-dominated states (Hughes 1994). More recent work has highlighted the important role of herbivorous reef fish in reversing algal phase shifts, and avoiding alternative stable states (Graham et al. 2013). Areas of reef cleared by herbivores act as important coral recruitment sites, and restoring trophic cascades previously lost through overfishing have been shown to increase coral recruitment rates (Mumby et al. 2007).

1.1.7 Herbivorous reef fish on MCEs

While herbivorous reef fish occur at lower abundances and relative abundances at increased depth within MCE fish communities, they are still believed to have important roles in benthic community structuring on upper-MCEs (30-60 m) (Kahng et al. 2010). For example, in the Marshall Islands herbivorous reef fish made up 40 % of the fish community at 30 m but declined to close to 0 % at 90 m (Thresher and Colin 1986). In the Caribbean herbivorous reef fish have been reported to comprise a similar proportion (36 %) of the community at 30 m compared to shallow reefs, but rapidly declined below 40 m (Bejarano et al. 2014). For upper-MCEs reef fish are likely to be the dominant herbivores, as the effects of the urchin *Diadema antillarum* (the primary herbivorous echinoderm in the Caribbean) declines with depth (Morrison 1988), even before taking into account the mass mortality event of 1983 which decimated their population throughout the region (de Ruyter van Steveninck and Bak 1986). For example, in Curaçao, following *Diadema* mass mortality there was less change in algal coverage at 40 m depth than at shallower sites (de Ruyter van Steveninck and Bak 1986; Nugues and Bak 2008). This suggests that despite lower herbivorous reef fish abundance on upper-MCEs than shallow reefs, they may have a crucial functional role in maintaining the reef. Several reasons have been suggested for the decline of herbivorous reef fish abundance with depth, including changes in algal abundance, growth rates and diversity, differences in reef structure and changes in sedimentation rates.

Changes in algal coverage and growth are known to occur with depth (Liddell and Avery 2000; Aponte and Ballantine 2001). Aponte & Ballantine (2001) conducted detailed studies of deep-water benthic algae composition and coverage in the Bahamas from 45–150 m. Algal coverage decreased from 57 % to 16 % over this depth range with broadly different species and groups dominating each depth zone. These abundances and declines seem to be similar to reports from upper-MCEs elsewhere, such as 70 % coverage at 50 m declining to 50 % at 70 m in Puerto Rico (Ballantine et al. 2011) and 20 % at 50 m to 15 % at 70 m in American Samoa (Bare et al. 2010). While these patterns correlate with a decline in light availability (Leichter et al. 2008), Brokovich et al. (2010a) found in the Red Sea that the decline in herbivorous reef fish biomass and grazing pressure from shallow reefs to MCEs is far greater than that of algal coverage. On shallow reefs 40-60 % of turf algae was grazed, compared to <20 % at 65 m. Studies of algal recruitment rates in Florida MCEs, however, have found high variability between sites (Leichter et al. 2008). At one study site, algal recruitment was greater at 50–60 m than at 30–40 m, while on another there was no significant difference between 30 m and 70 m. Therefore, while changes in algal coverage and growth rates could explain some of the decline in herbivorous reef fish seen with depth, there are likely additional factors affecting this.

Another driver of this change could be varying algal species composition. Aponte & Ballantine (2001) reported that *Lobophora* spp. and *Halimeda copiosa* dominated the upper mesophotic (45–60 m) in the Bahamas, with Lang (1974) reporting *Halimeda* spp. at these depths in Jamaica and Bongaerts et al. (2011) reporting similar *Halimeda* spp. “curtains” in the Coral Sea. From the few studies of MCE algae it appears an increased proportion of algae calcify with depth, making grazing harder (Hay 1997). Density of algae is also an important factor in affecting herbivory rates. Hoey & Bellwood (2011) showed that herbivorous reef fish avoid areas of high macrolgal density, preferentially grazing lower density stands and providing a positive feedback loop by enhancing the persistence of high density macroalgal patches on reefs. Both Aponte and Ballantine (2001) and Bongaerts & Bridge (2011) reported *Halimeda* spp. approaching 100 % coverage at some locations on upper-MCEs so, once established, avoidance by herbivorous reef fish could lead to these curtains persisting. At greater depths Aponte and Ballantine (2001) recorded abundant red coralline algae and *Peyssonnelia* spp. between 90–120 m, with green *Ostreobium* spp. present from 90-120 m and becoming dominant below 150 m. Algal turfs, however, are an important component of upper-MCEs with approximate coverage of 14 % between 60–75 m, but dropping off rapidly between 75–90 m (Aponte and Ballantine 2001). It is not clear whether depth has any effect on palatability, although it has been suggested that nutrient content may be more important (Clements et al. 2009). Algae on coral reefs are known to have chemical defences (Bolser and Hay 1996), which can lead to selectivity by fish (Coen and Tanner 1989), though these defences have been shown to be ineffective against some mesophotic reef fish (Slattery and Lesser 2014). There is mixed evidence for changes in chemical defence of an algal species with depth. High UV levels found in shallow water have been found to reduce the concentration of secondary metabolites in algae, making it more susceptible to grazing (Cronin and Hay 1996), while other studies have found no effects (Macaya et al. 2005; Pansch et al. 2008). Currently there is no clear consensus on the role of algal chemical ecology in structuring herbivorous reef fish populations.

Structural differences in the reef may also explain the decline in herbivores. A study off Florida using recruitment tiles in areas close to the reef at 30–35 m depth found reduced algal abundance and herbivorous reef fish were observed grazing (Leichter et al. 2008). At greater depths algal abundance increased, and herbivorous fish declined, except for around deeper artificial reefs (wrecks) at 50–60 m. Here herbivory halos 10–20 m wide surrounding the wrecks showed reduced algal abundance, suggesting that herbivorous reef fish were living within the wreck structure. These findings fit with shallow reef fish herbivory studies in western Australia which indicated that structural complexity best explains spatial patterns in herbivory (Vergés et al. 2011). Work on MCEs in Hawaii, however, suggest that carnivorous fish are most constrained to reef areas of higher complexity, but herbivorous reef fish were less so, suggesting that the latter venture out more to graze algae (Parrish and Boland 2004).

1.1.8 Invasive MCE fish species

Several invasive species have been recorded on MCEs, including invasive Indo-Pacific lionfish in the western Atlantic (Lesser and Slattery 2011), and both octocorals (Kahng and Grigg 2005) and invasive algal species in Hawaii (Spalding 2012). Within the western Atlantic, invasive lionfish have been highlighted as a major conservation concern (Sutherland et al. 2010). Invasive Indo-Pacific lionfish were first introduced into the western Atlantic in the 1980s and spread rapidly across the region in the mid-2000s (Schofield 2009; 2010; Aguilar-Perera and Tuz-Sulub 2010), they have been increasingly documented at mesophotic depths in the northern-Caribbean (Nuttall et al. 2014), though little is known about their ecology on MCEs. Lionfish are highly fecund and consume many small reef fish, which are naïve to their predation (Albins and Hixon 2013). Dahl et al. (2014) identified that by mass, lionfish diet consisted of 98.2 % reef-associated organisms, with 89.5 % small (<5 cm) demersal reef fish. This sudden arrival and high abundance of lionfish on MCEs is likely having a large ecological impact. Lesser & Slattery (2011) reported that following lionfish invasion of a Bahamian MCE there was a loss of herbivorous reef fish to 60 m. This led to a benthic phase shift to an algal-dominated mesophotic community and a loss of sponge and coral cover which was facilitated by chemical suppression of coral growth by algae (Slattery and Lesser 2014). Lionfish are known to have ontogenetic migrations, with new recruits settling in shallow mangrove, seagrass and back-reef habitats before migrating to shallow reef slopes as they mature (Claydon et al. 2012). It is not clear how widespread the invasion of MCEs by lionfish is within the western Atlantic, and whether MCE populations represent an extension of the shallow-water lionfish ontogenetic migration.

1.1.9 Deep Reef Refugia Hypothesis (DRRH)

Global and local stressors do not act homogeneously over shallow reefs and MCEs, with many anthropogenic impacts so far observed disproportionately affecting shallow reefs. This led to the Deep Reef Refugia Hypothesis (DRRH), first proposed by Glynn (1996) and first tested by Riegl and Piller (2003). Bongaerts et al. (2010) reviewed the two key concepts of the DRRH as follows:

1. Deeper reef systems are dampened from impacts affecting shallow reefs, allowing species lost from shallower reefs during impacts to survive on the deeper reefs.
2. Recruits or individuals that survived on deeper reef systems are able to colonise or migrate to shallower reef systems aiding recovery.

Therefore, the DRRH requires two critical attributes; common species between the shallow and deep reefs that are able to benefit from the deep refuge, and connectivity between populations of the same species across the depth gradient. If these critical attributes are met for many species, then MCEs would likely have a crucial role in coral reef ecosystem-level resilience and so should be more rapidly integrated into shallow reef conservation plans.

Deeper reefs being less affected by impacts was first proposed based on thermal stress events affecting coral communities (Glynn 1996), with less coral bleaching recorded at greater depths on reefs. Though this has recently been challenged, as in some cases deeper reefs can face greater thermal stress (Neal et al. 2014). Other stressors affecting corals, for example storm-induced waves may also decline with depth (Liddell and Ohlhorst 1988), though there are several cases of storm damage to MCEs (Menza et al. 2007; White et al. 2013; Bongaerts et al. 2013b). More recently the DRRH has been expanded from coral stressors to fish exploitation, with the recognition that many reef fisheries are currently shallow focused. Interactions between depth and fishing impacts have long been recognised, with a global trend of fishing shifting to deeper water species as shallower stocks become depleted (Morato et al. 2006).

1.1.10 Depth refuges in reef fisheries

Impacts of local coastal fisheries are increasingly being recognised as depth specific at fine scales. Studies have mostly been conducted as comparisons between commercially valuable fish species abundance inside and outside marine protected areas at varying depths, for example, on coral reefs in the Red Sea (Ashworth and Ormond 2005), Tanzania (Tyler et al. 2009), the Caribbean (Polunin and Roberts 1993) and rocky reefs in the Mediterranean (García-Rubies and Zabala 1990). Ashworth and Ormond (2005) found higher reef fish abundance in seven families in protected areas at 1 m depth when compared to fished areas, but only between two families at 10 m depth in the Red Sea. Tyler et al. (2009) looked at a 1-14 m depth range in protected areas and areas with active artisanal coral reef fisheries in Zanzibar, Tanzania. Commercially valuable fish species richness was depleted at shallower depths on fished sites compared to protected area controls, but increased with depth up to 7 m, when there was no longer a difference between fished and protected areas. Such clear effects across small depth gradients on shallow reefs illustrate that fishing impacts can be detected at fine depth resolutions in fish communities.

Depletion of shallow reef fish stocks puts increasing pressure onto fish at greater depths. For example, in Hawaii when recruitment of yellow tang (*Zebrasoma flavescens*) is low on shallow reefs fishers increase the depth of collection to try to maintain catches for the marine aquarium trade (Stevenson et al. 2011). Working in a large well-established Fijian

MPA, Goetze et al. (2011) found the effects of artisanal fishing could be detected in shallow species compositions (5–8 m), but not at near-MCE depths (25-30 m). Lindfield et al. (2014b) studied the effects of spearfishing in the southern Mariana Islands. Traditionally this was conducted by free divers (breath-hold diving), restricting maximum fishing depth, though increasingly SCUBA has allowed extension to greater depths (>30 m) (Myers 1993). Historically, in the free-dive fishery, the length of scarines and *Hipposcarus longiceps* found at depth was much greater than those on shallow reefs. Following twenty years of both fishing techniques, the length of scarines and *Hipposcarus longiceps* caught by both free diving and SCUBA has declined, however, the decline has been most severe at 30 m. Near-MCE depths therefore can act as biomass refuges for commercially valuable species when faced with shallow fisheries impact.

1.2 Study Site: Utila Island, Bay Islands, Honduras

1.2.1 Introduction to Utila

To improve our understanding of MCE fish communities, this thesis uses Utila island, part of the southern Mesoamerican Barrier Reef System (MBRS), Caribbean, as a representative reef system demonstrating a clear shallow to MCE gradient. Utila is the third largest island making up the Bay Islands, an archipelago of >70 islands, and has an area of 4160 ha and is approximately 29 km off the north shore of Honduras (Figure 1.2) (Luttinger 1997). The human population of Utila was approximately 8,000 in 2008 (Korda et al. 2008), and has been steadily growing. Extensive coral reefs and mangrove forests exist around Utila, including several offshore banks and mangrove lagoons (Harborne et al. 2001).

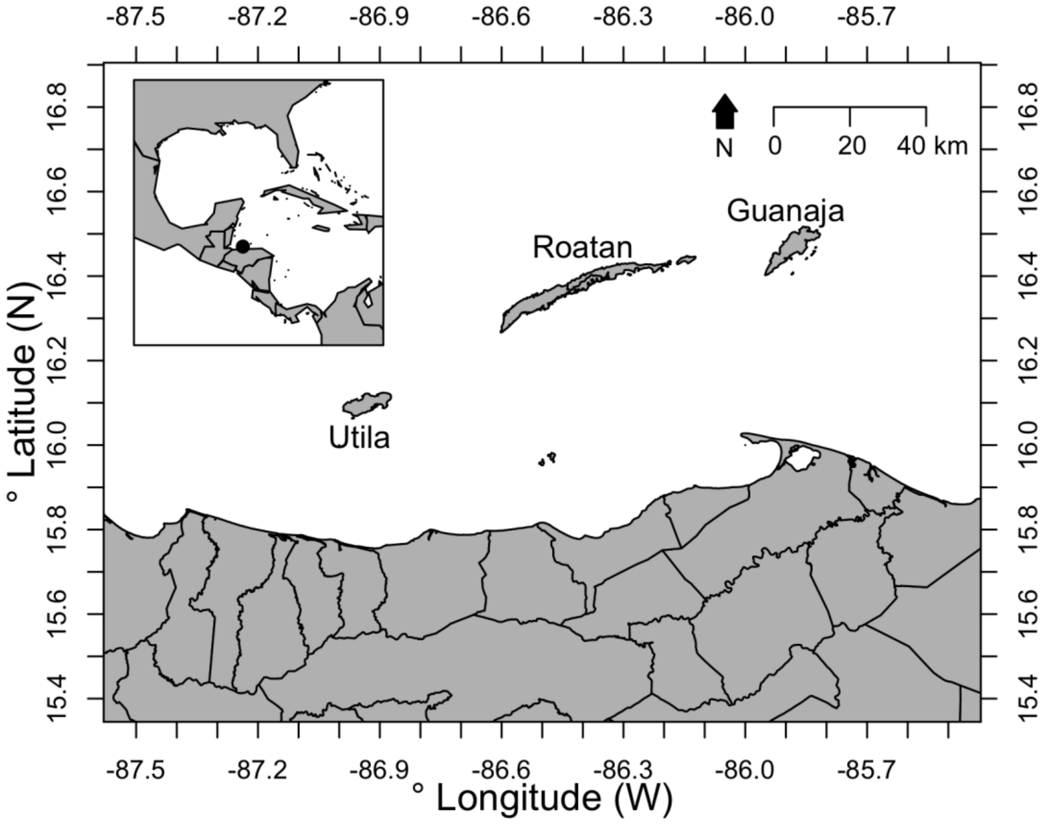

Figure 1.2 The Bay Islands Archipelago off the north Honduran coast.

The three principle islands are shown: Roatan, Guanaja and Utila. Inset – The location of Utila is indicated in relation to the western Caribbean and Gulf of Mexico.

Utila lies on the edge of the continental shelf (Gobert et al. 2005), approximately 60–80 m deep, which connects with mainland Honduras to the south, but leads onto a steep reef wall drop-off to >200 m on the island's north coast. This drops further to several thousand meters deep, ultimately reaching the western edge of the Cayman Trench (Heyman and Kjerfve 2001). The Bay Islands have a low tidal range, with changes of 30–35 cm reported (Mackenzie and Stehlik 1996). Sedimentation is a major problem for the MBRS as a whole, which is exposed to terrestrial run-off annually (Chérubin et al. 2008). At the southern end of the MBRS, however, reefs are subjected to twice-yearly pulses of terrestrial run-off, caused by both the initial run-off impact itself, but then also by this run-off being cycled in a gyre that returns the pollutants to the reefs several months later (Paris and Chérubin 2008).

1.2.2 Utila marine biodiversity and conservation

Ecological surveys have identified 113 fish species on Utilan reefs (Jaxion-Harm et al. 2012), although a detailed fish species list for the nearby Cayos Cochinos MPA identified 226 fish species (Clifton and Clifton 1998), so it is likely that actual fish richness is much higher. For zooxanthellate scleractinian corals, 46 species from 21 genera have been identified around Utila (Scaps and Saunders 2011), representing 93 % of known Caribbean shallow reef species. Work by Gerez (2013) identified an increase in coral species and genera diversity with depth from 0–16 m on the southern reefs of Utila. The diversity and distributions of most other taxonomic groups are poorly studied, although 23 octocoral species have been identified from nearby Roatan Island (Keith 1992). Much research effort on the island has focused around the extensive mangrove lagoons, exploring aspects including their role in supporting nearby reef fisheries (Harm et al. 2008; Jaxion-Harm et al. 2012; 2013), the role of lagoonal macroalgal beds in fish, crustacean and zooplankton distributions (Jaxion-Harm and Speight 2012) and how mangrove epibionts drive fish diversity and abundance (MacDonald and Weis 2013).

Utila and the wider Bay Islands' marine environment is being severely impacted by a range of threats. Utila's reefs have a low mean coral cover which declined, from 16 % in 2004-2005 to 11 % in 2007-2008 (García-Salgado et al. 2008), though more recent estimates suggest coral cover is around 12 % (Bodmer et al. 2015). Studies indicate that turtle populations are declining (Meylan 1999), reef fish communities are becoming dominated by herbviores with a loss of predatory fish (Clifton and Clifton 1998) and fisheries are in decline (Box and Canty 2011). Corals were known to be locally and severely impacted by the 1998 bleaching event (Carilli 2008) and took longer to recover than other reefs in the region (Carilli et al. 2009). Populations of long-spined urchins (*Diadema antillarum*), a key reef herbivore, declined following an epidemic that spread in the 1980s and have only shown limited recovery (Bologna et al. 2012; Bodmer et al. 2015). The first invasive Indo-Pacific lionfish was observed on shallow reefs in the Bay Islands during May 2009, and by August 2009 21 further lionfish had been observed including four on Utilan shallow reefs (Schofield 2009), with the Roatan Institute of Deep-Sea Exploration recording lionfish on mesophotic reefs down to 120 m in the Bay Islands shortly after (Schofield 2010).

While the entire Bay Islands makes up a National Marine Park, fishing is unrestricted in most areas. In response to reef threats several limited fishing zones have been established, including two around Utila. These Utila restricted fishing zones are located at Turtle Harbour on the north coast, and Raggedy Cay on the west coast. Officially the National Marine Park are supposed to collect entry fees from recreational divers diving within these restricted fishing zones to fund enforcement, although the stated entry fee at USD 3.00 per diver is well below the average entry fee of USD 6.56 for a two tank dive trip in a Caribbean MPA (Terk and Knowlton 2010). In addition, observations during this D.Phil. through working alongside Operation Wallacea suggested that some dive centres refuse to pay entry fees, possibly because of a lack of transparency on the uses of such funds, and because of poor enforcement to date. Other smaller-scale projects have been more successful, such as the installation and maintenance of mooring buoys at all regularly dived sites around Utila to improve dive access and minimise anchor damage to the reef (Moreno 2005). Other more novel ideas to respond to threats are also being trialled, including small-scale reef restoration projects (Young et al. 2012).

1.2.3 Economic development of Utila

Historically, crustacean and mollusc fisheries have been major sources of income for local people on Honduras' Caribbean coastline (Mackenzie and Stehlik 1996). Yet agriculture rapidly became a major occupation of settlers on Utila until the 1960s when fishing took over as the main source of income from coconut farming (Korda et al. 2008). Fishing remains the primary income source for many residents of the Utila Cays (located off the south-west tip of the island), however, during the past 20 years tourism has become the largest overall income source on Utila (Korda et al. 2008). Many former fishers became involved in tourism through developing a deep-water sport fishing industry, catching pelagic fish species such as tuna (*Thunnus atlanticus, T. albacares, T. obesus*), blue marlin (*Makaira nigricans*) and wahoo (*Acanthocybium solandri*) off the north coast of the island (Heyman and Kjerfve 2001).

In the 1990s and early 2000s the Bay Islands represented one of the most rapidly developing tourist destinations in the Western Hemisphere, with the number of tourists visiting the islands growing from less than 10,000 a year to more than 100,000 annually (Stonich 2003; Moreno 2005). The focus of many of the visitors to Utila is low-cost diving-based trips (Moreno 2005), with most tourists staying in basic backpacker accommodation (Cronk and Steadman 2002). Cronk and Steadman (2002) interviewed Utilan dive shop owners and found the dive industry very different to other locations. Dive shop owners reported that in a normal dive destination they would actively advertise internationally to attract dive tourists. On Utila, however, most visitors are long-term travellers seeking the cheapest place to learn to dive. With 12 dive centres on Utila competing on price, while offering near-identical entry-level diver training courses, overall profitability of the dive industry is low. This creates conflicting economic and environmental pressures, as developments clear coastal mangrove forests in order to increase capacity (Luttinger 1997). Much of the investment in these facilities is also foreign (Moreno 2005), providing limited opportunities for locals to gain.

Within the Bay Islands there are major governance barriers preventing stable development. Luttinger (1997) reviewed these as the economy transitioned between fishing-based to reef-tourism-based, identifying four major barriers: (i) legislation only covers some environmentally damaging activities and only very large scale development projects require environmental impact assessments, (ii) local government lacks ability to identify breaches of legislation, (iii) inadequate penalties exist for breach of environmental legislation, where it is often cheaper to pay penalties than comply with the rules, (iv) lack of technical expertise by decision makers to evaluate development proposals on sustainability. While this review was conducted in the 1990s, these same issues are repeatedly identified in more recent governance studies on Utila (Moreno 2005; Box and Canty 2011).

1.2.4 Utila fisheries

Current fisheries on Utila are focused on offshore banks surrounding the island, rather than the previously over-exploited fringing reefs that have become the centre of the dive tourist industry (Harborne et al. 2001; Gobert et al. 2005). The fishery is made up of industrial line fishers and small-scale fishers using targeted handlines (Gobert et al. 2005). Since 2004 spearfishing and trap fishing have been banned within the Bay Islands National Marine Park (Kramer et al. 2015). Most fishers who live on the Bay Islands use fiberglass boats with outboard engines and target fish families such as tuna, barracuda, snapper and grouper (Gobert et al. 2005).

Gobert et al. (2005) compared the relative contribution of the shallow- water and deep-water fisheries for snapper (Lutjanidae) and grouper (Serranidae) species throughout the Bay Islands. They found that the Utilan snapper fishery was dominated by shallow-water species, though deeper-water species are regularly being caught. This contrasts with other locations in the Bay Islands where deeper-water snapper species make up a greater proportion of the catch. The snapper fishery in 1999 on Utila produced approximately 207.1 tonnes of fish, of which 21.5 tonnes came from deeper reefs (Gobert et al. 2005). This meant that the overall snapper catch reported from Utila is much larger than that reported for any other area in the Bay Islands.

Utilan fishers are also becoming poorer, with the price paid per pound of fish in Honduran Lempira not changing between 1992 and 2011 (Box and Canty 2011). In addition to this price stagnation, many deep-water snappers were exported to the USA for sale, and during this period the Honduran Lempira has more than halved in value against the USA. On top of the decline in value, the costs of fishing have risen 6 % per annum on average (Box and Canty 2011). This has resulted in massive reductions in profitability for Utilan fishers at the same time that tourism has risen to become an increasingly lucrative opportunity. There is, however, a risk that those dependent on the fisheries who do not speak languages such as English will not be able to access the benefits of tourism and so will progressively be driven into poverty (Box and Canty 2011). This has led to resentment in some parts of the local population, where people unable to make a living from tourism are finding themselves pressurised by other more affluent locals with investments in sport fishing, hotels and dive centres to reduce fishing on the reef (Stonich 2003). Yet these more well-off locals are involved in coastal land clearance for development and so are also having less obvious negative environmental impacts (Stonich 2003).

Korda et al. (2008) identified key drivers behind overfishing on Utila. They found that the majority of fishers were aware that fish stocks had declined around Utila and that the market-orientated model of fisheries governance (or lack of governance) is likely to have exacerbated the decline. They also reported that fishers are increasingly catching ‘trash fish’, species such as triggerfish and parrotfish that had no commercial value until recently when larger more desirable fish stocks declined. Throughout all studies on fisheries on Utila, weak management and overexploitation have been consistently identified as the main causes behind fisheries decline (Gobert et al. 2005; Korda et al. 2008; Box and Canty 2011).

1.3 Statistical approaches used in this thesis

This thesis uses a range of statistical techniques to address questions related to fish ecology of MCEs. Different disciplines have different favoured methods for statistical analysis, and this thesis follows many methods used by marine community ecologists interested in discerning patterns in ecological communities between different locations and through time.

1.3.1 Types of analysis

Broadly three types of analysis throughout this thesis, (i) permutational tests and (ii) generalised linear (mixed) models and (iii) multivariate statistical approaches for visualisation of data. All approaches offer great flexibility in analysis, but have their own unique strengths and weaknesses.

Permutational multivariate analysis of variance (PERMANOVA) allows analysis of variance (ANOVA) type experimental designs to be analysed while incorporating univariate or multivariate data. PERMANOVA partitions sums of squares allowing the treatment of fixed or random experimental factors, including nested factors and interaction terms between factors (Anderson et al. 2008). This allows a pseudo-*F* statistic to be calculated along with an associated *p* value that can be interpreted in a similar way to the results of ANOVA analysis. PERMANOVA is a powerful analysis method as the only assumptions are that the data has a similar multivariate spread among groups (analogous to homogeneity of variance in ANOVA) and that samples are independent (though nested structures and repeated measures can be accounted for). Other assumptions of ANOVA (normality) and multivariate ANOVA (MANOVA) such as linearity, absence of multicollinearity and equality of covariance matrices do not apply (Anderson et al. 2008). These advantages, combined with the conceptual ease of using and understanding this method have made PERMANOVA widely used within the marine community ecology.

Other approaches are also widely used to analyse ecology data, with many researchers using Generalised Linear Models (GLMs) and Generalised Linear Mixed-Effects Models (GLMEMs). A major advantage of GLMs and GLMEMs over traditional linear modelling is that they allow more complex analysis, by simultaneously considering the effects of multiple variables. These techniques are appropriate for analysing count data from ecological communities with a model family and error structure (link function) selected based on the data type. The assumptions associated with these models allow the response variable to not be normally distributed, but instead is assumed to fit a distribution from a selected family (e.g. Poisson, binomial). A transformation (link function) is also applied to the response variable, with the model assuming a linear relationship between the response and the explanatory variables, addressing issues of homogeneity of variance.

GLMEMs specifically allow fixed and random effects within the same model. While good experimental design is essential, and field studies should be planned to use balanced independent sampling (Ruxton & Colegrave 2003), in some cases there are good reasons for planning experiments that require GLMEMs to analyse. For example, when conducting analysis of time series data with repeated measures taken from the same sampling points, with each sampling point receiving different treatments, and these groups of points spatially replicated in blocks (see Chapter 3) a GLMEM could be used. When testing for differences between the treatment types the repeated measures must be accounted for, while also accounting for differences between blocks. Ideally this experimental set up would be designed and analysed as a full factorial ANOVA, however in some cases this is not possible. GLMEMs allow analysis of ecological data from incomplete factorial designs, allowing insights to still be gained from experiments or surveys that cannot be analysed using traditional linear modelling methods.

1.3.2 Model simplification

This thesis follows the convention of Crawley (2007), with fitting the most complex model with all interactions initially, followed by model simplification. To simply a model, one interaction or factor was removed at a time, before testing whether the removal has a significant difference on model explanatory power. This approach works for all linear models and GLMs and GLMEMs used in this thesis. The philosophy behind this approach also translates when using balanced experimental designs for PERMANOVA analysis, with all factors and more complex interactions fitted initially before simplification.

In most cases during this thesis tries to use balanced experimental designs, with equal replication levels within each factor being tested. In some cases however that has not been possible, because of limited data available. In the case of Chapter 2, with a limited number of surveys possible on MCEs, the number of shallow reef surveys was intentionally increased (generating an unbalanced design) with the intention of increasing statistical power to discern differences in ecological communities between shallow reefs and MCE (Barker Bausell & Li 2002). However, when analysing unbalanced designs with PERMANOVA with more than one factor, the main effects of factors and interactions are not independent of each other (Anderson et al. 2008). This means that the order in which terms are fitted is important and affects the results. There are four different ways of partitioning variance for unbalanced designs, known as Types 1-4 Sums of Squares. In this thesis, because of the natural ordering of factors across spatial scales and hypothesise being tested, when using PERMANOVA for unbalanced experimental designs Type 1 Sum of Squares were used. This approach of sequential sum of squares fits each factor or interaction after taking account of any previous factors or interactions fitted (Anderson et al. 2008). Therefore changes in the ordering of factors and interactions potentially could alter results. However, this major factor first approach to statistics is considered appropriate when testing a factor or interaction of interest after controlling for the variation caused by another factor (Anderson et al. 2008).

1.4 D.Phil. Overview

The purpose of this D.Phil. was to increase understanding of the fish ecology of Caribbean MCEs. To address this, I mostly focus on the reefs of Utila as representative shallow and MCE reef systems for the MBRS and the Caribbean more widely, though I contrast my results with other studies and data from shallow reefs and MCEs elsewhere in the western Atlantic and globally. Specifically, I address how reef fish communities shift in trophic group composition across the shallow reef-MCE depth gradient and the resulting implications this has for reef fish as structuring agents for MCE benthic communities. I consider how recorded MCE fish communities are affected by different survey techniques, including behavioural responses of fish, and how choice of survey technique affects accuracy and interpretation of data. To conclude, I test the DRRH using lionfish as a model species, investigating whether the DRRH is allowing an invasive fish species to undermine current conservation management practices.

Based on these aims, this thesis is divided into six data chapters (Chapters 2–7), each written and formatted as a scientific publication, followed by an overall discussion and conclusion section (Chapter 8). In addition, three appendices (Chapters 9-11) contain further publications completed during this thesis that provide a more synthetic overview of threats to and challenges of surveying MCEs, or contain supplementary data supporting the thesis but not core to the main narrative. The six data chapters can be broken down as follows, each with a discrete set of hypotheses being tested:

1.4.1 Chapter 2 – Reef fish community biomass and trophic structure changes across shallow to upper-mesophotic reefs in the Mesoamerican Barrier Reef, Caribbean

This chapter presents the first surveys of fish communities across shallow to upper-mesophotic reefs in the Bay Islands, Honduras. Fish communities were surveyed at four depths (5, 15, 25 and 40 m) at seven sites around Utila using accelerated decompression open-circuit diving. I tested whether fish communities varied based on depth, in particular exploring variation between the sheltered southern and exposed northern shores. In addition, I investigated how fish community trophic structure across this depth gradient was affected by assessing fish communities based on abundance or biomass.

1.4.1.1 Hypotheses tested:

1. Fish communities shift away from being herbivore-dominated on shallow reefs to being planktivore-dominated on MCEs.
2. Because of the large number of small-bodied planktivores and herbivores present in the shallows, fish community patterns across the depth gradient based on abundance will overestimate community shifts compared to biomass.
3. South shore MCE fish communities at 40 m, which exist on a very gently sloping continental shelf, will be more similar to adjacent shallow reef fish communities, whereas north shore MCE fish communities at 40 m, which were on steep walls extending to >200 m maximum depth, will be more distinctive from their adjacent shallow reef communities.
4. Based on previously recorded increases in fish length caused by ontogenetic shifts between back reef habitats and shallow reef slopes, average individual fish lengths will be greater on MCEs than shallow reefs, indicating the extension of ontogenetic migrations onto MCEs.

1.4.2 Chapter 3 – Light availability appears more important than fish exposure in structuring upper-mesophotic reef benthic communities

This chapter uses light and fish exclusion cages with a factorial design to experimentally manipulate light and fish exposure for MCE benthic communities over a 16-month period. Herbivorous and spongivorous reef fish are present on Utilan MCEs, but it is not clear how much of a role they have in affecting benthic coverage of different MCE components. I assessed the relative role of light availability versus fish exposure to test which of these processes causes the greatest changes to cover of three key reef benthic groups: hard corals, macroalgae and sponges.

1.4.2.1 Hypotheses tested:

1. Hard coral and macroalgal coverage will decline with reduced light intensity.
2. Macroalgal and sponge cover will increase when herbivorous and spongivorous fish are excluded from the reef.

1.4.3 Chapter 4 – Assessing Caribbean shallow and mesophotic reef fish communities using baited-remote underwater video (BRUV) and diver-operated video (DOV) survey techniques

This chapter assesses whether fish community structure data on shallow reefs and MCEs are artificially biased by the choice of survey technique. I compare two widely used methods of stereo-video, baited remote underwater video (BRUV) and diver operated video (DOV) transects, with surveys conducted at two depths (5 and 40 m) at four sites around Utila. The species richness, abundance, biomass and length distribution of fish are compared across depths. My results highlight the importance of considering what component of the reef fish community surveyors are most interested in when deciding on a survey technique, as inconsistent responses were identified across families, species and trophic groups between methods across the depth gradient.

1.4.3.1 Hypotheses tested:

1. Carnivorous fish will represent a greater proportion of the BRUV surveyed community because of bait attraction effects.
2. Shallow fish communities will be more similar between DOV and BRUV because of previous habituation to divers on the reef, while MCE fish communities which are less frequently exposed to divers will show greater differences between DOV and BRUV (interaction between survey method and depth).

3. DOV will record more representative fish length distributions because of the differing nature of a mobile survey along the reef, compared to the static MaxN point count nature of a BRUV.

1.4.4 Chapter 5 – Wariness of reef fish to passive diver presence with varying dive gear type across a shallow to mesophotic depth gradient

This chapter builds on the findings of Chapter 4 to explore how different dive technologies used to survey shallow and MCE fish communities can bias survey results. I test the differences in recorded fish community structure between DOV surveys conducted using open-circuit (OC) SCUBA and a closed-circuit rebreather (CCR). CCR recycles breathing gas for divers, and so does not produce bubbles like OC, which are known to disturb fish communities and there could alter recorded fish abundance. In addition, fish responses to diver disturbance are known to be affected by body size, potentially biasing body sizes of recorded individuals.

1.4.4.1 Hypotheses tested:

1. Greater abundance of fish will be recorded by divers using CCR than OC because of the near-silent nature of CCR.
2. Smaller minimum approach distances (MADs) will be recorded by divers using CCR than OC, as the near-silent nature of CCR will allow divers to approach fish more closely without them fleeing.
3. Fish communities on shallow reefs will allow OC divers to approach more closely than those on MCEs because of habituation effects caused by the large number of shallow reef recreational tourist dives conducted on the reefs of Utila.

1.4.5 Chapter 6 – Large scale invasion of western Atlantic mesophotic reefs by lionfish potentially undermines culling-based management

In this chapter I focus on lionfish, an invasive fish species in the western Atlantic, which may be benefiting from MCEs acting as refuges in the face of shallow reef culling. As the lionfish invasion is a western Atlantic regional problem, and there was little published evidence of how widespread the lionfish invasion of MCEs has been, I contacted researchers based around the region specifically asking for any unpublished data they had from field surveys. Using this data, I conducted a meta-analysis of lionfish abundance and body size based on 63 sites in seven western Atlantic countries. I initially identified whether the lionfish invasion of MCEs is geographically widespread. Following this I tested whether invasive lionfish were more abundant and larger on MCEs than shallow reefs, and the impacts of hard substrata availability, the time since initial lionfish invasion and the presence or absence of lionfish culling on the relative abundance and body length distribution between shallow reefs and MCEs. I also compared the abundance and length distribution from the invaded sites with three Indo-Pacific sites with native lionfish populations.

1.4.5.1 Hypotheses tested:

1. Based on their rapid spread across shallow reef systems, MCEs have been widely invaded by lionfish across the western Atlantic region.
2. Invasive lionfish are more abundant on MCEs than shallow reefs because current management approaches (culling) are restricted to shallow reefs.
3. Lionfish are on average larger on MCEs than shallow reefs because of a possible continuation of their existing ontogenetic migration to include MCEs.
4. Lionfish abundance on MCEs will be unaffected by adjacent shallow reef lionfish culling because, if evidence for an ontogenetic migration is found, it suggests that there will be little movement of individual lionfish from MCEs to shallow reefs.

1.4.6 Chapter 7 – Testing ontogenetic migrations in invasive lionfish populations across shallow to mesophotic coral reefs

This chapter directly follows on from Chapter 6, using a detailed study at a single location, Utila, Honduras to explore lionfish ecology between shallow reefs and MCEs. With some uncertain patterns emerging from the meta-analysis in Chapter 6, I use lionfish dissection data to directly compare reproductive maturity down the depth gradient. In addition, I look at changes in lionfish health and feeding at multiple depths to identify dietary shifts.

1.4.6.1 Hypotheses tested:

1. Lionfish reproductive maturity will be greater on MCEs than shallow reefs, forming an extension of the previously reported ontogenetic migration on shallow reefs.
2. Because of the decline in reef fish abundance with depth, but no significant difference in invertebrate abundance with depth, we expect that MCE lionfish diets will shift towards containing a greater proportion of invertebrates.

1.5 References

- Aguilar-Perera A, Tuz-Sulub A (2010) Non-native, invasive Red lionfish (*Pterois volitans* [Linnaeus, 1758]: Scorpaenidae), is first recorded in the southern Gulf of Mexico, off the northern Yucatan Peninsula, Mexico. Aquatic Invasions 5:S9–S12
- Albins MA, Hixon MA (2013) Worst case scenario: potential long-term effects of invasive predatory lionfish (*Pterois volitans*) on Atlantic and Caribbean coral-reef communities. Environ Biol Fish 96:1151–1157
- Allen GR, Erdmann MV (2009) Reef fishes of the Bird’s Head Peninsula, West Papua, Indonesia. Check List 5:587–628
- Amado-Filho GM, Pereira-Filho GH, Bahia RG, Abrantes DP, Veras PC, Matheus Z (2012) Occurrence and distribution of rhodolith beds on the Fernando de Noronha Archipelago of Brazil. Aquatic Biology 101:41–45
- Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, U.K.
- Aponte NE, Ballantine DL (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 48:2185–2194
- Armstrong RA, Singh H (2012) Mesophotic Coral Reefs of the Puerto Rico Shelf. In: Harris P.T., Baker E.K. (eds) Seafloor Geomorphology as Benthic Habitat. Elsevier, London, pp 365–374
- Ashworth JS, Ormond RFG (2005) Effects of fishing pressure and trophic group on abundance and spillover across boundaries of a no-take zone. Biol Conserv 121:333–344
- Ballantine DL, Ruiz H, Aponte NE (2011) Algal composition and dynamics in mesophotic coral reefs, Southwest Puerto Rico. Journal of Phycology 47:15

- Bare AY, Grimshaw KL, Rooney JJ, Sabater MG, Fenner D, Carroll B (2010) Mesophotic communities of the insular shelf at Tutuila, American Samoa. Coral Reefs 29:369–377
- Barker Bausell R, Li YF (2002) Power Analysis for Experimental Research: A Practical Guide for the Biological, Medical and Social Sciences. Cambridge University Press, Cambridge, UK
- Beaman RJ, Bridge T, Done T, Webster JM, Williams S, Pizarro O (2012) Habitats and Benthos at Hydrographers Passage, Great Barrier Reef, Australia. In: Harris P.T., Baker E.K. (eds) Seafloor Geomorphology as Benthic Habitat. Elsevier, London, pp 425–434
- Beaman RJ, Bridge TCL, Lüter C, Reitner J, Wörheide G (2016) Spatial patterns in the distribution of benthic assemblages across a large depth gradient in the Coral Sea, Australia. Mar Biodiv 1–14
- Bejarano I, Appeldoorn RS, Nemeth M (2014) Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico. Coral Reefs 33:313–328
- Beldade R, Holbrook SJ, Schmitt RJ, Planes S, Malone D, Bernardi G (2012) Larger female fish contribute disproportionately more to self-replenishment. Proceedings of the Royal Society B: Biological Sciences 279:2116–2121
- Berkeley SA, Chapman C, Sogard SM (2004) Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85:1258–1264
- Bodmer MDV, Rogers AD, Speight MR, Lubbock N, Exton DA (2015) Using an isolated population boom to explore barriers to recovery in the keystone Caribbean coral reef herbivore *Diadema antillarum*. Coral Reefs 34:1011–1021
- Boland RC, Parrish FA (2005) Description of Fish Assemblages in the Black Coral Beds off Lahaina, Maui, Hawai'i. Pacific Science 59:411–420

- Bologna PA, Webb Wilson L, Connelly P, Saunders JE (2012) A new baseline for *Diadema antillarum*, *Echinometra viridis*, *E. lucunter*, and *Eucidaris tribuloides* populations within the Cayos Cochinos MPA, Honduras. Gulf and Caribbean Research 24:1–5
- Bolser RC, Hay ME (1996) Are Tropical Plants Better Defended? Palatability and Defenses of Temperate vs. Tropical Seaweeds. Ecology 77:2269
- Bongaerts P, Bridge TCL, Kline DI, Muir PR, Wallace CC, Beaman RJ, Hoegh-Guldberg O (2011) Mesophotic coral ecosystems on the walls of Coral Sea atolls. Coral Reefs 30:335–335
- Bongaerts P, Frade PR, Ogier JJ, Hay KB, van Bleijswijk J, Englebert N, Vermeij MJ, Bak RP, Visser PM, Hoegh-Guldberg O (2013a) Sharing the slope: depth partitioning of agariciid corals and associated *Symbiodinium* across shallow and mesophotic habitats (2-60 m) on a Caribbean reef. BMC Evol Biol 13:205
- Bongaerts P, Muir P, Englebert N, Bridge TCL, Hoegh-Guldberg O (2013b) Cyclone damage at mesophotic depths on Myrmidon Reef (GBR). Coral Reefs 32:935–935
- Bongaerts P, Ridgway T, Sampayo EM, Hoegh-Guldberg O (2010) Assessing the “deep reef refugia” hypothesis: focus on Caribbean reefs. Coral Reefs 29:309–327
- Box SJ, Canty SWJ (2011) The Long and Short Term Economic Drivers of Overexploitation in Honduran Coral Reef Fisheries Due to Their Dependence on Export Markets. Proceedings of the 63rd Gulf and Caribbean Fisheries Institute November 1 - 5, 2010 San Juan, Puerto Rico 63:43–51
- Bridge T, Scott A, Steinberg D (2012) Abundance and diversity of anemonefishes and their host sea anemones at two mesophotic sites on the Great Barrier Reef, Australia. Coral Reefs 31:1057–1062
- Bridge TCL, Hughes TP, Guinotte JM, Bongaerts P (2013) Call to protect all coral reefs. Nature Climate Change 3:528–530

- Brock VE, Chamberlain TC (1968) A Geological and Ecological Reconnaissance off Western Oahu, Hawaii, Principally by Means of the Research Submarine “Asherah”. Pacific Science 22:373–394
- Brokovich E, Ayalon I, Einbinder S, Segev N, Shaked Y, Genin A, Kark S, Kiflawi M (2010a) Grazing pressure on coral reefs decreases across a wide depth gradient in the Gulf of Aqaba, Red Sea. Marine Ecology Progress Series 399:69–80
- Brokovich E, Ben-Ari T, Kark S, Kiflawi M, Dishon G, Iluz D, Shashar N (2010b) Functional changes of the visual system of the damselfish *Dascyllus marginatus* along its bathymetric range. Physiology & Behavior 101:413–421
- Brokovich E, Einbinder S, Kark S, Shashar N, Kiflawi M (2007) A deep nursery for juveniles of the zebra angelfish *Genicanthus caudovittatus*. Environ Biol Fish 80:1–6
- Brokovich E, Einbinder S, Shashar N, Kiflawi M, Kark S (2008) Descending to the twilight-zone: changes in coral reef fish assemblages along a depth gradient down to 65 m. Marine Ecology Progress Series 371:253–262
- Brolund TM, Tychsen A, Nielsen LE, Arvedlund M (2004) An assemblage of the host anemone *Heteractis magnifica* in the northern Red Sea, and distribution of the resident anemonefish. Journal of the Marine Biological Association of the United Kingdom 84:671–674
- Bryan DR, Kilfoyle K, Gilmore RG Jr, Spieler RE (2013) Characterization of the mesophotic reef fish community in south Florida, USA. Journal of Applied Ichthyology 29:108–117
- Burke LM, Reytar K, Spalding M, Perry A (2011) Reefs at Risk Revisited. World Resources Institute,
- Carilli J (2008) Threshold Decline in Mesoamerican Coral Growth and Resiliency. Nature Precedings

- Carilli JE, Norris RD, Black BA, Walsh SM, McField M (2009) Local Stressors Reduce Coral Resilience to Bleaching. PLoS ONE 4:e6324
- Chérubin LM, Kuchinke CP, Paris CB (2008) Ocean circulation and terrestrial runoff dynamics in the Mesoamerican region from spectral optimization of SeaWiFS data and a high resolution simulation. Coral Reefs 27:503–519
- Claydon J, Calosso MC, Traiger SB (2012) Progression of invasive lionfish in seagrass, mangrove and reef habitats. Marine Ecology Progress Series 448:119–129
- Clements KD, Raubenheimer D, Choat JH (2009) Nutritional ecology of marine herbivorous fishes: ten years on. Functional Ecology 23:79–92
- Clifton KE, Clifton LM (1998) A survey of fishes from various coral reef habitats within the Cayos Cochinos Marine Reserve, Honduras. rev Biol Trop 46 Supl.(4):109–124
- Cocheret de la Morinière E, Pollux BJA, Nagelkerken I, van der Velde G (2002) Post-settlement Life Cycle Migration Patterns and Habitat Preference of Coral Reef Fish that use Seagrass and Mangrove Habitats as Nurseries. Estuarine, Coastal and Shelf Science 55:309–321
- Coen LD, Tanner CE (1989) Morphological Variation and Differential Susceptibility to Herbivory in the tropical brown alga *Lobophora variegata*. Marine Ecology Progress Series 54:287–298
- Cole AJ, Pratchett MS, Jones GP (2008) Diversity and functional importance of coral-feeding fishes on tropical coral reefs. Fish Fish 9:286–307
- Cole RG, Syms C, Davey NK, Gust N, Notman P, Stewart R, Radford CA, Carbines G, Carr MH, Jevs AG (2007) Does breathing apparatus affect fish counts and observations? A comparison at three New Zealand fished and protected areas. Marine Biology 150:1379–1395
- Colin PL (1974) Observation and collection of deep-reef fishes off the coasts of Jamaica and British Honduras (Belize). Marine Biology 24:29–38

- Colin PL (1976) Observations of Deep-Reef Fishes in the Tongue-of-the-Ocean, Bahamas. Bulletin of Marine Science 26:603–605
- Colin PL, Devaney DM, Hillis-Colinvaux L, Suchanek TH, Harrison JT (1986) Geology and biological zonation of the reef slope, 50-360 m depth at Enewetak Atoll, Marshall Islands. Bulletin of Marine Science 38:111–128
- Crawley M (2007) The R Book. Wiley-Blackwell. UK
- Cronin G, Hay ME (1996) Susceptibility to Herbivores Depends on Recent History of both the Plant and Animal. Ecology 77:1531
- Cronk L, Steadman S (2002) Tourists as a common-pool resource: A study of dive shops on Utila, Honduras. In: Cohen J., Dannhaeuser N. (eds) *Economic Development: An Anthropological Approach*. Altamira Press, Walnut Creek, CA, pp 51–68
- Cummings DO, Booth DJ, Lee RW, Simpson SJ, Pile AJ (2010) Ontogenetic diet shifts in the reef fish *Pseudanthias rubrizonatus* from isolated populations on the North-West Shelf of Australia. Marine Ecology Progress Series 419:211–222
- Dahl KA, Patterson WF III (2014) Habitat-Specific Density and Diet of Rapidly Expanding Invasive Red Lionfish, *Pterois volitans*, Populations in the Northern Gulf of Mexico. PLoS ONE 9:e105852
- de Ruyter van Steveninck ED, Bak RPM (1986) Changes in abundance of coral-reef bottom components related to mass mortality of the sea urchin *Diadema antillarum*. Marine Ecology Progress Series 34:87–94
- Downie RA, Babcock RC, Thomson DP, Vanderklift MA (2013) Density of herbivorous fish and intensity of herbivory are influenced by proximity to coral reefs. Marine Ecology Progress Series 482:217–225
- Earle JL, Pyle RL (1997) *Hoplolatilus pohle*, a New Species of Sand Tilefish (Perciformes: Malacanthidae) from the Deep Reefs of the D'Entrecasteaux Islands, Papua New Guinea. Copeia 1997:382

- Feitoza BM, Rosa RS, Rocha LA (2005) Ecology and Zoogeography of Deep-Reef Fishes in Northeastern Brazil. Bulletin of Marine Science 76:725–742
- Fowler AM, Booth DJ (2012) Evidence of sustained populations of a small reef fish on artificial structures. Does depth affect production on artificial reefs? Journal of Fish Biology 80:613–629
- Fox RJ, Bellwood DR (2007) Quantifying herbivory across a coral reef depth gradient. Marine Ecology Progress Series 339:49–59
- Fricke HW, Knauer B (1986) Diversity and spatial pattern of coral communities in the Red Sea upper twilight zone. Oecologia 71:29–37
- Garcia-Sais JR (2010) Reef habitats and associated sessile-benthic and fish assemblages across a euphotic–mesophotic depth gradient in Isla Desecheo, Puerto Rico. Coral Reefs 29:277–288
- García-Rubies A, Zabala M (1990) Effects of total fishing prohibition on the rocky fish assemblages of Medes Islands marine reserve (NW Mediterranean). Scientia Marina 54:317–328
- García-Salgado MA, Nava-Martínez GG, Vasquez M, Jacobs ND, Majil I, Molina-Ramírez A, Yañez-Rivera B, Cubas A, Dominguez-Calderon JJ, Hadaad W, Maldonado MA, Torres O (2008) Declining Trend on the Mesoamerican Reef System Marine Protected Areas. Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008 2:888–894 (Session number 18)
- Gerez MJA (2013) Differences in composition of territories in relation to behaviour, stage, and depth of the three-spot damselfish, *Stegastes planifrons*, in Caribbean coral reefs. Studies by Undergraduate Researchers at Guelph 6:52–57
- Gilmore GR, Jones RS (1992) Color Variation and Associated Behavior in the Epinepheline Groupers, *Mycteroperca Microlepis* (Goode and Bean) and *M. Phenax* Jordan and Swain. Bulletin of Marine Science 51:83–103

- Glynn PW (1996) Coral reef bleaching: facts, hypotheses and implications. Global Change Biol 2:495–509
- Gobert B, Berthou P, Lopez E, Lespagnol P, Turcios MDO, Macabiau C, Portillo P (2005) Early stages of snapper–grouper exploitation in the Caribbean (Bay Islands, Honduras). Fisheries Research 73:159–169
- Goetze JS, Jupiter SD, Langlois TJ, Wilson SK, Harvey ES, Bond T, Naisilisili W (2015) Diver operated video most accurately detects the impacts of fishing within periodically harvested closures. Journal of Experimental Marine Biology and Ecology 462:74–82
- Goetze JS, Langlois TJ, Egli DP, Harvey ES (2011) Evidence of artisanal fishing impacts and depth refuge in assemblages of Fijian reef fish. Coral Reefs 30:507–517
- Goreau TF, Graham EA (1967) A New Species of *Halimeda* from Jamaica. Bulletin of Marine Science 17:432–441
- Goreau TF, Wells JW (1967) The shallow-water Scleractinia of Jamaica: revised list of species and their vertical distribution range. Bulletin of Marine Science 17:442–453
- Graham NA, Bellwood DR, Cinner JE, Hughes TP, Norström AV, Nystrom M (2013) Managing resilience to reverse phase shifts in coral reefs. Frontiers in Ecology and the Environment 11:541–548
- Grassle JF (1991) Deep-sea benthic biodiversity. BioScience 41:464–469
- Harborne AR, Afzal DC, Andrews MJ (2001) Honduras: Caribbean Coast. Marine Pollution Bulletin 42:1221–1235
- Harm JH, Kearns E, Speight MR (2008) Differences in coral-reef fish assemblages between mangrove-rich and mangrove-poor islands of Honduras. Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008 1:296–300 (Session number 10)

- Harris PT, Bridge TCL, Beaman RJ, Webster JM, Nichol SL, Brooke BP (2013) Submerged banks in the Great Barrier Reef, Australia, greatly increase available coral reef habitat. ICES Journal of Marine Science 70:284–293
- Hay ME (1997) The ecology and evolution of seaweed-herbivore interactions on coral reefs. Coral Reefs 16:S67–S76
- Heyman WD, Kjerfve B (2001) The Gulf of Honduras. In: Seeliger U., Kjerfve B. (eds) Coastal Marine Ecosystems of Latin America. Springer Ecological Studies, Berlin, Heidelberg, pp 17–32
- Hillis-Colinvaux L (1986) Deep water populations of *Halimeda* in the economy of an atoll. Bulletin of Marine Science 38:155–169
- Hinderstein LM, Marr JCA, Martinez FA, Dowgiallo MJ, Puglise KA, Pyle RL, Zawada DG, Appeldoorn R (2010) Theme section on “Mesophotic Coral Ecosystems: Characterization, Ecology, and Management.” Coral Reefs 29:247–251
- Hixon MA, Johnson DW, Sogard SM (2014) BOFFFFs: on the importance of conserving old-growth age structure in fishery populations. ICES Journal of Marine Science 71:2171–2185
- Hoey AS, Bellwood DR (2011) Suppression of herbivory by macroalgal density: a critical feedback on coral reefs? Ecol Letters 14:267–273
- Hughes TP (1994) Catastrophes, Phase Shifts, and Large-Scale Degradation of a Caribbean Coral Reef. Science 265:1547–1551
- Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N, Pratchett MRS, Steneck RS, Willis B (2007) Phase Shifts, Herbivory, and the Resilience of Coral Reefs to Climate Change. Current Biology 17:360–365
- Itzkowitz M, Haley M, Otils C, Evers D (1991) A reconnaissance of the deeper Jamaican coral reef fish communities. Northeast Gulf Science 12:25–34

- Jaxion-Harm J, Pien C, Saunders JE, Speight MR (2013) Distribution of fish, crustacea and zooplankton at different distances from mangrove prop roots within a semi-isolated lagoon. Journal of the Marine Biological Association of the United Kingdom 93:1471–1479
- Jaxion-Harm J, Saunders J, Speight MR (2012) Distribution of fish in seagrass, mangroves and coral reefs: life-stage dependent habitat use in Honduras. rev Biol Trop 60:683–698
- Jaxion-Harm J, Speight MR (2012) Algal cover in mangroves affects distribution and predation rates by carnivorous fishes. Journal of Experimental Marine Biology and Ecology 414:19–27
- Kahng SE, Copus JM, Wagner D (2014) Recent advances in the ecology of mesophotic coral ecosystems (MCEs). Current Opinion in Environmental Sustainability 7:72–81
- Kahng SE, Garcia-Sais JR, Spalding HL, Brokovich E, Wagner D, Weil E, Hinderstein L, Toonen RJ (2010) Community ecology of mesophotic coral reef ecosystems. Coral Reefs 29:255–275
- Kahng SE, Grigg RW (2005) Impact of an alien octocoral, *Carijoa riisei*, on black corals in Hawaii. Coral Reefs 24:556–562
- Keith DE (1992) Shallow-water gorgonians (Octocorallia) of Roatan, Honduras. Bulletin of Marine Science 50:212–226
- Klumpp DW, McKinnon D, Daniel P (1987) Damselfish territories: zones of high productivity on coral reefs. Marine Ecology Progress Series 40:41–51
- Korda RC, Hills JM, Gray TS (2008) Fishery decline in Utila: Disentangling the web of governance. Marine Policy 32:968–979
- Kosaki RK, Pyle RL, Randall JE, Irons DK (1991) New Records of Fishes from Johnston Atoll, with Notes on Biogeography. Pacific Science 45:186–203

- Kramer P, McField M, Álvarez-Filip L, Drysdale I, Flores MR, Giró A, Pott R (2015) 2015 Report Card for the Mesoamerican Reef. healthyreefs.org
- Kulbicki M, Bozec Y-M, Labrosse P, Letourneur Y, Mou-Tham G, Wantiez L (2005) Diet composition of carnivorous fishes from coral reef lagoons of New Caledonia. Aquat. Living Resour. 18:231–250
- La Mesa G, Di Muccio S, Vacchi M (2006) Abundance, size distribution and habitat preferences in the grouper assemblage of the Ustica marine reserve (SW Mediterranean). Cybium 30:365–377
- Lang JC (1974) Biological Zonation at the Base of a Reef: Observations from the submersible Nekton Gamma have led to surprising revelations about the deep fore-reef and island slope at Discovery Bay, Jamaica. American Scientist 62:272–281
- Leichter JJ, Stokes MD, Genovese SJ (2008) Deep water macroalgal communities adjacent to the Florida Keys reef tract. Marine Ecology Progress Series 356:123–138
- Lesser MP, 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 13:1855–1868
- Lewis SM (1986) The Role of Herbivorous Fishes in the Organization of a Caribbean Reef Community. Ecological Monographs 56:183–200
- Liddell WD, Avery WE (2000) Temporal change in hard substrate communities 10-250 m, the Bahamas. Proceedings of the 9th International Coral Reef Symposium, Bali, Indonesia 23-27 October 2000 2:1053–1058
- Liddell WD, Ohlhorst SL (1988) Hard substrata community patterns, 1-120 M, North Jamaica. Palaios 413–423
- Lindfield SJ, Harvey ES, Halford AR, McIlwain JL (2016) Mesophotic depths as refuge areas for fishery-targeted species on coral reefs. Coral Reefs 35:125–137

- Lindfield SJ, Harvey ES, McIlwain JL, Halford AR (2014a) Silent fish surveys: bubble-free diving highlights inaccuracies associated with SCUBA-based surveys in heavily fished areas. Methods Ecol Evol 5:1061–1069
- Lindfield SJ, McIlwain JL, Harvey ES (2014b) Depth Refuge and the Impacts of SCUBA Spearfishing on Coral Reef Fishes. PLoS ONE 9:e92628
- Loh TL, Pawlik JR (2014) Chemical defenses and resource trade-offs structure sponge communities on Caribbean coral reefs. Proceedings of the National Academy of Sciences 111:4151–4156
- Loya Y, Eyal G, Treibitz T, Lesser MP, Appeldoorn R (2016) Theme section on mesophotic coral ecosystems: advances in knowledge and future perspectives. Coral Reefs 35:1–9
- Luck DG, Forsman ZH, Toonen RJ, Leicht SJ, Kahng SE (2013) Polyphyly and hidden species among Hawai‘i’s dominant mesophotic coral genera, *Leptoseris* and *Pavona* (Scleractinia: Agariciidae). PeerJ 1:e132
- Luttinger N (1997) Community-based coral reef conservation in the Bay Islands of Honduras. Ocean & Coastal Management 36:11–22
- Macaya EC, Rothäusler E, Thiel M, Molis M, Wahl M (2005) Induction of defenses and within-alga variation of palatability in two brown algae from the northern-central coast of Chile: Effects of mesograzers and UV radiation. Journal of Experimental Marine Biology and Ecology 325:214–227
- MacDonald JA, Weis JS (2013) Fish community features correlate with prop root epibionts in Caribbean mangroves. Journal of Experimental Marine Biology and Ecology 441:90–98
- Mackenzie CL, Stehlik LL (1996) The Crustacean and Molluscan Fisheries of Honduras. Marine Fisheries Review 58:33–44

- MacNeil MA, Connolly SR (2015) Multi-scale patterns and processes in reef fish abundance. In: Mora C. (eds) Ecology of Fishes on Coral Reefs. Cambridge University Press, Cambridge, pp 116–124
- Macpherson E, Duarte CM (1991) Bathymetric trends in demersal fish size: is there a general relationship? Marine Ecology Progress Series 71:103–112
- Marshall DJ, Heppell SS, Munch SB, Warner RR (2010) The relationship between maternal phenotype and offspring quality: Do older mothers really produce the best offspring? Ecology 91:2862–2873
- McClanahan T (2000) Bleaching damage and recovery potential of Maldivian coral reefs. Marine Pollution Bulletin 40:587–597
- Menza C, Kendall M, Hile S (2008) The deeper we go the less we know. rev Biol Trop 56:11–24
- Menza C, Kendall M, Rogers C, Miller J (2007) A deep reef in deep trouble. Continental Shelf Research 27:2224–2230
- Meylan AB (1999) Status of the Hawksbill Turtle (*Eretmochelys imbricata*) in the Caribbean Region. Chelonian Conservation and Biology 3:177–184
- Mitchell SJ, Doolette DJ (2013) Recreational technical diving part 1: an introduction to technical diving methods and activities. Diving and Hyperbaric Medicine 43:86–93
- Moffitt RB, Parrish FA, Polovina JJ (1989) Community structure, biomass and productivity of deepwater artificial reefs in Hawaii. Bulletin of Marine Science 44:616–630
- Morato T, Watson R, Pitcher TJ, Pauly D (2006) Fishing down the deep. Fish Fish 7:24–34
- Moreno PS (2005) Ecotourism Along the Meso-American Caribbean Reef: The Impacts of Foreign Investment. Hum Ecol 33:217–244

- Morrison D (1988) Comparing Fish and Urchin Grazing in Shallow and Deeper Coral Reef Algal Communities. Ecology 69:1367
- Mumby PJ, Harborne AR, Williams J, Kappel CV, Brumbaugh DR, Micheli F, Holmes KE, Dahlgren CP, Paris CB, Blackwell PG (2007) Trophic cascade facilitates coral recruitment in a marine reserve. Proceedings of the National Academy of Sciences 104:8362–8367
- Mumby PJ, Steneck RS, Edwards AJ, Ferrari R, Coleman R, Harborne AR, Gibson JP (2012) Fishing down a Caribbean food web relaxes trophic cascades. Marine Ecology Progress Series 445:13–24
- Myers RF (1993) Guam’s Small-Boat-based Fisheries. Marine Fisheries Review 55:117–128
- Neal BP, Condit C, Liu G, Santos S, Kahru M, Mitchell BG, Kline DI (2014) When depth is no refuge: cumulative thermal stress increases with depth in Bocas del Toro, Panama. Coral Reefs 33:193–205
- Nugues MM, Bak RPM (2008) Long-term dynamics of the brown macroalga *Lobophora variegata* on deep reefs in Curaçao. Coral Reefs 27:389–393
- Nuttall MF, Johnston MA, Eckert RJ, Embesi JA, Hickerson EL, Schmahl GP (2014) Lionfish (*Pterois volitans* [Linnaeus, 1758] and *P. miles* [Bennett, 1828]) records within mesophotic depth ranges on natural banks in the Northwestern Gulf of Mexico. BioInvasions Records 3:111–115
- Pansch C, Gómez I, Rothäusler E, Veliz K, Thiel M (2008) Species-specific defense strategies of vegetative versus reproductive blades of the Pacific kelps *Lessonia nigrescens* and *Macrocystis integrifolia*. Marine Biology 155:51–62
- Paris CB, Chérubin LM (2008) River-reef connectivity in the Meso-American Region. Coral Reefs 27:773–781
- Parker FO, Ross SW (1986) Observing reef fishes from submersibles off North Carolina. Northeast Gulf Science 8:31–49

- Parrish FA, Boland RC (2004) Habitat and reef-fish assemblages of banks in the Northwestern Hawaiian Islands. Marine Biology 144:1065–1073
- Parrish FA, Pyle RL (2002) Field Comparison of Open-Circuit Scuba to Closed-Circuit Rebreathers for Deep Mixed-Gas Diving Operations. Marine Technology Society Journal 36:13–22
- Pereira-Filho GH, Amado-Filho GM, Guimarães SMPB, Moura RL, Sumida PYG, Abrantes DP, Bahia RG, Güth AZ, Jorge RR, Filho RBF (2011) Reef fish and benthic assemblages of the Trindade and Martin Vaz Island group, Southwestern Atlantic. Brazilian Journal of Oceanography 59:201–212
- Pinheiro HT, Goodbody-Gringley G, Jessup ME, Shepherd B, Chequer AD, Rocha LA (2016) Upper and lower mesophotic coral reef fish communities evaluated by underwater visual censuses in two Caribbean locations. Coral Reefs 35:139–151
- Polunin NVC, Roberts CM (1993) Greater biomass and value of target coral-reef fishes in two small Caribbean marine reserves. Marine Ecology Progress Series 100:167–176
- Pyle RL (1998) Use of Advanced Mixed-Gas Diving Technology to Explore the Coral Reef “Twilight Zone.” In: Tanacredi J.T., Loret J. (eds) Ocean Pulse: A Critical Diagnosis. Springer, New York, pp 71–88
- Pyle RL (2000) Assessing Undiscovered Fish Biodiversity on Deep Coral Reefs Using Advanced Self-Contained Diving Technology. Marine Technology Society Journal 34:82–91
- Pyle RL, Chave EH (1994) First Record of the Chaetodontid Genus *Prognathodes* from the Hawaiian Islands. Pacific Science 48:90–93
- Quattrini AM, Ross SW, Sulak KJ, Necaise AM, Casazza TL, Dennis GD (2004) Marine fishes new to continental United States waters, North Carolina, and the Gulf of Mexico. Southeastern Naturalist 3:155–172

- Radford CA, Jeffs AG, Tindle CT, Cole RG, Montgomery JC (2005) Bubbled waters: The noise generated by underwater breathing apparatus. Marine and Freshwater Behaviour and Physiology 38:259–267
- Ralston S, Gooding RM, Ludwig GM (1986) An ecological survey and comparison of bottom fish resource assessments (submersible versus handline fishing) at Johnston Atoll. Fishery Bulletin 84:141–155
- Randall JE, Lobel PS, Chave EH (1985) Annotated Checklist of the Fishes of Johnston Island. Pacific Science 39:24–80
- Randall JE, Pyle RL (2001) Four new serranid fishes of the anthiine genus *Pseudanthias* from the South Pacific. Raffles Bulletin of Zoology 49:19–34
- Rickel S, Genin A (2005) Twilight transitions in coral reef fish: the input of light-induced changes in foraging behaviour. Animal Behaviour 70:133–144
- Riegl B, Piller WE (2003) Possible refugia for reefs in times of environmental stress. International Journal of Earth Sciences 92:520–531
- Russ GR (1987) Is rate of removal of algae by grazers reduced inside territories of tropical damselfishes? Journal of Experimental Marine Biology and Ecology 110:1–17
- Ruxton GD, Colegrave N (2002) Experimental design for the life sciences. Oxford University Press. Oxford, UK
- Sammarco PW, Williams AH (1982) Damselfish territoriality: Influence on *Diadema* distribution and implications for coral community structure. Marine Ecology Progress Series 8:53–59
- Sazima I, Grossman A, Sazima C (2010) Deep cleaning: a wrasse and a goby clean reef fish below 60 m depth in the tropical south-western Atlantic. Mar. Biodivers. Rec. 3:e60

- Scaps P, Saunders J (2011) Shallow Water Stony Corals (Scleractinia, Milleporidae, and Stylasteridae) from Utila and Cayos Cochinos, Honduras. ISRN Zoology 2011:1–9
- Schofield PJ (2009) Geographic extent and chronology of the invasion of non-native lionfish (*Pterois volitans* [Linnaeus 1758] and *P. miles* [Bennett 1828]) in the Western North Atlantic and Caribbean Sea. Aquatic Invasions 4:473–479
- Schofield PJ (2010) Update on geographic spread of invasive lionfishes (*Pterois volitans* [Linnaeus, 1758] and *P. miles* [Bennett, 1828]) in the Western North Atlantic Ocean, Caribbean Sea and Gulf of Mexico. Aquatic Invasions 5:S117–S122
- Shinn EA, Wicklund RI (1989) Artificial Reef Observations from a Manned Submersible off Southeast Florida. Bulletin of Marine Science 44:1041–1050
- Sieber A, Pyle R (2010) A review of the use of closed-circuit rebreathers for scientific diving. Underwater Technology 29:73–78
- Slattery M, Lesser MP (2014) Allelopathy in the tropical alga *Lobophora variegata* (Phaeophyceae): mechanistic basis for a phase shift on mesophotic coral reefs? Journal of Phycology 50:493–505
- Slattery M, Lesser MP, Brazeau D, Stokes MD, Leichter JJ (2011) Connectivity and stability of mesophotic coral reefs. Journal of Experimental Marine Biology and Ecology 408:32–41
- Smith TB, Blondeau J, Nemeth RS, Pittman SJ, Calnan JM, Kadison E, Gass J (2010) Benthic structure and cryptic mortality in a Caribbean mesophotic coral reef bank system, the Hind Bank Marine Conservation District, U.S. Virgin Islands. Coral Reefs 29:289–308
- Spalding HL (2012) Ecology of mesophotic macroalgae and *Halimeda kanaloana* medows in the main Hawaiian Islands. University of Hawai‘i at Mānoa

- Sparks JS, Gruber DF (2012) A New Mesophotic Clingfish (Teleostei: Gobiesocidae) from the Bahamas. Copeia 2012:251–256
- Starck WA, Colin PL (1978) *Gramma linki*: a new species of grammid fish from the tropical western Atlantic. Bulletin of Marine Science 28:146–152
- Stevenson TC, Tissot BN, Dierking J (2011) Fisher behaviour influences catch productivity and selectivity in West Hawaii's aquarium fishery. ICES Journal of Marine Science 68:813–822
- Stonich SC (2003) The Political Ecology of Marine Protected Areas: The Case of the Bay Islands. In: Gossling S. (eds) Tourism and Development in Tropical Islands. Edward Elgar Publishing Limited, Cheltenham, UK, pp 121–147
- Strasburg DW, Jones EC, Iversen RTB (1968) Use of a Small Submarine for Biological and Oceanographic Research. J. Cons. perm. int. Explor. Mer 31:410–426
- Sutherland WJ, Clout M, Côté IM, Daszak P, Depledge MH, Fellman L, Fleishman E, Garthwaite R, Gibbons DW, De Lurio J, Impey AJ, Lickorish F, Lindenmayer D, Madgwick J, Margerison C, Maynard T, Peck LS, Pretty J, Prior S, Redford KH, Scharlemann JPW, Spalding M, Watkinson AR (2010) A horizon scan of global conservation issues for 2010. Trends in Ecology & Evolution 25:1–7
- Terk E, Knowlton N (2010) The role of SCUBA diver user fees as a source of sustainable funding for coral reef marine protected areas. Biodiversity 11:78–84
- Thresher RE, Colin PL (1986) Trophic structure, diversity and abundance of fishes of the deep reef (30-300m) at Enewetak, Marshall Islands. Bulletin of Marine Science 38:253–272
- Titus BM, Daly M, Exton DA (2015) Do Reef Fish Habituate to Diver Presence? Evidence from Two Reef Sites with Contrasting Historical Levels of SCUBA Intensity in the Bay Islands, Honduras. PLoS ONE 10:e0119645

- Trianni MS, Tenorio MC (2012) Gross diest composition and trophic level estimation of the spotcheek emperor, *Lethrinus rubrioperculatus* (ACTINOPTERYGII: PERCIFORMES: LETHRINIDAE), in the commonwealth of the Northern Mariana Islands, Micronesia. Acta Ichthyologica et Piscatoria 42:89–99
- Tyler E, Speight M, Henderson P, Manica A (2009) Evidence for a depth refuge effect in artisanal coral reef fisheries. Biol Conserv 142:652–667
- Vergés A, Vanderklift MA, Doropoulos C, Hyndes GA (2011) Spatial Patterns in Herbivory on a Coral Reef Are Influenced by Structural Complexity but Not by Algal Traits. PLoS ONE 6:e17115
- Vinogradova NG (1962) Vertical zonation in the distribution of deep-sea benthic fauna in the ocean. Deep Sea Research 8:245–250
- Wagner D, Kosaki RK, Spalding HL, Whitton RK, Pyle RL, Sherwood AR, Tsuda RT, Calcinai B (2014) Mesophotic surveys of the flora and fauna at Johnston Atoll, Central Pacific Ocean. Mar. Biodivers. Rec. 7:e68
- Watson DL, Harvey ES (2007) Behaviour of temperate and sub-tropical reef fishes towards a stationary SCUBA diver. Marine and Freshwater Behaviour and Physiology 40:85–103
- Watson DL, Harvey ES, Anderson MJ, Kendrick GA (2005) A comparison of temperate reef fish assemblages recorded by three underwater stereo-video techniques. Marine Biology 148:415–425
- Watson DL, Harvey ES, Fitzpatrick BM, Langlois TJ, Shedrawi G (2010) Assessing reef fish assemblage structure: how do different stereo-video techniques compare? Marine Biology 157:1237–1250
- Weaver DC, Dennis GD III, Sulak KJ (2001) Northeastern Gulf of Mexico Coastal and Marine Ecosystem Program: Community structure and Trophic Ecology of

Demersal Fishes on the Pinnacles Reef Tract. US Geological Survey Biological Sciences Report USGS BSR 92

- Wenner CA (1983) Species associations and day-night variability of trawl-caught fishes from the inshore sponge-coral habitat, South Atlantic bight. Fishery Bulletin 80:537–552
- White KN, Ohara T, Fujii T, Kawamura I, Mizuyama M, Montenegro J, Shikiba H, Naruse T, McClelland TY, Denis V, Reimer JD (2013) Typhoon damage on a shallow mesophotic reef in Okinawa, Japan. PeerJ 1:e151
- Young CN, Schopmeyer SA, Lirman D (2012) A Review of Reef Restoration and Coral Propagation Using the Threatened Genus *Acropora* in the Caribbean and Western Atlantic. Bulletin of Marine Science 88:1075–1098

2 REEF FISH COMMUNITY BIOMASS AND TROPHIC STRUCTURE CHANGES ACROSS SHALLOW TO UPPER-MESOPHOTIC REEFS IN THE MESOAMERICAN BARRIER REEF, CARIBBEAN

2.1 Abstract

Mesophotic coral ecosystems (MCEs; reefs 30-150 m depth) are of increased research interest because of their potential role as depth refuges from many shallow reef threats. Yet few studies have identified patterns in fish species composition and trophic group structure between MCEs and their shallow counterparts. Here we explore reef fish species and biomass distributions across shallow to upper-MCE Caribbean reef gradients (5-40 m) around Utila, Honduras, using a diver-operated stereo-video system. Broadly, we found reef fish species richness, abundance and biomass declining with depth. At the trophic group level we identified declines in herbivores (both total and relative community biomass) with depth, mostly driven by declines in parrotfish (Scaridae). Piscivores increased as a proportion of the community with increased depth while, in contrast to previous studies, we found no change in relative planktivorous reef fish biomass across the depth gradient. In addition, we also found evidence of ontogenetic migrations in the blue tang (*Acanthurus coeruleus*), striped parrotfish (*Scarus iserti*), blue chromis (*Chromis cyanea*), creole wrasse (*Clepticus parrae*), bluehead wrasse (*Thalassoma bifasciatum*) and yellowtail snapper (*Ocyurus chrysurus),* with a higher proportion of larger individuals at mesophotic and near-mesophotic depths than on shallow reefs. Our results highlight the importance of using biomass measures when considering fish community changes across depth gradients, with biomass generating different results to simple abundance counts.

Chapter 2: Reef fish community biomass and trophic structure changes across shallow to upper-mesophotic reefs in the Mesoamerican Barrier Reef, Caribbean

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2.2 Introduction

Mesophotic coral ecosystems (MCEs; zooxanthellate coral reefs from 30 m to approximately 150 m depth) are understudied (Menza et al. 2008), yet may have a linear extent as great as shallow coral ecosystems (Puglise et al. 2009). Mapping of MCEs has led to suggestions that identifying their full distribution may double overall reef area at regional scales (Harris et al. 2013). Much recent interest in MCEs has focused on whether they act as refuges for threatened shallow-reef species (Bongaerts et al. 2010). Many threats faced by reefs, such as bleaching, direct storm damage, local pollution and overfishing have previously been assumed to decline in severity with depth (Bridge et al. 2013). However, this proposed importance as a depth refuge has recently been challenged, as it has become clear that MCEs face many of these threats as well (Chapter 9).

Zooxanthellate coral reefs in tropical and subtropical areas can broadly be divided into three depth zones: (i) shallow coral reefs (0-30 m), (ii) the upper mesophotic zone (30-60 m) and (iii) the lower mesophotic zone (60 m-maximum local depth of zooxanthellate corals, typically between 150 and 200 m) (Kahng et al. 2010; 2014). MCEs are often found in association with shallow coral reefs, as reefs extend down the depth gradient from shallow to mesophotic depths. Armstrong and Singh (2012) observed that temperature and light availability are important for MCE communities to establish, but sediment channelling at depth separated highly developed MCEs from poorly established communities at very close proximity.

Reef fish and coral distributions from shallow reefs to MCEs exhibit similar patterns in species richness, abundance and biomass, although there is geographical variation in exact transition depths between communities. For example, in the Caribbean, over 75 % of shallow coral species extend into MCEs (Bridge et al. 2013). Bejarano *et al.* (2014) compared fish populations on MCEs to 70 m and found approximately 75 % of fish species on Puerto Rican MCEs could also be found on shallower reefs, although many of these did not occur past 60 m. Research in the Marshall Islands reported a shallow fore reef fish community that extended from the surface to approximately 75-90 m, after which many shallow species were lost (Thresher and Colin 1986). In the Red Sea, shallower fish species were found to drop off rapidly below 30 m (Brokovich et al. 2008). Many of these differences in transition points between shallow and mesophotic fish communities are likely to be driven by local and regional variations in environmental conditions, although the general trend of the upper mesophotic zone harbouring shallow fish species appears widespread.

Trophic groups also respond differently to depth across the shallow to mesophotic gradient. Generally reef fish communities shift from herbivore dominance at shallow depths to carnivore dominance (mainly zooplanktivores) at mesophotic depths (Kahng et al. 2010). This trend has been observed in the Caribbean (Garcia-Sais 2010; Kahng et al. 2010), Brazil (Feitoza et al. 2005; Pereira-Filho et al. 2011), the Marshall Islands (Thresher and Colin 1986) and the Red Sea (Brokovich et al. 2008; 2010). Regionally in the Caribbean, planktivores account for 22 % of fish species recorded on MCEs (Kahng et al. 2010). In the Gulf of Mexico Weaver et al. (2001) found fish from the subfamily Anthiinae (small planktivores in the family Serranidae) were so abundant on MCEs that they considered them keystone species, transferring energy from the water column to the reef.

At a local scale, the structural complexity provided by corals and other benthic species is critical for MCE fish communities to thrive. For example, large grouper abundance in Puerto Rico has been observed to peak at 25 m depth, but only in association with *Montastrea spp.,* the dominant scleractinian coral at those depths (Garcia-Sais 2010). Other studies have reported the grouper *Mycteroperca phenax* associated with high structural complexity reefs at 70-100 m depth in Puerto Rico (Gilmore and Jones 1992). In the Red Sea, turnover in fish community composition at mesophotic depths appears highly correlated with a reduction in branching coral abundance (Brokovich et al. 2008). Other fish distributions are directly restricted based on benthic associations. Anemonefish on MCEs, both in the Red Sea (Brolund et al. 2004) and the Great Barrier Reef (Bridge et al. 2012), are likely to be driven by the distribution of their host anemones rather than reaching any physiological depth limit for the fish themselves.

Many fish species incorporate depth into their life histories, migrating bathymetrically as they mature or for spawning (Macpherson and Duarte 1991). These ontogenetic migrations are driven by the trade-off between maximising food availability and minimising predation risk (Dahlgren and Eggleston 2000). Ontogenetic migrations have been observed in many Caribbean fish species with young juveniles typically found in mangroves, seagrass beds and back reefs, before migrating to reef crests and slopes as they mature (Cocheret de la Morinière et al. 2002). However, depth distributions in species life histories are far from consistent. In the Gulf of Aqaba, Red Sea, juvenile zebra angelfish (*Genicanthus caudovittatus*) abundance peaks at 60-65 m, where 80 % of juveniles were found, compared to adult peak abundance at 30 m (Brokovich et al. 2007).

Within the Caribbean and western Atlantic, existing research is generally limited to a few locations such as Puerto Rico (Garcia-Sais 2010; Bejarano et al. 2014), Brazil (Feitoza et al. 2005; Pereira-Filho et al. 2011; Rosa et al. 2016), Curaçao (Pinheiro et al. 2016), Bermuda (Pinheiro et al. 2016) and the Gulf of Mexico (Weaver et al. 2001), and has mostly focused on comparing visual in-water or video counts of species abundance. Using fish biomass provides a better understanding of ecosystem functions across the depth gradient, and the dominant trophic groups driving these patterns. Switching community analysis from abundance to biomass could significantly affect our understanding of tropic structuring as, for example, piscivorous fish tend to be larger bodied than planktivorous or herbivorous reef fish (Kulbicki et al. 2015), so simple abundance comparisons are unlikely to determine the relative ecological functions performed by different groups. Length information also allows patterns in fish life history to be identified, including ontogenetic migrations across the depth gradient. In this study we investigate how reef fish communities change across a shallow to upper mesophotic reef gradient on the southern Mesoamerican Barrier Reef by addressing both abundance and biomass. We use a diver-operated stereo-video system to enable accurate reef fish biomass estimates to be made along a Caribbean shallow-MCE depth gradient for the first time. Specifically we address changes in dominant fish trophic groups, to test whether previously reported patterns elsewhere on MCEs occur in the southern Mesoamerican Barrier Reef, and the use of the biomass metric rather than abundance affects interpretation of results.

2.3 Materials and methods

2.3.1 Data Collection

Surveys were conducted at seven fringing coral reef sites around Utila, Bay Islands, Honduras (see Table S2.1 for GPS locations). Utila is located at the southern end of the Mesoamerican Barrier Reef. Two sites (The Maze and Spotted Bay) were located on the island’s exposed north shore, and five were located on the more sheltered south shore (Stingray Point, Little Bight, Black Coral Wall, Coral View and Lighthouse Reef) (Fig 2.1). On the south shore the reef slope reaches a maximum depth of 40-60 m where the seabed levels off stretching to mainland Honduras. South shore MCEs tend to be extensive patch reef systems, with large areas of reef separated by areas of sand. On the north shore of Utila, the reef slope continues to >100 m. Shallow reefs at south shore sites consist of a spur and groove system, whereas shallow reefs on the north shore are on steeper reef walls. Field Permits were issued by the Instituto de Conservacion Forestal (ICF), Honduras.

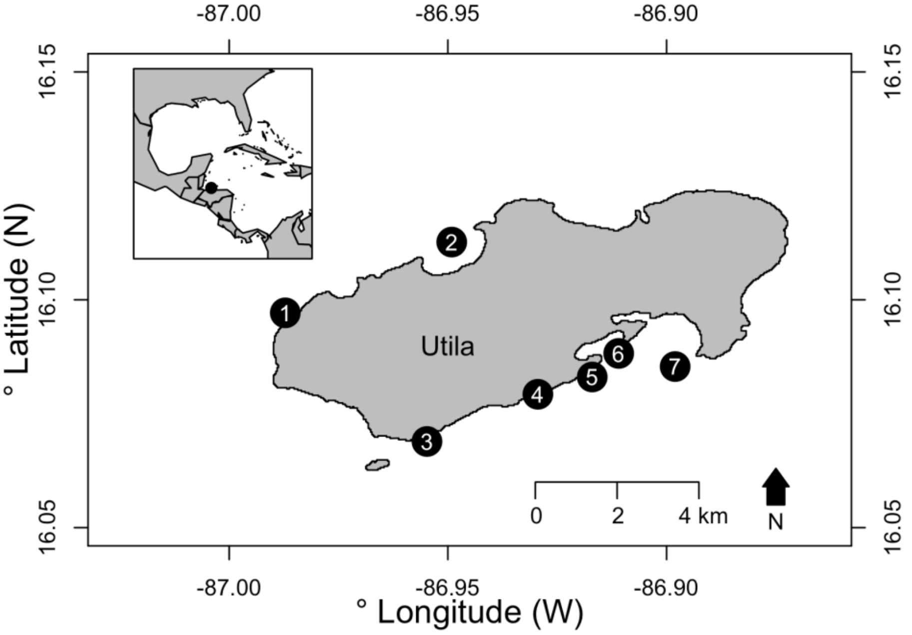

Figure 2.1 Map of study sites around Utila, Honduras.

Survey locations are marked, with numbers indicating sites as follows: (1) Spotted Bay, (2) The Maze, (3) Stingray Point, (4) Little Bight, (5) Black Coral Wall, (6) Coral View and (7) Lighthouse Reef. Inset map shows the location of Utila relative to the Caribbean region. Map sourced from GADM database of Global Administrative Areas under a CC BY licence with permission.

Transects were conducted using a diver-operated stereo-video system (SVS; SeaGIS, Melbourne, Australia), composed of two Canon HFS21 high definition video cameras (see following for system overview: Harvey et al. 2001; 2004; Watson et al. 2010). SVS allows fish communities to be recorded more quickly in water than traditional underwater visual census (UVC) technique, and for fish biomass to be estimated more accurately (Holmes et al. 2013). At each site, six transects were conducted at 5 m and 15 m and four transects were conducted at 25 m and 40 m. Fewer transects were conducted at 25 m and 40 m depths because of increased logistical challenges associated with depth. However, using an unbalanced study design such as this increases overall statistical power to discern ecological changes across the depth gradient (Barker Bausell and Li 2002).

Transects were 50 m long following the reef contour, with a 10 m interval between replicates, and were surveyed by an SVS operator and a second diver responsible for distance measurement. To minimise potential disturbance to the fish community while cameras were set to record and synchronised, transects were begun with the cameras pointing vertically down, and at 10 m the SVS operator was alerted via a fin tug to indicate the start of the transect proper. At this point cameras were pointed along the reef, with another fin tug indicating the transect end after a further 50 m. Cameras were angled at approximately 20 degrees downwards, and kept approximately 0.5 m above the seabed, filming the reef scape along the transect. Transects took approximately 4 minutes to film with divers using open circuit accelerated decompression SCUBA diving.

Footage was analysed in EventMeasure software v3.51 (SeaGIS, Melbourne, Australia), allowing the calibrated SVS footage to be synchronised and fish total lengths to be measured. EventMeasure also resolved centre points of each individual fish encountered into distances on a three-dimensional coordinate system, allowing us to exclude fish outside 2.5 m either side and 5 m in front of the camera system. Side distance restriction maintains a consistent belt along the transect, while front distance restriction prevents variations in visibility (e.g. turbidity, light intensity) from influencing data.

After completing the fish surveys, benthic videos were filmed by the SVS operator as the survey team returned along each 50 m transect using a Cannon HFS21 or GoPro Hero 3 Silver video camera. This allowed the SVS operator to swim slowly along the transect with the camera pointing vertically down and held approximately 0.3 m above the benthos, keeping the transect tape within the frame at all times. Point-intercept analysis was conducted, recording the benthic substrate at 0.25 m intervals following English et al. (1997).

SVS and benthic video analyses were conducted by a volunteer team of undergraduate students. Volunteers were extensively trained prior to participation, having specifically (i) completed a week long Caribbean coral reef ecology course combining lectures with in-water practicals where experienced researcher pointed out fish species for them to observe, (ii) passed Caribbean coral, macroalgae and reef fish species identification written exams (pass grade 80%) and had any incorrectly identified reef fish discussed with them, (iii) completed a training tutorial in EventMeasure analysis, and (iv) conducted 12 training stereo-video transect analyses in EventMeasure where a fish identification expert reviewed each video on completion allowing commonly mistaken fish species to be highlighted to the volunteers. While conducting the actual transect video analysis, volunteers had a copy of Humann and Deloach (2008) and were able to discuss or confirm any fish they were unsure of with other volunteers or an experienced researcher. All fish were identified to species level following Humann and Deloach (2008).

2.3.2 Analysis

Fish length measurements were converted into biomass using Equation 1.

Equation 1: $W = aL^b$

Where L represents the fish length in centimetres, W the weight in grams and a and b published species-specific conversion constants from Fishbase (accessed September 2014). Where conversion constants were not available, the genus mean was used. Where fish length measurements were not possible (e.g. because of the angle of an individual to the cameras), the individual was recorded and the mean length for that species at that depth and site applied or, where no site and depth con-specifics were available, the mean across all depths and sites. These fish were excluded from all analysis of median fish lengths. Variation in coverage of key benthic groups (hard coral, soft coral, macroalgae, sponge and sand) were calculated as percentage cover. Fish species richness, abundance and biomass were calculated as mean per transect (250 m^2).

To compare benthic habitats between north and south shores, sites and depths, a non-parametric permutational analysis of variance (PERMANOVA) was used (Anderson et al. 2008). For fish biomass, a Bray-Curtis dissimilarity matrix was constructed based on fourth root transformed fish species biomass for both non-metric multidimensional scaling (NMDS) and PERMANOVA. MDS plots were constructed to illustrate the relationship between fish communities and the availability of hard substrata using the vegan package (Oksanen et al. 2013) in R (R Core Team 2013). For comparisons between fish communities we included percentage hard substratum as a variable in the PERMANOVA model. Hard substratum was defined as benthic cover that was not mud, sand or rubble following Gratwicke and Speight (2005). Fish community permutations were constrained by site. All PERMANOVAs were run for 99999 permutations using the ‘adonis’ function in vegan. Because of the multifactor and unbalanced nature of our data, when testing interactions sums of squares for model terms are non-independent (Anderson et al. 2008). To address this ‘adonis’ uses Type I (sequential) sums of squares, with each term sequentially fitted after taking account of the previously fitted terms. This approach is considered appropriate for nested experimental designs where terms naturally exhibit a hierarchical ordering (Anderson et al. 2008).

To investigate variation in the proportion of fish feeding guilds we used the Caribbean fish feeding guild classification by Micheli et al. (2014) to allocate each species to a trophic group. Trophic groups were: herbivores, planktivores, piscivores, omnivores, invertebrate feeders and fish species that feed as both piscivores and invertebrate feeders, which we call carnivores. We calculated the proportion of the overall fish community each trophic group composed at each depth. We used a univariate PERMANOVA based on Euclidean distances and constrained by site to test for differences between the total biomass at 5 m and

40 m and the proportion of biomass at 5 m and 40 m each trophic group comprised. Only 5 m and 40 m data were used to allow a simple comparison between the fish community on shallow reefs at the top of the reef slope, with those found deeper in the upper-mesophotic zone. Proportions were calculated by transect to allow permutations within sites, with summary mean proportions calculated by averaging across sites. To test whether changes in trophic groups along the depth gradient were caused by species gains, losses or turnover we conducted separate Principal Components Analysis (PCA) for each trophic group using the fourth root transformed multivariate species biomass data (Shedrawi et al. 2014). Pearson's correlation coefficients between each species' biomass in the trophic group and the first PCA axis for that trophic group were calculated. For species that exhibited a correlation coefficient $|r| \geq 0.3$ we further tested whether the species biomass or proportion of trophic group biomass changed between 5 m and 40 m with a univariate PERMANOVA using Euclidean distances.

Kernel density estimates (KDEs) were used for fish length distribution visualisation at each depth and to test differences in the body length distributions for species identified as correlating with the PCA analysis. Bandwidths for the KDEs were chosen by the Sheather-Jones selection procedure (Sheather and Jones 1991) using the 'dpik' function in the 'KernSmooth' package (Wand 2013), following Langlois et al. (2012). KDEs are used when the relatedness between samples is not known and the data are not normally distributed. Differences in length distributions were tested by a permutation test (n= 10,000) using the function 'sm.density.compare' in the R package 'sm' (Bowman and Azzalini 2014). This uses permutation to test for differences in area between the two probability density distributions.

In order to have enough replicate length measurements from individual species to allow identification of whether they exhibited differing body size with depth, we grouped all lengths across all sites and from 5 m and 15 m as shallow and from 25 m and 40 m as deep. For the resulting seven species with 20 or greater individuals measured in both shallow and deep groups we calculated KDEs.

2.4 Results

2.4.1 Fish species richness, abundance and biomass

Fish species richness gradually declined with depth from an average per transect of 12.7 ± 1.0 (mean ± standard error) species at 5 m to 4.0 ± 0.9 species at 40 m (Fig 2.2A), whereas abundance and biomass rapidly declined between 5 m and 15 m and then did not change with further increases in depth (Fig 2.2B & 2C). Hard substratum availability declined with depth from 92% at 5 m, to 20% at 40 m. Many hard substratum-associated benthic groups such as hard corals, soft corals and sponge were found to decline across the depth gradient (Fig 2.3). Macroalgae coverage shows a less clear pattern at shallower depths, but is lower at mesophotic than shallow reef depths (Fig 2.3). When visualising fish communities at each site and depth using NMDS it becomes apparent that the fish communities are highly correlated with the availability of hard substratum (Fig 2.4A), yet when standardised on hard substratum availability fish communities still group on depth (Fig 2.4B), suggesting differences in hard substratum availability with depth was not the only driver of fish community structure. Hard substratum availability explained a significant amount of the variation along with individual sites (Table 2.1). Hard substratum effects varied with shore, with higher hard substrate at MCEs at the Maze on the north shore (59.8 ± 14.19 %) compared to Spotted Bay and the south shore sites (13.8 ± 2.0 %). Depth was also significant in explaining fish communities. Particularly interesting is both the site:depth and shore:depth interactions, implying that while sites had different patterns in fish community with depths, the two north shore sites' fish communities vary with depth differently to those on the south shore. Three-way interactions were tested when the model was run, but were found not to be significant.

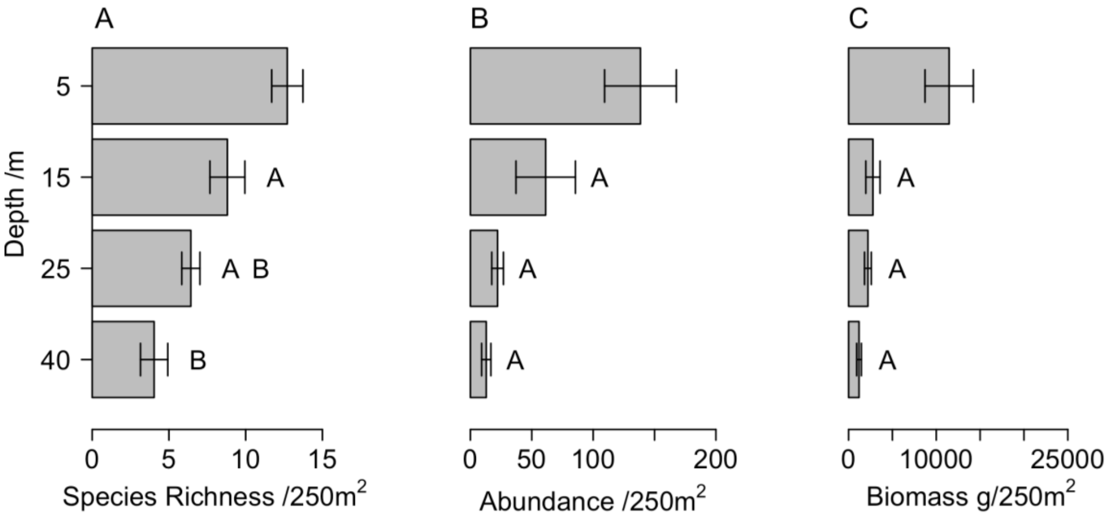

Figure 2.2 Changes in fish communities with depth.

(A) Species richness, (B) abundance, (C) biomass down the depth gradient. Figure shows mean and SE. Letters indicate statistically different groups at the p<0.05 level.

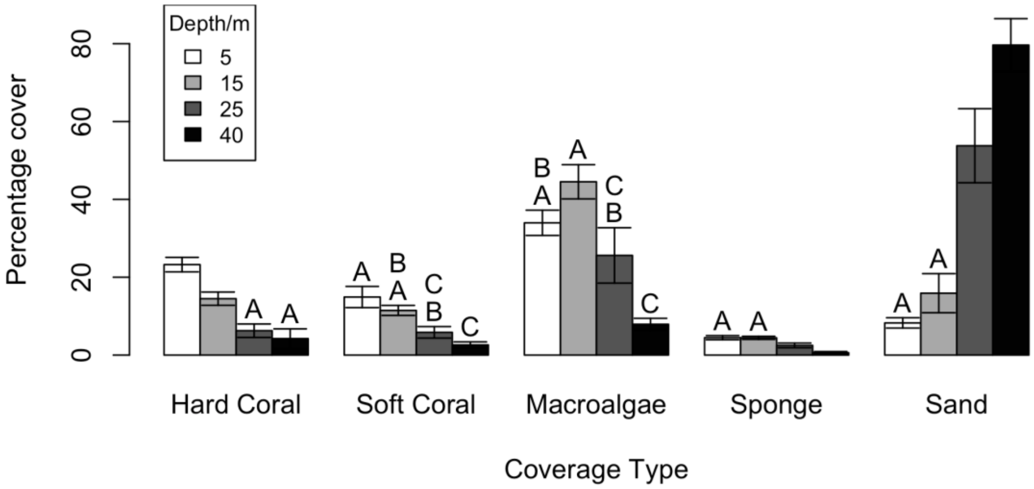

Figure 2.3 Change in the percentage cover of key benthic groups with depth.

Figure shows mean and SE, letters indicate differences (p>0.05 with one-way ANOVA).

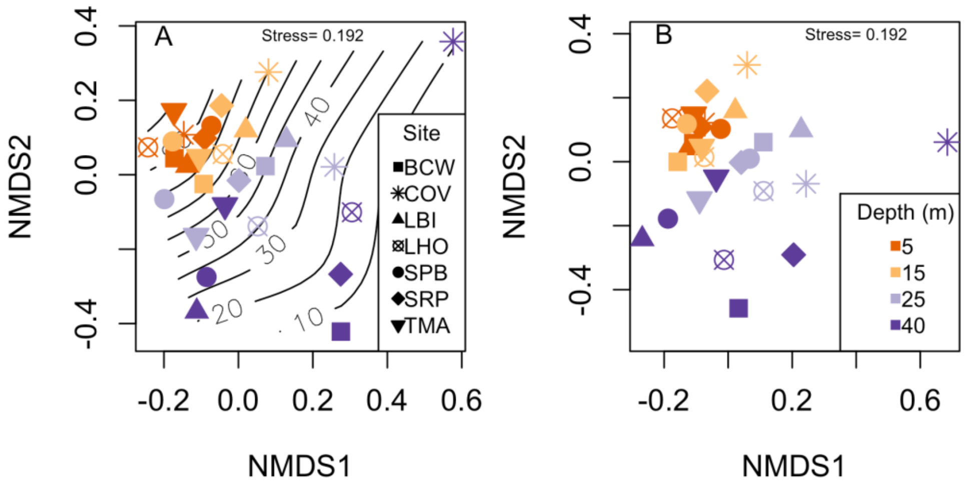

Figure 2.4 Non-metric multidimensional scaling plot of fish biomass weighted communities for each depth and site.

(A) Overall NMDS plot showing community clustering by depth, contour lines indicate percentage cover of hard substrata and (B) fish community biomass standardised by percentage hard substrata availability at each site. Different colours indicate survey depths, while different symbols indicate different survey sites. Survey sites were: BCW – Black Coral Wall, COV – Coral View, LBI – Little Bight, LHO – Lighthouse Reef, SPB – Spotted Bay, SRP – Stingray Point and TMA – The Maze.

Table 2.1 Testing the effects of hard substrata, shore, site and depth on the recorded fish community using PERMANOVA.

Analysis conducted on a Bray-Curtis dissimilarity matrix based on fourth-root transformed fish biomass data. Permutations were run 9999 times and constrained by site.

	df	SS	MS	Pseudo-F	p(perm)
Hard substrata	1	2.304	2.30399	6.6586	<0.001
Shore	1	1.028	1.02797	2.9709	0.344
Site	5	2.205	0.44094	1.2743	<0.001
Depth	3	2.629	0.87641	2.5329	<0.001
Hard substrate:Shore	1	0.557	0.55716	1.6102	0.013
Hard substrate:Site	5	2.460	0.49191	1.4216	0.001
Hard substrate:Depth	3	1.376	0.45881	1.3260	0.014
Shore:Depth	3	1.564	0.52144	1.5070	<0.001
Site:Depth	15	6.450	0.43003	1.2428	<0.001
Hard substrate:Shore:Depth	3	1.179	0.39309	1.1360	0.128
Hard substrate:Site:Depth	15	5.806	0.38707	1.1187	0.071
Residuals	79	27.335	0.34602		
Total	134	54.894			

2.4.2 Fish trophic structuring

Grouping fish into trophic groups allowed changes in dominant feeding strategies with depth to be investigated. Within all trophic groups fewer species were observed at 40 m than at 5 m (Table 2.2). Invertebrate feeders had the greatest species richness at both 5 m and 40 m, with 25 and 17 species respectively, followed by herbivores with 17 and 12 species. Grouping communities based on abundance (Fig 2.5A) or biomass (Fig 2.5B) was found to have major effects on the dominant trophic groups recorded. Planktivorous fish were found to be the dominant trophic group at all depths when calculating community proportion based on abundance, representing 63 % of all fish recorded at 5 m (3619 individuals recorded in total across all transects) and 35 % of fish recorded at 40 m (121 individuals recorded). When using biomass weighted communities, however, the planktivores composed a much smaller percentage of the community, only 18 % at 5 m and 6 % at 40 m, and the biomass was more evenly distributed between trophic groups (Fig 2.5). At shallow depths in the biomass-weighted community herbivores were the largest group, making up 27 % of the community at 5 m, whereas on MCEs piscivores became the largest trophic group at 37 % of the community (Fig 2.5). Fish biomass was significantly lower at 40 m than 5 m for herbivores, invertebrate feeders and planktivores, while carnivores, omnivores and piscivores has similar biomass between 5 m and 40 m (Table 2.2). Considering each trophic group biomass as a percentage of the total biomass recorded per transect at each depth, we found only herbivores had a decline in proportion of the community (38.97% ± 5.78 at 5 m to 18.96% ± 2.96 at 40 m), while piscivores increased their proportion of the community (6.44 ± 1.86 at 5 m to 20.14 ± 8.62 at 40 m) (Table 2.2).

Table 2.2 Comparison of fish trophic groups between 5 m and 40 m depths across all sites.

Species richness is the total number of unique species observed at all transects at all sites in the respective depth band. Mean biomass and mean percentage biomass are given per transects, along with the results of univariate PERMANOVAs constrained by site to test for differences with depth.

Trophic Group	Species Richness		Mean Biomass per transect/g ± SE			
	5 m	40 m	5 m	40 m	Test statistic	p(perm)
Herbivore	17	12	2972.95 ± 724.14	300.73 ± 112.14	8.24	<0.001
Invertebrate feeder	25	17	2594.35 ± 1318.35	194.74 ± 51.60	5.10	0.003
Carnviore	6	3	1471.81 ± 658.08	175.67 ± 117.27	2.00	0.114
Omnivore	1	0	3085.71 ± 1261.70	0.00 ± 0.00	1.66	0.280
Piscivore	6	4	788.34 ± 393.47	429.07 ± 250.11	0.38	0.611
Planktivore	6	4	2053.30 ± 792.09	74.83 ± 56.06	4.66	0.001

Trophic Group	Mean Percentage Biomass per transect ± SE			
	5 m	40 m	Test statistic	p(perm)
Herbivore	38.97 ± 5.78	18.96 ± 2.96	9.06	0.004
Invertebrate feeder	19.60 ± 5.13	22.42 ± 8.46	0.21	0.618
Carnviore	12.92 ± 2.43	9.59 ± 5.91	0.40	0.540
Omnivore	7.92 ± 0.97	0.00 ± 0.00	2.56	0.182
Piscivore	6.44 ± 1.86	20.14 ± 8.62	4.58	0.034
Planktivore	18.67 ± 2.56	14.61 ± 5.26	0.50	0.503

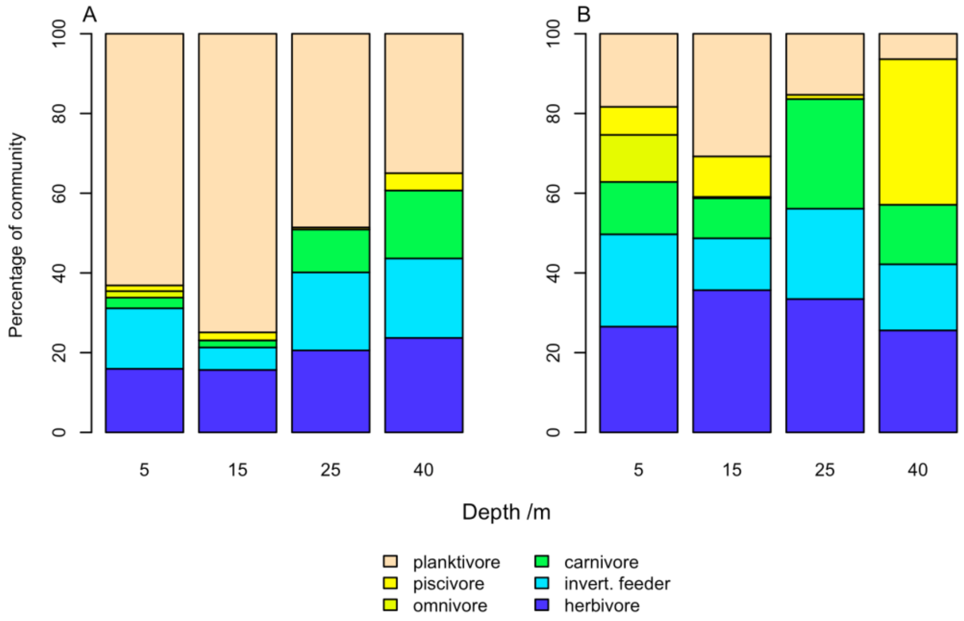

Figure 2.5 Change in percentage of different trophic groups with depth.

Fish community was weighed by (A) abundance and (B) biomass. Percentage abundance and biomass of each trophic group was calculated by summing all fish identified on all transects at all sites in a depth band.

Within each trophic group we identified which species were most likely to explain the patterns observed by the trophic group as a whole based on correlations with a PCA (Table 2.3). Within herbivores we found *Acanthurus* spp. did not change in total biomass or the proportion of herbivore biomass they composed between 5 m and 40 m. All parrotfish (genus *Scarus* and *Sparisoma*) declined in biomass with depth, with four species (*Sparisoma aurofrenatum*, *Sparisoma chrysopterum*, *Sparisoma rubripinne* and *Sparisoma viride*) also declining as a proportion of the herbivore community. The two damselfish species (*Microspathodon chrysurus* and *Stegastes adustus*) declined in biomass with depth, but did not change in the proportion of the herbivore community they made up. This suggests the decline in herbivores observed between 5 m and 40 m is caused both by a loss of species with depth, and a decline in biomass as a proportion of the herbivore community at 40 m. In piscivores, the only trophic group to increase as a proportion of the community between 5 m and 40 m, there were no changes in mean biomass or percentage of piscivore biomass made up by any of the three species correlating with the PCA (*Aulostomus* *maculatus, Caranx ruber* and *Sphyraena barracuda*). Within the planktivores we identified two species (*Clepticus parrae* and *Chromis cyanea*) that significantly declined as a proportion of total community biomass, suggesting the changes observed in proportion of planktivores observed with depth was also driven by species loss.

Table 2.3 Species correlating with changes in their trophic group with depth.

Results for species exhibiting a >0.3 absolute value correlation with the first Principle Components Analysis axis for their trophic group. Correlations were tested using a PERMANOVA based on biomass per transect, and the percentage of biomass contribution to total biomass of that guild per transect. Significance of changes are indicated and the direction of change shown.

Trophic Group		**Mean Biomass per transect**			**Mean Percentage of Trophic Group Biomass per transect**		
		Pseudo F	p(perm)	Change	Pseudo F	p(perm)	Change
Herbivores							
	Acanthurus bahianus	2.87	0.051	-	0.67	0.405	-
	Acanthurus coeruleus	1.66	0.178	-	1.12	0.306	-
	Microspathodon chrysurus	7.93	0.002	↓	0.06	0.874	-
	Stegastes adustus	18.22	<0.001	↓	0.10	0.786	-
	Scarus iserti	5.01	0.010	↓	0.32	0.580	-
	Sparisoma aurofrenatum	3.63	0.012	↓	5.12	0.012	↓
	Sparisoma chrysopterum	6.13	0.006	↓	4.77	0.011	↓
	Sparisoma rubripinne	6.05	0.009	↓	4.67	0.023	↓
	Sparisoma viride	18.19	<0.001	↓	12.61	0.001	↓
Invertebrate Feeders							
	Abudefduf saxatilis	4.23	0.012	↓	33.27	<0.001	↓
	Lutjanus apodus	2.47	0.073	-	6.77	0.010	↓
	Lutjanus jocu	0.63	0.904	-	0.00	1.000	-
	Lutjanus mahogoni	2.20	0.053	-	9.84	0.003	↓
Omnivores							
	Kyphosus	1.59	0.280	-	2.86	0.142	-

	sectatrix						
Piscivores							
	Aulostomus maculatus	1.91	0.253	-	2.09	0.252	-
	Caranx ruber	0.82	0.567	-	0.00	1.000	-
	Sphyraena barracuda	0.89	0.471	-	0.91	0.568	-
Planktivores							
	Clepticus parrae	0.93	0.401	-	6.96	0.008	↓
	Chromis cyanea	5.60	0.001	↓	25.99	<0.001	↓

2.4.3 Fish length distributions

To better understand the differences between abundance- and biomass-weighted fish communities (Fig 2.5) we plotted total lengths for all fish at each depth (Fig 2.6), finding that at shallow depths a larger proportion of the community comprises smaller reef fish. When grouping the 5 m and 15 m data together as shallow and the 25 m and 40 m data as deep across all sites only seven species were measured more than 20 times at both shallow and deep depths. These species were: the herbivores the blue tang, (*Acanthurus coeruleus*), the stoplight parrotfish, (*Sparisoma viride*) and the striped parrotfish (*Scarus iserti*), three planktivores, the blue chromis (*Chromis cyanea*), the creole wrasse (*Clepticus parrae*), and blueheaded wrasse (*Thalassoma bifasciatum*) and a carnivore, the yellowtail snapper (*Ocyurus chrysurus*). Six of these seven species exhibited significant differences in their fish length structure between shallow and deep locations, with larger individuals found deeper when tested using a permutational test of distribution (Fig 2.7). The stoplight parrotfish was the only species that did not exhibit a change in length distribution with depth (Fig 2.7F). The striped parrotfish exhibited a bimodal length distribution at deeper depths (Fig 2.7G). We tested whether this was caused by combining the length distributions from 25 m and 40 m but found no difference between fish recorded at these two depths (permutational test of equality, n=75, p=0.76).

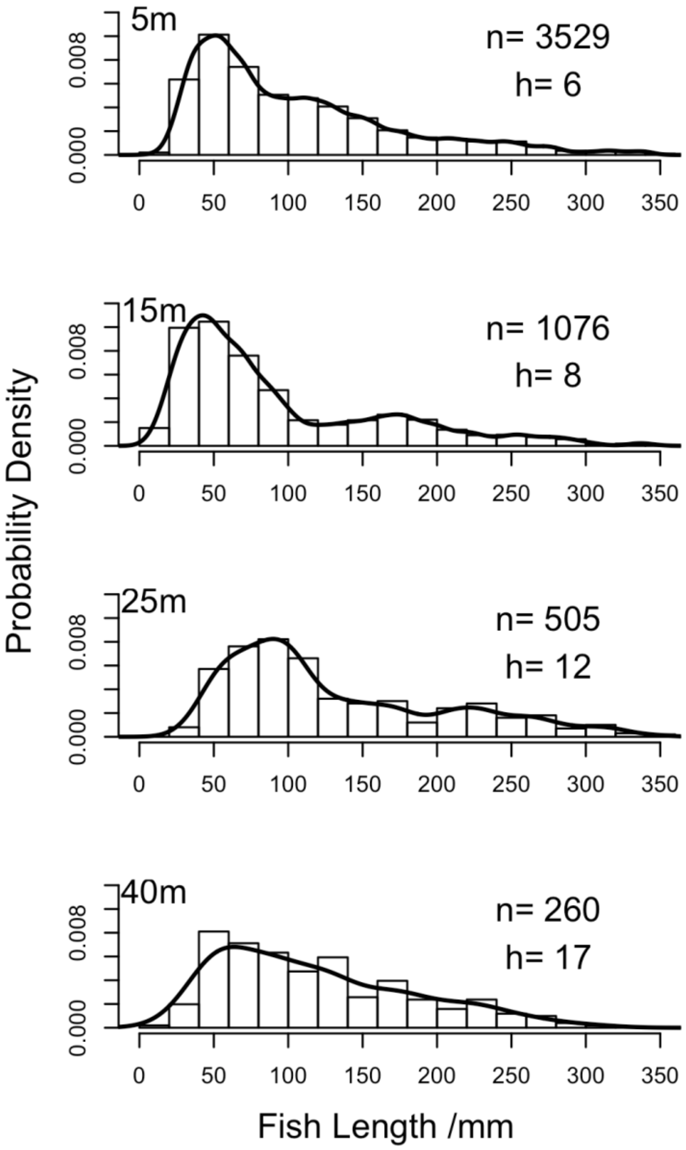

Figure 2.6 Whole fish community length distributions at each depth across all transects and sites.

Number of fish lengths recorded (n), and the separation bandwidths to produce kernel density estimates (h) calculated by Sheather-Jones selection procedure are shown.

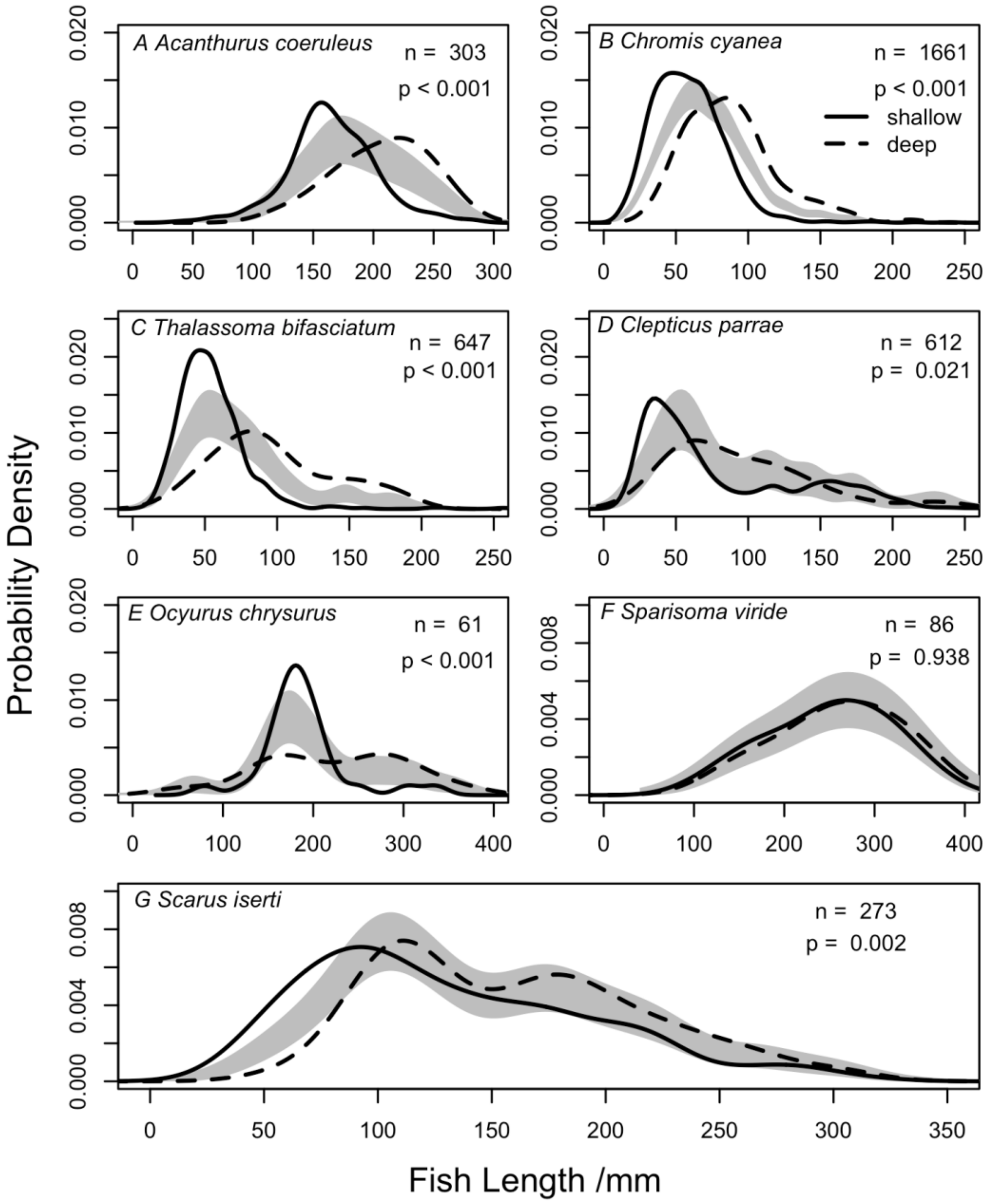

Figure 2.7 Comparison of kernel density estimates for seven fish species between shallow and deep reefs.

Comparisons based on all fish individuals measured on shallow (5 and 15 m) and deep reefs (25 and 40 m) at all sites. Grey shaded regions represent one standard error either side of the null model, n= number of individual fish measured, p indicates whether the length distributions are significantly different based on permutation tests.

2.5 Discussion

2.5.1 Fish species richness, abundance and biomass changes across the depth gradient

Our results showed a continuous decline in fish species richness with increased depth, which contrasts with findings from many other Caribbean locations. For example across a 15-50 m depth gradient in Puerto Rico the greatest fish species richness was recorded at 25 m (Garcia-Sais 2010), whereas off North Carolina this peak in species richness occurred deeper in the 52-98 m range (Parker and Ross 1986). Outside of the western-Atlantic, in the Red Sea species richness declined from shallow to mesophotic reefs, but with a small peak at 30 m; the boundary between the shallow and mesophotic, caused by a mix of deep and shallow specialist and depth generalist species (Brokovich et al. 2008). In Hawaii the greatest richness was observed in the 31-60 m range, though in this study it was likely the 0-30 m range was under sampled (Strasburg et al. 1968).

The differences between our findings and those of previous work may be explained by the maximum extent of the reefs at our study sites. The south shore of Utila has a maximum depth range of 40-60 m, with low hard substratum availability at 25 m and 40 m. This suggests that observed fish communities at these sites represent the shallow-water community in the absence of fully developed deeper MCEs, reducing the number of deep specialist fish species present. Reefs at our two north shore sites extend to >100 m and we did see a significant shore:depth interaction (Table 2.1). This suggests a difference in the turnover of fish communities with depth at sites on the north shore of Utila compared to the south shore. Many other factors could also in part explain this interaction; the north shore sites have larger shallow reef communities by area, with extensive back reefs behind the reef crest, and greater wave exposure for much of the year than the south shore sites, and so shallow reef differences could be an important driver. Coastal development has also focused on the south shore of Utila, resulting in the south shore reefs facing increased sedimentation from mangrove forest clearance and south shore lagoon dredging.

Declines in abundance of fish with depth are consistent with previous work (Thresher and Colin 1986; Feitoza et al. 2005; Bejarano et al. 2014), though Garcia-Sais et al. (2010) found a small increase in abundance at 25 m associated with a peak in species richness. These fine-scale changes in abundance are most likely driven by patterns in reef structural complexity, and so are locally driven. Unlike the shallow reef, most deep reef areas were not continuous reef, but large patch reef systems that the majority of fish were observed to aggregate around. Both reef fish recruitment and survival is known to be affected by density dependence (Tolimieri 2015), therefore lower fish densities on south shore MCEs could be caused by density dependence acting through reduced habitat availability. With lower habitat availability on MCEs compared to shallow reefs, reef fish populations may face greater competition and predation when at lower densities than those found on shallow reefs, leading to lower survival rates and so reduced population sizes. Studies on shallow Caribbean coral reefs have identified density dependence effects in survival, fecundity and mortality in reef fish (Hixon et al. 2012), with these populations persisting at stable population sizes through time. This suggests that some of our abundance patterns could be driven by this changing reef structure with depth.

2.5.2 Changes in fish trophic groups

Many of the fish trophic groups found on shallow reefs were also recorded on MCEs, with surprisingly little change in proportion of the community. While we did not directly measure environmental variables across the depth gradient, some of this consistency may result from similar environmental conditions. While factors such as reef slope angle and visibility varied by site, they were fairly consistent across the depth gradient. The only exception was reef slope angle on the south shore, which was similar for 5, 15 and 25 m depth bands within sites, but the seabed became much flatter allowing patch reef systems to develop at 40 m depth. In other locations larger shifts in trophic guilds have been observed with depth. For example planktivores increased from 16 % of individual fish recorded at 30 m to 31 % at 40 m in Puerto Rico (Garcia-Sais 2010), whereas they did not significantly change on Utila, making up 49 % and 36 % of fish recorded at 25 m and 40 m respectively. Most previous work has shown planktivores to increase as a proportion of the community with depth (Thresher and Colin 1986; Feitoza et al. 2005), while herbivores decline (Brokovich et al. 2010; Kahng et al. 2010). It is not clear why planktivorous fish were observed to decline with depth on Utila, but many previous studies used simple abundance measures to assess changes in fish community. It is possible that these increases in planktivore abundance as a proportion of the total community may actually reflect a decline in planktivore biomass as a proportion of the community. Many of these other studies crossed the shallow to upper mesophotic, and upper mesophotic to lower mesophotic boundaries, so an increase in planktivores may be associated with deeper fish communities than we observed. These greater depths may be associated with larger differences in environmental variables, such as temperature and luminosity affecting habitat types and driving species distributions. As previously mentioned, the 40 m transects at the majority of our sites were close to the maximum bathymetric extent of reefs at the site. Therefore, this increase in planktivorous species could require a deeper reef extent than at our surveyed sites. In addition, smaller-bodied reef fish such as planktivores often require benthic habitat complexity for shelter (Gratwicke and Speight 2005). We found lower hard coral and sponge cover and greater sand cover on MCEs than shallower reefs, suggesting that reduced habitat availability could play a role in planktivore decline. While the proportion of the community composed by planktivores at different depths is variable, many studies have recorded an overall decline in planktivorous fish abundance with depth (Thresher and Colin 1986; Bejarano et al. 2014) which agrees with our results on Utila.

Declines in herbivores shown here are more consistent with previous studies, with herbivores representing 36 % and 26 % of fish at 25 m and 40 m on Utila compared to 31 % (30 m) and 21 % (40 m) in Puerto Rico (Bejarano et al. 2014) and 40 % (30 m) and close to 0 % (90 m) in the Marshall Islands (Thresher and Colin 1986). These patterns followed similar declines in macroalgal coverage with depth. While herbivorous reef fish occur at lower abundances (relative and proportional) on MCEs compared to shallow reefs they are still ecologically important. Lesser et al. (2011) reported that following lionfish invasion of a Caribbean MCE there was a decline in herbivorous reef fish to 60 m which led to a benthic phase shift to an algal-dominated mesophotic community. Reef fish, despite their lower abundance, are likely to be the dominant herbivores on MCEs, as the influence of *Diadema antillarum* urchins (the Caribbean's primary herbivorous echinoderm) declines with depth (Morrison 1988). For example in Curacao, following a mass *Diadema* mortality event in 1983, there was less change in algal coverage at 40 m depth than at shallower sites (de Ruyter van Steveninck and Bak 1986; Nugues and Bak 2008). This suggests that despite low herbivorous reef fish abundance in upper-MCEs they have a crucial functional role in maintaining benthic reef health. Exact reasons for declines in herbivorous reef fish with depth are not clear, although there have been suggestions that changes in algal species, abundance or growth rates may be responsible (Itzkowitz et al. 1991; Liddell and Avery 2000; Aponte and Ballantine 2001). Algal growth rates correlate with the availability of light which decreases along the depth gradient (Leichter et al. 2008), and so a decline in algal productivity per unit area of substrate can be expected with depth.

Other suggested drivers of this bathymetric herbivore variation include differences in reef structure and changes in sedimentation rates. A study investigating algal recruitment and growth dynamics in the Caribbean using recruitment tiles found areas within close proximity (30-50 m distance) to reefs at 30-35 m depth had reduced algal abundance and herbivorous reef fish were observed grazing (Leichter et al. 2008). At greater depths algal abundance increased, and herbivorous fish declined except around artificial reefs (wrecks) at 50-60 m (Leichter et al. 2008). Here, herbivory halos 10-20 m wide surrounding the wrecks could be found with much lower algal abundance than nearby areas, suggesting that herbivorous reef fish were living within the wreck structure, implying reef structural complexity is important for deep-water herbivorous fish communities.

The two herbivorous acanthurid and two pomacentrid species interestingly did not significantly change in their proportion of the overall biomass-weighted fish community at shallow and MCE depths. This suggests that much of the decline in herbivores at mesophotic depths comes from a decline in scarids. Detailed diet studies conducted in the southern Caribbean suggest differences in diets, digestion and feeding of the acanthurid and scarid species encountered in our study might explain why scarids are lost more quickly with depth (Ferreira and Gonçalves 2006). Scarid species generally feed on more detritus than acanthurids (Ferreira and Gonçalves 2006), although the Atlantic blue tang (*Acanthurus coeruleus*) also has a diet similar to many scarid species and does not decline with depth in our results. Previous work has identified rapid scarid declines across shallow depth gradients in the Caribbean (Nemeth and Appeldoorn 2009), with declines in coral cover with depth impacting their supplementary feeding on coral polyps. For example, abundance of the redband parrotfish (*Sparisoma aurofrenatum*), one of the scarids we observed declining with depth, is positively correlated with coverage of the boulder star coral (*Orbicella franksi*) (Rotjan and Lewis 2006).

Piscivores were observed to increase in relative biomass with depth in a similar pattern to other locations (Thresher and Colin 1986; Feitoza et al. 2005). This reflects the shift away from herbivory in the community, as well as potential refuge effects. For example, studies have observed that large-bodied piscivorous species targeted by fisheries are often found in higher abundance at mesophotic depths (Bejarano et al. 2014), with depth acting as a refuge. Despite this increase in piscivores relative biomass on MCEs, it has been suggested that a large proportion of feeding carried out by large bodied piscivores and carnivores found on MCEs is actually conducted on shallow reefs (Papastamatiou et al. 2015). This implies movement of individual large bodied piscivores and carnivores between the depth bands, and suggests these piscivores may therefore play an important role in transporting nutrients between shallow reefs and MCEs. Invertebrate feeder results are also broadly consistent on Utila with those found in Puerto Rico, with declines in abundance from 19 % (25 m) to 16 % (40 m) on Utila and 30 % (30 m) to 27 % (40 m) in Puerto Rico (Bejarano et al. 2014).

2.5.3 Changes in fish lengths

Length distributions varied between shallow and mesophotic depths across the whole community. This can be explained by the high numbers of small-bodied fish at shallow depths, and is supported by the differences in the proportion of trophic groups based on abundance or biomass weighting, with the most abundant small fish being planktivores. Six species with ≥20 individuals recorded on shallow and deep reefs (blue tang, striped parrotfish, blue chromis, creole wrasse, bluehead wrasse, yellowtail snapper) exhibited increased body length with depth. In the Caribbean, previous work has identified ontogenetic migrations in the striped parrotfish and yellowtail snapper, with movement from shallower water to deeper reef habitats as juveniles mature to adults (Cocheret de la Morinière et al. 2002). Finding larger body lengths of both these species at increased depth suggests that upper-MCEs are incorporated into this ontogenetic migration.

There could be several explanations for this effect of depth on body size, which likely varies by species. For larger roaming reef fish species, such as the striped parrotfish and yellowtail snapper, larger individuals may be less susceptible to predation, and so are able to roam to greater depths where there is reduced structural complexity and thus they are more exposed. It has been shown experimentally that, in the absence of predators, fish occupy the habitat allowing the best growth rate, whereas when predators are present fish are associated with more structural complexity as a refuge, thus reducing vulnerability (Werner et al. 1983a; 1983b). Work on Caribbean Nassau grouper (*Epinephelus striatus*) suggests that juveniles face a trade-off between maximising growth rates and minimising predation risk, and are associated with habitats that minimise the ratio of mortality risk to growth rate (Dahlgren and Eggleston 2000), while similar patterns have been observed for French grunt (*Haemulon flavolineatum*) (Grol et al. 2014). However, increases in blue chromis and blue-headed wrasse lengths with depth are harder to explain as both are small-bodied planktivores. Blue-headed wrasse are known to act as cleaner fish, with cleaner activity declining with depth on the reef (Johnson and Ruben 1988), suggesting increased reliance on planktivory at deeper sites. Blue chromis recruitment has also been observed to decline with depth across a 10-40 m depth gradient, with no recruits recorded below 30 m (Luckhurst and Luckhurst 1977). This suggests that larger individuals at greater depth are the result of individuals moving deeper.

2.6 Conclusions

We investigated patterns in reef fish communities and fish trophic groups across shallow to mesophotic gradients on the southern Mesoamerican Barrier Reef, providing the most detailed study conducted in this region. We found reef fish species richness, abundance and biomass declined with increased depth. Importantly, we identify differences between fish community composition calculated based on abundance and those based on biomass. By using biomass, unlike many previous studies, we found no difference in planktivorous fish relative community composition across the depth gradient, while we detected declines in herbivores with increased depth and an increase in piscivores. This suggests future fish community comparisons across depth gradients should incorporate fish biomass assessments to provide better estimates of trophic composition.

2.7 Acknowledgments

We wish to thank Richard Ashley, Sarah Laverty and all the staff and volunteers at Coral View Research Centre and Operation Wallacea Utila during summer 2014.

2.8 Supporting Information

S1 Table. Coordinates of survey sites.

All GPS points recorded in WGS84 and represent the centre of all transects conducted at the site. Site numbers indicate site location on Fig 2.1.

Site Number	Site	Latitude	Longitude
1	Spotted Bay	16.09713416	-86.98717118
2	The Maze	16.11266214	-86.94911793
3	Stingray Point	16.06890550	-86.95477948
4	Little Bight	16.07926302	-86.92942222
5	Black Coral Wall	16.08305968	-86.91699554
6	Coral View	16.08823274	-86.91094506

2.9 Reference List

- Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, U.K.
- Aponte NE, Ballantine DL (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 48:2185–2194
- Armstrong RA, Singh H (2012) Mesophotic Coral Reefs of the Puerto Rico Shelf. In: Harris P.T., Baker E.K. (eds) Seafloor Geomorphology as Benthic Habitat. Elsevier, London, pp 365–374
- Barker Bausell R, Li YF (2002) Power Analysis for Experimental Research: A Practical Guide for the Biological, Medical and Social Sciences. Cambridge University Press, Cambridge, UK

- Bejarano I, Appeldoorn RS, Nemeth M (2014) Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico. Coral Reefs 33:313–328
- Bongaerts P, Ridgway T, Sampayo EM, Hoegh-Guldberg O (2010) Assessing the “deep reef refugia” hypothesis: focus on Caribbean reefs. Coral Reefs 29:309–327
- Bowman AW, Azzalini A (2014) R package “sm”: nonparametric smoothing methods.
- Bridge T, Scott A, Steinberg D (2012) Abundance and diversity of anemonefishes and their host sea anemones at two mesophotic sites on the Great Barrier Reef, Australia. Coral Reefs 31:1057–1062
- Bridge TCL, Hughes TP, Guinotte JM, Bongaerts P (2013) Call to protect all coral reefs. Nature Climate Change 3:528–530
- Brokovich E, Ayalon I, Einbinder S, Segev N, Shaked Y, Genin A, Kark S, Kiflawi M (2010) Grazing pressure on coral reefs decreases across a wide depth gradient in the Gulf of Aqaba, Red Sea. Marine Ecology Progress Series 399:69–80
- Brokovich E, Einbinder S, Kark S, Shashar N, Kiflawi M (2007) A deep nursery for juveniles of the zebra angelfish *Genicanthus caudovittatus*. Environ Biol Fish 80:1–6
- Brokovich E, Einbinder S, Shashar N, Kiflawi M, Kark S (2008) Descending to the twilight-zone: changes in coral reef fish assemblages along a depth gradient down to 65 m. Marine Ecology Progress Series 371:253–262
- Brolund TM, Tychsen A, Nielsen LE, Arvedlund M (2004) An assemblage of the host anemone *Heteractis magnifica* in the northern Red Sea, and distribution of the resident anemonefish. Journal of the Marine Biological Association of the United Kingdom 84:671–674
- Cocheret de la Morinière E, Pollux BJA, Nagelkerken I, van der Velde G (2002) Post-settlement Life Cycle Migration Patterns and Habitat Preference of Coral Reef

Fish that use Seagrass and Mangrove Habitats as Nurseries. Estuarine, Coastal and Shelf Science 55:309–321

- Dahlgren CP, Eggleston DB (2000) Ecological processes underlying ontogenetic habitat shifts in a coral reef fish. Ecology 81:2227–2240
- de Ruyter van Steveninck ED, Bak RPM (1986) Changes in abundance of coral-reef bottom components related to mass mortality of the sea urchin *Diadema antillarum*. Marine Ecology Progress Series 34:87–94
- English S, Wilkinson C, Baker V (1997) Survey manual for tropical marine resources. Australian Institute of Marine Sciences, Townsville
- Feitoza BM, Rosa RS, Rocha LA (2005) Ecology and Zoogeography of Deep-Reef Fishes in Northeastern Brazil. Bulletin of Marine Science 76:725–742
- Ferreira CEL, Gonçalves JEA (2006) Community structure and diet of roving herbivorous reef fishes in the Abrolhos Archipelago, south-western Atlantic. Journal of Fish Biology 69:1533–1551
- Garcia-Sais JR (2010) Reef habitats and associated sessile-benthic and fish assemblages across a euphotic–mesophotic depth gradient in Isla Desecheo, Puerto Rico. Coral Reefs 29:277–288
- Gilmore GR, Jones RS (1992) Color Variation and Associated Behavior in the Epinepheline Groupers, *Mycteroperca Microlepis* (Goode and Bean) and *M. Phenax* Jordan and Swain. Bulletin of Marine Science 51:83–103
- Gratwicke B, Speight MR (2005) The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. Journal of Fish Biology 66:650–667
- Grol MGG, Rypel AL, Nagelkerken I (2014) Growth potential and predation risk drive ontogenetic shifts among nursery habitats in a coral reef fish. Marine Ecology Progress Series 502:229–244

- Harris PT, Bridge TCL, Beaman RJ, Webster JM, Nichol SL, Brooke BP (2013) Submerged banks in the Great Barrier Reef, Australia, greatly increase available coral reef habitat. ICES Journal of Marine Science 70:284–293
- Harvey E, Fletcher D, Shortis M (2001) A comparison of the precision and accuracy of estimates of reef-fish lengths determined visually by divers with estimates produced by a stereo-video system. Fishery Bulletin 99:63–71
- Harvey E, Fletcher D, Shortis MR, Kendrick GA (2004) A comparison of underwater visual distance estimates made by scuba divers and a stereo-video system: implications for underwater visual census of reef fish abundance. Mar. Freshwater Res. 55:573–580
- Hixon MA, Anderson TW, Buch KL, Johnson DW, McLeod JB, Stallings CD (2012) Density dependence and population regulation in marine fish: a large-scale, long-term field manipulation. Ecological Monographs 82:467–489
- Holmes TH, Wilson SK, Travers MJ, Langlois TJ, Evans RD, Moore GI, Douglas RA, Shedrawi G, Harvey ES, Hickey K (2013) A comparison of visual- and stereo-video based fish community assessment methods in tropical and temperate marine waters of Western Australia. Limnol. Oceangr. Methods 11:337–350
- Humann P, Deloach N (2008) Reef Fish Identification Florida Caribbean Bahamas. New World Publications Inc, U.S.
- Itzkowitz M, Haley M, Otils C, Evers D (1991) A reconnaissance of the deeper Jamaican coral reef fish communities. Northeast Gulf Science 12:25–34
- Johnson WS, Ruben P (1988) Cleaning behavior of *Bodianus rufus*, *Thalassoma bifasciatum*, *Gobiosoma evelynae*, and *Periclimenes pedersoni* along a depth gradient at Salt River Submarine Canyon, St. Croix. Environ Biol Fish 23:225–232
- Kahng SE, Copus JM, Wagner D (2014) Recent advances in the ecology of mesophotic coral ecosystems (MCEs). Current Opinion in Environmental Sustainability 7:72–81

- Kahng SE, Garcia-Sais JR, Spalding HL, Brokovich E, Wagner D, Weil E, Hinderstein L, Toonen RJ (2010) Community ecology of mesophotic coral reef ecosystems. Coral Reefs 29:255–275
- Kulbicki M, Parravicini V, Mouillot D (2015) Patterns and processes in reef fish body size. In: Mora C. (eds) Ecology of Fishes on Coral Reefs. Cambridge University Press, Cambridge, pp 104–115
- Langlois TJ, Fitzpatrick BR, Fairclough DV, Wakefield CB, Hesp SA, McLean DL, Harvey ES, Meeuwig JJ (2012) Similarities between Line Fishing and Baited Stereo-Video Estimations of Length-Frequency: Novel Application of Kernel Density Estimates. PLoS ONE 7:e45973
- Leichter JJ, Stokes MD, Genovese SJ (2008) Deep water macroalgal communities adjacent to the Florida Keys reef tract. Marine Ecology Progress Series 356:123–138
- Lesser MP, 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 13:1855–1868
- Liddell WD, Avery WE (2000) Temporal change in hard substrate communities 10-250 m, the Bahamas. Proceedings of the 9th International Coral Reef Symposium, Bali, Indonesia 23-27 October 2000 2:1053–1058
- Luckhurst BE, Luckhurst K (1977) Recruitment patterns of coral reef fishes on the fringing reef of Curaçao, Netherlands Antilles. Can. J. Zool. 55:681–689
- Macpherson E, Duarte CM (1991) Bathymetric trends in demersal fish size: is there a general relationship? Marine Ecology Progress Series 71:103–112
- Menza C, Kendall M, Hile S (2008) The deeper we go the less we know. rev Biol Trop 56:11–24

- Micheli F, Mumby PJ, Brumbaugh DR, Broad K, Dahlgren CP, Harborne AR, Holmes KE, Kappel CV, Litvin SY, Sanchirico JN (2014) High vulnerability of ecosystem function and services to diversity loss in Caribbean coral reefs. Biol Conserv 171:186–194
- Morrison D (1988) Comparing Fish and Urchin Grazing in Shallow and Deeper Coral Reef Algal Communities. Ecology 69:1367
- Nemeth M, Appeldoorn R (2009) The Distribution of Herbivorous Coral Reef Fishes within Fore-reef Habitats: the Role of Depth, Light and Rugosity. Caribbean Journal of Science 45:247–253
- Nugues MM, Bak RPM (2008) Long-term dynamics of the brown macroalga *Lobophora variegata* on deep reefs in Curaçao. Coral Reefs 27:389–393
- Oksanen J, Kindt R, Legendre P, OHara B, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) vegan: Community Ecology Package. cran.r-project.org
- Papastamatiou YP, Meyer CG, Kosaki RK, Wallsgrove NJ, Popp BN (2015) Movements and foraging of predators associated with mesophotic coral reefs and their potential for linking ecological habitats. Marine Ecology Progress Series 521:155–170
- Parker FO, Ross SW (1986) Observing reef fishes from submersibles off North Carolina. Northeast Gulf Science 8:31–49
- Pereira-Filho GH, Amado-Filho GM, Guimarães SMPB, Moura RL, Sumida PYG, Abrantes DP, Bahia RG, Güth AZ, Jorge RR, Filho RBF (2011) Reef fish and benthic assemblages of the Trindade and Martin Vaz Island group, Southwestern Atlantic. Brazilian Journal of Oceanography 59:201–212
- Pinheiro HT, Goodbody-Gringley G, Jessup ME, Shepherd B, Chequer AD, Rocha LA (2016) Upper and lower mesophotic coral reef fish communities evaluated by underwater visual censuses in two Caribbean locations. Coral Reefs 35:139–151

- Puglise KA, Hinderstein LM, Marr J, Dowgiallo MJ, Martinez FA (2009) Mesophotic Coral Ecosystems Research Strategy: International Workshop to Prioritize Research and Management Needs for Mesophotic Coral Ecosystems. coastalscience.noaa.gov 24
- R Core Team (2013) R: A language and environment for statistical computing. R-project.org
- Rosa MR, Alves AC, Medeiros DV, Coni EOC, Ferreira CM, Ferreira BP, de Souza Rosa R, Amado-Filho GM, Pereira-Filho GH, de Moura RL, Thompson FL, Sumida PYG, Francini Filho RB (2016) Mesophotic reef fish assemblages of the remote St. Peter and St. Paul’s Archipelago, Mid-Atlantic Ridge, Brazil. Coral Reefs 35:113–123
- Rotjan RD, Lewis SM (2006) Parrotfish abundance and selective corallivory on a Belizean coral reef. Journal of Experimental Marine Biology and Ecology 335:292–301
- Sheather SJ, Jones MC (1991) A Reliable Data-Based Bandwidth Selection Method for Kernel Density-Estimation. Journal of the Royal Statistical Society Series B-Methodological 53:683–690
- Shedrawi G, Harvey ES, McLean DL, Prince J, Bellchambers LM, Newman SJ (2014) Evaluation of the effect of closed areas on a unique and shallow water coral reef fish assemblage reveals complex responses. Coral Reefs 33:579–591
- Strasburg DW, Jones EC, Iversen RTB (1968) Use of a Small Submarine for Biological and Oceanographic Research. J. Cons. perm. int. Explor. Mer 31:410–426
- Thresher RE, Colin PL (1986) Trophic structure, diversity and abundance of fishes of the deep reef (30-300m) at Enewetak, Marshall Islands. Bulletin of Marine Science 38:253–272

- Tolimieri N (2015) Density dependence, independence and population dynamics. In: Mora C (ed) Ecology of Fishes on Coral Reefs. Cambridge University Press, Cambridge, UK
- Wand M (2013) KernSmooth: Functions for kernel smoothing for Wand & Jones (1995).
- Watson DL, Harvey ES, Fitzpatrick BM, Langlois TJ, Shedrawi G (2010) Assessing reef fish assemblage structure: how do different stereo-video techniques compare? Marine Biology 157:1237–1250
- Weaver DC, Dennis GD III, Sulak KJ (2001) Northeastern Gulf of Mexico Coastal and Marine Ecosystem Program: Community structure and Trophic Ecology of Demersal Fishes on the Pinnacles Reef Tract. US Geological Survey Biological Sciences Report USGS BSR 92
- Werner EE, Gilliam JF, Hall DJ, Mittelbach GG (1983a) An Experimental Test of the Effects of Predation Risk on Habitat Use in Fish. Ecology 64:1540
- Werner EE, Mittelbach GG, Hall DJ, Gilliam JF (1983b) Experimental Tests of Optimal Habitat Use in Fish: The Role of Relative Habitat Profitability. Ecology 64:1525

3 LIGHT AVAILABILITY APPEARS MORE IMPORTANT THAN FISH EXPOSURE IN STRUCTURING UPPER-MESOPHOTIC REEF BENTHIC COMMUNITIES

3.1 Abstract

Both light availability and fish predation have been suggested as important drivers of mesophotic coral ecosystem (MCE; reefs from 30-150 m) benthic communities. However, both light availability and fish abundance decline with increased depth on MCEs, making it hard to disentangling their effects. To investigate MCE light and fish effects we used a factorial experiment manipulation, with fish exclusion and light reduction cages alongside control quadrats to track their impacts on benthic communities over 16 months. We found effects of light on hard coral, macroalgal and sponge cover, suggesting that light availability is important for benthic communities. While we identified effects of fish exposure on hard coral and sponge cover, the direction of observed patterns suggest these are most likely caused by cage artefacts. Our results suggest a crucial role of light in structuring MCE benthic communities.

Chapter 3: Light availability appears more important than fish exposure in structuring upper-mesophotic reef benthic communities

In preparation for submission:

Andradi-Brown DA, Exton DA, Laverick JH, Gress E, Rogers AD (in prep) Light availability appears more important than fish exposure in structuring upper-mesophotic reef benthic communities.

3.2 Introduction

Mesophotic coral ecosystems (reefs from 30–150 m; MCEs) are understudied (Menza et al. 2008), but could play an important role in supporting overall reef resilience (Bridge et al. 2013). MCEs support rich benthic communities including corals, sponges and algae alongside associated fish (Kahng et al. 2010), with many shared species between shallow reefs and upper-MCEs (30-60 m; Kahng et al. 2010; Bridge et al. 2013). Despite this overlap in species with the shallows, few studies have addressed basic ecological questions on MCEs to test whether previously identified shallow reef processes also occur on MCEs.

Shallow reef fish are known to play a crucial role in structuring benthic communities, with grazing aiding hard coral recruitment (Mumby et al. 2007) and herbivore declines associated with phase-shifts to algal domination (Hughes 1994). Although herbivorous fish occur at lower densities on MCEs compared to shallow reefs (Brokovich et al. 2010; Chapter 2), they are believed to contribute towards MCE benthic community structuring (Kahng et al. 2010). For example, around wrecks at 50–60 m depth off Florida herbivory halos suggest aggregating herbivores alter adjacent benthic communities (Leichter et al. 2008). On Caribbean MCEs, fish are likely the dominant herbivores, as effects of *Diadema* declines with depth (Morrison 1988). In addition, MCE herbivore predation by invasive lionfish is believed to cause trophic cascades favouring macroalgae (Lesser and Slattery 2011).

MCE macroalgal cover declines with increased depth, likely influenced by reduced light availability (Leichter et al. 2008). Light also affects MCE hard corals, though corals may use heterotrophy to survive in low light (Lesser et al. 2010).

As both light and herbivore abundance declines from shallow reefs to MCEs, it is unclear the relative importance of these factors on MCE benthic community structure. This study uses fish exclusion and light reduction cages alongside control quadrats to identify how changes in fish and light exposure impact MCE benthic communities over 16 months.

3.3 Materials and Methods

This study was conducted at Coral View reef, on Utila, Honduras (Fig. 3.1a). Here, the shallow reefs are a spur and groove system, with a gentle slope to approximately 30 m where the seabed flattens and a mesophotic patch reef system exists in the 30-40 m depth range. These patch reefs vary in area between approximately 3-15 m^2, are covered with a mixture of hard corals, algae, sponges and soft corals, and support a range of fish species including herbivores and spongivores (Chapter 2).

To investigate the role of fish communities and light in structuring the mesophotic benthic community fixed 0.5 x 0.5 m quadrats were established on patch reefs between 33-38 m depth. We used a blocked design with 7 blocks each comprised of 2 adjacent patch reefs. On one patch reef within each block we established three quadrats, with the locations chosen to contain hard corals, macroalgae and sponges. Quadrats were marked with reef nails and photographed, before being randomly allocated to a treatment: fish exclusion cage also reducing light (full cage), light reduction cage (partial cage) and a quadrat frame (open) (Fig. 3.1b). Because of concerns fish would aggregate around the cages, on the second patch reef in each block we established an additional open quadrat (control). Cages and quadrat frames were built from ¼ inch diameter PVC piping, and cages a height of 0.3 m. Nylon mesh (0.5 cm x 0.5 cm square mesh aperture) was used to cover the top and sides of each cage frame for fish exclusion (full), or just the top for light exclusion leaving the sides open for fish access (partial).

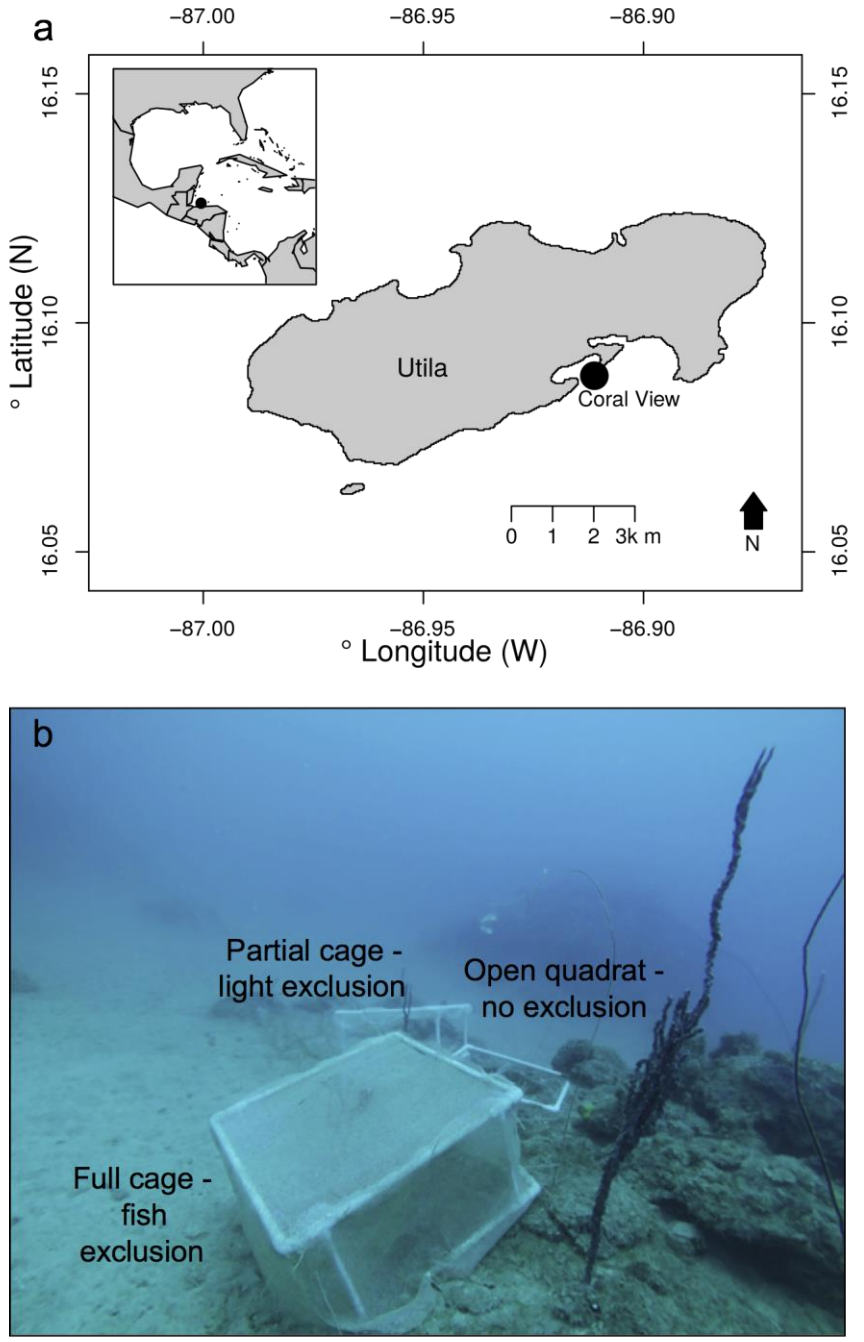

Figure 3.1. (a) Map of study location and (b) photograph of the experimental set up.

(a) Map of study location, Coral View, on the south shore of Utila, Honduras (WGS84 coordinates: 16.0882327 N, 86.910945 W). Inset – the location of Utila is shown relative the western Caribbean. (b) Photograph of the experimental set up at a patch reef with three quadrats illustrating three of the treatment types.

With four quadrats per block (three experimental on one patch reef, and one control on the other), and seven blocks, this gave 28 permanent quadrats in total across 14 patch reefs. We had two experimental factors: light exposure, coded as low (full and partial cages) and normal (open and control quadrats), and fish exposure, coded as low (full cages), normal (control quadrats) and artificial (partial cages and open quadrats). Quadrats were photographed at the start of the experiment (March 2015) and then after 5 (August 2015), 12 (March 2016) and 16 (July 2016) months. The camera (GoPro Hero 3+ Silver) was held in a photo-quadrat frame ensuring consistent camera angle to the reef. At these times mesh on cages were cleaned, though fouling was low.

Photographs were analysed with Coral Point Count with Excel Extensions (CPCe) (Kohler and Gill 2006), recording hard coral, macroalgae, sponge and soft coral area. All statistical analyses were conducted in R (R Core Team 2013). We calculated mean percentage cover of each benthic type for all quadrats at each time point and used Nonmetric Multidimensional Scaling (NMDS) to visualise benthic community changes through time. NMDS was run with Bray-Curtis dissimilarities with 'MetaMDS' in the package vegan (Oksanen et al. 2013). As we did not have a full factorial design, we used linear mixed-effects models to test for differences in benthic groups and interactions between light and fish exposures. To aid model fitting, benthic variables were square rooted, mean centered and scaled to two standard deviations of their mean. Models were fitted using 'lmer' set to optimize the log-likelihood criterion in the package lme4 (Bates et al. 2015) in the following form: Benthic cover ~ Fish + Light * Month + Fish * Month + (1|Quadrat) + (1|Block) + (1|Initial benthic cover). Where Fish, Light and Block were factors, Month was a continuous variable, Quadrat represented a unique identification for each permanent quadrat to account for the repeated measures. We started with this full model with all predictors and simplified by using Likelihood Ratio Tests to remove non-significant interactions (Bolker et al. 2009). P-values were generated using Kenward-Roger Approximation in the pbkrtest package (Halekoh and Højsgaard 2014). Predictions were plotted to investigate interactions between different predictors.

3.4 Results

The control and open quadrats maintained similar benthic MCE communities throughout the experiment (Fig. 3.2a), and when visualised with NMDS, with the exception of the month=0 control quadrats, all time points were highly clustered throughout the experiment (Fig. 3.2b). Combined fish exclusion and light reduction caused benthic communities to progressively transition away from their original composition, and did not appear to reach a stable state within the 16-month period (Fig. 3.2b). Partial cages (light reduction only) initially showed a community shift in a similar direction in multivariate space to full cages up to 12 months, when the direction changed, likely representing a move towards a new stable state in response to the adjusted light levels, although more time would be needed to confirm this (Fig. 3.2b).

Regardless of treatment or control, we found hard coral cover was decreasing on MCEs throughout the experiment (Table 3.1, Table 3.2), while macroalgae cover showed inconsistent responses with increases in some quadrats and declines in others generating high variability (Table 3.1, Table 3.2). However, when controlling for different initial benthic coverage in each quadrat and within blocks, we found reductions in both hard coral (Fig. 3.3a) and macroalgal cover (Fig. 3.3c) and gains in sponge cover (Fig. 3.3e) were associated with low light levels. For hard corals, we found a significant light:month interaction (Table 3.2), indicating that hard coral cover declines more severely in low light conditions than under normal light levels (Fig. 3.3a).

Table 3.1 Percentage cover of key benthic groups at the start (0 months) and end (16 months) of the study.

Quadrats types were: Control quadrats = quadrats on patch reefs with no cages, Open quadrats = quadrats on the same patch reef as cages but with no direct treatment, Partial quadrats = quadrats with partial cages (light only exclusion) and Full quadrats = quadrats with full cages (light and fish exclusion).

Benthic group	Quadrat type	Initial percentage cover		End percentage cover	
		Mean	SE	Mean	SE
Hard Coral	Control	10.92	2.38	8.19	2.20
Hard Coral	Open	8.40	2.33	8.34	3.05
Hard Coral	Partial	12.75	3.43	5.64	2.92
Hard Coral	Full	4.77	1.54	0.93	0.41
Macroalgae	Control	18.02	6.81	32.59	5.97
Macroalgae	Open	36.70	9.02	44.80	11.96
Macroalgae	Partial	37.22	9.90	6.56	2.40
Macroalgae	Full	18.38	5.14	1.90	1.38
Sponge	Control	2.18	1.10	1.42	0.74
Sponge	Open	1.77	0.75	0.68	0.41
Sponge	Partial	3.56	0.88	3.33	0.92
Sponge	Full	2.62	0.58	1.66	0.51

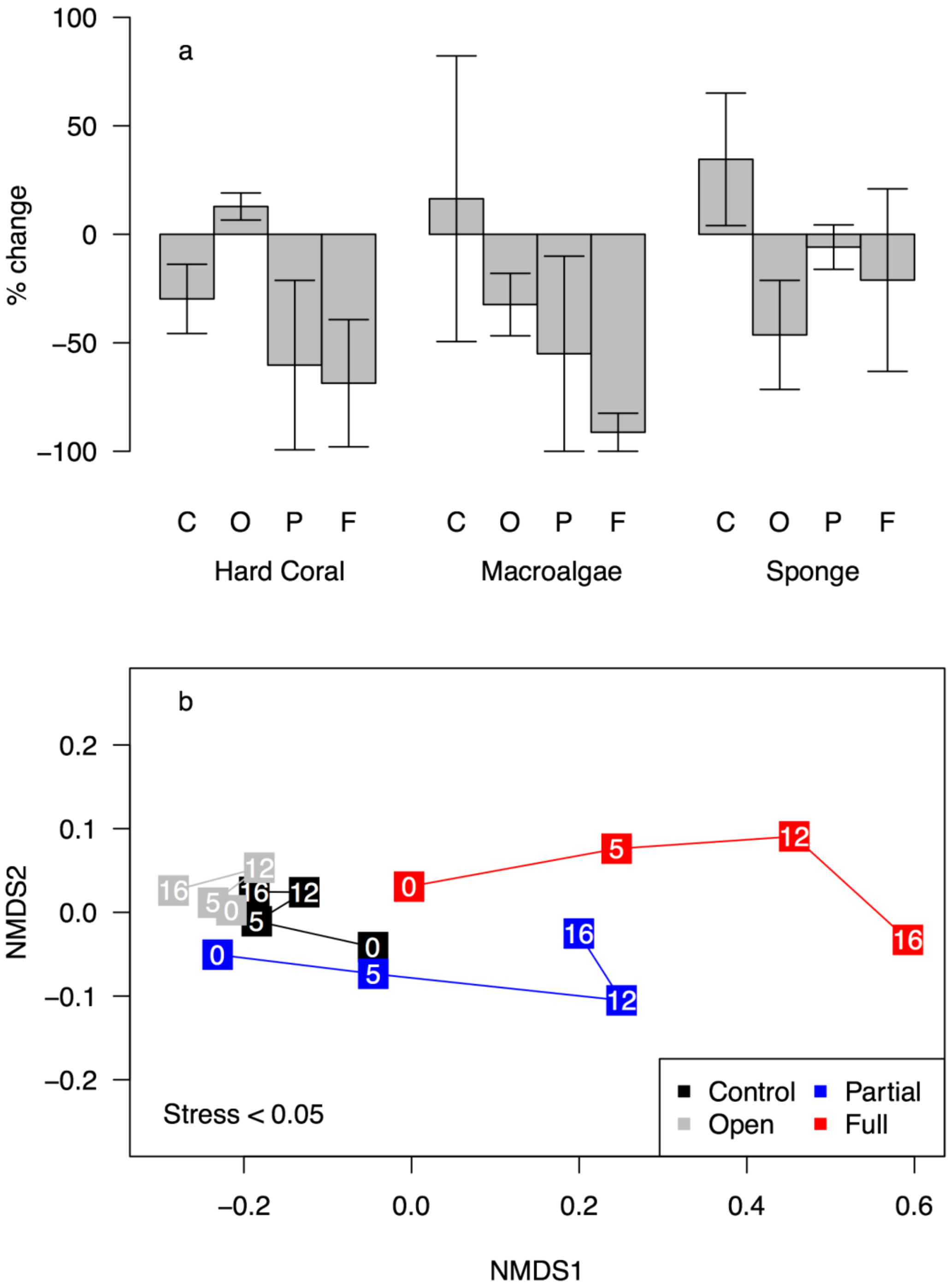

Figure 3.2 (a) Percentage change for key benthic groups between the start and end of the experiment and (b) NMDS plot showing changes in benthic community structure through time.

a Percentage change for key benthic groups between the start and end of the experiment (16 months). C= control quadrats, O= open quadrats on same patch reef as cages, P= partial cages (light only exclusion) and F= full cages (light and fish exclusion). Error bars show one standard error. b NMDS plot showing changes in benthic community structure based on hard coral, macroalgae, sponge and soft coral cover through time. Numbers on each point represent the time point (months) since the start of the experiment.

Table 3.2 Summary of the linear mixed-effect models. The intercept row represents the combination: low light and excluded fish, which the other factor levels are compared to. (N=normal, A=artificial)

	Estimate	**SE**	***t* value**	**Pr(>|t|)**
Hard Coral				
(Intercept)	-0.09	0.16	-0.55	0.585
lightN	-0.22	0.22	-0.99	0.331
month	-0.04	0.01	-6.08	<0.001
fishN	0.54	0.28	1.89	0.071
fishA	0.46	0.20	2.30	0.030
lightN:month	0.03	0.01	3.64	0.001
Macroalgae				
(Intercept)	-0.36	0.13	-2.82	0.008
lightN	0.64	0.13	5.11	<0.001
month	-0.01	0.01	-2.23	0.032
fishN	0.14	0.18	0.79	0.432
fishA	0.18	0.13	1.47	0.152
Sponge				
(Intercept)	0.02	0.17	0.12	0.907
lightN	-0.59	0.20	-3.01	0.006
month	-0.01	0.01	-0.82	0.419
fishN	0.59	0.28	2.12	0.045
fishA	0.30	0.20	1.53	0.139

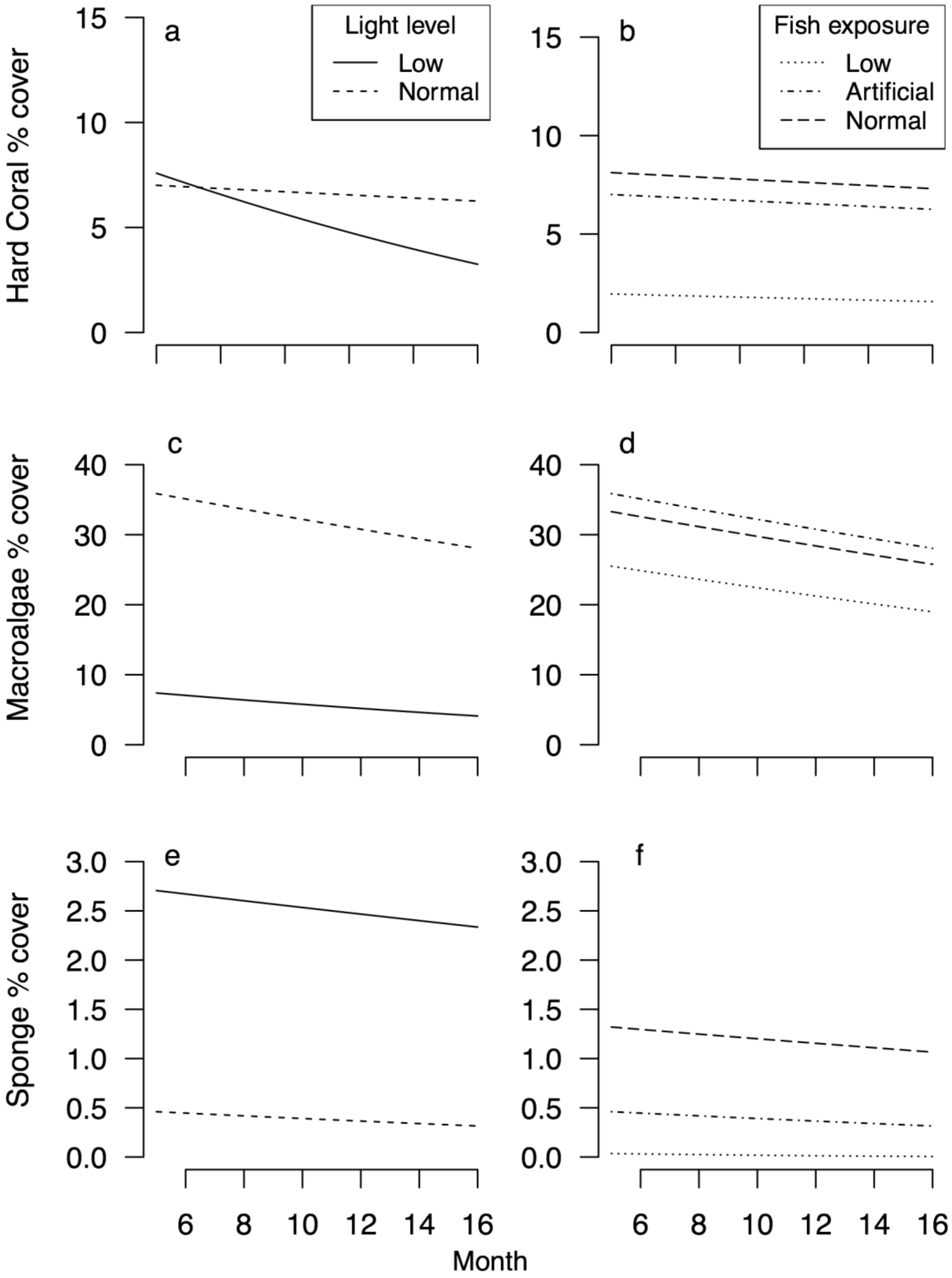

Figure 3.3 Prediction plots showing the effects of: (a) light levels and (b) fish exposure on hard corals; (c) light levels and (d) fish exposure on macroalgae; and (e) light levels and (f) fish exposure on sponges through time.

3.5 Discussion

We found low light associated with more rapid declines in hard coral and macroalgal coverage. This is unsurprising as both hard corals and macroalgae are photosynthetic, so a reduction in light availability would be expected to reduce growth rates. However, we detected a significant light:month interaction for hard coral, while no significant interaction between light and month for macroalgae (Table 3.2, Fig. 3.3). This suggests the declines in hard coral cover associated with light reduction is a gradual process over many months, while declines in macroalgae caused by light reduction are very rapid. This explains why when our model is visualised as a prediction plot (Fig. 3.3), despite controlling for different starting values in macroalgal cover, the model predicts such extreme differences based on light after 6 months. Taken in context, this implies that macroalgal may be more sensitive to changes in light regime than hard corals, and so reef management decisions affecting water turbidity may be especially important in affecting MCEs. Sedimentation has been highlighted as a major reef management challenge MCEs (Chapter 9), and recent land use change in the Bay Islands combined with increased tourism it is likely to be affecting reef health.

We found that fish exclusion resulted in lower hard coral cover compared to fish accessible quadrats on the same reef, while no effect of fish exposure was detected in macroalgal cover (Table 3.2). Brokovitch et al. (2010) studied algal growth and herbivory in the Red Sea using artificial settlement plates and bioassays, finding declines in both algal growth rates and herbivory with increased depth, but that herbivory was occurring on MCEs. In addition, their studies directly compared herbivory between algal growth plates inside and outside cages, with no control for the effect of light. Our results suggest a significant effect of light reduction on algal communities, implying that their results may have underestimated the levels of herbivory occurring on MCEs in the Red Sea. In contrast to this, our results suggest that light is the primary driver of macroalgal cover on the MCEs of Utila.

Drivers for the patterns observed in sponge cover are less clear. Similar to hard corals, we observed lowest sponge cover in quadrats with fish excluded, with highest sponge cover in our control quadrats away from the cage structures (Table 3.2, Fig. 3.3f). This contrasts with a previous sponge caging experiment carried out in Florida at 30 m, which suggested fish predation was the major factor affecting sponge growth (Pawlik et al. 2013). In Florida, sponges without chemical defences grew more within cages than outside them, with sponges outside the cages showing high numbers of bite marks, while chemically defended sponges showed no difference based on fish exposure (Pawlik et al. 2013). Finding an effect of fish exposure on sponge cover between quadrats accessible to fish close to the artificial complexity of the cages and control quadrats may indicate that spongivorous fish were aggregating around the exclusion cages causing increased spongivory. However, this does not explain why excluding fish caused the greatest decline in sponge cover, especially as many important spongivorous fish are large bodied (e.g. angelfishes) so caging would effectively exclude them. Another possibility is that the cages were restricting water flow around the benthos (Miller and Gaylord 2007), with hard corals and sponges encased within cages suffering reduced water flow. For many sponges, feeding is enhanced by ambient water flows (Leys et al. 2011), so reduced water flow is likely to lead to reduced sponge survival. Heterotrophic feeding is important for many hard corals on MCEs (Lesser et al. 2010), and so it is possible that the decline in coral cover in enclosed cages was an artefact of restricting water flows and so reducing food availability to corals that were already facing reduced light levels.

We also identified changes in sponge cover caused by light exposure, with reduced light leading to greater sponge cover than normal light levels (Table 3.2, Fig 3.3e). While some sponges benefit from hosting cyanobacterial photosymbionts (Freeman and Thacker 2011), it appears unlikely for MCE sponges in our study because of their greater survival in lower light conditions. As lower light conditions were associated with declines in hard corals and macroalgae, this might reduce spatial competition between these groups and sponges (López-Victoria et al. 2006).

It was not possible to use a full factorial experimental design for this study, and we were limited to including control quadrats, open quadrats, light exclusion and combined fish and light exclusion cages. Because of the loss of light associated with placing a mesh over the reef, we were unable to include a treatment that allowed light in while excluding fish. While in shallow reef ecology it has been reported that cages have little effect on reducing light availability to benthic habitats (Miller & Hay 1998), the one previous caging study of algal communities on MCEs suggested that because of the low light availability, mesh placed over the benthos is likely to cause significant lowering of light levels (Brokovitch et al. 2010). This implies that studies wishing to use this treatment must use artificial lighting to maintain light levels on under any fish exclusion cage. Alternatively a larger mesh size could be used, allowing more light through while still excluding fish, however this would alter the accessibility of the quadrat to fish, introducing a fish size bias, with smaller fish able to access the quadrat. We therefore used a linear mixed-effects model to account for our unbalanced design, allowing disentanglement of the effects of fish presence/absence from the effects of light despite not having a full factorial design. We recommend that future studies should aim to include all treatment groups if possible.

This study serves as a first attempt to quantify the relative importance of light and fish exposure in structuring MCE benthic communities, with our results suggesting that light levels are more important than exposure to fish communities. However, despite using a factorial design, it is not clear whether the significant effects of fish exposure detected in this experiment reflect actual effects or cage artefacts. Future studies should therefore focus on disentangling cage artefacts to establish herbivory and spongivory rates on Mesoamerican Barrier Reef MCEs.

3.6 Acknowledgements

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3.7 References

- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127–135
- Bridge TCL, Hughes TP, Guinotte JM, Bongaerts P (2013) Call to protect all coral reefs. Nature Climate Change 3:528–530
- Brokovich E, Ayalon I, Einbinder S, Segev N, Shaked Y, Genin A, Kark S, Kiflawi M (2010) Grazing pressure on coral reefs decreases across a wide depth gradient in the Gulf of Aqaba, Red Sea. Marine Ecology Progress Series 399:69–80
- Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Marine Pollution Bulletin 50:125–146
- Freeman CJ, Thacker RW (2011) Complex interactions between marine sponges and their symbiotic microbial communities. Limnology and Oceanography 56:1577–1586
- Halekoh U, Højsgaard S (2014) A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models–the R package pbkrtest. Journal of Statistical Software 59:

- Hughes TP (1994) Catastrophes, Phase Shifts, and Large-Scale Degradation of a Caribbean Coral Reef. Science 265:1547–1551
- Kahng SE, Garcia-Sais JR, Spalding HL, Brokovich E, Wagner D, Weil E, Hinderstein L, Toonen RJ (2010) Community ecology of mesophotic coral reef ecosystems. Coral Reefs 29:255–275
- Kohler KE, Gill SM (2006) Coral Point Count with Excel extensions (CPCe): A Visual Basic program for the determination of coral and substrate coverage using random point count methodology. Computers & Geosciences 32:1259–1269
- Leichter JJ, Stokes MD, Genovese SJ (2008) Deep water macroalgal communities adjacent to the Florida Keys reef tract. Marine Ecology Progress Series 356:123–138
- Lesser MP, 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 13:1855–1868
- Lesser MP, Slattery M, Stat M, Ojimi M, GATES RD, Grottoli A (2010) Photoacclimatization by the coral *Montastraea cavernosa* in the mesophotic zone: light, food, and genetics. Ecology 91:990–1003
- Leys SP, Yahel G, Reidenbach MA, Tunnicliffe V, Shavit U, Reiswig HM (2011) The Sponge Pump: The Role of Current Induced Flow in the Design of the Sponge Body Plan. PLoS ONE 6:e27787
- López-Victoria M, Zea S, Weil E (2006) Competition for space between encrusting excavating Caribbean sponges and other coral reef organisms. Marine Ecology Progress Series 312:113–121
- Menza C, Kendall M, Hile S (2008) The deeper we go the less we know. rev Biol Trop 56:11–24

- Miller LP, Gaylord B (2007) Barriers to flow: The effects of experimental cage structures on water velocities in high-energy subtidal and intertidal environments. Journal of Experimental Marine Biology and Ecology 344:215–228
- Miller MW, Hay ME (1998) Effects of fish predation and sea- weed competition on the survival and growth of corals. Oecologia 113:231–238
- Morrison D (1988) Comparing Fish and Urchin Grazing in Shallow and Deeper Coral Reef Algal Communities. Ecology 69:1367
- Mumby PJ, Harborne AR, Williams J, Kappel CV, Brumbaugh DR, Micheli F, Holmes KE, Dahlgren CP, Paris CB, Blackwell PG (2007) Trophic cascade facilitates coral recruitment in a marine reserve. Proceedings of the National Academy of Sciences 104:8362–8367
- Oksanen J, Kindt R, Legendre P, OHara B, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) vegan: Community Ecology Package. cran.r-project.org
- Pawlik JR, Loh T-L, McMurray SE, Finelli CM (2013) Sponge Communities on Caribbean Coral Reefs Are Structured by Factors That Are Top-Down, Not Bottom-Up. PLoS ONE 8:e62573
- R Core Team (2013) R: A language and environment for statistical computing. R-project.org

4 ASSESSING CARIBBEAN SHALLOW AND MESOPHOTIC REEF FISH COMMUNITIES USING BAITED-REMOTED UNDERWATER VIDEO (BRUV) AND DIVER-OPERATED VIDEO (DOV) SURVEY TECHNIQUES

Chapter 4: Assessing Caribbean shallow and mesophotic reef fish communities using baited-remoted underwater video (BRUV) and diver-operated video (DOV) survey techniques

4.1 Abstract

Fish surveys form the backbone of reef monitoring and management initiatives throughout the tropics, and understanding patterns in biases between techniques is crucial if outputs are to address key objectives optimally. Often biases are not consistent across natural environmental gradients such as depth, leading to uncertainty in interpretation of results. Recently there has been much interest in mesophotic reefs (reefs from 30-150 m depth) as refuge habitats from fishing pressure, leading to many comparisons of reef fish communities over depth gradients. Here we compare fish communities using stereo-video footage recorded via baited remote underwater video (BRUV) and diver-operated video (DOV) systems on shallow and mesophotic reefs in the Mesoamerican Barrier Reef, Caribbean. We show inconsistent responses across families, species and trophic groups between methods across the depth gradient. Fish species and family richness were higher using BRUV at both depth ranges, suggesting that BRUV is more appropriate for recording all components of the fish community. Fish length distributions were not different between methods on shallow reefs, yet BRUV recorded more small fish on mesophotic reefs. However, DOV consistently recorded greater relative fish community biomass of herbivores, suggesting that studies focusing on herbivores should consider using DOV. Our results highlight the importance of considering what component of reef fish community researchers and managers are most interested in surveying when deciding which survey technique to use across natural gradients such as depth.

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4.2 Introduction

When conducting ecological monitoring programs it is important to select an appropriate sampling method. Within coral reef fish assessments it is well known that all sampling methods suffer from biases (Harvey et al. 2004; Edgar et al. 2004), yet it is often crucial to have accurate data on fish species abundance and biomass to inform management decisions. Therefore it is important to consider the appropriateness of different methods to assess fish populations based on individual target species, dominance of key trophic groups within the community, biogeographic region and locally-influenced fish behavioural adaptations (Kulbicki 1998; Watson et al. 2010; Januchowski-Hartley et al. 2012; Goetze et al. 2015).

Historically it was common to use destructive sampling to minimise bias when surveying reefs, with ichthyocides such as rotenone released over a small area of reef to allow collection of most individuals for identification (Ackerman and Bellwood 2000). In many cases, contemporary fish community assessments are informing conservation management or ecological research, making such destructive sampling techniques largely unethical and counterproductive. Using ichthyocides for sampling also makes long term monitoring challenging by their very nature. In place of destructive sampling, research ultimately moved to a reliance on underwater visual census (UVC) methods (English et al. 1997), with divers swimming transects and recording abundance and in some cases length estimates for fish within estimated transect boundaries. While cheap and easy to conduct, UVC has been extensively criticised for a lack of repeatability resulting from inconsistencies between observers (Sanderson and Solonsky 1986; Thompson and Mapstone 1997), such as a large variation in transect boundary and visual fish length estimation (Harvey et al. 2004). These problems reduce statistical power for UVC surveys making it harder to separate genuine changes from observer differences, particularly in long-term monitoring with inevitable turnover in observers through time (Thompson and Mapstone 1997).

As a result there have been widespread calls to use video methods for fish community monitoring (Pelletier et al. 2011; Tessier et al. 2013). Videography allows observers to pause footage during analysis to consult fish identification guides or experts for help with identification. They also make monitoring programs involving multiple observers over several years easier to standardise, as identical videos can be used to train observers and control for biases. The development of stereo-video camera systems (SVS) facilitates length measurements, useful for estimating both fish lengths and transect boundaries (Harvey et al. 2004), further reducing observer bias. Video is not without challenges though, as despite advances in underwater imaging systems, videos reduce clarity and present a restricted field of view compared to observers in the water (Holmes et al. 2013).

Both in-water stereo diver-operated video (DOV) and stereo baited video camera drops (baited remote underwater video; BRUV) are widely used for reef fish surveys. Despite this, however, studies assessing differences between these contrasting SVS systems on tropical reefs are geographically limited to western Australia (Watson et al. 2010; Langlois et al. 2010) and Fiji (Goetze et al. 2015). Patterns observed in the Indo-Pacific may not be true for tropical reefs in the western Atlantic, which has differing fish species richness, trophic structures and taxonomic groups providing ecosystem functions (Roff and Mumby 2012). In addition, previous comparison studies have not considered differential effects of depth on the results obtained. Yet understanding any variation with depth between DOV and BRUV is crucial, as technique choice is often influenced by survey depth. Scientific divers conducting research within the recreational diving range are normally limited to 30 m maximum depth, with little scope to conduct in-water reef fish surveys deeper than 20 m because of breathing gas and no-decompression limit restrictions (Sieber and Pyle 2010). However, researchers are becoming increasingly interested in mesophotic reefs (light dependent reefs 30-160 m; Hinderstein et al. 2010) as potential refuges for fish from shallow-focused fishing pressure and other anthropogenic threats (Bejarano et al. 2014; Lindfield et al. 2014b). In addition, it is increasingly recognised that mesophotic reefs face many threats in their own right (Chapter 9). To survey deeper reefs many researchers use BRUV systems, because of the low cost and ease of surveying compared to the significant resources required for divers to safely work at mesophotic depths via technical diving (Sieber and Pyle 2010). On shallow reefs (<20 m) where monitoring programs historically have conducted UVC transects, DOV surveys are the logical video equivalent.

Many factors are known to affect fish detection in video surveys, and their impacts are likely to vary between BRUV and DOV, and also with depth. Between survey type, Watson & Harvey (2007) found the presence of a diver in the water caused changes in the recorded abundance and approach distance for several fish species, while other species may be attracted to divers (Cole et al. 2007). These biases are unlikely to be random with respect to fish trophic group, for example carnivorous snappers were under-detected by divers compared to remote camera surveys (Willis and Babcock 2000), and baiting camera systems may bias fish community surveys towards larger predatory fish (Watson et al. 2005). This has lead to the suggestion that other trophic groups may appear at lower abundances on BRUVs, although bait has been shown to have little impact on recorded abundance of herbivores (Harvey et al. 2007).

Many marine protected areas now have substantial recreational dive-based tourism on their shallow reefs. Reef fish have been shown to partially habituate to passive diver presence (Titus et al. 2015), suggesting differences between DOV surveys conducted on shallow heavily-dived reefs and deeper, less-frequently dived reefs may partially be caused by differing fish responses to divers. A recent study surveying protected and spear-fished areas found large differences in fish biomass detected by DOV, based on the diver equipment use

(Lindfield et al. 2014a). Transects filmed using normal recreational open-circuit equipment recorded lower fish diversity and abundance when compared to near-silent closed-circuit rebreathers in the fished areas, suggesting fish evade detection in fished areas through diver avoidance. If deeper reefs are below the limit of fishers, this would not only lead to a biomass refuge (Bejarano et al. 2014; Lindfield et al. 2014b), but also potentially make resident fish less evasive of diver surveys when compared to their shallow counterparts. In addition, many reef fish species exhibit ontogenetic migrations with new fish recruits settling in shallow marine habitats (i.e. mangroves, seagrass beds and shallow coral reefs) and moving to deeper reefs as they mature (Cocheret de la Morinière et al. 2002). Maturation in many fish species is associated with changes in diet (Cocheret de la Morinière et al. 2003), suggesting individual fish species responses to bait could be dependent on individual fish maturity, which is correlated with depth. Maturation is also associated with changes in behaviour, as fish try to minimise predation risk while maximising feeding (Helfman et al. 1982) and so potentially affecting detection ability by divers across the shallow to mesophotic reef gradient.

To test these questions, we compare BRUV and DOV assessments of fish community structure on shallow and mesophotic reefs in the western Atlantic, and assess whether differences between techniques are consistent across the depth range. Specifically, we test whether the baited nature of BRUV leads to a greater proportion of carnivorous fish in the community compared to DOV and contrast this with herbivorous reef fish detection differences between techniques and depths.

4.3 Methods

4.3.1 Study sites

Study sites were located on Utila, Honduras, approximately 29 km north of the Honduran mainland (Fig 4.1). Utila is at the southern extent of the Mesoamerican Barrier Reef, and extensive coral reefs and mangrove forests exist around the island. The reefs of Utila are contained within the Bay Islands National Marine Park, and there is a large recreational dive tourism industry (Doiron and Weissenberger 2014), with tens of thousands of dives conducted on the shallow reefs annually. Despite this, there is a large fishery around the island (Gobert et al. 2005; Box and Canty 2011), although increasingly this fishery focuses on offshore rather than fringing reefs, and so fishing is only conducted at low levels by hand lines at our study sites. Surveys were conducted at four sites on the south shore of Utila (Fig 4.1), where the shallow reef slopes down to the continental shelf (approximately 60-80 m depth), which extends to the Honduran mainland. All surveys were conducted in June-August 2014, with research permits issued by Instituto de Conservación Forestal (ICF), Honduras.

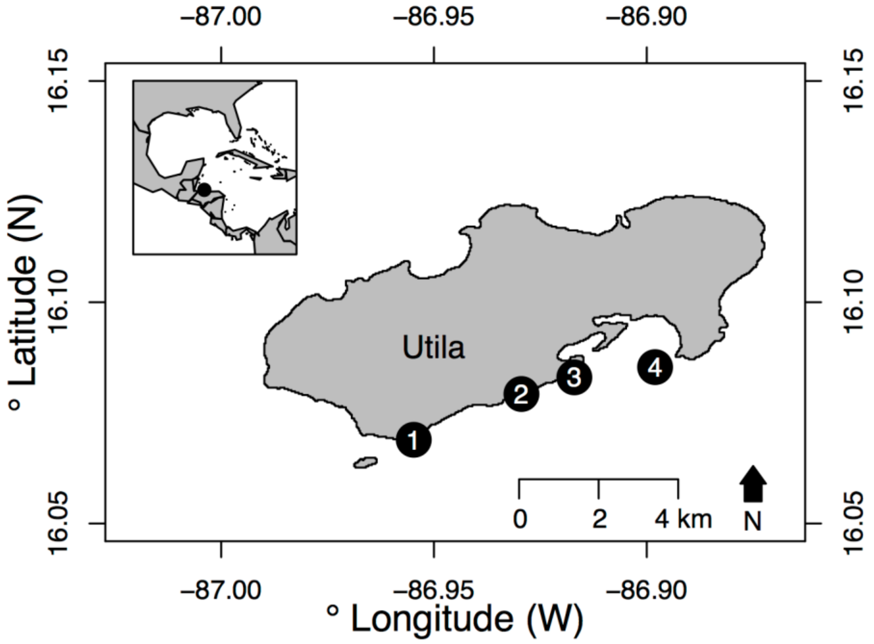

Figure 4.1 The four survey sites on the south shore of Utila, Bay Islands, Honduras.

Sites were: (1) Stingray Point, (2) Little Bight, (3) Black Coral Wall and (4) Lighthouse Reef. Inset – The location of Utila is indicated with a black circle relative to the western Caribbean and Gulf of Mexico.

4.3.2 Survey techniques

Surveys made use of stereo-video systems (SVS). SVS consist of two video cameras at fixed angles to allow footage of fish to be filmed simultaneously from two different positions. Using these dual images it is possible to accurately estimate the length of fish. These lengths can be converted into biomass estimates using standardised length-weight relationships.

4.3.3 Diver-operated video (DOV)

Transects were conducted using a commercially available stereo-DOV (SeaGIS, Melbourne, Australia), fitted with two Cannon HFS21 cameras (see Watson et al. (2010) for a full system overview). To minimise disturbance to the recorded fish community while setting up the cameras, transects were conducted as follows: the DOV operator started the cameras recording, synchronised them using a handheld torch and then angled the cameras downwards while their dive buddy attached a transect tape to the reef. The team then swam 10 m along the reef with the cameras pointing downwards as the buddy laid the transect tape, after 10 m the buddy signalled to the DOV operator. The DOV operator then started the 50 m transect by angling the cameras to film across the reef slope at the target survey depth. The DOV operator then swam along the reef with their buddy laying the transect tape and signalling when 50 m had been covered. Transects took approximately three minutes to film, with divers using open-circuit dive equipment. Four 50 m long transects were conducted at both 5 m (shallow) and 35-40 m (mesophotic) at each site, with each transect separated by a 10 m interval and following the respective depth contour.

4.3.4 Baited remote underwater video (BRUV)

We used a custom made BRUV consisting of two GoPro Hero 3+ Silver Cameras mounted in ScoutPro H3 deep-sea housings on an aluminium bar 0.8 m apart and inwardly converging at 10 degrees. This was built into a plastic chassis with a weight. The BRUV was deployed for 50 minutes in two depth bands; 5 m (shallow) and 30-55 m (mesophotic).

Five BRUV drops were conducted at each depth at each site, with the shallow BRUVs placed along the same reef crest location as the shallow DOV transects were conducted (see S4.1 Table for more details). The shallow BRUV deployments were assisted by divers to minimise damage to the reef and were placed at approximately 20 m intervals along the reef crest facing outwards towards open water. Mesophotic BRUVs were deployed by boat over the location of the mesophotic DOV transects, with a depth sounder used to find the target depth of 40 m prior to deployment, though actual depths recorded by the BRUV varied between 30-55 m (see S4.1 Table for BRUV GPS locations and depths). Each BRUV was baited with approximately 1.5 Kg of tuna heads and guts, suspended in a wire mesh bag 80 cm in front of the cameras. Tuna parts used were sourced from a local fisher, and were waste material that would normally be disposed of following filleting. Only one BRUV was deployed at a time, and multiple BRUV deployments at the same site were separated by a minimum of 2 hours to avoid overlaps of bait plumes.

The ordering of DOV transects and BRUV drops at each depth at each site was randomly selected, with no more than two BRUV drops or two DOV transects conducted in a depth band at a site on one day.

4.3.5 SVS analysis

Both BRUV and DOV camera systems were calibrated using a calibration cube and CAL software (http://www.seagis.com.au/bundle.html). All fish length measurements were conducted in EventMeasure software (http://www.seagis.com.au/event.html). Fish lengths were measured as fork lengths, the distance from the tip of the snout to end of the centre of the caudal fin rays.

For BRUVs, the MaxN biomass for each species was recorded. Videos were watched for 50 minutes from the BRUV camera system arriving on the seabed, and video frames were annotated in EventMeasure to indicate the paired frames containing the maximum number of individuals of each species during this period. The length of all the fish in this frame was then measured. MaxN avoids repeatedly counting the same individual, as fish often enter, exit and then re-enter the view for static cameras (Priede et al. 1994).

To ensure consistency within the DOV analysis, only fish that had their mid-point within a 5 m transect width (2.5 m either side of the camera centre) were included, and only fish within 5 m linearly in-front of the cameras were included. Fish with a three-dimensional location outside these specifications when calculated in EventMeasure were ignored, enabling us to standardise the DOV survey area. This generated a total survey area of 250 m^2 of reef per DOV transect. Care was taken to watch fish swimming behaviour on transects to minimise risk of double counting individuals that moved along the reef as we swam. Because of the relatively short survey time of the DOV transect (3 min), and the linear distance covered along the reef while surveying (50 m), the risk of double counting fish was low. The total abundance of each fish species and all their individual lengths across the whole transect were then used to estimate the fish community.

For both BRUV and DOV it was sometimes not possible to measure the length of a fish as it appeared on one camera only of the stereo pair, normally caused by being close in front of one of the cameras. In these cases, the mean length of all other individuals of that species recorded on that BRUV drop or DOV transect was applied. If no other individuals of the species were recorded on that drop or transect, the mean of all other individuals of the species across all BRUV drops or DOV transects at that site and depth band were used. All lengths were converted into weight estimates using Equation 4.1, where W is the fish weight (g), L is the fish length (cm) and a and b species-specific conversion constants.

Equation 4.1: $W=aL^b$

Conversion constants were obtained from fishbase (accessed September 2014; Froese and Pauly 2015).

4.3.6 Data analysis

Fish species were allocated into three trophic groups based on the feeding guild classification by Micheli et al. (2014). Trophic groups were: herbivores, carnivores (grouping ‘piscivores’ and ‘invert. feeder/pisciv.’ from the classification) and others. Permutational multivariate analyses of variance (permutational MANOVA Anderson et al. 2008) were used because of their lack of assumptions about data distributions. To test for differences in species richness, abundance, total biomass and relative biomass between trophic groups we used Euclidian distances in a permutational analysis of variance (permutational ANOVA). As DOV records all individual fish along transects, while BRUV records the MaxN of each fish species in a fixed location, comparing the raw recorded fish abundance or biomass between the techniques makes interpretation of patterns difficult. As our focus was on broader fish community differences between methods across depths we standardised fish abundance and biomass data by calculating relative abundance and biomass for each fish family and species on each transect or drop. This was done by dividing the total abundance or biomass of each fish family or species in turn by the total fish abundance or biomass recorded on the transect or drop. For community analysis, where many species were recorded as zero abundance and biomass we used Bray-Curtis dissimilarities on relative abundance or biomass made up by each species. Permutational ANOVA/MANOVA was conducted using the ‘adonis’ function, while principle coordinates analysis (PCO) was conducted using the ‘cmdscale’ function, both from the package vegan (Oksanen et al. 2013) in R (R Core Team 2013). All permutational tests were run for 9999 permutations, constrained within survey site, and simplified to remove non-significant interactions between model terms. As our study had multiple factors and unbalanced numbers of samples between the two survey methods, when testing for interactions the sums of squares for model terms are non-independent (Anderson et al. 2008). We used Type I (sequential) sums of squares, where each term was fitted sequentially after the previous fitted terms, meaning the order terms were fitted was important. This approach is appropriate when there is a logical order to fit terms based on the research question being addressed (Anderson et al. 2008), in this study we wanted to identify the effect of depth after the effect of site, and the effect of survey method after the effects of site and depth. We therefore fitted terms in the following prioritisation order: site, depth and survey method, with interactions in the order: site:depth, site:method, depth:method, site:depth:method.

A constrained analysis of principal coordinates (CAP) was run for all shallow and mesophotic data separately using the function 'capscale' in vegan (Oksanen et al. 2013). We tested for fish species likely to be driving differences between the two methods and depths by identifying Pearson correlations between individual fish species' relative community biomass and the canonical axis. Correlations of $|r| \geq 0.3$ were used to highlight potential species which might be driving differences. We also plotted relative community biomass for each species assessed by BRUV against that assessed by DOV, allowing species showing large differences in community composition between the two methods to be identified.

Kernel density estimates (KDE) were used to calculate length distributions for fish recorded by each method. KDEs were calculated using the 'dpik' function in the package KernSmooth (Wand 2013). The 'dpik' function selects KDE bandwidths using the Sheather-Jones selection procedure, which selects the optimal bandwidth for constructing a

KDE based on the distribution of the lengths (Sheather and Jones 1991). We followed Langlois et al. (Langlois et al. 2012) in using the function ‘sm.density.compare’ from the R package sm (Bowman and Azzalini 2014) to test whether KDEs generated for fish communities surveyed by the two methods in each depth band were significantly different.

4.4 Results

4.4.1 Species richness, abundance and biomass

On shallow reefs DOV recorded 26 % more individual fish than BRUV, while on mesophotic reefs BRUV recorded 339 % more individuals than DOV (Table 4.1). For both shallow and mesophotic reefs BRUV recorded more species than DOV, with 72 % and 88 % respectively, and many species unique to each method (Fig 4.2a). BRUV at both shallow and mesophotic depths also recorded more families than DOV. The top five most commonly recorded fish families are identical for shallow reefs between the two methods (Labridae, Pomacentridae, Acanthuridae, Scaridae and Lutjanidae), but differ for mesophotic reefs (Fig 4.3). For mesophotic reefs both techniques recorded Labridae, Serranidae and Lutjanidae in their top five families, but BRUVs also recorded Carangidae and Sparidae while DOV recorded Scaridae and Acanthuridae. We tested for differences in species richness between sites, depths and methods (S4.2 Table), finding that there were significant site:method interactions and depth:method interactions, suggesting that identified differences in fish species richness between sites is affected by survey method, but more importantly that the difference in species richness recorded by BRUV and DOV is affected by depth. Mean species richness was greatest on shallows reefs for both methods, declining 64 % for BRUV and 63 % for DOV when compared to mesophotic reefs (Fig 4.2). We found fish biomass was significantly affected by site, depth and the survey method, with a significant site:depth interaction, suggesting that fish biomass changes differently across the depth gradient depending on site (S4.3 Table). Mean fish biomass recorded per replicate was greater for BRUVs than DOV at both depths (Fig 4.2b), but declined with depth.

Table 4.1 Summary of collected fish data on shallow and mesophotic reefs by diver-operated (DOV) and baited-remote (BRUV) stereo-video systems.

	Shallow reefs			Mesophotic reefs		
	BRUV	DOV	Total	BRUV	DOV	Total
Individuals	1792	2251	4043	514	117	631
Species	74	43	80	60	32	69
Species unique to method	37	6		37	9	
Families	22	15	23	25	16	26
Families unique to method	8	1		10	1	

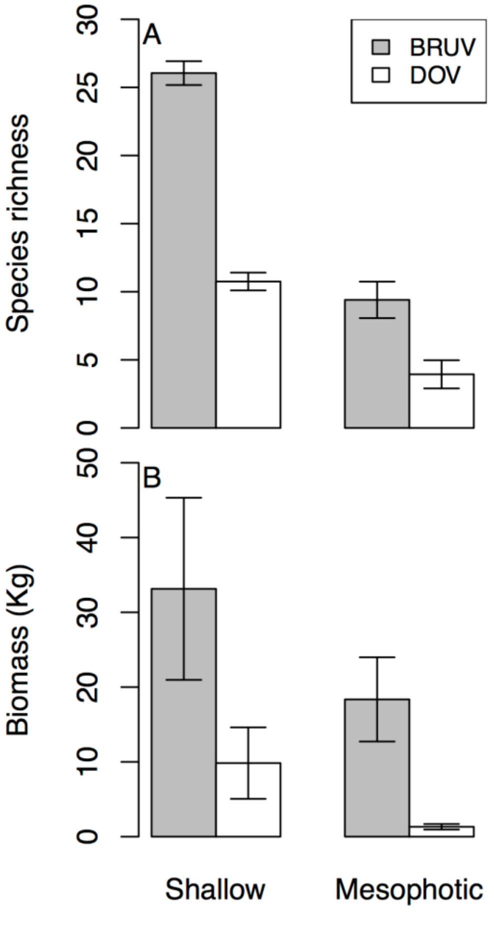

Figure 4.2 Mean (A) fish species richness and (B) fish biomass of shallow and mesophotic reefs per site using the two different sampling methods (BRUV and DOV). Species richness and biomass is per 250 m^2 transect for DOV, and per BRUV drop for BRUV.

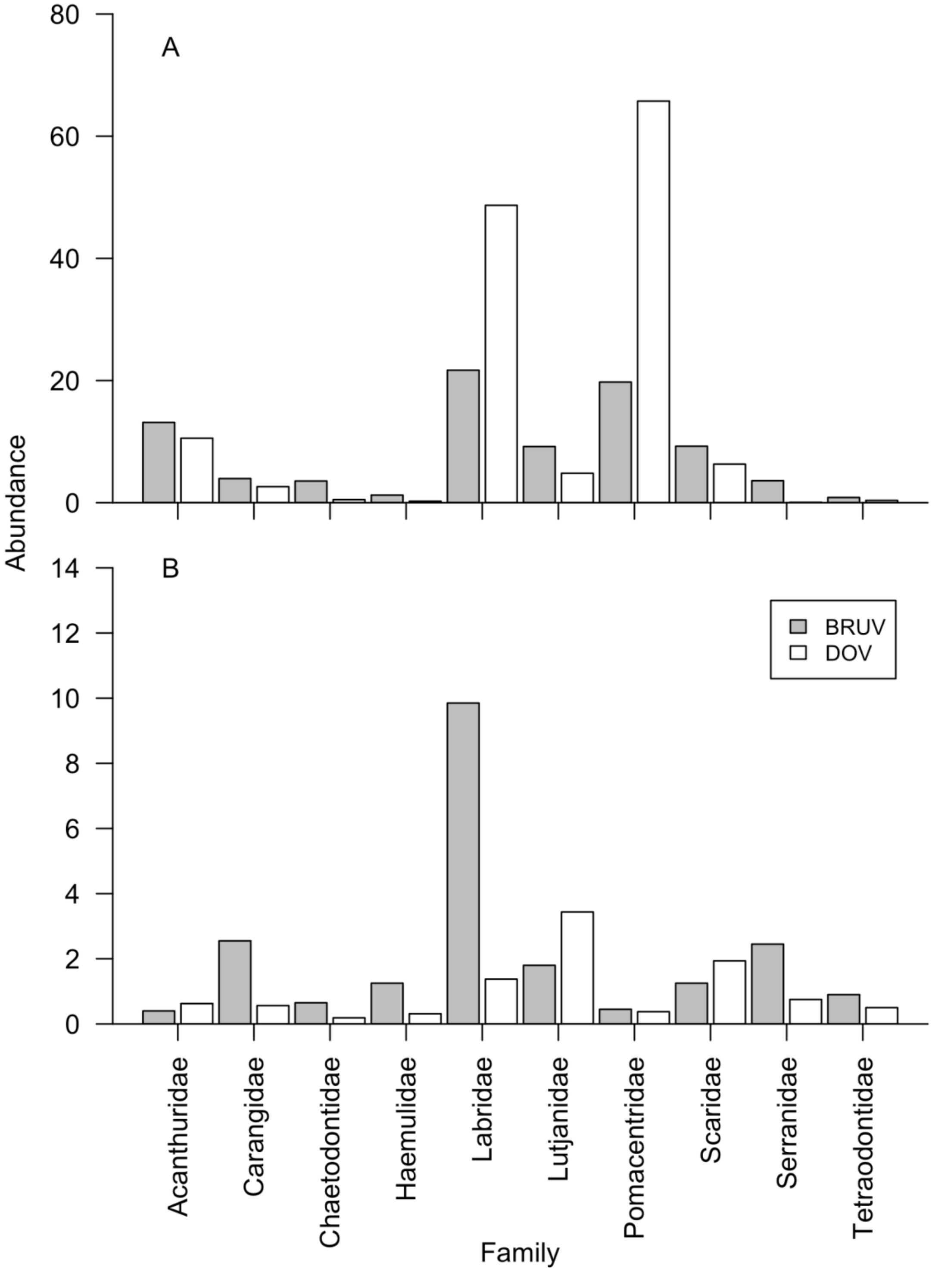

Figure 4.3 Fish abundance per family on (A) shallow reefs, and (B) mesophotic reefs, using BRUV and DOV methods calculated across all sites.

Only families comprising >1 % of the total number of fish recorded are shown. Abundance represents the mean number of fish recorded per 250 m^2 DOV transect, or per BRUV drop for DOV and BRUV surveys respectively.

4.4.2 Fish community structure

For fish families on shallow reefs, DOV recorded a greater mean abundance of Labridae and Pomacentridae per transect than BRUV recorded per drop (Fig 4.3a). This is reflected in greater relative community abundances for Labridae and Pomacentridae on DOV than BRUV at 35 % and 47 % versus 24 % and 22 % respectively. BRUV recorded greater abundances per drop than per DOV transects for Chaetodontidae and Serranidae on shallow reefs, with other fish families appearing at similar abundances from both methods (Fig 4.3a). On mesophotic reefs the pattern was reversed for Labridae, with BRUV recording greater abundance than DOV (Fig 4.3b), and Labridae comprising 38 % of the community by abundance. Herbivorous families such as Acanthuridae and Scaridae were recorded at similar abundances by BRUV and DOV on both shallow and mesophotic reefs (Fig 4.3). Interestingly, the carnivorous family Lutjanidae was recorded at higher abundances on mesophotic reefs by DOV than BRUV. While Carangidae, another carnivorous family, showed the reverse of this pattern with greater BRUV abundance than DOV on mesophotic reefs.

At the species level, several different species dominate the communities at shallow and mesophotic depths (Fig 4.4), driving the previously reported method:depth interactions. The most common shallow water fish recorded by DOV was the damsel *Chromis cyanea*, making up 32 % of the fish community compared to only 8 % on BRUV (Fig 4.4). *Clepticus parrae* was also recorded at greater relative abundance on DOV (16 %) than BRUV (2 %) on shallow reefs. At mesophotic depths, however, *C. parrae* was the most abundant fish species recorded by BRUV, composing 37 % of the fish community. Other notably abundant species at mesophotic depths are the snapper *Lutjanus synagris* and the parrotfish *Scarus iseri*, both of which were recorded at high abundances by DOV.

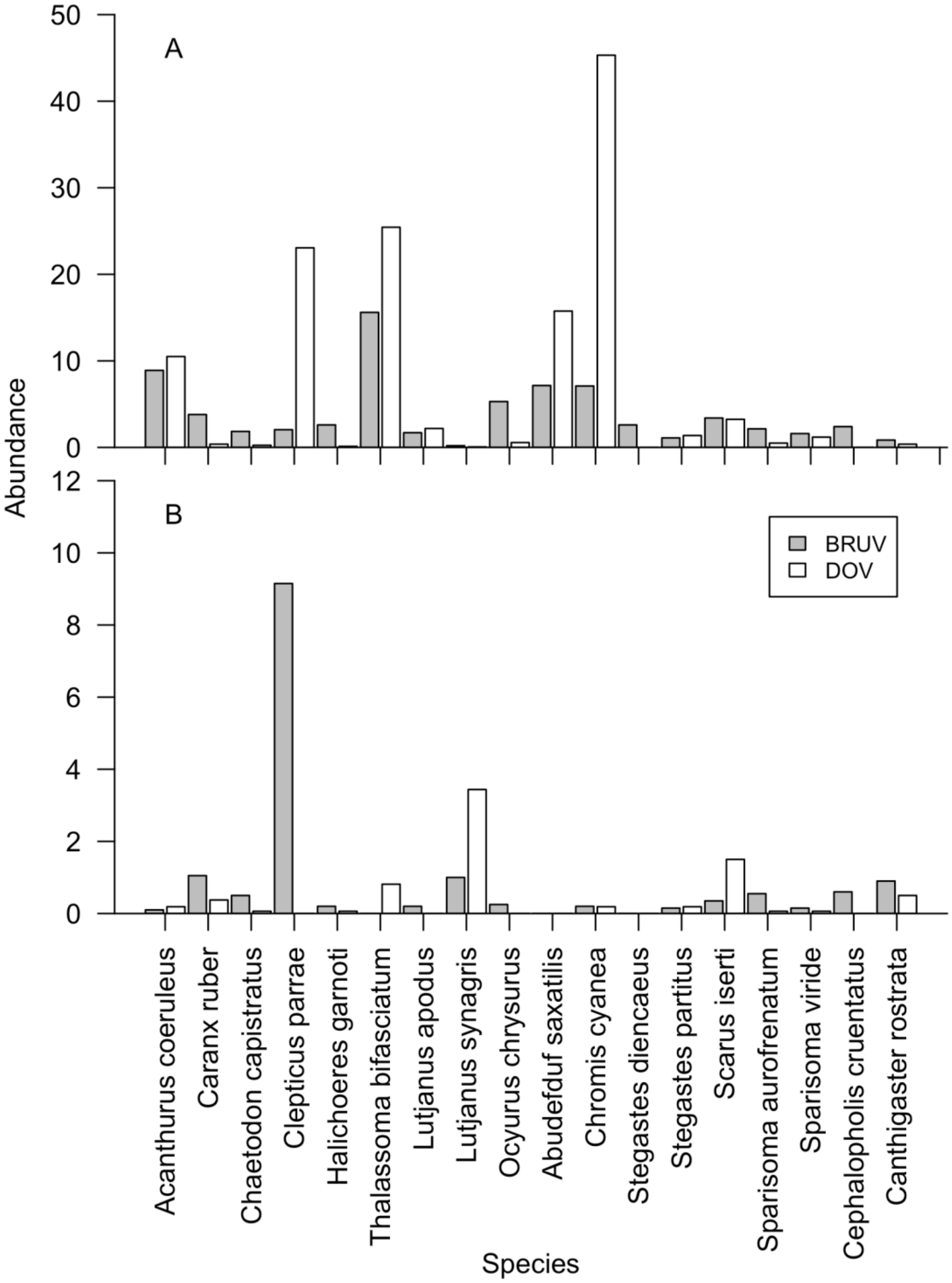

Figure 4.4 Fish abundance per species on (A) shallow reefs, and (B) mesophotic reefs, using BRUV and DOV methods calculated across all sites.

Only species comprising >1 % of the total number of fish recorded are shown. Abundance represents the mean number of fish recorded per 250 m^2 DOV transect, or per BRUV drop for DOV and BRUV surveys respectively.

We calculated relative biomass of each fish species recorded to investigate patterns in fish community structure. When visualising the relative community biomass of each species using PCO, at most sites the communities grouped primarily based on depth, with some grouping based on method within depth bands (S4.1 Fig). When formally tested we found differences in fish community structure between different sites, depths and methods and also site:method and depth:method interactions (S4.4 Table). We conducted CAP analyses to identify families and species driving this pattern. On shallow reefs our CAP analysis indicated BRUVs tended to be correlated with greater relative biomasses of families that contain carnivores such as Haemulidae, Lutjanidae and Serranidae, though also surprisingly Chaetodontidae (Table 4.2). For both shallow and deep reefs we found that DOV detected a greater relative community biomass of Scaridae. On deep reefs BRUV detected Dasyatidae, Ostraciidae and Pomacanthidae that were absent from DOV surveys. Unsurprisingly many species correlating with our species level CAP axis (Table 4.3) belonged to families correlated with our family level CAP axis (Table 4.2). Five species of Pomacentridae (*Abudefduf saxatilis, Chromis cyanea, Chromis multilineata, Stegastes adustus* and *Stegastes partitus*) and three species of Scaridae (*Scarus coeruleus, Scarus iserti* and *Sparisoma viride*) made up a larger proportion of the shallow DOV biomass weighted community than BRUV. We found the carnivores *Lutjanus analis, Lutjanus jocu, Cephalopholis cruentatus* and *Mycteroperca venenosa* all as a greater proportion of the community biomass on shallow reef BRUV, alongside two Labridae species: *Bodianus rufus* and *Halichoeres radiatus*. One Labridae species, *Thalassoma bifasciatum,* was recorded as a greater relative biomass on DOV on both shallow and mesophotic reefs. At mesophotic depths we found five species (*Dasyatis americana, Haemulon aurolineatum, Gymnothorax moringa, Pomacanthus arcuatus* and *Cephalopholis cruentatus*) from five different families making up a greater proportion of relative community biomass on BRUV than DOV. With the exception of *Pomacanthus arcuatus* (a spongivore) these species are all carnivorous.

Table 4.2 Fish families with relative community biomass correlating (|r|≥0.3) with the constrained analysis of principle coordinates axis.

Fish families with relative community biomass correlating (|*r*|≥0.3) with the constrained analysis of principle coordinates axis indicating greater relative community biomass recorded for the fish family on one survey method over the other (DOV vs BRUV). Mean (± 1 standard error) and the median relative community biomass recorded for each family by both methods are reported.

	r	**BRUV**		**DOV**	
		Mean±SE	Median	Mean±SE	Median
Shallow					
BRUV > DOV					
Chaetodontidae	-0.31	0.01 ± 0.01	0.01	0.01 ± 0.01	0.01
Haemulidae	-0.39	0.04 ± 0.01	0.05	0.04 ± 0.02	0.03
Lutjanidae	-0.52	0.25 ± 0.04	0.27	0.14 ± 0.03	0.13
Serranidae	-0.45	0.04 ± 0.01	0.04	0 ± 0	0
DOV > BRUV					
Pomacentridae	0.69	0.06 ± 0.02	0.05	0.24 ± 0.11	0.16
Scaridae	0.43	0.15 ± 0.04	0.13	0.24 ± 0.07	0.27
Deep					
BRUV > DOV					
Dasyatidae	-0.34	0.59 ± 0.01	0.59	0 ± 0	0
Ostraciidae	-0.30	0.07 ± 0.01	0.07	0 ± 0	0
Pomacanthidae	-0.31	0.12 ± 0.05	0.14	0 ± 0	0
Sparidae	-0.53	0.19 ± 0.05	0.16	0.08 ± 0	0.08
DOV > BRUV					
Labridae	0.31	0.02 ± 0.01	0.00	0.12 ± 0.07	0.10
Scaridae	0.40	0.03 ± 0	0.03	0.16 ± 0.04	0.12
Tetraodontidae	0.35	0 ± 0	0.00	0.13 ± 0.07	0.13

We compared overall fish length distributions recorded by the two techniques on shallow and mesophotic reefs. On shallow reefs there was no difference between BRUV and DOV (Fig 4.5a), though DOV surveys generated several peaks, with many small fish. For mesophotic reefs there were more small fish recorded by BRUV than DOV surveys (Fig 4.5b).

Table 4.3 Fish species with relative community biomass correlating (|r|≥0.3) with the constrained analysis of principle coordinates axis.

Fish species with relative community biomass correlating (|*r*|≥0.3) with the constrained analysis of principle coordinates axis, indicating greater relative community biomass recorded for the fish species on one survey method over the other (DOV vs BRUV). Mean (± 1 standard error) and the median relative community biomass recorded for each species by both methods are reported.

	Species	r	BRUV		DOV	
			Mean±SE	Median	Mean±SE	Median
Shallow						
BRUV > DOV						
Acanthuridae	*Acanthurus chirurgus*	-0.36	0.01 ± 0	0.01	0 ± 0	0
Chaetodontidae	*Chaetodon striatus*	-0.40	0 ± 0	0	0 ± 0	0
Labridae	*Bodianus rufus*	-0.32	0.01 ± 0	0.01	0.01 ± 0.01	0
Labridae	*Halichoeres radiatus*	-0.32	0 ± 0	0	0 ± 0	0
Lutjanidae	*Lutjanus analis*	-0.35	0.07 ± 0.03	0.04	0.01 ± 0.01	0
Lutjanidae	*Lutjanus jocu*	-0.30	0.1 ± 0.02	0.11	0.08 ± 0.05	0.08
Pomacentridae	*Stegastes diencaeus*	-0.60	0 ± 0	0	0 ± 0	0
Serranidae	*Cephalopholis cruentatus*	-0.55	0.04 ± 0.01	0.04	0 ± 0	0
Serranidae	*Mycteroperca venenosa*	-0.34	0.01 ± 0	0.01	0 ± 0	0
DOV > BRUV						
Labridae	*Thalassoma bifasciatum*	0.44	0.01 ± 0	0.01	0.02 ± 0	0.02
Pomacentridae	*Abudefduf saxatilis*	0.37	0.03 ± 0.01	0.02	0.11 ± 0.08	0.04
Pomacentridae	*Chromis cyanea*	0.74	0 ± 0	0	0.12 ± 0.04	0.11
Pomacentridae	*Chromis*	0.49	0 ± 0	0	0.01 ± 0	0.01

	multilineata					
Pomacentridae	*Stegastes adustus*	0.62	0 ± 0	0	0 ± 0	0.01
Pomacentridae	*Stegastes partitus*	0.40	0 ± 0	0	0 ± 0	0
Scaridae	*Scarus coeruleus*	0.31	0 ± 0	0	0.01 ± 0	0.01
Scaridae	*Scarus iserti*	0.37	0.01 ± 0	0.01	0.06 ± 0.02	0.05
Scaridae	*Sparisoma viride*	0.49	0.02 ± 0	0.02	0.12 ± 0.05	0.12
Deep						
BRUV > DOV						
Dasyatidae	*Dasyatis americana*	-0.47	0.59 ± 0.59	0.01	0 ± 0	0
Haemulidae	*Haemulon aurolineatum*	-0.32	0.01 ± 0.01	0	0 ± 0	0
Muraenidae	*Gymnothorax moringa*	-0.31	0.02 ± 0	0.01	0 ± 0	0
Pomacanthidae	*Pomacanthus arcuatus*	-0.32	0.2 ± 0.2	0.01	0 ± 0	0
Serranidae	*Cephalopholis cruentatus*	-0.4	0.01 ± 0.01	0	0 ± 0	0
DOV > BRUV						
Labridae	*Thalassoma bifasciatum*	0.30	0 ± 0	0	0.11 ± 0.07	0.10
Scaridae	*Scarus iserti*	0.51	0.01 ± 0	0	0.12 ± 0.02	0.12
Tetraodontidae	*Canthigaster rostrata*	0.31	0 ± 0	0	0.13 ± 0.07	0.13

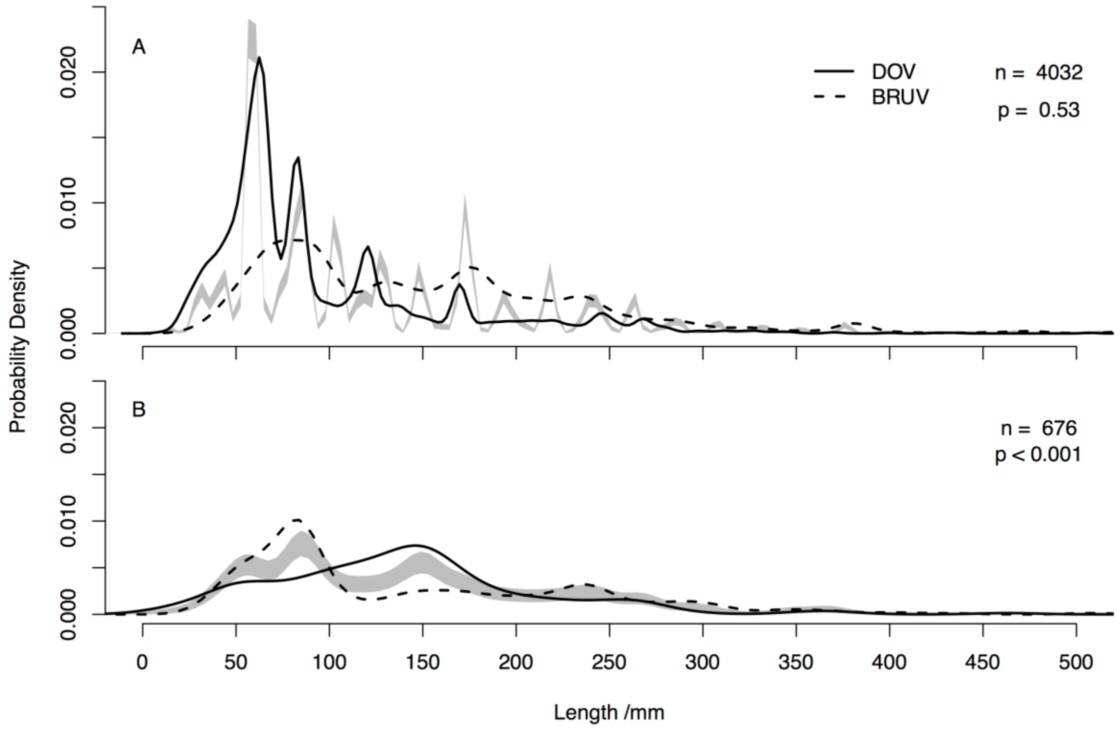

Figure 4.5 Fish length frequency distribution for (A) shallow and (B) mesophotic reefs recorded by DOV and BRUV across all sites.

Grey shaded areas represent one standard error either side of the null model, *n*= number of individual fish measured, *p* indicates whether the length distributions are significantly different based on permutation tests.

4.4.3 Herbivores and carnivores

As it has previously been suggested that using bait may bias recorded fish communities towards carnivores at the expense of other trophic groups, we conducted an analysis of just carnivores and herbivores. For both groups greater relative biomass was detected by DOV at mesophotic depths than BRUV (S4.2 Fig, S4.5 Table). Interestingly, there was no difference in relative biomass between techniques on shallow reefs for carnivores but DOV recorded higher relative biomass for herbivores (S4.2 Fig).

On shallow reefs we found differences in the herbivore length distribution (Fig 4.6a), with DOV generating a more distinctive modal peak, while on mesophotic reefs no difference was detected (Fig 4.6c). For carnivores differences in fish length distribution were detected at both shallow and mesophotic depths. On shallow reefs the mode was shifted to the right for DOV (Fig 4.6b), indicating larger carnivorous fish were more commonly detected when surveying by DOV, yet on mesophotic reefs the reverse was true, with larger carnivorous fish more commonly recorded on the BRUV (Fig 4.6d).

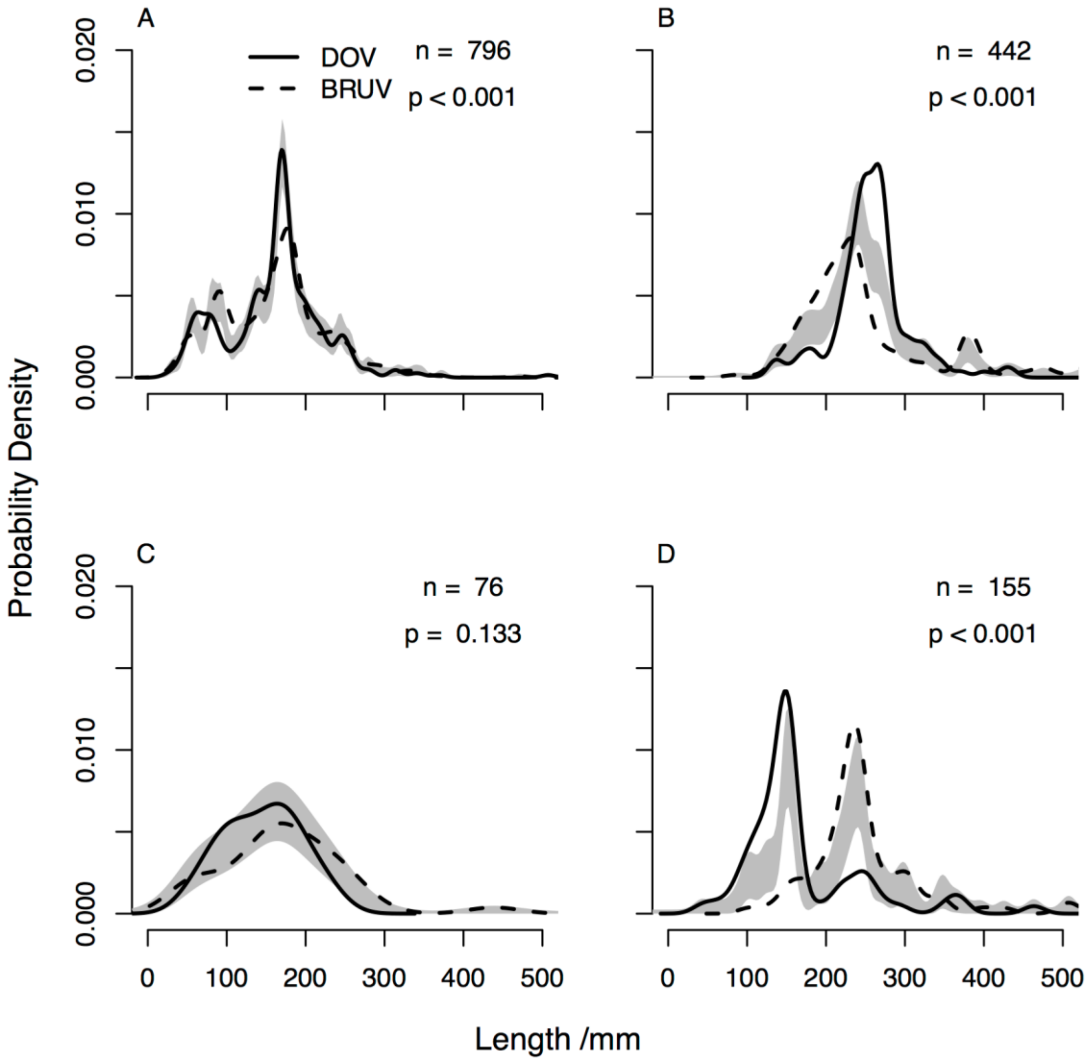

Figure 4.6 Length distributions for shallow (A) herbivores, (B) carnivores and mesophotic (C) herbivores and (D) carnivores comparing BRUV and DOV.

Grey shaded areas represent one standard error either side of the null model, *n*= number of individual fish measured, *p* indicates whether the length distributions are significantly different based on permutation tests.

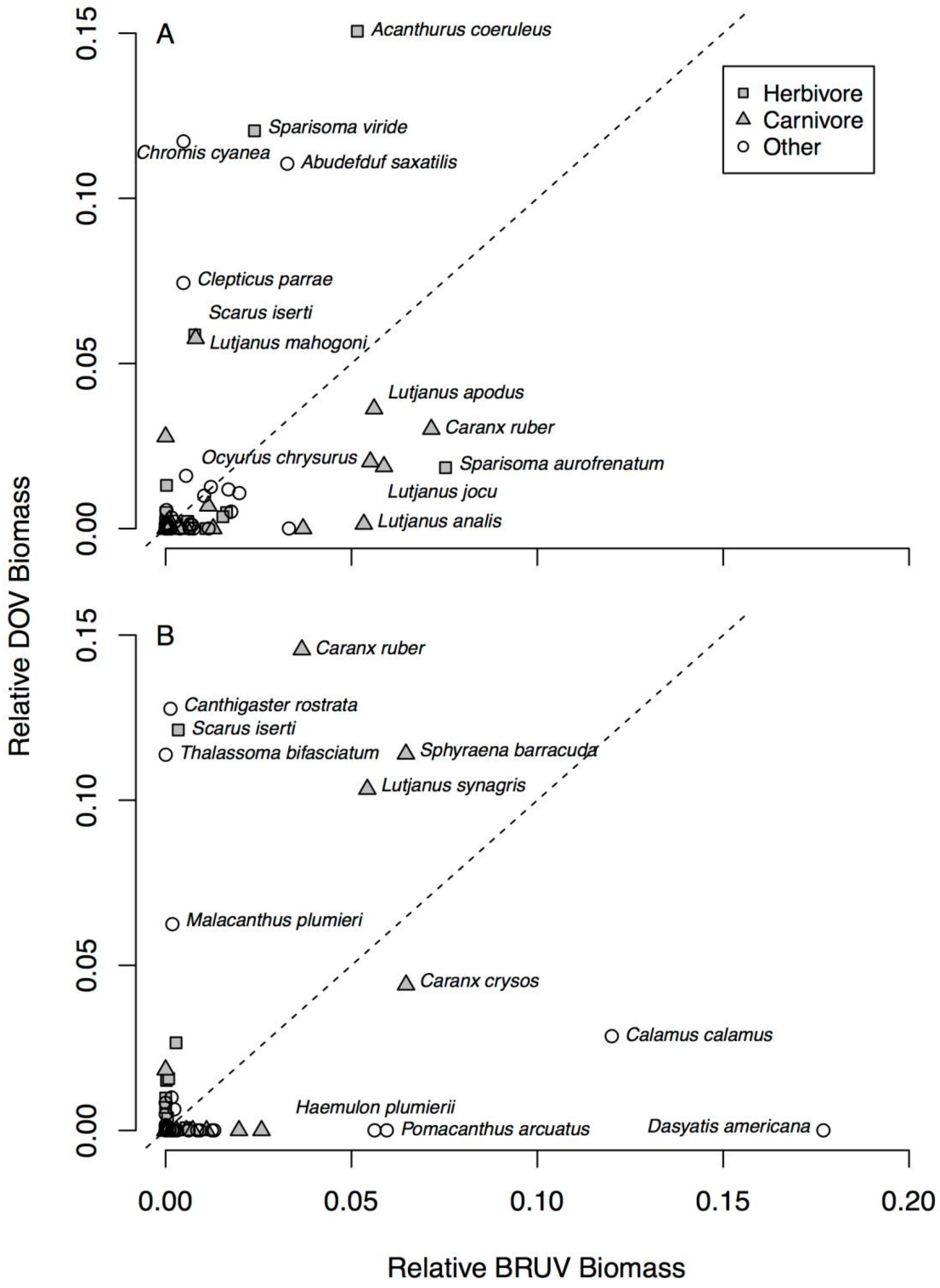

Figure 4.7 Relative community biomass measured by BRUV compared to DOV for (A) shallow and (B) mesophotic reefs.

Species composing the same proportion of the community when assessed by both methods should be located on the dashed line, with species furthest from the dashed line showing significant bias towards one survey method.

To further identify which herbivore and carnivore species may be driving differences between the two survey techniques we plotted relative biomass surveyed by BRUV against that from the DOV (Fig 4.7). Fig 4.7a shows a group of carnivorous fish (*Lutjanus spp.*, *Ocyurus chrysurus* and *Caranx ruber*), which were all recorded at greater proportions of the community on BRUV than DOV on shallow reefs. Herbivore species show less clear patterns in the shallows. At mesophotic depths the patterns are less clear (Fig 4.7b), though several carnivores appear to make up a larger proportion of the community on DOV than BRUV (*Caranx ruber*, *Sphyraena barracuda* and *Lutjanus synagris*).

4.5 Discussion

In this study we compared diver-operated stereo-video surveys (DOV) and baited remote underwater video surveys (BRUV) on shallow and mesophotic coral reefs to identify differences in relative community biomass for fish species. Our results show the choice of sampling method affects the reef fish community results obtained, but crucially that these effects are not consistent with depth. This suggests fish families and species show differences in response to, or detection by, specific survey techniques depending on depth. Our results are particularly important for researchers assessing patterns in fish communities across depth gradients.

There are several possible explanations for inconsistent differences between BRUV and DOV surveys with depth, including differing fish responses to divers based on (i) historical diver exposure (Titus et al. 2015), (ii) fishing pressures (Lindfield et al. 2014a) and (iii) ontogenetic changes with depth (Cocheret de la Morinière et al. 2002). This study was conducted in the Bay Islands National Marine Park, though the sites we surveyed are not no-take zones. In practice, there is a large recreational dive tourism industry on Utila and multiple dive boats visit these sites in a typical day. This means fishing only occurs at low levels at the sites, but exposes the shallow reef fish community to intensive diver contact.

Historically, however, fishing has been extensively conducted on the reefs of Utila (Gobert et al. 2005; Box and Canty 2011).

Regardless of depth, and in line with other studies (Watson et al. 2010; Langlois et al. 2010), we recorded more species on BRUV surveys than DOV. DOV recorded many more individual fish on shallow reefs than BRUV, reflecting the difference in counting fish between the two methods, with all fish counted on DOV transects, but only the maximum number of individuals seen in a single frame (MaxN) for BRUV (Priede et al. 1994). Surprisingly, this pattern reversed on mesophotic reefs, with more individuals counted on BRUV than DOV. Species richness and abundance generally increases with increased sampling effort, therefore these patterns are likely to be influenced by our choice of BRUV drop time and size of DOV transect. However, fish abundances are known to be lower on mesophotic reefs than shallow reefs around Utila (Chapter 2). Therefore, greater fish abundance recorded by BRUVs on mesophotic reefs than DOVs might be caused by: the baited nature of BRUV, combined with the longer survey duration, allowing fish to be attracted from a larger area than that covered by DOV transects, or that BRUVs are better at detecting individual fish on mesophotic reefs. While it is possible to estimate the visible distance in a BRUV frame (Bernard and Götz 2012), this is not a good estimate of the survey area for many fish species, as bait plumes will spread varying distances and directions based on tides and currents. Studies looking at bait dispersal have found differences in fish community composition based on water flow rates past the BRUV (Taylor et al. 2013). Without better modelling of this, it is difficult to estimate bait plume area, especially with the BRUV left in place for 50 minutes.

On shallow reefs we found many carnivorous families (e.g. Lutjanidae and Serranidae) and species (e.g. *Lutjanus analis, Lutjanus jocu, Cephalopholis cruentatus and Mycteroperca* *venenosa*) significantly correlated with our CAP analysis indicating greater relative community biomass on BRUV than DOV. When looking at outlying species with greater relative biomass on BRUV than DOV on shallow reefs (Fig 4.7a) many *Lutjanus* species were identified alongside *Ocyurus chrysurus* and *Caranx ruber*. This increased biomass of carnivorous fish on BRUV fits with our hypothesis and with previous studies (Harvey et al. 2007; Langlois et al. 2010). This is likely to be caused by a range of factors, including bait attraction (Harvey et al. 2007), large bodied carnivorous species avoiding the bubbles produced by recreational divers particularly in areas with previous spearfishing (Lindfield et al. 2014a), and the long BRUV deployment times (Taylor et al. 2013; Harasti et al. 2015).

Surprisingly we found greater carnivore biomass on mesophotic reefs on DOV than BRUV, but no particular carnivorous families or species correlated with our CAP analysis making it harder to identify specific carnivorous families or species driving this trend. However, three carnivorous species had greater relative biomass on DOV surveys than BRUV; *Caranx ruber*, *Lutjanus synagris* and *Sphyraena barracuda* (Fig 4.7b), while the snapper *Lutjanus synagris* also had high relative abundance on mesophotic reefs (Fig 4.4). This pattern could in part be explained by factors such as behavioural differences in response to divers (Lindfield et al. 2014a), fish identification challenges in low light environments (Holmes et al. 2013), or habitat heterogeneity at mesophotic depths (Harvey et al. 2007).

Despite large bodied carnivores having been shown to avoid divers in fished areas (Lindfield et al. 2014a), few dives are conducted to mesophotic depths on Utila, and mesophotic fish biomass is known to be retained despite shallow fisheries (Bejarano et al. 2014; Lindfield et al. 2014b). The increased relative biomass of carnivores could reflect reduced diver avoidance if fishing pressure in this depth range has been limited. Studies have identified that flight initiation distance (FID), the distance at which a fish flees from a diver, can be greater in areas with current or previous fishing (Januchowski-Hartley et al.

2012), and FID is naturally higher for larger individuals in many fish families (Januchowski-Hartley et al. 2011). However, FID variation with fishing pressure does not appear to occur consistently in all families, having been shown to be present in Scaridae (Gotanda et al. 2009; Januchowski-Hartley et al. 2012), but not in Lutjanidae or Serranidae (Januchowski-Hartley et al. 2011).

Another explanation for increased carnivore relative community biomass on DOV is declining light intensity with depth. Identification of small fish using DOV is harder on mesophotic than shallow reefs, while video has also been reported to make identification harder through reduced clarity compared to the human eye (Holmes et al. 2013). The lack of resolution using video could bias DOV at depth to larger bodied fish that are more readily distinguishable during analysis. With the BRUV system static on the seabed for 50 minutes the likelihood of visually identifying small-bodied species is increased through their movement. Although not used here, artificial lighting has been used to good effect on night BRUV surveys (Harvey et al. 2012), and its use on all BRUV and DOV surveys could help increase detection of small individuals.

Habitat heterogeneity is another factor that might drive observed differences between techniques with depth. Harvey et al. (2007) reports significant habitat:technique interactions when testing un-baited against baited remote video systems on the Great Barrier Reef. They found no differences between techniques on coarse sand and rubble patches or where the camera system landed a short distance away from more structurally complex benthic habitats. However, they did find differences when surveying fine sand/mud and more complex habitats (reefs and macroalgal, sponge and gorgonian beds). While the shallow reefs surveyed here were continuous spur and groove systems, mesophotic reefs were a large patch reef system with reefs separated by areas of fine sand and mud. By the linear nature of DOV surveys, following a depth contour ensured that transects incorporated both the patch reefs and the areas separating them. This level of habitat heterogeneity presents significant challenges to remotely deployed point count methods such as BRUVs. As the BRUV is deployed from a boat, guiding it onto a reef patch is not easily possible. We used a depth sounder to measure depth, and dropped BRUVs in areas known to have reef from exploratory dive surveys, but it was not possible to ensure that direct reef contact would be made, meaning replicates include non-reef habitats adjacent to reefs. While we would expect overall fish biomass to be lower from BRUVs on non-reef areas, large carnivores are some of the most mobile fish on reefs (Chapman and Kramer 2000). Therefore we would expect relative community biomass of carnivores to increase in BRUV data from non-reef areas compared to reef areas, caused by large carnivores swimming off patch reefs attracted by the bait, while other fish trophic groups less readily leave the reef. This suggests that mesophotic BRUVs would be biased to carnivores, however, we found greater relative biomass of carnivores via DOVs.

While herbivorous fish relative community biomass was consistently greater on both shallow and mesophotic reefs when surveyed by DOV than BRUV (S4.2 Fig), the responses we saw to method and depth were often family specific and varied based on whether weighting the community on biomass or abundance. Acanthuridae and Scaridae were both recorded at higher relative abundances by BRUV than DOV on shallow reefs, with the reverse on mesophotic reefs. This pattern is likely to be caused by the high abundance of Labridae and Pomacentridae on shallow reefs detected by DOV. Scaridae, which has a crucial role in algal grazing on western Atlantic reefs (Mumby 2009), was consistently recorded at greater relative community biomass on DOV than BRUV (Table 4.2, Table 4.3). As herbivores are unlikely to be attracted by the bait plume of BRUVs, they are likely to make up a lower proportion of BRUV surveyed community biomass. This effect may be particularly accentuated with depth, as previous studies have shown that herbivorous reef fish biomass generally declines with depth (Kahng et al. 2010), including on Utila based on DOV surveys (Chapter 2). This suggests that for studies specifically interested in herbivorous reef fish on mesophotic reefs DOV would be the preferred survey method.

Some of the species level relative biomass variation with depth and between methods might be caused by individuals being at different stages in their life cycle. Many tropical western Atlantic fish species undergo ontogenetic migrations, normally from shallow to deeper marine habitats (Cocheret de la Morinière et al. 2002). Chapter 2 looked at length distributions across the depth gradient in reef fish on Utila, and identified several species occurring at larger body sizes at mesophotic depths than shallow depths. Two of these species were the herbivore *Scarus iserti*, and the planktivore *Thalassoma bifasciatum,* both of which were consistently recorded at greater relative biomass at mesophotic depths on DOV than BRUV (Fig 4.7), and greater or similar abundances on DOV (Fig 4.4). A third species, the carnivore *Ocyurus chrysurus*, as previously mentioned, was recorded at increased abundance (Fig 4.4) and relative community biomass (Fig 4.7) on the BRUV. While we did not test specifically for individual species length differences between the two depths and methods, our results suggest future studies should look at length distributions generated by the two techniques. As BRUV uses MaxN to assess each species, in species that highly aggregate as juveniles, such as *Thalassoma bifasciatum* (Feddern 1965), MaxN is likely to represent juvenile aggregations generating a biased length distribution for the species despite more mature individuals potentially appearing at different times on the BRUV drop. DOV is likely to provide a more uniform length distribution as all individuals of a species within the transect area are measured. This will be particularly apparent for non-aggregating common planktivorous species such as *Chromis cyanea*, making up >30 % of the observed individual fish on shallow reefs by DOV, but not attracted to the BRUV. This is supported by much greater abundance of Labridae and Pomacentridae on DOV than

BRUV on shallow reefs, with all other families similar between the two methods or more abundant on BRUV (Fig 4.3). Studies interested in fish length distributions should therefore consider individual fish species behaviour and ontogeny both on the reef and in response to divers when deciding whether to use BRUV or DOV, as both methods have biases that will likely affect length distributions.

In addition to the differences in recorded fish community between the two techniques, there are other practical constraints to consider when deciding whether to use BRUV or DOV for surveys of shallow or mesophotic reefs. Regardless of the method selection, SVS video analysis is time consuming and labour intensive. Video processing times vary greatly based on fish abundance, resulting in shallow reef surveys typically taking longer than mesophotic surveys to analyse. The three minutes of DOV footage per transect took approximately 30-60 minutes to analyse, while the equivalent 50 minutes of BRUV took approximately 240-300 minutes to analyse. Therefore, despite BRUV capturing extra species (Table 4.1), there is a trade-off with the extra analysis required. If species richness is of interest, the addition of more DOV replicates might be preferable over BRUV to reduce video analysis time. However, while DOV surveys may be quicker to analyse, conducting mesophotic DOV surveys, requires advanced dive training and equipment, specialist dive safety management and detailed planning (Chapter 10). This makes DOV surveys, particularly for the lower mesophotic (reefs from 60-150 m), very costly and logistically challenging to conduct, especially in remote field settings. Therefore, while the comparisons outlined in this paper focus on differences in recorded fish community between techniques, the practical logistics of fieldwork may play a major role in dictating which technique should be used.

4.6 Conclusion

We compared the recorded fish community by diver-operated stereo-video (DOV) and baited remote underwater stereo-video (BRUV) surveys on shallow and mesophotic reefs on Utila, Honduras. We detected differences between both techniques in recorded fish communities, but importantly we found differences between techniques varied with depth. We show these differences affect recorded relative community biomass of different trophic groups, including large carnivorous fish often targeted by fisheries. BRUV recorded greater species richness at both shallow and mesophotic depths, making it most appropriate for recording all components of the fish community. DOV however recorded greater relative community biomass of herbivorous reef fish, suggesting studies interested in herbivores specifically should consider using DOV. It is therefore important for researchers and those designing reef fish monitoring programs that span depth gradients to carefully consider what attributes of the reef fish community they are most interested in surveying, and how these various biases in survey technique will affect the ultimate interpretation of their results.

4.7 Acknowledgments

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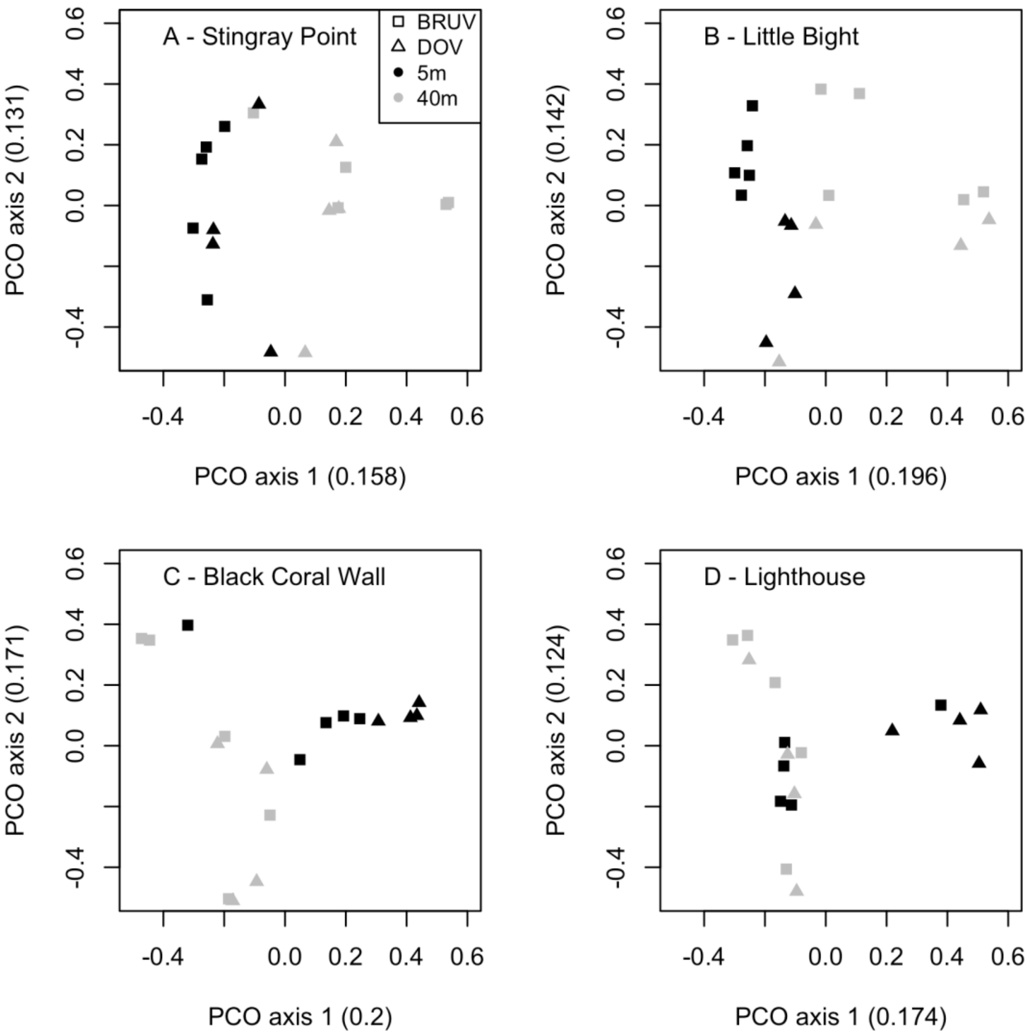
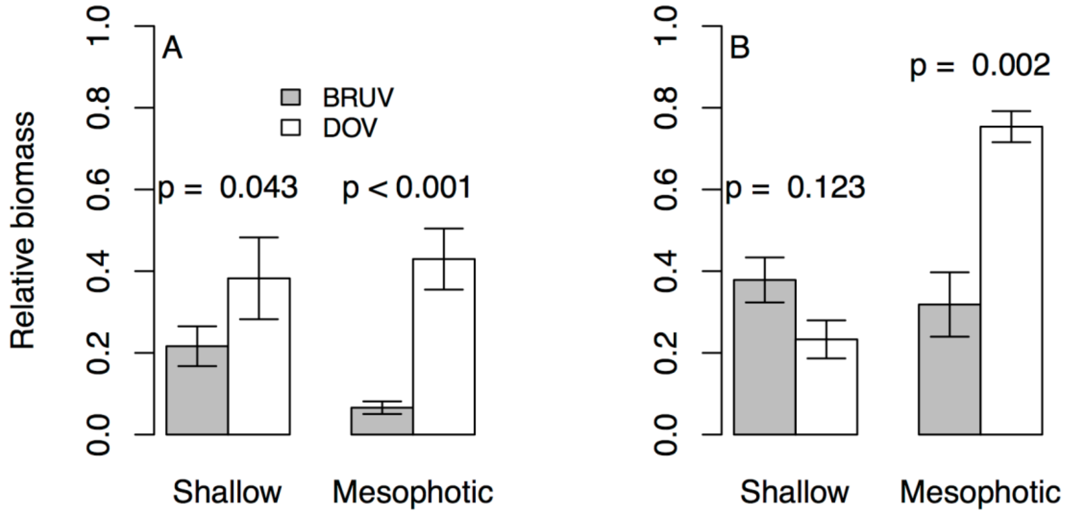
4.8 Supporting Information



S1

S4.1 Fig. Fish relative biomass community structure principal coordinates analysis plot for each site: (A) Stingray Point, (B) Little Bight, (C) Black Coral Wall and (D) Lighthouse reef. The proportion of variation explained by each axis is shown in brackets.



S4.2 Fig. Differences in relative community biomass recorded by the two survey techniques (BRUV and DOV) at each depth for (A) herbivores and (B) carnivores. Mean ± 1 SE shown, p values indicate whether differences are significant, calculated by a Euclidian permutational ANOVA between the two methods at each depth (see S5 Table for full permutational ANOVA results).

S4.1 Table. GPS Coordinates for survey locations. Points listed under the column Depth as 'Shallow/Mesophotic' represent GPS coordinates of fixed mooring buoys on the reef crest at the sites. For shallow and mesophotic DOV surveys divers descended from these mooring buoys to the survey depth (5 m or 40 m) and conducted two transects east and two transects west from the mooring line. Transects in both directions were started 10 m along the reef from the indicated GPS point. In addition, shallow BRUV surveys were conducted both east and west of these fixed mooring buoys at 5 m depth spaced at approximately 20 m intervals on the reef crest. Mesophotic BRUV drops were deployed by boat, with GPS coordinates for each drop recorded. Mesophotic replicates are named in the form Site_Depth_Day_Month_Year. All GPS coordinates collected on a Garmin GPS unit and recorded in WGS 84.

Depth	Site	Replicate	Latitude	Longitude
Shallow/ Mesophotic	Stingray Point	Shallow DOV and BRUV and Mesophotic DOV	16.0689055	-86.95477948
Shallow/ Mesophotic	Little Bight	Shallow DOV and BRUV and Mesophotic DOV	16.07926302	-86.92942222
Shallow/ Mesophotic	Black Coral Wall	Shallow DOV and BRUV and Mesophotic DOV	16.08305968	-86.91699554

Shallow/ Mesophotic	Lighthouse Reef	Shallow DOV and BRUV and Mesophotic DOV	16.08534778	-86.89803786
Mesophotic	Stingray Point	SR_45m_07_08_14	16.069732	-86.95252
Mesophotic	Stingray Point	SR_55m_11_08_14	16.067316	-86.952449
Mesophotic	Stingray Point	SR_50m_11_08_14	16.069242	-86.95097
Mesophotic	Stingray Point	SR_45m_09_07_14	16.067022	-86.955091
Mesophotic	Stingray Point	SR_40m_11_07_14	16.065148	-86.95621
Mesophotic	Little Bight	LB_40m_01_08_14	16.074428	-86.92763
Mesophotic	Little Bight	LB_50m_18_07_14	16.075742	-86.926418
Mesophotic	Little Bight	LB_50m_13_07_14	16.075578	-86.928331
Mesophotic	Little Bight	LB_55m_28_06_14	16.075331	-86.929724
Mesophotic	Little Bight	LB_49m_27_06_14	16.077433	-86.929066
Mesophotic	Black Coral Wall	BCW_45m_28_06_14	16.082718	-86.917268
Mesophotic	Black Coral Wall	BCW_44m_27_06_14	16.083609	-86.915567
Mesophotic	Black Coral Wall	BCW_45m_11_07_14	16.082366	-86.917158
Mesophotic	Black Coral Wall	BCW_40m_31_07_14	16.080342	-86.915708
Mesophotic	Black Coral Wall	BCW_50m_12_07_14	16.081651	-86.916106
Mesophotic	Lighthouse Reef	LH_30m_11_08_14	16.079121	-86.902081
Mesophotic	Lighthouse Reef	LH_45m_09_08_14	16.079937	-86.897165
Mesophotic	Lighthouse Reef	LH_45m_09_08_14	16.079163	-86.900483
Mesophotic	Lighthouse Reef	LH_30m_19_07_14	16.08375	-86.89688
Mesophotic	Lighthouse Reef	LH_30m_13_07_14	16.083395	-86.898666

S4.2 Table. Euclidian permutational ANOVA testing differences in species richness recorded by the two methods (DOV and BRUV) across both sites and depths. Permutations were constrained within Site and the model simplified to remove non-significant interactions.

Source	df	MS	F	p
Site	3	36.70	2.15	<0.001
Depth	1	2713.39	158.85	<0.001
Method	1	1915.92	112.16	<0.001
Site:Method	3	54.17	3.17	0.034
Depth:Method	1	430.12	25.18	<0.001
Residuals	62	17.08		
Total	71			

S4.3 Table. Euclidian permutational ANOVA testing differences in total fish biomass recorded by the two methods (DOV and BRUV) across both sites and depths. Permutations were constrained within Site and the model simplified to remove non-significant interactions.

Source	df	MS	F	p
Site	3	1364.80	2.58	<0.001
Depth	1	2591.40	4.89	0.026
Method	1	7233.70	13.66	<0.001
Site:Depth	3	1721.20	3.25	0.027
Residuals	63	529.40		
Total	71			

S4.4 Table. Permutational MANOVA of Bray-Curtis relative fish species community biomass dissimilarities recorded by the two methods (DOV and BRUV) across both sites and depths. Permutations were constrained within Site and the model simplified to remove non-significant interactions.

Source	df	MS	F	p
Site	3	0.53	1.44	<0.001
Depth	1	2.32	6.28	<0.001
Method	1	1.75	4.74	<0.001
Site:Method	3	0.56	1.53	0.004
Depth:Method	1	0.91	2.47	<0.001
Residuals	62	0.37		
Total	71			

S4.5 Table. Full statistical analysis results for S2 Fig. Euclidian permutational ANOVA testing differences in relative fish biomass recorded by the two methods (DOV and BRUV) for (A) shallow herbivores, (B) mesophotic herbivores, (C) shallow carnivores and (D) mesophotic carnivores.

A. Shallow Herbivores:

Source	df	MS	F	p
Method	1	0.25	4.18	0.041
Residuals	34	0.06		
Total	35			

B. Deep herbivores:

Source	df	MS	F	p
Method	1	0.42	8.21	0.006
Residuals	12	0.05		
Total	13			

C. Shallow Carnivores:

Source	df	MS	F	p
Method	1	0.13	2.59	0.129
Residuals	31	0.05		
Total	32			

D. Mesophotic Carnivores:

Source	df	MS	F	p
Method	1	1.08	14.02	<0.001
Residuals	25	0.08		
Total	26			

4.9 References

- Ackerman JL, Bellwood DR (2000) Reef fish assemblages: a re-evaluation using enclosed rotenone stations. Marine Ecology Progress Series 206:227–237
- Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, U.K.
- Bejarano I, Appeldoorn RS, Nemeth M (2014) Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico. Coral Reefs 33:313–328
- Bernard A, Götz A (2012) Bait increases the precision in count data from remote underwter video for most subtidal reef fish in the warm-temperate Agulhas bioregion. Marine Ecology Progress Series 471:235–252
- Bowman AW, Azzalini A (2014) R package “sm”: nonparametric smoothing methods.
- Box SJ, Canty SWJ (2011) The Long and Short Term Economic Drivers of Overexploitation in Honduran Coral Reef Fisheries Due to Their Dependence on Export Markets. Proceedings of the 63rd Gulf and Caribbean Fisheries Institute November 1 - 5, 2010 San Juan, Puerto Rico 63:43–51
- Chapman MR, Kramer DL (2000) Movements of Fishes Within and Among Fringing Coral Reefs in Barbados - Springer. Environ Biol Fish 57:11–24
- Cocheret de la Morinière E, Pollux B, Nagelkerken I, van der Velde G (2003) Diet shifts of Caribbean grunts (Haemulidae) and snappers (Lutjanidae) and the relation with nursery-to-coral reef migrations. Estuarine, Coastal and Shelf Science 57:1079–1089
- Cocheret de la Morinière E, Pollux BJA, Nagelkerken I, van der Velde G (2002) Post-settlement Life Cycle Migration Patterns and Habitat Preference of Coral Reef

Fish that use Seagrass and Mangrove Habitats as Nurseries. Estuarine, Coastal and Shelf Science 55:309–321

- Cole RG, Syms C, Davey NK, Gust N, Notman P, Stewart R, Radford CA, Carbines G, Carr MH, Jevs AG (2007) Does breathing apparatus affect fish counts and observations? A comparison at three New Zealand fished and protected areas. Marine Biology 150:1379–1395
- Doiron S, Weissenberger S (2014) Sustainable dive tourism: Social and environmental impacts—the case of Roatan, Honduras. Tourism Management Perspectives 10:19–26
- Edgar GJ, Barrett NS, Morton AJ (2004) Biases associated with the use of underwater visual census techniques to quantify the density and size-structure of fish populations. Journal of Experimental Marine Biology and Ecology 308:269–290
- English S, Wilkinson C, Baker V (1997) Survey manual for tropical marine resources. Australian Institute of Marine Sciences, Townsville
- Feddern HA (1965) The spawning, growth, and general behavior of the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). Bulletin of Marine Science 15:896–941
- Froese R, Pauly D (2015) FishBase. fishbase.org
- Gobert B, Berthou P, Lopez E, Lespagnol P, Turcios MDO, Macabiau C, Portillo P (2005) Early stages of snapper–grouper exploitation in the Caribbean (Bay Islands, Honduras). Fisheries Research 73:159–169
- Goetze JS, Jupiter SD, Langlois TJ, Wilson SK, Harvey ES, Bond T, Naisilisili W (2015) Diver operated video most accurately detects the impacts of fishing within periodically harvested closures. Journal of Experimental Marine Biology and Ecology 462:74–82

- Gotanda KM, Turgeon K, Kramer DL (2009) Body size and reserve protection affect flight initiation distance in parrotfishes. Behav Ecol Sociobiol 63:1563–1572
- Harasti D, Malcolm H, Gallen C, Coleman MA, Jordan A, Knott NA (2015) Appropriate set times to represent patterns of rocky reef fishes using baited video. Journal of Experimental Marine Biology and Ecology 463:173–180
- Harvey E, Fletcher D, Shortis MR, Kendrick GA (2004) A comparison of underwater visual distance estimates made by scuba divers and a stereo-video system: implications for underwater visual census of reef fish abundance. Mar. Freshwater Res. 55:573–580
- Harvey ES, Cappo M, Butler JJ, Hall N, Kendrick G (2007) Bait attraction affects the performance of remote underwater video stations in assessment of demersal fish community structure. Marine Ecology Progress Series 350:245–254
- Harvey ES, Dorman SR, Fitzpatrick C, Newman SJ, McLean DL (2012) Response of diurnal and nocturnal coral reef fish to protection from fishing: an assessment using baited remote underwater video. Coral Reefs 31:939–950
- Helfman GS, Meyer JL, McFarland WN (1982) The ontogeny of twilight migration patterns in grunts (Pisces: Haemulidae). Animal Behaviour 30:317–326
- Hinderstein LM, Marr JCA, Martinez FA, Dowgiallo MJ, Puglise KA, Pyle RL, Zawada DG, Appeldoorn R (2010) Theme section on “Mesophotic Coral Ecosystems: Characterization, Ecology, and Management.” Coral Reefs 29:247–251
- Holmes TH, Wilson SK, Travers MJ, Langlois TJ, Evans RD, Moore GI, Douglas RA, Shedrawi G, Harvey ES, Hickey K (2013) A comparison of visual- and stereo-video based fish community assessment methods in tropical and temperate marine waters of Western Australia. Limnol. Oceangr. Methods 11:337–350

- Januchowski-Hartley FA, Graham NAJ, Feary DA, Morove T, Cinner JE (2011) Fear of Fishers: Human Predation Explains Behavioral Changes in Coral Reef Fishes. PLoS ONE 6:e22761
- Januchowski-Hartley FA, Nash KL, Lawton RJ (2012) Influence of spear guns, dive gear and observers on estimating fish flight initiation distance on coral reefs. Marine Ecology Progress Series 469:113–119
- Kahng SE, Garcia-Sais JR, Spalding HL, Brokovich E, Wagner D, Weil E, Hinderstein L, Toonen RJ (2010) Community ecology of mesophotic coral reef ecosystems. Coral Reefs 29:255–275
- Kulbicki M (1998) How the acquired behaviour of commercial reef fishes may influence the results obtained from visual censuses. Journal of Experimental Marine Biology and Ecology 222:11–30
- Langlois T, Harvey E, Fitzpatrick B, Meeuwig J, Shedrawi G, Watson D (2010) Cost-efficient sampling of fish assemblages: comparison of baited video stations and diver video transects. Aquatic Biology 9:155–168
- Langlois TJ, Fitzpatrick BR, Fairclough DV, Wakefield CB, Hesp SA, McLean DL, Harvey ES, Meeuwig JJ (2012) Similarities between Line Fishing and Baited Stereo-Video Estimations of Length-Frequency: Novel Application of Kernel Density Estimates. PLoS ONE 7:e45973
- Lindfield SJ, Harvey ES, McIlwain JL, Halford AR (2014a) Silent fish surveys: bubble-free diving highlights inaccuracies associated with SCUBA-based surveys in heavily fished areas. Methods Ecol Evol 5:1061–1069
- Lindfield SJ, McIlwain JL, Harvey ES (2014b) Depth Refuge and the Impacts of SCUBA Spearfishing on Coral Reef Fishes. PLoS ONE 9:e92628
- Micheli F, Mumby PJ, Brumbaugh DR, Broad K, Dahlgren CP, Harborne AR, Holmes KE, Kappel CV, Litvin SY, Sanchirico JN (2014) High vulnerability of

ecosystem function and services to diversity loss in Caribbean coral reefs. Biol Conserv 171:186–194

- Mumby PJ (2009) Herbivory versus corallivory: are parrotfish good or bad for Caribbean coral reefs? Coral Reefs 28:683–690
- Oksanen J, Kindt R, Legendre P, OHara B, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) vegan: Community Ecology Package. cran.r-project.org
- Pelletier D, Leleu K, Mou-Tham G, Guillemot N, Chabanet P (2011) Comparison of visual census and high definition video transects for monitoring coral reef fish assemblages. Fisheries Research 107:84–93
- Priede IG, Bagley PM, Smith A, Creasey S, Merrett NR (1994) Scavenging deep demersal fishes of the Porcupine Seabight, north-east Atlantic: observations by baited camera, trap and trawl. Journal of the Marine Biological Association of the United Kingdom 74:481–498
- R Core Team (2013) R: A language and environment for statistical computing. R-project.org
- Roff G, Mumby PJ (2012) Global disparity in the resilience of coral reefs. Trends in Ecology & Evolution 27:404–413
- Sanderson SL, Solonsky AC (1986) Comparison of a rapid visual and a strip transect technique for censusing reef fish assemblages. Bulletin of Marine Science 39:
- Sheather SJ, Jones MC (1991) A Reliable Data-Based Bandwidth Selection Method for Kernel Density-Estimation. Journal of the Royal Statistical Society Series B-Methodological 53:683–690
- Sieber A, Pyle R (2010) A review of the use of closed-circuit rebreathers for scientific diving. Underwater Technology 29:73–78

- Taylor MD, Baker J, Suthers IM (2013) Tidal currents, sampling effort and baited remote underwater video (BRUV) surveys: Are we drawing the right conclusions? Fisheries Research 140:96–104
- Tessier A, Pastor J, Francour P, Saragoni G, Crec'hriou R, Lenfant P (2013) Video transects as a complement to underwater visual census to study reserve effect on fish assemblages. Aquatic Biology 18:229–241
- Thompson AA, Mapstone BD (1997) Observer effects and training in underwater visual surveys of reef fishes. Marine Ecology Progress Series 154:53–63
- Titus BM, Daly M, Exton DA (2015) Do Reef Fish Habituate to Diver Presence? Evidence from Two Reef Sites with Contrasting Historical Levels of SCUBA Intensity in the Bay Islands, Honduras. PLoS ONE 10:e0119645
- Wand M (2013) KernSmooth: Functions for kernel smoothing for Wand & Jones (1995).
- Watson DL, Harvey ES (2007) Behaviour of temperate and sub-tropical reef fishes towards a stationary SCUBA diver. Marine and Freshwater Behaviour and Physiology 40:85–103
- Watson DL, Harvey ES, Anderson MJ, Kendrick GA (2005) A comparison of temperate reef fish assemblages recorded by three underwater stereo-video techniques. Marine Biology 148:415–425
- Watson DL, Harvey ES, Fitzpatrick BM, Langlois TJ, Shedrawi G (2010) Assessing reef fish assemblage structure: how do different stereo-video techniques compare? Marine Biology 157:1237–1250
- Willis TJ, Babcock RC (2000) A baited underwater video system for the determination of relative density of carnivorous reef fish. Mar. Freshwater Res. 51:755–763

5 WARINESS OF REEF FISH TO PASSIVE DIVER PRESENCE WITH VARYING DIVE GEAR TYPE ACROSS A SHALLOW TO MESOPHOTIC DEPTH GRADIENT

5.1 Abstract

Both active and passive human interactions with reef fish communities are increasingly recognized to cause fish behavioural changes. However, few studies have considered how these behavioural adaptations impact standard reef survey techniques, particularly across natural gradients of interest to ecologists and reef managers. Here we measure abundance and minimum approach distance at the family level using stereo-video surveys to compare the effects of bubble-producing open-circuit SCUBA versus silent closed-circuit rebreathers. Surveys extended across a shallow-mesophotic gradient on the fringing reefs of Utila, Honduras, to explore how the effects of diver presence also vary with depth. For most fish families we recorded similar abundances with the two diving techniques, suggesting that open-circuit transects are generally appropriate for surveying in western Atlantic reefs. Despite no overall difference in recorded fish abundance, we identified many fish families (Carangidae, Labridae, Pomacentridae, Scaridae) that allowed closed-circuit rebreather divers to approach more closely than open-circuit divers. Smaller fish generally allowed divers to approach more closely than larger fish. In addition, the detected effects in diver approach distance for Haemulidae, Mullidae and Serraninae interacted with depth, with larger fish allowing divers to approach less closely at mesophotic depths than shallower depths. Our findings stress the importance of considering variation in fish behavioural adaptations caused by different levels of historical human exposure along natural gradients such as depth, which otherwise may lead to risk of biases when surveying by traditional monitoring programs.

Chapter 5: Wariness of reef fish to passive diver presence with varying dive gear type across a shallow to mesophotic depth gradient

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5.2 Introduction

Many studies have assessed the effectiveness of marine protected areas, including different management forms such as no-take zones and partial protection, generally finding that marine protected areas are effective in maintaining fish density and biomass (Sciberras et al. 2013). However, assessments rarely consider whether varying fish behaviours across the study area may bias their survey techniques. Fish behaviour is known to be impacted by previous exposure to humans (Januchowski-Hartley et al. 2015), yet locations with direct human interactions with the marine environment tend to be those reef managers are most interested in assessing. Even on a local scale, the exposure of fish communities to these effects can be highly variable along natural gradients such as depth. With much recent interest in mesophotic coral ecosystems (MCEs; reefs 30-150 m depth) acting as refuges from fishing (Bejarano et al. 2014; Lindfield et al. 2015), gaining a better understanding of fish behavioural survey biases across depth gradients is crucial.

While bias in some form is an unavoidable symptom of all survey methods, stakeholders rely heavily on data pertaining to fish populations and their responses to management interventions to inform decision-making. On tropical coral reefs this tends to involve baseline fish community data collected using underwater visual census (UVC) by surveyors in the water (English et al. 1997). To conduct UVC open-circuit (OC) SCUBA divers swim along a fixed-length transect recording individuals of all (or target) fish species, additionally estimating lengths in some cases (English et al. 1997). As a result of concerns about repeatability between surveyors due to observer bias (Thompson and Mapstone 1997), video surveys have begun to replace in-water observations for many surveys (Mallet and Pelletier 2014). While the use of video removes many errors associated with in-water data collection, the differing response behaviours of reef fish to diver presence raises new concerns (Chapman et al. 1974; Cole 1994; Watson and Harvey 2007). The operation and bubble release of traditional OC SCUBA generates high levels of noise at the low frequencies fish are most sensitive to (Radford et al. 2005). This has led to the suggestion fish may be able to audibly detect diver presence before divers can visually identify fish (Radford et al. 2005).

Despite these known effects, few UVC studies acknowledge the bias that OC SCUBA is likely to cause to their results (Dickens et al. 2011), instead most biases are assumed to be consistent across survey sites and thus mitigated. However, in areas with regular spearfishing, flight initiation distance (FID; the minimum distance a diver can approach a fish before it flees) of many fish families have repeatedly been found to be higher than in protected areas (Gotanda et al. 2009; Januchowski-Hartley et al. 2011; 2014). Therefore, despite standardised methodologies, varying fishing pressure may bias survey results and artificially inflate the apparent effectiveness of marine protected areas (Lindfield et al. 2014a). Even within protected areas, other effects may significantly bias results, for example fish habituation to the presence of divers in areas with intensive dive tourism (Titus et al. 2015), which can be enhanced when divers feed fish populations (Cole 1994), or the presence of fish ontogenetic migrations, with more mature and thus larger individuals found at greater depths (Grol et al. 2014). Many fish families exhibit greater FID in larger individuals than smaller individuals (Gotanda et al. 2009; Januchowski-Hartley et al. 2011), suggesting for species with well-defined ontogenetic migrations, divers might be able to approach individuals more closely on shallow reefs than deeper reefs.

Fishing pressure in many locations is highly depth biased, being generally most intense at shallower depths (Lindfield et al. 2014b). The nature of artisanal fisheries means fish tend to be harvested at shallow depths using simple gear types before depletion forces fishers deeper or technology improves access. For example, in Hawaii when collecting live fish for the marine aquarium trade, fishers move to deeper reefs when shallow juvenile recruitment is perceived as weak (Stevenson et al. 2011). In the southern Mariana Islands, which has had a historic shallow-reef freediving spear fishery, following the introduction of SCUBA, the mean length of Scaridae and Acanthuridae caught by SCUBA spear fishers was initially higher but rapidly declined (Lindfield et al. 2014b). This suggests SCUBA facilitated access to and exploitation of a depth refuge. There is increasing evidence that MCEs may act as depth refuges in historically heavily-fished areas (Bejarano et al. 2014; Lindfield et al. 2015). Despite potential refuge effects, MCEs are themselves threatened and require integration into marine protected areas (Chapter 9).

The effects of recreational dive tourism are also highly depth biased, being typically limited to a maximum depth of 30 m, and in reality generally much shallower because of training limitations and no-decompression limits. This subsequently skews the opportunity for fish habituation to OC divers towards shallower reefs. Even when surveying across depth gradients, the increased distance for bubbles to travel to the surface when completing deeper transects is likely to produce greater total noise (Radford et al. 2005), and thus impacts on fish behaviour, than shallower transects, leading to unintended bias in the data. Recent advances have made technical diving more accessible, including the emergence of commercially available closed-circuit rebreathers (CCR). CCR systems recycle gas rather than releasing bubbles into the water column, see Sieber and Pyle (2010) for a detailed system overview, and are therefore significantly quieter than their OC counterparts. When it comes to their impact on fish behaviour, fish can detect the sounds of OC divers >200 m away over a range of typical background noise levels, while CCR divers can only be detected between 0.3-15.9 m away depending on background underwater noise (Radford et al. 2005).

It therefore seems likely that some patterns detected in fish surveys using OC divers across depth gradients may be influenced by fish behavioural biases that change with depth. Yet no studies have directly investigated how detection bias in OC SCUBA changes across the depth gradient. To investigate this question we conducted fish community assessments across a shallow to mesophotic reef gradient at three sites in the Bay Islands Marine Park, Utila, Honduras using OC SCUBA and CCR. By conducting surveys using CCR, fish disturbance effects caused by bubbles and sounds of OC SCUBA regulators were absent, reducing these biases on fish community assessment. We test whether any differences observed between methods is affected by depth, though with differing effects depending on whether a fish family is commercially exploited. As the shallow reefs of Utila are still exposed to occasional fishing, we expect commercially fished Lutjanidae and Serranidae species will be more wary of OC divers at shallow depths than on MCEs. For non-commercially fished families, mesophotic populations are likely to be less habituated to diver presence than shallow reef fish, suggesting that individuals could be more wary of OC divers on MCEs than shallow reefs.

5.3 Materials and Methods

5.3.1 Study sites

Surveys were conducted on the south shore of Utila, Bay Islands, Honduras (Figure 5.1). Utila is located approximately 29 km off the Caribbean coast of Honduras, forming the southern extent of the Mesoamerican Barrier Reef (Harborne et al. 2001). The majority of reef fishing is carried out by handlines targeting Lutjanidae and Serranidae (Box and Canty 2011). Historically fishers carried spears to opportunistically shoot large fish they encountered, however, the island’s fringing reefs have been absent of spearfishing for almost 20 years (Kramer et al. 2015). Recently tourism has replaced fishing as the dominant source of income (Cronk and Steadman 2002; Doiron and Weissenberger 2014), primarily consisting of recreational diving (Doiron and Weissenberger 2014), with >10 dive centres operational on Utila and tens of thousands of recreational dives completed annually.

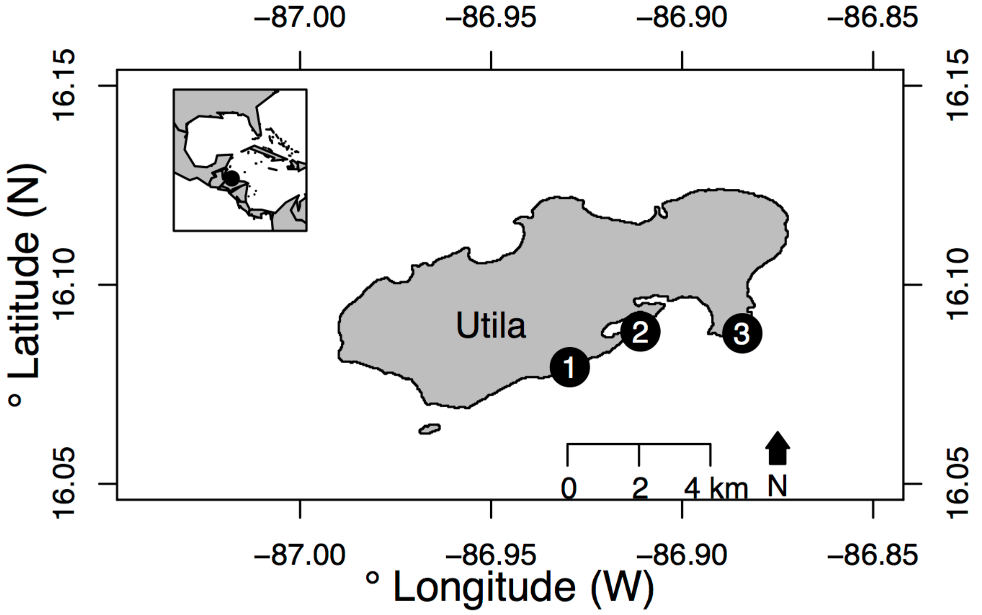

Figure 5.1 Map of Utila with the three survey sites marked.

Sites were: (1) Little Bight, (2) Coral View and (3) Rocky Point. Inset map indicates location of Utila relative to the Western Atlantic region.

5.3.2 Video surveys of fish communities

Fish community surveys were conducted along 50 m transects using a diver operated stereo-video system (SVS), made up of two Canon HFS21 video cameras (see: Chapter 2 for more detailed camera specifications). By recording with two cameras simultaneously, SVS allows post-dive analysis of fish abundance and accurate measurements of body length and distance from camera, all within automatically defined transect boundaries. Four replicate transects separated by 10 m were conducted following the reef contour at four depths (5, 15, 25, 40 m) at three sites (Figure 5.1). Each transect was surveyed twice on different days by an SVS operator using two different dive gear types, once using OC SCUBA equipment and once using CCR with the order randomised for each transect. All transects were conducted during daytime between the hours of 8 am and 4 pm. Transects were surveyed by an SVS operator followed by a second diver a several meters behind laying a transect tape to measure distance. The second diver normally used the same dive gear as the SVS operator, though because of dive scheduling logistics this was not always possible, however, they never used OC SCUBA while the SVS operator was conducting CCR transects. To minimise disturbance to the fish community prior to recording, a 60 m transect tape was used, with the cameras set recording and synchronised with a hand torch before swimming 10 m along the reef with the cameras filming directly downwards below the diver. After the SVS operator had swum 10 m, the transect diver signalled for them to lift the cameras and begin the transect proper, signalling again once the full 50 m had been swum. Cameras were held to film along the reef following the depth contour. Each transect took approximately 3 min to film. CCR surveys were conducted using a mixture of Hollis Prism 2 (Hollis, San Leandro, CA, USA), rEvo X micro (rEvo rebreathers, Bruges, Belgium), or Sentinel (Vobster Marine Systems, Somerset, UK) rebreathers.

5.3.3 Video analysis

Transect videos were blinded to survey method and analysed with EventMeasure software v3.51 (SeaGIS, Melbourne, Australia). Transect boundaries were defined as 2.5 m either side of the transect giving a 5 x 50 m survey area for each transect. All fish were identified to species level or the highest taxonomic level possible if not identifiable to species. If visible on both cameras, fish length and distance from cameras was recorded using the built in measurement tools in EventMeasure. Measurements were taken when each individual fish was at its closest to the SVS, thus recording the minimum approach distance (MAD; the minimum distance the SVS operator could approach the fish before it moved away). Where MADs were below the minimum distance needed to appear simultaneously on both cameras, we recorded the fish at the closest point while visible on both cameras. Where fish appeared on the transect but were never visible on both cameras simultaneously, fish lengths and MAD were unable to be recorded and thus these were only included in abundance data. Data analyses were conducted at the family level. However, because of the intense historical large bodied grouper fishery around Utila we split the Serranidae into three groups by removing the Serraninae and Grammistinae sub-families for separate analysis, and thus only leaving larger bodied grouper species of commercial value in the

Serranidae group we analyse here. These sub-families contain small-bodied fish species that have not been targeted by fisheries around Utila (Gobert et al. 2005; Box and Canty 2011),

5.3.4 Abundance analysis

As a result of the non-normal nature of fish abundance data we used permutational multivariate analyses of variance (PERMANOVA) because of their lack of assumptions about the distribution of the data (Anderson et al. 2008). A univariate PERMANOVA was run for each fish family individually based on a Euclidian dissimilarity matrix constructed with the abundance data, testing for differences between sites, depth and the site:depth interaction. Transects where a fish family was not recorded by either CCR or OC were removed from the analysis so that identical abundance records for both techniques on a transect represent the same number of individuals recorded and not common absence. As many fish families did not appear on transects we restricted analysis to fish families that appeared on >20 transects across all sites and depths. All PERMANOVAs were conducted in R (R Core Team 2013) using the ‘adonis’ function in the package vegan (Oksanen et al. 2013) and run for 99999 permutations.

5.3.5 Minimum Approach Distance (MAD) analysis

To investigate MAD for each fish family we used analysis of covariance (ANCOVA) with four explanatory variables: site and survey method as factors and depth and fish length as continuous variables. To meet normality and homogeneity assumptions we natural log-transformed raw MAD data. We also tested for interactions between survey method, depth and fish length on MAD. Models were fitted in R (R Core Team 2013) and the function ‘step’ was used to simplify models starting with the full model with all interactions, and iteratively removing one variable or interaction at a time from the model starting with the most complex. If removing a variable or interaction resulted in a lower model Akaike information criterion (AIC) the variable or interaction was dropped, if not it was replaced and another variable or interaction tested. Where significant two-way interactions were identified we examined them while controlling for the other explanatory variables by following a partial correlation approach. This involved plotting relationships between the centred residuals from linear models of the variables of interest with the other explanatory variables. During model checking two *Lachnolaimus maximus* records were removed as they had an undue influence on the fitted model. *Lachnolaimus maximus* is a large-bodied Labridae species and these two records, the only times we recorded this species, were both of individual fish substantially larger than any other Labridae in our dataset. With these two individuals removed the remaining 379 Labridae MAD and length measurements met the model assumptions.

5.4 Results

5.4.1 Abundance

Median fish abundance was greater for CCR transects than OC at all surveyed depths (Figure 5.2), however there was no significant effect of survey method on total fish abundance per transect (PERMANOVA, Pseudo-F=2.17, p=0.08). However, we found abundance differences between CCR and OC when considering specific fish families. Method effects were recorded for Acanthuridae and Tetraodontidae and significant method:depth interactions for large bodied Serranidae, Sparidae and Tetraodontidae across the shallow to mesophotic depth gradient (Table 5.1). Acanthuridae median abundance per transect was similar between methods (Figure 5.3a), but shallow (5 m) OC transects detected several large schools of *Acanthurus coeruleus* increasing the mean abundance estimates from 1.83 ±0.58 per 250 m^2 for CCR to 6.67 ±5.05 per 250 m^2 for OC (Mean ± SE). Approximately equal abundances of large-bodied Serranidae were recorded by both methods at 5 and 15 m, with more individuals recorded by OC at 25 and 40 m. In a similar way, greater abundance of Sparidae was recorded on shallow reefs (5 and 15 m) by CCR than OC, with this pattern reversed at depth and greater abundance recorded by OC at 40 m

(Figure 5.3c). Tetraodontidae displayed similar abundance recorded by OC and CCR in the shallows, but greater abundance recorded by CCR at 25 and 40 m (Figure 5.3d). The majority of fish families tested did not show any effect of survey method on abundance, though many exhibited effects of depth and survey site on abundance (Table 5.1). No difference in abundance between the two methods was detected for Chaetodontidae, Haemulidae, Lutjanidae, Pomacanthidae, Pomacentridae and Scaridae (Table 5.1).

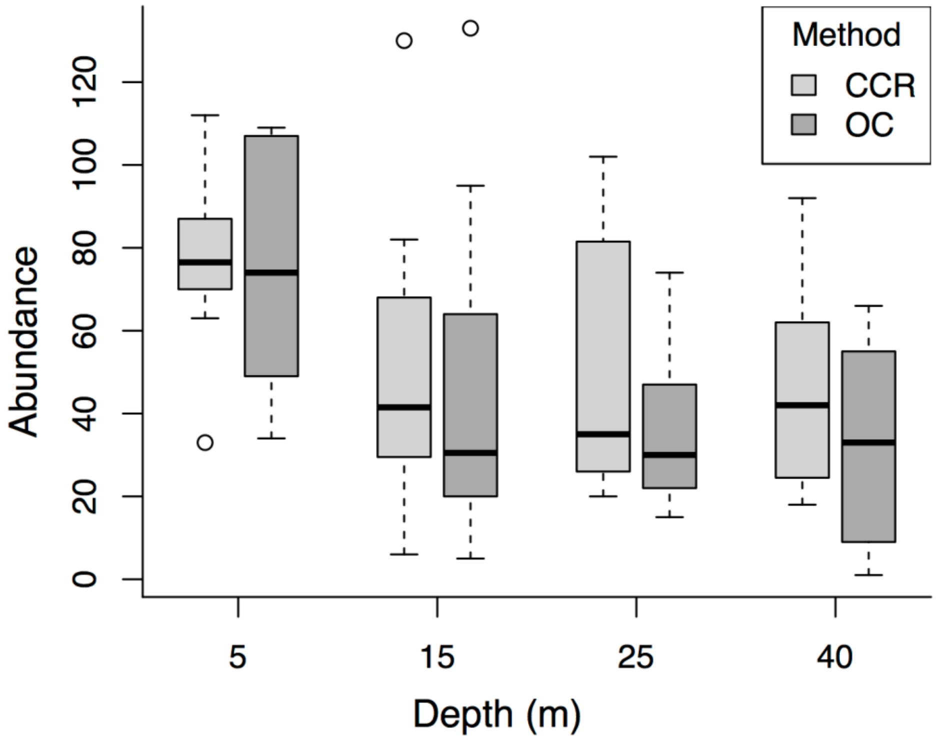

Figure 5.2 Comparison of the two survey methods across the depth gradient for total fish abundance per transect for all sites. Boxes represent inter-quartile range with the median (dark bar) marked.

Table 5.1 Euclidian PERMANOVA results for difference in abundance recorded on the same transect for each fish family by OC and CCR. Only fish families recorded on >20 out of the 48 transects are shown.

	DF	**MS**	**Pseudo-F**	**P(perm)**
Acanthuridae				
site	2	31.76	0.63	0.063
method	1	160.51	3.18	0.041
depth	1	191.43	3.79	0.067
method:depth	1	46.44	0.92	0.384
Residuals	64	50.45	0.87	
Total	69	1.00		
Chaetodontidae				
site	2	0.23	0.25	0.732
method	1	0.17	0.18	0.691
depth	1	0.85	0.90	0.353
method:depth	1	0.20	0.21	0.648
Residuals	48	0.94	0.96	
Total	53	1.00		
Haemulidae				
site	2	23.82	3.16	0.034
method	1	3.91	0.52	0.491
depth	1	30.96	4.11	0.031
method:depth	1	25.78	3.42	0.053
Residuals	68	7.54	0.83	
Total	73	1.00		
Labridae				
site	2	1029.87	6.53	0.007
method	1	24.54	0.16	0.698
depth	1	1986.09	12.59	<0.001
method:depth	1	20.08	0.13	0.726
Residuals	84	157.76	0.76	
Total	89	1.00		
Lutjanidae				
site	2	0.10	0.02	0.453
method	1	1.01	0.24	0.615
depth	1	10.08	2.39	0.128
method:depth	1	0.14	0.03	0.856
Residuals	74	4.22	0.96	
Total	79	1.00		
Pomacanthidae				
site	2	3.52	2.37	0.772
method	1	0.94	0.63	0.487
depth	1	0.23	0.15	0.694
method:depth	1	0.46	0.31	0.572

Residuals	46	1.49	0.89	
Total	51	1.00		
Pomacentridae				
site	2	548.60	4.33	<0.001
method	1	182.00	1.44	0.233
depth	1	11189.60	88.27	<0.001
method:depth	1	452.90	3.57	0.060
Residuals	84	126.80	0.45	
Total	89	1.00		
Scaridae				
site	2	152.32	13.74	<0.001
method	1	4.44	0.40	0.523
depth	1	276.18	24.92	<0.001
method:depth	1	1.05	0.09	0.763
Residuals	84	11.08	0.61	
Total	89	1.00		
Serranidae				
site	2	3.00	2.15	0.014
method	1	3.31	2.38	0.123
depth	1	1.67	1.20	0.272
method:depth	1	9.80	7.04	0.007
Residuals	62	1.39	0.81	
Total	67	1.00		
Sparidae				
site	2	1.03	1.23	0.032
method	1	0.02	0.03	0.776
depth	1	1.01	1.21	0.264
method:depth	1	6.38	7.65	0.005
Residuals	38	0.83	0.77	
Total	43	1.00		
Tetraodontidae				
site	2	1020.75	11.91	<0.001
method	1	1917.39	22.36	<0.001
depth	1	1867.40	21.78	<0.001
method:depth	1	1326.63	15.47	<0.001
Residuals	86	85.74	0.51	
Total	91	1.00		

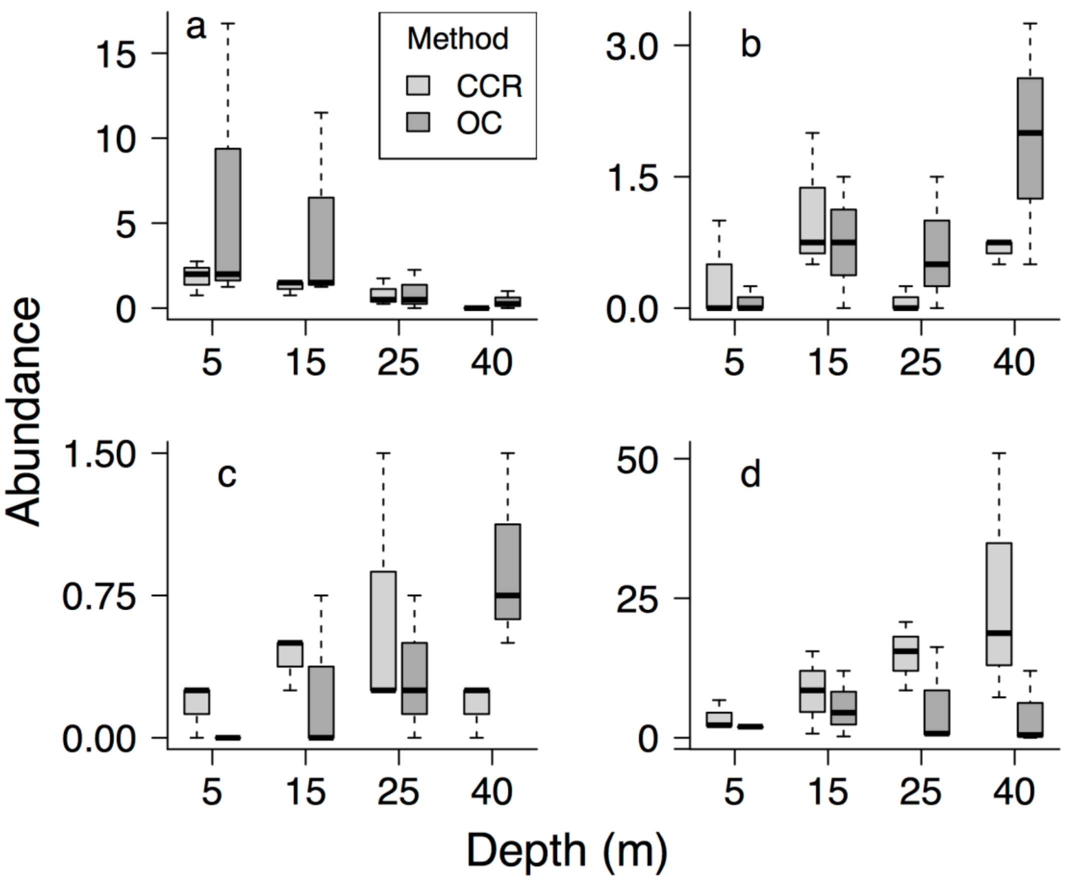

Figure 5.3 Fish family mean abundance at each site recorded by CCR (light grey) and OC (dark grey) across the depth gradient. Fish families are (a) Acanthuridae, (b) Serranidae, (c) Sparidae and (d) Tetradontidae.

5.4.2 Minimum approach distance (MAD)

Across all fish families and depths we recorded smaller mean MAD scores with CCR (2.51 m) than OC (2.71 m), representing CCR divers being able to get 7.4 % closer to fish than OC divers (paired t test, t=-2.57, df=46, p=0.01). Despite this broad pattern, some fish families exhibited different MAD patterns in relation to survey method, depth and individual fish length. MAD was greater for OC for Carangidae, Labridae, Pomacentridae and Scaridae, meaning individual fish could be more closely approached 12 %, 10 %, 6 % and 9 % respectively by divers when using CCR (Table 5.2). The sub-family Serraninae showed the opposite pattern, with divers able to approach 31 % closer on OC than CCR (Table 5.2). Interactions between method and depth were also found for Labridae, with divers able to approach fish 15 % more closely on CCR than OC at 5 and 15 m. However, this difference decreased as depth increased, with little difference (<1 %) between methods at 25 and 40 m (Figure 5.4a). Many fish families did not show a difference in MAD between the two survey methods, including Acanthuridae and Tetraodontidae (Table 5.2), despite exhibiting differences in abundance between techniques (Table 5.1). The two commercially fished families, Lutjanidae and larger bodied Serranidae, exhibited no effects of method on MAD, though for both families MAD decreased with depth (Table 5.2), resulting in divers more closely able to approach individuals 24 % and 17 % respectively at 25 and 40 m than 5 and 15 m.

Many families were observed to have higher MADs for larger individuals, including Acanthuridae, Labridae, Pomacentridae and Scaridae, meaning larger fish allowed divers to approach them less closely than smaller fish (Table 5.2). Haemulidae, Mullidae and Serraninae all had significant depth and length interactions, though Serraninae was only recorded at 25 m and 40 m depth (Table 5.2). When visualised, these depth:length interactions showed the greatest effect of fish length on MAD at mesophotic depths, with less effect in the shallows (Figure 5.5). However, regardless of depth all showed a positive relationship between fish length and MAD. This suggests that for these families larger individuals are more tolerant of allowing divers closer on shallow reefs than they are at mesophotic depths.

We also detected interactions between fish length and survey method for Labridae and Pomacentridae (Table 5.2), with small individuals appearing more wary of OC divers than CCR divers, whereas larger individuals show less difference between the two methods (Figure 5.4b-c).

Table 5.2 ANCOVA model results for log minimum approach distance (MAD) for fish families following simplification based on model AIC.

Only fish families that >40 individual fish range measurements were made for are shown. Depth and length estimates represent the slope of the fitted regression line. Method estimates represent the change in MAD associated with a switch from CCR to OC.

Family/term	DF	MS	F	P	Estimate
Acanthuridae					
Site	2	1.20	7.48	0.001	
Depth	1	0.03	0.21	0.646	0.0144
Method	1	0.37	2.31	0.133	0.1515
Length	1	1.18	7.36	0.008	0.0046
Depth:Length	1	0.43	2.66	0.107	-0.0001
Residuals	71	0.16			
Carangidae					
Site	2	2.59	19.98	<0.001	
Depth	1	0.16	1.25	0.271	0.0709
Method	1	1.25	9.63	0.004	0.0795
Length	1	0.41	3.13	0.086	0.3180
Depth:Length	1	0.44	3.40	0.074	0.1107
Method:Length	1	0.24	1.83	0.185	0.1847
Residuals	34	0.13			
Haemulidae					
Depth	1	1.52	14.84	<0.001	-0.0217
Length	1	0.00	0.03	0.875	-0.0018
Depth:Length	1	0.43	4.23	0.042	0.0001
Residuals	114	0.10			
Labridae					
Site	2	0.42	3.97	0.020	
Depth	1	5.58	52.28	<0.001	-0.0060
Method	1	1.79	16.82	<0.001	0.5437
Length	1	3.51	32.92	<0.001	0.0033
Depth:Method	1	0.52	4.85	0.028	-0.0181
Depth:Length	1	0.03	0.31	0.580	0.0000
Method:Length	1	0.76	7.12	0.008	-0.0052
Depth:Method:Length	1	0.71	6.68	0.010	0.0002
Residuals	363	0.11			
Lutjanidae					
Site	2	0.91	9.61	<0.001	
Depth	1	1.01	10.68	0.001	-0.0079
Residuals	109	0.09			
Mullidae					
Depth	1	0.31	2.91	0.094	-0.0403

Method	1	0.05	0.48	0.493	0.1267
Length	1	0.21	1.95	0.169	-0.0052
Depth:Length	1	0.99	9.39	0.003	0.0002
Residuals	55	0.11			
Pomacentridae					
Site	2	0.55	4.71	0.009	
Depth	1	2.31	19.81	<0.001	-0.0143
Method	1	1.56	13.37	<0.001	0.3006
Length	1	1.62	13.93	<0.001	0.0013
Depth:Length	1	0.41	3.52	0.061	0.0001
Method:Length	1	1.33	11.41	0.001	-0.0032
Residuals	751	0.12			
Scaridae					
Site	2	0.44	3.50	0.032	
Depth	1	0.70	5.58	0.019	-0.0047
Method	1	1.00	7.96	0.005	0.1349
Length	1	3.98	31.84	<0.001	0.0015
Residuals	287	0.13			
Serranidae					
Site	2	0.07	0.95	0.396	
Depth	1	0.57	8.21	0.007	-0.0119
Method	1	0.00	0.04	0.844	-0.2067
Length	1	0.00	0.03	0.869	-0.0007
Method:Length	1	0.18	2.59	0.116	0.0017
Residuals	37	0.07			
Serraninae					
Depth	1	0.19	1.99	0.163	-0.0231
Method	1	0.87	8.92	0.004	-0.4320
Length	1	0.21	2.18	0.144	-0.0202
Depth:Length	1	0.39	4.02	0.049	0.0007
Method:Length	1	0.38	3.86	0.054	0.0046
Residuals	66	0.10			
Tetraodontidae					
Depth	1	1.00	11.53	0.001	-0.0046
Method	1	0.18	2.07	0.151	-0.1204
Length	1	0.18	2.10	0.148	-0.0044
Method:Length	1	0.29	3.31	0.070	0.0048
Residuals	291	0.09			

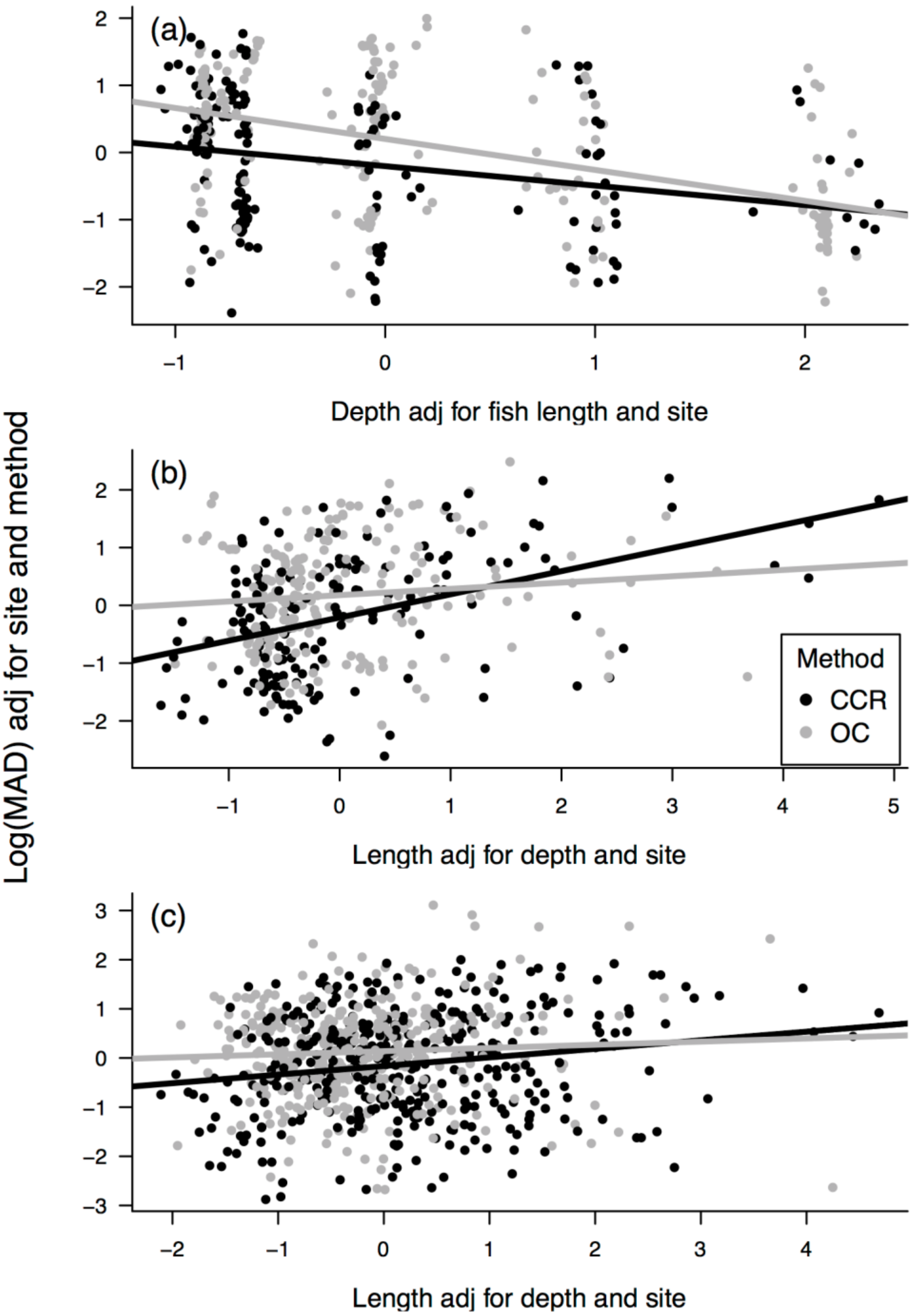

Figure 5.4 Visualisation of the depth:method interaction for (a) Labridae and length:method interactions for (b) Labridae and (c) Pomacentridae.

Minimum approach distance and fish length and depth have been standardised for the effects of site and depth/fish length then centred.

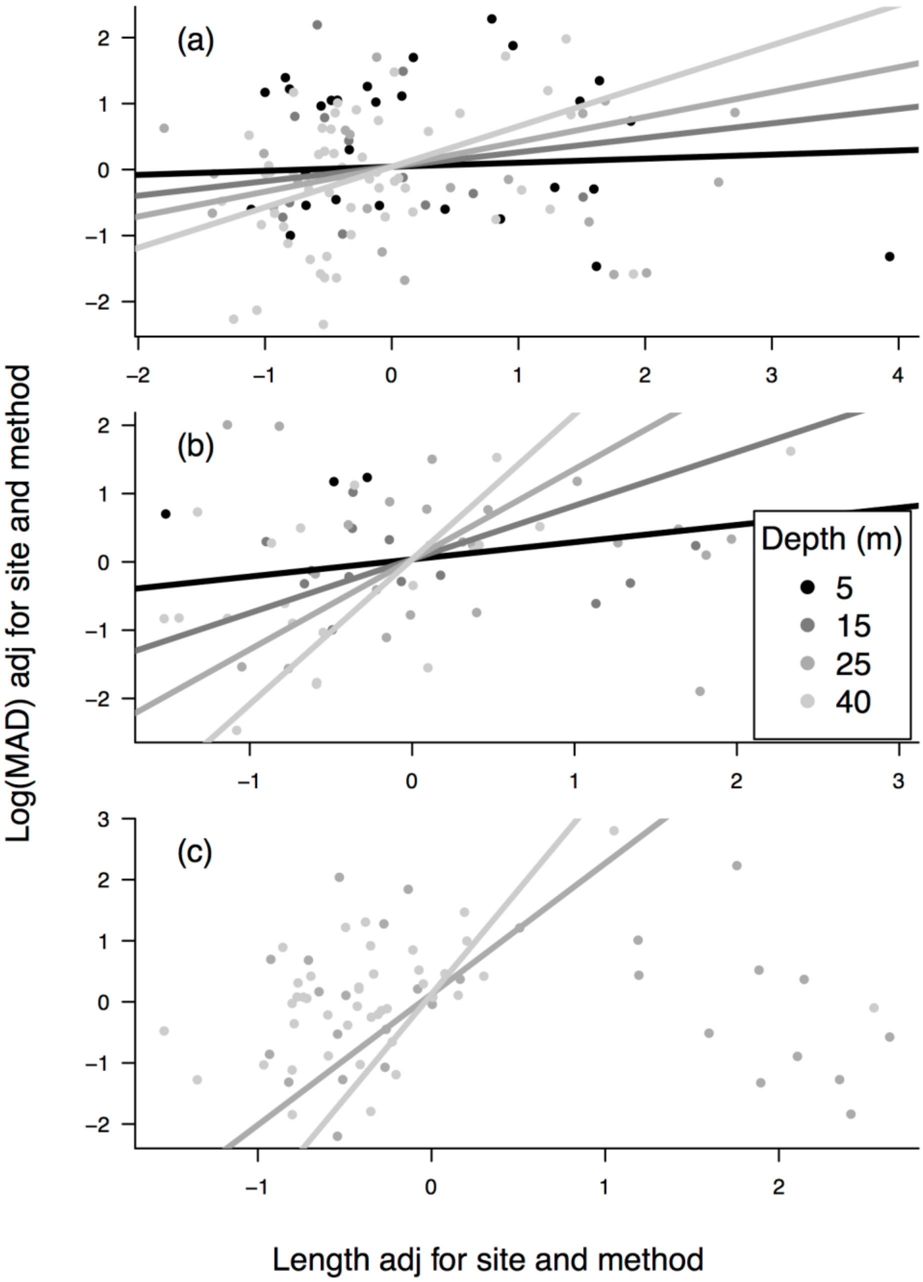

Figure 5.5 Visualising the depth:length interaction for (a) Haemulidae, (b) Mullidae and (c) Serraninae.

Plots show fish length against minimum approach distance with the fitted model for different depth bands. Length and minimum approach distance have been adjusted for effects of site and survey method and the data standardised.

5.5 Discussion

5.5.1 Detectability variation between OC and CCR

Of the 11 families encountered on more than 20 transects, only four showed variations in overall abundance between OC and CCR, and we found no difference in total fish abundance between the two methods. This suggests generally OC SCUBA surveys are appropriate for reef fish community monitoring on Utila. While we did detect some differences between OC SCUBA and CCR techniques, particularly in minimum approach distance (MAD), our results suggest these differences are unlikely to undermine typical reef monitoring programs. Of the differences in abundance we identified between techniques: large-bodied Serranidae and Sparidae had similar or greater abundance on CCR surveys than OC surveys on shallow reefs (5 and 15 m), but this pattern reversed at 40 m where OC detected greater abundances than CCR. Tetraodontidae were recorded at similar numbers on shallow reefs by both methods, but with CCR recording increasingly greater abundances than OC at greater depths. Acanthuridae were recorded at greater abundance on OC than CCR at all depths. To our knowledge, only one study has previously compared fish surveys between OC and CCR, conducted in Micronesia and demonstrating a clear negative effect of OC on recorded fish biomass in areas with spearfishing (Lindfield et al. 2014a). Our data suggest many fish families around Utila are not sufficiently affected by OC divers as to influence abundance surveys.

Despite no significant differences in abundance between the two techniques for all but four families, we did detect the effect of technique on MAD when grouping all fish families, and for five of the 11 families we tested individually. This leads us to conclude that OC does causes direct effects on fish communities, through driving active avoidance or attraction behavioural responses in some key fish families. This agrees with previous research demonstrating increased OC diver avoidance in areas with spearfishing pressure (Gotanda et al. 2009; Januchowski-Hartley et al. 2011), and in response to passive diver presence (Watson and Harvey 2007). Most in-water diver surveys of shallow-reef communities are conducted by OC (English et al. 1997), while those on MCEs are generally conducted by CCR (Bejarano et al. 2014; Pinheiro et al. 2016), potentially raising concerns over direct depth comparisons. This, combined with our own data on MAD changes with depth, reinforces the need to account for differing behavioural responses before comparing community data collected by different dive techniques.

5.5.2 Commercially targeted species

Utilan reef fisheries have historically been focused on two reef fish families, the Lutjanidae and Serranidae (Box and Canty 2011). After removing the smaller-bodied sub families Serraninae and Grammistinae, larger-bodied Serranidae were recorded at greater abundances using CCR on shallow reefs (5 and 15 m), and using OC on MCEs. However, we found no difference in Lutjanidae abundance between the two survey techniques at any depth. This suggests that at shallow depths Serranidae exhibit an avoidance response to OC divers, whereas below the range of most dive tourists and historical spearfishing they are less fearful of, and potentially even attracted to, divers. Lindfield et al. (2014a) observed fish species targeted by spearfishers avoid OC divers, and this was significant enough to reduce abundance estimates compared with surveys conducted in the same location using CCR. However in contrast, Feary et al. (2011) compared areas with intensive spearfishing to those that were protected in Papua New Guinea. They found that while fish were significantly more wary of divers in spearfished areas, these differences were not enough to alter abundance estimates. They estimated that differences in estimated abundance would only occur if visibility was reduced to below 6 m (Feary et al. 2011). These patterns appear consistent with our observed results, with similar abundances recorded for many species regardless of survey technique, yet differences in MAD detected. While large scale spearfishing no longer occurs on Utilan fringing reefs, occasional fishing by other techniques does occur, so this light fishery could be influencing the recorded Serranidae abundance. Previous research has identified that large carnivorous reef fish can be attracted to divers (Cole 1994), particularly in areas where divers are likely to have been feeding fish. Occasional fish feeding has been conducted around Utila by divers, but it is not known to regularly occur. This suggests, for Serranidae, the predation risk from the fishery could be overcoming habituation effects from regular divers in the water.

For both Serranidae and Lutjanidae we detected a decrease in minimum approach distance (MAD) with depth, suggesting regardless of survey technique divers could approach individual fish more closely on MCEs than shallow reefs. Previous studies of fish responses to divers have highlighted species-specific effects (Watson and Harvey 2007), so patterns could be explained by species turn-over with depth within the families. However, we believe the differences we identified represent genuine differences in Serranidae and Lutjanidae behaviour across the depth gradient as there has been little species turn-over reported in these families with depth. Andradi-Brown et al (Chapter 2) studied fish communities across shallow to MCE gradients on Utila, including at two of our study sites, finding that no Serranidae and only one Lutjanidae species significantly changed in relative community biomass across the 5-40 m depth gradient. Several previous studies have looked at changes in flight initiation distance (FID), the minimum distance that a diver can approach a fish before it actively swims away, finding that for targeted fish species FID increased following the introduction of fishing (Gotanda et al. 2009; Januchowski-Hartley et al. 2011), though most studies have focused specifically on spearfishing. Our results are surprising, as no regular spearfishing has been reported on the shallow reefs of Utila for close to two decades (Kramer et al. 2015). Although it has been suggested that, in the absence of regular spearfishing, individual fish that have previously experienced hunting by spearfishers are likely to flee in response to future encounters with divers, regardless of whether they are spearfishers or tourists (Januchowski-Hartley et al. 2015), historical spearfishing on Utila likely falls beyond the lifespan of most reef fish. However, Januchowski-Hartley et al (2015) suggested this could potentially influence behaviour of conspecifics, as it has been demonstrated that reef fish are capable of learning predator recognition from conspecific responses to predator stimuli (Manassa and McCormick 2012). Therefore on shallow reefs it is possible the high number of tourist divers may help maintain a diver avoidance behavioural response even in the absence of continued spearfishing. In addition, while spearfishing has been absent from Utila for close to two decades as a broad multiple-species fishery, extensive hand spearing is regularly conducted at our survey sites for invasive lionfish (Chapter 7). The presence of regular in-water lionfish culling by divers potentially could act as predator stimuli to reef fish in general. This is far from clear, as fish have been shown to habituate to diver presence on the reefs of Utila (Titus et al. 2015), and studies in other taxa suggest that in the absence of targeted spearfishing we would quickly expect these habituation effects to lead to reduced MADs (Cooper and Whiting 2007).

Although we detected a difference in Serranidae abundance between OC and CCR we detected no effect of survey method on Serranidae MAD. Because of the difference in abundance recorded between techniques for Serranidae we would expect this to be reflected in differing approach distances between methods. However, our results could also be explained if Serranidae exhibit strong avoidance effects to OC in the shallows such that we were unable to film them on video transects, causing the observed difference in abundance, but preventing us from measuring their MAD and so excluding these individuals from MAD analysis.

5.5.3 Non-commercially targeted species

We found few differences in abundance between OC and CCR for non-commercially targeted fish species. The three exceptions were Acanthurdiae, Sparidae and Tetraodontidae. This lack of major differences between survey methods for many fish families' abundance was surprising, as it is in contrast to previous studies such as Watson and Harvey (2007). However, these effects were calculated by fish point counts using a static camera system with an OC diver either present or absent. Habituation to OC divers has been recorded in reef fish on Utilan fringing reefs, though fish still exhibited diminished behaviours compared to surveys without divers present (Titus et al. 2015). Previous studies have also found few differences when comparing fish communities surveyed by OC and semi-closed rebreathers (Cole et al. 2007), though unlike CCR semi-closed rebreathers produce bubbles. Our lack of differences in observed abundance between survey techniques for many fish families fits with results of Lindfield et al (2014a), who found that their non-fished family control, Chaetodontidae, did not show a significant difference in biomass between survey techniques. While our abundance results suggest that choice of dive technique did not affect abundance count on transects across the depth gradient for many fish families, our MAD results show that many fish families did in fact exhibit differences in their responses to the two dive techniques. MADs were lower for CCR surveyed fish than OC for several families (Carangidae, Labridae, Pomacentridae, Scaridae), though many showed no effect. Only the sub-family Serraninae had smaller MAD for OC transects than CCR transects. This means considering fish behavioural effects is important when deciding which dive technique to use.

Several possible explanations are likely for the fish families for which we did observe different abundances between techniques. Higher abundances recorded for Acanthuridae by OC are likely to be caused by shallow OC transects encountering several large schools of Atlantic blue tang (*Acanthurus coeruleus*) that were not encountered when the transects were conducted by CCR. While we cannot rule out attraction effects of OC divers to these schools, we did not observe any other non-commerically targeted fish species attracted to OC on shallow reefs. Sparidae showed similar responses to depth and survey method to large-bodied Serranidae, suggesting that they may have been harvested in the past.

Tetraodontidae abundance patterns are harder to explain, but most individuals recorded belonged to one species, the Caribbean sharpe-nose pufferfish (*Canthigaster rostrata*). As these are a small-bodied species, habituation effects to the sounds of shallow OC divers could explain the observed differences in abundance between techniques. If this were the case, it is not clear why similar abundance patterns were not detected in many other non-commercially targeted reef fish species. Tetraodontidae were, however, detected on the most transects of all fish families tested, and are one of the most abundant fish detected on Utila across both shallow reefs and upper-MCEs (Chapter 2). This high number of transects combined with large numbers of individuals recorded means we have greater power to detect differences in abundance between techniques and across the depth gradient in Tetraodontidae than most other non-fished families we tested. However, our MAD results for Tetraodontidae run counter to this explanation. We found no differences in MAD between OC and CCR, and MAD declined with increased depth, therefore MCE Tetraodontidae allowed divers to approach more closely than shallow reef ones. This pattern was also observed in several other non-commercially fished species, including the Haemulidae, Labridae, Pomacentridae and Scaridae. This is the reverse pattern expected if these species were habituated to the presence of OC divers on shallow reefs, suggesting despite the high numbers of divers on shallow reefs there is little effect of diver habituation. This lack of habituation is further supported by the method:depth interaction we detected in Labridae, with divers able to get closer in the shallows on CCR, but not much difference on MCEs. MAD measurements with stereo-video are most effective for larger fish species that we can observe with both cameras in the stereo-video pair in order to measure distance.

Therefore caution is required when interpreting MAD values for small-bodied species that do not respond to divers by swimming away, but instead hide in the reef structure. If the majority of disturbed fish hide before we observe them on both cameras then we are unable to record this high MAD distance, potentially biasing our comparison. However, we observed evidence of a lack of habituation in the shallows in several larger-bodied reef fish families, such as Haemulidae and Scaridae, lending strength to our results.

We recorded greater MADs for larger individuals in the families Acanthuridae, Haemulidae, Labridae, Mullidae, Pomacentridae, Serraninae and Scaridae. These results are consistent with previous FID studies, which have found larger Acanthuridae and Scaridae do not allow divers to approach as closely before fleeing (Gotanda et al. 2009; Januchowski-Hartley et al. 2011). No large bodied Labridae species were included in our analysis, and all Pomacentridae species recorded around Utila were small bodied. This makes it unlikely the increased MADs for larger individuals are driven only by fishing. Another explanation is proposed by Gotanda et al (2009) based on ecological processes unrelated to fishing pressure: generally as reproductive value increases it is predicted that risk taking should decrease (Clark 1994). As mortality rates decline with increased size in marine fish (Sogard 1997), larger individuals would therefore be predicted to reduce risk taking.

Different responses by fish of the same length were observed across the depth gradient for Haemulidae, Mullidae and Serraninae, with stronger effects of fish length on MAD at greater depths. This suggests larger fish are more likely to flee on MCEs than shallow reefs, and we would expect less variation in MAD values from fish of differing lengths on shallow reefs when compared to MCEs. Previous work has hypothesised that light levels may interact with fish length in affecting FIDs (Januchowski-Hartley et al. 2011), as generally there is an improvement in fish vision with increasing body size (Fernald 1985). This could explain these results, as fish of all body sizes are more likely to be able to detect divers approaching in shallow sites with high light availability, whereas at deeper depths with lower light levels it may be harder for small fish to identify divers approaching than large fish. However, a study of reef fish visual acuity across shallow to mesophotic reefs suggests high plastic adaptive ability of fish visual systems to compensate for lower light levels (Brokovich et al. 2010). These adaptations were sufficient for a zooplanktivorous species from the family Pomacentridae to show little change in foraging behaviour across a shallow reef to upper-MCE gradient (Brokovich et al. 2010). This adaptive plasticity makes it hard to disentangle effects of depth from those of changing light levels, and requires further research.

This study highlights the importance of considering changes in fish behaviour when conducting reef fish surveys across depth gradients. While for many fish families we did not detect differences in abundance between open circuit and closed circuit rebreather surveys, we did identify family level differences in how close divers could approach individual fish between the methods. Some differences in approach distance varied with depth, with the direction of response family specific. This study highlights the need for reef fish community studies making comparisons across natural gradients such as depth to control for changes in fish behaviour that may affect their fish detection results.

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5.7 References

- Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, U.K.
- Bejarano I, Appeldoorn RS, Nemeth M (2014) Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico. Coral Reefs 33:313–328
- Box SJ, Canty SWJ (2011) The Long and Short Term Economic Drivers of Overexploitation in Honduran Coral Reef Fisheries Due to Their Dependence on Export Markets. Proceedings of the 63rd Gulf and Caribbean Fisheries Institute November 1 - 5, 2010 San Juan, Puerto Rico 63:43–51
- Brokovich E, Ben-Ari T, Kark S, Kiflawi M, Dishon G, Iluz D, Shashar N (2010) Functional changes of the visual system of the damselfish *Dascyllus marginatus* along its bathymetric range. Physiology & Behavior 101:413–421
- Chapman CJ, Johnstone ADF, Dunn JR, Creasey DJ (1974) Reactions of fish to sound generated by divers' open-circuit underwater breathing apparatus. Marine Biology 27:357–366
- Clark CW (1994) Antipredator behavior and the asset-protection principle. Behavioral Ecology 5:159–170
- Cole RG (1994) Abundance, size structure, and diver-oriented behaviour of three large benthic carnivorous fishes in a marine reserve in Northeastern New Zealand. Biol Conserv 70:93–99

- Cole RG, Syms C, Davey NK, Gust N, Notman P, Stewart R, Radford CA, Carbines G, Carr MH, Jevs AG (2007) Does breathing apparatus affect fish counts and observations? A comparison at three New Zealand fished and protected areas. Marine Biology 150:1379–1395
- Cooper WE, Whiting MJ (2007) Effects of risk on flight initiation distance and escape tactics in two southern African lizard species. Acta Zoologica Sinica 53:446–453
- Cronk L, Steadman S (2002) Tourists as a common-pool resource: A study of dive shops on Utila, Honduras. In: Cohen J., Dannhaeuser N. (eds) *Economic Development: An Anthropological Approach*. Altamira Press, Walnut Creek, CA, pp 51–68
- Dickens LC, Goatley CHR, Tanner JK, Bellwood DR (2011) Quantifying Relative Diver Effects in Underwater Visual Censuses. PLoS ONE 6:e18965
- Doiron S, Weissenberger S (2014) Sustainable dive tourism: Social and environmental impacts—the case of Roatan, Honduras. Tourism Management Perspectives 10:19–26
- English S, Wilkinson C, Baker V (1997) Survey manual for tropical marine resources. Australian Institute of Marine Sciences, Townsville
- Feary DA, Cinner JE, Graham NAJ, Januchowski-Hartley (2011) Effects of Customary Marine Closures on Fish Behavior, Spear-Fishing Success, and Underwater Visual Surveys. Conservation Biology 25:341–349
- Fernald RD (1985) Growth of the teleost eye: novel solutions to complex constraints. Environ Biol Fish 13:113–123
- Gobert B, Berthou P, Lopez E, Lespagnol P, Turcios MDO, Macabiau C, Portillo P (2005) Early stages of snapper–grouper exploitation in the Caribbean (Bay Islands, Honduras). Fisheries Research 73:159–169

- Gotanda KM, Turgeon K, Kramer DL (2009) Body size and reserve protection affect flight initiation distance in parrotfishes. Behav Ecol Sociobiol 63:1563–1572
- Grol MGG, Rypel AL, Nagelkerken I (2014) Growth potential and predation risk drive ontogenetic shifts among nursery habitats in a coral reef fish. Marine Ecology Progress Series 502:229–244
- Harborne AR, Afzal DC, Andrews MJ (2001) Honduras: Caribbean Coast. Marine Pollution Bulletin 42:1221–1235
- Januchowski-Hartley FA, Cinner JE, Graham NAJ (2014) Fishery benefits from behavioural modification of fishes in periodically harvested fisheries closures. Aquatic Conserv: Mar. Freshw. Ecosyst. 24:777–790
- Januchowski-Hartley FA, Graham NAJ, Cinner JE, Russ GR (2015) Local fishing influences coral reef fish behavior inside protected areas of the Indo-Pacific. Biol Conserv 182:8–12
- Januchowski-Hartley FA, Graham NAJ, Feary DA, Morove T, Cinner JE (2011) Fear of Fishers: Human Predation Explains Behavioral Changes in Coral Reef Fishes. PLoS ONE 6:e22761
- Kramer P, McField M, Álvarez-Filip L, Drysdale I, Flores MR, Giró A, Pott R (2015) 2015 Report Card for the Mesoamerican Reef. healthyreefs.org
- Lindfield SJ, Harvey ES, Halford AR, McIlwain JL (2015) Mesophotic depths as refuge areas for fishery-targeted species on coral reefs. Coral Reefs
- Lindfield SJ, Harvey ES, McIlwain JL, Halford AR (2014a) Silent fish surveys: bubble-free diving highlights inaccuracies associated with SCUBA-based surveys in heavily fished areas. Methods Ecol Evol 5:1061–1069
- Lindfield SJ, McIlwain JL, Harvey ES (2014b) Depth Refuge and the Impacts of SCUBA Spearfishing on Coral Reef Fishes. PLoS ONE 9:e92628

- Mallet D, Pelletier D (2014) Underwater video techniques for observing coastal marine biodiversity: A review of sixty years of publications (1952–2012). Fisheries Research 154:44–62
- Manassa RP, McCormick MI (2012) Social learning and acquired recognition of a predator by a marine fish. Anim Cogn 15:559–565
- Oksanen J, Kindt R, Legendre P, OHara B, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) vegan: Community Ecology Package. cran.r-project.org
- Pinheiro HT, Goodbody-Gringley G, Jessup ME, Shepherd B, Chequer AD, Rocha LA (2016) Upper and lower mesophotic coral reef fish communities evaluated by underwater visual censuses in two Caribbean locations. Coral Reefs 35:139–151
- R Core Team (2013) R: A language and environment for statistical computing. R-project.org
- Radford CA, Jeffs AG, Tindle CT, Cole RG, Montgomery JC (2005) Bubbled waters: The noise generated by underwater breathing apparatus. Marine and Freshwater Behaviour and Physiology 38:259–267
- Sciberras M, Jenkins SR, Kaiser MJ, Hawkins SJ, Pullin AS (2013) Evaluating the biological effectiveness of fully and partially protected marine areas. Environmental Evidence 2:4
- Sieber A, Pyle R (2010) A review of the use of closed-circuit rebreathers for scientific diving. Underwater Technology 29:73–78
- Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. Bulletin of Marine Science 60:1129–1157
- Stevenson TC, Tissot BN, Dierking J (2011) Fisher behaviour influences catch productivity and selectivity in West Hawaii's aquarium fishery. ICES Journal of Marine Science 68:813–822
- Thompson AA, Mapstone BD (1997) Observer effects and training in underwater visual surveys of reef fishes. Marine Ecology Progress Series 154:53–63

- Titus BM, Daly M, Exton DA (2015) Do Reef Fish Habituate to Diver Presence? Evidence from Two Reef Sites with Contrasting Historical Levels of SCUBA Intensity in the Bay Islands, Honduras. PLoS ONE 10:e0119645
- Watson DL, Harvey ES (2007) Behaviour of temperate and sub-tropical reef fishes towards a stationary SCUBA diver. Marine and Freshwater Behaviour and Physiology 40:85–103

6 LARGE-SCALE INVASION OF WESTERN ATLANTIC MESOPHOTIC REEFS BY LIONFISH POTENTIALLY UNDERMINES CULLING-BASED MANAGEMENT

Chapter 6: Large-scale invasion of western Atlantic mesophotic reefs by lionfish potentially undermines culling-based management

6.1 Abstract

The detrimental effects of invasive lionfishes (*Pterois volitans* and *Pterois miles*) on western Atlantic shallow reefs are well documented, including declines in coral cover and native fish populations, with disproportionate predation on critically endangered reef fish in some locations. Yet despite individuals reaching depths >100 m, the role of mesophotic coral ecosystems (MCEs; reefs 30-150 m) in lionfish ecology has not been addressed. With lionfish control programs in most invaded locations limited to 30 m by diving restrictions, understanding the role of MCEs in lionfish distributions remains a critical knowledge gap potentially hindering conservation management. Here we synthesise unpublished and previously published studies of lionfish abundance and body length at paired shallow reef (0-30 m) and MCE sites in 63 locations in seven western Atlantic countries and eight sites in three Indo-Pacific countries where lionfish are native. Lionfish were found at similar abundances across the depth gradient from shallow to adjacent MCEs, with no difference between invaded and native sites. Of the five invaded countries where length data were available three had larger lionfish on mesophotic than shallow reefs, one showed no significant difference, while the fifth represented a recently invaded site. This suggests at least some mesophotic populations may represent extensions of natural ontogenetic migrations. Interestingly, despite their shallow focus, in many cases culling programs did not appear to alter abundance between depths. In general, we identify widespread invasive lionfish populations on MCE that could be responsible for maintaining high densities of lionfish recruits despite local shallow-biased control programs. This study highlights the need for management plans to incorporate lionfish populations below the depth limit of recreational diving in order to address all aspects of the local population and maximise the effectiveness of control efforts.

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6.2 Introduction

Fewer than 16 % of marine environments are now considered to be free of invasive species and the threat to biodiversity they represent (Molnar et al. 2008). The major impacts they cause to natural ecosystem health and functioning, whether as predators, competitors, pathogens or parasites are well documented (Simberloff et al. 2013) and present an enormous dilemma for conservation practitioners. The ability to cross environmental barriers combined with high reproductive output facilitates the rapid spread of many marine invasive species (Côté et al. 2013) while the difficulties associated with removing individuals makes complete eradication almost impossible once invasives become established across a large geographical range (Thresher and Kuris 2004). Instead managers are often forced to limit their efforts to local control, and to focus on mitigating the most severe impacts rather than seeking complete eradication (Williams and Grosholz 2008).

The appearance of lionfish in the western Atlantic has led to one of the fastest marine invasions ever recorded (Schofield 2010). Native to the Indo-Pacific, the lionfishes *Pterois volitans* and *Pterois miles* (Family: Scorpaenidae) were introduced to the western Atlantic in the early 1980s and have since become a widespread conservation concern in the region (Côté et al. 2013). Lionfish have proved to be highly effective predators on invaded shallow coral reefs benefiting from young age of maturity combined with high fecundity, highly generalist feeding strategy, lack of natural predators and parasites, and prey naivety (Côté et al. 2013). Invasive population densities are consequently five times greater than those of lionfish in their native range, although the presence of additional co-occurring Indo-Pacific lionfish species may partially reduce this overall disparity (Darling et al. 2011). Invasive lionfish have been reported to reduce native fish recruitment by up to 79 % (Albins and Hixon 2008) and drive declines in overall prey fish biomass of up to 65 % (Green et al. 2012) on shallow patch reef systems. Their impacts on mesophotic reefs in The Bahamas have shown their potential to cause declines in coral cover (Lesser and Slattery 2011), while dietary analysis has suggested disproportionate predation on critically-endangered reef fish species in some locations (Rocha et al. 2015). Lionfish density appears limited by food availability (Benkwitt 2013), with invasive individuals 1.5 times longer and three times heavier on average than those in their native range (Darling et al. 2011).

Invasive lionfish are now established from North Carolina, USA in the north, throughout most of the Greater Caribbean and Gulf of Mexico (Schofield 2010), with an individual collected as far south as Brazil (Ferreira et al. 2015). This wide spatial distribution makes complete eradication impossible with current methods that focus on active culling to reduce local population densities. Culling efforts are often managed/regulated by government and conducted by volunteer recreational SCUBA divers using Hawaiian sling hand spears or pole spears (Morris et al. 2009). Evidence suggests that culling successfully reduces lionfish abundance on shallow reefs (Frazer et al. 2012), and such reductions aid the recovery of native fish communities (Green et al. 2014). However, continued culling is crucial as lionfish populations quickly recover after culling ceases (Arias-González et al. 2011).

It is vital that the ecology and distribution of the invasive species is fully understood to design the most effective form of control. If invasive species are widely dispersed habitat generalists, such as lionfish, then they will likely exhibit source-sink population dynamics, with individuals in certain areas disproportionately contributing to recruitment (Travis and Park 2004). Depth is recognised as a fish refuge from harvesting pressure, with major differences in biomass and species composition detected across even small depth gradients (Tyler et al. 2009; I. Bejarano et al. 2014). Yet few studies have considered how control programs are affected by depth, despite several major shallow reef marine invasive species being recorded across wide depth ranges (Chapter 9).

Lionfish are found on mesophotic coral ecosystems (MCEs; reefs from 30 m to 150 m) in their native range, having been reported at 65 m in the Red Sea (Brokovich et al. 2008), from trawl surveys at 75 m in New Caledonia (Kulbicki et al. 2012) and remotely operated vehicle (ROV) observations below 80 m in American Samoa (Wright 2005). Lionfish have also been anecdotally observed on MCEs throughout much of their invaded range, although comprehensive sampling at mesophotic depths remains lacking. For example, lionfish have been recorded down to 55 m in Puerto Rico (I. Bejarano et al. 2014), over 100 m in The Bahamas (Lesser and Slattery 2011), 112 m in the northwestern Gulf of Mexico (Nuttall et al. 2014), and 120 m in Honduras (Schofield 2010). Even though many western Atlantic MCE observations report high abundances, logistical difficulties associated with accessing mesophotic reefs (Pyle 1998) mean that culling is largely restricted to shallow reefs. With active management interventions absent from most MCEs it is therefore feasible that deeper reefs are providing a refuge environment for invasive lionfish.

Many coral reef fish species are known to undergo ontogenetic migrations, typically from shallow habitats (mangroves, seagrass beds and nearshore reefs) to deeper reef habitats (Appeldoorn et al. 2003). These ontogenetic movements are believed to be driven by trade-offs between maximising food availability (and thus growth rates), while minimising predation risk (Kimirei et al. 2013). Some fish even demonstrate multiple stages of ontogenetic habitat shifts, incorporating transition habitats during intermediate life stages (Grol et al. 2014). Ontogenetic inter-habitat migrations by lionfish have been previously reported, with juveniles found in mangroves, seagrass beds and shallow sheltered reefs before migrating to reef slopes (10-30 m) (Claydon et al. 2012). It is not clear whether lionfish populations on MCEs represent a continuation of this ontogenetic migration, with lionfish primarily moving from the shallows to MCEs, or a less depth-structured population with lionfish movement in both directions between shallow reefs and MCEs.

This study synthesises data from multiple studies throughout the invaded and native range of lionfish, encompassing both shallow and mesophotic depths, in order to explore the role of deeper reef environments in invasive lionfish ecology and to inform future management efforts. We use a meta-analysis approach to compare adjacent shallow-mesophotic paired reefs to identify whether anecdotal large-scale patterns in lionfish distributions are supported by available evidence. Specifically, we address: (i) the relative abundance of lionfish across the depth gradient in both native and invaded ranges; (ii) whether body size varies between shallow and mesophotic reefs; and, (iii) how these patterns with depth are affected by the availability of hard substrata, time since site invasion, and active management via culling.

6.3 Materials and Methods

6.3.1 Study design

Study sites were identified through discussions at the Second International Mesophotic Workshop (Eilat, Israel, October 2014). Mesophotic researchers at the workshop, along with key collaborators, provided lionfishes (*P. volitans* and/or *P. miles*) abundance and/or body length data from reef sites shallower than 30 m (shallow) and adjacent deep reefs between 30-150 m (mesophotic). These depth zones follow the widely accepted definition of shallow and mesophotic reefs (Hinderstein et al. 2010), allowing broad patterns between the two to be tested. MCEs are broadly defined as reef communities harbouring zooxanthellate corals, though the dominant reef organisms can also be non-zooxanthellate corals, sponges or algae, that occur at depths from 30 m to >150 m (Hinderstein et al. 2010). Lionfish abundance measures were based on a variety of visual and video-transect techniques, but were consistent within each shallow-mesophotic pairing (summarised in Table 6.1). Fish length measurements used a combination of underwater visual estimates (broad size categories), post-dive measurement after spearing, and stereo-video in situ measurements (Table 6.1).

Table 6.1 Numbers of individual shallow-mesophotic paired sites used in analysis for each country, along with the mean shallow and mesophotic survey depth, maximum mesophotic survey depth, and methods used for lionfish abundance and length surveys.

Country	Abundance				
	Number of sites	Mean shallow depth (m)	Mean mesophotic depth (m)	Maximum mesophotic depth (m)	Methods
Western Atlantic					
Bahamas	2	13	54	91	visual transects
Bermuda	4	15	50	60	visual transects
Bonaire	33	19	35	35	visual transects
Cayman Islands	3	20	61	91	visual transects
Curaçao	12	19	38	90	visual transects
Honduras	5	13	45	91	visual transects, stereo-video transects
Puerto Rico	4	25	55	70	visual transects
Indo-Pacific					
Israel	2	7	46	65	visual transects
Federated States of Micronesia	3	17	46	61	visual transects
Philippines	3	12	46	61	visual transects

Table 6.1 continued

Country	Length				
	Number of sites	Mean shallow depth (m)	Mean mesophotic depth (m)	Maximum mesophotic depth (m)	Methods
Western Atlantic					
Bahamas	2	13	54	91	visual estimation
Bermuda	1	15	70	65	spearing
Bonaire	21	19	35	35	in water measurement
Cayman Islands	0	-	-	-	-
Curaçao	9	21	35	35	in water measurement, spearing
Honduras	2	14	57	110	spearing, stereo-video camera drops
Puerto Rico	4	25	50	70	visual estimation
Indo-Pacific					
Israel	1	5	49	65	visual estimation
Federated States of Micronesia	3	17	46	61	visual estimation
Philippines	3	12	46	61	visual estimation

For inclusion, data from each site were required to include the following information for both shallow and mesophotic depths: (i) mean abundance and/or body length of lionfish; (ii) standard deviation/standard error of mean; (iii) number of replicates; (iv) depths for all replicates; (v) survey year; and, (vi) survey methodology. Where possible, we also recorded the following: (i) year of lionfish site invasion (non-native sites); (ii) shallow and mesophotic percentage hard-substrata cover; and, (iii) presence/absence of regular lionfish culling. Hard substrata included all benthic habitat types excluding mud, sand and rubble following Gratwicke and Speight (2005). Presence/absence of regular lionfish culling was based on information available from the data providers and, because of a general lack of intensity data, culling was treated as a categorical variable defined as regular local spearing of lionfish by divers.

In total, data incorporated lionfish abundance from 63 sites in seven western Atlantic (invasive) countries and eight sites from three Indo-Pacific/Red Sea (native) countries, with lionfish length data from 39 sites in six invasive countries and seven sites in three native countries (Figure 6.1, Table 6.1). Shallow data ranged 5-30 m, while mesophotic data ranged 30-110 m. While the majority of data are previously unpublished, published data were also extracted from (i) broader fish community studies with no direct comparison of lionfish populations across depth gradients, specifically for The Bahamas (Lesser and Slattery 2011), Israel (Brokovich et al. 2008) and Puerto Rico (I. Bejarano et al. 2014), and (ii) a study on lionfish culling effectiveness for Bonaire and some Curaçao sites (de León et al. 2013). When a site had multiple survey depths within a single zone, data were combined and mean depth (weighted by replication) used. Western Atlantic studies were totals of both invasive lionfishes (*P. volitans* and *P. miles*), while Indo-Pacific studies were for *P. volitans* (Micronesia, Philippines) and *P. miles* (Israel) separately, as these species do not co-occur in these locations. While *P. volitans* and *P. miles* are distinct species, they seem to be ecologically and morphologically analogous (Freshwater et al. 2009).

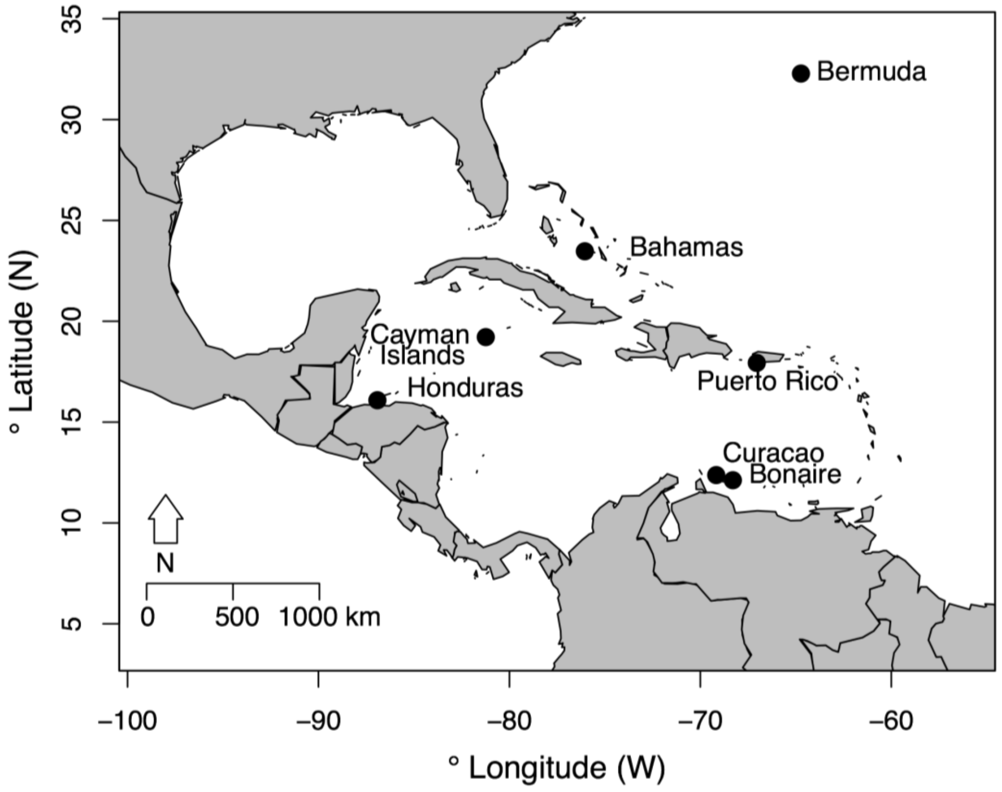

Figure 6.1 Western Atlantic lionfish survey locations included in this study, and surveyed between 2009-2014. Multiple paired shallow-mesophotic sites were located within the marked region for each country.

6.3.2 Data analysis

To compare sites surveyed using different methods, summary data from each site were converted into a standardised effect size, Hedges' *d* (Hedges and Olkin 1985; Koricheva et al. 2013). Hedges' *d* (Equation 6.1) is based on the standardised mean difference (SMD) between two groups (here depth zones), where $\overline{\mathrm{X_s}}$ and $\overline{\mathrm{X_m}}$ represent mean lionfish abundance or body length (respectively) at a single shallow-mesophotic pairing. Hedges' *d* values can be positive or negative indicating the direction and magnitude of effect, with d=0 representing no difference. Negative effect sizes indicate greater abundances or larger lionfish at mesophotic depths, while a positive effect size indicates the reverse. Pooled standard deviation (s_{pooled}) was calculated from the standard deviation of means for shallow and mesophotic depths (Equation 6.2), where $\mathrm{s_s}$ and $\mathrm{s_m}$ represent standard deviation, and $\mathrm{n_s}$ and $\mathrm{n_m}$ represent number of replicates within a site for shallow and mesophotic reefs respectively. *J* (Equation 6.1 and 6.3) is a correction for small sample sizes, allowing Hedges' *d* to be reliable with small numbers of replicates.

$$d = \frac{\overline{X_s}\text{-}\overline{X_m}}{s_{pooled}} J \qquad \text{Equation 6.1}$$

$$s_{pooled} = \sqrt{\frac{(n_s\text{-}1)(s_s)^2+(n_m\text{-}1)(s_m)^2}{n_s+n_m\text{-}2}} \qquad \text{Equation 6.2}$$

$$J = 1\text{-}\frac{3}{4(n_s+n_m\text{-}2)\text{-}1} \qquad \text{Equation 6.3}$$

The variance of each Hedges' *d* estimate was calculated using Equation 6.4.

$$v_d = \frac{n_s+n_m}{n_s n_m} + \frac{d^2}{2(n_s+n_m)} \qquad \text{Equation 6.4}$$

When fitting models using data obtained using different methods, between-site heterogeneity in effect sizes must be considered (Koricheva et al. 2013). Under standardised methods, heterogeneity between replicates tends to be from sampling variance alone. To account for sampling variation within and between sites, we fitted random-effects models based on Hedges' *d* estimates and variance. Random-effects models were fitted to calculate summary effects for sites with and without lionfish culling for each country, and overall for all sites within the invasive and native ranges. Mixed-effects models were then used to test the influence of potential effect modifiers on effect sizes and direction. Mixed and random-effects models were fitted using the DerSimonian-Laird estimator (Koricheva et al. 2013), with sites weighted by the inverse of their effect size variance for calculations of summary effects. To identify within-study heterogeneity I^2 and H^2 statistics were calculated. I^2 quantifies the percentage of heterogeneity that can be explained by between-study variance. By calculating heterogeneity as a percentage, it is possible to directly compare I^2 values between meta-analyses with different numbers of studies and effect metrics (Higgins and Thompson 2002). H^2 is another related measure of heterogeneity with H^2=1 indicating homogeneity of treatment effects and larger values providing increasing evidence of heterogeneity (Higgins and Thompson 2002). For testing the amount of heterogeneity accounted for when fitting mixed-effects models, a *pseudo*-R^2 value was calculated following Raudenbush (2008). All analyses were conducted in the statistical program R (R Core Team 2013) with effect sizes calculated using the 'escalc' function and models fitted and heterogeneity estimated with the 'rma.uni' function in the metafor package (Viechtbauer 2010).

6.4 Results

6.4.1 Variation in lionfish abundance with depth

We found no overall difference in the abundance of lionfish between shallow and mesophotic depths at invaded sites without culling (Figure 6.2a, SMD=-0.06, 95 % CI= -0.20, 0.32), or those with culling (Figure 6.2b, SMD=-0.20, 95 % CI=-0.43, 0.03). No individual invaded country exhibited differences in abundance between depth ranges at sites without culling (Figure 6.2a). Differences were found in Bermuda where culling takes place (Figure 6.2b), with abundance higher at mesophotic depths (SMD=-1.16, 95 % CI=-1.72, -0.61).

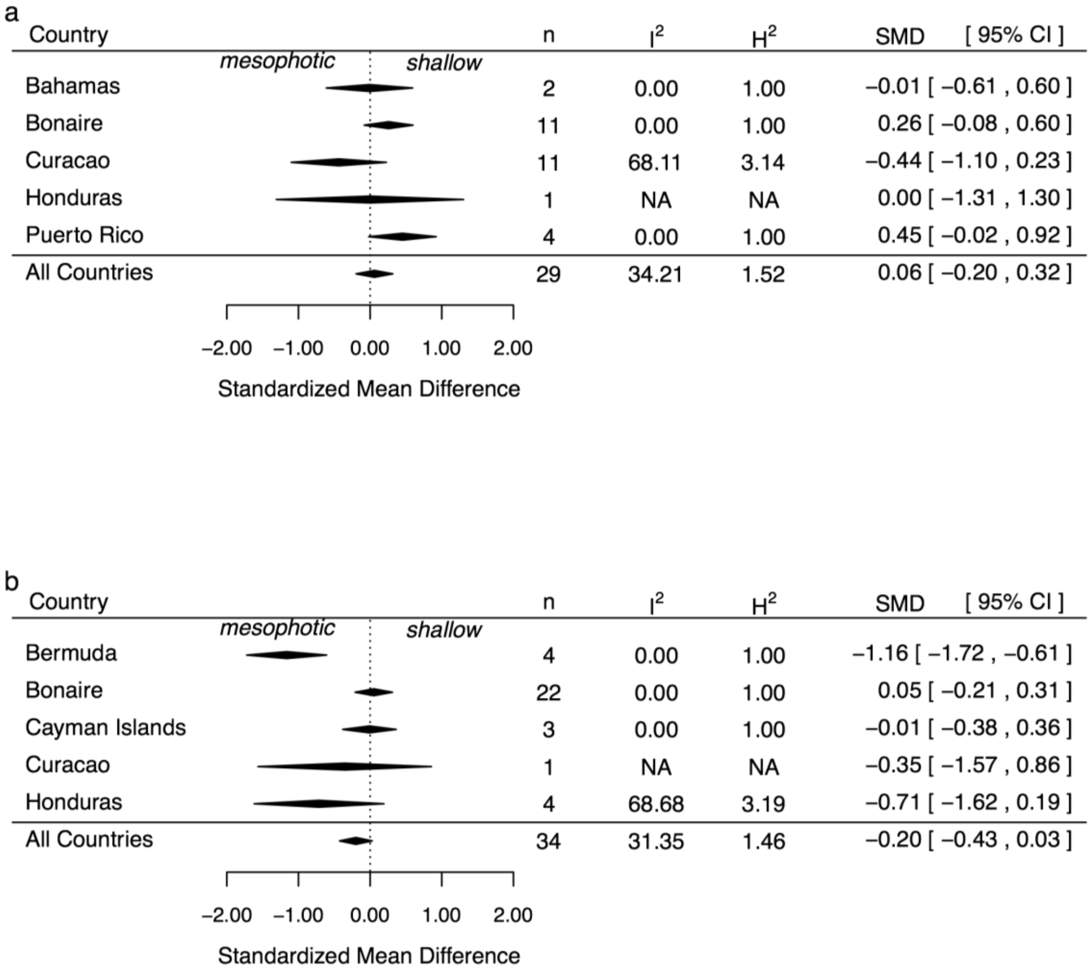

Figure 6.2 Summary random-effect models for lionfish abundance at western Atlantic sites with countries presented individually and grouped, showing (a) sites without active lionfish culling and, (b) sites with active lionfish culling.

Diamonds centre represent the mean summary effect, and the width the 95 % confidence interval (CI). Diamonds intersecting the dashed vertical line indicate the summary effect is not significantly different to zero, while *n*= number of study sites (each site contains multiple replicate surveys – see Electronic Supplementary Materials for individual site information), I^2= residual heterogeneity/unaccounted variability, H^2= unaccounted variability/sampling variability, SMD= Standardized Mean Difference, and NA=values not able to be calculated due to small sample sizes. Positive effect sizes (SMD) indicate greater lionfish abundance on shallow reefs than mesophotic reefs, while negative effect sizes indicate the reverse.

Native range sites exhibited similar abundance patterns to the western Atlantic (Figure 6.3a; SMD=-0.14, 95 % CI=-0.46, 0.17). When tested in a fixed-effects model, there was no difference between the overall summary effect for Indo-Pacific sites and either non-culled western Atlantic sites (*z*=-1.32, *p*=0.19) or culled western Atlantic sites (*z*=0.38, *p*=0.70). Invasive lionfish populations therefore appear to exhibit a similar depth-distribution pattern to those in their native range in terms of abundance.

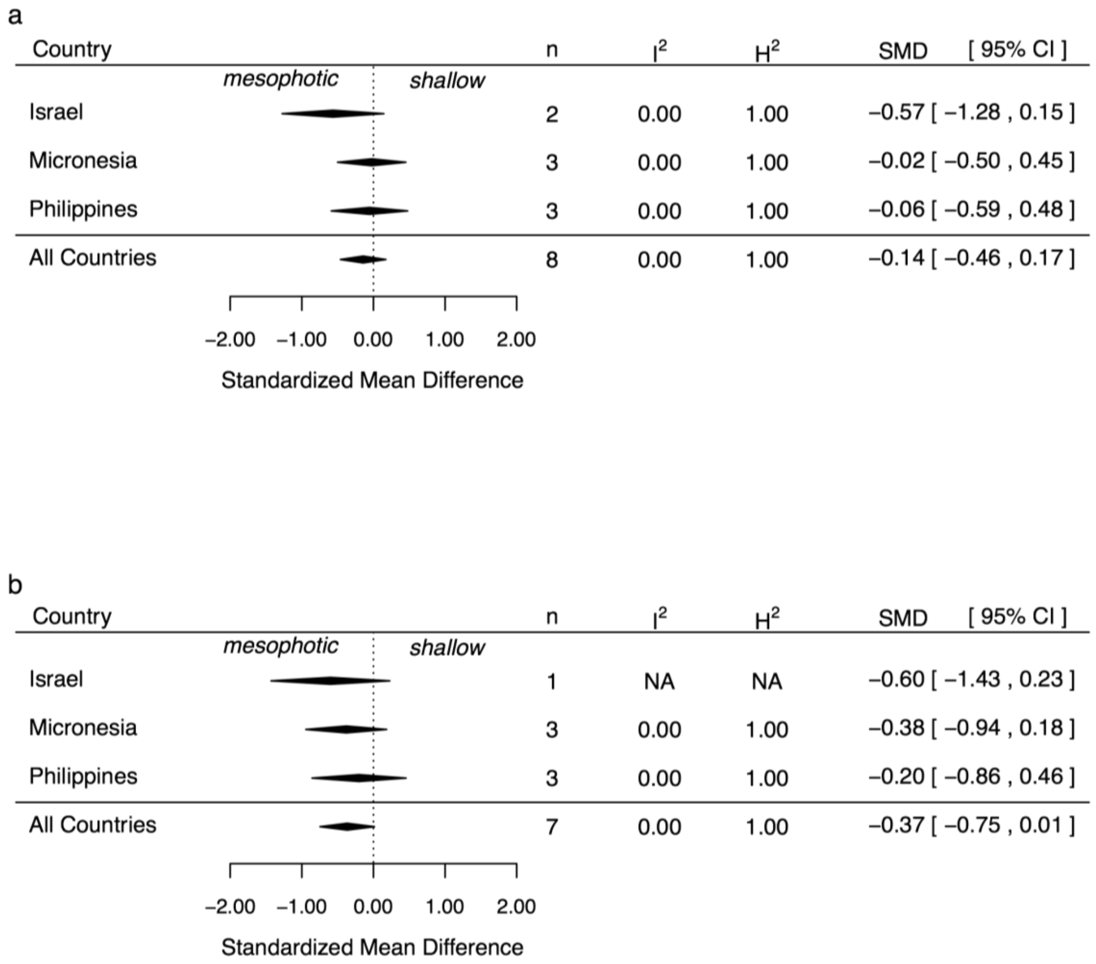

Figure 6.3 Summary random-effect models for (a) lionfish abundance and (b) mean lionfish body length at Indo-Pacific reef sites, with countries presented individually and grouped.

Positive effect sizes (SMD) indicate greater abundance or larger mean body length of lionfish on shallow reefs than mesophotic reefs, while negative effect sizes indicate the reverse. See Figure 2 legend for full description.

Despite no overall difference in lionfish abundance between shallow and mesophotic reefs, the magnitude and direction of each site's observed effect size (SMD) was significantly correlated with both time since invasion and availability of hard substrata (Table 6.2). There was a negative relationship between SMD and time since invasion (t=-2.07, p=0.038), meaning surveys conducted soon after initial invasion found a greater abundance of lionfish on shallow reefs compared to those conducted at increased time intervals after invasion. Time since first lionfish observation at our study sites ranged from 2-14 years. Shallow hard-substrata cover also negatively correlated with effect size (t=-2.14, p=0.032), indicating that at sites with greater shallow hard-substrata availability a greater abundance of lionfish was found at mesophotic depths. Mesophotic hard-substrata cover was positively correlated with effect size (t=2.76, p=0.006), suggesting that at sites with lower mesophotic hard-substrata availability, a greater abundance of lionfish was found at mesophotic depths. The presence of culling (Table 6.2) had no impact on the mean difference in abundance between shallow and mesophotic sites. The addition of these effect modifiers to the model accounts for 27.79 % of the between-study variation observed, with I^2 reduced from 40.79 % (all sites random-effect model with no effect modifiers) to 21.50 %.

Table 6.2 Mixed-effect model testing effect modifiers impacting western Atlantic lionfish abundance effect sizes.

Effect modifiers fitted as fixed-effects in the random-effects model. Positive estimates indicate a positive correlation between individual studies' Hedges' *d* and the variable, where *n*=54, I^2=21.50 %, H^2=1.27 and R^2=27.79 %. Asterisks indicate significance level, with * indicating *p<0.05* and ** indicating *p<0.01*.

Parameter	Estimate	Standard Error	t-value	p-value
Intercept	0.904	0.517	1.749	0.080
Time since first lionfish observation	-0.204	0.099	-2.073	0.038*
Culling	0.001	0.191	0.004	0.997
Shallow hard substrata % cover	-0.019	0.009	-2.140	0.032*
Mesophotic hard substrata % cover	0.018	0.007	2.764	0.006**

6.4.2 Variation in lionfish body size with depth

We found no overall difference in mean lionfish body length between shallow and mesophotic invaded sites for all sites without culling combined (Figure 6.4a). However, at the country level, those sites without culling in The Bahamas, Curaçao and Honduras showed larger lionfish at mesophotic depths than shallow depths (Figure 6.4a). Bonaire data showed no difference (SMD=-0.32, 95 % CI= -1.24, 0.61), while Puerto Rico had larger lionfish in the shallows (SMD=0.90, 95 % CI=-0.36, 1.43). At sites where culling takes place, there was no difference in mean body length between depth ranges (Figure 6.4b, SMD=0.18, 95 % CI=-0.21, 0.57). Comparisons within their native-range found no difference in body length between shallow and mesophotic depths (Figure 6.3b, SMD=-0.37, 95 % CI=-0.75, 0.01). This native range summary effect was not different to non-culled invaded sites (*z*=-0.45, *p*=0.65), but was different to invaded sites with culling (*z*=-2.19, *p*=0.028).

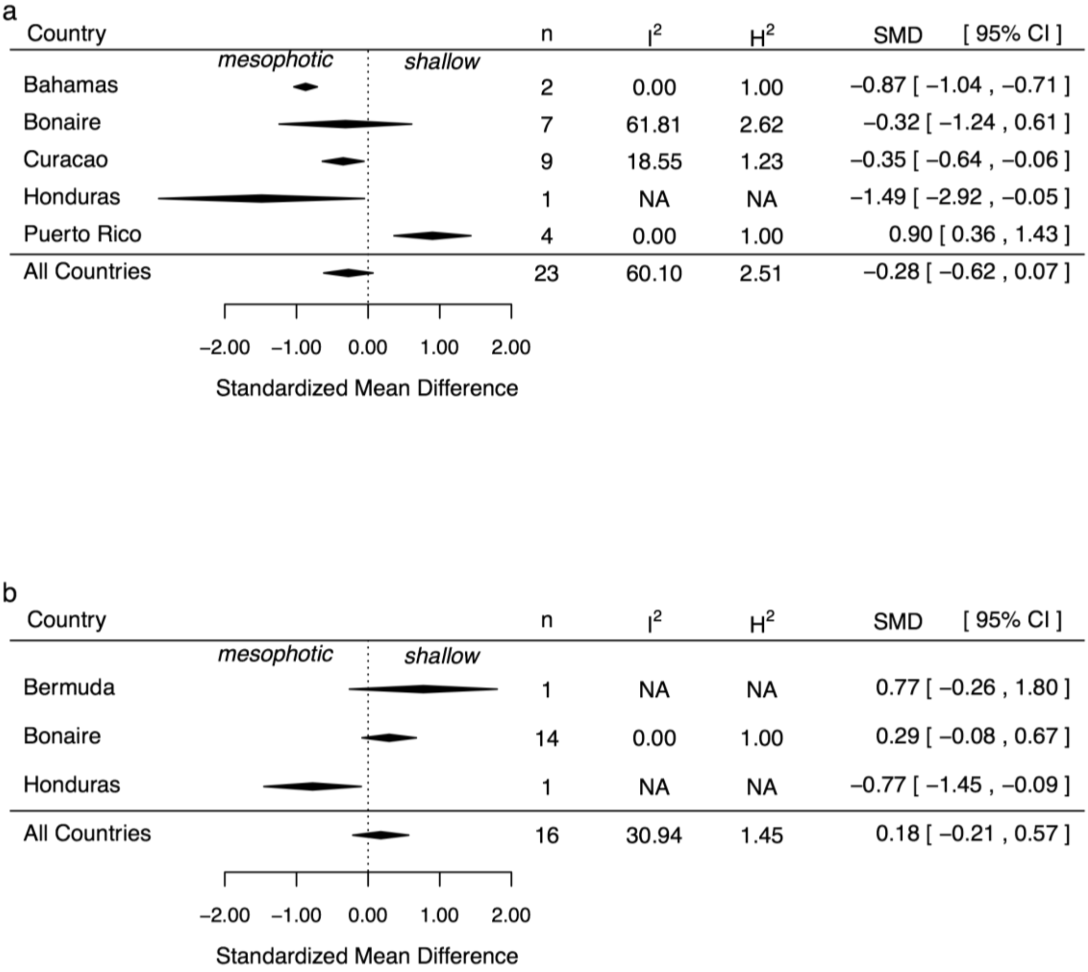

Figure 6.4 Summary random-effect models for lionfish body length at western Atlantic sites with countries presented individually and grouped, showing (a) sites without active culling and (b) sites with active lionfish culling.

Positive effect sizes (SMD) indicate larger mean lionfish body length on shallow reefs than mesophotic reefs, while negative effect sizes indicate the reverse. See Figure 2 legend for full description

The differences in mean lionfish body length between invaded depth ranges were affected by the time since first lionfish observation and the presence/absence of culling (Table 6.3). Time since invasion had a negative relationship with effect size (t=-3.42, p=0.001), indicating more initial similarity in fish lengths between depths, but progressively larger fish on mesophotic and/or smaller fish on shallow reefs with time. Presence or absence of culling was also highlighted as an important modifier affecting length distributions (t=3.05, p=0.002), with different overall summary effects for invaded sites with and without culling (*z*=-2.03, *p*=0.04). Neither shallow nor mesophotic hard-substrata cover had an effect on lionfish body lengths. Including these factors in the model accounted for 83.39 % of the between-study heterogeneity, with I^2 in a random-effects model for all sites without effect modifiers reduced from 44.75 % to 10.57 %.

Table 6.3 Mixed-effect model testing effect modifiers impacting western Atlantic lionfish body length effect sizes.

Effect modifiers fitted as fixed-effects in the random-effects model. Positive estimates indicate a positive correlation between individual studies' Hedges' *d* and the variable, where n=30, I^2=10.57 %, H^2=1.12 and R^2=83.39 %. Stars indicate significance level, with * indicating *p<0.05* and ** indicating *p<0.01*.

Parameter	Estimate	Standard Error	t-value	p-value
Intercept	0.778	0.626	1.243	0.214
Time since first lionfish observation	-0.343	0.100	-3.415	0.001**
Culling	0.721	0.236	3.054	0.002**
Shallow hard substrata % cover	-0.010	0.011	-0.944	0.345
Mesophotic hard substrata % cover	0.007	0.007	0.968	0.333

Lionfish length distributions have generally been found to be unimodal, however, cohort effects can occasionally be observed, particularly as new invasions occur (Trégarot et al. 2015). To incorporate lionfish length data from all sites, mean length had to be used. To test for potential bimodality, we tested the variance of lionfish lengths against depth for all shallow and mesophotic western Atlantic sites using Spearman's rank correlation coefficient. Our results do not suggest any consistent difference in unimodal or bimodal length distributions in either depth zone, as we found no relationship between length variance and depth for non-culled (*rho*=<0.01, *p*=0.99, *n*=92) or culled (*rho*=-0.191, *p*=0.13, *n*=64) western Atlantic sites.

6.5 Discussion

Invasive lionfish have widely colonised mesophotic reefs throughout the western Atlantic and at the time of surveying exist at densities similar to those on nearby shallow reefs, which have to date received the vast majority of research and management attention. Importantly, we also show that individual lionfish in some locations are larger on mesophotic reefs than their shallow counterparts. These findings raise important questions about the role of MCEs in lionfish life history, such as whether MCEs are acting as depth refuges for invasive populations, and so providing a local source of recruits despite removal efforts. Our results also indicate that without direct human intervention via culling the relative abundance and length distributions between shallow and mesophotic depths appear similar for invaded and native sites, suggesting a similar role for MCEs between the two regions.

6.5.1 Mesophotic reefs as a potential refuge for invasive lionfish

Across the western Atlantic region, we found no difference in lionfish abundances between shallow and mesophotic depths, highlighting an urgent need to integrate deeper reefs into lionfish management plans, which currently focus almost exclusively on removal from shallow environments. Supported by previous shallow-reef research (e.g. Claydon et al. 2012), combined with our results of (i) no difference in lionfish abundance between depth zones in most locations, (ii) larger lionfish on MCEs in three locations, and (iii) increasing proportional abundance and mean length of lionfish found on MCEs with greater time since invasion, we propose that lionfish population resilience benefits from substantial mature mesophotic lionfish populations (Figure 6.5). Specifically, lionfish invade new sites at shallow depths via larval settlement from floating egg bundles, with juveniles and then adults subsequently migrating to greater depths, which happen to be below the range of lionfish control programs.

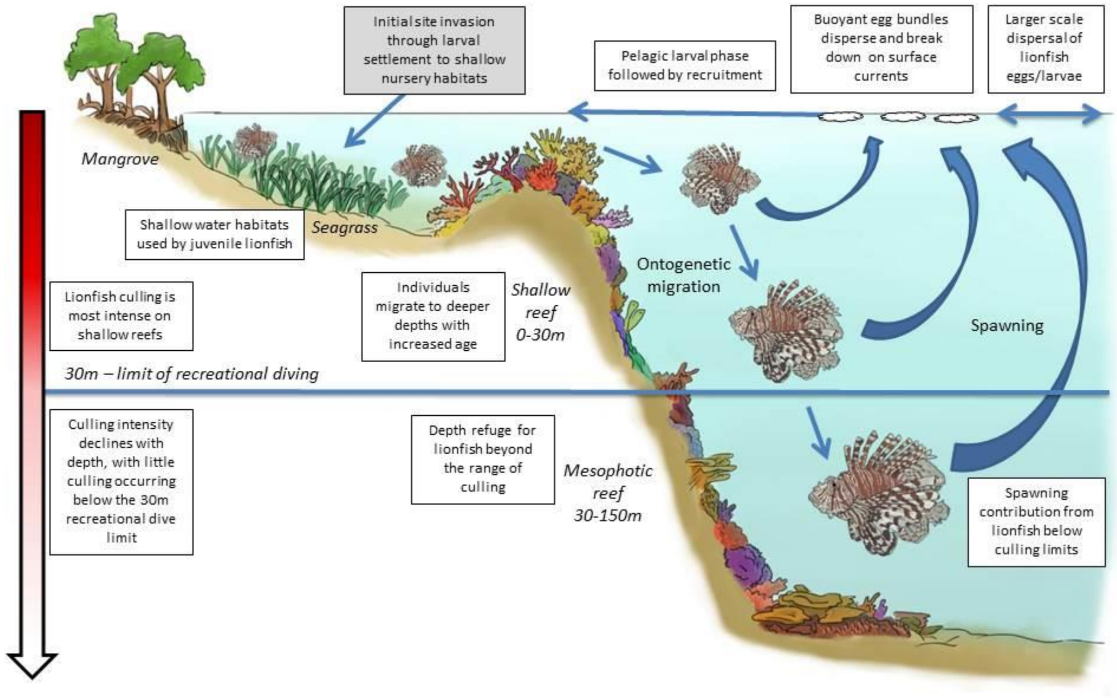

Figure 6.5 Schematic diagram of the proposed lionfish distribution across the shallow-mesophotic depth gradient. Lionfish initially invade new sites at shallow depths, before moving to deeper reef areas beyond the range of lionfish control measures (culling).

At first glance, this presents a major challenge to lionfish managers, as culling programs are often restricted to shallower depths through a reliance on volunteer recreational divers to achieve sufficient effort and by the financial and logistical complications of technical diving (Pyle 1998). Lionfish culling is most effective if adults of all age classes are targeted (Arias-González et al. 2011), with shallow culling resulting in reduced average lengths and abundance of shallow individuals (Frazer et al. 2012), while work on other species has shown that fishing at restricted depth zones can cause fine-scale length-distribution changes (Lindfield et al. 2014).

Previous research into lionfish depth distributions at invaded locations absent of culling has proved inconclusive. Claydon et al. (2012) reported greater sightings of lionfish, per unit effort, at 10-30 m than at 0-5 m in the Turks and Caicos Islands, and Lee et al. (2012) found greater abundances of lionfish with larger body lengths on surveys deeper than, compared to shallower than, 13 m in Jamaica. In contrast, in their native ranges, McTee et al. (2014) found lionfish abundance to be highest at shallow depths in the Red Sea, as did Kulbicki et al. (2012) in their review of Indo-Pacific literature. Our data suggest these differing patterns are likely partially driven by varying availability of hard substrata and linked factors (e.g. structural complexity), which have been shown to be important in lionfish aggregations (S. Bejarano et al. 2014). Although lionfish abundance is significantly higher in the western Atlantic than in their native range (Darling et al. 2011; Kulbicki et al. 2012), we identified similar patterns in relative abundance and mean body length distributions across the depth gradient at native sites as we found at invaded sites without culling. This suggests lionfish are using MCEs in their invaded range in similar ways as their native counterparts and, thus native-range studies can support and inform our understanding of the western Atlantic invasion.

Lionfish populations in their native and invaded geographical range limits are likely to face different structuring processes affecting their abundance and distribution. In Bermuda, it has been noted that lionfish are rarely observed in shallow reef habitats, but are much more common on deep fore-reef slopes (Smith et al. 2013). While all sites in our study from Bermuda were culled, it is possible that, at higher latitudes, physical factors such as temperature and wave energy may be the most significant factors driving lionfish-abundance distributions across depth gradients. For example, work by Whitfield et al. (2014) in North Carolina, USA, at the northern limit of the established populations, suggests that lionfish abundance across the shallow-mesophotic depth gradient correlates strongly with minimum winter water temperatures. In Bermuda, inshore shallow habitats experience lower winter water temperatures than deeper sites further offshore (Coates et al.

2013). These low winter inshore temperatures are below the temperature threshold at which experimental lab studies have suggested lionfish cease feeding (Kimball 2004). This potentially drives the larger abundances observed on MCEs compared to shallow habitats. We suggest that further studies on lionfish ecology in Bermuda should focus on the interaction between culling-based management and seasonal water-temperature variation in order to better understand whether temperature has a more significant role at the invasion range limit.

6.5.2 Lionfish body size and depth

The idea of a deep refuge for invasive lionfish was previously modelled by Arias-González et al. (2011), who found that if larger lionfish exist below depths accessible to culling programs, it will be harder to reduce and control the overall population. There are several possible explanations for larger lionfish being found below culling depths. When spawning, lionfish produce buoyant egg bundles that drift on the ocean's surface for several days before breaking down (Morris et al. 2009), leaving the embryos/larvae free floating with a pelagic larval duration of between 20-35 days (Ahrenholz and Morris 2010). Settlement is then believed to primarily occur in mangroves, seagrass beds, and shallow reef environments (<5 m) (Claydon et al. 2012). These shallow ecosystems are used by juvenile lionfish, before individuals migrate to intermediate depth reefs (10-30 m) as they mature (Claydon et al. 2012) (see Figure 6.5), and this migration could extend into MCEs. Alternatively, the presence of larger lionfish on deeper reefs could be caused by culling biases at shallow depths, disproportionately reducing the number of larger lionfish and so giving the appearing of increasing body size with depth. This could be driven by higher detection rates for larger lionfish than smaller lionfish in culling programs (Green et al. 2013)

While we did not find consistent patterns in lionfish body size with depth, additional factors such as time since invasion are likely to explain this discrepancy. In three invaded countries we found larger lionfish at mesophotic depths (The Bahamas, Curaçao, Honduras), in Bonaire, we found no difference in lionfish body lengths with depth, while in Puerto Rico, we found larger lionfish in the shallows. Puerto Rican studies were conducted two years after lionfish invasion, the shortest time interval after invasion of any included studies, so it is possible these studies do not represent the natural length distribution with depth for an established lionfish population. We found a negative correlation between time since invasion and the SMD in lionfish body length, suggesting this short time since invasion explains why we did not find larger lionfish at mesophotic depths in Puerto Rico. Individual country results must be interpreted with caution before generalisation, as some countries are only represented by several sites within the analysis (see Table 6.1). However, these broad country-level results provide support to the hypothesis that invasive lionfish on MCEs are an extension of the established mangrove, seagrass and shallow reef (>5 m) to intermediate depth reef (10-30 m) ontogenetic migrations (Claydon et al. 2012). However, research studying movement patterns through tagging, stable isotope or otolith age-structure analysis in lionfish across the depth gradient are needed to confirm these ontogenetic movements. These studies should be conducted to allow comparisons between areas with regular culling and those without to establish the influence on culling at finer scales than has been possible in this analysis.

6.5.3 Culling changes lionfish movement and behaviour

Our data suggest shallow culling (<30 m) leads to increased similarity in lionfish body lengths across the depth gradient, or even to an increase in larger individuals at shallow depths compared to MCEs. As we treated culling as a categorical variable, many subtle impacts of differing culling effort may be hidden. Future work would benefit from detailed culling records (e.g. frequency, intensity, time of day, experience of cullers) to better understand its impacts. A likely response to shallow culling would be changes in movement patterns of lionfish. It has previously been hypothesised that ontogenetic fish migrations may be reduced when competition between individuals in shallow habitats is limited (Appeldoorn et al. 2003), meaning those lionfish that avoid culling may mature at shallower depths. Yet this hypothesis is based on consistent mortality rates, whereas applying culling increases shallow lionfish mortality. However, we found similar lionfish abundance effects when comparing shallow and mesophotic reefs regardless of the presence of culling. This could suggest that culling on shallow reefs did not cause a significant decline in shallow lionfish abundance, but there is strong evidence to suggest this is not the case (Frazer et al. 2012). There are several biological explanations possible, for example: (i) individuals may be re-colonising shallow reefs from adjacent deeper reefs; (ii) an established mesophotic lionfish population may have been absent before shallow culling was effectively implemented; or alternatively (3) this was simply caused by low power in our analysis. Previous studies of lionfish movement have focused within the shallows and, while the majority were found to have high site fidelity, there are multiple records of individuals moving more than 1km (Côté et al. 2013; Akins et al. 2014). This would suggest that the relatively small distance between adjacent shallow and mesophotic reefs is within their natural movement range. However, movement from deeper areas without culling into shallow areas under continued culling runs counter to expected fish behaviour, as we would expect individuals to avoid areas with increased mortality risk (Werner and Gilliam 1984; Kimirei et al. 2013), although a large reduction in competition could outweigh the potential mortality risk. Further research is required to address this, focusing on specific movement studies of individual fish in culled areas across the depth gradient.

Lionfish culling reduces the number of lionfish in culled areas (Frazer et al. 2012), but also affects lionfish behaviour, making lionfish more cautious during daylight hours (Côté et al. 2014). Therefore, this combination of active removal combined with increasingly cryptic behaviour suggests we should observe greater relative lionfish abundances at deeper depths at culled sites than sites without culling. However, we do not see an effect of culling on lionfish relative abundance distribution across the depth gradient. When lionfish engage in increased cryptic behaviour, smaller lionfish become more easily hidden than larger individuals leading to greater detection rates for larger lionfish on lower structural complexity sites (Green et al. 2013). This process could explain our observations that higher abundance was correlated with areas of lower hard substrata, and that following culling we found similar mean body sizes at shallow and mesophotic depths, yet in the absence of culling we saw three countries with larger lionfish at depth.

While organised culling has been the focus of most lionfish management efforts, there is increasing interest in developing fisheries for lionfish and promoting market demand for invasive lionfish consumption (Bogdanoff et al. 2014). Switching focus from a government-led invasive species control effort to a market-based fisheries approach could bring substantial increases in lionfish removal (Bogdanoff et al. 2014). For example, in The Bahamas at the Cape Eleuthera Institute, a small marine research centre, their 2011-2015 invasive lionfish-culling program resulted in the collection of 55 kg of lionfish, whereas a locally developed lionfish fishery collected 680 kg of lionfish in 2015 alone (J Curtis-Quick personal communication). Larger lionfish on MCEs could potentially be a target for future fisheries, especially as fishers have previously reported them from MCEs in lobster traps and by hook and line (Bogdanoff et al. 2014). While hook and line is not considered appropriate for widespread lionfish collection (Bogdanoff et al. 2014), there has been much interest in developing a lionfish trap that can be deployed while minimizing bycatch (Pitt and Trott 2015). Traps can remove substantial numbers of MCE lionfish – for example, over 1,200 lionfish were removed from 40-80 m depth around Bermuda as bycatch in commercial lobster traps between September 2013-March 2014 (Pitt and Trott 2015).

However, in many locations, lionfish trapping has not been achieved effectively at a large scale (Morris et al. 2011) and further research and development of traps is required before trapping can be widely adopted. When developing lionfish fisheries as a conservation management strategy it is important to concentrate on keeping lionfish populations low, as there is a risk that established fisheries could become focused on managing for continued lionfish harvests. With these current technological limits most current MCE lionfish removal is carried out by technical divers using hand spears. With recent reduced costs, and increased interest from the recreational dive community in technical diving (Mitchell and Doolette 2013), it is becoming possible to incorporate deeper culling depths into lionfish management programs. For example, in 2016 the Bay Islands Conservation Association (the organisation with responsibility for lionfish management on Utila, Honduras) began to include a technical diving category in their annual lionfish derby to encourage technical divers to become involved in lionfish removal efforts and specifically target MCEs (M Arteaga personal communication).

Our results raise critical questions about the role of MCEs as a refuge for lionfish from culling and whether shallow culling program impacts are limited to shallow reefs or able to affect deeper populations. With increased interest from technical divers combined with technological development in lionfish traps, it is becoming possible to incorporate deeper culling depths into lionfish management programs. In addition, if mesophotic lionfish populations are dependent on lionfish recruitment in shallow marine habitats for new individuals, then infrequent deep reef culling combined with intense regular shallow culling could be sufficient to reduce the overall population and maximise the chances of complete localised eradication where culling intensity is sufficient.

6.6 Acknowledgements

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6.7 References

- Ahrenholz DW, Morris JA (2010) Larval duration of the lionfish, *Pterois volitans* along the Bahamian Archipelago. Environ Biol Fish 88:305–309. doi: 10.1007/s10641-010-9647-4
- Akins JL, Morris JA Jr, Green SJ (2014) In situ tagging technique for fishes provides insight into growth and movement of invasive lionfish. Ecol Evol 4:3768–3777. doi: 10.1002/ece3.1171
- Albins MA, Hixon MA (2008) Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. Marine Ecology Progress Series 367:233–238.

- Appeldoorn RS, Friedlander A, Sladek Nowlis J, et al (2003) Habitat connectivity in reef fish communities and marine reserve design in Old Providence-Santa Catalina, Colombia. Gulf and Caribbean Research 14:61–77.
- Arias-González JE, González-Gándara C, Luis Cabrera J, Christensen V (2011) Predicted impact of the invasive lionfish *Pterois volitans* on the food web of a Caribbean coral reef. Environmental Research 111:917–925. doi: 10.1016/j.envres.2011.07.008
- Bejarano I, Appeldoorn RS, Nemeth M (2014) Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico. Coral Reefs 33:313–328. doi: 10.1007/s00338-014-1125-6
- Bejarano S, Lohr K, Hamilton S, Manfrino C (2014) Relationships of invasive lionfish with topographic complexity, groupers, and native prey fishes in Little Cayman. Marine Biology 1–14. doi: 10.1007/s00227-014-2595-3
- Benkwitt CE (2013) Density-Dependent Growth in Invasive Lionfish (*Pterois volitans*). PLoS ONE 8:e66995. doi: 10.1371/journal.pone.0066995
- Bogdanoff AK, Akins JL, Morris JA, 2013 GCFI Lionfish Workgroup (2014) Invasive Lionfish in the Marketplace: Challenges and Opportunities. Proceedings of the 66th Gulf and Caribbean Fisheries Institute November 4 - 8, 2013 Corpus Christi, Texas USA 66:140–147.
- Brokovich E, Einbinder S, Shashar N, 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 371:253–262. doi: 10.3354/meps07591
- Claydon J, Calosso MC, Traiger SB (2012) Progression of invasive lionfish in seagrass, mangrove and reef habitats. Marine Ecology Progress Series 448:119–129. doi: 10.3354/meps09534
- Coates KA, Fourqurean JW, Kenworthy WJ, et al (2013) Introduction to Bermuda: Geology, Oceanography and Climate. In: Sheppard CRC (ed) Coral Reefs of the

United Kingdom Overseas Territories, Coral Reefs of the World 4. Springer, pp 115–133

- Côté IM, Darling ES, Malpica-Cruz L, et al (2014) What Doesn't Kill You Makes You Wary? Effect of Repeated Culling on the Behaviour of an Invasive Predator. PLoS ONE 9:e94248. doi: 10.1371/journal.pone.0094248
- Côté IM, Green SJ, Hixon MA (2013) Predatory fish invaders: Insights from Indo-Pacific lionfish in the western Atlantic and Caribbean. Biol Conserv 164:50–61.
- Darling ES, Green SJ, O’Leary JK, Côté IM (2011) Indo-Pacific lionfish are larger and more abundant on invaded reefs: a comparison of Kenyan and Bahamian lionfish populations. Biol Invasions 13:2045–2051. doi: 10.1007/s10530-011-0020-0
- de León R, Vane K, Bertuol P, et al (2013) Effectiveness of lionfish removal efforts in the southern Caribbean. Endang Species Res 22:175–182. doi: 10.3354/esr00542
- Ferreira CEL, Luiz OJ, Floeter SR, et al (2015) First Record of Invasive Lionfish (*Pterois volitans*) for the Brazilian Coast. PLoS ONE 10:e0123002. doi: 10.1371/journal.pone.0123002.g002
- Frazer TK, Jacoby CA, Edwards MA, et al (2012) Coping with the Lionfish Invasion: Can Targeted Removals Yield Beneficial Effects? Rev Fish Sci 20:185–191. doi: 10.1080/10641262.2012.700655
- Freshwater DW, Hamner RM, Parham S, Wilbur AE (2009) Molecular evidence that the lionfishes *Pterois miles* and *Pterois volitans* are distinct species. Journal of the North Carolina Academy of Science 125:39–46.
- Gratwicke B, Speight MR (2005) The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. Journal of Fish Biology 66:650–667. doi: 10.1111/j.0022-1112.2005.00629.x

- Green SJ, Akins JL, Maljković A, Côté IM (2012) Invasive Lionfish Drive Atlantic Coral Reef Fish Declines. PLoS ONE 7:e32596. doi: 10.1371/journal.pone.0032596
- Green SJ, Dulvy NK, Brooks AML, et al (2014) Linking removal targets to the ecological effects of invaders: a predictive model and field test. Ecol Appl 24:1311–1322. doi: 10.1890/13-0979.1
- Green SJ, Tamburello N, Miller SE, et al (2013) Habitat complexity and fish size affect the detection of Indo-Pacific lionfish on invaded coral reefs. Coral Reefs 32:413–421. doi: 10.1007/s00338-012-0987-8
- Grol MGG, Rypel AL, Nagelkerken I (2014) Growth potential and predation risk drive ontogenetic shifts among nursery habitats in a coral reef fish. Marine Ecology Progress Series 502:229–244.
- Hedges LV, Olkin I (1985) Statistical Method for Meta-Analysis. Academic Press, Orlando, FL
- Higgins JPT, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. Statist Med 21:1539–1558. doi: 10.1002/sim.1186
- Hinderstein LM, Marr JCA, Martinez FA, et al (2010) Theme section on "Mesophotic Coral Ecosystems: Characterization, Ecology, and Management." Coral Reefs 29:247–251. doi: 10.1007/s00338-010-0614-5
- Kimball ME, Miller J, Whitfield PE, Hare JA (2004) Thermal tolerance and potential distribution of invasive lionfish (*Pterois volitans/miles* complex) on the east coast of the United States. Marine Ecology Progress Series 283:269–278.
- Kimirei IA, Nagelkerken I, Trommelen M, et al (2013) What Drives Ontogenetic Niche Shifts of Fishes in Coral Reef Ecosystems? Ecosystems 16:783–796. doi: 10.1007/s10021-013-9645-4
- Koricheva J, Gurevitch J, Mengersen K (2013) Handbook of Meta-analysis in Ecology and Evolution, 1st edn. Princeton University Press

- Kulbicki M, Beets J, Chabanet P, et al (2012) Distributions of Indo-Pacific lionfishes *Pterois spp*. in their native ranges: implications for the Atlantic invasion. Marine Ecology Progress Series 446:189–205. doi: 10.3354/meps09442
- Lee S, Buddo DSA, Aiken KA (2012) Habitat Preference in the Invasive Lionfish (*Pterois volitans/miles*) in Discovery Bay, Jamaica: Use of GIS in Management Strategies. Proceedings of the 64rd Gulf and Caribbean Fisheries Institute October 31 - November 5, 2011 Puerto Morelos, Mexico 64:39–48.
- Lesser MP, Slattery M (2011) Phase shift to algal dominated communities at mesophotic depths associated with lionfish (*Pterois volitans*) invasion on a Bahamian coral reef. Biol Invasions 13:1855–1868. doi: 10.1007/s10530-011-0005-z
- Lindfield SJ, McIlwain JL, Harvey ES (2014) Depth Refuge and the Impacts of SCUBA Spearfishing on Coral Reef Fishes. PLoS ONE 9:e92628. doi: 10.1371/journal.pone.0092628
- McTee SA, Grubich JR (2014) Native densities, distribution, and diurnal activity of Red Sea lionfishes (Scorpaenidae). Marine Ecology Progress Series 508:223–232. doi: 10.3354/meps10847
- Mitchell SJ, Doolette DJ (2013) Recreational technical diving part 1: an introduction to technical diving methods and activities. Diving and Hyperbaric Medicine, 43:86–93.
- Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat of invasive species to marine biodiversity. Frontiers in Ecology and the Environment 6:485–492.
- Morris JA, Akins JL, Barse A, et al (2009) Biology and ecology of the invasive lionfishes, *Pterois miles* and *Pterois volitans*. Proceedings of the Gulf and Caribbean Fisheries Institute 61:409–414.

- Morris JA, Thomas A, Rhyne AL, Breen N, Akins L, Nash B (2011) Nutritional Properties of the Invasive Lionfish: A Delicious and Nutritious Approach for Controlling the Invasion. AACL Bioflux, 4:21–26.
- Nuttall MF, Johnston MA, Eckert RJ, et al (2014) Lionfish (*Pterois volitans* [Linnaeus, 1758] and *P. miles* [Bennett, 1828]) records within mesophotic depth ranges on natural banks in the Northwestern Gulf of Mexico. BioInvasions Records 3:111–115.
- Pitt JM, Trott TM (2015) Trapping Lionfish in Bermuda, Part II: Lessons learned to date. In Proceedings of the 67th Gulf and Caribbean Fisheries Institute November 3-7 2014. Christ Church, Barbados, 67:221–224.
- Pyle RL (1998) Use of Advanced Mixed-Gas Diving Technology to Explore the Coral Reef "Twilight Zone." In: Tanacredi JT, Loret J (eds) Ocean Pulse: A Critical Diagnosis, 1st edn. Springer, New York, pp 71–88
- R Core Team (2013) R: A language and environment for statistical computing. R-project.org
- Raudenbush SW (2008) Analyzing effect sizes: Random effects models. In: Cooper H, Hedges LV, Valentine JC (eds) The Handbook of Research Synthesis and Meta-Analysis, 2nd edn. Russell Sage Foundation, New York, p 632
- Rocha LA, Rocha CR, Baldwin CC, et al (2015) Invasive lionfish preying on critically endangered reef fish. Coral Reefs 34:803–806. doi: 10.1007/s00338-015-1293-z
- Schofield PJ (2010) Update on geographic spread of invasive lionfishes (*Pterois volitans* [Linnaeus, 1758] and *P. miles* [Bennett, 1828]) in the Western North Atlantic Ocean, Caribbean Sea and Gulf of Mexico. Aquatic Invasions 5:S117–S122.

- Simberloff D, Martin J-L, Genovesi P, et al (2013) Impacts of biological invasions: what's what and the way forward. Trends in Ecology & Evolution 28:58–66. doi: 10.1016/j.tree.2012.07.013
- Smith SR, Sarkis S, Murdoch TJ, et al (2013) Threats to Coral Reefs of Bermuda. In: Sheppard CRC (ed) Coral Reefs of the United Kingdom Overseas Territories, Coral Reefs of the World 4. Springer, pp 173–188
- Thresher RE, Kuris AM (2004) Options for Managing Invasive Marine Species. Biol Invasions 6:295–300. doi: 10.1023/B:BINV.0000034598.28718.2e
- Travis JMJ, Park KJ (2004) Spatial structure and the control of invasive alien species. Anim Conserv 7:321–330. doi: 10.1017/S1367943004001507
- Trégarot E, Fumaroli M, Arqué A, et al (2015) First records of the red lionfish (*Pterois volitans*) in Martinique, French West Indies: monitoring invasion status through visual surveys. Mar Biodivers Rec 8:e1. doi: 10.1017/S1755267214001341
- Tyler E, Speight M, Henderson P, Manica A (2009) Evidence for a depth refuge effect in artisanal coral reef fisheries. Biol Conserv 142:652–667. doi: 10.1016/j.biocon.2008.11.017
- Viechtbauer W (2010) Conducting Meta-Analyses in R with the metafor Package. In: Journal of Statistical Software. http://www.jstatsoft.org/v36/i03/. Accessed 30 May 2013
- Werner EE, Gilliam JF (1984) The Ontogenetic Niche and Species Interactions in Size-Structured Populations. Annual Review of Ecology and Systematics 15:393–425.
- Whitfield PE, Muñoz RC, Buckel CA, et al (2014) Native fish community structure and Indo-Pacific lionfish *Pterois volitans* densities along a depth-temperature gradient in Onslow Bay, North Carolina, USA. Marine Ecology Progress Series 509:241–254. doi: 10.3354/meps10882

- Williams SL, Grosholz ED (2008) The Invasive Species Challenge in Estuarine and Coastal Environments: Marrying Management and Science. Estuaries and Coasts 31:3–20. doi: 10.1007/s12237-007-9031-6
- Wright DJ (2005) Report of HURL Cruise KOK0510: Submersible Dives and Multibeam Mapping to Investigate Benthic Habitats of Tutuila, American Samoa. Technical Report, NOAA's Office of Undersea Research Submersible Science Program, Hawai’i Undersea Research Lab

7 TESTING ONTOGENETIC MIGRATIONS IN INVASIVE LIONFISH POPULATIONS ACROSS SHALLOW TO MESOPHOTIC CORAL REEFS

7.1 Abstract

Invasive lionfishes (*Pterois volitans* and *P. miles*) are recognised as a major threat to native western Atlantic marine biodiversity. Although lionfish inhabit both shallow reefs and mesophotic coral ecosystems (MCEs; reefs from 30-40 to 150 m depth), the primary management response implemented by many countries has been culling limited to shallow reefs (<30 m). Here we study lionfish populations from the surface down to 85 m around Utila, Honduras. We found lionfish at increased densities, body size, weight and maturity on MCEs compared to shallow reefs, with the greatest proportion of immature lionfish at shallower depths. In addition, we found lionfish diets remarkably consistent regardless of depth. Importantly, our results support the previously proposed ontogenetic migration of invasive lionfish onto MCEs, suggesting that MCE lionfish are likely to be disproportionately contributing towards lionfish recruitment onto the shallow reefs of Utila, potentially undermining current culling-based management.

Chapter 7: Testing ontogenetic migrations in invasive lionfish populations across shallow to mesophotic coral reefs

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7.2 Introduction

Lionfish, native to the Indian and Pacific Oceans and Red Sea, were first recorded in the western Atlantic in the 1980s, and have since become a major invasive species (Schofield 2009; 2010). Two lionfish species have been recorded in this region, *Pterois volitans* and *P. miles*, though it is believed that *P. volitans* is responsible for much of the invasion, with *P. miles* mostly restricted to the waters around the US mainland (Freshwater et al. 2009). On western Atlantic shallow reefs, lionfish have been reported 1.5 times larger and three times heavier than in their native range (Darling et al. 2011). Lionfish are carnivores, and their arrival on shallow patch reef systems has been associated with declines in native fish recruitment of up to 79 % (Albins and Hixon 2008) and prey fish declines of up to 65 % (Green et al. 2012a). The most common management response to the invasion has been the introduction of lionfish culling programs, where volunteer divers use handheld spears to actively remove lionfish from the reef (Morris 2012). Culling programs in some areas have been effective in reducing lionfish densities (Frazer et al. 2012), but as they are a highly fecund species, culling rates must remain high or populations will quickly recover (Arias-González et al. 2011).

Mesophotic coral ecosystems (MCEs; reefs from 30-150 m depth) are highly understudied (Menza et al. 2008), with most of the limited research focused on their native biodiversity (Kahng et al. 2010; 2014), although a recent review has highlighted the potential threat posed to MCEs by invasive species (Chapter 9). Lionfish now appear to have widely invaded MCEs throughout the western Atlantic region (Schofield 2010; Lesser and Slattery 2011; Nuttall et al. 2014). This widespread invasion is unsurprising, as they have been reported from MCEs in several locations across their native range including in the Red Sea (Brokovich et al. 2008), the Philippines and Micronesia (Chapter 6), New Caledonia (Kulbicki et al. 2012), and American Samoa (Wright 2005). While lionfish densities in the invaded range are much higher than in the native range (Darling et al. 2011), their relative abundance across adjacent shallow reef to MCE gradients appear similar between invaded and native range sites (Chapter 6) . In contrast to shallow reefs, the impacts of invasive lionfish on MCEs are poorly understood, though there is evidence that their consumption of trophic fish groups such as herbivores can lower grazing pressure on algal communities, leading to supressed scleractinian coral growth and algal dominated reefs (Lesser and Slattery 2011; Slattery and Lesser 2014).

Many tropical reef-fish species exhibit ontogenetic migrations, with juveniles recruited into shallow nursery habitats such as mangroves and seagrass beds before moving onto more exposed reef as they mature (Cocheret de la Morinière et al. 2002). Female lionfish spawn buoyant egg masses that float to the surface before breaking apart (Fishelson 1978), with juveniles recruiting into shallow marine habitats. Analysis using proxy measures for maturity, such as fish weight or length, have suggested ontogenetic migrations between these shallow marine habitats and reef slopes (<30 m) (Claydon et al. 2012). Previous studies investigating whether lionfish ontogenetic migrations extend onto MCEs have relied on similar proxy measures for maturity, and found mixed results; for example in the Leeward Antilles, some locations had heavier lionfish on deeper reefs than shallow reefs, while other locations showed no correlation between depth and weight (de León et al. 2013). A recent meta-analysis across the western Atlantic identified lionfish with larger body lengths on MCEs than shallow reefs in three countries; The Bahamas, Curaçao and Honduras, with one country showing no relationship and another showed the reverse trend (Chapter 6). Therefore, it is not clear whether the previously documented ontogenetic migration to shallow reef slopes extends to MCEs, and no studies have directly assessed lionfish maturity across the depth gradients.

As most lionfish culling is conducted by volunteer recreational divers, removals are generally limited to <30 m, with the majority concentrated even shallower. MCEs therefore have the potential to act as lionfish refuges, undermining culling efforts on shallow reefs (Arias-González et al. 2011). This refuge effect could act through several mechanisms, including: (i) large lionfish densities remaining on MCEs following shallow reef focused lionfish culling, or (ii) larger, and therefore more fecund lionfish present on MCEs than shallow reefs, driven by onogenetic migration, which could be disproportionately responsible for new recruitment.

In this study we used the reefs surrounding Utila, Honduras, to represent a contemporary Caribbean post-invasion reef system that extends across the shallow reef to MCE depth range and experiences significant shallow culling. We surveyed lionfish population from the surface down to 85 m depth and speared 1,392 lionfish to conduct detailed fish measurements and dissections, in particular to record lionfish maturity directly from the gonad developmental stage. We specifically tested whether (i) lionfish densities remain high on MCEs despite shallow reef culling, and (ii) lionfish are more mature at increased depths, suggesting a refuge role for MCEs enabling invasive lionfish to persist despite invasive species management interventions.

7.3 Methods

7.3.1 Study site

Lionfish surveys and collections were conducted around the island of Utila, Honduras, on the southern Mesoamerican Barrier Reef (Figure 7.1). Utila is located off the north coast of mainland Honduras within the Bay Islands National Marine Park, and is surrounded by fringing coral reefs. On the south shore, shallow reefs exist as a spur and groove system, sloping to approximately 30-40 m where the seabed flattens and an MCE patch reef system exists. On the north shore reefs are characterised by steep walls dropping from the crest at

6–10 m to >100 m. North shore walls are broken with several narrow ledges approximately 20 m wide at various depths depending on the site, and large back reef areas exist behind the reef crest. Off the south-west of Utila there are a series of cays surrounded by reef, and throughout the Bay Islands there are extensive off-shore reef banks rising from the seabed with established shallow and MCE communities. Lionfish were first reported in the Bay Islands in May 2009 with numerous sightings throughout late 2009 and 2010 (Schofield 2010), followed by widespread colonisation of reef habitats.

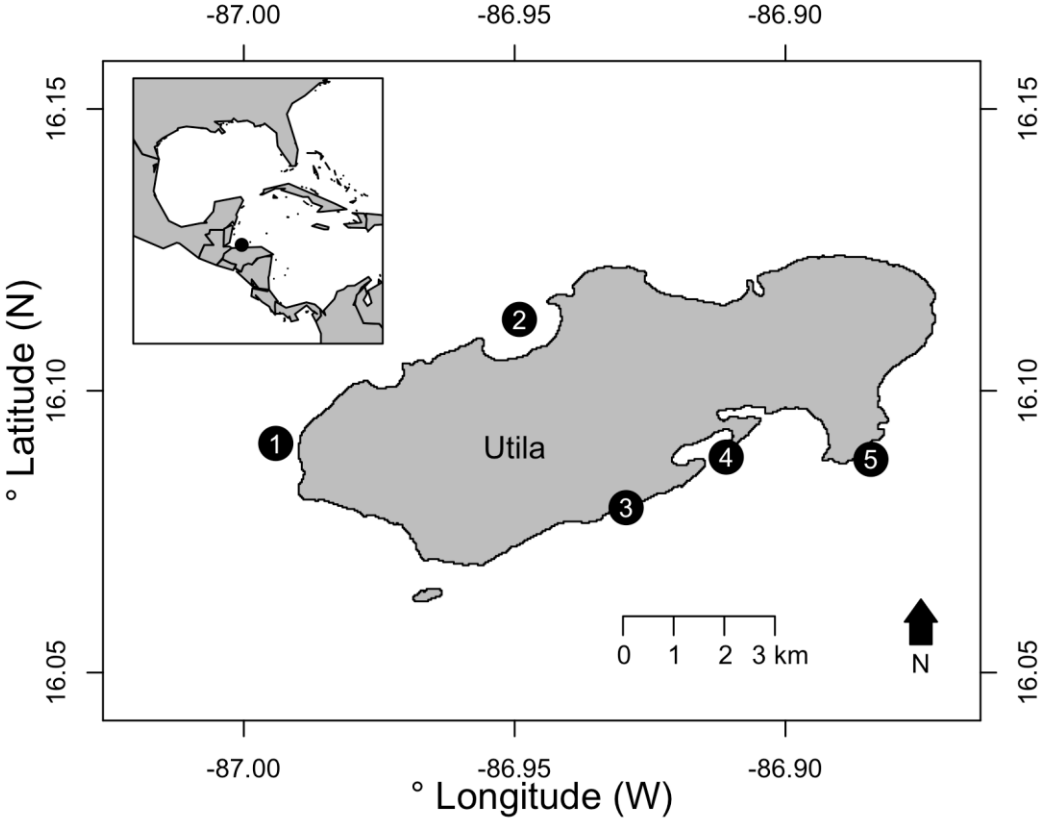

Figure 7.1 Lionfish density survey locations around Utila, Honduras.

Survey locations are marked, with numbers indicating sites as follows: (1) Raggedy Cay, (2) The Maze, (3) Little Bight, (4) Coral View, (5) Rocky Point. Inset map shows the location of Utila relative to the western Atlantic region.

7.3.2 Lionfish density

Lionfish population densities were surveyed during July–September 2015 using video transects with a diver-operated stereo-video system (Chapter 5). Transects were conducted on shallow reefs and MCEs at five sites (Figure 7.1) at the following depths: 5, 15, 25, 40, 55, 70 and 85 m. Transects were completed to a maximum depth of 40 m at Little Bight and Coral View, 55 m at Raggedy Cay and Rocky Point and 85 m at The Maze. These maximum survey depths represent the maximum depth of each site, with the exception of Raggedy Cay and The Maze, where they represent the approximate depth limit of light dependent scleractinian corals based on preliminary dive surveys. All transects were carried out by divers using Hollis Prism 2 (Hollis, San Leandro, CA, USA) or rEvo X micro (rEvo rebreathers, Bruges, Belgium) closed-circuit rebreathers (Chapter 5). Four 50 m long transects were conducted along each of the respective depth contours at each site following the survey protocol outlined in Chapter 10. Transects were analysed using EventMeasure software v3.51 (SeaGIS, Melbourne, Australia), with boundaries defined 2.5 m either side of the transect giving a total number of lionfish per 250 m^2 survey area for each transect.

7.3.3 Lionfish collection and dissection

To enable the detailed study of changes in lionfish biology across the depth gradient we used data collected from lionfish hand speared by divers around Utila. Lionfish data came from four sources: (i) Bay Islands Conservation Association (BICA) lionfish derbies, (ii) Utila Whale Shark and Oceanic Research Center lionfish culling program, (iii) Operation Wallacea lionfish monitoring program and (iv) the University of Oxford Thinking Deep MCE expedition. The contributions to this study from each source are shown in Table 7.1, with lionfish collected between 2014–2016. All spearing was conducted under permits issued by BICA (the local lionfish management authority), with ethical approval granted to the work by the University of Oxford Department of Zoology Local Ethical Review Committee. Research permits for Operation Wallacea and the University of Oxford were issued by the Instituto de Conservación Forestal (ICF), Honduras (permit number: ICF-261-16).

Table 7.1 Source of lionfish used in the study, collection year and number of lionfish.

Source	Month(s)	Year	Number of lionfish
Bay Islands Conservation Association Lionfish Derby	Jul	2015	118
	Jul	2016	195
Whale Shark and Oceanic Research Center Lionfish Culling Program	Apr - Dec	2015	526
	Jan - Aug	2016	365
Operation Wallacea Lionfish Monitoring	Jun - Aug	2014	60
	Jun - Aug	2015	44
	Jun - Aug	2016	53
University of Oxford Thinking Deep Expedition	Aug - Sep	2015	31

Lionfish derbies are a community approach to lionfish removal in the western Atlantic, with divers forming teams to compete against each other to remove the most lionfish from the reefs (Malpica-Cruz et al. 2016). Dive teams collecting lionfish in the BICA lionfish derbies were briefed on the importance of collecting all lionfish regardless of size. To minimize risk of biases in size of fish collected, the derby awarded prizes for the team with the most fish collected, and to the team collecting the largest, smallest and median sized fish. The depth of collection was recorded for all lionfish within three depth intervals; shallow: 0–25 m, intermediate: 25–40 m and MCE: >40 m. Lionfish collected from sources other than the derby were collected on a mixture of dedicated lionfish spearing dives and opportunistically on other survey and recreational dives during the time period indicated in Table 7.1, with exact depth of collection recorded, or in the case of many fish being collected, using the same depth bands as the derby.

All collected lionfish were dissected following the standardized dissection techniques outlined by Green et al. (2012b). In summary: recording total length (length from the tip of snout to the end of the caudal fin), weight, sex and gonad weight. Female gonads were staged on a 5 level score as follows: (1) immature, (2) early developing, (3) developing, (4) spawning capable and (5) actively spawning. Males were staged as (1) immature and (2) spawning capable. See Green et al. (2012b) for images and detailed descriptions of each gonad stage. Fat tissue was removed by hand from around internal organs and weighed and used as indicator of lionfish health, with greater fat suggesting the lionfish were healthier. The proportion of lionfish body fat was calculated as the fat tissue weight divided by total weight for each lionfish. Lionfish stomachs were opened and the contents were counted and identified into broad categories: fish, shrimp, crab, other invertebrates and algae. If possible, consumed fish were identified to family level.

7.3.4 Data analysis

To identify changes in lionfish length, weight, maturity and proportion of body fat with depth we used analysis of variance (ANOVA) on the untransformed variables based on three depth categories: 0–25, 25–40 and >40 m. We assessed differences between groups using Tukey's Honest Significant Differences, considering results significant if $p<0.05$. To identify differences in the feeding rates at different depths we divided lionfish stomachs into successes (food items present), and failures (food items absent) and fitted a binomial generalized linear model (GLM) with a logit link function. We examined patterns in lionfish prey fish consumption across the depth bands using principle components analysis. Vectors were then plotted to indicate the relationship between fish families and the depth bands. The principle components analysis was constructed using the functions 'rda' in the package 'vegan' (Oksanen et al. 2013) in R (R Core Team 2013).

7.4 Results

7.4.1 Lionfish distribution

When conducting lionfish density surveys across the depth gradient, no lionfish were found at 5, 15 or 25 m at any of the five sites surveyed (Figure 7.2A). Lionfish were recorded at all depths ≥40 m, with highest densities recorded at The Maze at 70 m, where mean density was 3 individuals per 250m^2 (Figure 7.2). Our lionfish dissection data incorporated lionfish collected from just below the surface (<1 m) through to the deepest lionfish collected at 72 m. Lionfish body length was found to change with depth ($F_{2,1352}=5.0$, $p=0.007$), though no difference in mean lionfish length was identified by the post-hoc Tukey's Honest Significant Difference (THSD) test between the shallow reef (0 – 25 m) and intermediate depth band (25 – 40 m), with mean lengths (± standard error) of 25.8 ± 0.26 cm and 25.4 ± 0.7 cm respectively (Figure 7.2B). However, lionfish found on MCEs (40 – 72 m) were larger on average than shallow (THSD: $p=0.008$) and intermediate (THSD: $p=0.013$) groups, at 28.1 ± 0.7 cm. Similar to body length, lionfish weight increased with depth (Figure 7.2C, $F_{2,737}=39.1$, $p<0.001$). Shallow lionfish weighed on average 0.28 ± 0.01 Kg, which was less than the intermediate (0.43 ± 0.02 Kg, THSD: $p<0.001$) or the MCE (0.40 ± 0.02 Kg, THSD: $p<0.001$), with no difference in weight between intermediate and MCE depth lionfish.

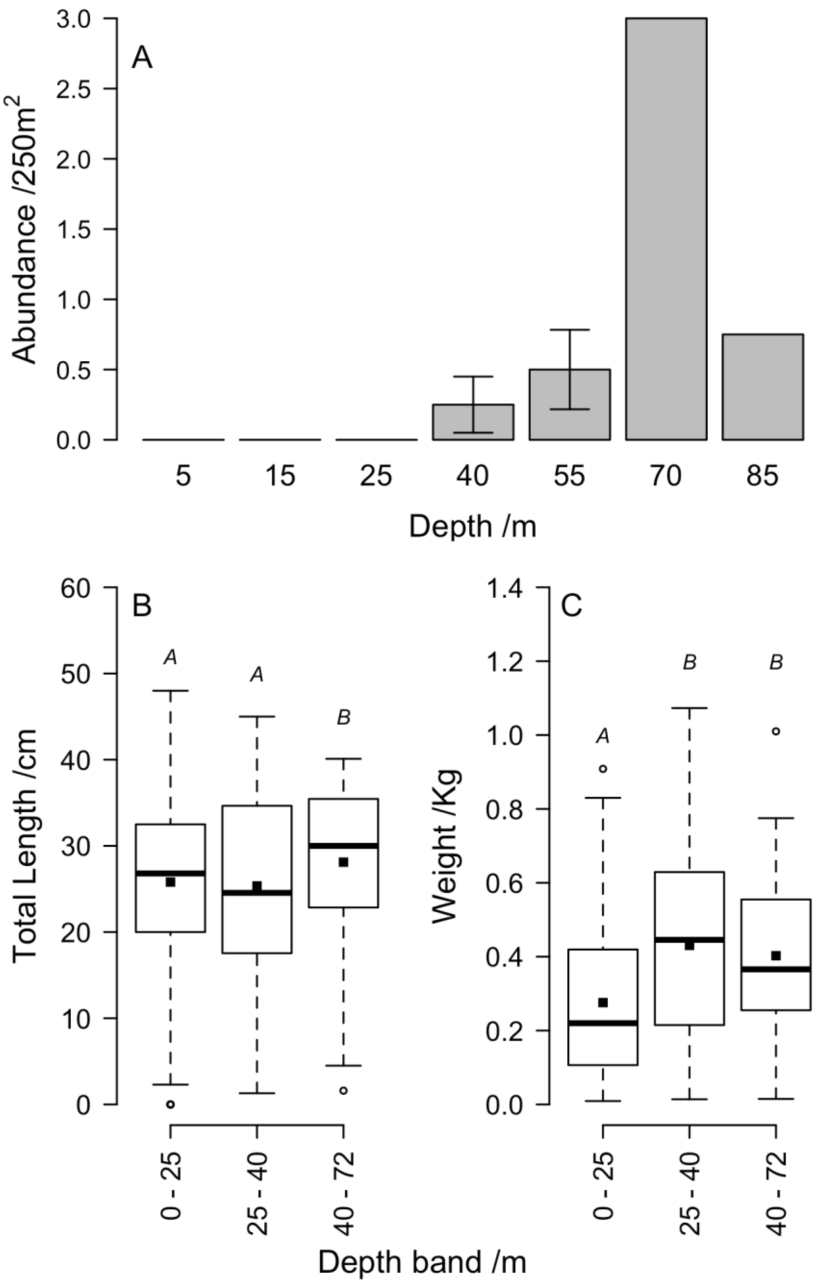

Figure 7.2 Lionfish (A) abundance, (B) body size and (C) weight changes.

(A) Lionfish abundance recorded across the depth gradient. Lionfish abundance was recorded at all five sites for 5, 15, 25 and 40 m, at Raggedy Cay, The Maze and Rocky Point for 55 m, and only at The Maze for 70 and 85 m. Error bars indicate 95 % confidence intervals. No lionfish were recorded on any transects at 5, 15 or 25 m. (B) Lionfish length and (C) lionfish weight, where the solid black line represents the median, with the box indicating the upper and lower quartiles and whiskers representing the maximum or minimum observed value that is within 1.5 times the interquartile range of the upper or lower quartile respectively. Squares represent the mean. Letters designate significantly different groupings indicated by a post-hoc Tukey Honest Significant Difference Test.

Female lionfish maturity changed with depth, both in terms of mean gonad stage (Figure 7.3A, $F_{2,441}$=3.7, p=0.027), and mean gonad weight (Figure 7.3C, $F_{2,190}$=11.1, p<0.001). In both cases, MCE female lionfish had higher average gonad stages and gonad weight than shallow or intermediate depth female lionfish. While male mean gonad stages were different between depth groups ($F_{2,657}$=3.8, p=0.022), these did not indicate a consistent pattern across the shallow to MCE depth gradient. Patterns in gonad stages were likely influenced by the declining proportion of immature lionfish with increased depth (Figure 7.3B). However, only 37 % of immature lionfish could be sexed, with these sexed immature individuals representing 10 % of all males and 5 % of all females staged. While the male gonad staging did not indicate a clear relationship with depth, the male gonad weight at MCEs was greater than that recorded in the shallows (Figure 7.3D, THSD: p=0.049).

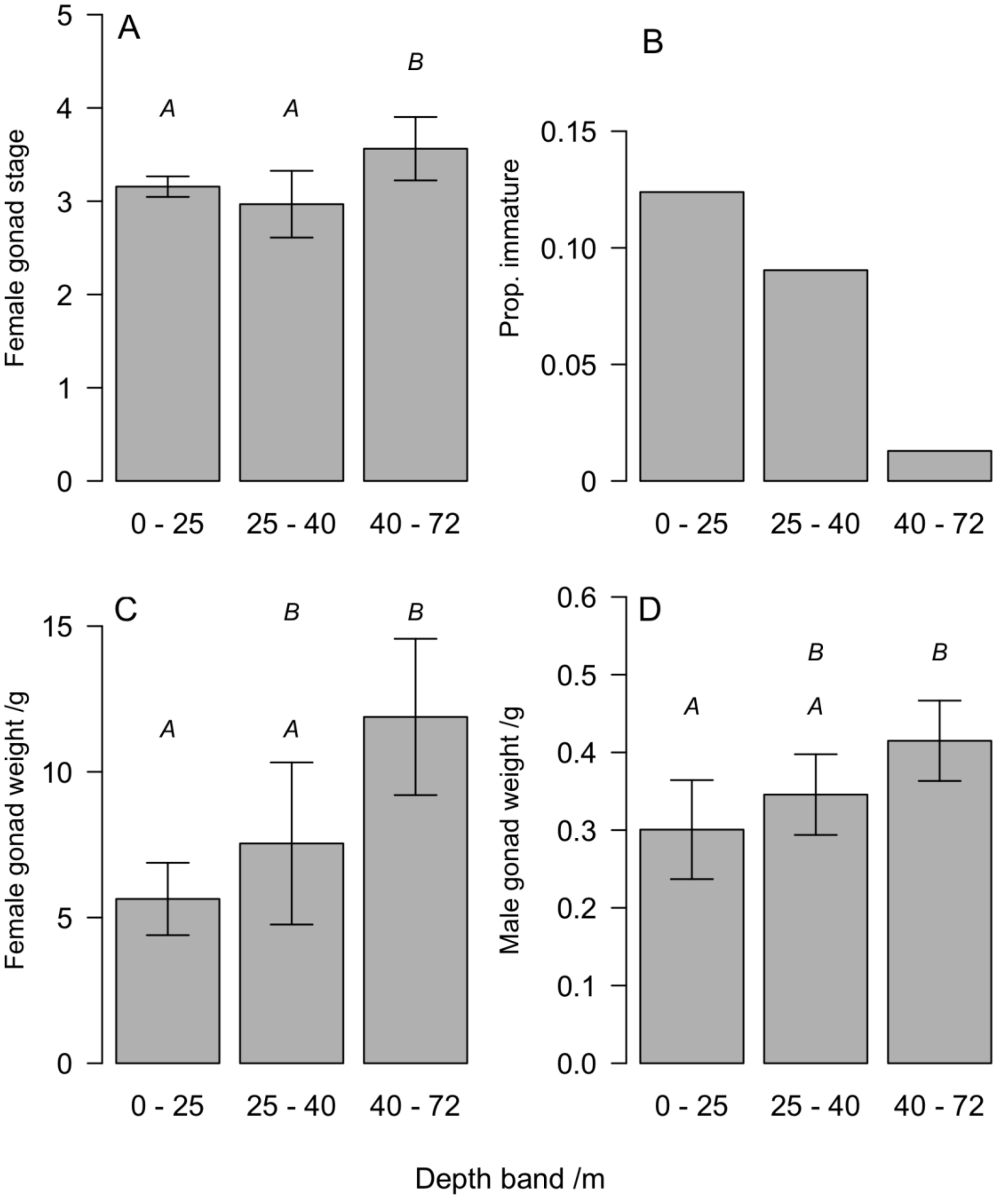

Figure 7.3 Lionfish maturity and gonad weight with depth.

(A) Female gonad stage, (B) immature lionfish as a proportion of the total lionfish recorded in each depth band, (C) female gonad weight and (D) male gonad weight. Error bars indicate 95 % confidence intervals. Letters designate significantly different groupings indicated by a post-hoc Tukey Honest Significance Difference Test.

7.4.2 Lionfish condition and feeding

The proportion of body fat changed significantly with depth (Figure 7.4A, $F_{2,482}$=8.9, p<0.001), with MCE lionfish having the lowest proportion of body fat at 0.0030 ± 0.0005, significantly lower than lionfish on intermediate depth reefs (0.0060 ± 0.0007, THSD:

p=0.005), or shallow reefs (0.0063 ± 0.0004, THSD: *p*<0.001). There was no difference in the proportion of body fat between lionfish on the shallow or intermediate depth reefs (Figure 7.4A). Because we previously identified changes in lionfish body size with depth, to ensure patterns in the proportion of body fat were not driven by changes in body size we tested for a correlation between lionfish body length and proportion of body fat and found no relationship (Pearson correlation coefficient: -0.01, *t*=-0.35, df=686, *p*=0.73). For female lionfish we tested for a relationship between gonad weight and the proportion of body fat controlling for maturity level, fish weight and depth. We found that females with a lower proportion of body fat had increased gonad weight (Table 7.2).

Table 7.2 ANCOVA results for the effect of proportion of body fat, maturity, weight and depth on female lionfish gonad weight.

Lionfish gonad weight was fourth root transformed to meet ANCOVA assumptions. Depth was fitted as a categorical variable, with the intercept representing Depth: 0-25 m, and other depth values given as a difference from the intercept. Maturity and Weight estimates represent slopes of the model line.

	Estimate	**Standard Error**	***t* value**	**P**
Intercept	0.282	0.06	4.64	<0.001
Proportion of fat	-9.737	3.14	-3.10	0.002
Maturity	0.235	0.02	11.89	<0.001
Weight	0.002	0.00	8.54	<0.001
Depth: 25-40 m	0.107	0.05	2.08	0.040
Depth: 40-72 m	0.132	0.05	2.88	0.004

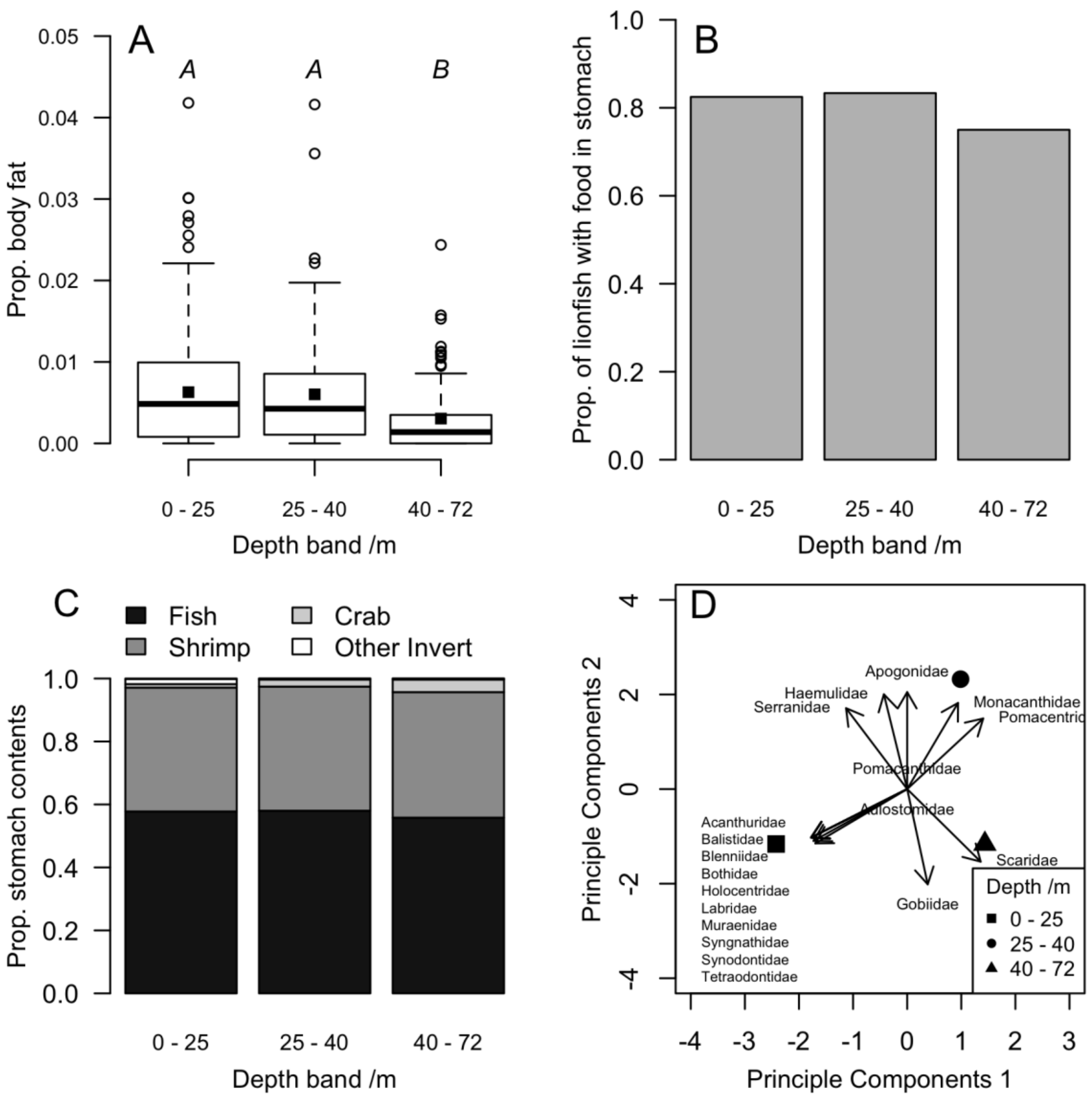

Figure 7.4 Lionfish condition and diet with depth.

(A) Proportion of lionfish body weight comprised of fat. The solid black line represents the median, with the box indicating the upper and lower quartiles and whiskers representing the maximum or minimum observed value that is within 1.5 times the interquartile range of the upper or lower quartile respectively. Squares represent the mean. Letters designate significantly different groupings indicated by a post-hoc Tukey Honest Significant Difference Test. (B) Proportion of lionfish with food in the their stomachs. No difference was found between depth bands (Table 7.3). (C) Proportion of lionfish stomach contents comprised of fish, shrimp, crab and other invertebrates. (D) Principle components analysis indicating similarities between fish family abundance found in lionfish stomachs at different depths. Plot based on mean fish family abundance across all lionfish stomachs in each depth band. Arrows indicate relationships with different fish families. The list of families in the bottom left of the plot represent the families associated with shallow reefs (0-25 m) and the overlapping arrows pointing towards the lower left.

We found no difference in the proportion of lionfish stomachs containing food with depth (Figure 7.4B, Table 7.3), and the diet composition of lionfish remained remarkably consistent across the depth gradient (Figure 7.4C). Fish were the dominant component, accounting for between 56–58 % of all stomach items across all depths. Shrimp were the second most common item, comprising 39–40 % of all items at all depths. All other items combined accounted for <4 % of lionfish diets, though crabs did show a slight increase in lionfish diets with depth, comprising 1.1 % in the shallows, 2.2 % at intermediate depths and 4.0 % on MCEs. Algae of the genus *Halimeda* and *Dictyota* was found in the stomachs of seven shallow lionfish, but only accounted for 0.1 % of the shallow lionfish stomach items overall. The principle components plot of fish family abundance found in lionfish stomachs suggests each depth band has a unique assemblage (Figure 7.4D). The intermediate and MCE depth assemblages were placed closer to one another than to the shallows, implying that while they are different assemblages, there is more similarity between the fish families consumed at these two depths. Gobiidae were the most consumed fish family in the shallows and on MCEs, making up 31 % and 43 % of fish found in lionfish stomachs respectively (Table 7.4). Both Pomacentridae and Scaridae made up a large proportion of fish consumed on MCEs by lionfish, yet made up a much smaller proportion in the shallows (Table 7.4).

Table 7.3 GLM results for differences in the proportion of lionfish stomachs containing food with depth.

Binomial GLM was fitted with a logit link function. The intercept represents the shallow group (0–25 m), with other estimates given as the difference from the intercept. Residual deviance: 1135.4 on 1201 degrees of freedom.

	Estimate	Standard Error	*z* value	P
Intercept	1.55	0.08	18.28	<0.001
25 – 40 m	0.06	0.25	0.25	0.806
40 – 72 m	-0.45	0.23	-1.89	0.059

Table 7.4 Proportion of fish by family recorded within lionfish stomachs within each depth band.

Family	Proportion of fish within depth band /m		
	0 - 25	25 - 40	40 - 72
Acanthuridae	0.02	0.00	0.00
Apogonidae	0.00	0.07	0.00
Balistidae	0.01	0.00	0.00
Blenniidae	0.26	0.07	0.07
Gobiidae	0.31	0.15	0.43
Haemulidae	0.01	0.04	0.00
Holocentridae	0.01	0.00	0.00
Labridae	0.17	0.07	0.07
Monacanthidae	0.03	0.19	0.07
Pomacentridae	0.05	0.22	0.14
Scaridae	0.08	0.11	0.21
Serranidae	0.03	0.07	0.00
Synodontidae	0.01	0.00	0.00
Tetraodontidae	0.01	0.00	0.00

7.5 Discussion

We identified that MCEs surrounding Utila are home to greater densities of lionfish than nearby shallow reefs, suggesting that MCEs act as a refuge for invasive lionfish from the current range of culling in Utila. Lionfish populations on deeper reefs contained higher proportions of mature individuals, with higher mean gonad weights, and females displayed higher maturity scores than shallow lionfish. This suggests that this MCE population refuge not only allows individual lionfish to evade culling, but is also a preferred habitat of mature fish. Our findings highlight the importance of incorporating lionfish populations on MCEs into lionfish management decision-making.

7.5.1 Greater lionfish density on MCEs than shallow reefs

Lionfish densities on MCEs was greater than shallow reefs, with no lionfish recorded on our transects at 5, 15, or 25 m. Considering the high shallow reef lionfish densities reported from many western Atlantic locations without culling (Darling et al. 2011; Frazer et al. 2012), these results suggest that the Utila lionfish culling is effectively reducing shallow lionfish numbers. Our results indicate, however, that reefs below the range of lionfish removal retain substantial lionfish populations. The high lionfish densities at 70 m compared to 85 m at the Maze were likely influenced by reef habitat availability, with MCEs at this site characterised by steep walls broken by ledges approximately 20 m wide (Chapter 10). Our 70 m transects at the Maze coincided with one such ledge, providing a large area of reef habitat, while our 85 m transects were on a wall. Therefore differences in lionfish density within MCEs (40–85 m) should be treated with caution because fewer sites were surveyed at 55 m, and only one site at 70 and 85 m. Regardless of variation in lionfish density on MCEs, our results clearly indicate the refuge role played by MCEs for lionfish in the presence of shallow culling.

7.5.2 Potential lionfish ontogenetic movements across shallow reef to MCE depth gradients

We detected declining proportions of immature lionfish with depth, supporting previous observations of ontogenetic movements in lionfish (Claydon et al. 2012). In addition to our results agreeing with previously documented increases in lionfish body size with depth (de León et al. 2013; Chapter 6), we also found female lionfish to be more mature at increased depths, and the mean gonad weight for both male and female lionfish to be higher with depth (Figure 7.3). The lack of significant relationship between male maturity and depth was likely caused by our staging system for lionfish gonads, combined with our inability to sex many immature individuals. Because of limited visual differences in male lionfish gonads (Green et al. 2012b), males were only categorised as immature or mature. Although only 37 % of immature lionfish could be sexed, immature lionfish were more common at shallower depths. Had it been possible to sex all immature lionfish, it is likely that we would observe an increase in male maturity with increased depth alongside the trend of increased male gonad weight with increased depth. Detecting differences in female lionfish maturity across depths is also likely to suffer from under-inclusion of immature females, however, as maturity was assessed on a scale of 1–5, significant differences with depth were still apparent.

We analysed lionfish stomachs and found no difference in the proportion containing food with depth. Despite this there were differences in the proportion of body fat, with MCE lionfish having lower body fat levels than shallow counterparts. We found female lionfish with lower body fat levels have greater gonad weights while controlling for lionfish body size and maturity. This suggests that lower fat stores could be indicative of energy being partitioned to gonad development and reproductive output, with the most reproductively active lionfish found on MCEs. Our results also suggest that other changes in lionfish condition could be occurring across the depth gradient. For example, total length of lionfish was not different between shallow and intermediate reefs, yet MCEs had longer lionfish. In contrast, lionfish on shallow reefs weight less than lionfish on both intermediate depth reefs and MCEs. These differences suggest that lionfish body depth could also be increasing across the depth gradient, with deeper lionfish becoming larger. While we did not directly measure body depth, we recommend future studies consider this metric.

Our results found that individual lionfish on MCEs are larger and more mature than those in the shallows. This pattern fits with previous studies documenting increased maturity at greater depths across shallow reef to MCE gradients for native western Atlantic reef fish (Goldstein et al. 2016). It is well established in many fish species that larger older females have greater fecundity than smaller younger females, and that larger older females produce larvae with greater survival rates than younger females (Birkeland and Dayton 2005; Hixon et al. 2014). These differing survival rates can be caused by decreased egg size in smaller females (Hislop 1988), or by increased energy reserve provision in eggs produced by older mothers (Berkeley et al. 2004), leading to faster growth rates in larvae and thus enhanced recruitment success (Bergenius et al. 2002). This suggests that in locations such as Utila, where MCE lionfish are larger and more mature than their shallow reef conspecifics they may be disproportionately responsible for continued new shallow reef lionfish recruitment than their population densities alone would suggest.

While increases in mean length, weight, maturity and gonad weight at increased depth are indicators of ontogenetic migrations in other western Atlantic reef fish species (Cocheret de la Morinière et al. 2002; Goldstein et al. 2016), they do not conclusively indicate an ontogenetic migration occurring in lionfish. Previous shallow reef culling has been found to result in decreased mean lionfish body length in lionfish populations (Frazer et al. 2012), possibly because of bias by divers to remove larger, more easily detectable lionfish (Green et al. 2013). Despite observing greater mean lionfish length, weight and gonad weight on MCEs there were high levels of variation in the data at all depths. For example, some large mature fish and some small immature fish were recorded at all depths. Therefore, while our results are consistent with the lionfish depth patterns expected from ontogenetic migrations, these results could be explained by the presence of culling. To establish whether these patterns are caused by ontogenetic migrations or culling we recommend future studies compare age estimates from lionfish otoliths across the depth gradient between locations with and without culling. However, regardless of the mechanism causing the observed differences in lionfish size and maturity across the depth gradient, our results clearly still highlight that MCEs are acting as depth refuges for lionfish on Utila, and that these MCE lionfish may be disproportionately responsible for new lionfish recruits entering the population.

7.5.3 Differences in lionfish diet across the depth gradient

Stomach content analysis found that fish were the dominant diet component for all depths. While fish dominating the diet is consistent with shallow reef findings across the invaded and native lionfish range (Muñoz et al. 2011; Green et al. 2011; Cure et al. 2012), it has been suggested that lionfish diets are mostly determined by the local abundance of fish and invertebrate species (Muñoz et al. 2011). Previous studies on Utila have identified declines in native fish abundance and biomass with increased depth (Chapter 2), and that abundance of large planktonic reef-associated invertebrates (>2 mm body size) increases with depth (Chapter 11). We therefore may have expected to see a shift to increased invertebrate composition of lionfish diets on MCEs. However, when considering fish families consumed by lionfish, we identified that Gobiidae comprised 43 % of fish in MCE lionfish diets. As Gobiidae are small benthic associated cryptic fish, it is likely that as previous Utila fish surveys across the depth gradient have used video based transects (Chapter 2), they have under-recorded Gobiidae abundance on reefs (Holmes et al. 2013). A study from Curaçao identified greatest numbers of Gobiidae recruits in the 20–30 m range when compared to 10 m and 40 m (Luckhurst and Luckhurst 1977). Yet we found Gobiidae made up the lowest proportion of fish in lionfish stomachs in the 25–40 m range, with greater proportions in the 0–25 m and 40–72 m depth bands. While our principle components analysis was limited to three groups, it does suggests that lionfish diets at the intermediate depths (25-40 m) and on MCEs (40-72 m) are more similar than shallow diets. This pattern fits with recorded fish community change-over in prey items across the depth gradient (Chapter 2, Chapter 11). Previous work has suggested that lionfish culling can lead to a decrease in the number of stomach items, and in some cases can lead to a shift in diet from fish to invertebrates (Frazer et al. 2012). Despite culling restricted to shallow reefs, we found no evidence of reduced feeding at shallow depths, or a greater invertebrate contribution to lionfish diets in the shallows.

7.5.4 Management approaches for MCE lionfish

In most western Atlantic countries affected by the lionfish invasion, the most commonly used approach for lionfish population management has been culling by divers using hand spears (Côté et al. 2014). Culling has been found to be effective in reducing lionfish abundance on shallow reefs (Frazer et al. 2012), which in turn can help shallow native reef fish communities recover (Green et al. 2014). Culling is normally managed by governments, but typically carried out by volunteer recreational divers, limiting removal efforts in many locations to <30 m. Technical diving requirements has meant lionfish removal on many western Atlantic MCEs has not been possible (Mitchell and Doolette 2013). However, within the dive community there is increased interest in technical diving (Mitchell and Doolette 2013), leading some lionfish management organisations to specifically engage technical divers in community based lionfish management activities. For example, on Utila, during the July 2016 annual lionfish derby a technical diving category was included for the first time. Public lionfish derbies are a widely used management tool to increase lionfish removal from reefs while increasing public engagement and awareness of marine conservation, with teams competing for prizes based on the number and sizes of lionfish collected (Malpica-Cruz et al. 2016). By incorporating a technical diving category, and specifically encouraging dive centres with technical diving capabilities to compete, it was possible to expand lionfish removal efforts onto MCEs.

Other methods that have collected lionfish from MCEs include hook-and-line fishing and trapping (Bogdanoff et al. 2014). As lionfish are ambush predators, most lionfish collection by hook-and-line has been as bycatch, typically with low numbers of lionfish collected, making hook-and-line unsuitable for extensive MCE lionfish removal (Bogdanoff et al. 2014). In contrast, lionfish traps have shown promise in some areas. For example, in Bermuda over 1,200 lionfish were removed between September 2013 and March 2014 in traps from 40-80 m depth (Pitt and Trott 2015). While these lionfish represented bycatch in commercial lobster traps, it has shown that substantial numbers of lionfish can be removed by trapping. This has driven interest in developing lionfish specific traps that can be more widely used for MCE lionfish removal (Pitt and Trott 2015).

7.6 Conclusion

We studied invasive lionfish populations across the shallow reef to MCE depth gradient, identifying a high density of lionfish below the maximum depth of most culling. These deeper reef lionfish were found to be larger, had heavier gonad weights, and females were more mature than those found on shallow reefs. This raises the possibility that deep reef lionfish may be undermining current shallow reef focused culling efforts. Our results highlight the need for lionfish management plans to consider the importance of MCEs to lionfish lifecycles, thereby improving the effectives of culling programs.

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7.8 References

- Albins MA, Hixon MA (2008) Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. Marine Ecology Progress Series 367:233–238
- Arias-González JE, González-Gándara C, Luis Cabrera J, Christensen V (2011) Predicted impact of the invasive lionfish *Pterois volitans* on the food web of a Caribbean coral reef. Environmental Research 111:917–925
- Bergenius M, Meekan M, Robertson R, McCormick M (2002) Larval growth predicts the recruitment success of a coral reef fish. Oecologia 131:521–525
- Berkeley SA, Chapman C, Sogard SM (2004) Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology 85:1258–1264
- Birkeland C, Dayton P (2005) The importance in fishery management of leaving the big ones. Trends in Ecology & Evolution 20:356–358
- Bogdanoff AK, Akins JL, morris JA, 2013 GCFI Lionfish Workgroup (2014) Invasive Lionfish in the Marketplace: Challenges and Opportunities. Proceedings of the 66th Gulf and Caribbean Fisheries Institute November 4 - 8, 2013 Corpus Christi, Texas USA 66:140–147
- Bridge TCL, Hughes TP, Guinotte JM, Bongaerts P (2013) Call to protect all coral reefs. Nature Climate Change 3:528–530
- Brokovich E, Einbinder S, Shashar N, Kiflawi M, Kark S (2008) Descending to the twilight-zone: changes in coral reef fish assemblages along a depth gradient down to 65 m. Marine Ecology Progress Series 371:253–262

- Claydon J, Calosso MC, Traiger SB (2012) Progression of invasive lionfish in seagrass, mangrove and reef habitats. Marine Ecology Progress Series 448:119–129
- Cocheret de la Morinière E, Pollux BJA, Nagelkerken I, van der Velde G (2002) Post-settlement Life Cycle Migration Patterns and Habitat Preference of Coral Reef Fish that use Seagrass and Mangrove Habitats as Nurseries. Estuarine, Coastal and Shelf Science 55:309–321
- Côté IM, Akins L, Underwood E, Curtis-Quick J, Green SJ (2014) Setting the record straight on invasive lionfish control: Culling works. PeerJ PrePrints 2:e398v1
- Cure K, Benkwitt CE, kinson, Pickering EA, Pusack TJ, McIlwain JL, Hixon MA (2012) Comparative behavior of red lionfish *Pterois volitans* on native Pacific versus invaded Atlantic coral reefs. Marine Ecology Progress Series 467:181–192
- Darling ES, Green SJ, O'Leary JK, Côté IM (2011) Indo-Pacific lionfish are larger and more abundant on invaded reefs: a comparison of Kenyan and Bahamian lionfish populations. Biological Invasions 13:2045–2051
- de León R, Vane K, Bertuol P, Chamberland VC, Simal F, Imms E, Vermeij M (2013) Effectiveness of lionfish removal efforts in the southern Caribbean. Endang. Species. Res. 22:175–182
- Fishelson L (1978) Oogenesis and spawn-formation in the pigmy lion fish *Dendrochirus brachypterus* (Pteroidae). Marine Biology 46:341–348
- Frazer TK, Jacoby CA, Edwards MA, Barry SC, Manfrino CM (2012) Coping with the Lionfish Invasion: Can Targeted Removals Yield Beneficial Effects? Rev Fish Sci 20:185–191
- Freshwater DW, Hines A, Parham S, Wilbur A, Sabaoun M, Woodhead J, Akins L, Purdy B, Whitfield PE, Paris CB (2009) Mitochondrial control region sequence analyses indicate dispersal from the US East Coast as the source of the invasive Indo-Pacific lionfish *Pterois volitans* in the Bahamas. Marine Biology 156:1213–1221

- Goldstein ED, D’Alessandro EK, Sponaugle S (2016) Demographic and reproductive plasticity across the depth distribution of a coral reef fish. Scientific Reports 6:34077
- Green SJ, Akins JL, Coté IM (2011) Foraging behaviour and prey consumption in the Indo-Pacific lionfish on Bahamian coral reefs. Marine Ecology Progress Series 433:159–167
- Green SJ, Akins JL, Maljković A, Côté IM (2012a) Invasive Lionfish Drive Atlantic Coral Reef Fish Declines. PLoS ONE 7:e32596
- Green SJ, Akins JL, Morris JA Jr (2012b) Lionfish Dissection: Techniques and applications. reef.org 24
- Green SJ, Dulvy NK, Brooks AML, Akins JL, Cooper AB, Miller S, Côté IM (2014) Linking removal targets to the ecological effects of invaders: a predictive model and field test. Ecol Appl 24:1311–1322
- Green SJ, Tamburello N, Miller SE, Akins JL, Coté IM (2013) Habitat complexity and fish size affect the detection of Indo-Pacific lionfish on invaded coral reefs. Coral Reefs 32:413–421
- Hislop JRG (1988) The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, *Melanogrammus aeglefinus*, in British waters. Journal of Fish Biology 32:923–930
- Hixon MA, Johnson DW, Sogard SM (2014) BOFFFFs: on the importance of conserving old-growth age structure in fishery populations. ICES Journal of Marine Science 71:2171–2185
- Holmes TH, Wilson SK, Travers MJ, Langlois TJ, Evans RD, Moore GI, Douglas RA, Shedrawi G, Harvey ES, Hickey K (2013) A comparison of visual- and stereo-video based fish community assessment methods in tropical and temperate marine waters of Western Australia. Limnol. Oceangr. Methods 11:337–350

- Kahng SE, Copus JM, Wagner D (2014) Recent advances in the ecology of mesophotic coral ecosystems (MCEs). Current Opinion in Environmental Sustainability 7:72–81
- Kahng SE, Garcia-Sais JR, Spalding HL, Brokovich E, Wagner D, Weil E, Hinderstein L, Toonen RJ (2010) Community ecology of mesophotic coral reef ecosystems. Coral Reefs 29:255–275
- Kulbicki M, Beets J, Chabanet P, Cure K, Darling E, Floeter SR, Galzin R, Green A, Harmelin-Vivien M, Hixon M, Letourneur Y, de Loma TL, McClanahan T, McIlwain J, MouTham G, Myers R, O'Leary JK, Planes S, Vigliola L, Wantiez L (2012) Distributions of Indo-Pacific lionfishes *Pterois* spp. in their native ranges: implications for the Atlantic invasion. Marine Ecology Progress Series 446:189–205
- Lesser MP, 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 13:1855–1868
- Luckhurst BE, Luckhurst K (1977) Recruitment patterns of coral reef fishes on the fringing reef of Curaçao, Netherlands Antilles. Can. J. Zool. 55:681–689
- Malpica-Cruz L, Chaves LCT, Côté IM (2016) Managing marine invasive species through public participation: Lionfish derbies as a case study. Marine Policy 74:158–164
- Menza C, Kendall M, Hile S (2008) The deeper we go the less we know. rev Biol Trop 56:11–24
- Mitchell SJ, Doolette DJ (2013) Recreational technical diving part 1: an introduction to technical diving methods and activities. Diving and Hyperbaric Medicine 43:86–93
- Morris JA (2012) Invasive Lionfish: A Guide to Control and Management. Gulf and Caribbean Fisheries Institute Special Publication Series Number 1, Marathon, Florida, USA

- Muñoz RC, Currin CA, Whitfield PE (2011) Diet of invasive lionfish on hard bottom reefs of the Southeast USA: insights from stomach contents and stable isotopes. Marine Ecology Progress Series 432:181–193
- Nuttall MF, Johnston MA, Eckert RJ, Embesi JA, Hickerson EL, Schmahl GP (2014) Lionfish (*Pterois volitans* [Linnaeus, 1758] and *P. miles* [Bennett, 1828]) records within mesophotic depth ranges on natural banks in the Northwestern Gulf of Mexico. BioInvasions Records 3:111–115
- Oksanen J, Kindt R, Legendre P, OHara B, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) vegan: Community Ecology Package. cran.r-project.org
- Pitt JM, Trott TM (2015) Trapping Lionfish in Bermuda, Part II: Lessons learned to date. Proceedings of the 67th Gulf and Caribbean Fisheries Institute November 3-7 2014 67:221–224
- R Core Team (2013) R: A language and environment for statistical computing. R-project.org
- Schofield PJ (2009) Geographic extent and chronology of the invasion of non-native lionfish (*Pterois volitans* [Linnaeus 1758] and *P. miles* [Bennett 1828]) in the Western North Atlantic and Caribbean Sea. Aquatic Invasions 4:473–479
- Schofield PJ (2010) Update on geographic spread of invasive lionfishes (*Pterois volitans* [Linnaeus, 1758] and *P. miles* [Bennett, 1828]) in the Western North Atlantic Ocean, Caribbean Sea and Gulf of Mexico. Aquatic Invasions 5:S117–S122
- Slattery M, Lesser MP (2014) Allelopathy in the tropical alga *Lobophora variegata* (Phaeophyceae): mechanistic basis for a phase shift on mesophotic coral reefs? Journal of Phycology 50:493–505

- Wright DJ (2005) Report of HURL Cruise KOK0510: Submersible Dives and Multibeam Mapping to Investigate Benthic Habitats of Tutuila, American Samoa. ir.library.oregonstate.edu

8 DISCUSSION

8.1 Overview

This thesis focused on changes in reef fish community composition, behaviour and maturity across shallow to mesophotic coral ecosystem (MCE) depth gradients, with the primary data collected around Utila, Honduras, but also with broader pan-tropical data introduced in a meta-analysis (Chapter 7). Utila is located on the southern Mesoamerican Barrier Reef, the second largest barrier reef globally, and its reefs are considered a typical representation of the contemporary Caribbean region. For example, the reefs of Utila have suffered *Diadema antillarum* population declines and face many anthropogenic impacts from an increasing human population (Harborne et al. 2001), such as costal development, reef fisheries and sedimentation. Therefore, the detailed study of MCE fish ecology around Utila in this thesis provides insights applicable to the western Atlantic region more generally, especially with limited previous research in much of the region. The six core data chapters and three appendices comprising this thesis combine to address three broad themes:

i. Understanding fish community ecological processes on MCEs (Chapters 2, 3)
ii. Evaluating effective survey techniques for MCE fish research (Chapters 4, 5, 10)
iii. Exploring the role of MCEs in the western Atlantic lionfish invasion (Chapters 6, 7, 9)

Here, I place the findings of this thesis within the broader research framework of each of these themes, and suggest further future research questions that could build upon the work presented here.

8.2 Understanding fish community ecological process on MCEs

This thesis contains two studies of fish ecology on MCEs, in Chapter 2 the fish community is characterized across shallow to upper-MCE depth gradients based on trophic composition and biomass, while in Chapter 3, an experimental manipulation looks at the relative role of fish predation and light levels in altering MCE benthic communities.

In Chapter 2 I found a decline in richness, abundance and biomass of reef fish with increased depth, as well as declines in key benthic groups, including hard coral, macroalgae and sponge. These patterns in abundance and biomass are consistent with studies elsewhere in the Caribbean (e.g. Thresher and Colin 1986; Feitoza et al. 2005; Bejarano et al. 2014), although declines in fish species richness contrast other similar studies within the western Atlantic. For example, in Puerto Rico the greatest fish species richness was observed at 25 m when comparing the 15–50 m depth gradient (Bejarano et al. 2014), and in Bermuda it increased with depths between 45-80 m (Pinheiro et al. 2016). However, the western Atlantic is a geographically large area, with distinctive fish assemblages based on reef area, island areas, level of isolation and extent of near shore productivity (Sandin et al. 2008). The results presented in Chapter 2 appear more similar to recently published data from islands of similar size and geographical location to Utila. On the northern Mesoamerican Barrier Reef, a recent study comparing reef fish communities at 15 m and 55 m using similar stereo-video survey methods found that species richness was lower on MCEs than shallow reefs (Gress et al. 2017), while in Curaçao, species richness declined with increased depth between 45–80 m (Pinheiro et al. 2016).

Fine-scale changes in species richness, abundance and biomass of reef fish are likely driven by local benthic habitat availability including structural complexity (Bejarano Rodríguez 2013). In the case of Utila, we used the availability of hard substrate as a proxy for structural complexity (Chapter 2), which declined with depth. On the south shore of Utila, the shallow reefs are made up of a developed patch and groove reef system, which transitions into a reef slope, and then a mesophotic patch reef community separated by large areas of sand and silt on a relatively flat seabed. In contrast, off the north shore of Utila the reefs are composed of steep walls broken by a series of ledges (Chapter 11). In addition to these geological features, complexity can change within the coral community directly as mesophotic corals adapt to increased depth by becoming flatter (Kahng et al. 2010). There are now several examples of anecdotal observations suggesting correlations between proxies for structural complexity and increased fish abundance on MCEs. For example, a study using remote operated vehicles (ROVs) off the Florida coast reported that the majority of MCE fish were associated with wrecks, and in the naturally low complexity areas the few fish observed were associated with small artificial structures such as tyres (Bryan et al. 2013). Published studies on MCE fish communities have so far only provided limited quantification of structural complexity as an explanatory factor, and future MCE work should address this through direct assessments. While the normal method for quantifying structural complexity on reefs is using a rugosity chain and tape (Luckhurst and Luckhurst 1978), because of the limited dive times at mesophotic depths (Chapter 11), new technology may provide more appropriate solutions for complexity assessments. For example, recent studies have quantified structural complexity using 3D modelling from single camera filming (Burns et al. 2015). Therefore, future studies could directly assess shifts in fish community composition while controlling for both the quantity and quality of available complexity.

Previous fish community surveys in the western Atlantic have documented declines in herbivorous fish with increased depth across the shallow to MCE gradient (see reviews in: Kahng et al. 2010; 2014). However, with the exception of Pinhero et al. (2016), these studies have been based on fish abundance, despite mean fish body size being known to be greater on MCEs in many cases (Chapter 2; Goldstein et al. 2016). Therefore, estimates of fish community shift based on abundance alone are unlikely to accurately reflect differences in ecological processes such as herbivory. In Chapter 2, I quantify biomass using a stereo-video system (SVS) to enable biomass of herbivores to be estimated in addition to abundance, finding that the overall biomass of herbivores also declined with increased depth. On shallow reefs, herbivorous reef fish have been demonstrated to have crucial roles in maintaining coral cover, and reducing the growth of fleshy macroalgae (Hughes et al.

2007; Graham et al. 2013). No detailed studies have been conducted to assess herbivory rates on MCEs in the western Atlantic, though a study in the Red Sea using artificial algal growth tiles found that while both algal cover and herbivorous fish biomass declined with increased depth, herbivore biomass declined more steeply (Brokovich et al. 2010). This would imply that herbivores are not the primary constraint on algal growth on Red Sea MCEs, though herbivory was detected to some degree down to 65 m. In the western Atlantic, however, there have been three observations that suggest that herbivorous reef fish may play an important role in structuring MCE benthic communities. Firstly, Leichter et al. (2008) reported observing herbivory grazing halos around wrecks in the Florida Keys at 50–60 m depth. These halos extended approximately 10-20 m around the wreck structure, suggesting that herbivorous fish were using the artificial structures as a refuge and most heavily grazing around them. Secondly, Leichter et al. (2008) also studied algal recruitment on tiles deployed from 30-70 m, and observed reduced algal abundance on tiles at 30 and 40 m compared to 50 and 60 m, combined with herbivorous reef fish feeding in the 30-40 m range. Thirdly, it has been suggested that phase shifts from coral to macroalgal dominated states that were observed on MCEs in the Bahamas following lionfish invasion were caused by a trophic cascades, with lionfish feeding on herbivorous reef fish, and so reducing grazing pressure (Lesser and Slattery 2011). This then allowed the macroalga, *Lobophora variegata*, to use allelopathy to competitively exclude hard corals from the reef (Slattery and Lesser 2014). Therefore, when combined, these anecdotal western Atlantic observations indicate that herbivorous reef fish may play a more significant a role in structuring MCE benthic communities on western Atlantic MCEs than was observed in the Red Sea.

As I observed declines in herbivorous fish biomass across the shallow-MCE gradient in Chapter 2, I tested whether herbivorous fish had a role in structuring MCE benthic communities. I used a factorial experimental design in Chapter 3 to experimentally manipulate light and fish exposure levels for permanent benthic quadrats. I observed declines in hard coral and algal cover in benthic communities in all treatments, with the experimental controls indicating these were mostly driven by reductions in light availability rather than changes in fish exposure. While some effects of fish were detected, the direction of these effects on hard coral and sponges indicated that these most likely represented cage artefacts, possibly caused by reduced water flows associated with exclusion cages being placed on the reef. Despite these challenges, Chapter 3 represents the first in-situ factorial experimental test of changes in fish and light levels on MCE benthic communities. While the experiment was limited in spatial scale, occurring at one site on Utila, the results clearly indicate the importance of light. However, because the logistical challenges of caging to exclude fish precluded the ability to manipulate fish exposure without changing light exposure, in this experiment light was factorial with only two levels, ‘normal’ and ‘low’. Based on previous anecdotal evidence (Leichter et al. 2008; Lesser and Slattery 2011), it is likely that herbivores do play a role in shaping in benthic communities, but that any signal of this was dwarfed by the effects of light loss. Additional future studies could use artificial lighting to enable fish exclusion with finer controls of light levels such that light levels could be maintained while removing fish. Studies of reef herbivory on shallow reefs have separated herbivores into different feeding groups, enabling studies of functional redundancy in algal removal from reefs (Burkepile and Hay 2008; 2011). These studies have used experimental manipulations to expose reefs to feeding by controlled densities of different herbivorous reef fish (Burkepile and Hay 2011). Similar approaches, using the herbivores detected on MCEs (Chapter 2) to test feeding complementarity verses redundancy amongst MCE herbivores in an experimental set up with maintained light levels, would provide interesting comparisons to shallow reefs.

8.3 Evaluating effective survey techniques for MCEs

Understanding how biases in survey techniques affect survey results is crucial for correct ecological interpretation, especially when combining data from different survey techniques, or when comparing survey data across natural ecological gradients such as depth where biases may vary. With such little research previously conducted on MCEs, it is not clear whether established shallow reef survey techniques remain consistent on MCEs. I consider these issues by, (i) comparing results from diver-operated video (DOV) and baited remote underwater video (BRUV) SVS systems on shallow reefs and MCEs (Chapter 4), and (ii) comparing how fish abundance and minimum approach distance (MAD) changes between open-circuit (OC) SCUBA and closed-circuit rebreather (CCR) conducted surveys across the shallow-MCE depth gradient (Chapter 5). These issues are particularly of importance, as the choice of survey technique and/or dive gear is often influenced by depth, rather than a decision about which technique is best suited for the research or monitoring questions of interest (Chapter 11). For example, many reef managers interested in MCEs chose to conduct BRUV surveys in part because of the high levels of technical diving required for in-water surveys, yet in parallel they conduct their shallow reef monitoring using diver swum UVC or DOV transects. Therefore disentangling biases between techniques is crucial to allow appropriate comparisons to be made.

This thesis shows that the choice of technique affects recorded fish community abundance and biomass, and crucially that differences between DOV/BRUV and OC/CCR transects are not consistent with depth. Specifically, when considering BRUVs, it is likely the longer deployment times, typically around one hour, allow for better recording of smaller cryptic fish that are disturbed by divers on MCEs. BRUVs are also baited; leading to the expectation that greater biomass of carnivorous fish would be detected. This was observed for shallow reefs, yet DOV recorded more carnivorous fish biomass on MCEs (Chapter 4). Herbivores, however, made up a larger proportion of the community biomass, and were recorded at greater abundance, on DOV than BRUVs, suggesting that surveys with a focus on herbivores should use DOV. This effect is particularly relevant to MCEs, which have low herbivore densities (Chapter 2; Kahng et al. 2010), meaning techniques that are optimal for herbivore detection are likely to allow more precise monitoring.

In addition to the differences between survey techniques, we found that the choice of dive gear when conducting DOV surveys affects abundance results for Acanthurdiae, Serranidae, Sparidae and Tetraodontidae (Chapter 5). However, these differences in abundance were relatively small, and transects conducted by OC and CCR were broadly comparable on Utila. Despite this, for many fish families we detected differences in the distance that divers could approach individual fish based on whether they were conducted using OC or CCR. In the case of Labridae, interactions between dive gear type and depth were identified, with larger differences caused by dive gear in the shallows than on MCEs. While this was only one family out of the 11 tested, suggesting that around Utila these interactions are not common, it does highlight the impacts of dive gear on fish surveys may not remain consistent with depth. We also found in general, regardless of dive gear type, that larger fish were more wary of divers which fits observations from other studies (Gotanda et al. 2009; Januchowski-Hartley et al. 2011). As several reef fish are known to increase in body size with increased depth around Utila (Chapter 2), these differences could lead to fewer fish being reported on MCE surveys if they avoid divers.

The comparisons between survey method (BRUV vs DOV) and dive gear type (OC vs CCR) were only conducted on the south shore of Utila. Current fishing on these reefs is lower than historic levels, though does occur, as the economy of Utila has shifted to increased dive tourism (Box and Canty 2011). This exposes the shallow reef fish at surveyed sites to high levels of OC recreational diver contact, while divers rarely visit MCEs. In the past spearfishing has been used, though has been near absent from the fringing reefs for approximately 20 years (Kramer et al. 2015). The two studies comprising Chapters 4 and 5 would be interesting to repeat in areas without regular diving, and in locations with more active reef fisheries to see if results are consistent. Previous studies have shown strong effects of OC dive gear in reducing observed fish abundance in areas where OC dive gear is used by spearfishers (Lindfield et al. 2014), while other studies have shown that protected areas can act as behavioural refuges, containing naïve fish, which are less fearful of divers (Januchowski-Hartley et al. 2013). It would be interesting to see whether MCEs below the depth of OC spear fisheries function in a similar way as behavioural refuges.

8.4 Understanding the role of MCEs in the western Atlantic lionfish invasion

Invasive lionfishes (*Pterois volitans* and *P. miles*), native to the Indian and Pacific Oceans and the Red Sea were introduced to the western Atlantic in the 1980s (Schofield 2009; 2010). They have since spread throughout much of the region, reaching their northern latitudinal temperature limit (Whitfield et al. 2014) and currently progressing south towards their southern limit, with recent records from Brazil (Ferreira et al. 2015). The damage lionfish cause to shallow reefs is well documented, with declines in native fish recruitment of up to 79 % (Albins and Hixon 2008), reduced native prey fish biomass up to 65 % (Green et al. 2012) and in some areas have a feeding preference for critically endangered reef fish (Rocha et al. 2015). There have been several observations of lionfish on MCEs in their invaded range, but prior to this thesis, only two studies had quantified lionfish populations on invaded MCEs (Slattery et al. 2011; de León et al. 2013). In Chapter 6 I conducted a meta-analysis, which showed that MCEs across the western Atlantic region have been widely invaded by invasive lionfish. I also found that while lionfish abundance was greater in the invaded than native range, the relative abundance across the shallow to MCE gradient was similar between them. This suggests that lionfish are behaving naturally in their movement patterns across the depth gradient in invaded range sites, and that studies of lionfish ecology in their native range can help inform understanding of their invasive population. In the meta-analysis I found no difference in lionfish abundance between shallow reefs and MCEs in most locations, however there was much variation in the data, combined with different intensities of culling. This chapter raised important questions about whether lionfish were undertaking an ontogenetic migration and gaining from a deep reef refuge in some locations, therefore limiting the effectiveness of culling on lionfish populations. I attempted to address these questions in Chapter 7 by conducting a detailed study of lionfish on the reefs around Utila. I found greater lionfish densities on MCEs than shallow reefs, contrasting with the meta-analysis results, and showing that on Utila MCEs act as refuge habitats for lionfish populations in the face of local shallow reef focused culling.

Previous work has identified that lionfish undergo ontogenetic migrations (Claydon et al. 2012), with lionfish recruits settling into shallow back reef environments, mangroves and seagrass beds, and then migrating to reef crests followed by reef slopes in the 12-24 m depth range as they progressively mature. No previous studies had documented whether lionfish on MCEs were an extension of their ontogenetic migration, or whether the population was of similar maturity across shallow reef slopes and MCEs. In most fish species female fecundity is related to body size and maturity (Hixon et al. 2014), so if larger, more mature lionfish were found on MCEs than shallower reef slopes, this could mean lionfish on MCEs are disproportionately responsible for new lionfish recruits. In addition, if lionfish management practices are limited to shallow reef culling, the resulting depth refuge for lionfish will contain the most fecund fish. In Chapter 7 maturity of lionfish across the depth gradient was specifically assessed, with the results suggesting that lionfish on MCEs are more mature than those on shallow reef slopes, supporting the idea that the ontogenetic migration extends onto MCEs. These patterns in increased maturity also correlate with increasing body size in lionfish, lending support to our conclusions from Chapter 2 where we identified several fish species that might incorporate MCEs into ontogenetic migrations based on changes in length distribution. Future reef fisheries management approaches should consider changes in maturity with depth, and in the case of lionfish, removal of individuals from MCEs to help reduce future recruitment.

In Chapter 5 I discussed the behavioural impacts of historical fisheries when testing whether fish behaviour changes in response to divers across the depth gradient. It has been suggested that in response to culling, lionfish may become more cautious, and hide in the reef, and that this could lead to lower feeding rates of lionfish providing additional benefits of culling beyond just lionfish removal (Côté et al. 2014). As current lionfish management is focused on shallow water culling this raises crucial questions on whether behavioural response to spearfishing interacts with depth. Behavioural adaptations to culling may be retained across the depth gradient, because of the lionfish ontogenetic migration exposing MCE individuals to culling risk at earlier life history stages when in the shallows. However, lionfish below the culling depth limit may be bolder, as they would not face regular culling. Future studies should attempt to disentangle these effects, as fish behavioural adaptations can be long lived (see discussion and references in Chapter 5).

8.5 Conclusion

MCEs represent a vastly under-studied ecosystem, and I use Utila, on the southern Mesoamerican Barrier Reef, as a representative example of a Caribbean MCE to investigate fish ecology. This thesis highlights that upper-MCEs around Utila have high fish species overlap with shallow reef communities, existing as an extension of them. However, unlike shallow reefs, light availability appears likely to have a more crucial limiting role in structuring MCE benthic communities than top-down processes such as herbivory, though herbivorous reef fish are present on upper-MCEs. When assessing fish communities, the choice of assessment metric (e.g. abundance or biomass), survey method (e.g. BRUV or DOV) and even dive gear type (e.g. OC SCUBA or CCR) if doing in-water diver surveys, all have effects on interpretation of results. With so few MCE studies previously conducted, future work should try to standardise techniques as much as possible to aid comparisons between studies. I also tested the role of MCEs as fish refuges, using invasive lionfish as a case-study species. Using a meta-analysis, allowing studies with different experimental designs to be compared, I found that lionfish have similar relative abundance distributions across the depth gradient between their invaded and native ranges. Despite high lionfish culling around Utila, I identified high densities of lionfish on MCEs at 40-85 m. These individuals were the most mature, suggesting that these deeper populations may be disproportionately responsible for new lionfish recruits. The results of this thesis also support the idea of ontogenetic migrations extending from shallow reefs to MCEs for several fish species, including lionfish. Future reef-fisheries management approaches should therefore consider changes in maturity with depth. In conclusion, this thesis has increased understanding of fundamental ecological processes occurring on MCEs and highlighted the importance of considering MCEs in reef management decision-making.

8.6 References

- Albins MA, Hixon MA (2008) Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. Marine Ecology Progress Series 367:233–238
- Bejarano I, Appeldoorn RS, Nemeth M (2014) Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico. Coral Reefs 33:313–328
- Bejarano Rodríguez I (2013) Deep Reef Fishes off La Parguera Insular Slope, Puerto Rico, and their Connectivity with Shallow Reefs. University of Puerto Rico, p 1–115
- Box SJ, Canty SWJ (2011) The Long and Short Term Economic Drivers of Overexploitation in Honduran Coral Reef Fisheries Due to Their Dependence on

Export Markets. Proceedings of the 63rd Gulf and Caribbean Fisheries Institute November 1 - 5, 2010 San Juan, Puerto Rico 63:43–51

- Brokovich E, Ayalon I, Einbinder S, Segev N, Shaked Y, Genin A, Kark S, Kiflawi M (2010) Grazing pressure on coral reefs decreases across a wide depth gradient in the Gulf of Aqaba, Red Sea. Marine Ecology Progress Series 399:69–80
- Bryan DR, Kilfoyle K, Gilmore RG Jr, Spieler RE (2013) Characterization of the mesophotic reef fish community in south Florida, USA. Journal of Applied Ichthyology 29:108–117
- Burkepile DE, Hay ME (2008) Herbivore species richness and feeding complementarity affect community structure and function on a coral reef. Proceedings of the National Academy of Sciences 105:16201–16206
- Burkepile DE, Hay ME (2011) Feeding complementarity versus redundancy among herbivorous fishes on a Caribbean reef. Coral Reefs 30:351–362
- Burns J, Delparte D, Gates RD, Takabayashi M (2015) Integrating structure-from-motion photogrammetry with geospatial software as a novel technique for quantifying 3D ecological characteristics of coral reefs. PeerJ 3:e1077
- Claydon J, Calosso MC, Traiger SB (2012) Progression of invasive lionfish in seagrass, mangrove and reef habitats. Marine Ecology Progress Series 448:119–129
- Côté IM, Darling ES, Malpica-Cruz L, Smith NS, Green SJ, Curtis-Quick J, Layman C (2014) What Doesn't Kill You Makes You Wary? Effect of Repeated Culling on the Behaviour of an Invasive Predator. PLoS ONE 9:e94248
- de León R, Vane K, Bertuol P, Chamberland VC, Simal F, Imms E, Vermeij M (2013) Effectiveness of lionfish removal efforts in the southern Caribbean. Endang. Species. Res. 22:175–182
- Feitoza BM, Rosa RS, Rocha LA (2005) Ecology and Zoogeography of Deep-Reef Fishes in Northeastern Brazil. Bulletin of Marine Science 76:725–742

- Ferreira CEL, Luiz OJ, Floeter SR, Lucena MB, Barbosa MC, Rocha CR, Rocha LA (2015) First Record of Invasive Lionfish (*Pterois volitans*) for the Brazilian Coast. PLoS ONE 10:e0123002
- Goldstein ED, D’Alessandro EK, Sponaugle S (2016) Demographic and reproductive plasticity across the depth distribution of a coral reef fish. Scientific Reports 6:34077
- Gotanda KM, Turgeon K, Kramer DL (2009) Body size and reserve protection affect flight initiation distance in parrotfishes. Behav Ecol Sociobiol 63:1563–1572
- Graham NA, Bellwood DR, Cinner JE, Hughes TP, Norström AV, Nystrom M (2013) Managing resilience to reverse phase shifts in coral reefs. Frontiers in Ecology and the Environment 11:541–548
- Green SJ, Akins JL, Maljković A, Côté IM (2012) Invasive Lionfish Drive Atlantic Coral Reef Fish Declines. PLoS ONE 7:e32596
- Gress E, Arroyo-Gerez M, Wright G, Andradi-Brown DA (2017) Mesophotic Coral Ecosystems (MCEs) act as deep reef refuges for fish populations in Cozumel, Mexico. Proceedings of the 69th Gulf and Caribbean Fisheries Institute November 7 - 11, 2016 Grand Cayman, Cayman Islands
- Harborne AR, Afzal DC, Andrews MJ (2001) Honduras: Caribbean Coast. Marine Pollution Bulletin 42:1221–1235
- Hixon MA, Johnson DW, Sogard SM (2014) BOFFFFs: on the importance of conserving old-growth age structure in fishery populations. ICES Journal of Marine Science 71:2171–2185
- Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N, Pratchett MRS, Steneck RS, Willis B (2007) Phase Shifts, Herbivory, and the Resilience of Coral Reefs to Climate Change. Current Biology 17:360–365

- Januchowski-Hartley FA, Graham NAJ, Cinner JE, Russ GR (2013) Spillover of fish naïveté from marine reserves. Ecol Letters 16:191–197
- Januchowski-Hartley FA, Graham NAJ, Feary DA, Morove T, Cinner JE (2011) Fear of Fishers: Human Predation Explains Behavioral Changes in Coral Reef Fishes. PLoS ONE 6:e22761
- Kahng SE, Copus JM, Wagner D (2014) Recent advances in the ecology of mesophotic coral ecosystems (MCEs). Current Opinion in Environmental Sustainability 7:72–81
- Kahng SE, Garcia-Sais JR, Spalding HL, Brokovich E, Wagner D, Weil E, Hinderstein L, Toonen RJ (2010) Community ecology of mesophotic coral reef ecosystems. Coral Reefs 29:255–275
- Kramer P, McField M, Álvarez-Filip L, Drysdale I, Flores MR, Giró A, Pott R (2015) 2015 Report Card for the Mesoamerican Reef. healthyreefs.org
- Leichter JJ, Stokes MD, Genovese SJ (2008) Deep water macroalgal communities adjacent to the Florida Keys reef tract. Marine Ecology Progress Series 356:123–138
- Lesser MP, 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 13:1855–1868
- Lindfield SJ, Harvey ES, McIlwain JL, Halford AR (2014) Silent fish surveys: bubble-free diving highlights inaccuracies associated with SCUBA-based surveys in heavily fished areas. Methods Ecol Evol 5:1061–1069
- Luckhurst BE, Luckhurst K (1978) Analysis of the influence of substrate variables on coral reef fish communities. Marine Biology 49:317–323
- Pinheiro HT, Goodbody-Gringley G, Jessup ME, Shepherd B, Chequer AD, Rocha LA (2016) Upper and lower mesophotic coral reef fish communities evaluated by underwater visual censuses in two Caribbean locations. Coral Reefs 35:139–151

- Rocha LA, Rocha CR, Baldwin CC, Weigt LA, McField M (2015) Invasive lionfish preying on critically endangered reef fish. Coral Reefs 34:803–806
- Sandin SA, Vermeij MJA, Hurlbert AH (2008) Island biogeography of Caribbean coral reef fish. Global Ecology and Biogeography 17:770–777
- Schofield PJ (2009) Geographic extent and chronology of the invasion of non-native lionfish (*Pterois volitans* [Linnaeus 1758] and *P. miles* [Bennett 1828]) in the Western North Atlantic and Caribbean Sea. Aquatic Invasions 4:473–479
- Schofield PJ (2010) Update on geographic spread of invasive lionfishes (*Pterois volitans* [Linnaeus, 1758] and *P. miles* [Bennett, 1828]) in the Western North Atlantic Ocean, Caribbean Sea and Gulf of Mexico. Aquatic Invasions 5:S117–S122
- Slattery M, Lesser MP (2014) Allelopathy in the tropical alga *Lobophora variegata* (Phaeophyceae): mechanistic basis for a phase shift on mesophotic coral reefs? Journal of Phycology 50:493–505
- Slattery M, Lesser MP, Brazeau D, Stokes MD, Leichter JJ (2011) Connectivity and stability of mesophotic coral reefs. Journal of Experimental Marine Biology and Ecology 408:32–41
- Thresher RE, Colin PL (1986) Trophic structure, diversity and abundance of fishes of the deep reef (30-300m) at Enewetak, Marshall Islands. Bulletin of Marine Science 38:253–272
- Whitfield PE, Muñoz RC, Buckel CA, Degan BP, Freshwater DW, Hare JA (2014) Native fish community structure and Indo-Pacific lionfish *Pterois volitans* densities along a depth-temperature gradient in Onslow Bay, North Carolina, USA. Marine Ecology Progress Series 509:241–254

9 APPENDIX: THREATS TO MESOPHOTIC CORAL ECOSYSTEMS AND MANAGEMENT OPTIONS

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9.1 Introduction

MCEs are often considered to be buffered from many large-scale impacts known to directly affect shallow coral ecosystems, such as warm water bleaching and tropical storms. Furthermore, by virtue of their depth, and in many cases, their remote offshore locations, some MCEs are afforded greater protection from direct human impacts, such as overfishing and land-based runoff. However, there is increasing evidence to suggest that MCEs are vulnerable to environmental disturbances, such as climate change impacts, overfishing, bottom fishing gear, capture of aquarium fish, precious coral trade, land-based pollution and invasive species (Table 9.1). Despite these threats, many MCEs are in good condition in comparison to their shallower counterparts. Strong management responses could mitigate at least some documented threats, thereby preventing future degradation of MCEs.

Table 9.9.1 Comparison of the major anthropogenic threats facing shallow-water reefs and MCEs

Shallow-water reefs	Mesophotic coral ecosystems
• Fishing (overfishing, destructive fishing with dynamite and poison, and damage from lost fishing gear) • Thermal stress (bleaching) from ocean warming • Diseases • Pollution (land-based) • Invasive species • Tourism and recreation • Anchor damage • Coral mining (for aggregate and lime)	• Fishing (overfishing and damage from lost fishing gear) • Thermal stress (bleaching) - reduced exposure to warm water stress • Diseases • Pollution - reduced exposure to land-based sources; exposed to deep-water sewage outfalls and dredging spoils • Invasive species • Tourism and recreation (reduced exposure)

• Coastal development • Marine aquarium trade	• Anchor damage (reduced exposure) • Coral mining (reduced to negligible exposure) • Marine aquarium trade • Oil and gas exploration • Cable and pipelines

9.2 Fisheries

The pressure from fisheries is considered by marine experts to be the greatest threat to all benthic habitats (Baker and Harris 2011). Fishing also represents a major threat to MCEs, both through the perturbation of trophic relationships and through physical damage to the reef structure. Whilst in general many MCEs have not been exploited to the same extent globally as shallower reefs (due in part to their relative geographic isolation and morphology, which impedes many fishing methods), overexploitation of shallower reefs and a resulting reduction in catches is leading increasingly to expansion of fishing activities into MCEs.

Commercially valuable fish species have been identified within MCEs, including species of high conservation concern (such as sharks and groupers). It has been suggested that following the onset of fishing, most commercially-valuable fish species suffer large population declines (Bejarano Rodríguez 2013; Williams et al. 2015). For example, many large-bodied grouper species form annual spawning aggregations at the edge of continental shelves, which - depending on the location - can occur at mesophotic depths (Baker et al. 2016). The predictability in time and space of these grouper spawning aggregations have made them particularly vulnerable to overexploitation (De Mitcheson et al. 2008). As a result, many are now functionally extinct in large parts of their range. To combat this, Caribbean countries have instigated seasonal fisheries closures during spawning (Starr et al. 2007). In Cuba, fisheries studies have indicated that species with greater predictability in aggregation location and timing show greater declines in fisheries landings than species with less predictable and concentrated aggregations (Claro et al. 2006).

As shallow reefs come under increasing pressure, many MCEs in areas accessible to artisanal fishing communities are likely to be fished. These fisheries often involve traditional fishing techniques such as hook and line, which allow communities to catch mesophotic reef fish. For example in the Maldives, grouper are fished (mostly for export) down to 80 m using this technique (Sattar and Adam 2005). Similarly in Malaysia, where fishing is carried out from small boats, artisanal fishers use mostly hook and line with a small number using longlines (Wood et al. 2006). Longlines are either deployed near the surface to catch pelagic fish such as tuna, or directly onto mesophotic reef slopes for demersal species such as sharks and groupers (Wood et al. 2006). These techniques are fairly typical of many Southeast Asian mesophotic reef fisheries.

Satellite location systems and depth sounders allow easier identification of offshore MCEs as potential new fishing grounds. The arrival of even relatively established technologies into new areas can open up MCEs for fishing. For example, spearfishing in the Mariana Islands was traditionally limited to free diving on shallow reefs but following the introduction of SCUBA diving, fishing has expanded into the mesophotic zone (Myers 1993; Lindfield et al. 2014).

Trawling is a major threat to many mesophotic communities, with benthic trawl nets designed to be dragged close to the seafloor. In addition to destroying MCEs through the direct impacts of their large metal balls or plates on the bottom, trawls often stir up sediment that smother coral, causing their death (Erftemeijer et al. 2012).

Documented examples of trawling impacts to MCEs are limited. Thus, to demonstrate the devastating effects that bottom trawling can have on MCEs, we must look to the azooxanthellate ivory tree coral reefs, *Oculina varicosa*, found at mesophotic depths off Florida, U.S.A. (70-90 m; Koenig et al. 2005; Reed et al. 2007). The *Oculina* reefs were first discovered in the 1970s with high coral cover and high densities of grouper spawning aggregations(Koenig et al. 2000). A small portion was set aside as a marine protected area in 1984, but the remaining reefs were left unprotected and open to trawling for rock shrimp. Chains were dragged through the reefs to provide tracks for the rock shrimp trawls. Submersible surveys showed the devastation caused by the trawls as the unprotected coral was crushed to rubble (Figure 9.1; Reed et al. 2007). Live coral areas have over 70 species of fish, while the dead coral areas are virtually devoid of fish with the few recorded being smaller bodied groups of low commercial value, such as damsels and butterflyfish (Koenig et al. 2000; 2005). This information led to the enactment of new legislation in 2015 that doubled the size of the *Oculina* marine protected area to approximately 600 nmi^2 and prohibits all bottom-tending gear such as trawls, longlines and traps.

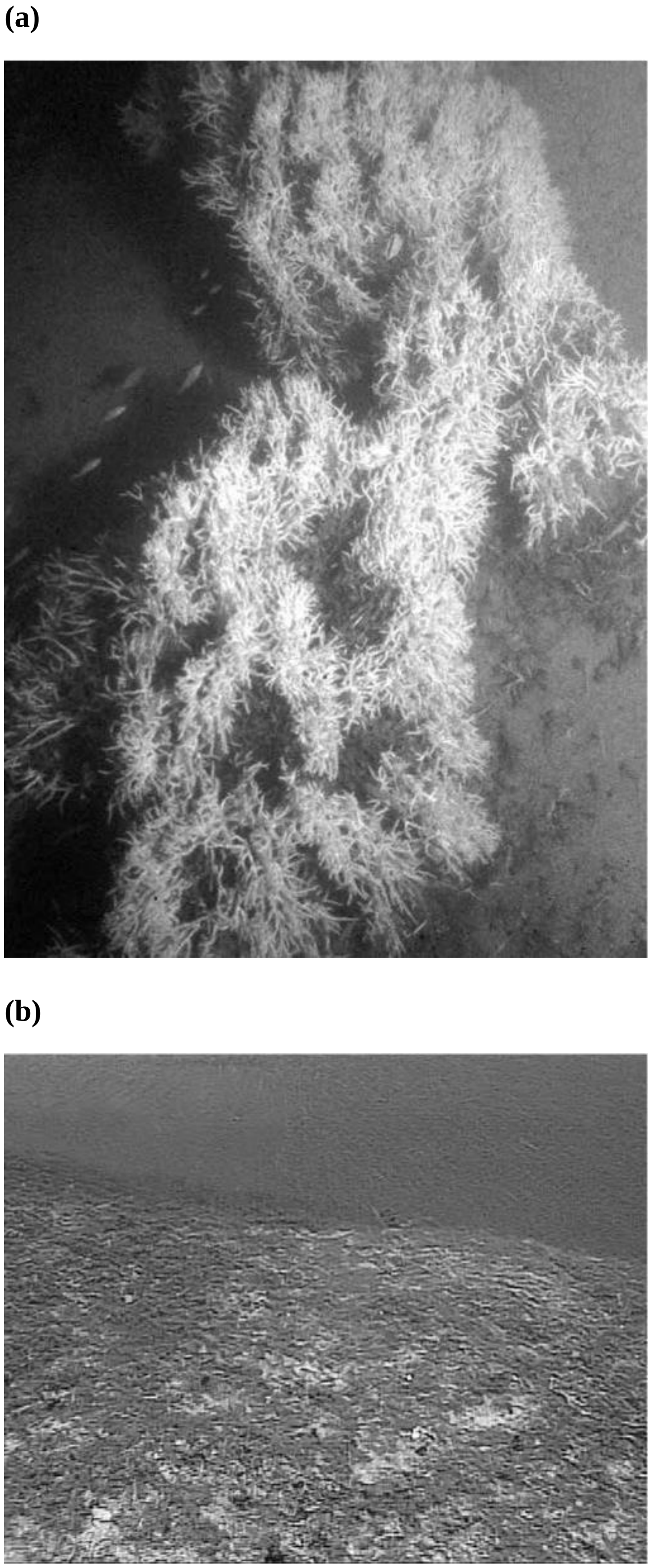


Figure 9.1 Deep-water *Oculina* at Cape Canaveral (67 m depth).

(a) Historical photo from submersible dive (JSL II-063) in 1976; (b) Same site (Clelia 613) in 2001 reduced to rubble from apparent bottom trawling. (Photo: Reed et al. 2007, used with permission).

9.2.1 Case Study: Commercially-important mesophotic fish in La Parguera, Southwest Puerto Rico

The shallow reef fishery in La Parguera, Puerto Rico, is an important activity shaping the ecosystem. It has a direct impact on species, stocks, and habitats present in the region (Valdés-Pizzini and Schärer-Umpierre 2014). Many commercially important species that historically supported a productive fishery have significantly decreased over the last 50 years. For example, fishing pressure has resulted in the loss of large bodied species and spawning aggregations (Kimmel 1985), the depletion of some species, and reductions in body size (Kimmel 1985; Pittman et al. 2010). Keystone commercial species, such as the rainbow (*Scarus guacamaia*) and midnight (*S. coelestinus*) parrotfishes, and Nassau (*Epinephelus striatus*; Figure 9.2) and tiger (*Mycteroperca tigris*) groupers are no longer found in the area. Other commercially important reef fishes are less frequently observed (e.g., red hind, *E. guttatus*), and only the smaller grouper species are still common in shallow areas (e.g., graysby, *Cephalopholis cruentata*) (Pittman et al. 2010). The low abundance of predators and the poor state of herbivores suggests that overfishing has impacted the food web (Guénette and Hill 2009; Pittman et al. 2010).

A recent study assessing the fishes associated with MCEs off La Parguera found that many of these formally abundant species on the shelf are now only found, or are only present in abundance, off the steep insular slope at a depth of 60-70 m (Bejarano et al. 2014). Some examples include, the goliath grouper (*E. itajara*), black grouper (*M. bonaci*; Figure 9.3), yellowfin grouper (*M. venenosa*), rainbow parrotfish (*S. guacamaia*), nurse shark (*Ginglymostoma cirratum*), dog snapper (*Lutjanus jocu*), cubera snapper (*L. cyanopterus*), and Caribbean reef shark (*Carcharhinus perezii*). The more structurally complex MCEs have greater abundances of these species, as fish can shelter under steep overhangs and in caves and crevices.

The presence of many common shallow reef species at mesophotic depths (76% of 103 species), the variability in fish assemblage composition, relative abundance and specific fish life-stages, suggests that connectivity occurs between shallow and mesophotic reefs via multiple mechanisms such as swimming, larval dispersal, and ontogenetic and spawning migrations. Although shelf-edge MCEs support a richer and more diverse fishery resource than nearshore habitats, these areas are subject to a lower impact from fisheries due to distance from the coast (10 km), steep sloping environment, narrow area, and prevailing onshore winds and currents. The connectivity between the less impacted MCEs and the shallow reefs of La Parguera may help support the conservation of commercially fished species by providing a refuge from the heavily exploited shallow water areas.

Figure 9.2 Nassau grouper, *Ephinephelus striatus,* on a MCE in La Parguera, Puerto Rico. (photo Héctor Ruiz)

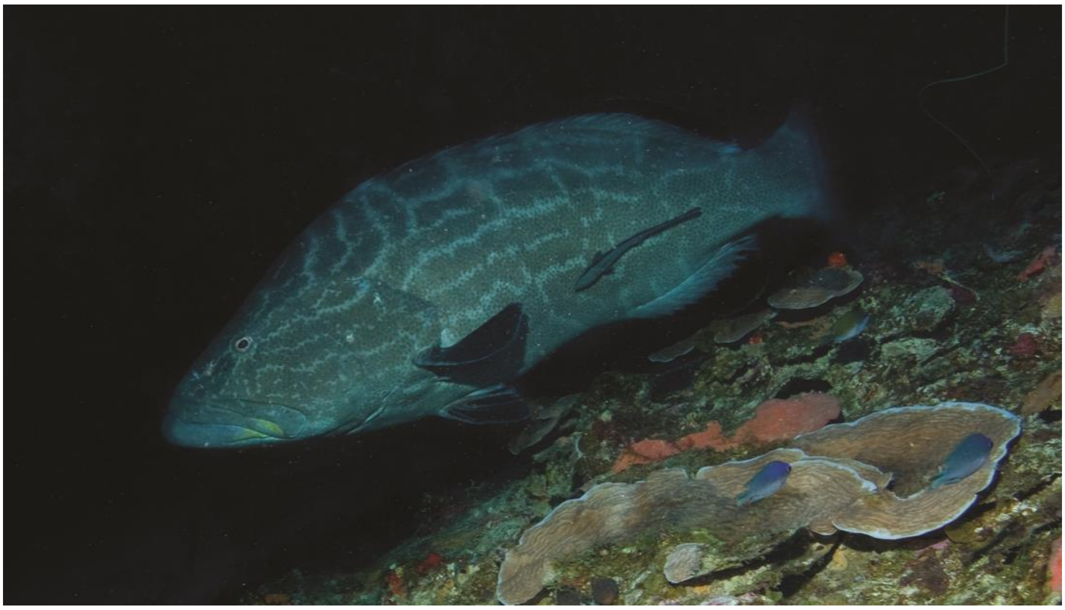


Figure 9.3 Black grouper, *Mycteroperca bonaci*, on a MCE in La Parguera, Puerto Rico. (photo Héctor Ruiz) Many large bodied groupers such as this are now only found in high abundance on MCEs because of overfishing on shallow reefs.

9.3 Climate Change

Increasingly stressful conditions from natural and anthropogenic factors are producing significant population declines and community shifts in shallow coral reef communities. Increasing ocean temperatures as a consequence of increasing atmospheric carbon dioxide levels (IPCC 2014) have led to mass coral bleaching, an increase in the number and prevalence of diseases (produced by pathogens) and ocean waters becoming more acidic, which is predicted to severely affect growth and survivorship of calcifying marine organisms by mid-century (Hoegh-Guldberg et al. 2007; Hoegh-Guldberg and Bruno 2010; Pandolfi et al. 2011; van Hooidonk et al. 2013; Maynard et al. 2015). Bleaching and disease can cause substantial mortality in corals, and can result in shifts in the composition and structure of coral communities over relatively short timescales (Miller et al. 2009; Weil et al. 2009a; 2009b). Ocean acidification, a decrease in the pH level in seawater caused by increasing carbon dioxide levels, will reduce calcification rates in reef-building organisms, accelerate reef erosion problems and directly impact the growth rates of coral and calcifying algae (Feely et al. 2004). Additionally, as ocean temperatures rise, it is expected that the intensity of tropical storms will increase (IPCC 2013), leading to increases in both direct (e.g., damaging storm waves) and indirect (e.g., sedimentation) impacts on MCEs.

The extent of the impact of increasing ocean temperatures and carbon dioxide levels on mesophotic reefs is poorly understood due to the difficulties of sampling at depth and a lack of long-term monitoring records for most MCEs. Like shallow reefs, MCEs are likely to show considerable spatial variability in susceptibility to climate change, depending on factors such as local-scale environmental conditions, historical exposure to extreme conditions, and differing susceptibility to stressors among different reef species. Locations where long-term data do exist, including Palau and the U.S. Virgin Islands, provide valuable insights into environmental processes affecting MCEs that can help guide monitoring, research and management programs. For example, from long-term data sets we have now learnt that MCEs are not protected from all threats, and that oceanographic processes are perhaps more important than depth in terms of buffering corals from the impact of thermal stress (Nir et al. 2014; Neal et al. 2014).

9.3.1 Coral Bleaching

MCEs are not immune to thermal stress (Neal et al. 2014) and coral bleaching. Coral bleaching, as evidenced by coral skeletons that are white or bleached in appearance, occurs when zooxanthellate corals become stressed and lose or expel their symbiotic algae (Figure 9.4). Corals can regain their algae and recover from coral bleaching, but prolonged stressful conditions cause corals to die from starvation.

Coral bleaching often results from an interaction between high water temperatures and high light irradiance (Baird et al. 2009). The thresholds at which bleaching occurs vary among individual species, populations and geographic locations (Baker et al. 2008). In some cases previous exposure to stressors is also a factor affecting resilience. For example, corals that inhabit areas with fluctuating environmental conditions are often more tolerant than those from stable environments (Oliver and Palumbi 2011). The mechanisms influencing thermal tolerance include photo-protective pigments, a natural coral sunscreen (Salih et al. 2000), and switching of algal symbionts to more high light tolerant strains (Bongaerts et al. 2015). However, since corals are thermally adapted or acclimated to their local environment, their ultimate response to fluctuating temperatures will be dictated by how quickly the external environment changes relative to their recent history (Oliver and Palumbi 2011). Such a dependence is evident in the seasonal bleaching observed of mesophotic corals in the Red Sea as summer months bring higher light levels and temperatures (Nir et al. 2014). Consequently, mesophotic corals may exhibit lower bleaching thresholds than corals in shallower habitats. Lower bleaching thresholds at mesophotic depths have been observed in the U.S. Virgin Islands (Tyler B. Smith pers. com.). However, other mesophotic corals occur in dynamic environments exposed to large temperature fluctuations on daily or even hourly scales due to local-scale oceanographic factors such as internal waves. In these cases the local oceanography could reduce the vulnerability of mesophotic corals to bleaching both through mitigating maximum temperatures and exposing corals to large fluctuations in temperature to which they have become adapted (e.g. Buerger et al. 2015). Water temperatures on MCEs may vary with changes in the depth of the thermocline, particularly due to the El Niño Southern Oscillation, upwelling and internal waves. Thermocline depth appears to exert a significant influence on mesophotic reefs, and a deepening of the thermocline associated with El Niño events has been implicated in bleaching of corals in Palau (Patrick L. Colin pers. obs.).

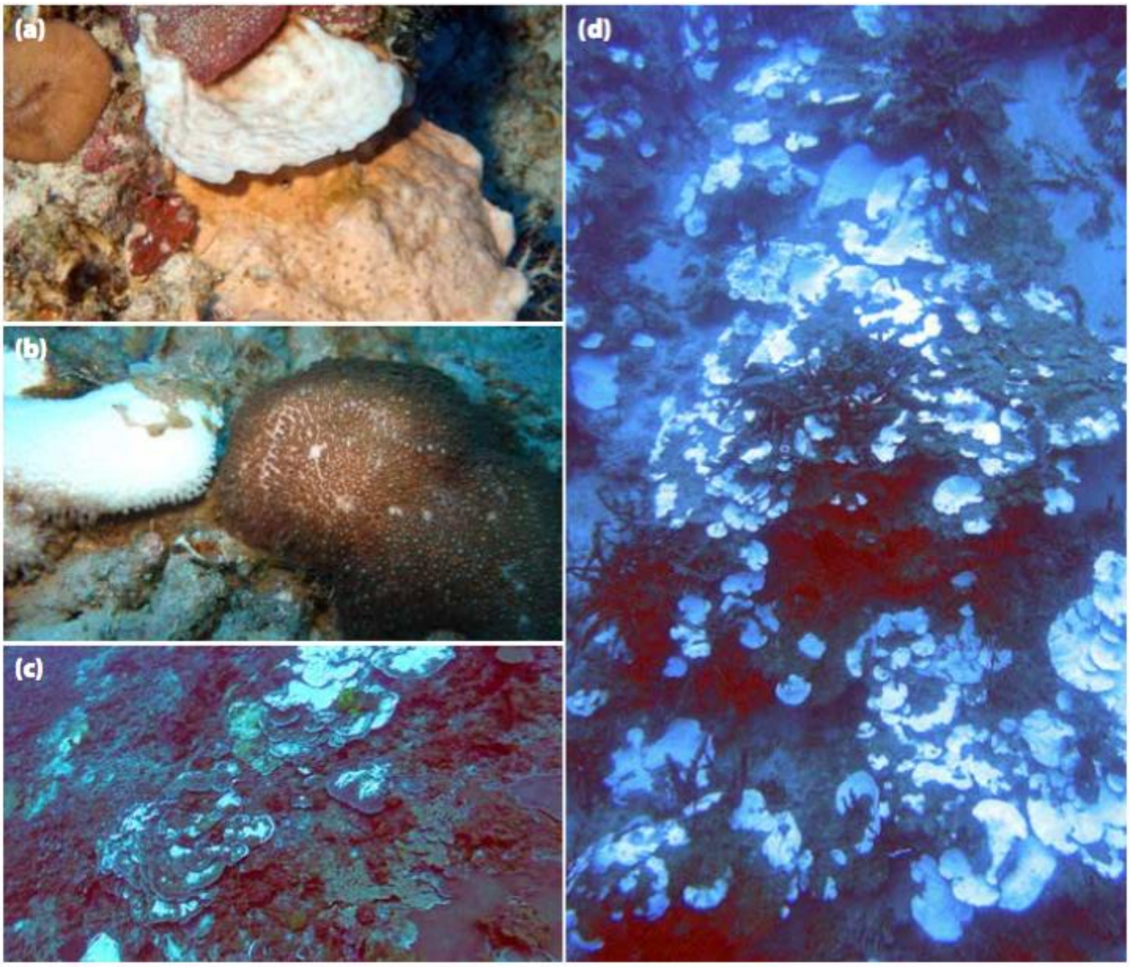
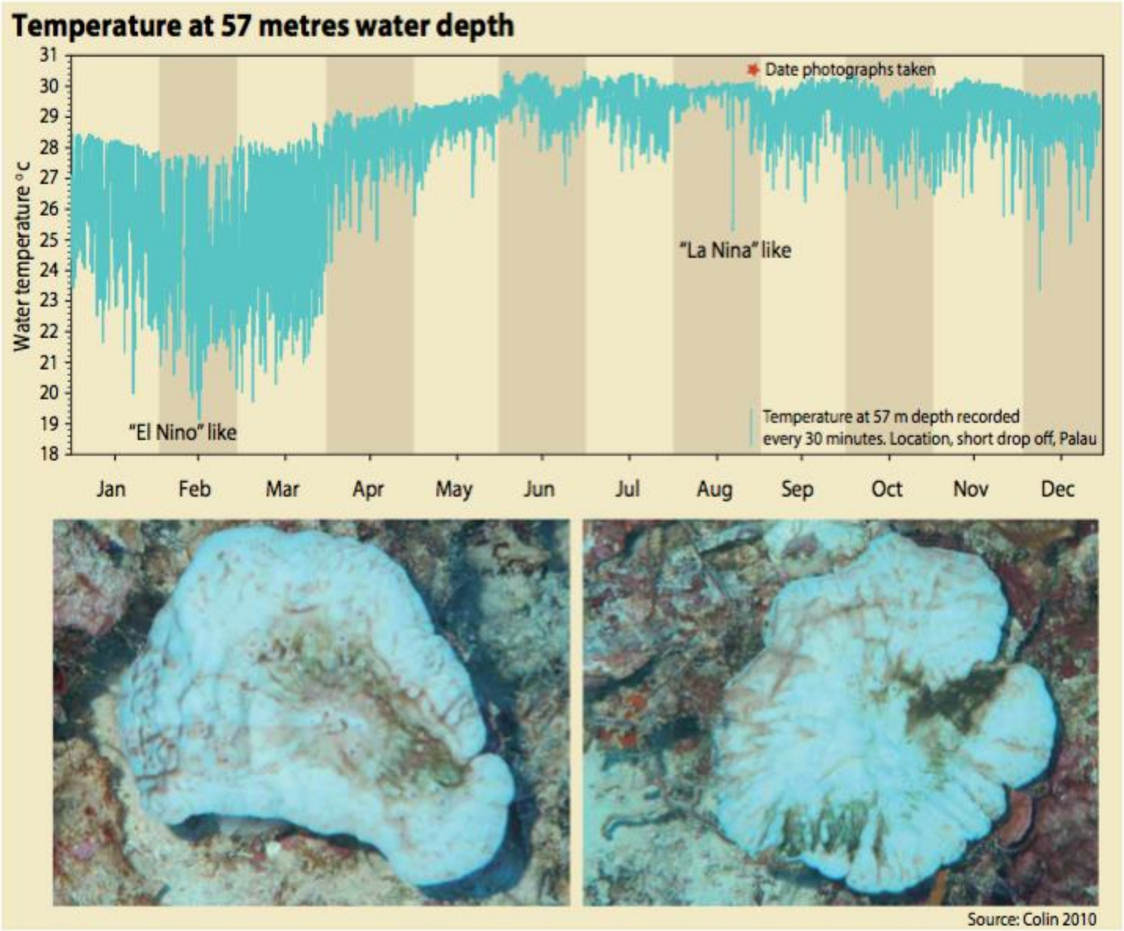


Figure 9.4 The loss of colour from these coral colonies is indicative of coral bleaching on MCEs. (a) *Leptoseris* sp. in Palau at 90 m depth (photo Patrick L. Colin), (b) *Goniopora* sp. at 60 m depth in Eilat, Israel (photo Gal Eyal), (c) and (d) *Agaricia* sp. in the U.S. Virgin Islands (photos Tyler Smith).

9.3.2 Impact of Highly Variable Temperature Regimes on MCEs

The El Niño Southern Oscillation is a naturally occurring phenomenon that produces varying ocean temperatures in the equatorial Pacific. It generally involves fluctuations between two states (El Niño and La Niña) that can last for several seasons. For equatorial reefs in the Western Pacific, El Niño events are associated with cool surface waters, and shallow thermoclines and nutriclines. La Niña events cause the opposite effect, with warm surface waters, and deep thermoclines and nutriclines. This is in contrast to conditions in the Central and Eastern Pacific, where warmer than normal ocean temperatures are associated with El Niño and cooler than normal temperatures are associated with La Niña events. The oscillation between El Niño and La Niña conditions can lead to rapidly fluctuating conditions that may pose a serious threat to MCEs (Glynn and D'Croz 1990).

During a bleaching event in Palau in August 2010, constantly high temperatures to a depth of 90 m were observed for periods of hours to days (Figure 9.5). Overall, the 2010 bleaching event was moderate and limited in duration, so mortality of corals was low (unlike the sustained bleaching event in 1998; Bruno et al. 2001).



Source: Colin 2010

Figure 9.5 Temperature at 57 m depth recorded every 30 minutes during 2010 off Palau.

The rapid shift from a highly stratified El Niño type water column to a uni-thermal (at near 30 °C) water column, with associated coral bleaching on the deep reef, can be seen. The bleached corals are platy agaricid corals within 30 m of the thermograph station. These and other similar corals did not survive the bleaching event of 2010.

9.3.3 Disease

Disease in corals and other organisms has increased in shallow coral reef ecosystems in the past decade along with increasing seawater temperatures (Burge et al. 2014). Increased seawater temperatures render organisms more susceptible to disease outbreaks, as well as bleaching events. Recent surveys of MCEs in Puerto Rico and the U.S. Virgin Islands indicate that mesophotic coral communities are indeed susceptible to biotic diseases (Smith et al. 2010; Baker et al. 2016) down to at least 100 m in depth. Coral bleaching and disease were observed in at least six of the 23 mesophotic scleractinian species (Figure 9.6) – *Agaricia undata*, *A. lamarcki*, *Undaria agaricites*, *Mycetophyllia aliciae*, *Montastraea cavernosa* and *Stephanocenia intersepta*. Most of the "disease signs" observed fall into a general category known as "white syndromes" (Figure 9.6) due to their characteristic white area of recent tissue-cleared skeletal material, with some signs resembling typical white plague disease (Figure 9.6). In 2014, at Pulley Ridge, a mesophotic reef in the eastern Gulf of Mexico, a total of 7,329 individual plate corals (*Agaricia* spp. and *Helioseris cucullata*) were counted from transect photos of which 247 were noted to be bleached, partially bleached, totally bleached, partly dead, recently dead, or diseased, resulting in 4.0% morbidity of the total population measured (Reed et al. 2015).

An example of white plague disease-like signs was observed in a single medium sized (832 cm^2) coral colony of *M. aliciae* at 55 m off La Parguera, Puerto Rico. This colony was healthy in October 2009, but showed white plague disease-like signs in December 2009, and was dead by March 2010 (Figure 9.7). Rate of tissue mortality varied approximately between 6-10 cm/month, one-third slower than the maximum tissue rate mortality reported for this disease type in shallower waters, but still faster than all other shallow coral diseases except black band disease (Weil 2004).

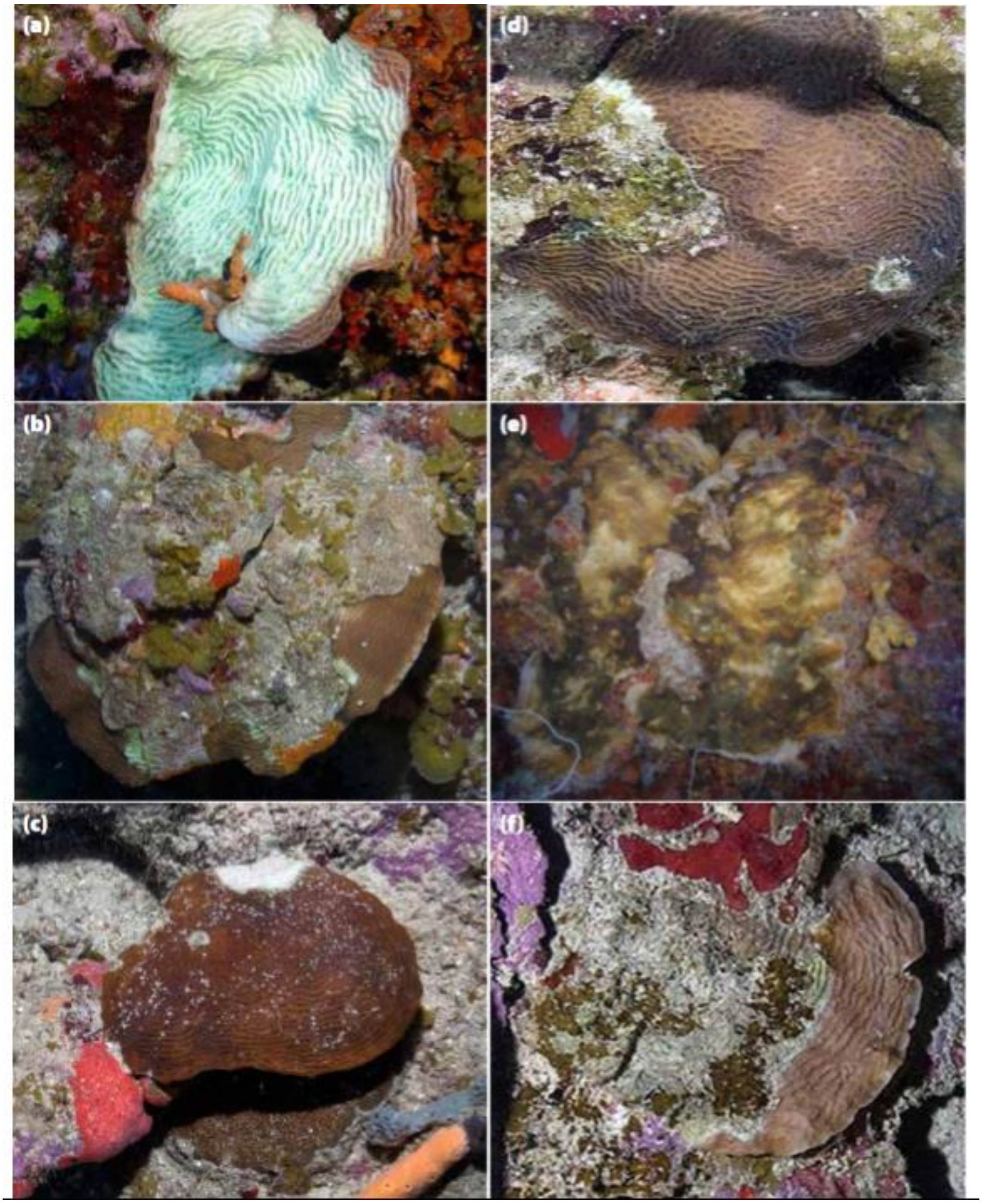


Figure 9.6 Bleached and diseased coral colonies in MCEs off La Parguera, Puerto Rico.

White plague-like signs were observed in agaricids (A and C) and *Mycetophyllia* sp. White band syndromes were observed in *Agaricia grahamae* (B) and *Undaria agaricites* (D). Bleaching was observed in *A. grahamae* (E), *Stephanocenia intersepta* and *U. agaricites*. Many colonies show signs of recent mortality (F), (photos Héctor Ruiz).

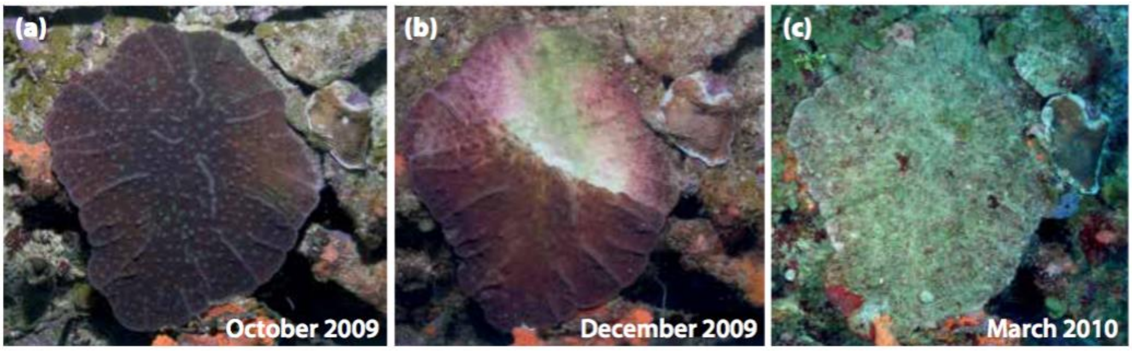


Figure 9.7 A time-series showing a colony of *Mycetophyllia aliciae* at 50 m off La Parguera, Puerto Rico.

The colony was healthy in October 2009, developed signs of white-plague-like disease and suffered rapid tissue mortality (white skeleton without tissue and secondary algae colonization in middle photograph), and was dead by March of 2010 (photos Héctor Ruiz).

9.3.4 Ocean acidification

Rising levels of carbon dioxide in the atmosphere, caused in large part by the burning of fossil fuels, has led to an increase in the levels of carbon dioxide in the ocean. When carbon dioxide is absorbed by seawater, it causes the seawater to become more acidic and carbonate ions to be less abundant. Corals and other calcifying organisms require calcium carbonate to build skeletons and shells. Therefore, increasing ocean acidification may inhibit growth, including reef building (Langdon and Atkinson 2005; Albright et al. 2010; Fabricius et al. 2011). Ocean acidification can also impact organisms in other ways, such as the ability of fish to detect predators (Munday et al. 2014) and a decrease coral settlement rates (Doropoulos et al. 2012). Perhaps the most consistent and pronounced effects observed for coral reef ecosystems in response to ocean acidification are enhanced rates of bioerosion (whereby hard substrata is eroded by living organisms; Andersson and Gledhill 2013).

Little information exists regarding the effects of ocean acidification on MCEs. One study that examined the precious coral, *Corallium rubrum*, can be used as an example of what could happen to mesophotic corals as it occurs at mesophotic depths in the Mediterranean Sea. In controlled studies simulating ocean acidification conditions anticipated by the end of the century *C. rubrum* exhibited reduced calcification and polyp activity (Cerrano et al. 2013). It has been suggested that calcification in the Mediterranean Sea may have already declined (by 50 %) as a consequence of anthropogenic-induced ocean acidification (Maier et al. 2012). In some regions, red corals are a component of MCEs; therefore, it is plausible that these populations currently inhabiting mesophotic habitats will be directly impacted ocean acidification over the course of the century.

9.4 Tropical storms

Hydrodynamic disturbances associated with storms (hurricanes in the Atlantic and Eastern Pacific, typhoons in the North Pacific and Indian Ocean, cyclones in the South Pacific) affect many coral reef regions, and play a significant role in structuring shallow reefs (Gardner et al. 2003; De'ath et al. 2012). Water velocities from storm waves (maximum orbital velocities) decline exponentially with depth, and mesophotic reefs are therefore afforded some protection from hydrodynamic disturbances (e.g. Woodley et al. 1981). However, organisms living in the upper mesophotic zone (30-50 m) may experience direct impacts from storms (White et al. 2013). Indirect effects of storms, such as debris avalanches, can affect MCEs (Harmelin-Vivien and Laboute 1986), while very severe storms can damage reefs to depths of at least 70 m (Bongaerts et al. 2013). The typical plating and foliose morphologies of many mesophotic coral species leaves them prone to degradation following physical disturbance. Significant impacts to large foliose coral communities were detected on MCEs off Okinawa combined with large increase in rubble following a typhoon in 2012 (White et al. 2013). Submerged banks not exposed to breaking waves are likely to be less vulnerable than lower-reef slopes (Roberts et al. 2015). In any case, predicted changes in the location, frequency and particularly the intensity of storms, will likely affect MCEs.

9.5 Sedimentation and pollution

Coral reefs normally require warm, clear, nutrient-poor water for development and survivorship (Veron 2000). Sedimentation smothers corals causing them to compromise energetic efficiency (Riegl and Branch 1995), abrasion to coral tissues (Loya 1976), impacts to calcification rates and coral growth, interference with feeding, photosynthesis of the symbiotic algae, respiration and settlement processes and lowers fecundity of the corals (reviewed on shallow reefs by Fabricius 2005). Sedimentation, nutrient enrichment and turbidity can degrade shallow and mesophotic coral reefs at local scales (Fabricius 2005; Vega Thurber et al. 2013; Katz et al. 2015).

MCE environments adjacent to shallow reef systems are usually influenced by sediment from the shallow reefs above (Figure 9.8). MCEs further offshore can also be impacted by sediment, especially from anthropogenic origins (e.g. sewage outflows and dredge dump sites; Armstrong and Singh 2012).

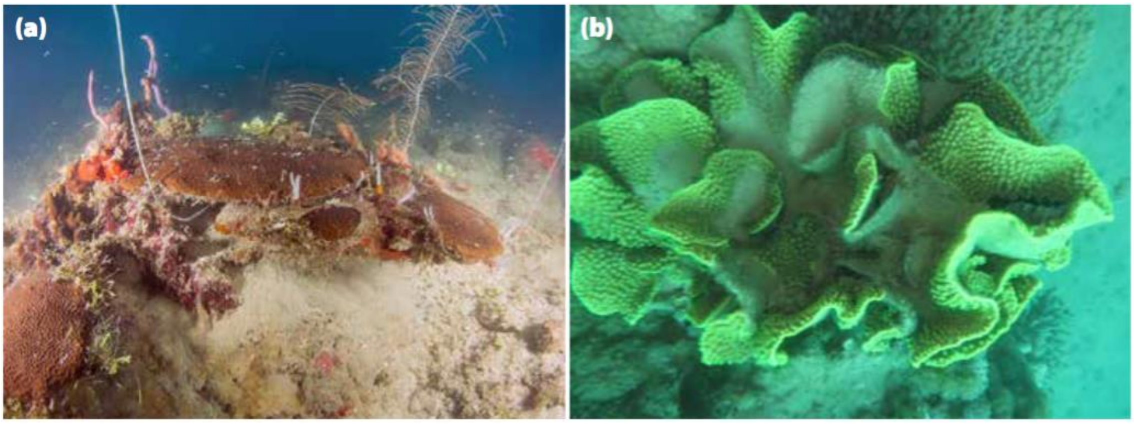


Figure 9.8 Sedimentation on mesophotic reefs.

(a) Large, easily disturbed sediment beds surround mesophotic reefs at 50 m off the south shore of Utila, Honduras. (b) *Turbinaria* sp. at 40 m off Eilat, Israel, with sediment deposited within the foliose structure, smothering the coral polyps (photos (a) Ally McDowell (b) Gal Eyal).

9.5.1 Case study: Anthropogenic stressors on MCEs in Ponce, Puerto Rico

MCEs located in a narrow insular shelf off the south coast of Puerto Rico are subject to a range of stressors such as outflow from several rivers, a sewage treatment plant with a deep sewer outfall, three deep dredge dump sites, and shipping activities.

A 2010 study examined the MCEs off Ponce (Appeldoorn et al. 2016). These MCEs were found to receive high rates of sedimentation and enriched ^{15}N (an indicator of anthropogenic nitrogen) concentrations. The most impacted sites, which showed a progressive increase in sedimentation and a decline in live benthos, were located close to shore, associated with dredge disposal sites (Figures 9.9 a, b). Higher sedimentation rates and reduced light penetration resulted in poor MCE development, as well as shallower maximum depths at which mesophotic corals, algae and other photosynthetic organisms could survive.

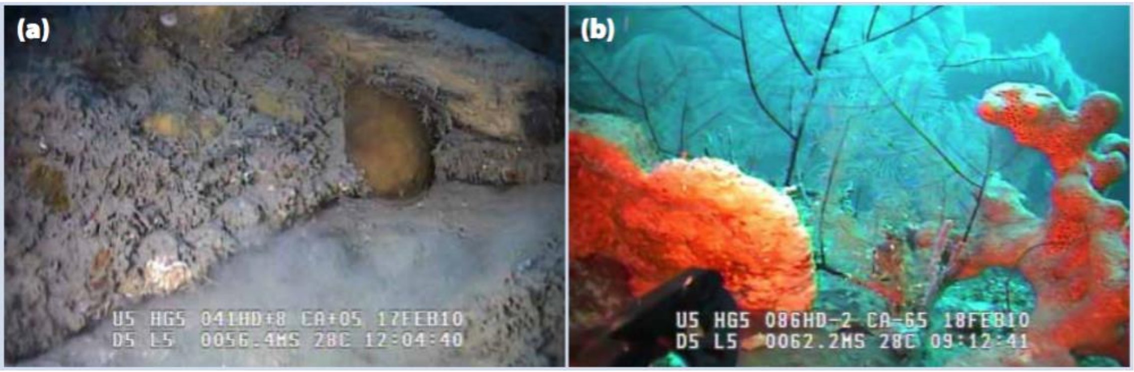


Figure 9.9 Remotely operated vehicle video captures from the area off Ponce, Puerto Rico.

(a) An impacted site close to shore at a former dredge disposal site, Ratones at 56 m, showing heavy siltation over the reef structure. (b) A less impacted offshore site, Caja de Muertos at 62 m, showing large sponges, *Antipathes* spp., black corals and an *Agaricia* sp. colony (photos (a) Francisco Pagan, (b) Richard S. Appeldoorn).

9.6 Marine Aquarium Trade

The international marine aquarium trade involves the collection, breeding and supply of many shallow and mesophotic reef fishes, corals and other invertebrates for home and public aquaria. Marine life in the trade is supplied from 40-45 countries (Rhyne et al. 2012). Estimates suggest that approximately 150 hard coral species and 1,500 fish species (across 50 families) are actively targeted by the marine aquarium trade (Wabnitz et al. 2003). Recent research has highlighted the role of the Philippines and Indonesia as the global dominant exporters, responsible for 55 % and 31 %, respectively, of all live fish imports into the United States for 2005 (Rhyne et al. 2012).

The United States imported approximately 11 million fish during the year 2004-05 (Rhyne et al. 2012), and the European market is of a similar size (Wood 2001). This suggests a global trade of over 22 million fish annually. Currently, most monitoring of the marine aquarium trade is based on the Convention on International Trade in Endangered Species (CITES) permits or packing lists for international shipping of live fish.

It is unclear what proportion of the traded marine life is collected from mesophotic reefs. However, there is trade in mesophotic reef fish species with countries such as Japan, where there is an enthusiastic market for ‘rare’ mesophotic fish species. Traded mesophotic species include anthias (Subfamily: Anthiinae, Figure 9.10), dottybacks (Family: Pseudochromidae), butterflyfish (Family: Chaetodontidae), wrasse (Family: Labridae) and pygmy angelfish (Genus: Centropyge). In particular, within the pygmy angelfish, the Peppermint angelfish (*Centropyge boylei*) and the Narcosis angelfish (*Centropyge narcosis*) are highly prized (see case study). Mesophotic fish are commonly labelled as ‘rare’ by the marine aquarium trade, but this is likely to reflect their limited supply due to the difficulties of collection rather than true ecological rarity. With current collection pressure quite dispersed over large reef areas, it is unlikely that the marine aquarium trade is having a large impact at mesophotic depths. However, populations of many mesophotic reef fish species lack quantitative assessments, making sustainable collection quotas hard to set.

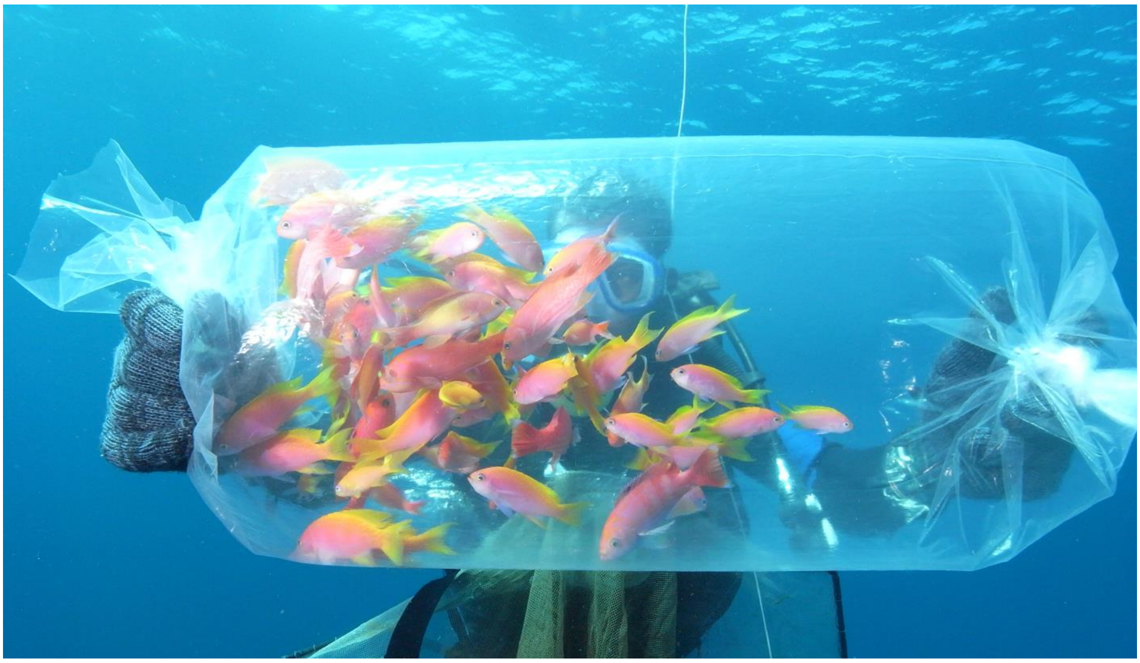


Figure 9.10 Anthias, *Pseudanthias bimaculatus* (Subfamily: Anthiinae), collected at 50 m in the Maldives for the marine aquarium trade. (photo Elizabeth Wood)

As many mesophotic fish species in the aquarium trade have gas filled swim bladders, careful planning is required to bring them to the surface without injury. Swim bladders expand and burst if fish are brought too rapidly to the surface (Munday et al. 2015). Traditionally, collectors have carefully punctured the swim bladder of the fish using a needle on ascent, though this may result in increased infection risk later. Many collectors still use ‘needling’ to bring collected fish to the surface (Figure 9.11), although, increasingly collectors are using decompression stops to allow fish time for gas to move out of the swim bladder during ascent. Work on Yellow Tang (*Zebrasoma flavescens*) on Hawaiian shallow reefs found venting excess gas from the swim bladder using a needle reduced immediate mortality post-dive, but lead to elevated serum cortisol (a proxy for fish stress), whereas the addition of decompression stops lead to lower increases in serum cortisol (Munday et al. 2015).

Figure 9.11 Collector using a needle to puncture the swim bladder of an anthias, *Pseudanthias bimaculatus*, to prevent the swim bladder bursting during ascent. (photo Elizabeth Wood)

In terms of scleractinian corals, it is unlikely that many are being collected from mesophotic depths, as most coral species in high commercial demand are found at greatest abundance on shallow reefs or reef slope environments less than 30 m in depth. With increased availability of advanced diving technology allowing access to greater depths, there could be an increase in demand for mesophotic species in the future.

9.6.1 Case study: Peppermint and Narcosis angelfish, jewels in the aquarium trade

The Peppermint (*Centropyge boylei*) and Narcosis (*Centropyge narcosis*) angelfishes (Figure 9.12) are two of the most prized fishes for home aquariums. They are both found in the Cook Islands and the Peppermint angelfish has also been observed in French Polynesia. Peppermint angelfish have been recorded at depths of 55-120 m (Pyle et al. 2009), while Narcosis angelfish have been reported deeper at 100-120 m (Pyle and Myers 2010). Both species ranges are likely to be severely under reported due to the difficulties in surveying these depths. Due to the rarity of collection in the marine aquarium trade, these species command extremely high prices. In 2012, private buyers offered up to 30,000 USD for a single Peppermint angelfish (Kaneshiro 2012), while a single Narcosis angelfish sold for 5,000 USD within seconds of being offered for sale online (Adams 2012).

Figure 9.12 (a) Peppermint angelfish (*Centropyge boylei*) and (b) the Narcosis angelfish (*Centropyge narcosis*) photographed at approximately 90 m in the Cook Islands. (photos Richard Pyle)

9.7 Precious Coral Fishery

Precious corals do not require sunlight and are generally referred to as deep-sea corals. They are found all over the world, down to the deep ocean depths. However, the range of precious corals overlaps with MCEs, and in some areas, such as around Hawai‘i, they can significantly contribute to habitat complexity and provide stabilisation to substrates at mesophotic depths (Tsounis et al. 2010).

Many of the species used in jewellery manufacture, including the prized black coral *Antipathes griggi* (Figure 9.13), are collected at mesophotic depths. In contrast with precious red corals (*Corallium spp.*), which have historically been collected by trawling in the Mediterranean, in Hawai’i less destructive techniques were utilized. Scuba diving was principally used for Hawaiian black coral collection starting in the 1960s, with manned submersibles and remotely operated vehicles used since the 1980s (Tsounis et al. 2010).

Considering the ecological role of precious corals in some mesophotic habitats, it is important to ensure that harvesting is sustainably managed to ensure ecosystem functions such as habitat complexity and substrate stabilisation provided by precious corals are maintained.

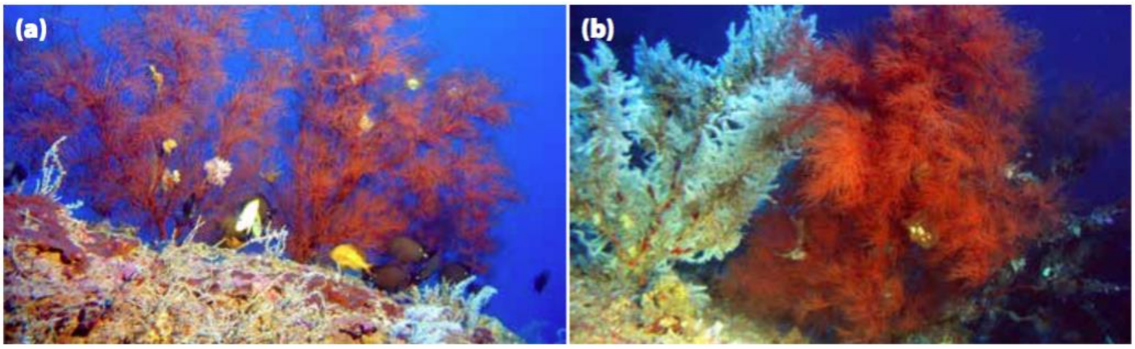


Figure 9.13 Black corals (Antipatharia) from mesophotic depths (70-100 m) in the ‘Au‘au Channel, Hawai‘i. (photos NOAA Hawai‘i Undersea Research Laboratory)

9.8 Invasive species

Invasive species are introduced species that are not native to a particular ecosystem. They may rapidly reproduce, causing detrimental ecological effects. Several invasive species have been recorded on MCEs, with the snowflake coral (*Carijoa riisei*), Lionfishes (*Pterois volitans* and *P. miles*) and green algae (*Avrainvillea*) being best documented.

9.8.1 Snowflake coral

The invasive parasitic snowflake octocoral (*Carijoa riisei*) was first recorded on the island of O‘ahu, in the Hawaiian Archipelago in the 1960s. Native to the West Pacific, it is not clear how the snowflake coral was first transported to Hawai‘i (Concepcion et al. 2010). Since its arrival, the snowflake coral has spread around most of the main Hawaiian Islands. It settles on black corals and smothers them (Figure 9.14; Kahng and Grigg 2005). In 2001, on the Maui Black Coral bed, it was found to cover more than 60 % of black corals between 80-105 m in depth, as well as settling on the undersides of mesophotic scleractinian corals and overgrowing them (Kahng and Grigg 2005). Like many invasive species, snowflake coral has a high reproductive rate. It employs continuous spawning of negatively buoyant eggs, as well as being able to spread vegetatively using ‘runners’ (Kahng et al. 2008).

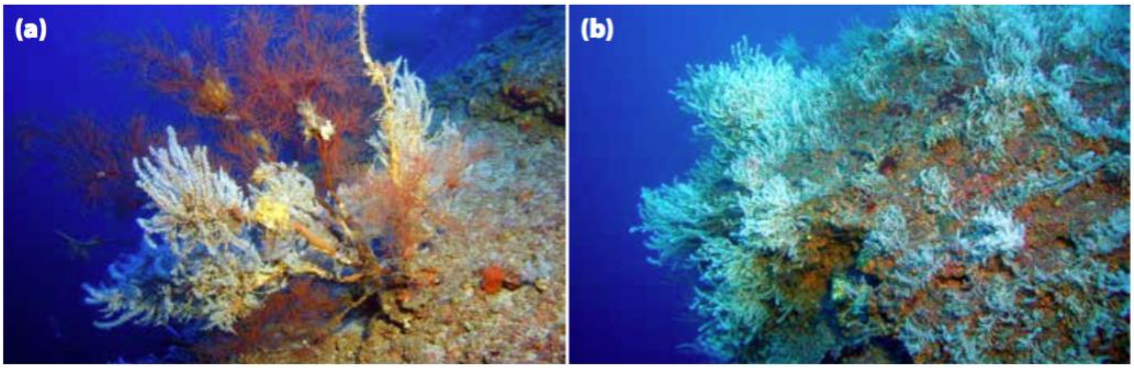


Figure 9.14 The ‘Au‘au Channel, Hawai‘i at 70-100 m showing (a) the snowflake coral, *Carijoa riisei*, smothering a black coral colony and (b) a *C. riisei* dominated mesophotic habitat. (photos NOAA Hawai‘i Undersea Research Laboratory)

9.8.2 Lionfish

Lionfish, *Pterois volitans* (Figure 9.15) and *P. miles*, originally native to the Indian and Pacific Oceans and Red Sea, are believed to have been introduced into waters around Florida, U.S. in the 1980s from home aquariums (Whitfield et al. 2002; Schofield 2009). Initially there were few sightings, but over the past decade they have rapidly spread across the western Atlantic colonising shallow and mesophotic reef habitats where they can reach at least five times their natural population densities (Darling et al. 2011; Kulbicki et al. 2012). Their current western Atlantic range stretches from Bermuda and North Carolina in the north, to Brazil in the south, encompassing the Caribbean Sea and Gulf of Mexico (Schofield 2009; 2010). Lionfish have been observed at 55 m in Puerto Rico (Bejarano et al. 2014), greater than 100 m in the Bahamas (Lesser and Slattery 2011), 112 m in the northwestern Gulf of Mexico (Nuttall et al. 2014) and 120 m in Honduras (Schofield 2010).

Lionfish are voracious, gape-limited predators, feeding on a wide variety of fish and invertebrate species. As invasive species, their presence on reefs has been observed to cause declines in prey fish biomass of up to 65 % (Green et al. 2012) and reduce native fish recruits by up to 79 % (Albins and Hixon 2008). Lionfish are highly successful invaders due to a combination of prey naivety (native prey species do not recognise lionfish as predators), a lack of predators, defensive venomous spines and a broad thermal tolerance (Morris et al. 2009). Furthermore, lionfish are highly fecund, spawning all year round at a maximum rate of once every 2-3 days (Gardner et al. 2015). Lionfish produce buoyant egg bundles, aiding dispersal and new site colonisation by drifting in surface currents.

Figure 9.15 Invasive lionfish (*Pterois volitans*) on a mesophotic reef at 60 m off Utila, Honduras. (photo Ally McDowell)

In addition to the direct effects on native reef fish and invertebrate abundance and recruitment, lionfish have also been linked with indirect MCE shifts. In the Bahamas, it is suggested that lionfish are associated with a shift in MCEs from coral- to algal-dominated states by altering the balance in the food chain, known as a trophic cascade. Lionfish feed upon mesophotic herbivorous and omnivorous fish, leading to a reduction in grazing pressure on the alga *Lobophora variegata*, and hence, an increase in algal abundance relative to corals and sponges occurs (Lesser and Slattery 2011; Slattery and Lesser 2014).

Thus, the lionfish invasion may contribute to major MCE shifts over relatively short (several years) timescales.

Lionfish exhibit ontogenetic migrations, with older lionfish found at greater depths on reef habitats (Claydon et al. 2012). Juvenile lionfish tend to use shallow reef environments, mangroves and seagrass beds as nursery grounds (Barbour et al. 2010; Claydon et al. 2012). It is likely that lionfish populations on MCEs form an extension of this ontogenetic migration, although this requires further research.

Current control measures for lionfish focus on hand culling with spears, often using dive volunteers (Figure 9.16). Culling is known to reduce the abundance of lionfish on shallow reefs (Frazer et al. 2012), and has been shown to aid the recovery of native shallow reef fauna (Green et al. 2014), although continuous culling is necessary to maintain low lionfish populations. There has been limited culling on MCEs, and there is no assessment of its effect. Across the western Atlantic, the International Coral Reef Initiative has a regional lionfish strategy aimed at fostering collaboration between governments, scientists and reef-reliant industries in the management of lionfish.

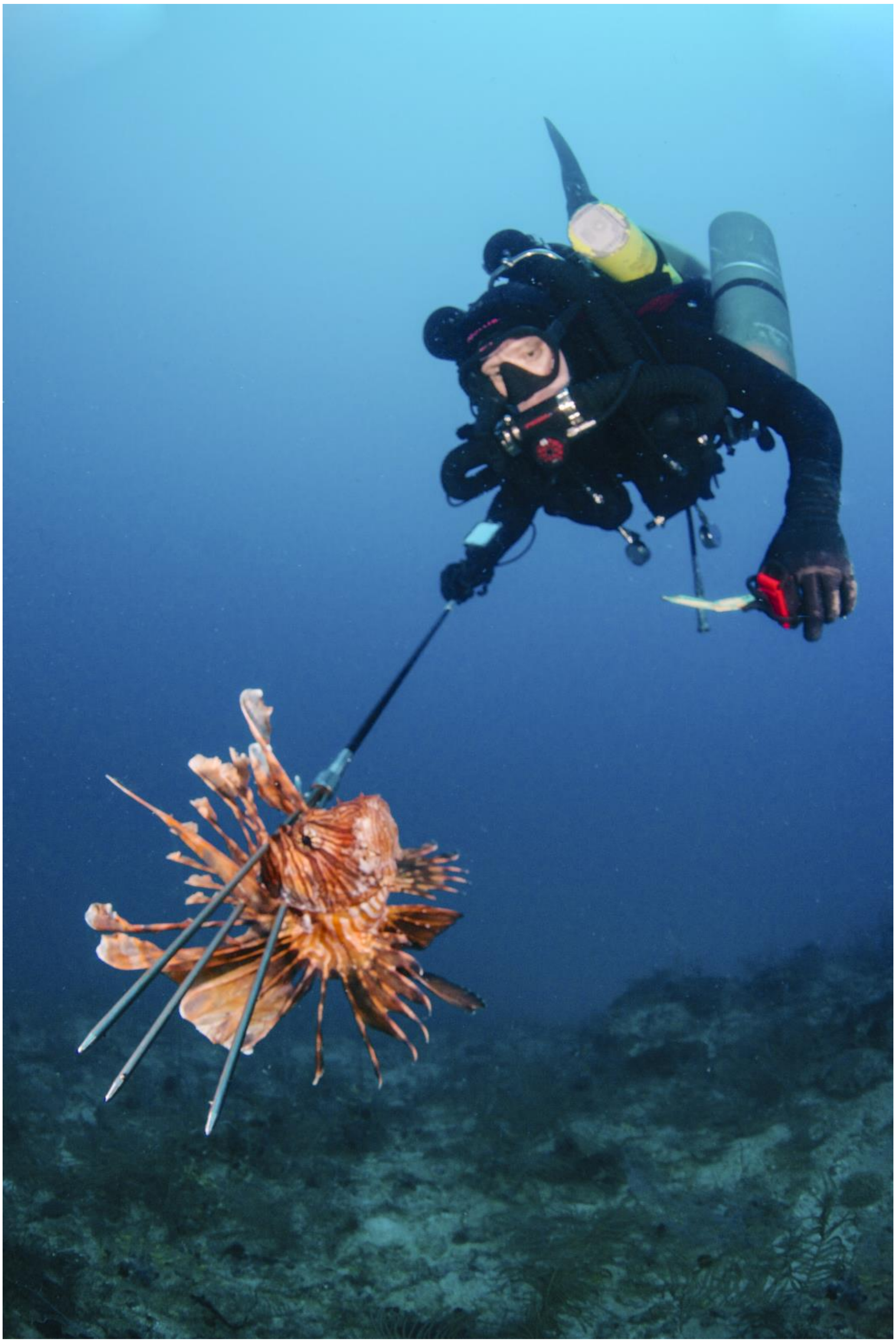


Figure 9.16 A diver using a closed-circuit rebreather to spear invasive lionfish at 50 m off Utila, Honduras. (photo Brian Sullivan)

9.8.3 Case study: Lionfish on the Pulley Ridge Mesophotic Reef in the Gulf of Mexico

Lionfish are now common throughout the western Atlantic Ocean, the Gulf of Mexico and Caribbean. First sightings in the Gulf of Mexico occurred in 2010 and lionfish populations have increased dramatically since then on many shallow and mesophotic reefs. During submersible dives in 2010, only a few lionfish were documented along the West Florida shelf MCEs in the Gulf of Mexico (Reed and Rogers 2011) and none were reported in the same region in 2003 (by a United States Geological Survey study). However, by 2013, a total of 703 lionfish were observed during remotely operated vehicle surveys at Pulley Ridge, a mesophotic reef (60-80 m depth) on the West Florida shelf (Figure 9.17; Reed et al. 2015). Of the 33 1-km^2 blocks surveyed at Pulley Ridge, 72 % had lionfish and most of the fish were associated with active red grouper burrows. From multibeam sonar maps of Pulley Ridge, over 136,000 red grouper burrows are visible in the region, which could mean the possibility of tens of thousands of lionfish.

Figure 9.17 The invasive lionfish has been found in increasing numbers at red grouper burrows at Pulley Ridge at 70 m. (photo John Reed, NOAA Coral Ecosystem Connectivity Expedition 2014)

9.8.4 Green Alga Avrainvillea

Avrainvillea sp., an invasive green alga (Figure 9.18) known for its propensity to displace native seagrass populations in the intertidal zone (Peyton 2009), was first reported in Hawai‘i in 1981 in 13 m of water off Kahe Point, O‘ahu (Brostoff 1989), and is now found offshore of O‘ahu at depths to 90 m (Peyton 2009; Spalding 2012). Molecular studies are still in the process of determining whether it is a new species to Hawai‘i, or an introduction from elsewhere. *Avrainvillea* spread from its first collection site at Kahe Point to Maunalua Bay (50 km distance) within about six years (Brostoff 1989). Presently, the distribution in Hawai‘i extends along most of O‘ahu’s southern and western shores, and has been documented in a one-time collection from Kaua‘i (Smith et al. 2002). The mechanism(s) of its rapid propagation is not known. The species’ high abundance at mesophotic depths introduces the possibility that it may have originated in mesophotic waters and then moved into shallower waters (Spalding 2012).

Avrainvillea forms dense mats on either hard or soft substrate. It sequesters fine sediments under its holdfast (Littler et al. 2005), creating anoxic mounds of soft sediment in otherwise hard-substrate dominated environments. Within its natural range, *Avrainvillea* has been found at mesophotic depths in Mauritius (*A. amadelpha*) at 90 m (Gepp and Gepp 1911), and on a Bahamian seamount (*A. levis*) at 125 m (Littler and Littler 1992). Despite its high abundance around west and south O‘ahu, *Avrainvillea* has not yet been found in the nearby ‘Au‘au Channel, which contains extensive MCEs, and its competitive impact on dominant native mesophotic algae, such as *Halimeda kanaolana* meadows, in this region is unknown. The high abundance and ability of *Avrainvillea* to grow on a diversity of substrate types increases the likelihood of its transport between the islands by potentially contaminating construction materials for underwater development, such as pipelines for an energy network, or by attachment to boat anchors. Removal efforts for mesophotic populations of

Avrainvillea would be costly and difficult, highlighting the need to limit the spread of this highly adaptable and invasive algal species.

Figure 9.18 *Avrainvillea* sp., an invasive green alga at 50 m, is found offshore of O‘ahu's southern and western shores from shallow to mesophotic depths.

The diver is measuring the sediment depth under the *algal mat*. This species has the potential to transform a diverse, coral-dominated area into dense mats of algae over anoxic mounds of sediment (photo Heather Spalding).

9.9 Management options

While some pressures on MCEs are global in origin, and require a global response, others are regional or local. It is important that measures to protect an individual MCE take an adaptive, ecosystem-based management approach to address the cumulative impacts, considering both global and local pressures. MCEs are vulnerable to the same threats and pressures as near-surface reefs stemming from land-sourced inputs (ridge to reef), but they are rarely included in the design of marine spatial plans and marine protected areas. Lack of awareness about the existence of MCEs and a limited understanding of their spatial distribution, are the main reasons for their exclusion from management plans. The good news is that the same management approaches used for shallow-water reefs can be used for MCEs (Table 9.2).

Table 9.2 Management options used for shallow-water reefs (Mumby and Steneck 2008) that could address comparable issues affecting MCEs.

	Shallow coral reef ecosystems	MCEs
Management Options	• Fishing closures • Marine protected areas (MPAs) • Wastewater treatment and management to reduce pollution • Shipping industry guidelines to curb introduced species • Shipping industry guidelines to restrict discharge of oil • Tourism guidelines to reduce reef damage • Placement of fixed mooring buoys to reduce anchor damage • Coral reef rehabilitation for damaged areas • Public education and involvement	• Fishing closures • MPAs (MCEs aren’t considered in most countries) • Wastewater treatment and management to reduce pollution (potential) • Shipping industry guidelines to curb introduced species (potential) • Shipping industry guidelines to restrict discharge of oil (potential) • Diving guidelines to reduce reef damage (potential) • Placement of fixed mooring buoys to

		reduce anchor damage (potential) • Guidelines for oil and gas exploration, alternative energy, cable and pipelines (potential)

9.10 References

- Adams J (2012) LiveAquaria sells $5000 narcosis angelfish almost immediately. http://reefbuilders.com/2012/10/18/liveaquaria-narcosis-angelfish/
- Albins MA, Hixon MA (2008) Invasive Indo-Pacific lionfish *Pterois volitans* reduce recruitment of Atlantic coral-reef fishes. Marine Ecology Progress Series 367:233–238
- Albright R, Mason B, Miller M, Langdon C (2010) Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. Proceedings of the National Academy of Sciences 107:20400–20404
- Andersson AJ, Gledhill D (2013) Ocean Acidification and Coral Reefs: Effects on Breakdown, Dissolution, and Net Ecosystem Calcification. Annu. Rev. Marine. Sci. 5:321–348
- Appeldoorn R, Ballantine D, Bejarano I, Carlo M, Nemeth M, Otero E, Pagan F, Ruiz H, Schizas N, Sherman C, Weil E (2016) Mesophotic coral ecosystems under anthropogenic stress: a case study at Ponce, Puerto Rico. Coral Reefs 35:63–75
- Armstrong RA, Singh H (2012) Mesophotic Coral Reefs of the Puerto Rico Shelf. In: Harris P.T., Baker E.K. (eds) Seafloor Geomorphology as Benthic Habitat. Elsevier, London, pp 365–374
- Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2009) Coral bleaching: the role of the host. Trends in Ecology & Evolution 24:16–20

- Baker AC, Glynn PW, Riegl B (2008) Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. Estuarine, Coastal and Shelf Science 1–37
- Baker EK, Harris PT (2011) Habitat mapping and marine management. Seafloor geomorphology as benthic habitat - GeoHAB atlas of seafloor geomorphic features and benthic habitats 23–38
- Baker EK, Puglise KA, Harris PT (2016) Mesophotic coral ecosystems - a lifeboat for coral reefs? The United Nations Environment Programme and GRID-Arendal, Nairobi and Arendal
- Barbour AB, Montgomery ML, Adamson AA, Díaz-Ferguson E, Silliman BR (2010) Mangrove use by the invasive lionfish *Pterois volitans*. Marine Ecology Progress Series 401:291–294
- Bejarano I, Appeldoorn RS, Nemeth M (2014) Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico. Coral Reefs 33:313–328
- Bejarano Rodríguez I (2013) Deep Reef Fishes off La Parguera Insular Slope, Puerto Rico, and their Connectivity with Shallow Reefs. University of Puerto Rico, p 1–115
- Bongaerts P, Frade PR, Hay KB, Englebert N, Latijnhouwers KRW, Bak RPM, Vermeij MJA, Hoegh-Guldberg O (2015) Deep down on a Caribbean reef: lower mesophotic depths harbor a specialized coral-endosymbiont community. Scientific Reports 5:7652
- Bongaerts P, Muir P, Englebert N, Bridge TCL, Hoegh-Guldberg O (2013) Cyclone damage at mesophotic depths on Myrmidon Reef (GBR). Coral Reefs 32:935–935
- Brostoff WN (1989) *Avrainvillea amadelpha* (Codiales, Chlorophyta) from Oahu, Hawaii. Pacific Science 43:166–169
- Bruno J, Siddon C, Witman J, Colin P, Toscano M (2001) El Niño related coral bleaching in Palau, Western Caroline Islands. Coral Reefs 20:127–136

- Buerger P, Schmidt GM, Wall M, Held C, Richter C (2015) Temperature tolerance of the coral *Porites lutea* exposed to simulated large amplitude internal waves (LAIW). Journal of Experimental Marine Biology and Ecology 471:232–239
- Burge CA, Mark Eakin C, Friedman CS, Froelich B, Hershberger PK, Hofmann EE, Petes LE, Prager KC, Weil E, Willis BL, Ford SE, Harvell CD (2014) Climate Change Influences on Marine Infectious Diseases: Implications for Management and Society. Annu. Rev. Marine. Sci. 6:249–277
- Cerrano C, Cardini U, Bianchelli S, Corinaldesi C, Pusceddu A, Danovaro R (2013) Red coral extinction risk enhanced by ocean acidification. Scientific Reports 3:1457
- Claro R, Sadovy Y, Lindeman KC, García-Cagide AR (2006) Effects of Fishing on Spawning Aggregations in Cuba strongly influenced by Fish and Fisher behaviour and Fish habitat. http://www.gcfi.org/proceedings/proceedings/effects-fishing-spawning-aggregations-cuba-strongly-influenced-fish-and-fisher-behaviour
- Claydon J, Calosso MC, Traiger SB (2012) Progression of invasive lionfish in seagrass, mangrove and reef habitats. Marine Ecology Progress Series 448:119–129
- Concepcion GT, Kahng SE, Crepeau MW, Franklin EC, Coles SL, Toonen RJ (2010) Resolving natural ranges and marine invasions in a globally distributed octocoral (genus *Carijoa*). Marine Ecology Progress Series 401:113–127
- Darling ES, Green SJ, O’Leary JK, Côté IM (2011) Indo-Pacific lionfish are larger and more abundant on invaded reefs: a comparison of Kenyan and Bahamian lionfish populations. Biological Invasions 13:2045–2051
- De Mitcheson YS, Cornish A, Domeier M, Colin PL, Russell M, Lindeman KC (2008) A Global Baseline for Spawning Aggregations of Reef Fishes. Conservation Biology 22:1233–1244
- De'ath G, Fabricius KE, Sweatman H, Puotinen M (2012) The 27-year decline of coral cover on the Great Barrier Reef and its causes. Proceedings of the National Academy of Sciences 109:17995–17999

- Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby PJ (2012) Ocean acidification reduces coral recruitment by disrupting intimate larval- algal settlement interactions. Ecol Letters 15:338–346
- Erftemeijer PLA, Riegl B, Hoeksema BW, Todd PA (2012) Environmental impacts of dredging and other sediment disturbances on corals: a review. Marine Pollution Bulletin 64:1737–1765
- Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Marine Pollution Bulletin 50:125–146
- Fabricius KE, Langdon C, Uthicke S, Humphrey C, Noonan S, De’ath G, Okazaki R, Muehllehner N, Glas MS, Lough JM (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nature Climate change 1:165–169
- Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO2 on the CaCO3 system in the oceans. Science 305:362–366
- Frazer TK, Jacoby CA, Edwards MA, Barry SC, Manfrino CM (2012) Coping with the Lionfish Invasion: Can Targeted Removals Yield Beneficial Effects? Rev Fish Sci 20:185–191
- Gardner PG, Frazer TK, Jacoby CA, Yanong RPE (2015) Reproductive biology of invasive lionfish (*Pterois* spp.). Front. Mar. Sci. 2:
- Gardner TA, Coté IM, Gill JA, Grant A, Watkinson AR (2003) Long-Term Region-Wide Declines in Caribbean Corals. Science 301:958–960
- Gepp A, Gepp ES (1911) The Codiaceae of the Siboga Expedition including a monograph of Flabellarieae and Udoteae (No. 62). EJ Brill,
- Glynn PW, D'Croz L (1990) Experimental evidence for high temperature stress as the cause of El Niño-coincident coral mortality. Coral Reefs 8:181–191

- Green SJ, Akins JL, Maljković A, Côté IM (2012) Invasive Lionfish Drive Atlantic Coral Reef Fish Declines. PLoS ONE 7:e32596
- Green SJ, Dulvy NK, Brooks AML, Akins JL, Cooper AB, Miller S, Côté IM (2014) Linking removal targets to the ecological effects of invaders: a predictive model and field test. Ecol Appl 24:1311–1322
- Guénette S, Hill RL (2009) A trophic model of the coral reef ecosystem of La Parguera, Puerto Rico: synthesizing fisheries and ecological data. Caribbean Journal of Science 45:317–337
- Harmelin-Vivien ML, Laboute P (1986) Catastrophic impact of hurricanes on atoll outer reef slopes in the Tuamotu (French Polynesia). Coral Reefs 5:55–62
- Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science 328:1523–1528
- Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral Reefs Under Rapid Climate Change and Ocean Acidification. Science 318:1737–1742
- Kahng SE, Benayahu Y, Wagner D, Rothe N (2008) Sexual reproduction in the invasive octocoral *Carijoa riisei* in Hawaii. Bulletin of Marine Science 82:1–17
- Kahng SE, Grigg RW (2005) Impact of an alien octocoral, *Carijoa riisei*, on black corals in Hawaii. Coral Reefs 24:556–562
- Kaneshiro V (2012) The $30,000 Peppermint Angelfish at Waikiki Aquarium. http://www.honolulumagazine.com/Honolulu-Magazine/May-2012/The-30000-Peppermint-Angelfish-at-Waikiki-Aquarium/
- Katz T, Ginat H, Eyal G, Steiner Z, Braun Y, Shalev S, Goodman-Tchernov BN (2015) Desert flash floods form hyperpycnal flows in the coral-rich Gulf of Aqaba, Red Sea. Earth and Planetary Science Letters 417:87–98

- Kimmel J (1985) A characterization of Puerto Rican fish assemblages. University of Puerto Rico
- Koenig CC, Coleman FC, Grimes CB, Fitzhugh GR, Scanlon KM, Gledhill CT, Grace M (2000) Protection of fish spawning habitat for the conservation of warm-temperate reef-fish fisheries of shelf-edge reefs of Florida. Bulletin of Marine Science 66:593–616
- Koenig CC, Shepard AN, Reed JK, Coleman FC, Brooke SD, Brusher J, Scanlon KM (2005) Habitat and Fish Populations in the Deep-Sea *Oculina* Coral Ecosystem of the Western Atlantic. American Fisheries Society Symposium 41:795–805
- Kulbicki M, Beets J, Chabanet P, Cure K, Darling E, Floeter SR, Galzin R, Green A, Harmelin-Vivien M, Hixon M, Letourneur Y, de Loma TL, McClanahan T, McIlwain J, MouTham G, Myers R, O'Leary JK, Planes S, Vigliola L, Wantiez L (2012) Distributions of Indo-Pacific lionfishes *Pterois* spp. in their native ranges: implications for the Atlantic invasion. Marine Ecology Progress Series 446:189–205
- Langdon C, Atkinson MJ (2005) Effect of elevated pCO2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J. Geophys. Res. 110:C09S07
- Lesser MP, 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 13:1855–1868
- Lindfield SJ, McIlwain JL, Harvey ES (2014) Depth Refuge and the Impacts of SCUBA Spearfishing on Coral Reef Fishes. PLoS ONE 9:e92628
- Littler DS, Littler MM (1992) Systematics of *Avrainvillea* (Bryopsidales, Chlorophyta) in the tropical western Atlantic. Phycologia 31:375–418
- Littler MM, Littler DS, Brooks BL (2005) Extraordinary mound building *Avrainvillea* (Chlorophyta): the largest tropical marine plants. Coral Reefs 24:555–555

- Loya Y (1976) Effects of Water Turbidity and Sedimentation on the Community Structure of Puerto Rican Corals. Bulletin of Marine Science 26:450–466
- Maier C, Watremez P, Taviani M, Weinbauer MG, Gattuso JP (2012) Calcification rates and the effect of ocean acidification on Mediterranean cold-water corals. Proceedings of the Royal Society B: Biological Sciences 279:1716–1723
- Maynard J, van Hooidonk R, Eakin CM, Puotinen M, Garren M, Williams G, Heron SF, Lamb J, Weil E, Willis B, Harvell CD (2015) Projections of climate conditions that increase coral disease susceptibility and pathogen abundance and virulence. Nature Climate Change 5:688–694
- Miller J, Muller E, Rogers C, Waara R, Atkinson A, Whelan KRT, Patterson M, Witcher B (2009) Coral disease following massive bleaching in 2005 causes 60% decline in coral cover on reefs in the US Virgin Islands. Coral Reefs 28:925–937
- Morris JA, Akins JL, Barse A, Cerino D, Freshwater DW, Green SJ, Muñoz RC, Paris C, Whitfield PE (2009) Biology and ecology of the invasive lionfishes, *Pterois miles* and *Pterois volitans*. Proceedings of the Gulf and Caribbean Fisheries Institute 61:409–414
- Mumby PJ, Steneck R (2008) Coral reef management and conservation in light of rapidly evolving ecological paradigms. Trends in Ecology & Evolution 23:555–563
- Munday ES, Tissot BN, Heidel JR, Miller-Morgan T (2015) The effects of venting and decompression on Yellow Tang (*Zebrasoma flavescens*) in the marine ornamental aquarium fish trade. PeerJ 3:e756
- Munday PL, Cheal AJ, Dixson DL, Rummer JL, Fabricius KE (2014) Behavioural impairment in reef fishes caused by ocean acidification at CO_2 seeps. Nature Climate Change 4:487–492
- Myers RF (1993) Guam’s Small-Boat-based Fisheries. Marine Fisheries Review 55:117–128

- Neal BP, Condit C, Liu G, Santos S, Kahru M, Mitchell BG, Kline DI (2014) When depth is no refuge: cumulative thermal stress increases with depth in Bocas del Toro, Panama. Coral Reefs 33:193–205
- Nir O, Gruber DF, Shemesh E, Glasser E, Tchernov D (2014) Seasonal Mesophotic Coral Bleaching of *Stylophora pistillata* in the Northern Red Sea. PLoS ONE 9:e84968
- Nuttall MF, Johnston MA, Eckert RJ, Embesi JA, Hickerson EL, Schmahl GP (2014) Lionfish (*Pterois volitans* [Linnaeus, 1758] and *P. miles* [Bennett, 1828]) records within mesophotic depth ranges on natural banks in the Northwestern Gulf of Mexico. BioInvasions Records 3:111–115
- Oliver TA, Palumbi SR (2011) Do fluctuating temperature environments elevate coral thermal tolerance? Coral Reefs 30:429–440
- Pandolfi JM, Connolly SR, Marshall DJ, Cohen AL (2011) Projecting coral reef futures under global warming and ocean acidification. Science 333:418–422
- Peyton KA (2009) Aquatic invasive species impacts in Hawaiian soft sediment habitats. University of Hawaiʻi at Mānoa
- Pittman SJ, Hile SD, Jeffrey C, Clark R, Woody K, Herlach B, Caldow C, Monaco M, Appeldoorn R (2010) Coral reef ecosystems of Reserva Natural La Parguera (Puerto Rico): Spatial and temporal patterns in fish and benthic communities (2001-2007). NOAA Technical Memorandum NOS NCCOS 107 202
- Pyle R, Myers R (2010) *Centropyge narcosis*. http://www.iucnredlist.org/details/165903/0
- Pyle R, Myers R, Craig MT (2009) *Centropyge boylei*. http://www.iucnredlist.org/details/165824/0
- Reed J, Farrington S, Harter S, Moe H, Hanisak D, David A (2015) Characterization of the Mesophotic Benthic Habitat and Fish Assemblages from ROV Dives on

Pulley Ridge and Tortugas during 2014 R/V Walton Smith Cruise. coastalscience.noaa.gov 133

- Reed J, Rogers S (2011) Final Cruise Report Flordia Shelf-Edge Expedition (FLoSEE) Deepwater Horizon Oil Spill Response: Survey of Deepwater and Mesophotic Reef Ecosystems in the Gulf of Mexico and Southeastern Florida. docs.lib.noaa.gov 82
- Reed JK, Koenig CC, Shepard AN (2007) Impacts of bottom trawling on a deep-water *Oculina* coral ecosystem off Florida. Bulletin of Marine Science 81:481–496
- Rhyne AL, Tlusty MF, Schofield PJ, Kaufman Les, Morris JA Jr, Bruckner AW (2012) Revealing the appetite of the marine aquarium fish trade: the volume and biodiversity of fish imported into the United States. PLoS ONE 7:e35808
- Riegl B, Branch GM (1995) Effects of Sediment on the Energy Budgets of 4 Scleractinian (Bourne 1900) and 5 Alcyonacean (Lamouroux 1816) Corals. Journal of Experimental Marine Biology and Ecology 186:259–275
- Roberts TE, Moloney JM, Sweatman HPA, Bridge TCL (2015) Benthic community composition on submerged reefs in the central Great Barrier Reef. Coral Reefs 34:569–580
- Salih A, Larkum A, Cox G, Kühl M, Hoegh-Guldberg O (2000) Fluorescent pigments in corals are photoprotective. Nature 408:850–853
- Sattar SA, Adam MS (2005) Review of Grouper Fishery of the Maldives with additional notes on the Faafu Atoll Fishery. broffice.gov.mv 54
- Schofield PJ (2009) Geographic extent and chronology of the invasion of non-native lionfish (*Pterois volitans* [Linnaeus 1758] and *P. miles* [Bennett 1828]) in the Western North Atlantic and Caribbean Sea. Aquatic Invasions 4:473–479
- Schofield PJ (2010) Update on geographic spread of invasive lionfishes (*Pterois volitans* [Linnaeus, 1758] and *P. miles* [Bennett, 1828]) in the Western North

Atlantic Ocean, Caribbean Sea and Gulf of Mexico. Aquatic Invasions 5:S117–S122

- Slattery M, Lesser MP (2014) Allelopathy in the tropical alga *Lobophora variegata* (Phaeophyceae): mechanistic basis for a phase shift on mesophotic coral reefs? Journal of Phycology 50:493–505
- Smith JE, Hunter CL, Smith CM (2002) Distribution and Reproductive Characteristics of Nonindigenous and Invasive Marine Algae in the Hawaiian Islands. Pacific Science 56:299–315
- Smith TB, Blondeau J, Nemeth RS, Pittman SJ, Calnan JM, Kadison E, Gass J (2010) Benthic structure and cryptic mortality in a Caribbean mesophotic coral reef bank system, the Hind Bank Marine Conservation District, U.S. Virgin Islands. Coral Reefs 29:289–308
- Spalding HL (2012) Ecology of mesophotic macroalgae and *Halimeda kanaloana* medows in the main Hawaiian Islands. University of Hawaiʻi at Mānoa
- Starr RM, Sala E, Ballesteros E, Zabala M (2007) Spatial dynamics of the Nassau grouper *Epinephelus striatus* in a Caribbean atoll. Marine Ecology Progress Series 343:239–249
- Tsounis G, Rossi S, Grigg R, Santangelo G, Bramanti L, Gili J-M (2010) The exploitation and conservation of precious corals. Oceanography and Marine Biology: An Annual Review 48:161–212
- Valdés-Pizzini M, Schärer-Umpierre MT (2014) People, Habitats, Species, and Governance: An Assessment of the Social-Ecological System of La Parguera, Puerto Rico. University of Puerto Rico, Mayagüez, Puerto Rico
- van Hooidonk R, Maynard JA, Planes S (2013) Temporary refugia for coral reefs in a warming world. Nature Climate Change 3:508–511

- Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, McMinds R, Zaneveld JR (2013) Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Global Change Biol 20:544–554
- Veron JEN (2000) Corals of the World. The Australian Institute of Marine Science, Townsville, Australia
- Wabnitz C, Taylor M, Green E, Razak T (2003) From ocean to aquarium: The global trade in marine ornamental species.
- Weil E (2004) Coral Reef Diseases in the Wider Caribbean. In: Rosenberg E., Loya Y. (eds) Springer Berlin Heidelberg, Berlin, Heidelberg, pp 35–68
- Weil E, Croquer A, Urreiztieta I (2009a) Temporal variability and impact of coral diseases and bleaching in La Parguera, Puerto Rico from 2003–2007. Caribbean Journal of Science 45:221–246
- Weil E, Cróquer A, Urreiztieta I (2009b) Yellow band disease compromises the reproductive output of the Caribbean reef-building coral *Montastraea faveolata* (Anthozoa, Scleractinia). Dis. Aquat. Org. 87:45–55
- White KN, Ohara T, Fujii T, Kawamura I, Mizuyama M, Montenegro J, Shikiba H, Naruse T, McClelland TY, Denis V, Reimer JD (2013) Typhoon damage on a shallow mesophotic reef in Okinawa, Japan. PeerJ 1:e151
- Whitfield PE, Gardner T, Vives SP, Gilligan MR, Courtenay Ray WR Jr, Ray GC, Hare JA (2002) Biological invasion of the Indo-Pacific lionfish *Pterois volitans* along the Atlantic coast of North America. Marine Ecology Progress Series 235:289–297
- Williams ID, Baum JK, Heenan A, Hanson KM, Nadon MO, Brainard RE (2015) Human, Oceanographic and Habitat Drivers of Central and Western Pacific Coral Reef Fish Assemblages. PLoS ONE 10:e0120516
- Wood E (2001) Global advances in conservation and management of marine ornamental resources. Aquarium Sciences and Conservation 3:65–77

- Wood E, Suliansa MS, Mustapa I (2006) Fishing practices, status of coral reef resources and tactics for reversing unsustainable use on the Semporna Island reefs (Sabah, Malaysia). Proceedings of 10th International Coral Reef Symposium 1211–1217
- Woodley JD, Chornesky EA, Clifford PA, Jackson JBC, Kaufman LS, Knowlton N, Lang JC, Pearson MP, Porter JW, Rooney MC, Rylaarsdam KW, Tunnicliffe VJ, Wahle CM, Wulff JL, Curtis ASG, Dallmeyer MD, Jupp BP, Koehl MAR, Neigel J, Sides EM (1981) Hurricane Allen's Impact on Jamaican Coral Reefs. Science 214:749–755

10 APPENDIX: CHALLENGES AND OPPORTUNITIES IN CONDUCTING MESOPHOTIC REEF RESEARCH

Chapter 10: Appendix: Challenges and opportunities in conducting mesophotic reef research

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10.1 Introduction

Mesophotic coral ecosystems (MCEs) are of increased interest because of the degradation of shallow coral ecosystems as a result of regional and global stressors, and the possibility that MCEs may be more protected by virtue of their depth. MCEs occur between depths of 30 to 150 m, and are characterized by light-dependent zooxanthellate coral, macroalgae and sponge communities (Hinderstein et al. 2010). Historically, MCEs have been poorly studied because of the logistical challenges associated with surveying this depth range (Pyle 2000; Hinderstein et al. 2010). Recent advances in technology are now making it possible to conduct research at these depths.

Much interest in MCEs has been driven by the deep-reef refugia hypothesis, first proposed by Glynn (1996) based on decreased thermal stress with depth, but now expanded to other reef stressors. In short this states: many of the stressors causing damage to shallow reefs decline with depth, therefore allowing species threatened on shallow reefs to survive in deeper waters. These deep populations can then recolonize shallow reefs following disturbance, forming a local source of recruits (Bongaerts et al. 2010). There is evidence that MCEs are buffered from the impacts of storm damage, chronic pollution, sedimentation and overfishing with increased depth (Bridge et al. 2013). For example, in a 30 year time series on permanent photo quadrats in Curaçao and Bonaire, shallow reefs (<20 m), appear to have experienced the greatest change, while MCEs appear to have maintained their coral communities (Bak et al. 2005).

It is increasingly being recognized, however, that MCEs themselves are under threat (Chapter 9). For example, fishing has caused declines in fish biomass on MCEs (Lindfield et al. 2014). Damage from invasive species has also been recorded on MCEs, with high densities of lionfish (*Pterois volitans* and *P. miles*) in the western Atlantic, and snowflake coral (*Carijoa riisei*) in Hawaii, leading to changes in the benthic community (Chapter 9).

Research to understand the refuge potential of MCEs and their resilience to stressors is therefore a high priority.

Here we discuss how the University of Oxford Thinking Deep expedition overcame some of the logistical challenges associated with diver surveying of MCEs and conducted ecological research to 85m in Utila, Honduras, in September 2015.

10.2 Use of Closed-Circuit Rebreathers (CCR)

We used closed-circuit rebreathers (CCR) to conduct decompression dives on MCEs. CCRs re-cycle the expired gas from the diver by absorbing the exhaled carbon dioxide, allowing the diver to breathe this gas again, giving much greater efficiency than traditional open-circuit (OC) SCUBA, as no gas is released (Sieber and Pyle 2010). Therefore with each breath the only gas addition required is a small amount of oxygen to maintain the oxygen partial pressure in the breathing gas, or a small amount of diluent gas with a lower percentage of oxygen - typically air or a helium-based gas mix, to dilute down the breathing gas if it contains too much oxygen given the depth. The CCR unit contains multiple independent oxygen detectors (cells) to measure the partial pressure of oxygen being breathed. A specialized dive computer can then adjust the partial pressure of oxygen automatically or the diver can manually adjust, through adding oxygen or diluent to maintain the breathing gas at the desired oxygen level. This gas recycling allows divers to use far smaller cylinders than traditional open circuit diving, while substantially extending dive times by maintaining optimal breathing mixes. As a back-up measure, divers always carry extra cylinders to provide sufficient breathing gas to end the dive should their rebreather fail. Despite the need for back up cylinders, using a rebreather substantially reduces the number of cylinders required compared to traditional OC mesophotic reef dives of similar depth and duration (Figure 10.1). For example, carrying out 30 minutes of research at 60 m depth using trimix breathing gas (mix of oxygen, helium and nitrogen) followed by required decompression results in similar total dive times for OC and CCR, of 100 minutes and 102 minutes respectively, yet vastly different cylinder requirements. A CCR diver conducting this work would need to carry two back up cylinders in addition to their rebreather, whereas an OC diver requires seven cylinders to make the dive safely (Table 10.1). This difference is crucial, as it gives CCR divers the space to carry camera systems, photo-quadrat frames, transect tapes and other required scientific survey equipment to mesophotic depths. In addition the available gas resources to the diver allow a much more conservative reserve than could possibly be carried by a diver using OC.

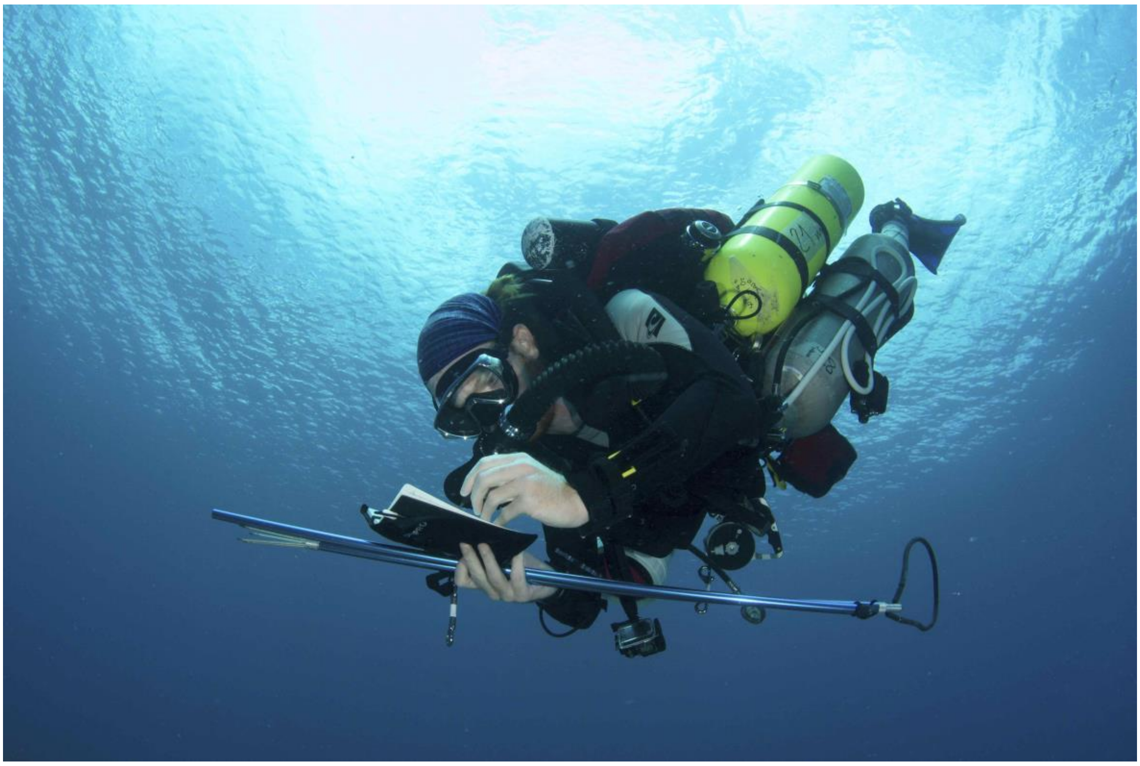

Figure 10.1 Closed-circuit rebreather (CCR) in use for scientific research.

Note the small cylinder immediately behind the diver's head, and the two larger back up cylinders at the aide of the diver. (photo: Ally McDowell)

Table 10.1 Number of cylinders a diver must carry to conduct 30 minutes of reef survey work at 60 m depth followed by required decompression.

Calculated assuming a: cylinder size of 11.1 L filled to 200 bar, breathing rate of 20 L/min, rule of thirds for gas management, gradient factor conservatism of 30/70, CCR set point of 1.3, CCR diluent of 16/45.

Gas mix	**Open Circuit cylinders**	**Rebreather back up cylinders**
18/45 trimix (18 % oxygen, 45 % helium, 37 % nitrogen)	4	1
50 % nitrox (50 % oxygen, 50 % nitrogen)	2	1
100 % oxygen	1	0
Total cylinders	7	2

When diving in relativity remote locations, helium and oxygen can often be in limited supply and expensive. CCR maximizes gas efficiency; for example on our reef survey dives to 85 m each diver consumed less than 600 L of oxygen and 600 L of trimix diluent per dive. This reduced breathing gas consumption meant we could conduct approximately eight mesophotic CCR dives using the same amount of trimix that a mesophotic OC diver would use on a similar single dive, allowing helium and oxygen usage and costs to be minimized while maximizing survey dive time.

In addition, we also used CCRs for shallow survey dives. While these dives were within the range of recreational OC SCUBA diving (<30 m), the use of CCRs allowed us to markedly increase efficiency. Our team was able to execute single dives up to four-hours long, far beyond the gas and obligatory decompression limits of recreational OC diving. Table 10.2 shows the potential for CCR diving at 15 m depth, dramatically increasing survey time without the bulk of additional cylinders and the build up of decompression debt. After three hours of diving at 15 m using an air diluent on CCR only one minute of mandatory decompression is required before surfacing, whereas over one hour of mandatory decompression is required using OC air. Switching to OC nitrox (mix of oxygen and nitrogen) reduces the decompression obligation but still leaves divers overburdened by a large number of cylinders. Application of rebreather technology enables researchers to substantially increase data collection time over the course of a fieldwork trip, especially in the recreational dive range, making it a tool that should be considered by reef scientists who conduct fieldwork even in the depth ranges of recreational scuba diving.

While earlier CCR technology gained a reputation of being dangerous, to the point of not being worth the risk, the main causes of these issues have been overcome with more modern systems, testing and rigorous diver training. Although there are higher inherent risks when using CCR rather than OC (Fock 2013), the accident analysis research related to both training and manufacturing has led to a greatly improved safety record. Rebreather training courses regularly emphasize constant monitoring of the CCR unit, and involve extensively practicing drills to simulate possible unit failures. For example, following each day’s diving during the Thinking Deep expedition, the Dive Safety Officer downloaded dive profile data for each rebreather. From this it was possible to check the oxygen cell readings for the unit for any inconsistencies indicating possible degradation or faulty connections, with the aim of identifying and solving potential problems before they occur. Should the diver encounter a problem, CCRs provide far more failure management options and available gas resources than OC systems, and divers always have a fully isolated back up option. With proper maintenance, diving practices, and training, CCRs can be used safely for research.

Table 10.2 Dive plan for 180 minutes survey work at 15 m depth, showing time required to complete the survey work followed by required decompression.

Comparisons are made for open circuit air, open circuit recreational nitrox (40 % oxygen and 60 % nitrogen) and closed circuit rebreather. The total number of cylinders of the required open circuit gas is indicated. Total dive time includes time to ascend between indicated depths. Calculated assuming a: cylinder size of 11.1L filled to 200 bar, breathing rate of 20 L/min, rule of thirds for gas management, gradient factor conservatism of 30/70, CCR set point of 1.3, air CCR diluent.

Depth	Air OC diver (21 % oxygen, 79 % nitrogen)	40 % nitrox OC diver (40 % oxygen, 60 % nitrogen)	CCR diver
Survey time at 15m (min)	180	180	180
Decompression time at 9m (min)	1	0	0
Decompression time at 6m (min)	13	0	1
Decompression time at 3m (min)	45	1	0
Total dive time (min)	240	183	182
Total cylinders required	7	6	1 (back up cylinder)

10.3 Use of photogrammetry

Even with the use of rebreathers, there are limits on how much the time for data collection can be extended, making it crucial to maximize efficiency. In coral reef ecological research and monitoring, most data are collected by divers in the water recording visual observations. While this process allows direct observations of reef organisms in situ, it is time consuming, resulting in long fieldwork trips to collect even small datasets. Yet the careful use of photogrammetric methods (photo/video) can enable vast amounts of data to be collected with minimal time in the water.

With improvements in camera quality and reliability it is now possible for many visual observation studies to get data of similar, and in some cases superior quality, from photogrammetric methods. For example, we used stereo-video systems for accurate fish community biomass assessments (Figure 10.2). This method allows more accurate fish length measurements than traditional diver visual length estimations (Harvey et al. 2001). A typical 50 m fish visual transect takes 20 minutes to complete in a high-fish diversity reef environment, whereas a stereo-video transect can be filmed in less than 4 minutes. This shifts the bulk of the workload to post field trip analysis of video footage, but enables vast quantities of data to be collected in short, highly efficient field trips. Photogrammetric methods also have the advantage of creating images and videos that can be archived as permanent records, allowing examination at a later date for additional information. Archived photos and videos may also be used in future studies that are not yet envisioned. In addition, the use of photogrammetry allowing blinding of survey location during image/video analysis, this is particularly important when addressing questions researchers may exhibit unconscious bias on (e.g. comparing MPAs to fished areas). Specifically for mesophotic reef surveys, the use of photogrammetry overcomes the restricted dive times at greater depths, while simultaneously allowing the insights from in-water observation and first-hand experience that is not possible using remote survey techniques.

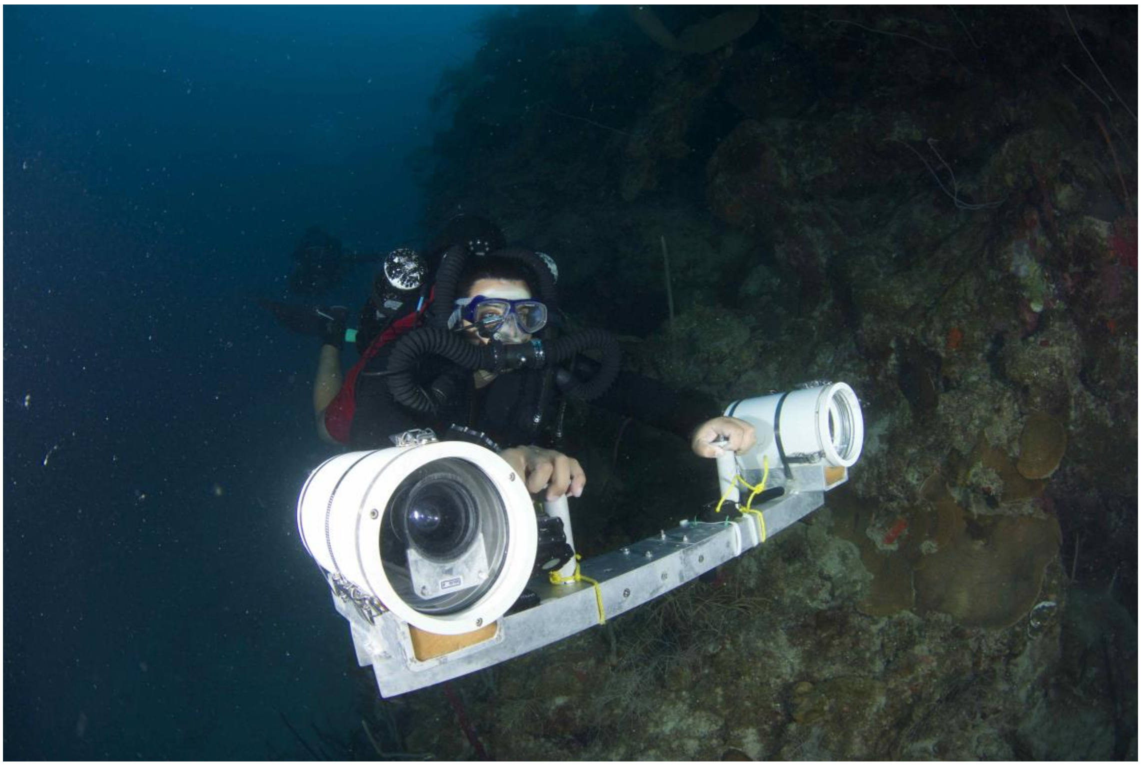

Figure 10.2 A CCR diver using a stereo-video system to conduct fish biomass surveys along reef transects.

Post dive the footage from the two cameras are synchronized, allowing fish lengths to be accurately estimated. (Photo: Ally McDowell)

10.4 Use of team-oriented diving

Teams of technical and recreational support divers working together improved the overall dive safety for deep dives, while increasing data collection ability. Support divers were able to collect bulky equipment (e.g. stereo-video systems, photo quadrats), delicate samples (e.g. water samples, sediment traps) and spare back up gas cylinders from the deep dive team early during their decompression schedule (Figure 10.3). Divers completing long decompression schedules (>2 hours) were therefore not overladen with equipment, reducing physical exertion and increasing comfort, and so reducing a risk factor contributing to decompression complications. It was important for each team to have a clear dive plan with accurate time estimates to allow teams to stagger their water entry and meet underwater. In addition, by taking research equipment (e.g. a stereo-video system) from the deep divers early in their decompression schedule, it was possible for an additional dive team to use this equipment while the deeper team was decompressing. In some cases, this allowed several hours of additional data collection on shallow reefs that would otherwise have been wasted.

Support divers also acted as a standby response team that could assist the deep divers in an emergency situation. We used pre-arranged color coded delayed surface marker buoys (DSMB) to signal to the boat and support divers on the surface, allowing support divers to quickly descend down the DSMB line to meet the deep divers. A single orange DSMB indicated the deep dive team had begun decompression, that all was well, and for the support divers to carry out the pre-planned equipment or scientific samples collection from the deep dive team. A single yellow DSMB indicated an emergency situation requiring immediate assistance. In this situation the support divers would immediately descend while the boat radioed the dive center informing them an emergency situation was underway and to standby for more information. Both an orange and a yellow DSMB on the same line indicated the deep divers required additional back up decompression gas, which the support divers were able to descend with.

Figure 10.3 Support diver receiving unused deep back up cylinders from the deep dive team during their decompression stops.

Good teamwork is necessary to efficiently conduct mesophotic dive surveys. (photo: Jack Laverick)

10.5 Preliminary mesophotic observations

Utila is located on the edge of the continental drop off, with steep coral covered walls off the north shore and gentler reef slopes on the south. Several ledges broke the steep north shore walls at approximately 30 m, 55 m and 70 m depths. These ledges were approximately 20 m wide and covered with established reef communities, providing a source of structural complexity that was associated with higher fish densities. Common fish observed in the 70-85 m depth range included the sunshine fish (*Chromis insolata*) and blackfin snapper (*Lutjanus buccanella*). In addition, on several occasions we observed Caribbean reef sharks (*Carcharhinus perezii*) at depths >50 m. Because of the historic shark fishing around Utila, divers on shallow reefs very rarely report seeing Caribbean reef sharks. Despite over 350 shallow reef dives conducted on Utila during the past three years we had never previously observed them. Utila has an intensive shallow reef lionfish culling program, and we saw few lionfish on the fringing reefs shallower than 30 m. However, large aggregations of lionfish were seen at mesophotic depths, with multiple occasions where >10 individuals were observed aggregated around single coral bommies. Full benthic and fish video analysis is currently underway, and we plan to shortly publish full research findings from this expedition.

10.6 Conclusion

When planning mesophotic research it is important to carefully consider which survey techniques to use based on the specific research objectives. Many established shallow reef techniques can be adapted to mesophotic depths depending on the resources available and logistics of the study site. For our research questions, many of the changes required to adapt shallow reef techniques for mesophotic depths were technological, such as using closed circuit rebreathers rather than open circuit scuba and using photo and video methods. It is important to remember that shifting research diving to greater depths requires a step-up in team awareness, training and orientation to ensure all divers remain safe and surveys can be conducted efficiently.

10.7 Acknowledgments

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10.8 References

- 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
- Bongaerts P, Ridgway T, Sampayo EM, Hoegh-Guldberg O (2010) Assessing the “deep reef refugia” hypothesis: focus on Caribbean reefs. Coral Reefs 29:309–327
- Bridge TCL, Hughes TP, Guinotte JM, Bongaerts P (2013) Call to protect all coral reefs. Nat Clim Chang 3:528–530
- Fock AW (2013) Analysis of recreational closed-circuit rebreather deaths 1998–2010. Diving Hyperb Med 43:78–85
- Glynn PW (1996) Coral reef bleaching: facts, hypotheses and implications. Glob Chang Biol 2:495–509
- Harvey E, Fletcher D, Shortis M (2001) Improving the statistical power of length estimates of reef fish: a comparison of estimates determined visually by divers with estimates produced by a stereo-video system. Fish. Bull. 99:72–80
- Hinderstein LM, Marr JCA, Martinez FA, Dowgiallo MJ, Puglise KA, Pyle RL, Zawada DG, Appeldoorn R (2010) Theme section on “Mesophotic Coral Ecosystems: Characterization, Ecology, and Management.” Coral Reefs 29:247–251
- Lindfield SJ, McIlwain JL, Harvey ES (2014) Depth Refuge and the Impacts of SCUBA Spearfishing on Coral Reef Fishes. PLoS ONE 9:e92628
- Pyle RL (2000) Assessing Undiscovered Fish Biodiversity on Deep Coral Reefs Using Advanced Self-Contained Diving Technology. Marine Technology Society Journal 34:82–91

- Sieber A, Pyle R (2010) A review of the use of closed-circuit rebreathers for scientific diving. Underwater Technology 29:73–78

11 APPENDIX: IDENTIFYING ZOOPLANKTON COMMUNITY CHANGES BETWEEN SHALLOW AND UPPER-MESOPHOTIC REEFS ON THE MESOAMERICAN BARRIER REEF, CARIBBEAN

11.1 Abstract

Mesophotic coral ecosystems (MCEs, reefs 30-150 m) are understudied, yet the limited research conducted has been biased towards large sessile taxa, such as scleractinian corals and sponges, or mobile taxa such as fishes. Here we investigate zooplankton communities on shallow reefs and MCEs around Utila on the southern Mesoamerican Barrier Reef using planktonic light traps. Zooplankton samples were sorted into broad taxonomic groups. Our results indicate similar taxonomic zooplankton richness and overall biomass between shallow reefs and MCEs. However, the abundance of larger bodied (>2 mm) zooplanktonic groups, including decapod crab zoea, mysid shrimps and peracarid crustaceans, was higher on MCEs than shallow reefs. Our findings highlight the importance of considering zooplankton when identifying broader reef community shifts across the shallow reef to MCE depth gradient.

Chapter 11: Appendix: Identifying zooplankton community changes between shallow and upper-mesophotic reefs on the Mesoamerican Barrier Reef, Caribbean

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11.2 Introduction

Tropical coral ecosystems are some of the most diverse ecosystems on the planet (Sala and Knowlton 2006), with light-dependent scleractinian corals extending from the surface down to approximately 150-165 m depth (Maragos and Jokiel 1986; Kahng and Maragos 2006). Yet most coral reef research is heavily biased towards the shallows (<30 m) (Menza et al. 2008) because of the difficulties associated with accessing deeper reefs. Mesophotic coral ecosystems (MCEs; Hinderstein et al. 2010), reefs from 30 m to approximately 150 m, are increasingly recognized as containing important components of reef diversity both as refuge habitats for shallow reefs and also as unique ecological assemblages that need protection in their own right (Bongaerts et al. 2010; Bridge et al. 2013; Chapter 9).

Most of the limited work on MCEs has focused on large sessile taxa, such as scleractinian corals and sponges, or large mobile taxa such as fishes (Kahng et al. 2010; Kahng et al. 2014). It has, however, been estimated that 168,000 invertebrate species have been described on coral reefs (Ruppert et al. 2003; Stella et al. 2011), far greater than the approximately 5,000 fish species and 700 scleractinian coral species currently recognized (Veron 2000; Bellwood et al. 2012). Most biodiversity on reefs is therefore comprised of small mobile invertebrates, many of which are cryptic and found associated with other sessile reef fauna or in the zooplankton (Fautin et al. 2010; Plaisance et al. 2011; Head et al. 2015).

Zooplankton is comprised of a diverse range of organisms with different components normally classified into coarse groups based on size, for example mesozooplankton range from 0.2–20 mm and macrozooplankton from 2–20 cm (Johnson and Allen 2012). In the Caribbean, zooplankton recorded adjacent to coral reefs has generally been found to be dominated by copepods with amphipods, isopods, polychaetes, shrimp larvae and crab larvae all present but at lower abundances (Heidelberg et al. 2004; Heidelberg et al. 2010).

Previous studies have suggested major factors affecting zooplankton abundance on reefs include currents, active zooplankton avoidance behaviour of reef habitats, and differing exploitation patterns by predators (Motro et al. 2005; Yahel et al. 2005; Heidelberg et al. 2010). Approximately 20 % of reef invertebrates are crustaceans, making them one of the largest and most speciose groups on coral reefs (Kramer et al. 2014) and an important component of reef fish diets (Randall 1967). Many zooplankton can exhibit active swimming behavior to avoid predation or areas with higher risk of predation (Haury et al. 1980; Ohman 1988). Planktivorous fish predation pressure is thought to lead to depleted plankton abundance over reefs, however, planktivorous fishes tend to feed <1.5 m over the reef and so this effect is limited to close to the reef surface (Motro et al. 2005).

On MCEs zooplankton is particularly important as a food source, because zooplanktivores are widely observed as the dominant fish trophic group globally, for example in the Red Sea (Brokovich et al. 2008), in Hawaii (Pyle et al. 2016) and in the Marshall Islands (Thresher and Colin 1986). These patterns are also widely found in the Caribbean (Garcia-Sais 2010; Bejarano et al. 2014; Chapter 2), with a study suggesting that approximately 60 % of MCE reef fish feed on zooplankton and mobile-invertebrates on the Mesoamerican Barrier Reef, Caribbean (Chapter 2). Shallow-reef corals also feed on zooplankton, which provides an important additional energy source to that provided by their symbionts (Ferrier-Pagès et al. 2003). On MCEs corals are believed to increase heterotrophic feeding because of low light availability, likely making them more dependent on zooplankton than their shallow counterparts (Fricke et al. 1987; Mass et al 2007; Lesser et al. 2010). In addition, invertebrate groups such as decapods have important functional roles in maintaining fish health. For example, cleaning fish of parasites, e.g. cleaner shrimp (Becker and Grutter 2004), and defending coral colonies from predators and clearing excess sediment thus preventing smothering of coral polyps, e.g. *Trapezia* crabs (McKeon and Moore 2014).

Despite the important roles zooplankton are likely to have on MCEs, few studies have documented MCE zooplankton communities and how they differ from those on shallow reefs. Here, we investigate the mesozooplankton and macrozooplankton community on shallow reefs and upper-MCEs on the Mesoamerican Barrier Reef, Caribbean, to identify differences in abundance, biomass, and community structure across the depth gradient.

11.3 Methods

Surveys were conducted on the south shore of Utila, Bay Islands, Honduras. Utila is located off the north shore of Honduras, with its reefs forming the southern extent of the Mesoamerican Barrier Reef. Off the south shore of Utila, shallow reefs form a spur and groove system, with a reef slope down to approximately 35 m where the seabed flattens and a patch reef MCE is formed. From these MCE patch reefs the south shore seabed continues to gently slope to approximately 70-80 m before rising to the Honduran mainland. Three replicate light trap deployments were conducted at 15 m (shallow) and 40 m (MCE) at three sites: Coral View, Black Coral Wall and Little Bight (Figure 11.1) during July - September 2015. Light traps were built following Jones (2006). Traps were modified from these specifications to use twelve 12 V light-emitting diodes (LEDs) powered by a 12 V, 4,800 mAh rechargeable lithium ion battery as the light source in each trap. LEDs were white light emitting with each LED having a luminous intensity of 12,000 – 14,000 mcd and wavelength of 5,000 – 6,500 nm. Light traps were deployed by divers 0.5 m above the reef at each depth during the afternoon. They were activated with a digital timer set to illuminate the trap 30 minutes before sunset and remain lit until sunrise the following morning. Divers recovered the light traps at 7:30 am the morning following deployment. Sites and depths were surveyed over multiple nights, with no more than two traps deployed at a site in a single night. All light traps were placed a minimum of 20 m distance from previous light trap deployments, and where two traps were placed at the same site on the same night these were separated by a minimum of 50 m. Research permits for this work were issued to Operation Wallacea by the Instituto de Conservación Forestal (ICF), Honduras, permit number: ICF-261-16. As the focus of the work was on invertebrates, and no higher vertebrates were involved, ethical review was not required.

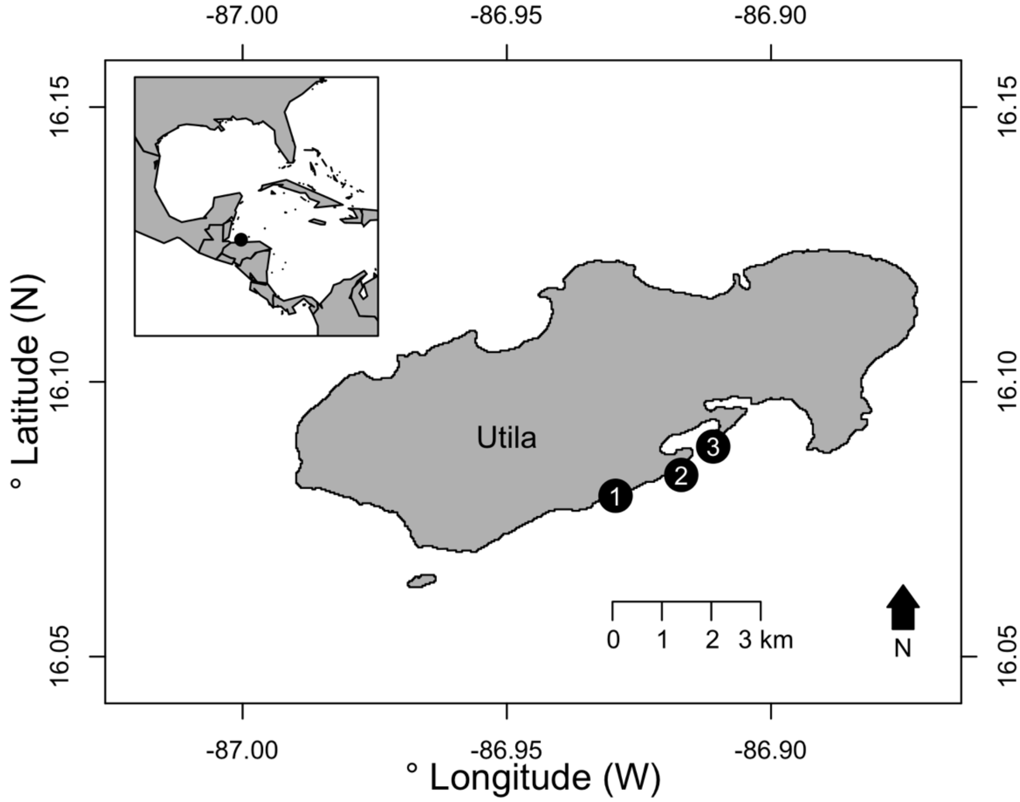

Figure 11.1 The three survey sites in the south shore of Utila, Bay Islands, Honduras.

Sites were: (1) Little Bight, (2) Black Coral Wall and (3) Coral View. Inset – The location of Utila is indicated with a black circle relative to the western Caribbean and Gulf of Mexico.

Samples were sorted following groupings used in Johnson and Allen (2012) into broad taxonomic and developmental groups readily identifiable in the field with the use of a dissecting microscope. These groups were: (i) arrow worms, (ii) barnacle larvae, (iii) cladocerans, (iv) copepods, (v) decapod crab zoea, (vi) decapod shrimp zoea, (vii) decapod crab megalopae, (viii) lobster phyllosoma, (ix) mantis shrimp larvae, (x) mysid shrimps, (xi) peracarid crustaceans, (xii) oligochaetes, (xiii) polychaetes, (xiv) mites, (xv) urochordates and (xvi) fish larvae. All sampled individuals >2 mm were counted to give abundance, and all individuals regardless of size were sorted and dry weighed to record biomass.

Nonmetric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (PERMANOVA) were used to visualize and test for differences in abundance and biomass between the two depths based on Bray-Curtis dissimilarities on a fourth root transformed matrix (Anderson et al. 2008), while differences in richness were tested using a Euclidean PERMANOVA. Transformed data were used to reduce the influence of the most abundant taxonomic groups when assessing community differences in the PERMANOVA (Anderson et al. 2008). When processing samples, one 15 m Black Coral Wall light trap collected no taxonomic groups with sufficient biomass to register on our field scales (weight <0.01 g); this necessitated its removal from multivariate analysis of biomass data. All PERMANOVAs were run for 99999 permutations using the ‘adonis’ function in vegan (Oksanen et al. 2015) in R (R Core Team 2013). Constrained analysis of principal coordinates (CAP) was conducted for the abundance data using the ‘capscale’ function in vegan (Oksanen et al. 2015). All taxonomic groups with a Pearson correlation coefficient |>0.5| with either of the first two CAP axes were identified as potential drivers of community difference with depth. The abundance of these taxonomic groups was then individually tested using a Euclidian PERMANOVA to identify whether they changed with depth.

11.4 Results

Overall we found similar richness of taxonomic groups on shallow and mesophotic reefs (Figure 11.2A), with much variation in the overall abundance and biomass at both depths (Figure 11.2B-C). We used an NMDS to visualise differences in the community sampled by the light traps at shallow and mesophotic depths. Abundance data appeared to show a difference in the community between shallow and mesophotic reefs (Figure 11.3A), while biomass-weighted data showed no clear patterns (Figure 11.3B). We tested these patterns in a PERMANOVA (Table 11.1), finding differences in the recorded light trap community taxonomic richness, abundance and biomass between survey sites. Abundance of taxonomic groups also changed between the two depths, but no pattern with depth was found for taxonomic richness or biomass.

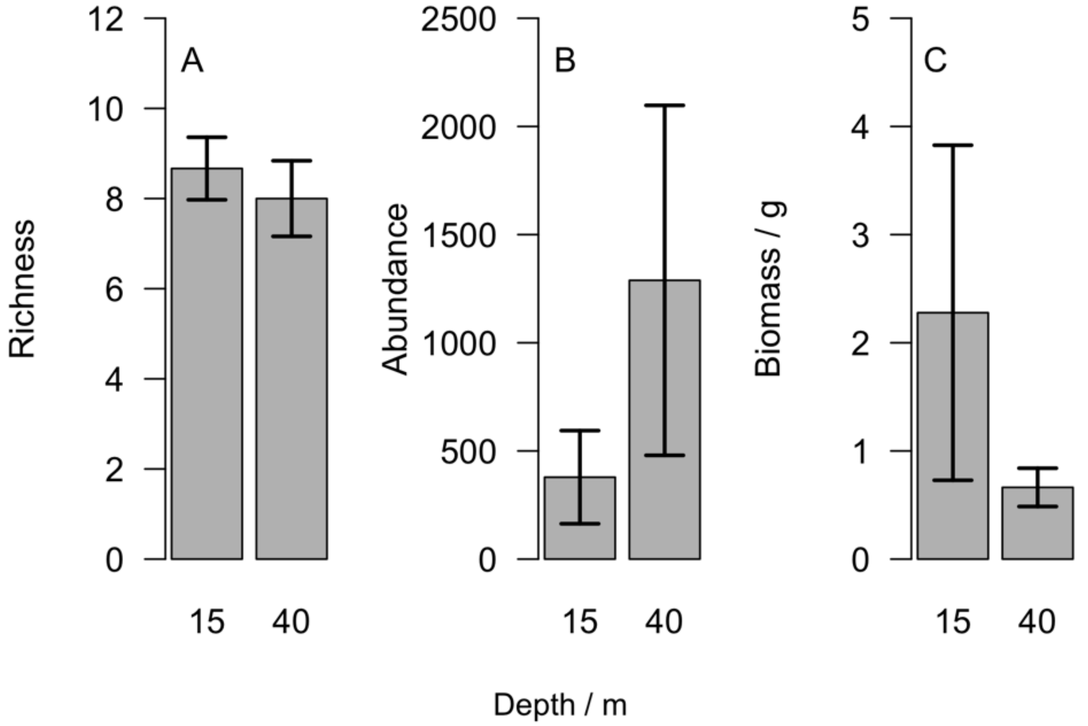

Figure 11.2 (A) Number of different taxonomic groups recorded, (B) mean abundance per light trap and (C) total biomass across all taxa groups comparing reefs at 15 m and 40 m. Bars show mean ± 1 standard error.

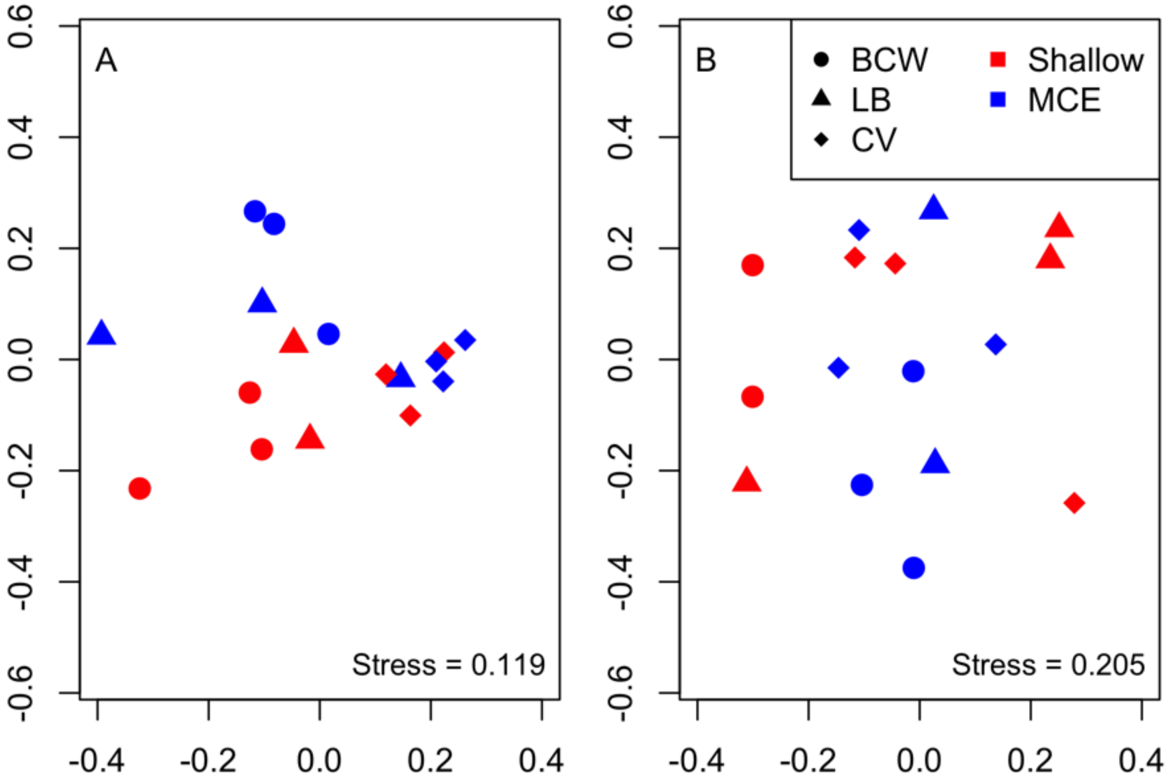

Figure 11.3 Nonmetric multidimensional scaling plot for (A) abundance and (B) biomass of the invertebrate and fish fish larvae.

Colours indicate different depths, while shapes indicate different survey sites. Sites were: BCW – Black Coral Wall, LB – Little Bight and CV – Coral View.

Table 11.1 PERMANOVA results testing for differences in the recorded light trap community between sites and depth for abundance and biomass data.

		Richness		**Abundance**		**Biomass**	
	DF	pseudo-F	P	pseudo-F	P	pseudo-F	P
Survey Site	2	3.97	0.04	5.58	<0.0001	2.33	0.01
Depth	1	0.76	0.41	2.71	0.03	1.42	0.24
Residual	13						
Total	16						

We conducted Euclidian PERMANOVAs on abundance results correlating with the CAP axis to identify taxonomic groups varying with depth (Table 11.2). We identified decapod crab zoea, mysid shrimps, peracarid crustaceans and oligochaete abundance as increasing on MCEs compared to shallow reefs, with no oligochaetes recorded on shallow reefs. We did not detect any zooplanktivorous groups at greater abundance on shallow reefs than MCEs, nor any changes in fish larvae abundance between shallow reefs and MCEs.

Table 11.2 Euclidian PERMANOVA results for abundance of taxonomic groups that correlate |>0.5| with the first of second CAP axis.

Taxonomic Group	**Shallow (15 m)**		**Mesophotic (40 m)**		**Pseudo-F**	**P**
	Mean	SE	Mean	SE		
First axis						
Decapod crab megalopae	25.00	18.92	15.78	14.13	0.47	0.24
Decapod crab zoea	17.89	17.39	69.11	56.53	2.31	<0.01
Decapod shrimp zoea	52.00	19.66	38.22	26.72	0.27	0.53
Mysid shrimps	84.11	63.12	637.44	600.61	1.52	0.04
Peracarid crustaceans	68.22	44.79	216.11	93.51	5.04	<0.01
Urochordates	29.11	29.11	83.89	74.11	1.32	0.15
Second axis						
Cladocerans	4.33	4.33	0.33	0.33	2.44	0.10
Oligochaetes	0.00	0.00	93.67	93.67	2.28	<0.01

11.5 Discussion

While MCEs are of increased interest because of their potential role as refuges for threatened shallow-reef taxa (Bongaerts et al. 2010; Bridge et al 2013; Lindfield et al. 2016), almost all existing research has focused on large sessile benthic taxa such as hard corals, macroalgae and sponges, or large mobile organisms such as fishes (Kahng et al. 2010; Kahng et al. 2014). Few studies have considered changes in small mobile invertebrates making up reef cryptofauna and zooplankton. We found significant differences in zooplankton richness, abundance and biomass between study sites and, interestingly, higher abundances on MCEs than on shallow reefs while biomass did not change.

Previous work has suggested zooplankton, in particular larger planktonic individuals such as mysids, isopods, and decapod larvae, are particularly important prey items for planktivorous fish (Hobson and Chess 1978). Unlike in many locations (see Kahng et al. 2010; Kahng et al. 2014), a previous study on Utila at our study sites found no difference in relative planktivorous reef fish biomass between shallow reefs and MCEs, and a decline in actual biomass of planktivorous reef fish with increased depth (Chapter 2). This pattern is surprising as we identified greater abundance of zooplankton >2 mm size and no change in biomass of zooplankton across the depth gradient, suggesting similar or potentially greater food resources for planktivorous fish on MCEs. In addition, planktivorous reef fish exhibit high visual system plastic adaptive ability, and show few differences in feeding ability caused by changes in light levels across depth gradients, suggesting they should be able to feed efficiently at MCE depths included in this study (Brokovich et al. 2010). However, the previous planktivorous fish study from Utila was conducted during daylight hours (Chapter 2), whilst light traps in this study were deployed overnight. Therefore, as zooplankton are known to have diurnal movement patterns (Angel 1985), the abundance of zooplankton we recorded here may not be available to planktivorous fish during daylight hours. MCEs on the south shore of Utila exist as a gently sloping patch reef system on the continental shelf that remains within mesophotic depths before rising to become the mainland of Honduras. Therefore, unlike other MCEs adjacent to deep-sea habitats, at the sites we surveyed there is unlikely to be a large diurnal migration of zooplankton from deeper water at night.

Our finding of greater abundance of some zooplanktivorus groups on MCEs, and no change in zooplankton biomass between shallow reefs and MCEs contrast with previously identified zooplankton depth patterns. In Jamaica, Ohlhorst (1985) studied zooplankton across a 6–24 m depth gradient using traps placed over the reef, finding that both the abundance of zooplankton and the volume of zooplankton per trap declined with depth. When looking at specific taxonomic groups, we identified greater abundance of mysid shrimps and peracarid crustaceans on MCEs (40 m) than shallow reefs (15 m). Whereas, no difference was identified in mysid shrimp or peracarid crustacean abundance between 15 m and 24 m in Jamaica (Ohlhorst 1985). In Hawaiian reef cryptofauna, brachyuran crab abundance has been reported to decline across a 12–90 m depth gradient (Hurley et al. 2016). However much of this pattern was caused by one crab genus, which when excluded led to crab abundance increasing with depth. While these Hawaiian brachyuran crabs had settled on the reef, we identified increased abundance of decapod crab zoea on MCEs on Utila, though no difference in decapod crab megalopae with depth. In addition, we found similar abundances of fish larvae between shallow reefs and MCEs. However, fish larval recruitment is known to be seasonal, and previous studies have identified abundance differences in fish larval recruits across 10–40 m in the Caribbean (Luckhurst and Luckhurst 1977). These fish recruitment patterns were highly species specific, with fish recruits more abundant for some species at 40 m than 10 m, whilst the reverse is true for other species (Luckhurst and Luckhurst 1977).

In this study we found no change in taxa richness between shallow reefs and MCEs, however, we only classified invertebrates into broad taxonomic groups, lacking the resolution needed to detect fine scale richness patterns. In Jamaica, Ohlhorst (1985) reported an increase in taxonomic richness across depths from 6–24 m but used higher resolution taxonomic groupings. In contrast, in Hawaii, Hurley et al. (2016) reported the greatest brachyuran crab reef cryptofauna diversity on shallow reefs, with 40 % of species at 12 m and declining richness with depth to 90 m. Differences in richness patterns with depth between these studies are likely caused by different reef habitats, taxonomic resolution, biogeographic regions, and sampling techniques.

Patterns in species richness across the shallow to mesophotic gradient has been a major focus of research (Kahng et al. 2010; Kahng et al. 2014), potentially being used to inform conservation management and in defining MCE ecology (Laverick et al. 2016). The current upper depth limit of MCEs is defined at 30–40 m based on the limits of recreational SCUBA diving (Menza et al. 2008; Hinderstein et al. 2010; Loya et al. 2016). However, there is disagreement within the mesophotic research community over whether this upper limit should be redefined based on biological community turnover with depth (Laverick et al. 2016). Recently a consensus formed that MCEs can be divided into upper and lower zones based on a species transition commonly observed in scleratinian corals and fishes at approximately 60 m depth in many locations (Loya et al. 2016). At present, with MCE biodiversity so poorly documented, including the near absence of studies on zooplankton and mobile reef invertebrates, further investigative work is necessary to test whether the recorded patterns in reef communities with depth are consistent across many other taxonomic groups on reefs.

This study provides a first glimpse of the patterns in zooplankton communities associated with MCEs in the Caribbean. Further research is necessary to determine fine-scale patterns across the depth gradient in zooplankton communities to help identify depth transition zones between communities and areas with unique biodiversity assemblages.

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11.7 References

- Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, U.K.
- 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 (2016a) Threats to mesophotic coral ecosystems and management options. In: Baker EK, Puglise KA, Harris PT (eds) *Mesophotic Coral Ecosystems – A Lifeboat For Coral Reefs?* The United Nations Environment Programme and GRID-Arendal, Nairobi and Arendal, pp 67–82
- Andradi-Brown DA, Gress E, Wright G, Exton DA, Rogers AD (2016b) Reef Fish Community Biomass and Trophic Structure Changes across Shallow to Upper-Mesophotic Reefs in the Mesoamerican Barrier Reef, Caribbean. PLoS ONE 11:e0156641. doi: 10.1371/journal.pone.0156641
- Angel MV (1985) Vertical migrations in the oceanic realm: possible causes and probable effects. Contributions in Marine Science 27:45–70.
- Becker JH, Grutter AS (2004) Cleaner shrimp do clean. Coral Reefs 23:515–520. doi: 10.1007/s00338-004-0429-3
- Bejarano I, Appeldoorn RS, Nemeth M (2014) Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico. Coral Reefs 33:313–328. doi: 10.1007/s00338-014-1125-6
- Bellwood DR, Renema W, Rosen BR (2012) Biodiversity hotspots, evolution and coral reef biogeography. In: Gower D, Johnson K, Richardson J, et al. (eds). Cambridge University Press, Cambridge, pp 216–245
- Bongaerts P, Ridgway T, Sampayo EM, Hoegh-Guldberg O (2010) Assessing the “deep reef refugia” hypothesis: focus on Caribbean reefs. Coral Reefs 29:309–327. doi: 10.1007/s00338-009-0581-x

- Bridge TCL, Hughes TP, Guinotte JM, Bongaerts P (2013) Call to protect all coral reefs. Nature Climate Change 3:528–530. doi: 10.1038/nclimate1879
- Brokovich E, Ben-Ari T, Kark S, Kiflawi M, Dishon G, Iluz D, Shashar N (2010) Functional changes of the visual system of the damselfish *Dascyllus marginatus* along its bathymetric range. Physiology & Behavior 101:413–421. doi: 10.1016/j.physbeh.2010.07.006
- Brokovich E, Einbinder S, Shashar N, Kiflawi M, Kark S (2008) Descending to the twilight-zone: changes in coral reef fish assemblages along a depth gradient down to 65 m. Marine Ecology Progress Series 371:253–262. doi: 10.3354/meps07591
- Fautin D, Dalton P, Incze LS, Leong JAC, Pautzke C, Rosenberg A, Sandifer P, Sedberry G, Tunnell Jr JW, Abbott I, Brainard RE, Brodeur M, Eldredge LG, Feldman M, Moretzsohn F, Vroom PS, Wainstein M, Wolff N (2010) An Overview of Marine Biodiversity in United States Waters. PLoS ONE 5:e11914. doi: 10.1371/journal.pone.0011914
- Ferrier-Pagès C, Witting J, Tambutt E, Sebens KP (2003) Effect of natural zooplankton feeding on the tissue and skeletal growth of the scleractinian coral *Stylophora pistillata*. Coral Reefs 22:229–240. doi: 10.1007/s00338-003-0312-7
- Fricke HW, Vareschi E, Schlichter D (1987) Photoecology of the coral *Leptoseris fragilis* in the Red Sea twilight zone (an experimental study by submersible). Oecologia 73:371–381. doi: 10.1007/BF00385253
- Garcia-Sais JR (2010) Reef habitats and associated sessile-benthic and fish assemblages across a euphotic–mesophotic depth gradient in Isla Desecheo, Puerto Rico. Coral Reefs 29:277–288. doi: 10.1007/s00338-009-0582-9
- Haury LR, Kenyon DE, Brooks JR (1980) Experimental evaluation of the avoidance reaction of *Calanus finmarchicus*. Journal of Plankton Research 2:187–202. doi: 10.1093/plankt/2.3.187

- Head CEI, Bonsall MB, Koldewey H, Pratchett MS, Speight M, Rogers AD (2015) High prevalence of obligate coral-dwelling decapods on dead corals in the Chagos Archipelago, central Indian Ocean. Coral Reefs 34:905-915
- Heidelberg KB, O'Neil KL, Bythell JC, Sebens KP (2010) Vertical distribution and diel patterns of zooplankton abundance and biomass at Conch Reef, Florida Keys (USA). Journal of Plankton Research 32:75–91. doi: 10.1093/plankt/fbp101
- Heidelberg KB, Sebens KP, Purcell JE (2004) Composition and sources of near reef zooplankton on a Jamaican forereef along with implications for coral feeding. Coral Reefs 23:263–276. doi: 10.1007/s00338-004-0375-0
- Hinderstein LM, Marr JCA, Martinez FA, Dowgiallo MJ, Puglise KA, Pyle RL, Zawada DG, Appeldoorn R (2010) Theme section on “Mesophotic Coral Ecosystems: Characterization, Ecology, and Management.” Coral Reefs 29:247–251. doi: 10.1007/s00338-010-0614-5
- Hobson ES, Chess JR (1978) Trophic relationships among fishes and plankton in the lagoon at Enewetak Atoll, Marshall Islands. Fishery Bulletin 76:133–153.
- Hurley KKC, Timmers MA, Godwin LS, Copus JM, Skillings DJ, Toonen RJ (2016) An assessment of shallow and mesophotic reef brachyuran crab assemblages on the south shore of O‘ahu, Hawai‘i. Coral Reefs 35:103–112. doi: 10.1007/s00338-015-1382-z
- Johnson WS, Allen DM (2012) Zooplankton of the Atlantic and Gulf Coasts A Guide to their Identification and Ecology, 2nd edn. The John Hopkins University Press, Baltimore
- Jones DL (2006) Design, construction, and use of a new light trap for sampling larval coral reef fishes. NOAA Technical Memorandum NMFS-SEFSC-544

- Kahng SE, Copus JM, Wagner D (2014) Recent advances in the ecology of mesophotic coral ecosystems (MCEs). Current Opinion in Environmental Sustainability 7:72–81. doi: 10.1016/j.cosust.2013.11.019
- Kahng SE, Garcia-Sais JR, Spalding HL, Spalding HL, Brokovich E, Wagner D, Weil E, Hinderstein L, Toonen RJ (2010) Community ecology of mesophotic coral reef ecosystems. Coral Reefs 29:255–275. doi: 10.1007/s00338-010-0593-6
- Kahng SE, Maragos JE (2006) The deepest, zooxanthellate scleractinian corals in the world? Coral Reefs 25:254–254. doi: 10.1007/s00338-006-0098-5
- Kramer MJ, Bellwood DR, Bellwood O (2014) Benthic Crustacea on coral reefs: a quantitative survey. Marine Ecology Progress Series 511:105–116. doi: 10.3354/meps10953
- Laverick, JH, Andradi-Brown DA, Exton DA, Bongaerts P, Bridge TCL, Lesser MP, Pyle RL, Slattery M, Wagner D, Rogers AD (2016) To what extent do mesophotic coral ecosystems and shallow reefs share species of conservation interest? Environmental Evidence 5:16. doi**:** 10.1186/s13750-016-0068-5
- Lesser MP, Slattery M, Stat M, Ojimi M, Gates RD, Grottoli A (2010) Photoacclimatization by the coral *Montastraea cavernosa* in the mesophotic zone: light, food, and genetics. Ecology 91:990–1003. doi: 10.1890/09-0313.1
- Lindfield SJ, Harvey ES, Halford AR, McIlwain JL (2016) Mesophotic depths as refuge areas for fishery-targeted species on coral reefs. Coral Reefs 35:125–137. doi: 10.1007/s00338-015-1386-8
- Loya Y, Eyal G, Treibitz T, Lesser MP, Appeldoorn R (2016) Theme section on mesophotic coral ecosystems: advances in knowledge and future perspectives. Coral Reefs 35:1–9. doi: 10.1007/s00338-016-1410-7
- Luckhurst BE, Luckhurst K (1977) Recruitment patterns of coral reef fishes on the fringing reef of Curaçao, Netherlands Antilles. Can J Zool 55:681–689. doi: 10.1139/z77-089

- Maragos JE, Jokiel PL (1986) Reef corals of Johnston Atoll: one of the world's most isolated reefs. Coral Reefs 4:141–150. doi:10.1007/BF00427935
- Mass T, Einbinder S, Brokovich E, Shashar N, Vago R, Erez J, Dubinsky Z (2007) Photoacclimation of *Stylophora pistillata* to light extremes: metabolism and calcification. Marine Ecology Progress Series 334:93–102. doi: 10.3354/meps334093
- McKeon CS, Moore JM (2014) Species and size diversity in protective services offered by coral guard-crabs. PeerJ 2:e574. doi: 10.7717/peerj.574
- Menza C, Kendall M, Hile S (2008) The deeper we go the less we know. rev Biol Trop 56:11–24.
- Motro R, Ayalon I, Genin A (2005) Near-bottom depletion of zooplankton over coral reefs: III: vertical gradient of predation pressure. Coral Reefs 24:95–98. doi: 10.1007/s00338-004-0451-5
- Ohlhorst, SL (1985) Reef zooplankton collected along a depth gradient at Discovery Bay, Jamaica. In Reaka M (ed.) The Ecology of Coral Reefs. NOAA Symposia Series for Undersea Research 3:101–116.
- Ohman MD (1988) Behavioral responses of zooplankton to predation. Bulletin of Marine Science 43:530–550.
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2015). vegan: Community Ecology Package. R package version 2.2-1. http://CRAN.R-project.org/package=vegan
- Plaisance L, Caley MJ, Brainard RE, Knowlton N (2011) The Diversity of Coral Reefs: What Are We Missing? PLoS ONE 6:e25026. doi: 10.1371/journal.pone.0025026
- Pyle RL, Boland R, Bolick H, Bowen BW, Bradley CJ, Kane C, Kosaki RK, Langston R, Longenecker K, Montgomery A, Parrish FA, Popp BN, Rooney J,

Smith CM, Wagner D, Spalding HL (2016) A comprehensive investigation of mesophotic coral ecosystems in the Hawaiian Archipelago. PeerJ 4:e2475. doi: 10.7717/peerj.2475

- R Core Team (2013) R: A language and environment for statistical computing. R-project.org
- Randall JE (1967) Food habits of reef fishes of the West Indies. Studies in Tropical Oceanography 5:665–847.
- Ruppert EE, Fox R, Barnes RD (2003) Invertebrate Zoology: a Functional Evolutionary Approach, 7 edn. Brooks Cole, Belmont, CA
- Sala E, Knowlton N (2006) Global Marine Biodiversity Trends. Annu Rev Environ Resourc 31:93–122. doi: 10.1146/annurev.energy.31.020105.100235
- Stella JS, Pratchett MS, Hutchings PA, Jones GP (2011) Coral-associated invertebrates: diversity, ecological importance and vulnerability to disturbance. Oceanography and Marine Biology: An Annual Review 49:43–104.
- Veron JEN (2000) Corals of the World. The Australian Institute of Marine Science, Townsville, Australia
- Yahel R, Yahel G, Genin A (2005) Near- bottom depletion of zooplankton over coral reefs: I: diurnal dynamics and size distribution. Coral Reefs 24:75–85. doi: 10.1007/s00338-004-0449-z