Diving into the deep-end: Investigating tropical deep-reef fish assemblages

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Statement of the Contribution of Others

Chapter Two: TS, Mike Cappo (MC) and Michael Kingsford (MK) conceived the study. TS and MK conducted the fieldwork. TS analyzed the videos, compiled the dataset, and performed statistical analyses. MC checked species identification and offered assistance with the database and statistical methods. MC and TS commissioned the illustrations drawn by Juliet Corley. All authors contributed to the manuscript.

Chapter Three: TS, James Daniell (JD), Tom Bridge (TB), Rob Beaman (RB), MC and MK planned the sampling design and data collection. TS, TB and JD conducted the fieldwork (BRUVS and multibeam concurrently). RB and MC gave technical advice. TS conducted the video and statistical analysis. JD provided multibeam data and figures. JD and TB contributed to the Methods section based on their respective technical expertise. All authors contributed to the final manuscript.

Chapter Four: Multibeam data from JD. Statistical advice from MC. Analysis and write-up by TS.

Chapter Five: Multibeam data from JD. Review, analysis and write-up by TS.

Chapter Six: Otolith collection by Ashley Williams (AW) and the Pacific Community fisheries researchers. TS prepped solution-based ICP-MS and LA-ICP-MS samples. TS completed the LA-ICP-MS and Yi Hu completed the solution-based ICP-MS. Analysis and write-up by TS with input from AW and MK.

Chapter Seven: Otolith collection by AW (the Pacific Community) and Corey Wakefield (Fisheries Western Australia), and fisheries researchers in Tonga and Papua New Guinea. Samples provided by Peter Mous (The Nature Conservancy Indonesian Fisheries Program). Labwork, statistical analysis and write-up by TS.

Thesis Abstract

Increasing demand paired with declining catch rates from traditional fisheries has caused fishers from across the tropical Indian and Pacific Oceans to shift their focus towards deep-reef species. This trend is also seen in Australia; however, little is known about the local biology and ecology of these newly targeted species. Therefore, my objective was to combine multiple techniques, including underwater video, multibeam analysis of habitat, and otolith microchemistry, to examine the distribution, abundance, and species composition of a commercially important assemblage of deep-reef fishes. The information gathered from this project will assist in the resource management of these unique fish assemblages.

In this project I examined the biodiversity and ecology of deep-reef fishes at multiple spatial scales. I considered large depth gradients along the continental shelf-break to look at shifts in assemblage structure, but also broad geographic scales extending thousands of kilometres that had the potential to encapsulate multiple stocks. My specific aims were: (1) to describe deep-reef fish assemblages and examine fish-habitat associations for shelf-break environments in the Great Barrier Reef (GBR), Chapters 2 through 5; (2) to determine the utility of otolith microchemistry to identify regional stock structure, and then to apply the technique to fish populations across the Indian Ocean to the Central Pacific (Chapters 6 and 7).

In Chapter 2, I demonstrated that depth was a strong predictor of the distribution of fishes. Individual species had different depth distributions and few fish species overlapped between adjacent depth strata, indicating that these are unique assemblages that change with respect to depth. In general, species richness and abundance decreased with increasing depth. New species location records were found for *Chromis circumaurea*, *Chromis okamurai*, *Chromis mirationis*, *Hoplolatilus marcosi* and *Bodianus bennetti* in the GBR at lower mesophotic depths. After consulting various fish experts, three potentially new species from the genera *Selenanthias*, *Chromis*, and *Bodianus* species were detected. This was the first research project to use underwater video stations at multiple reefs down to 260 m depths in the GBR and in doing so this research has re-defined depth distributions of some fish assemblages and increased maximum depth records for a number of species.

Habitat was also important in predicting where deep-reef fish occur and there was high variation within depth strata (Chapter 3). Although species were often only found within a certain depth range, species' distribution and abundance was determined by localized habitat features. Furthermore, species distribution was dependent on the trophic group and degree of habitat specialization. Shelf-break slope environments had decreasing structural complexity with depth, such as greater proportion of plants and calcified reefs at shallower and middle depths and more mud, sand and rubble at the deepest depths. Depth, relative steepness, topographical relief and

hardness of substrate differentiated where these species were distributed. Epibenthic cover and substrate were important factors in influencing fish distributions and the presence of encrusting organisms and calcified reef translated to higher abundance and diversity (Chapter 4). Deeper fishes had varying degrees of habitat specialization and these habitat preferences can have important management implications (Chapter 5). Closely related species (in the same genus) had varying levels of habitat association; these differences likely reflected their species-specific ecology and behaviour (i.e. what they eat, degree of movement). Species with stronger associations may be more easily targeted and directly or indirectly impacted by environmental changes.

I hypothesized that environmental variation among species would be reflected in the hard structures of the fish themselves and give some insight to population structure at multiple spatial scales. I investigated otolith elemental composition for commercially-valuable deep-reef fishes of the Pacific: *Etelis coruscans* (flame snapper) and *Etelis sp.* (ruby snapper, recently distinguished from the pygmy ruby snapper) to determine the most robust approach to elemental chemistry that would assist in revealing population structure (Chapter 6). Overlapping and non-overlapping elemental fingerprints clarified where deepwater fish resources should be considered a continuous stock or separate stocks between locations. I compared the two major methods of otolith chemistry; laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) had better discriminatory accuracy than solution-based inductively coupled plasma mass spectrometry. Using a smaller ablation spot size had greater temporal resolution when I used a transect of the cross-section of the otolith, from the core to the edge to represent the timeline, or life history, of the fish. Using specific locations of the otolith transect also increased the spatial discrimination of the elemental fingerprints. It was concluded that the spatial separation of the otolith edge was better for stock discrimination.

Fishery management decisions rely on accurate information of where natural boundaries in fish populations occur (i.e. stock structure), and it was predicted that the chemistry of otoliths could help in discriminating distinct groups or management units. Based on the outcomes of Chapter 6, I then extended LA-ICP-MS chemical analyses to assess fish populations from otolith samples collected by fisheries researchers from the Pacific Community (New Caledonia) and Fisheries Western Australia. Otoliths were from three broad regions (Indian Ocean, West Pacific and Central Pacific) and included multiple Pacific Island nations: New Caledonia, Tonga, Vanuatu, Samoa, Fiji, Papua New Guinea, Wallis and Futuna, and Monowai Seamount (international waters). Combined with samples I collected from the Indonesia, the GBR and Coral Sea (Australia), this sampling design included ten international Exclusive Economic Zones (EEZ), and three fishery management zones in Australia (Kimberley, Pilbara/Gascoyne and GBR/Coral Sea). This is the first project that applied otolith chemical analyses of multiple deep-

reef species (*E. coruscans*, *E. sp.* and *Etelis carbunculus*, the pygmy ruby snapper) across a broad area (most of their distribution), for which identifying stock structure could assist management decisions and promote cooperation between adjoining nations. The potentially robust stocks identified were smaller than previously suggested, which is cause for concern. Smaller stocks may be more vulnerable to fishing pressure and local extirpation. For these locations precautionary management measures should be put in place that recognises these biological units until further evidence suggests otherwise.

My PhD research suggests that due to narrow depth distributions, deep-reef assemblages of fishes are vulnerable to overexploitation. Further, deep-reef fish depend on certain habitats and this can add an extra level of vulnerability if these depths and preferred habitat are isolated or uncommon. Deep reefs are critical ecological habitats and unique from shallower environments. Deep-reef ecosystems are still poorly understood, but they are an increasingly threatened component of the GBR and mesophotic reefs worldwide. Tropical deep-reef fish stocks are at risk of over-exploitation in the Indo-Pacific without sufficient information for fisheries management. Sensible protection of deeper areas will be critical if stocks are to be sustainably managed before they are lost. Deep-reef fisheries have been managed by EEZ rather than biological stocks. Here, I used elemental chemistry to identify biological units that could be useful for management strategies. Greater resolution of stock identity and pathways of connectivity in large biological stocks, is required to conserve the unique resources and unappreciated biodiversity of deep-reef fishes.

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Table 1-1: A list of commonly used abbreviations.

Abbreviation	
EEZ	Exclusive Economic Zone
GBR	Great Barrier Reef
GBRWHA	Great Barrier Reef World Heritage Area
GBRMPA	Great Barrier Reef Marine Park Authority
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LA-ICP-MS	Laser Ablation Inductively Coupled Plasma Mass Spectrometry
MCE	Mesophotic Coral Ecosystem (tropical reefs 30-150 m)
Me:Ca	Metal:Calcium ratio

Table 1-2: Definitions of terminology used in this thesis.

Term	***Definition***
Assemblage	A collection of species that overlap in space and time within a given area (e.g. habitat or depth range)
Deep reefs	Reefs at depths >50 m (below typical SCUBA diving limits); deep-reef *adj.*
Shelf-break	The edge of the continental shelf where it begins to drop off into the continental slope
Habitats	The physical and biological components that make up an organism's surrounding environment habitat or the environmental conditions that influence responses in the presence, abundance, growth and other important life-history traits of an organism (i.e. environmental niche, Hutchinson 1957)
Mesophotic	'Middle light' or depths approximately 50-150 m with typically lower light levels
Mesophotic Coral Ecosystems	Deeper reef-based ecosystems typically defined as the depths 30-150 m.
Mesopopulation	A 'medium scale' population level, usually describes most closely what is known as the functional definition of a stock. Immigration and emigration minimal (Kingsford & Battershill 1998).
Metapopulation	A 'population of populations' (Smedbol et al. 2002). Describes the broadest population level, often multiple stocks may be nested in a metapopulation.
Population	At the local level, there may be sufficient differentiation in demographic, life history, trophic or habitat requirements.
Sub-mesophotic	Depths below ~150 m
Rariphotic	Depths below ~100 m with higher levels of new species records and descriptions (Baldwin 2018)

Region	A broad area encompassing multiple possible stocks and often defined at the sub-ocean basin level (e.g. Western Pacific, Central Pacific, East Pacific)
Stock	Unit of convenience for fishery managers (i.e. stock identification), also a collection of local populations that equates with the definition of a mesopopulation.

Chapter 1 General Introduction

Global fisheries

Global fishing pressure is increasing and fishermen are targeting deeper habitats (Morato et al. 2006, FAO 2018). Technological advancements have changed commercial and even recreational fishing. Global positioning units, 'fish-finders', and three-dimensional acoustic mapping software programs (e.g. WASSP multibeam, Shelmerdine et al. 2014; RoxAnn, Bejarano et al. 2011) have a competitive market, and there are more economic incentives to invest in specialized gear such as hydraulic or mechanized reels and renewed interest to further develop deep-reef fisheries (Dalzell et al. 1996, Stone 2003, Adams & Chapman 2004, Newman et al. 2016). In the 1950s, average fishing depth was 40 m, now average depths are 150 m (Morato et al. 2006). This shift in fishing pressure, combined with the biological characteristics of fishes that live in deep environments, make them particularly susceptible to the effects of fishing (Morato et al. 2006, Cheung et al. 2007). Fishing may more detrimentally affect species with life history traits such as slower growth and maturation rates, long lifespans and low natural mortality rates, resulting in changes to the exploited communities (Jennings et al. 1999, Cheung et al. 2007). While coral reefs are structurally complex and diverse ecosystems, they are especially at risk and vulnerable to collapse as often the full consequences of greater fishing pressure may be considerably delayed (Jackson et al. 2001). Over a third of worldwide coral reefs are expected to be lost within the next few decades, which will have significant impacts for the 500 million people that rely on coral reef resources (Wilkinson 2008). For instance, as human population growth in the Pacific increases, it is projected that fish production needs to increase 46% in the next 20 years (Chin et al. 2011). This high demand for marine resources means 75% of Pacific island coastal fisheries will not be able to meet their food security needs by 2030 (Bell 2009).

Coral reefs worldwide have experienced dramatic changes due to intensified anthropogenic disturbances, which is apparent in the Great Barrier Reef (GBR), Australia, despite strengthened protection measures in recent decades (Kenchington 1990, Jackson et al. 2001). Fishing practices are among the anthropogenic stressors that combine to alter the structure and ecosystem functioning of marine environments (Lubchenco et al. 2003). Industrialized fishing can rapidly affect communities, leading to reduced stocks of larger predatory fish and changes to the ecosystem structure and function (Myers & Worm 2003). It is important to have 'baseline' estimates for unexploited communities, but for many offshore benthic communities this information is lacking. Newly fished areas initially show very high catch rates, but can decline to lower catch rates in a few years, often posing challenges for setting sustainable fishing targets, and causing economic uncertainty for fishers (Stone 2003, Adams & Chapman 2004). Often a large (~80%) decline can occur within 15 years of industrialized fishing effort, which is usually before scientific monitoring is established (Myers & Worm 2003). In some cases, the decline can

be surprisingly rapid, with stocks depleting within a few years or seasons of targeted fishing pressure (e.g. orange roughy; Koslow et al. 2000, Clark 2001). Current information on deeper fish assemblages is insufficient and precautionary measures should be taken to ensure there are adequate levels of spatial protection of deep-reef habitats (Sumpton et al. 2013).

In some locations fishing pressure, overfishing and localized extirpations, may already exist as a precursor to many scientific ecological studies. Current available information on deeper fishes and habitats is limited and coarse in many fished locations, and sampling deeper environments poses extra challenges. What we understand of deepwater fish and fisheries is limited compared to the majority of studies that focus on shallower depths (<30 m). There is limited information on the composition of deep-reef habitats, their relation to fish ecology, and overall ecosystem dynamics (Friedlander & Parrish 1998, Pearson & Stevens 2015). Tropical fisheries, especially those in developing nations that operate on smaller industrial scales, have had considerably less attention than larger commercial and temperate fisheries (Nash & Graham 2016, Newman et al. 2017) and often have higher species diversity (Pauly 1979). Tropical deep-reef fisheries are among the data-poor fisheries lacking biological and ecological information, and this translates to uncertainty in fisheries management (Newman 2003, Williams et al. 2012, Newman et al. 2015, Hill et al. 2016, Newman et al. 2016, Newman et al. 2017). Overall there is poor understanding of stock structure due to unknown recruitment dynamics, long dispersal potential, and spatially patchy reef habitats (Richards & Lindeman 1987). Many deepwater species have life history characteristics that make them especially vulnerable to fishing mortality (Wakefield et al. 2013, Newman et al. 2015). Typically, these benthopelagic fishes exhibit longer lifespans, slow growth rates and late maturity (Andrews et al. 2012, Williams et al. 2013, Newman et al. 2016), which can augment the setbacks of local population extirpations. Lastly, stocks of commercial deep-reef fishes may have low natural mortality and low production potential (Williams et al. 2013, Newman et al. 2016). It is with these factors in mind that we should quickly address key knowledge gaps.

Investigating deeper fish population ecology

There is a substantial body of information on reef fish assemblages from shallow water due to the accessibility of SCUBA diving, and it is only recently that diving has been used to explore mesophotic depths (Pyle 2000). Ecologists have demonstrated that shallow water assemblages are highly variable at multiple spatial scales due to complex links to environmental and ecological processes, such as habitat associations (Connell & Jones 1991), environmental gradients (Williams 1982), and competitive interactions (Robertson 1996, Bonin et al. 2015), but for deeper reefs worldwide, many of these links are not well-defined. Coral reef ecosystems are 'multi-scalar' with different ecological processes and in-built environmental patchiness that affect fish ecology (Sale 1998). In contrast, little is known about deeper reefs, which is sometimes

referred to as the 'Twilight Zone' for the fading light levels at mesophotic and sub-mesophotic depths, but also for the paucity of knowledge of these ecosystems (Pyle 1998). For Mesophotic Coral Ecosystems (MCEs), less is known about what scales environmental gradients influence the fish assemblages that inhabit them (Kahng et al. 2010, Kahng et al. 2014). Similar studies in temperate regions have commonly demonstrated how unique deep-slope fish assemblages are, and how depth and habitat are important explanatory variables (Stein et al. 1992, Yoklavich et al. 2000, Nasby-Lucas et al. 2002, Tissot et al. 2007, Love et al. 2009).

There is a consensus from ecological studies in terrestrial and marine environments that there is much information to be gained by designing projects that incorporate multiple spatial scales (Levin 1992, Sale 1998, Williams et al. 2003, Palumbi 2004, Hixon et al. 2012, Anderson et al. 2013, Taylor et al. 2015). Furthermore, structuring a hierarchical design can provide more accurate comparisons among distant locations and improve the generality of the results (Sale 1998). Species distributions will reflect the importance of preferred or suitable habitats as well as the 'seascape' configuration that structures fish assemblages (Grober-Dunsmore et al. 2007, Anderson et al. 2009). Fish-habitat associations explain how habitat features influence the spatial distribution of species, highlighting what are defining patterns and processes, and at what spatial scales they are relevant.

Spatial dynamics and distribution are central to the hierarchy of population units and defining effective boundaries within a species' range. The terms 'population' and 'stock' can be vague and not useful from a management perspective because of unknowns such as larval dispersal capacities, adult movements and migrations. The metapopulation concept considers a species' throughout its range to be a 'population of populations' (Levins 1969) with differing levels of connectivity. The broad metapopulation may be made up of 'mesopopulations', or stocks, which should be largely self-replenishing with little dependence on recruitment from other stocks (Fig. 1-1). These stocks may experience localised extirpation and rely on founder effects from neighbouring populations to become re-established. Accordingly, the internal spatial structure of a metapopulation has the potential to vary through time (Sinclair & Iles 1989). Fishery stocks may be spatially discrete but not necessarily isolated. Stocks may incorporate local populations with some differences in ontogenetic traits, species' interactions, and associations with the environment (Hanski & Gilpin 1991). Pelagic larval dispersal and limited adult mobility often reinforce reef fish metapopulations (Kritzer & Sale 2004) and these two traits operate on broad and narrow spatial scales of habitat use (Sale 1998). Therefore, an understanding of the linkages between population units is critical for the management of fisheries.

In order to investigate possible stock structure, multiple methods are useful, each with varying degrees of spatial and temporal resolution. Genetic analyses can be used to accurately

define species and identify population units with tools such as genetic markers or genetic variation (e.g. Ovenden et al. 2004, Salini et al. 2006). However, even limited immigration can 'homogenize the genetic structure' in larger populations or fail to detect stock structure when species have potentially high dispersal (Ovenden et al. 2015). Fish parasites can help to define stock structure as similar parasite communities infer shared histories (e.g. Hutson et al. 2011, Barton et al. 2018). Similarly, otolith chemical analyses provides stock structure information as the uptake of elements into the otolith reflect similarities in the environment or physiology experienced by individual fish (e.g. Kalish et al. 1996, Campana et al. 2000, Thresher & Proctor 2007, Macdonald et al. 2013, Wright et al. 2018). The natural composition of fish ear-bones, absorbed from environmental and physiological differences individual fish experience (Campana 1999, Campana et al. 2000), translates to the geographic separation of metapopulations. By comparing elemental concentrations found in trace amounts it is possible to delineate the structure of fishery stocks using these concentrations as environmental cues (Campana et al. 2000). Comparing multiple approaches (that each provide a layer of information) helps to resolve stock structure in fisheries and provides useful insight into marine populations (Begg & Waldman 1999).

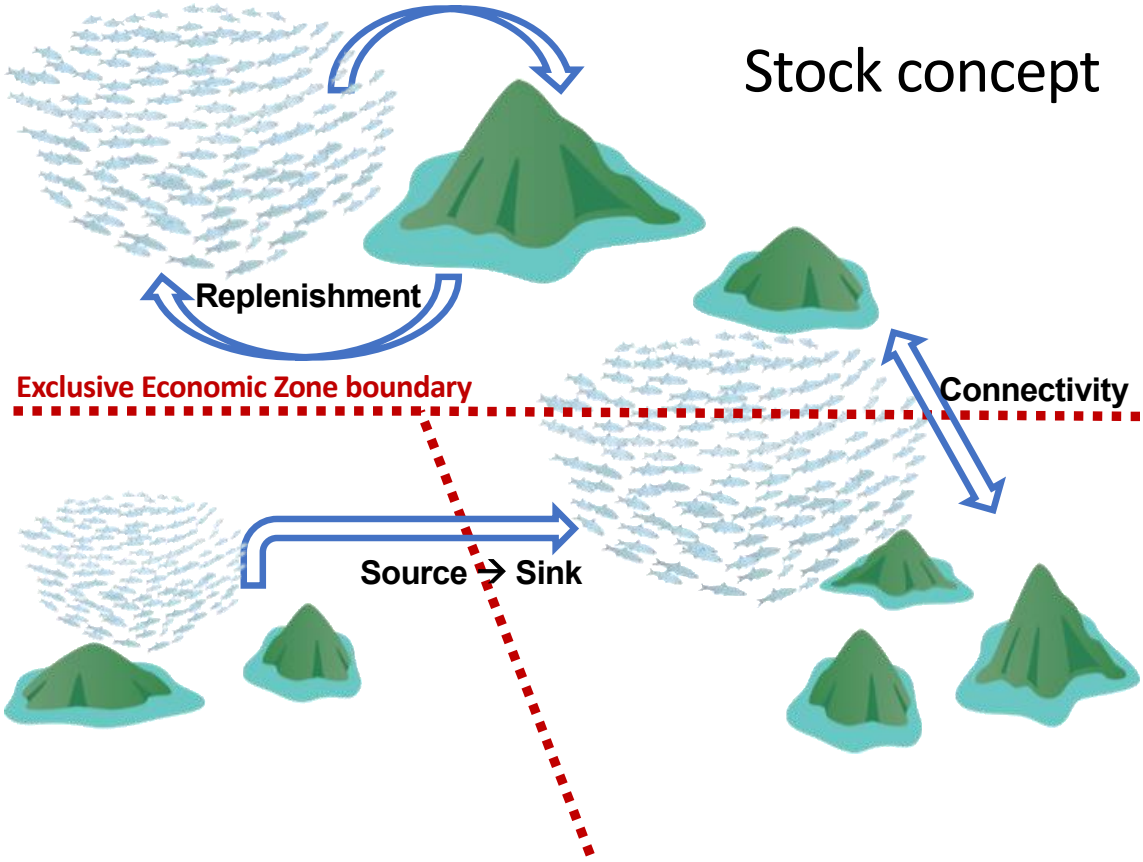

Figure 1-1: Illustration of some metapopulation dynamics. Arrows indicate possible movement and migration patterns for theoretical stocks in a marine metapopulation model. Figure includes illustrations modified from (IAN 2018).

Broad objectives and research significance

My research focused on the deep-reef fishes and fisheries ecology of the Indo-Pacific, layering information from multiple scales on the spatial distribution of fish inhabiting deeper environments. To do this, I focused on two major spatial scales: describing local populations of deeper fishes and habitats along the GBR shelf-break, and then moving to spatial scales that could correspond with stocks within a metapopulation for three potentially vulnerable species of eteline snappers. Conclusions on population structure at broader spatial scales were based on analyzing trace element otolith signatures from deepwater snappers throughout their Indo-Pacific distribution.

The shelf-break fish assemblages were largely unexplored at the greater depths (>100 m) of the Great Barrier Reef Marine Park (GBRMP) despite many broad-scale studies. The 'deep shoal' habitats (generally >20 m deep patches of hard substratum) and representative shallower habitats throughout the GBRMP have been included in previous underwater video surveys (e.g. Cappo et al. 2009, Espinoza et al. 2014). While the depth range of these studies extended into mesophotic depths (~80 m), no research had the specific intent to document the deep-reef fishes and habitats of the shelf-break. Past studies used manned submersibles (e.g. Harris & Davies

1989) and autonomous underwater vehicles (AUV) to document habitats at deeper depths (e.g. (Williams et al. 2010b, Bridge et al. 2011a). Exploratory fishing studies documented some deeper fish using hook-and-line (Kramer et al. 1994) and scientific trawl (Last et al. 2014), but these studies were limited, opportunistic endeavours. Only recently has there been greater systematic and collaborative sampling effort to describe the geomorphology (e.g. Webster et al. 2008, Abbey et al. 2011, Harris et al. 2013, Puga-Bernabéu et al. 2013) and faunal communities (e.g. Bongaerts et al. 2011, Bridge et al. 2011a, Bridge et al. 2011b, Bridge et al. 2012b, Englebert et al. 2017) but clearly absent was a characterization of the deeper fish assemblage of the GBR (>100 m).

We now know that deeper reefs and submerged shoals greatly extend the outer GBR area, and presumably create ample habitats for deeper fish assemblages. The shelf-break, or the eastern edge of the GBR, varies in distance offshore over the latitudinal length of the GBR. The shelf is narrow in the northern section and widens at the southern end. Deeper reefal habitats are comprosed of corals, sponges, whips, sea-fans and macroalgae (Pitcher et al. 2007) and can have substantial reef architecture below the surface-visible reefs (Harris et al. 2013). The benthos has been well-described in some sections (e.g. Bongaerts et al. 2011, Bridge et al. 2011a, Bridge et al. 2011b, Bridge & Guinotte 2012, Bridge et al. 2012b, Englebert et al. 2014, Englebert et al. 2017). Demersal fishes are an important economic component of the GBR fauna and it is increasingly recognized that many reef fishes strongly associate with habitat features like live coral, complex topography, substratum type, and depth (Newman & Williams 1996, Connell & Kingsford 1998, Munday et al. 2007, Kingsford 2009). Internationally this concept is referred to as 'Essential Fish Habitat' (EFH) or the habitats and waters necessary for fish to fulfil growth, feeding, and reproduction (Rosenberg et al. 2000). There is strong evidence of EFH requirements for deeper commercial fish assemblages in some locations worldwide (Moffitt & Parrish 1996, Parrish et al. 1997, Kelley et al. 2006, Misa 2013, Moore et al. 2013), but more research is needed to verify whether this is similar throughout the species' distribution. Shelf-break habitats might have similar roles for GBR mesophotic and sub-mesophotic fish assemblages (Cappo et al. 2009) and anecdotal information provided during the 2004 GBRMP re-zonation suggested deep shoal and submerged habitat features were important for commercial and recreational fishing (Cappo et al. 2012).

Shallower fish assemblages need biologically and structurally complex habitats (Wilson et al. 2008, Anderson et al. 2009). These habitats provide either access to more resources or reduced competition and predation (Friedlander & Parrish 1998). This results in the co-occurrence of more species and greater abundance of those species (Almany 2004). Research from deeper habitats worldwide demonstrate that often fish assemblages are highly influenced by depth (e.g. Brokovich et al. 2008, Garcia-Sais 2010, Zintzen et al. 2012, Bejarano et al. 2014). The predominant influence of depth on fish assemblages may be due to the various gradients of

temperature, light levels and water movement (Garrabou et al. 2002). These environmental variables can determine the benthic flora, fauna and reef architecture – comprised of available microhabitats – possibly resulting in greater niche availability, structural complexity, or diversity of benthic habitats (e.g. Pitcher 2004, Levin et al. 2010, Messmer et al. 2011). Depth and habitat factors may co-vary (Malcolm et al. 2010b), which may also be confounded by the abundance and distribution of habitats. These differences will be reflected in the functional groups of fishes and overall trophic ecology (Thresher & Colin 1986, Bulman et al. 2002, Fox & Bellwood 2007). However, competitive interactions may also be important processes structuring fish assemblages in deeper habitats as they are in shallower environments (e.g. Connell 1983, Bonin et al. 2015). Therefore, assemblage patterns can result from complex interactions between depth and other environmental or ecological variables, and it is sometimes difficult to separate the relative influence of specific variables (Malcolm et al. 2011). Since fishing alters the species composition, population structure and trophic structure of fished assemblages (Cheung et al. 2007, Norse et al. 2012, Watson & Morato 2013), it will be important to establish baselines for deeper fishes, which is useful information for resource management.

Deeper marine habitats have additional sampling challenges (e.g. limited light, greater ambient pressure, time and cost of sampling), and often this has led to more 'basic' research questions being answered as the scale of what is not known far outweighs what is known. Direct fish observations via diving beyond 100 m had been limited before the more widespread use of mixed gases and closed-circuit rebreather technology, which allows for safer dives but with significant decompression time. This has been the most successful tool for taxonomic studies, and newer innovations are allowing the successful capture of living specimens (Pyle 2000, Rocha et al. 2014, Shepherd et al. 2018b). Manned submersibles have been used where these research tools were available, but the expensive of operating and maintaining submersibles precludes their more widespread use. Some of the most explicit information on deeper reefs comes from these direct observations (e.g. Colin 1974, Colin 1976, Chave & Mundy 1994, Starr et al. 1996, Kelley et al. 2006, Tissot et al. 2007, Laidig et al. 2013, Baldwin et al. 2018). The use of Remotely Operated Vehicles (ROVs) has the potential to significantly add to deeper exploration (e.g. Cánovas-Molina et al. 2016, McLean et al. 2017, Bond et al. 2018) but high costs and logistics are limiting. Similarly, drop-cameras (e.g. Easton et al. 2017) also have the potential to add to deeper habitat studies but often a larger research vessel is necessary to deploy ROVs and drop-cameras. Stationary Baited Remote Underwater Video Stations (BRUVS) can be deployed simultaneously, for greater observation time, replication, and efficiency of sampling. BRUVS can be used over a variety of habitats, are not extractive, and do not require fish experts to be present for species identification, reducing many of the observer biases associated with other visual methods (Cappo

2010). Further, archived video and images could be used to measure and compare changes over time.

My PhD sought to improve the existing knowledge of deeper fish and habitats and addresses some of the challenges of managing fisheries from a global perspective. I used BRUVS and multibeam echo-sounders to gather information on fish assemblages and habitat on local populations (Fig. 1-2). I documented the diversity and abundance of fishes on multiple reefs along a depth gradient 50-260 m (Chapter 2). I predicted that depth would drive fish assemblage structure. In cases where narrow depth distributions were found, I hypothesized that these would be more vulnerable fish populations. Accordingly, I described variation in assemblages with depth and key indicator species that were representative of those assemblages. To further discriminate patterns due to depth from distributions influenced by habitat features, I investigated fish-habitat associations (Chapter 3-5). Due to the complexities of environmental gradients and natural variability in fish assemblages, I first explored how assemblage composition changes with respect to habitat features (Chapter 3), followed by an investigation on how habitat affects overall species richness and abundance patterns (Chapter 4), and then I took a closer look at how single-species habitat associations vary (Chapter 5). These descriptions form 'stepping stones' to understand broader species distribution patterns and contributes a firm foundation of basic ecological information for local populations of deep-reef fish assemblages.

In Chapters 6 and 7, I investigated the multi-scale complexity of potential stocks within metapopulations of different species from fishery samples. From otoliths collected from Indonesia to Tonga, this study represents that largest dataset of trace element otolith chemistry for tropical deep-reef fishes. I conducted a preliminary study using otolith samples from two species of deepwater snappers collected from 5-6 Exclusive Economic Zones (EEZs) comparing fine-scale resolution information from both solution-based inductively coupled plasma mass spectrometry and laser ablation inductively coupled plasma mass spectrometry (Chapter 6). I extended these methodological findings into a larger sample comparing the otolith chemistry of three sympatric species of deepwater snappers across ten EEZs and twelve regions of fishing interest, including three management zones in Australia (Chapter 7). By looking at regional and local elemental otolith compositions, we can learn about the distribution of deeper ecosystems and evaluate whether the spatial boundaries between metapopulations of fish align with regional management strategies.

My PhD research has direct application to fisheries management. I describe variation in abundance and assemblage composition for local populations together with data on depth and habitat as well as stock structure within metapopulations of deep-reef fishes. My major research outputs can contribute to better population models and stock assessments with the outcome of

improving management decisions. Effective management of these new fisheries requires high resolution information regarding the distribution of fishes. This study is the first to evaluate deep-reef fish metapopulations over multiple spatial scales along the GBR and across the Pacific.

Figure 1-2: T. Sih and M. Kingsford deploying Baited Remote Underwater Video Stations to survey deep habitats of the Great Barrier Reef off of the R/V *James Kirby* (James Cook University).

Chapter 2 Deep-reef fish assemblages of the Great Barrier Reef shelf-break (Australia)

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Abstract

Tropical mesophotic and sub-mesophotic fish ecology is poorly understood despite increasing vulnerability of deeper fish assemblages. Worldwide there is greater fishing pressure on continental shelf-breaks and the effects of disturbances on deeper fish species have not yet been assessed. Difficult to access, deeper reefs host undocumented fish diversity and abundance. Baited Remote Underwater Video Stations (BRUVS) with lights were used to sample deeper habitats (54-260 m), in the Great Barrier Reef (GBR), Australia. Here I described fish biodiversity, relative abundance and richness, assessing the prediction that depth would drive assemblage structure in the GBR. Distinct groups of fishes were found with depth whilst overall richness and abundance decreased steeply between 100 and 260 m. Commercially-valuable Lutjanidae species from *Pristipomoides* and *Etelis* genera, were absent from shallower depths. Few fish species overlapped between adjacent depth strata, indicating unique assemblages with depth. I also detected new location records and potential new species records. The high biodiversity of fish found in shelf-break environments is poorly appreciated and depth is a strong predictor of assemblage composition. This may pose a challenge for managers of commercial fisheries as distinct depth ranges of taxa may translate to more readily targeted habitats, and therefore, an inherent vulnerability to exploitation.

Introduction

Fishes occupying deeper shelf-break environments are susceptible to increasing threats as the condition of many shallower coral reefs is in decline due to the effects of anthropogenic and environmental disturbances (e.g. fishing, pollution, coral bleaching and warming temperatures; Hoegh-Guldberg 1999, Hughes et al. 2003). Deeper mesophotic reefs are extensions of shallow habitats and can play a critical role in maintaining the health of the greater ecosystem (Lesser et al. 2009). Deeper environments may be refuges for shallow-reef fishes threatened by fishing pressure (Feitoza et al. 2005, Lindfield et al. 2016) and warming temperatures (Currey et al. 2015). Worldwide, fishers are fishing deeper and more efficiently with better technology and gear (Roberts 2002, Morato et al. 2006, Cheung et al. 2007). The value of these ecosystems must be evaluated in the face of potential rapid future exploitation. What are critical – or irreplaceable – components to protect for future resources? Only by pushing the depth boundaries of ecological studies can we understand if deeper benthic habitats have similar or different patterns and processes. Further, to what degree are shallow and deep habitats connected? We need methods that can be used in both shallower and deeper habitats for comparisons over a broad geographic range.

There is a paucity of ecological information on the distribution and abundance of deep-reef fishes worldwide (Pyle 1998, 2000), though this information has increased in the past decade (Baldwin et al. 2018). The light-limited depths of the mesophotic and sub-mesophotic, which traditionally has remained a mystery due to the greater logistics (Gage & Tyler 1991) and costs (Pyle 2000, Kahng et al. 2010) of sampling deeper, and often remote, habitats. Mesophotic coral reefs can extend to 150 m in clear waters (Hinderstein et al. 2010, Kahng et al. 2014) and this depth is thought to be the lower distribution of many reef-based species (Colin 1974, Feitoza et al. 2005, Brokovich et al. 2008, Garcia-Sais 2010), including fishes. Studies on mesophotic fish ecology may not sample the greater taxonomic diversity available (Pearson & Stevens 2015) because time, cost and expertise are often limited. However, deep-reefs may have a disproportionally high number of novel or endemic species (Pyle et al. 2008, Kane et al. 2014, Last et al. 2014). The current information on deeper fish distribution is also not evenly distributed worldwide; it is currently unclear whether deep-reef fishes are found in broad geographic ranges but so far are only found in a few explored locations (Pyle 2000, Brokovich et al. 2008, Pyle et al. 2008).

The greatest proportion of reef fish biodiversity studies are limited to depths shallower than 30 m (Kahng et al. 2010, Kane et al. 2014). This presents a large bathymetric gradient of reef communities that have not been explicitly described. Mesophotic fish and coral assemblages may change along depth gradients (Kahng et al. 2010, Kahng et al. 2014, Kane et al. 2014) and may include shallower-occurring species, but also deep-specialist species restricted to certain depths

(Feitoza et al. 2005, Brokovich et al. 2008, Baldwin & Robertson 2014, Bejarano et al. 2014, Baldwin & Robertson 2015, Rosa et al. 2015, Tornabene et al. 2016a). The Great Barrier Reef (GBR) comprises 2,500 reefs and represents the world's largest continuous coral reef ecosystem covering approximately 344,400 km^2 (GBRMPA 2016). With over 1500 known fish species in the Great Barrier Reef Marine Park (GBRMP; Choat & Russell 2008), few studies include the mesophotic depths along the edge of the continental shelf (Last et al. 2011b). This shelf-break may potentially have greater species diversity than mesophotic reefs in other study locations (Last et al. 2005, Last et al. 2011b, Kane et al. 2014) as follows: (1) the western Pacific and Australia is close to the 'centre of reef biodiversity' (Bellwood & Hughes 2001, Allen 2002, Allen 2008); (2) the broad shelf of the GBR harbours greater diversity (Allen 2008); and (3) the amount of deeper reef habitat may have been previously underestimated (Harris et al. 2013). The continental shelf-edge can exhibit steep environmental gradients, subject to a wide range of environmental drivers that can significantly change over tens of meters and affect the faunal diversity (e.g. light availability, temperature, benthic substrate, and food availability; Zintzen et al. 2012) and I predicted that there would be distinct fish assemblages along this gradient.

Depth is likely a key driver of assemblage structure (Gaston 2000, Cappo et al. 2007, Baldwin & Robertson 2014, Pearson & Stevens 2015) and evidence in the mesophotic so far concurs with this paradigm. Bathymetric breaks have been established for the GBR for coral species, including a transition at 60 m between distinct upper and lower mesophotic tropical assemblages (Bridge et al. 2011a) and at subtropical latitudes around 50 m (Malcolm et al. 2010a). Fish species richness appears to increase to a maximum at 25-30 m, then decreases to 50-65 m (Pearson & Stevens 2015), however, these studies did not investigate deeper, to the maximum extent of these light-limited reef environments. Understanding how species richness is distributed across environmental gradients, such as the shallow-to-deep reef transition zone, is key to understanding how species in both zones may respond to future environmental changes. Further, bathymetric distribution data can improve conservation and management efforts and reduce bycatch, by encouraging fisheries to target depth ranges with a high proportion of target species relative to unwanted species.

Monitoring techniques often focus on economically important fishes, limiting the ability to detect changes in whole fish assemblages (Depczynski & Bellwood 2003, Maxwell & Jennings 2005, Magurran et al. 2010). Underwater video has great potential to document and monitor deep-reef assemblages of fish and can be constructed to survey deeper depths with adequate light. Specifically, Baited Remote Underwater Videos Stations (BRUVS) have been used to monitor fish and benthic assemblages of the GBR, but not fish assemblages in deeper mesophotic and sub-mesophotic reef and inter-reefal habitats (Cappo et al. 1998, Cappo et al. 2007). BRUVS are useful for studying deep-reef fishes, as they can withstand pressures associated with greater

depths and are easily replicated for repeatable ecological studies (see reviews Murphy & Jenkins 2010, Harvey et al. 2013, Mallet & Pelletier 2014). Surveys with similar baited video equipment have assessed mesophotic fish assemblages in other locations, investigating abundance and size distributions (Merritt et al. 2011, Fitzpatrick et al. 2012, Moore et al. 2013), habitat associations (Fitzpatrick et al. 2012, Misa 2013), and the efficacy of Marine Protected Areas for fisheries management (Sackett et al. 2014, Moore et al. 2016a). However, no studies have investigated below the 80 m isobath in the GBRMP (Cappo et al. 2007). BRUVS have inherent biases that have to be carefully considered, such as the presence of a bait plume, which can alter the behavior of fishes and preferentially attract larger, more mobile fishes (see reviews (Murphy & Jenkins 2010, Harvey et al. 2013, Mallet & Pelletier 2014). However, an advantage of this method is that it is not intrusive or destructive, thus BRUVS are permitted in most zones of the GBRMP. BRUVS are a good method in baseline and longterm deep-reef studies in the GBR as the images and video are geo-referenced and can be kept as a permanent record to validate fish identifications, or to compare species compositions over temporal and spatial scales with controlled sampling effort along a great depth range.

The objective of this study was to use BRUVS to investigate tropical fish assemblages in mesophotic to sub-mesophotic depths at a number of reefs along the shelf-edge of the central GBR (Fig. 2-1). I hypothesized that abundance of fishes and related diversity would vary with depth and that the patterns would be consistent by reef. This is the first comprehensive fishery-independent survey of mesophotic fish biodiversity within the GBR at depths of 50-300 m. Specifically, I aimed to: (a) determine how species richness and abundance vary with depth; (b) describe fish assemblages and identify key depth-indicator species; and (c) provide critical baseline information, which is archived for future comparisons; (d) measure thermal profiles of the water column, in multiple years where I hypothesized that temperature/depth strata may correlate with the distribution of fishes.

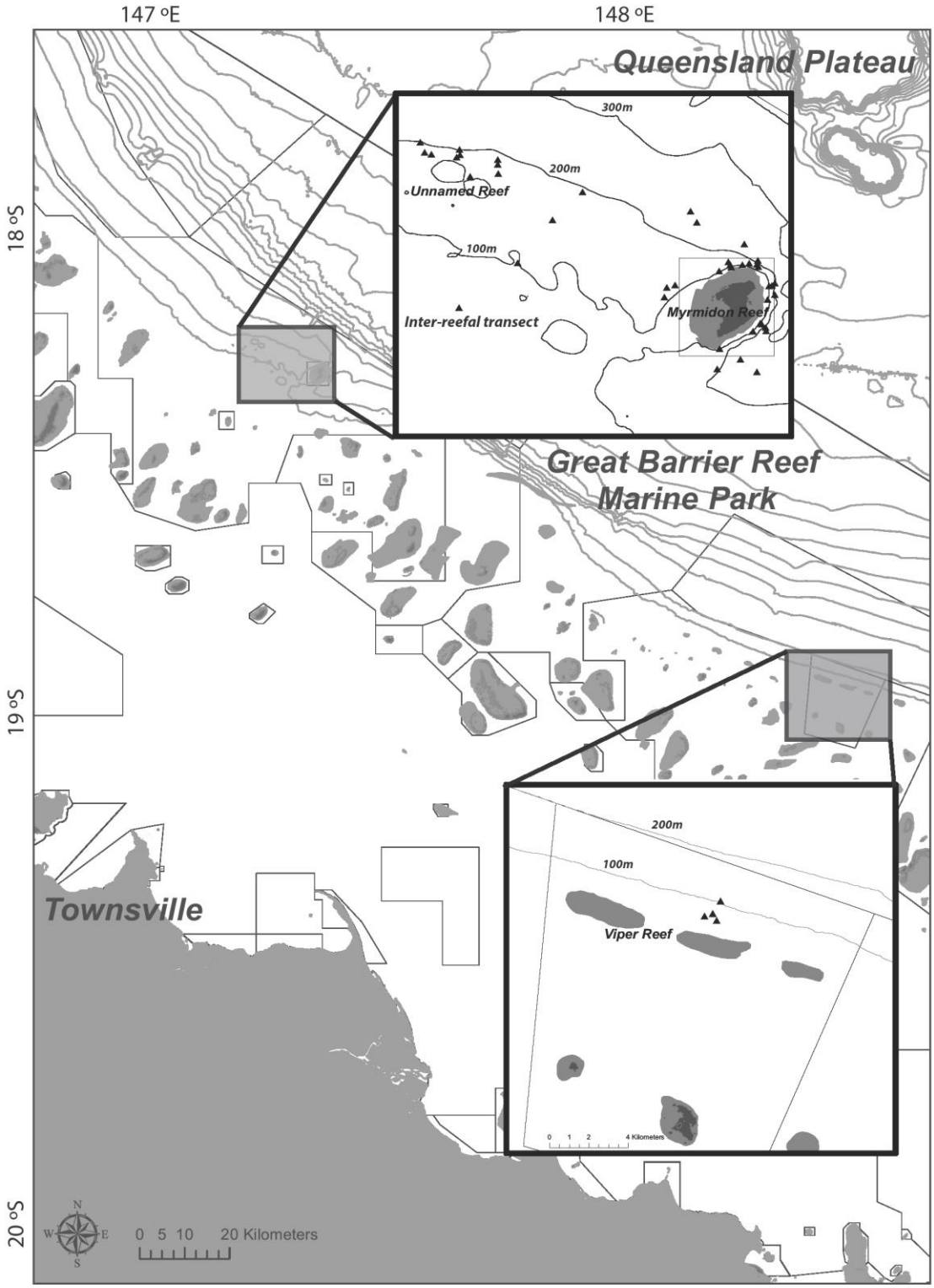

Figure 2-1: Map of Baited Remote Underwater Video Station surveys along the outer shelf-break of the Great Barrier Reef, Australia. Forty-eight BRUVS deployments (triangles) across three reefs (Unnamed, Myrmidon, and Viper) and an inter-reefal transect. Map components: bathymetric contour lines (100 m) from deepreef.org and shapefiles provided by the Great Barrier Reef Marine Park Authority. The edge of the continental shelf is over 100 km offshore around the Central Great Barrier Reef.

Methods

BRUVS deployment

Three reefs were sampled along the shelf-edge (Myrmidon, Unnamed and Viper) and one inter-reefal transect using a depth-stratified sampling design (Fig. 2-1). Two identical BRUVS units rated to 300 m were used, with an aluminum elliptical roll-bar frame enclosing a camera-housing with a flat acrylic front port and battery-powered spotlight (white) mounted above the top roll-bar. Sony high-definition Handicams HDR-CX110 were used, with focus set to manual infinity to maximize the field of view. Using a bridle-rope configuration with twice the water depth of attached line per deployment, each BRUVS was marked by surface floats and flags for retrieval. The bait arm consisted of a plastic conduit to a plastic mesh bag filled with ~1 kg of crushed pilchards (*Sardinops sagax*; see review for the effect of bait, Westerberg & Westerberg 2011, Hardinge et al. 2013).

Forty-eight deployments were made in May, June and Sept 2014 on three cruises. All deployments were placed during daylight (50-300 m of water depth; 0700-1800) with most of the effort targeting 100-300 m in transects at each reef with three targeted depth strata. My hypothesis was that there would be differences in the fish assemblage with depth. BRUVS were deployed in shallow (~100 m), mid (~150 m) and deep (~200 m) strata at each reef. Viper Reef is on a shallower sloping shelf-edge, so depths of >200 m were not available without travelling substantially further offshore. Instead, BRUVS were deployed shallower to get a similar bathymetric depth gradient (50-150 m) over a similar spacing between deployments (i.e. differences would be due to depth, not increased distance from shore). Within depth-strata BRUVs were haphazardly-spaced several hundred meters apart.

Fish identification and analysis of video metrics

Underwater imagery was read using Australian Institute of Marine Science (AIMS) purpose-built software. The following details were noted: time on the sea-bed, time of first appearance of each species, and abundance *N* of each species until time MaxN (highest number of individuals of a species per frame) reached, until the end of sampling (when the video left the bottom or when the tape finished recording). MaxN is a conservative estimate of abundance to eliminate the possibility of re-counting fish swimming in and out of the field-of-view (Cappo 2010). Videos were read to its full length (27-84 minutes, average soak of 54 minutes) and later standardized for length of time of sampling (number of species present-absent per site for species richness, and number of fish per species for relative abundance, per 60 minute increment). Fish were identified to lowest possible taxa, with the assistance of fish experts, fish identification books and Fishbase.org (Froese & Pauly 2018). Every effort was made to identify large, conspicuous fish in addition to smaller, cryptic species. Videos, fish identification photographs, and BRUVS

deployment metadata are archived in the Australian Institute of Marine Science database and can be accessed by request.

Depth patterns

Species were summed across all sites for species richness and abundance. Where standardized values of total abundance and richness were used, the estimates were standardized by number of species per 60 minutes of sampling time. For these analyses two depth classification systems were used. For the one-way ANOVA, which required a balanced design, three depth categories 'Shallow' (50-115 m), 'Mid' (128-160 m) and 'Deep' (179-260 m) were used. For other analyses Shallow was further divided to two smaller categories to investigate the differences 50-115 m. These sites were categorized in four depth strata: 'upper mesophotic' (50-65 m), 'middle mesophotic' (85-115 m), 'lower mesophotic' (128-160 m) and 'sub-mesophotic' (179-260 m). These strata represented breaks in the depth-stratified sampling design, but also aligned with previously documented transitional boundaries, including the ~150 m lower depth-limit of Mesophotic Coral Ecosystems (MCEs; Kahng et al. 2010). Analyses were performed using several packages in R statistical software (R Core Development Team 2018, CRAN ver. 3.2.3) and Excel.

To evaluate the general trend of how species richness and abundance varied with depth, standardized richness and abundance were square-root transformed and data were tested for any significant deviation from normality (Shapiro-Wilks: species richness Wilks=0.98, p=0.66; abundance Wilks = 0.95, p=0.07) to meet the assumptions of ANOVA. In the original design I had the factors 'Depth' (a=3) and 'Reef' (b=3; Myrmidon, Unnamed, Viper) and site (n=4) with an interaction between depth and site. The interaction was weak ($p < 0.25$), therefore, the factors were pooled as recommended by Underwood (Underwood 1997). The factor 'Reef' was pooled for a stronger test for the factor 'Depth'. ANOVA was performed for Depth (a=3, n=14) for both richness and abundance and two-tailed t-tests between depth groups with a Bonferroni correction was applied.

Mean standardized richness and abundance were also plotted in relation to depth strata separately by reef (Myrmidon, Viper and Unnamed; varied number of replicates within stratum). In addition, deployments were made along an inter-reefal transect (60-200 m, one replicate per depth). Shallower BRUVS sets from Viper Reef, one from on top of the submerged unnamed deep reef and the inter-reefal transect were included as an additional (50-65 m depth strata, n=4). For analysis of separate families, I separated the Lutjanidae family into 'deep' members (*Etelis* and *Pristipomoides* genera) and 'other' (all other member species). Family analyses followed the one-way ANOVA for species richness and abundance.

Investigating fish assemblages

I also wanted to investigate species associations as they may be better predictors of environmental conditions than species individually. This is often difficult because of positively-skewed frequency distributions and the high frequency of zeros in larger community composition datasets (Legendre 2005). Species abundances (summed MaxN, maximum number of fish per species per site) were fourth-root transformed, which down-weights highly abundant species and reduces the skew in the distribution for each species (Borcard et al. 2011).

I used a Principle Coordinate Analysis (PCoA) ordination to visualise the differences between sites. Eliminating single-species occurrences (species only occurring at one site) from this analysis (58 of 130 species), I used 47 of the sites with 72 of the fish species in a Bray-Curtis dissimilarity matrix (packages vegan, Oksanen et al. 2013; ecodist, Goslee & Urban 2007). Agglomerative heirarchical unconstrained clustering revealed 12 significant clusters (SIMPROF; packages cluster (Maechler et al. 2015), clustersig (Whitaker & Christman 2014). For the ordination I color-coded the sites with the depth strata from the previous constrained univariate analyses and size-coded the symbols to correspond with species richness in the resulting biplot (functions capscale, vegdist). Capscale revealed ordination distances that were analogous to the original dissimilarities and is similar to redundancy analysis but can utilise non-Euclidean dissimilarities (Oksanen et al. 2013). To determine which fish species corresponded with the variance between sites, I plotted the 15 species with the highest species scores.

I used species abundance data to perform multi-level pattern analysis of species by depth (functions multipatt, package indicspecies, De Cáceres & Legendre 2009). This method first lists species associated with particular groups of sites and then indicator species analysis is independently conducted for each species (De Cáceres 2013). This method requires multiple testing, but can help to predict the likelihood of individual species to attribute to that depth assemblage (De Cáceres 2013). Statistical significance is interpreted based on the IndVal index, which is a measure of association between the species and that depth group and tested through a permutation test (Dufrêne & Legendre 1997). An advantage of the function multipatt is that it looks for both indicator species for individual depth strata as well as combinations of strata (De Cáceres 2013). I also repeated this analysis using presence-absence (occurrence) data using Pearson's phi coefficient of association, a measure of the correlation used between binary variables (values of 0 and 1, Borcard et al. 2011). Because this analysis is independently conducted for each species, I chose to include all species. Further, rare or single-species occurrences can be important for ecosystem functioning (Lyons et al. 2005, Poos & Jackson 2012). I considered the inclusion of all species to align with my objective of describing complete

assemblages, and rare species (*sensu* FishBase) are of higher conservation concern as they can be more sensitive to ecosystem stresses than common species (Cao et al. 1998).

Measurements of temperature with depth

On the outer shelf-edge off Myrmidon Reef, near the 300-m isopleth (Fig. 2-1), a *Seabird* Conductivity Temperature and Depth recording device was slowly lowered (<1 m/sec) by hand to an estimated maximum depth before retrieval. The instrument was calibrated for 60 seconds below the surface before deployment. Repeated samples were made in early August 2009, 2010 and 2013.

Results

A total of 1081 individual fish, sharks and rays were identified, representing 130 species from 29 families (48 BRUVS deployments, 42.35 hours of bottom-time/sampling-time). Species diversity varied with 1-40 species identified per deployment, average species richness was 9.44 species, and mean abundance of 22.5 fishes. Lutjanidae, Lethrinidae and Nemipteridae were the families most frequently sighted. The most speciose families were Labridae (23 spp), Carangidae (16 spp), Lutjanidae (16 spp), and Lethrinidae (11 spp). BRUVS allowed us to identify large-bodied fish such as groupers, jacks, snappers and apex predators such as sharks. Many commercially-valuable species were sighted including *Pristipomoides filamentosus*, *Pristipomoides multidens*, and *Plectropomus laevis*. Some smaller species and juveniles were only identified to genus (i.e. juvenile *Lethrinus sp.*).

Some of the species seen at these depths are of conservation concern according to IUCN criteria (IUCN 2018), these include: Scalloped Hammerhead and Humphead Maori Wrasse (*Sphyrna lewini* and *Cheilinus undulatus*, Endangered), Blotched Fantail Ray, Silvertip Shark and Sandbar Shark (*Taeniurops meyeni*, *Carcharhinus albimarginatus* and *Carcharhinus plumbeus*, Vulnerable), and Whitetip Reef Shark and Grey Reef Shark (*Triaenodon obesus* and *Carcharhinus amblyrhynchos,* Near Threatened).

Several of the species observations represent new geographic location records for Australia and specifically the GBR (Table 2-1). These include *Chromis okamurai* (143 m, Yamakawa & Randall 1989), *Chromis mirationis* (155-194 m, Tanaka 1917), *Chromis circumaurea* (115 m, Pyle et al. 2008), *Hoplolatilus marcosi* (100 m, Burgess 1978) and the recently described *Bodianus bennetti* (155-179, Gomon & Walsh 2016). Unrecognized species from *Selenanthias* (143-160 m), *Chromis* (155 m), and *Bodianus* (143 m) were also observed and may potentially be new species.

A number of small-bodied fishes were recorded and are likely an underestimate of true abundance and richness. Both *Terelabrus rubrovittatus* and *Cirrhilabrus roseafascia* appeared in a large proportion (17%) of the sites. Other frequently-sighted smaller fish include small *Bodianus* species (25% of sites) and *Pentapodus* species (19%).

Table 2-1: Fish species identified in deep-reef Baited Remote Underwater Video Station videos from the Central Great Barrier Reef shelf-break. Identifications to species designation where possible and taxonomic information based on the Australian Faunal Directory (ABRS 2009) and California Academy of Sciences' Catalog of Fishes (Eschmeyer et al. 2016). CAAB codes are the eight-digit Codes for Australian Aquatic Biota maintained by CSIRO Division of Marine and Atmospheric Research for species of research or commercial interest. Australian standard names are according to the Australian Faunal Directory or *FishBase (Froese & Pauly 2018) common name. FishBase, Fishes of Australia (Bray & Gomon 2018), IUCN Redlist (IUCN 2018), Randall's *Reef and Shore Fishes of the South Pacific* (Randall 2005) and Allen and Erdmann's *Reef Fishes of the East Indies* app (Allen & Erdmann 2013) were consulted for reported depth range. Where differences in these references occurred, the maximum depth range is reported. Climate and known distribution information from FishBase. New record information was compared to reported data from FishBase, Fishes of Australia and Atlas of Living Australia databases and cross-referenced with John Pogonoski (CSIRO).

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Carcharhinidae							
Carcharhinus albimarginatus (Rüppell, 1837)	37018027	Silvertip Shark	98-155 m (13)	1-800 m		Tropical Indo-Pacific	No
Carcharhinus amblyrhynchos (Bleeker, 1856)	37018030	Grey Reef Shark	54-156 m (10)	0-1000 m		Tropical Indo-West & Central Pacific	No
Carcharhinus plumbeus (Nardo, 1827)	37018007	Sandbar Shark	259 m (1)	0-500 m		Subtropical Atlantic & Indo-Pacific	No
Loxodon macrorhinus Müller & Henle, 1839	37018005	Sliteye Shark	107 m (1)	7-100 m	Marginal	Tropical Indo-West Pacific	No
Triaenodon obesus (Rüppell, 1837)	37018038	Whitetip Reef Shark	54-99.5 m (3)	1-330 m		Tropical Indo-Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Sphyrnidae							
Sphyrna lewini (Griffith & Smith, 1834)	37019001	Scalloped Hammerhead	105 m (1)	0-1000 m		Circumglobal, tropical and temperate seas	No
Dasyatidae							
Taeniurops meyeni (Müller & Henle, 1841)	37035017	Blotched Fantail Ray	54 m (1)	1-500 m		Tropical Indo-West Pacific	No
Muraenidae							
Gymnothorax berndti Snyder, 1904	37060089	Y-Patterned Moray*	150 m (1)	30-303 m		West Indo-Pacific	Yes, new to GBR
Gymnothorax elegans Bliss, 1883	37060090	Elegant Moray*	110-149 m (2)	92-450 m		Indo-West Pacific	No, known from unpublished records
Gymnothorax intesi (Fourmanoir & Rivaton, 1979)	37060076	Whitetip Moray	200 m (1)	200-400 m		Subtropical West Pacific	No
Gymnothorax prionodon Ogilby, 1895	37060049	Sawtooth Moray	150-194 m (2)	20-80 m	Yes	Subtropical to temperate West Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Fistulariidae							
Fistularia commersonii Rüppell, 1838	37278001	Smooth Flutemouth	54 m (1)	0-200 m		Tropical Indo-Pacific	No
Peristediidae							
Satyrichthys sp.	37288912		245 m (1)				
Serranidae							
Epinephelus cyanopodus (Richardson, 1846)	37311145	Purple Rockcod	99.5-102 m (2)	2-150 m		Tropical West Pacific	No
Epinephelus morrhua (Valenciennes, 1833)	37311151	Comet Grouper	115-194 m (6)	80-370 m		Tropical Indo-Pacific	No
Plectranthias kelloggi Jordan & Evermann, 1903	37311210	Eastern Flower Porgy*	155-179 m (2)	60-540 m		Temperate Pacific	Yes
Plectropomus leopardus (Lacépède, 1802)	37311078	Common Coral Trout	100-105 m (2)	3-100 m	Marginal	Tropical West Pacific	No
Plectropomus laevis (Lacépède, 1801)	37311079	Bluespotted Coral Trout	85-128 m (4)	4-100 m	Yes	Tropical Indo-Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Pseudanthias engelhardi (Allen & Starck, 1982)	37311115	Barrier Reef Basslet	100 m (1)	37-70 m	Yes	Tropical West-Central Pacific	No
Selenanthias sp.	37311947		143-179 m (6)	129-204 m		Subtropical to temperate West Pacific	Yes, new to GBR
Variola louti (Forsskål, 1775)	37311166	Yellowedge Coronation Trout	54-98 m (2)	3-300 m		Tropical Indo-Pacific	No
Malacanthidae							
Hoplolatilus marcosi Burgess, 1978	37331012	Redback Sand Tilefish*	100 m (1)	18-80 m	Yes	Tropical Indo-Pacific	Yes
Echeneidae							
Echeneis naucrates Linnaeus, 1758	37336001	Sharksucker	54-155 m (8)	0-200 m	Yes	Subtropical; Circumtropical	No
Carangidae							
Carangoides caeruleopinnatus (Rüppell, 1830)	37337021	Onion Trevally	54-129 m (12)	1-60 m	Yes	Tropical Indo-West Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Carangoides chrysophrys (Cuvier, 1833)	37337011	Longnose Trevally	54-60 m (2)	30-60 m		Indo-Pacific	No
Carangoides dinema Bleeker 1851	37337078	Shadow Trevally	54-102 m (4)	1-22 m	Yes	Tropical Indo-West Pacific	No
Carangoides ferdau (Forsskål, 1775)	37337068	Blue Trevally	57-100 m (2)	1-60 m	Yes	Tropical Indo-Pacific	No
Carangoides fulvoguttatus (Forsskål, 1775)	37337037	Turrum	99.5-102 m (2)	?-100 m	Marginal	Indo-West Pacific	No
Carangoides orthogrammus (Jordan & Gilbert, 1882)	37337057	Thicklip Trevally	85-129 m (3)	3-168 m		Tropical Indo-Pacific	No
Carangoides plagiotaenia Bleeker, 1857	37337070	Barcheek Trevally	106 m (1)	2-200 m		Tropical Indo-Pacific	No
Caranx ignobilis (Forsskål, 1775)	37337027	Giant Trevally	54-85 m (2)	10-188 m		Tropical Indo-Pacific	No
Caranx melampygus Cuvier, 1833	37337050	Bluefin Trevally	54-85 m (2)	0-190 m		Tropical Indo-Pacific	No
Gnathanodon speciosus (Forsskål, 1775)	37337012	Golden Trevally	102 m (1)	0-162 m		Tropical Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Pseudocaranx dentex (Bloch & Schneider, 1801)	37337062	Silver Trevally	99.5-155 m (2)	10-238 m		Tropical Atlantic and Indo-Pacific	No
Seriola dumerili (Risso, 1810)	37337025	Amberjack	146-260 m (11)	1-360 m		Sub-tropical, circumglobal	No
Seriola rivoliana Valenciennes, 1833	37337052	Highfin Amberjack	98-245 m (10)	5-250 m		Sub-tropical, circumglobal	No
Lutjanidae							
Aphareus rutilans Cuvier, 1830	37346001	Rusty Jobfish	85-245 m (23)	10-330 m		Tropical Indo-Pacific	No
Aprion virescens Valenciennes, 1830	37346027	Green Jobfish	54-105 m (2)	0-180 m		Tropical Indo-Pacific	No
Etelis carbunculus Cuvier, 1828	37346014	Ruby Snapper	226 m (1)	90-400 m		Tropical Indo-Pacific	No
Lipocheilus carnolabrum (Chan, 1970)	37346031	Tang's Snapper	194 m (1)	90-340 m		Indo-West Pacific	No
Lutjanus bohar (Forsskål, 1775)	37346029	Red Bass	85-128 m (10)	4-180 m		Tropical Indo-Pacific	No
Lutjanus sebae (Cuvier, 1816)	37346004	Red Emperor	99.5-103 m (2)	5-180 m		Tropical Indo-West Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Paracaesio kusakarii Abe, 1960	37346060	Saddleback Snapper	156-200 m (3)	100-310 m		Tropical West Pacific	No
Pristipomoides argyrogrammicus (Valenciennes, 1831)	37346054	Ornate Jobfish	193-245 m (6)	70-350 m		Tropical Indo-Pacific	No
Pristipomoides auricilla (Jordan, Evermann & Tanaka, 1927)	37346059	Goldflag Snapper	150-194 m (3)	90-360 m		Indo-Pacific	No
Pristipomoides filamentosus (Valenciennes, 1830)	37346032	Rosy Snapper	85-201 m (16)	40-400 m		Indo-Pacific	No
Pristipomoides multidens (Day, 1870)	37346002	Goldbanded Snapper	129-250 m (14)	40-350 m		Tropical & sub-tropical Indo-Pacific	No
Pristipomoides sieboldii (Bleeker, 1857)	37346064	Lavender Snapper	143 m (1)	100-500 m		Indo-Pacific	No
Pristipomoides typus Bleeker, 1852	37346019	Sharptooth Snapper	115-250m (18)	40-180m	Yes	Tropical Indo-Pacific	No
Symphorus nematophorus (Bleeker, 1860)	37346017	Chinamanfish	60-105 m (4)	20-100 m	Marginal	Tropical West Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Caesionidae							
Pterocaesio marri Schultz, 1953	37346068	Bigtail Fusilier	54 m (1)	1-35 m	Yes	Tropical Indo-Pacific	No
Symphysanodontidae							
Symphysanodon sp.	37346930		115 m (1)				
Nemipteridae							
Nemipterus balinensis (Bleeker, 1859)	37347039	Bali Threadfin Bream	194-240 m (2)	50-150 m	Yes	Tropical Indo-West Pacific	No
Pentapodus aureofasciatus Russell, 2001	37347029	Yellowstripe Threadfin Bream	54-106 m (7)	5-80 m	Yes	Tropical Pacific	No
Pentapodus nagasakiensis (Tanaka, 1915)	37347012	Japanese Threadfin Bream	100 m (1)	2-100 m		Tropical West Pacific	No
Scolopsis sp.	37347902		65 m (1)				
Lethrinidae							
Gymnocranius euanus (Günther, 1879)	37351022	Paddletail Seabream	54-156 m (10)	15-50 m	Yes	Tropical West Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Gymnocranius grandoculis (Valenciennes, 1830)	37351005	Robinson's Seabream	54-155 m (10)	20-170 m		Tropical Indo-Pacific	No
Lethrinus laticaudis Alleyne & Macleay, 1877	37351006	Grass Emperor	54 m (1)	5-35 m	Yes	Tropical West Pacific	No
Lethrinus miniatus (Forster, 1801)	37351009	Redthroat Emperor	54-128 m (8)	5-250 m		Tropical West Pacific	No
Lethrinus nebulosus (Forsskål, 1775)	37351008	Spangled Emperor	100-179 m (2)	0-90 m	Yes	Tropical Indo-West Pacific	No
Lethrinus olivaceus Valenciennes, 1830	37351004	Longnose Emperor	54-105 m (5)	1-185 m		Tropical Indo-West Pacific	No
Lethrinus ravus Carpenter & Randall, 2003	37351031	Drab Emperor	54-128 m (5)	5-35 m	Yes	Tropical West Pacific	No
Lethrinus rubrioperculatus Sato, 1978	37351012	Spotcheek Emperor	54-106 m (8)	8-198 m		Tropical Indo-Pacific	No
Lethrinus semicinctus Valenciennes, 1830	37351016	Blackblotch Emperor	54 m (1)	4-35 m	Yes	Tropical Indo-West Pacific	No
Wattsia mossambica (Smith, 1957)	37351027	Mozambique Seabream	105-160m (8)	100-300m		Tropical Indo-West Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Mullidae							
Mulloidichthys pfluegeri (Steindachner, 1900)	37355040	Orange Goatfish	54-103 m (3)	13-200 m		Tropical Indo-West Pacific	Yes
Parupeneus heptacantha (Lacépède, 1802)	37355004	Cinnabar Goatfish	54-103 m (4)	12-350 m		Tropical Indo-West Pacific	No
Parupeneus multifasciatus (Quoy & Gaimard, 1825)	37355026	Banded Goatfish	54 m (1)	3-161 m		Tropical Pacific	No
Parupeneus pleurostigma (Bennett, 1831)	37355027	Sidespot Goatfish	100 m (1)	1-120 m		Tropical Indo-Pacific	No
Chaetodontidae							
Heniochus diphreutes Jordan, 1903	37365005	Schooling Bannerfish	128 m (1)	5-210 m		Subtropical Indo-Pacific	No
Pomacanthidae							
Pomacanthus imperator (Bloch, 1787)	37365014	Emperor Angelfish	100-105 m (2)	1-100 m		Tropical Indo-Pacific	No
Pomacanthus semicirculatus (Cuvier, 1831)	37365080	Blue Angelfish	105 m (1)	1-40 m	Yes	Tropical Indo-West Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Cirrhitidae							
Cyprinocirrhites polyactis (Bleeker, 1875)	37374006	Lyretail Hawkfish	100 m (1)	10-132 m		Tropical Indo-West Pacific	No
Pomacentridae							
Chromis circumaurea Pyle, Earle & Greene, 2008	37372153	Gold-rim Chromis*	115 m (1)	?-100 m	Yes	Tropical West Pacific	Yes
Chromis mirationis Tanaka 1917	37372048	Japanese Puller	155-194 m (2)	40-208 m		Subtropical West Pacific	Yes, new to GBR
Chromis okamurai Yamakawa & Randall, 1989	37372154	Okinawa Chromis*	143 m (1)	135-175 m		Subtropical to temperate Northwest Pacific	Yes
Chromis sp.	37372155		155 m (1)				Potential new species
Labridae							
Bodianus anthioides (Bennett, 1832)	37384052	Lyretail Pigfish	54 m (1)	6-60 m		Tropical Indo-Pacific	No
Bodianus bimaculatus Allen, 1973	37384055	Twospot Pigfish	100-106 m (2)	30-70 m	Yes	Tropical Indo-Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Bodianus izuensis Araga & Yoshino, 1975	37384058	Striped Pigfish	98-105 m (2)	12-70 m	Yes	Subtropical West Pacific	Yes
Bodianus masudai Araga & Yoshino, 1975	37384221		115-155 m (2)	30-113 m	Yes	Subtropical: West Pacific anti-tropical distribution	Yes
Bodianus bennetti	37384219	Lemon-striped Pygmy Hogfish	155-179 m (4)	97-130 m	Yes	West Pacific	Yes, new to GBR, recently published record from the Coral Sea
Bodianus sp.	37384220		143 m (1)				Potential new species
Cheilinus undulates Rüppell, 1835	37384038	Humphead Maori Wrasse	54 m (1)	1-100 m		Tropical Indo-Pacific	No
Choerodon venustus (De Vis, 1884)	37384042	Venus Tuskfish	54 m (1)	10-95 m		Subtropical West Pacific	No
Cirrhilabrus punctatus Randall & Kuiter, 1989	37384083	Finespot Wrasse	54-85 m (2)	2-78 m	Yes	Tropical West Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Cirrhilabrus roseafascia Randall & Lubbock, 1982	37384218	Pink-Banded Fairy Wrasse*	85-155 m (8)	30-100 m	Yes	Tropical West Pacific	Yes, new to GBR, recently published record from the Coral Sea
Cirrhilabrus sp.	37384910		54-200 m (2)				
Coris dorsomacula Fowler, 1908	37384093	Pinklined Wrasse	60 m (1)	2-45 m	Yes	Tropical West Pacific	No
Halichoeres sp.	37384920		54 m (1)				
Labroides dimidiatus (Valenciennes, 1839)	37384028	Common Cleanerfish	54 m (1)	1-40 m	Yes	Tropical Indo-Pacific	No
Labridae sp.	37384000		54 m (1)				
Oxycheilinus digrammus (Lacépède, 1801)	37384065	Violetline Maori Wrasse	179-193 m (2)	3-120 m	Yes	Tropical Indo-Pacific	No
Oxycheilinus orientalis Günther, 1862	37384030	Oriental Maori Wrasse	99.5-110 m (2)	10-80 m	Yes	Tropical Indo-West Pacific	No
Oxycheilinus sp.	37384933		150 m (1)				

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Terelabrus rubrovittatus Randall & Fourmanoir, 1998	37384210	Yellowbar Hogfish*	100-179 m (8)	50-140 m	Yes	Tropical Western Central Pacific; Japan; Maldives	Yes
Pinguipedidae							
Parapercis nebulosa (Quoy & Gaimard, 1825)	37390005	Pinkbanded Grubfish	105-179 m (11)	11-120 m	Yes	Tropical Indo-West Pacific	No
Parapercis sp.	37390901		60-245 m (10)				
Blenniidae							
Meiacanthus luteus Smith-Vaniz, 1987	37408054	Yellow Fangblenny	100 m (1)	0-40 m	Yes	Tropical West Pacific	No
Acanthuridae							
Acanthurus xanthopterus Valenciennes, 1835	37437020	Yellowmask Surgeonfish	100 m (1)	1-120 m		Tropical Indo-Pacific	No
Naso caesius Randall & Bell, 1992	37437046	Silverblotched Unicornfish	100-106 m (4)	15-50 m	Yes	Tropical Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Scombridae							
Gymnosarda unicolor (Rüppell, 1836)	37441029	Dogtooth Tuna	85-260 m (17)	10-300 m		Tropical Indo-Pacific	No
Scomberomorus commerson (Lacépède, 1800)	37441007	Spanish Mackerel	54-155 m (4)	0-200 m		Tropical Indo-West Pacific	No
Balistidae							
Abalistes stellatus (Anonymous, 1798)	37465011	Starry Triggerfish	54-128 m (6)	7-350 m		Tropical Indo-West Pacific	No
Balistidae sp.	37465000		54 m (1)				
Balistoides conspicillum (Bloch & Schneider, 1801)	37465031	Clown Triggerfish	54-105 m (2)	1-75 m	Yes	Tropical Indo-Pacific	No
Sufflamen bursa (Bloch & Schneider, 1801)	37465078	Pallid Triggerfish	54 m (1)	3-90 m		Tropical Indo-Pacific	No
Sufflamen fraenatum (Latreille, 1804)	37465014	Bridled Triggerfish	98-107 m (4)	8-200 m		Tropical Indo-Pacific	No

Species	CAAB code	Australian standard name	Depths observed (Number of videos)	Reported depth range	Depth extension?	Climate and known distribution	New record to the Great Barrier Reef or Australia
Tetraodontidae							
Torquigener sp.	37467913		240 m (1)				
Trionodon macropterus Lesson, 1831	37991885	Threetooth Puffer*	245 m (1)	50-300 m		Tropical Indo-West Pacific	No

Species richness and abundance with depth

Strong depth-related patterns of relative species richness (number of species per 60 minutes of video) and total fish abundance (sum of MaxN of all species per deployment per 60 minutes of video) were detected and these differences were significant according to ANOVA (Table 2-2). There was no interaction between depth and site (@ > p = 0.25) and therefore the interaction was pooled into the factor depth. Species richness and abundance generally decreased from shallow to deep although patterns varied by reef (Fig. 2-2). Comparing Shallow (50-115 m), Mid (128-160 m) and Deep (179-260 m) fish assemblage groups for species richness (t-tests), Shallow-Mid (p=0.08, NS) and Mid-Deep (p=0.06, NS) were not significantly different groups, but Shallow-Deep was (p=0.02*). Tukey's HSD highlighted the same differences in overall species richness between the depth groups: Shallow-Mid (p=0.21, NS), Mid-Deep (p=0.13, NS), and Shallow-Deep (p=0.001*). Species abundance based on summed MaxN of all species present at each site showed a similar pattern, with non-significant differences Shallow-Mid (p=0.47, NS) and Mid-Deep (p=0.18, NS), and Shallow-Deep was a significant change (p=0.004*) in pairwise t-tests. Post-hoc Tukey's HSD Shallow-Mid (p=0.33, NS), Mid-Deep (p=0.14, NS) and Shallow-Deep (p=0.004*). Variation of relative species abundance within depth strata was high, as indicated by standard error (SE) of 27-63% of the mean abundance per depth (Fig. 2-2). There was also variation in relative species richness within depths, SEs 19-49% mean richness. For both richness and abundance there was a general decrease in the variation between sites from shallow to deep. However, the variation within strata was not great enough to obscure strong depth-related patterns. The decline in relative species abundance was mirrored in some families, with carangids, labrids and lethrinids decreasing in abundance with depth (Fig. 2-3). Lutjanidae exhibited depth-related zonation between species, with species *Lutjanus bohar* and *L. sebae* found at shallower depths and species from *Pristipomoides* and *Etelis* genera only in deeper depths. Lethrinidae species *Gymnocranius euanus*, *G. grandoculis* and *Wattsia mossambica* occurred at depths down to 150-160 m, other lethrinid species occurred in 128 m or shallower. Some fish species were only present at depths greater than 100 m (i.e. *Pristipomoides aureofasciatus*, *W. mossambica*, *Lipocheilus carnolabrum*, *Paracaesio kusakarii*; Table 2-1).

Table 2-1: Species richness and abundance decreased with depth across all reefs pooled (one-way ANOVA).

Richness	**Df**	**SS**	**MS**	**F-value**	**p**
Among depths	2	12.55	6.28	7.19	0.002*
Within depths	39	34.04	0.87		
Abundance	**Df**	**SS**	**MS**	**F-value**	**p**
Among depths	2	38.62	19.31	5.88	0.006*
Within depths	39	128.13	3.29		

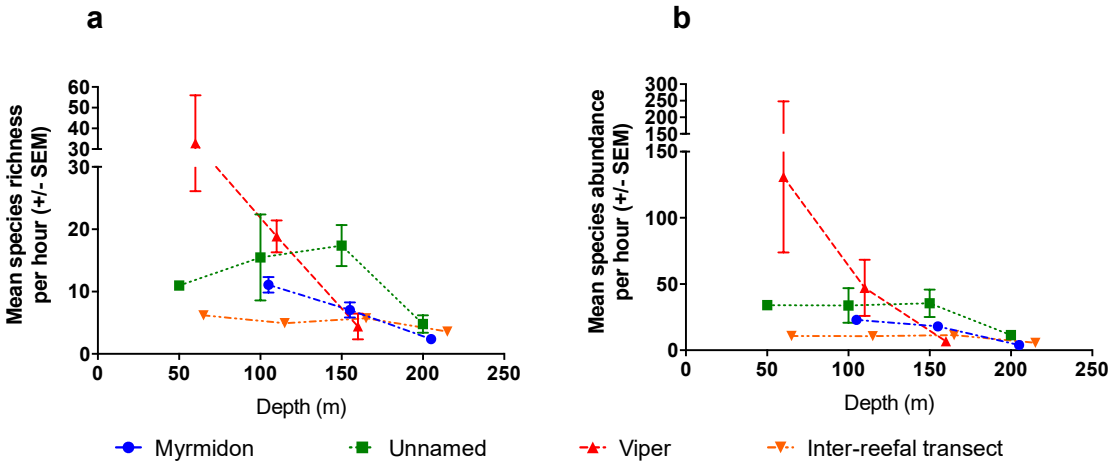

Figure 2-2: Species richness and abundance decline with increasing depth along the Great Barrier Reef shelf-break. a) Mean total species richness and b) Mean total species abundance (standardized per hour of sampling time). Symbols correspond to the three reefs and inter-reefal transect and are off-set for ease of interpretation. Sites were pooled into four depth strata: upper mesophotic (54-65 m, n = 4), middle mesophotic (85-115 m, n = 14), lower-mesophotic (128-160 m, n = 16), and sub-mesophotic (179-260 m, n = 15).

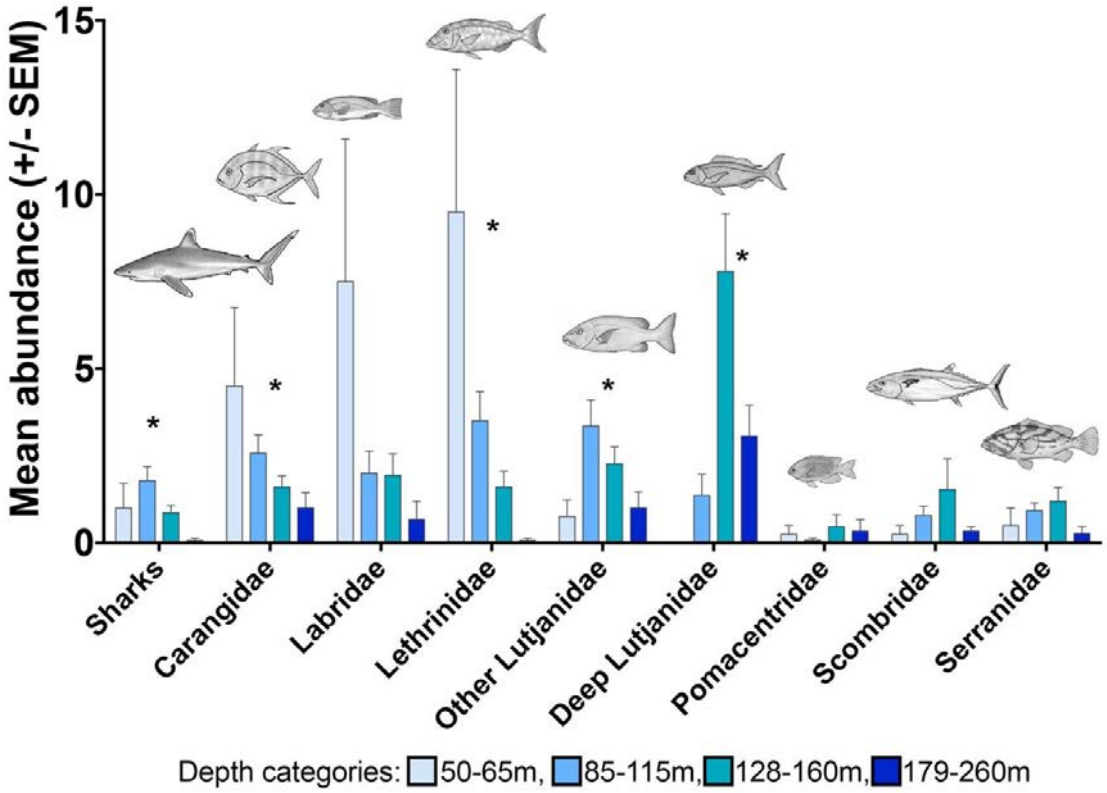

Figure 2-3: Mean total abundance of fish families sighted by Baited Remote Underwater Video Stations along the Great Barrier Reef shelf-break. Abundance was measured as MaxN per species per site, total abundance by family was the sum of all species relative abundance per site per depth category. Significantly different means (ANOVA) per depth are indicated by *. Illustrations drawn by Juliet Corley and commissioned by MC and TS.

Fish assemblages

Fish assemblages varied with depth. PCo1 explained 17.5% of the variance and separated the deepest and shallowest sites (Fig. 2-4a). PCo2 separated the middle sites and explained 11.9% of the variance. Shallower sites (<100 m) were more speciose. *Seriola dumerili*, *Pristipomoides* species and the lethrinid *Wattsia mossambica* associated with deeper sites. *Lethrinus rubrioperculatus*, *Gymnocranius euanus*, *Pentapodus aureofasciatus*, and *Carangoides caeruleopinnatus* frequented shallower sites (Fig. 2-4b).

There was high species variation within depth strata and a number of single-species occurrences (i.e. species only recorded at one site). Fifty-eight species identified were only present in one site, resulting in high among-site diversity. Of single species occurrences, MaxN (the maximum number of a species within a single video frame) ranged from 1-85 individuals.

There were great differences in group membership by depth. However, in some cases there was species overlap in group memberships with depth (Table 2-3). Indicator species analysis of four pre-defined depth groups and multi-level pattern analysis attributed 130 species to a group

or groups based on transformed species abundance. Twenty-three species were selected as having significant differences with depth: 13 were assigned to unique groups and ten species were assigned to two groups. No species were assigned to more than two groups. The upper mesophotic group (54-65 m) had a total of 36 unique species, of which seven were significantly attributed to only that depth strata ($p < 0.05$). The middle mesophotic group (85-115 m) was assigned 30 species with three significant. The lower mesophotic (128-160 m) had 18 species assigned, two were significant. The sub-mesophotic group (179-260 m) was assigned 13 species, only one was significant. There was a greater shared assemblage between the upper and middle mesophotic (11 species total), then between the upper and lower or the upper and sub-mesophotic groups. Middle and lower-mesophotic shared 11 species; the lower mesophotic and sub-mesophotic sites shared six species. The genus *Parapercis* (Pinguipedidae) was unusual in that it may be a depth-generalist genus, found in all three mesophotic groups (0.462, p=0.765). Further, the highly mobile *Gymnosarda unicolor* (Scombridae) was found throughout the deepest groups (0.622, p=0.363). Presence-absence data revealed almost identical results, out of 130 species 24 were selected: 12 were assigned to a unique group, 12 assigned to pairs of groups.

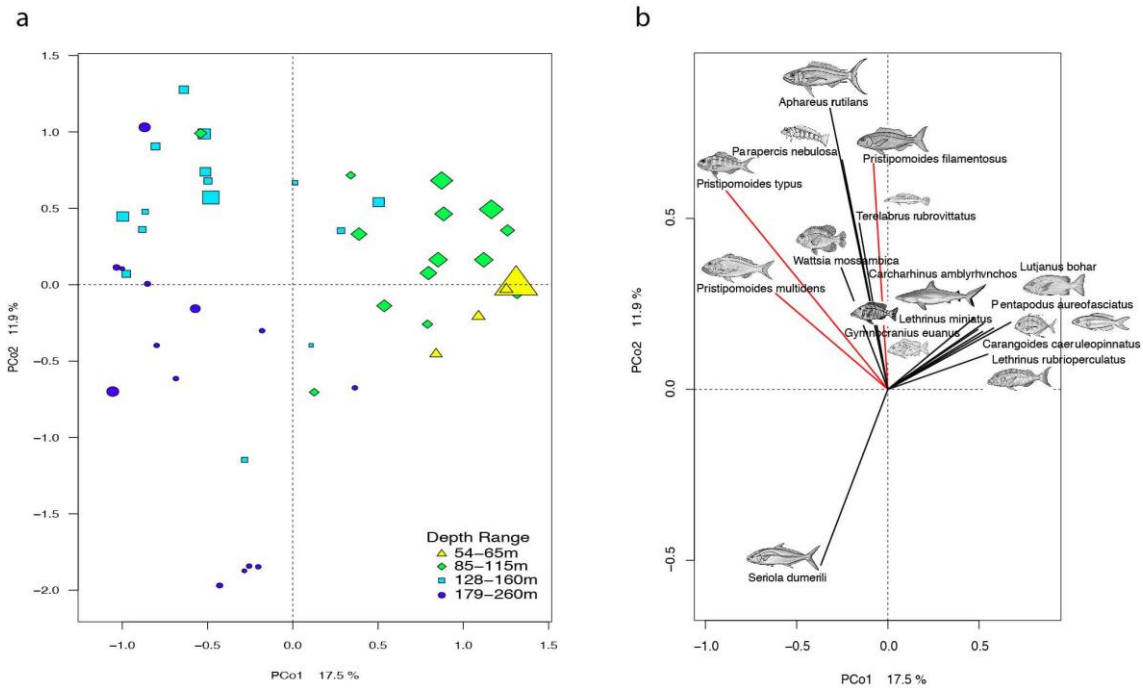

Figure 2-4: PCoA biplot of 47 Baited Remote Underwater Video Station sites: a) Sites are color-coded by depth range and the size of the symbol corresponds to the total species richness scaled by a tenth; b) 15 fish species scores are plotted that explain some of the variance between principle coordinates axes (scale of eigenvector is relative to the influence of that species to overall discrimination). Members of the *Pristipomoides* genus, prominent mesophotic fishes, are highlighted in red. Illustrations drawn by Juliet Corley and commissioned by MC and TS.

Table 2-2: Key fish indicator species per depth strata (multi-level pattern analysis). IndVal index (0-1) is accompanied by significance levels: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001; "a" for species abundance data, "o" for occurrence (presence-absence) data.

	Upper mesophotic (54-65 m, n = 4)	Middle mesophotic (85-115 m, n = 14)	Lower mesophotic (128-160 m, n = 15)	Sub-mesophotic (179-260 m, n = 15)
Species which contribute significantly to each group	*Abalistes stellatus* 0.957 *** a,o *Lethrinus rubrioperculatus* 0.752 ** a *Lethrinus sp.* 0.707 ** a,o *Carangoides chrysophyrys* 0.707 ** a,o *Mulloidichthys pfluegeri* 0.693 ** a,* o *Gymnocranius grandoculis* 0.672 * a,o *Carangoides dinema* 0.624 * a,o	*Lutjanus bohar* 0.774 ** a,o *Sufflamen fraenatum* 0.535 * a,o *Naso caesius* 0.535 * a,o	*Pristipomoides typus* 0.760 ** a *Wattsia mossambica* 0.657 * a,o *Selenanthias sp.* 0.449 * o	*Pristipomoides argyrogrammicus* 0.632 ** a,o

	Group 1+2	Group 2+3	Group 3+4
Species which contribute significantly to more than one group	*Carangoides caeruleopinnatus* 0.756 ** a,o *Lethrinus rubrioperculatus* 0.619 ** o *Carcharinus amblyrhyncos* 0.691 ** a, *o *Gymnocranius euanus* 0.690 * a,o *Pentapodus aureofasciatus* 0.624 * a,o *Lethrinus miniatus* 0.611 * a	*Aphareus rutilans* 0.756 ** a,o *Pristipomoides filamentosus* 0.679 * a,o *Carcharinus albimarginatus* 0.670 * a, ** o *Cirrhilabrus roseafascia* 0.402 * o	*Pristipomoides multidens* 0.683 * a, **o *Seriola dumerili* 0.606 * a,o *Pristipomoides typus* 0.579 * o

Temperature versus depth profiles

Seawater temperature varied greatly with depth (Fig. 2-5). At Myrmidon, CTD data from 2009-2013 indicated surface temperatures were about 25°C and well-mixed to approximately 100 m. Temperatures dropped by up to 10°C (i.e. 14-16°C) from ~100 m to a depth of ~250 m. The thermocline commenced at 70-100 m and in many years a decrease in temperature continued to the 200-250 m depth stratum with some evidence that the rate of change slowed at the greatest depths sampled. Although the steepness of the temperature change at the beginning and within the thermocline varied among years, the depth of the well-mixed shallow water layer was similar from year to year.

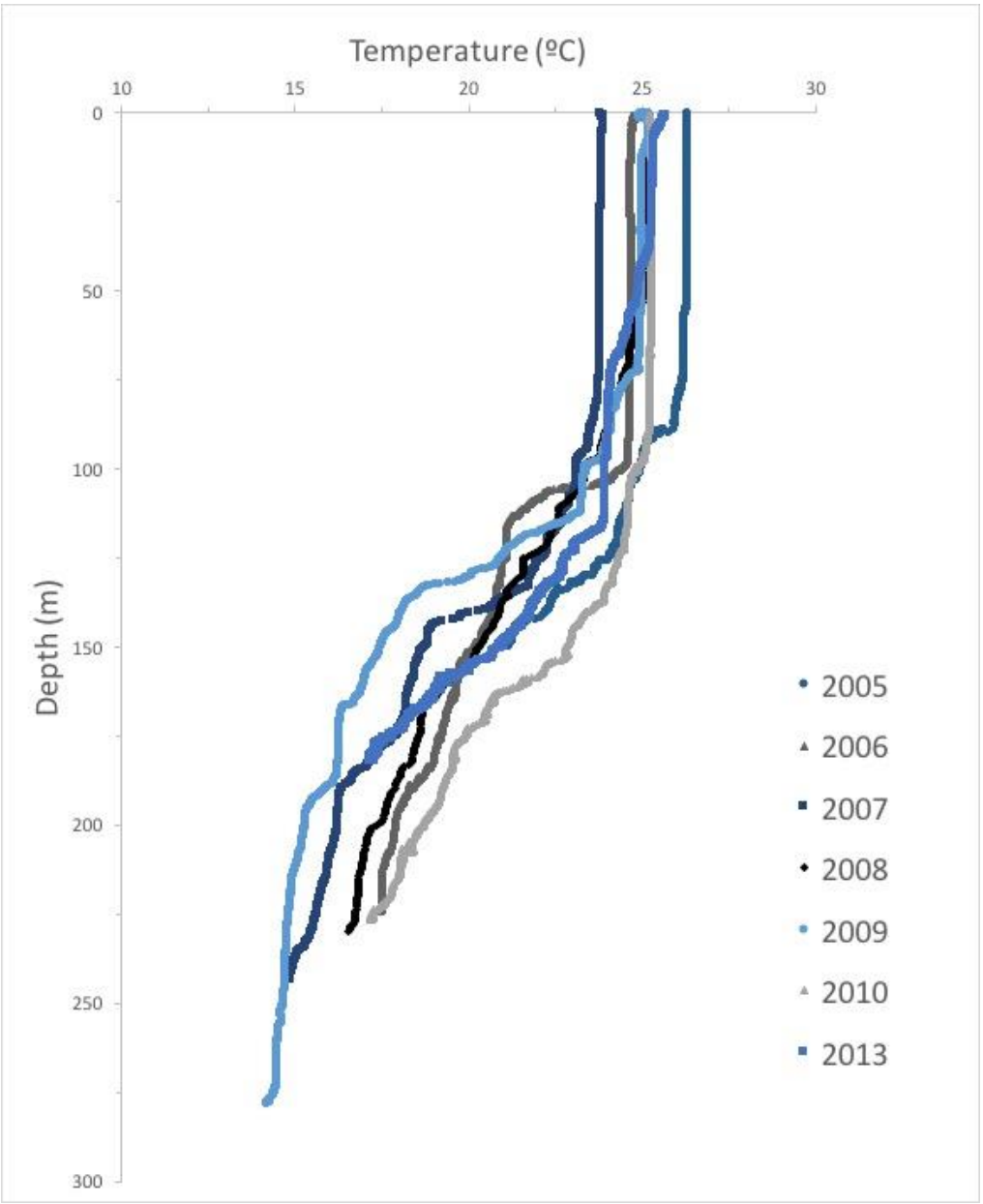

Figure 2-5: Position of the well-mixed layer and thermoclines in deep tropical waters off the shelf-break of the Great Barrier Reef, Australia. The data from 2005 to 2008 are re-drawn from Walther et al. (2013).

Discussion

I found strong differences in fish assemblages with depth with high variability among reefs and sites within reefs. Further, I found distinct assemblages of fishes in mesophotic and sub-mesophotic habitats of the GBR, and these contrasted greatly with those of shallower shelf-habitats (soft bottom 20-90 m; Cappo et al. 2007, Cappo 2010), including those of coral reefs (<30 m; Williams 1982, Alevizon et al. 1985, Russ 1989). There are few comprehensive datasets on tropical deep-reef fishes, however, there is a growing body of comparable work in disparate locations, such as Hawaii, Brazil, Puerto Rico and the Caribbean. This study is the first to characterize the diversity of deep-reef fish assemblages in the GBR. These depth patterns are similar to other deeper marine systems where the fish assemblage shows strong zonation and declining species richness and abundance with the depth gradient (e.g. Thresher & Colin 1986, Olavo et al. 2011, Fitzpatrick et al. 2012, Zintzen et al. 2012, Pearson & Stevens 2015, Pinheiro et al. 2016). Some species show narrower depth ranges, while others are less restricted, and this has important implications for the future management of these resources. For instance, conservation planners can set aside representative areas based on depth to maximize protection of mesophotic reefs and species. Fishery managers can better define optimal targeted fishing depths and designate 'Essential Fish Habitat' based on depth (Rosenberg et al. 2000), such as the designated Bottomfish Restricted Fishing Areas (BRFAs) implemented in the Hawaiian Bottomfish Fishery, for the protection of commercially important deep-reef fishes (Kelley et al. 2006, Moore et al. 2013, Sackett et al. 2014, Moore et al. 2016a, Moore et al. 2016b).

Fisheries are vulnerable to the effects of fishing if there is limited habitat or constrained depth-ranges for target species. Shallow waters have been heavily impacted by fishing (Jennings & Polunin 1996). In the tropics, where the food security of many countries is uncertain, deeper reefs may be next in-line for greater fishing pressure. Many tropical coastlines that have limited shallower fishing areas are targeting deeper fisheries (Crossland & Grandperrin 1980, Fry et al. 2006). This is concerning as deeper environments are thought to be vulnerable (Crossland & Grandperrin 1980, Fry et al. 2006, Cheung et al. 2007) and fish assemblages are poorly described (Hughes & Connell 1999, Bridge et al. 2013), which may compound the problem. In general, deeper fish assemblages are thought to be diverse, valuable and vulnerable (McKinnon et al. 2014). Since many of these species only occur at deeper depths, it is critical to consider these depth zones as distinct. Bycatch is one of the immeasurable impacts of fishing, therefore, it is important to inventory the biodiversity and value we may lose when we target deeper fisheries. High single-species occurrences can indicate the relative rarity of the fish taxa, but this can only be answered with future sampling and greater spatial replication. It is imperative, therefore, to obtain thorough baseline information on deeper tropical ecosystems before these species and habitats are compromised.

Some of the key indicator species per depth strata were commercially important species. Deep Lutjanidae (snappers from the genera *Aphareus*, *Etelis* and *Pristipomoides*), serranids, carangids and sharks are among the 'largely unexplored fauna' of the Townsville area and continental slope, and important for 'regional food futures' (Young et al. 2011). Australia shares fauna with the south-western Pacific islands and the larger Indo-Pacific region (Last et al. 2011b). As human populations increase across Australia and Indo-Pacific islands nations, pressure will be added to fish stocks throughout the region and sustainable fisheries management will increasingly become a major international political issue (Sainsbury et al. 1993, Garcia 1994, Young et al. 2011).

In many Pacific nations, there are long-standing or emerging deep bottomfish fisheries and there is growing concern that these data-limited fisheries are vulnerable to the effects of overfishing (Fry et al. 2006, Williams et al. 2012, Williams et al. 2013). In Hawaii, deep-reef lutjanids, serranids and carangids form the second largest fishery behind the tuna fishery (Moore et al. 2013). For the majority of these fishes, biological information is lacking, but limited life history information demonstrate overall low production (see review Newman et al. 2016). Essential Fish Habitat has been set aside to reduce the impacts from fishing in Hawaiian waters (Kelley et al. 2006) and in other countries where these species are targeted similar precautionary measures should be made.

In Australia, deep-reef fishes are targeted by multiple methods along an extensive tropical coastline spanning Queensland, Western Australia and the Northern Territory. In the Northern Territory and Western Australia, mixed gear is used to target *Pristipomoides* species, primarily *Pristipomoides multidens* (Lloyd et al. 1996, Newman et al. 2000b), however, often multiple species are marketed under the same common name 'Goldband snapper' (Lloyd 2005). In Western Australian waters deepwater demersal trawl gear is also used to target deep-reef fishes (Rodgers et al. 2010). Fishing methods which target >50 species in ~200 m depths unfortunately catch many species as bycatch. In Queensland, while fishing pressure in deeper habitats of the GBR is comparatively lower than in shallow waters, more comprehensive information on deeper habitats will help to extend conservation strategies for the GBR World Heritage Area (Harris et al. 2013, Bridge et al. 2016a) and the adjacent Coral Sea (Young et al. 2011, Bridge et al. 2013) to incorporate deeper habitats.

I found strong patterns of fish abundance with depth, but there was also some variation among reefs that may reflect depth-related patterns of habitat structural complexity (Bridge et al. 2011a, Bridge et al. 2011b, Amado-Filho et al. 2016). Decreases or changes in fish diversity within depth strata may be linked to differences in available habitat similar to shallow water environments (Crowder & Cooper 1982, Friedlander & Parrish 1998, Gratwicke & Speight 2005,

Suthers et al. 2011, Zintzen et al. 2012). Environmental drivers, such as currents and thermal stratification, will affect physical characteristics of the environment (i.e. temperature, sedimentation and food availability), which influence abundance and species diversity (Garrabou et al. 2002). These abiotic factors affect the benthic community (the biotic structures, e.g. hard coral), which combined with the geomorphology, constitutes the habitat available to fishes (Heyns et al. 2016). My results indicated inter-reefal habitats had lower relative species richness than those neighbouring reefs, suggesting the importance of the habitat type on diversity. Habitat quality may also explain some variation in relative species richness and abundance among reefs sampled.

Of the information necessary for conservation strategies, worldwide current species inventories and distributions are incomplete (Schultz et al. 2014). Further, data-poor locations inhibit the ability to monitor and record range extensions and distributional records. Analogous to the tropicalization of temperate waters (Last et al. 2011a, Vergés et al. 2014), shallower species may extend their range and begin to inhabit deeper depths (Munday et al. 2008). There is little information on how thermal tolerances may change fish distributions or behavior, such as changing spawning locations or moving deeper to avoid warm waters (Currey et al. 2015). Distributional records and documented range extensions can be used as a 'canary in a coalmine'; fishes as sentinel species can indicate the relative health of the broader ecosystems.

Shelf-break environments may be priority conservation hotspots, with high proportions of endemics (Kane et al. 2014, Last et al. 2014) or species with restricted depth-ranges (Roberts et al. 2002, Allen 2008). Australia has high total endemism and up to a third of its demersal fishes may be endemic (Last et al. 2011b), therefore, there may also be high endemism in its demersal shelf-break fish assemblages. We may also be underestimating the Australian shelf-break's conservation value, as key bioregions including the upper continental slope of Queensland and the inter-reefal areas of the GBR are missing comprehensive fish assemblage information (Last et al. 2005). As genetic tools are increasing the resolution of cryptic speciation, there are likely differences detected between eastern and western Australian populations, and within species-complexes from neighboring regions (Last et al. 2011b, DiBattista et al. 2018). Even without this information, Last et al. (2005, 2011) concluded that Australia-wide there were strong depth zonation patterns with characteristic and distinct demersal fish assemblages below 40 m. However, there was a 'disjunction' at the shelf-edge between the continental shelf and slope bathomes assemblages (>40 m and <200 m), possibly due to 'edge effects near the shelf break' (Last et al. 2005). I hypothesize that further investigation of shelf-edge habitats will demonstrate high diversity and distinctive communities. Shelf-break habitats should be considered intrinsically unique and a source of unforetold biodiversity and value.

There has been a rapid proliferation of reporting new species and new geographic records from mesophotic regions (e.g. Feitoza et al. 2005, Pyle et al. 2008, Randall & Heemstra 2008, Allen & Erdmann 2009, White 2011, Okamoto & Motomura 2012, Baldwin & Robertson 2013, Baldwin & Johnson 2014, Baldwin & Robertson 2014, Last et al. 2014, Allen & Walsh 2015, Baldwin & Robertson 2015, Baldwin et al. 2016, Tornabene et al. 2016a, Tornabene et al. 2016b). Even though underwater video cannot collect taxonomic samples (Bello et al. 2014, Rocha et al. 2014), it can be a useful method for identifying hotspots for conservation priorities (Allen 2002). There were species I was unable to identify. While these represent a small percentage (<5%) of fish species identified from BRUVS deployments, the observations indicate there are other new species at depths previously unrecorded in the GBR. In this study, fish identifications can be scrutinized as images are listed by CAAB (Codes for Australian Aquatic Biota) codes in the AIMS database for future re-assessments of these identifications.

In conclusion, I found that depth was a strong predictor of fish assemblages at mesophotic and sub-mesophotic depths of the GBR. My findings on the GBR align with other tropical and sub-tropical studies in deeper habitats. Distinct fish assemblages and high species diversity was found along the depth gradient and this potentially contributes to high levels of endemism in Australian fishes and other parts of the world. These narrow depth distributions may constitute an inherent vulnerability to targeted fishing pressures and should be incorporated in future regional management strategies.

Ethics statement

All methods in this study were carried out in accordance with local guidelines and regulations for the GBRMP. Experimental protocols were approved by the animal ethics committee at James Cook University. Methods were non-invasive and no animals were taken in this fieldwork.

Data availability statement

BRUVS deployment information, recorded species and linked images are available by request from the Australian Institute of Marine Science. Map bathymetric contour lines from Dr. Rob Beaman and Project 3DGBR (www.deepreef.org); map shapefiles provided by the Great Barrier Reef Marine Park Authority (http://www.gbrmpa.gov.au/resources-and-publications/spatial-data-information-services).

Chapter 3 Deep-reef fish assemblages of the Great Barrier Reef shelf-break: trophic structure and habitat associations

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A version of this chapter has recently been published in the journal *Diversity*.

Abstract

The ecology of deep-reef habitats along the Great Barrier Reef (GBR) shelf-break has rarely been investigated, as a result, there is little understanding of how associated fishes interact with these environments. Here, I examined the relationship between deep-reef fish assemblages and benthic habitat structure. I sampled 48 sites over a large depth gradient (54-260 m) in the central GBR using Baited Remote Underwater Video Stations and multibeam sonar. Fish assemblages differed both among multiple shelf-break reefs as well as habitats within reefs. While total epibenthic cover decreased with depth, deep-reef benthic communities still included sponges, corals and macroalgae, with macroalgae present to 194 m depths. Structural complexity also decreased with depth, with higher proportions of calcified reef, boulders and bedrock in shallower depths. Deeper sites were flatter and more homogeneous with greater proportions of soft substratum, such as mud and sand. Habitats were highly variable within depth strata, which was reflected in the differences in fish assemblages among sites and among locations. Overall, the trophic groups of the fishes present changed with depth; deeper assemblages included generalist and benthic carnivores, piscivores and planktivores while herbivores were exceptionally rare below 50 m. While depth influenced where trophic groups occurred, site orientation and local habitat morphology determined the species composition of these trophic groups within depths. Future conservation management strategies will need to consider the potential vulnerability of taxa with narrow depth distributions and habitat requirements in these unique shelf-break environments.

Introduction

In coastal oceans, the shelf-break is defined as the point where the continental shelf ends and the continental slope begins. It is characterized by steep increases in depth and associated changes in biotic and abiotic conditions. While tropical shelf-break ecosystems, such as deep reefs (>50 m depth), support a variety of ecologically and economically important fishes, there is a critical lack of information on the links between these fish assemblages, depth, and benthic composition, limiting our ability to effectively assess ecological impacts and manage stocks. While deep-reef fish assemblages include many species endemic to these habitats, they may also provide habitat extensions or 'refuges' for numerous shallow water fishes (Feitoza et al. 2005, Garcia-Sais 2010, Lindfield et al. 2016), and can supporting key ontogenetic stages (Jorgensen et al. 2009, Hoyos-Padilla et al. 2014) or large, highly fecund individuals (Cappo & Kelley 2010). Consequently, deeper habitats can represent critical reservoirs of biodiversity (Kane et al. 2014), helping to maintain fisheries resilience and safeguarding local and global biodiversity (Bejarano et al. 2014).

Despite their potential importance, the majority of deep reefs globally are afforded little or no protection (Heyns et al. 2016) with current management measures either insufficient or non-representative of geographic scope or ecological importance. One partial exception is Australia's Great Barrier Reef (GBR), where deep habitats are afforded some protection due to the comprehensive marine reserve network that includes continental shelf and slope habitats, in addition to the better-known shallow-water coral reefs. Indeed, the GBR marine reserve network was designed using conservation objectives that explicitly accounted for latitudinal and cross-shelf gradients in geophysical and environmental conditions likely to influence spatial patterns of biodiversity, an approach that resulted in reasonable representation of deepwater habitats despite a lack of biological data (Bridge et al. 2016a). Fish stocks within the GBR receive some additional protection from overlapping Queensland State and Commonwealth fishery regulations. Despite reasonable representation of deepwater habitats within the GBR marine reserve network, no information is currently available on finer scale biological or ecological factors that are critical for the management of particular species or ecosystems. For example, there is limited information on the ecology of deep reef ecosystems, the life history traits of associated fishes, and the role of deep habitat as a mediator of fish assemblage structure.

In shallow marine environments (<30 m depths), biotic and abiotic habitat characteristics that influence individual or population fitness impact the distribution and abundance of fish species. For instance, many fishes associate with structurally or biologically complex benthic habitats (Choat & Ayling 1987, Stein et al. 1992, Friedlander & Parrish 1998, Yoklavich et al. 2000, Harborne et al. 2011, Majewski et al. 2017) as these can provide a greater abundance of food resources, shelter, and reproductive opportunities. Increasing complexity can also mediate

important processes such as predator-prey interactions, recruitment, and competition (Heck & Orth 1980, Connell & Jones 1991, Johnson 2006, MacNeil & Connolly 2015), which in turn can promote greater fish assemblage diversity (Messmer et al. 2011). The widespread disturbance of shallow benthic habitats, as a result of climate change and other anthropogenic impacts, has led to decreased habitat complexity and loss of ecosystem function, and has corresponded with local and global declines in fish abundance and diversity (Alvarez-Filip et al. 2009, Graham 2014). While the significance of habitat complexity as a mediator of fish population structure and biodiversity is well documented for shallow reef systems, its role within deep reef ecosystems is poorly documented. However, given the potential economic and ecological value of these systems, and increasing and varied anthropogenic pressure applied to them (Andradi-Brown et al. 2016a, Rocha et al. 2018), understanding the importance of deep reef habitat composition for fish assemblages is critical for effective future management.

Our current understanding of shelf-edge reef fish communities and fish-habitat interactions is generally poor (e.g. Parker & Mays 1998, Kelley et al. 2006, Sink et al. 2006, Fitzpatrick et al. 2012, Starr et al. 2012, Heyns-Veale et al. 2016). Some studies have examined entire fish assemblages associated with deeper reefs, however, a number of potential interactions between habitat characteristics and the associated fish assemblage have been identified. For example, studies of fish assemblages from tropical Indo-Pacific and Atlantic shelf-breaks have reported the partitioning of trophic groups with depth (Thresher & Colin 1986, Brokovich et al. 2008, Garcia-Sais 2010, Fitzpatrick et al. 2012, Starr et al. 2012, Bejarano et al. 2014, Andradi-Brown et al. 2016b, Fukunaga et al. 2016, Kane & Tissot 2017). With increasing depth, abundance of herbivores decreases and abundance of planktivores increases (Kahng et al. 2010). However, the majority of these studies sampled depths <80 m, and the distribution of other groups, such as piscivores, showed no consistent depth-related patterns.

The abundance and composition of benthic fauna, especially habitat-forming species, such as corals, sponges and algae, are the primary drivers of fish assemblage composition (Dennis & Bright 1988). The distribution of these benthic organisms is often highly depth-dependent; for instance, scleractinian corals are generally the most important component of shallower mesophotic communities (Brokovich et al. 2008, Garcia-Sais 2010, Kane & Tissot 2017), while the representation of heterotrophic taxa such as sponges and gorgonians increase with depth and as light decreases (Bridge et al. 2011a, Fitzpatrick et al. 2012). Similarly, other studies have suggested that physical attributes of the underlying benthos that increase habitat complexity, such as overall rugosity or the presence of key elements such as boulders or bedrock, often affects fish abundance (Starr et al. 2012), even in the absence of habitat-forming sessile invertebrates and algae.

Our limited understanding of mesophotic fish-habitat relationships is largely due to the difficulty of studying them, with direct observations traditionally requiring the use of expensive and logistically restrictive equipment such as Remotely Operated Vehicles (ROVs; e.g. Cánovas-Molina et al. 2016, McLean et al. 2017), Autonomous Underwater Vehicles (AUVs; e.g. Williams et al. 2010b) or submarines (e.g. Starr et al. 1996, Tissot et al. 2007). However, Baited Remote Underwater Video Stations (BRUVS) and other single or stereo video systems (e.g. BotCam, stereo-BRUVS, stereo video-lander) are practical, cost-effective alternatives that can be deployed on complex topographies in a variety of habitats (Ellis & DeMartini 1995, Johansson et al. 2008, Merritt et al. 2011, Harvey et al. 2012, Langlois et al. 2012, Hannah & Blume 2014, Whitmarsh et al. 2017). Underwater video can effectively identify both assemblage patterns (species richness and abundance) and whole assemblage composition without depth restrictions, and can increase potential sampling time, replication rate, and sampling area relative to cost. Importantly, BRUVS are less selective or destructive than fishery-dependent methods (Cappo et al. 2007) and, as all deployments are filmed, images can be easily archived for future use. While BRUVS sample representative trophic groups and relative abundance at similar rates to diver-based surveys (Langlois et al. 2010), they can document higher species richness (Watson et al. 2010, Andradi-Brown et al. 2016c) as well as small fishes missed by divers (Andradi-Brown et al. 2016c). Shallower GBR BRUVS studies have identified strong cross-shelf gradients and weak latitudinal patterns, likely due to varying topographical complexity and the distribution of key habitats, as well as depth-related but variable changes to fish assemblages (Cappo 2010). In deeper deployments, baited units have greater sampling efficiency than unbaited units, recording a greater abundance of demersal species and allowing more accurate species identification (Hannah & Blume 2014). While BRUVS have been used extensively on the GBR (e.g. Cappo et al. 2007, Espinoza et al. 2014) they have rarely been deployed below 100 m depths. Deeper deployments have added challenges, including increased pressure at depth, low ambient light for cameras, strong currents, longer deployment and retrieval times, and substantial gear requirements. Since the field-of-view (FOV) of the BRUVS is limited, the parallel use of additional sampling techniques, such as multibeam echo-sounding technology, can rapidly gather complimentary high-resolution information on seafloor characteristics, such as substratum type, relief, rugosity, and complexity (Ierodiaconou et al. 2007) that can help further explain fish assemblage structure over multiple spatial scales.

I previously demonstrated that depth had a great influence on fish assemblages (Chapter 2). Here I predicted that an environmental mosaic of complex habitats would further affect the distribution patterns of fishes. Specifically, I examined how variation in fish assemblage composition related to benthic habitat among and within multiple locations along the GBR shelf-break. I described some reefal and inter-reefal deep habitats and investigated how multivariate

metrics of biotic and abiotic components may be responsible for assemblage patterns that may be masked by depth. I also assessed assemblage patterns of trophic groups and species co-occurrence, which could have important implications for future conservation management strategies of shelf-break habitats.

Methods

Study locations

Submerged shoals along the margin of the GBR support a wide range of ecosystems, largely due to the diverse range of shelf-edge reef morphologies that occur (Bridge et al. 2011b). The central GBR is particularly morphologically distinctive (Fig. 3-1, Hopley et al. 2007). In this region, very few reefs reach sea-level within eight kilometres landward of the shelf-edge, and only one emergent reef is found on the edge itself (Myrmidon Reef, Hopley et al. 2007). The shelf-edge here is characterized by one to three lines of submerged reefs, indicating periods of active development during lower historical sea levels (Harris & Davies 1989). The central GBR shelf-break is located >100 km from shore, a greater distance than in the northern GBR (north of Cairns), but much less than the southern GBR (up to 250 km). Gradients on the upper continental slope in the central GBR are also comparatively low compared to the northern GBR, with a combination of subsidence (Symonds et al. 1983) and sediment input (Puga-Bernabéu et al. 2013) the likely drivers for this morphology. The region commonly experiences nutrient enrichment as the seasonal thermocline of the adjacent Coral Sea shallows (Andrews & Gentien 1982), which in turn transports nutrient-rich waters to the continental shelf (Furnas & Mitchell 1996).

In order to assess variation in habitats along the upper continental slope environment, four distinct shelf-edge locations were targeted using multibeam sonar and BRUVS: Myrmidon Reef, a suite of unnamed shoals 15 km northwest of Myrmidon ('Northern Submerged Shoals'), an inter-reefal transect (Fig. 3-1a), and two submerged shoals 30 km east of Viper Reef ('Viper Reef', Fig. 3-1b). The mesophotic benthic communities of the central GBR are composed of a diverse range of habitat-forming taxa such as hard and soft corals (including Scleractinia, zooxanthellate and azooxanthellate Octocorallia), sponges, seagrasses and algae (Pitcher et al. 2007, Coles et al. 2009). Hard substratum above ~60 m is typically dominated by shallow-water zooxanthellate corals such as *Montipora*, *Porites*, *Seriatopora*, and Xeniidae. However, below 60 m communities are increasingly dominated by azooxanthellate octocorals (Bridge et al. 2011a, Bridge et al. 2011b). Inter-reef habitats between 50-80 m are generally composed of either bare sand or dense fields of calcareous *Halimeda* macroalgae, with this species becoming sparse below 80 m (Pitcher et al. 2007) but present where shelf-edge bathymetry allows nutrient upwelling to occur (Drew 2000). The shelf-edge between 90-140 m includes extensive hard reef substratum formed during lower Pleistocene sea levels that now supports dense forests of gorgonians (Bridge et al. 2011a, Bridge et al. 2012b). Beyond 140 m, this hard reef substratum is less abundant, with a correlated decline in the abundance of octocorals and other habitat-forming species. The one exception may be the eastern side of Myrmidon Reef, where a steep rocky slope extends to depths well below 150 m and continues to support azooxanthellate octocorals (T. Bridge pers. obs. from this study).

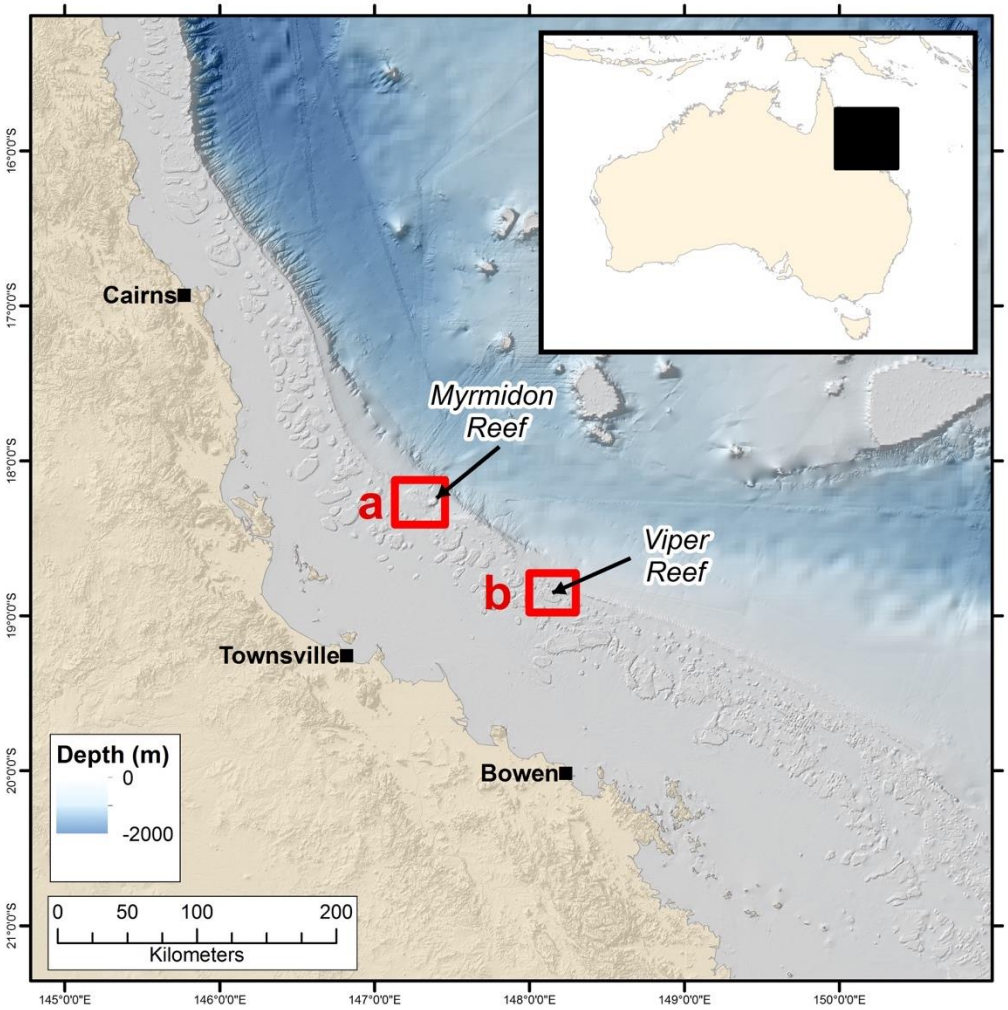


Figure 3-1: Map showing shelf-break areas of the central Great Barrier Reef sampled: a) Myrmidon Reef, Northern Submerged Shoals, an inter-reefal transect and b) Viper Reef. The shelf-break is over 100 km offshore and the adjacent continental slope drops off to depths of hundreds of metres. Map created by J. Daniell in ArcGIS.

Baited Remote Underwater Video Stations (BRUVS)

To sample fish assemblages and habitats *in situ*, 48 single BRUVS deployments were conducted over three research cruises (May, June and September 2014), all during daylight hours (0700-1800). BRUVS were depth-stratified targeting depths of ~100 m, ~150 m and over 200 m to investigate depth gradients (Chapter 2). Since Viper Reef has the shallowest slope environment, some deployments were placed at depths <100 m to ensure similar width of spacing between BRUVS at the other locations. All BRUVS were set at a minimum distance of 200 m between units to minimize the effects of bait plumes and reduce the likelihood of fish being re-sampled (Cappo et al. 2004). BRUVS were deployed at sites between 54-260 m depth, sampling a total of three reefs and one inter-reefal transect (Fig. 3-2).

A high-definition camera (Sony HDR-CX110E) was housed in an aluminium rollbar-frame for protection during deep deployments while also minimizing damage to benthic habitats (Fig. 3-3). The field-of-view of each BRUVS was illuminated by a white spotlight (550 lumen) to overcome diminished light with depth and aid in species identification. Camera focus was set to manual infinity to maximise the FOV. BRUVS were attached to a bridled rope configuration with sufficient rope (8-mm diameter polypropylene; approximately twice the water depth of the deployment because of the strong currents), ballast weights, and a float-flag assembly for retrieval. A plastic mesh bag filled with one kilogram of crushed pilchards (*Sardinops sagax*) was attached to the BRUVS via a flexible plastic conduit as attractant. BRUVS were left to soak for 45 min, but due to the time to reach the bottom, tapes were an average of 54 min (27-84 min). BRUVS units were retrieved from the surface using a hydraulic pot-hauler.

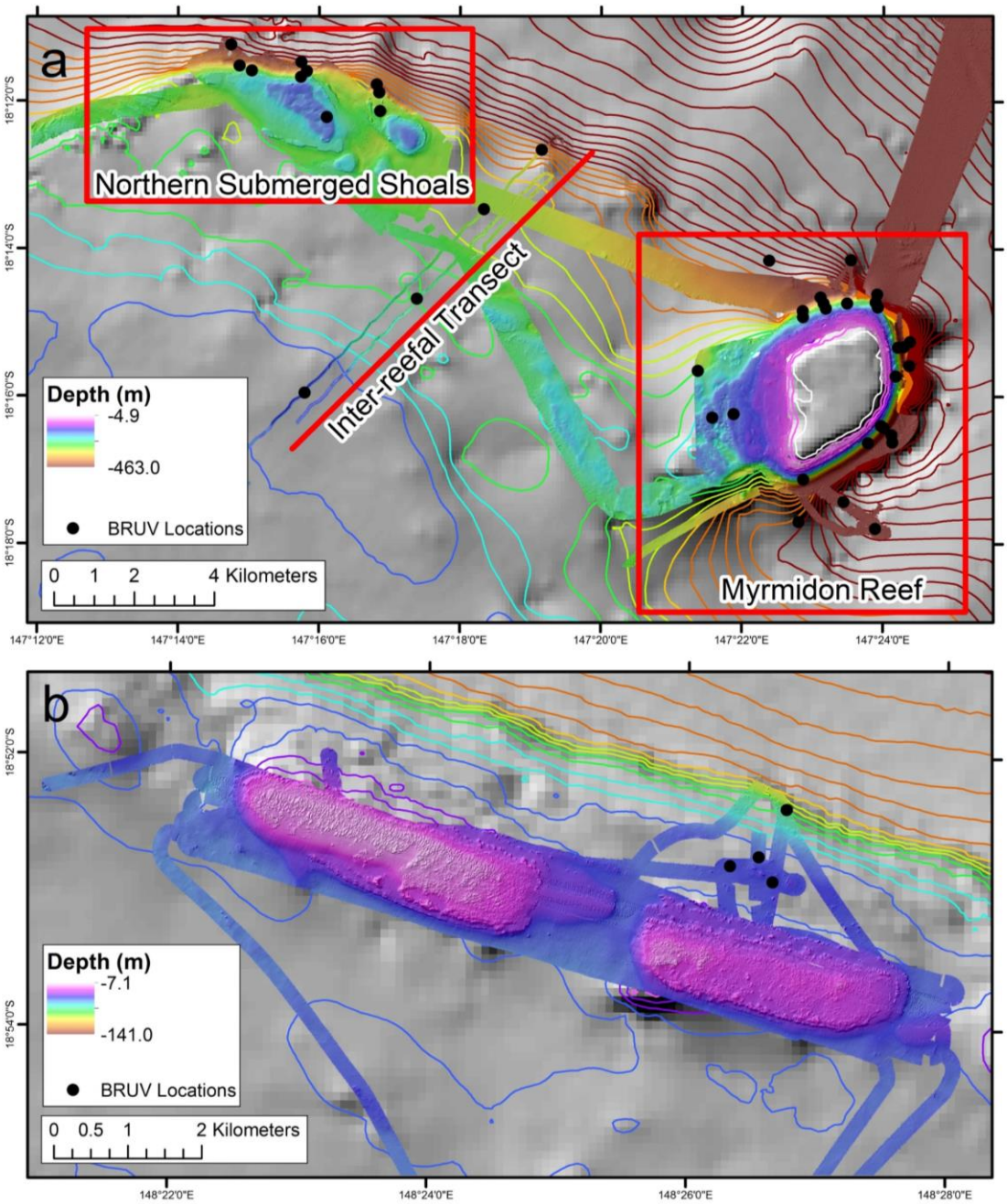


Figure 3-2: Regional and detailed multibeam bathymetry for a) the submerged shoals adjacent to Viper Reef, b) Myrmidon Reef, an inter-reefal transect, and the adjacent Northern Submerged Shoals. Sites of Baited Remote Underwater Video Station deployments are shown as black circles and depth (- metres below the surface from shallower to deeper depths) as a colour gradient (from high to low).

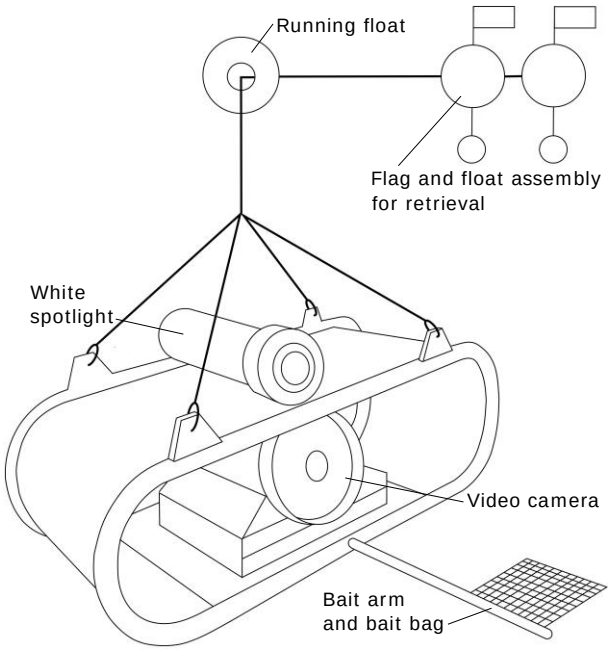


Figure 3-3: Illustration of Baited Remote Underwater Video Station unit for deepwater (<300 m) deployments. A high-definition video camera was in a water-tight housing and an additional white spotlight above the camera aided species identification. Bait arm of plastic mesh filled with ~1 kg of crushed pilchards extended into the camera's field-of-view. At surface-level there was a flag-float assembly for retrieval and a running float was used to keep track of slack line. This figure is a schematic not drawn to scale.

Videos were read to the full length, then standardized for number of fishes per hour, using purpose-built software developed by the Australian Institute of Marine Science (AIMS). Fishes were identified to the lowest possible taxonomic level with the help of multiple ichthyologists via correspondence. Time on the seabed, visibility, time of first appearance of each species, abundance *N* of each species until the time when MaxN was reached (i.e. the greatest number of individuals of a species per frame (Ellis & DeMartini 1995), and time of the end of sampling (i.e. when the video left the bottom or when the video camera stopped recording) were recorded. Video stills of all fish identified were indexed for inclusion in the AIMS reference image library. MaxN is a conservative estimate of abundance and is used to avoid recounting individuals that exit and re-enter the FOV (Cappo et al. 2003) and provides a minimum estimate of true abundance (Schobernd et al. 2013). Species richness and total abundance were summed for each deployment and standardized by effective sampling time to be estimates per hour filmed at the seabed. Individual BRUVS deployments were treated as independent sites and the sites sampled were divided into four locations (Myrmidon Reef, Viper Reef, Northern Submerged Shoals and the inter-reefal transect).

I hypothesized that some components of the epibenthos and substratum would affect the fish assemblage composition. Benthic habitat information at each site was estimated from the FOV. This included identifying major abiotic and biotic habitat characteristics based on a

standardized, tripartite, benthos classification scheme developed for a project that used similar methods to describe patterns in fish and fauna of deeper shoals on the GBR continental shelf with a range of habitat, spatial and temporal variables (Cappo et al. 2012). Substratum categories used were bedrock, boulder, calcareous reef, mud/silt, gravel (2-64 mm), rubble, sand and 'indeterminate' (i.e. where substratum could not be determined reliably due to the angle or visibility of the FOV). Bedform categories included qualitative descriptors such as bioturbated sand, boulder field, sand dunes, sand ripples, rubble field, flat gravel/sand/silt, *Halimeda* beds, high-relief reef, and low-outcrop reef. Benthic community categories included coral, gorgonian and sea-whip garden, low-relief rubble field, macroalgae bed, open sandy seabed, and seagrass bed. In addition, the following benthic community components were also qualitatively summarised in the same way: anemones, bryozoans/encrusting animals, coralline algae, gorgonian fans, forams, *Halimeda*, hard coral, hydroids, macroalgae, seagrass, soft coral, sponges, sea whips, zoanthids and 'none'. Each component was given a percentage score 0-100 in increments of 10. Rarer categories of substratum or epibenthos were pooled with related categories for fewer covariates (Table 3-1).

Multibeam sonar acquisition

Reef architecture can affect the distribution of fishes, and for this reason, I obtained a broader suite of information on the underlying habitat structure of shelf-break environments, with multibeam bathymetry and backscatter layers extracted for a number of neighbourhood characteristics. High-frequency multibeam sonar produces accurate, high-resolution topographic seabed models (Hughes-Clarke et al., 1996). While this technology is in wide use, it has only recently been applied to study shelf-break reefs and fish assemblages on the GBR (e.g. Webster et al. 2008, Beaman et al. 2016). Multibeam information has the potential to characterize fine-scale spatial relationships between deeper habitats and fishes (e.g. Stieglitz 2011). Multibeam sonar echo sounders collect bathymetry and backscatter information over a wide swath of the seafloor (Hughes-Clarke et al. 1996, Brown et al. 2011), with the relative acoustic backscatter, i.e. the 'acoustic reflectivity of the seabed', providing a useful proxy for seabed substratum (Brown et al., 2011). Multibeam sonar surveys using a Reson 8101 were conducted in 2014 onboard James Cook University's *RV James Kirby* (24-25 May) and Australian Institute of Marine Science's *RV Cape Ferguson* (03-09 Sept). Multibeam mapping in water depths of 10-250 m was conducted at a speed of 5-6 knots. The Reson 8101 emits 101 acoustic beams of 1.5º x 1.5º, covering an angular sector of up to 150º for a total swath (approximately seven times the water depth). A Kongsberg Seatex motion reference unit corrected for pitch, roll, and heave. A Fugro OmniSTAR 9200-XP differential GPS recorded positioning, with a quoted accuracy of 1.0 m RMS in the X and Y plane. Data from all peripheral sensors were recorded using QPS QINSy acquisition software. A Sontek CastAway CTD system corrected the acoustic sound

velocity profile. Predicted tides generated from XTide software (Flater 2005) corrected the bathymetric data by tidal datum over the survey period. Raw multibeam data files were converted to Extended Triton Format (XTF) and imported to Caris HIPS/SIPS post-processing software. All multibeam data post-processing included noise-editing, tide and sound velocity profile corrections. Bathymetry data were visually-inspected and data spikes removed to create a level and clean dataset relative to mean sea level. The error of estimation for vertical soundings reported is estimated to be a maximum of ± 0.2 m. The final digital elevation models were produced using Caris HIPS/SIPS software with a 5-m cell size.

Secondary datasets from multibeam

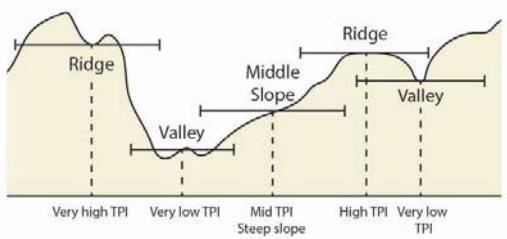
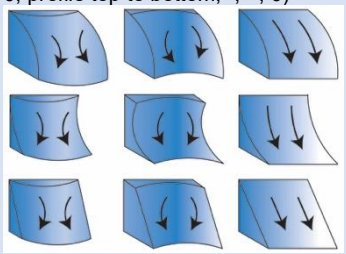
Multibeam sonar datasets provide measures of both seabed structure through bathymetry and seabed composition with acoustic backscatter (Hughes-Clarke et al. 1996). To improve the predictive power of the multibeam sonar datasets, a variety of secondary datasets, potentially correlating with seafloor properties, were produced from the raw bathymetry and backscatter data using neighbourhood-based statistics and terrain analysis techniques (Wilson et al. 2007, Brown et al. 2011). Neighbourhood operations produce an output raster dataset in which each cell location is a function of the input value at a cell location and the values of the cells in a specified kernel (i.e. neighbourhood) around that location. The configuration (size and shape) of the kernel determines which cells surrounding the input cell should be calculated in the output value. The most typical kernel size is 3 x 3 cells (i.e. a radius of 1 grid cell), which incorporates the processing cell and its closest eight neighbours.

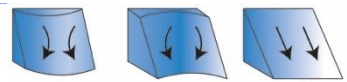
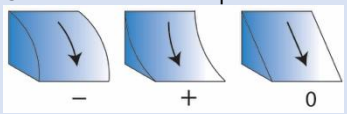
As multi-scale terrain analysis is predicted to be the most efficient method for characterizing features at multiple spatial scales (Fisher et al. 2004, Wilson et al. 2007, Heyward et al. 2011). A suite of derivative datasets that accounted for both high- and low-frequency variations in the multibeam data, and variations in the kernel (neighbourhood size), were included in the analyses. All derivatives of the bathymetry and backscatter were chosen because they have a potential influence on fish assemblage ecology (Table 3-1) and are commonly used within marine habitat and seabed characterisation (see Diesing et al. 2016) for a review and Appendix Fig. A1 for demonstrative examples of backscatter and bathymetry derivatives used in this study). Progressively lower frequency neighbourhood analyses were applied to the multibeam bathymetry and backscatter to investigate multiple spatial scales in two ways. Some neighbourhood functions (Easting, Northing, Slope, Topographic Position Index, Topographic Ruggedness Index, Surface Ratio, Total Curvature, Planar Curvature, and Profile Curvature) are used to quantify the ‘shape’ of the kernel, as a result, they are calculated from the surrounding eight pixels (a 3 x 3 kernel) and were applied to the bathymetry raster only. Therefore, to achieve progressively ‘lower frequency’ derivatives of these metrics, the bathymetry rasters were low-

pass filtered (5 times) using a 11 x 11 kernel-averaging filter. Each time the averaging low-pass filter was applied, the nine neighbourhood functions were then calculated to create derivative raster datasets at that resolution (designated '**' in Table 3-1). Neighbourhood functions that could be applied to larger kernel sizes were applied to both the bathymetry and backscatter grids using kernels with radius values of 1, 5, 10, 25, and 50 pixels (Range, Standard Deviation of Bathymetry, Average Backscatter and Standard Deviation of Backscatter, and these multiple spatial scales were designated with '***' in Table 3-1). Backscatter information can be interpreted as qualities of the substratum (i.e. 'hard' or 'soft'). Raster calculations were undertaken using the R software (R Core Development Team 2018) and the Raster package (Hijmans & van Etten 2011). Additional subroutines were written for Curvature measurement based on Zevenbergen and Thorne (1987).

Table 3-1: Explanatory covariates from multibeam echo sounding technology and estimates from the Baited Remote Underwater Video Station field-of-view (FOV). Some epibenthic and substratum categories were pooled for combined groups of benthos. Primary and secondary (derived) features from bathymetry and backscatter raster datasets. *Raw raster data. **Applied as a 3 x 3 kernel on bathymetry after it was averaged using kernels with a radius of 1, 5, 10, 25, 50 pixels. ***Applied kernels with a radius of 1, 5, 10, 25, 50 pixels. References where these multibeam derivatives are described are in parentheses. Example references where these factors have been highly influential on fish or benthic assemblages are noted in italics.

Covariate name (abbreviation)	Covariate type		Definition	Reference
Bedrock (bdrck)	*% composition of seafloor by substratum categories*		FOV estimated % Bedrock	
Boulder (bldr)	*% composition of seafloor by substratum categories*		FOV estimated % Boulder	*Moore et al. 2009*
Calcified reef (calc.rf)	*% composition of seafloor by substratum categories*		FOV estimated % Calcareous reef	*Moore et al. 2009*
Gravel (grvl)	*% composition of seafloor by substratum categories*		FOV estimated % Gravel (2-64mm)	*Haywood et al. 2008* *Holmes et al. 2008* *Malcolm et al. 2016*
Indeterminate (ind)	*% composition of seafloor by substratum categories*		FOV estimated % Indeterminate	
Mud (mud)	*% composition of seafloor by substratum categories*		FOV estimated % Mud/silt	*Haywood et al. 2008*
Rubble (rbbl)	*% composition of seafloor by substratum categories*		FOV estimated % Rubble	
Sand (snd)	*% composition of seafloor by substratum categories*		FOV estimated % Sand	*Malcolm et al. 2016* *Kane & Tissot 2017*
Filter feeders (fltrs)	*% composition of seafloor by epibenthic categories*		% combined Fans, Hydroids, Sponges, Whips	*Holmes et al. 2008*
Encrusting organisms (encr)	*% composition of seafloor by epibenthic categories*		FOV estimated % combined Bryozoans/encrusting animals, coralline algae	
Coral (crl)	*% composition of seafloor by epibenthic categories*		FOV estimated % combined Hard coral and Soft coral	*Garcia-Sais 2010* *Kane & Tissot 2017*
Bare (bare)	*% composition of seafloor by epibenthic categories*		FOV estimated % no epibenthic cover	
Plants (plants)	*% composition of seafloor by epibenthic categories*		FOV estimated % combined Macroalgae and Seagrass	*Holmes et al. 2008*
***Halimeda* (hal)**	*% composition of seafloor by epibenthic categories*		FOV estimated % *Halimeda*	
Name	**Source**	**Description**	**Possible ecological context**	**Reference**
Depth* (m)	Vessel depth sounder	Depth below sea-level	Location relative to Photic Zone Potential impact by waves and storms Location relative to thermoclines/haloclines	*Costa et al. 2014* *Oyafuso et al. 2017* *Kane & Tissot* *Moore et al. 2009* *Moore et al. 2011*
Easting**	Bathymetry derivative	Easterly component of the kernel azimuth	Level of exposure or protection from oceanographic processes	(Hirzel et al. 2002)
Northing**	Bathymetry derivative	Northerly component of the kernel azimuth	Level of exposure or protection from oceanographic processes	(Hirzel et al. 2002)
Slope (Degree)**	Bathymetry derivative	Change in elevation as a function of distance within the kernel	Indicate activity of gravity driven processes Indication of hard substratum	(Dartnell & Gardner 2004) *Misa et al. 2013* *Moore et al. 2009*

Topographic Position Index (TPI)**	Bathymetry derivative	Difference between centre kernel value and the average of all kernel values. *Example of TPI interpretation as defined in Weiss 2001 (SD is standard deviation of bathymetry):* *Ridge: z_0 > SD* *Upper slope: SD ≥ z_0 > 0.5 SD* *Middle slope: 0.5 SD ≥ z_0 ≥ -0.5 SD, slope > 5°* *Flat area: 0.5 SD ≥ z_0 ≥ -0.5 SD, slope ≤ 5°* *Lower slope: -0.5 SD > z_0 > -SD* *Valley: z_0 < -SD*	Relative topographic position in the neighbourhood: Positive TPI values are higher than their surroundings (i.e. ridges) and negative TPI values are lower than their surroundings (i.e. valleys). TPI values near zero are flat areas.  (re-drawn from (Jenness et al. 2011)	(Weiss 2001) *Iampietro et al. 2005* *Moore et al. 2009*
Terrain Ruggedness Index**	Bathymetry derivative	Average of the absolute difference between the centre kernel values and each of the other kernel values	Index of surface roughness indicating degree of structure complexity	(Riley et al. 1999)
Range***	Bathymetry derivative	Difference between the maximum and minimum values within the kernel	Index of surface roughness indicating degree of structure complexity	(Dartnell 2000) *Yates et al. 2016,* *Moore et al. 2009* *Holmes et al. 2008*
Surface Ratio**	Bathymetry derivative	Ratio of the kernel surface area and planimetric area	Relative vertical relief indicating degree of structure complexity	(Jenness 2004) *Moore et al. 2011*
Standard Deviation* (m)**	Bathymetry derivative	Standard deviation of values within the kernel	Index of surface roughness	(Costa et al. 2014)
Curvature (Degrees/m)**	Bathymetry derivative	Index of concavity/convexity measured within the kernel	Measure of overall curvature within kernel (planform left to right + -, 0; profile top to bottom, -, +, 0)  (re-drawn from "Curvature type" ArcGIS help files)	(Zevenbergen & Thorne 1987)
Planar Curvature (Degrees/m)**	Bathymetry derivative	Index of concavity/convexity measured perpendicular to slope within the kernel	Identifies ridges, valleys, and flat slopes	(Zevenbergen & Thorne 1987)

			 (re-drawn from "Curvature type" ArcGIS help files)	
Profile Curvature (Degrees/m)**	Bathymetry derivative	Index of concavity/convexity measured parallel to slope within the kernel	Concave or convex slopes  (re-drawn from "Curvature type" ArcGIS help files)	(Zevenbergen & Thorne 1987) *Moore et al. 2009*
Acoustic Backscatter* (Decibels)	Backscatter derivative	Acoustic backscatter	Proxy for seabed substratum	(Hughes-Clarke et al. 1996)
Ave Backscatter* (Decibels)**	Backscatter derivative	Average backscatter within the kernel	Proxy for seabed substratum	(Brown et al. 2011)
StdDev Backscatter* (Decibels)**	Backscatter derivative	Standard deviation of values within the kernel	Variation in substratum within kernel	(Brown et al. 2011)

Data Analysis

Habitats and fish assemblages separated by depth

Depth had a great influence on fish assemblage patterns; however, as numerous ecological factors vary with depth this can obscure the underlying drivers of fish distributions, including the influence of fish-habitat interactions (Chapter 2). Therefore, I investigated habitat differences within and among depth strata. I analysed patterns of fish and environmental covariates using non-metric multidimensional scaling (nMDS) and fitting environmental correlates on the ordination package 'vegan' (Oksanen 2015) in R. Fish abundance data was divided into 'Shallow' (54-115 m, n=18 sites), 'Middle' (128-160 m, n=14 sites) and 'Deep' (179-260 m, n=12 sites with no missing values) sites and fish species only occurring at one site were removed from the dataset, leaving 72 species. Sites (i.e. BRUVS deployments) were eliminated from the analyses if there were missing habitat values (some multibeam values were 'missing' if the kernels extended beyond the region where multibeam information was collected, which was more frequent at the deepest sites). One site was removed because it did not contain any of the remaining 72 species. Separating sites into three nMDS investigated the differences in habitats with the maximum separation between depth categories.

Ordination by nMDS separated the sites based on assemblage dissimilarities in relative abundances and composition. Separate nMDS identified what species and habitat variables contributed to similarities among locations (function metaMDS, k=2). Non-metric MDS is a flexible and robust ordination method for visualising patterns that preserves the ranks of dissimilarities in species abundance data. Relative abundances were transformed with a fourth-root to reduce the influence of highly abundant fishes, then scaled using a Wisconsin double-standardization with Hellinger method where species are standardized by the maxima and sites by the site total. Hellinger accounts for relative rarity and the 'horseshoe effect' where sites are considered more similar by what species are absent from those sites. Species abundance data was then incorporated into a Bray-Curtis resemblance matrix.

To see what environmental covariates were meaningful for distinguishing sites, correlating covariates were fitted as vectors overlaying the plotted sites if they were above the $p < 0.05$ significance level (function envfit, Pearson correlations with 999 permutations). This function estimated the strength of the correlation as well as the direction of the correlation among sites. Multibeam information and FOV information was first evaluated for variables that were highly correlated (>0.8) and those variables were removed. The absolute values of multibeam data were log(x+1)-transformed; FOV epibenthic/substratum measurements were arcsine-transformed. Environmental variables were scaled and converted into a Euclidean distance-based matrix.

I also investigated assemblage differences among deep reefs using similarity percentages (SIMPER, Primer v7), which estimated the contributions of fish species to the differences in assemblage composition variability between locations within depth strata. SIMPER analysis used presence/absence-transformed assemblage fish data, using a Bray-Curtis resemblance matrix with 70% as the cut-off level for low contributions.

Species-species associations

The occurrence and abundance of fishes may be explained by co-existence or competition with other species in the assemblage, thus I investigated between-species correlations. I plotted significant Pearson correlations ($p < 0.05$) for all the possible pairs of the 28 most frequently-occurring species using the packages 'corrplot' (Wei & Simko 2017) and 'Hmisc' (function rcorr, Harrell 2017). This subset of 28 species included the relative abundances of fishes observed at five or more of the total 48 sites. Significant negative correlations could indicate potentially competing species and significant positive correlations could indicate species co-existing in a similar ecological system.

Trophic assemblages

I hypothesized that fishes would have different levels of habitat association and that these levels were likely due to differences in ecological niche (i.e. what they eat). The degree of habitat specialization between fishes can even be different between closely related species (Wilson et al. 2008, Heupel et al. 2010b). An analysis was conducted to determine differences in the trophic assemblage (diversity of feeding groups) between deep-reef habitats. Each species was designated a trophic group based on diet or trophic ecology information according to Fishbase (herbivore, piscivore, planktivore, general carnivore, benthic carnivore, or unknown). The number of total species per trophic group (presence/absence) per site was summed as a measure of relative trophic richness. Some species' diets could be inferred to most likely category based on closely-related species (e.g. *Gymnothorax* species tend to be carnivores) but where there were different trophic niches within a family, these species were left as unknown.

Sites were plotted along the two primary axes (PC1 and PC2) accounting for most of the variation in trophic richness using a Principal Component Analysis (PCA) on Wisconsin-standardized trophic group richness. Wisconsin double-standardization first transformed data by 'species' maxima and then by 'site' totals for a more uniform comparison and common scale among sites with very different numbers of members, reducing the contribution of abundant taxa (Bray & Curtis 1957) and improving the gradient detection capability when comparing dissimilarities (Oksanen). Sites were grouped according to depth category and individual habitat measures were correlated to the variance explained in PC1 and PC2. I presumed broad trophic differences would be operating on larger spatial scales, so the multibeam measurements from the

50 x 50 kernel (i.e. largest sampling window). This approach compared each single predictor to the combined assemblage response of the principal component. This comparison reduced dimensionality, increasing the ability to identify how much assemblages respond directly to gradients in the environmental factors (Guyon & Elisseeff 2003). This method determined which habitat variables are most important in explaining the variation among sites.

Results

Description of deep-reef benthic shelf-break habitats

Epibenthic cover and substratum type varied with depth (54-260 m deep, Fig. 3-4 and 3-5). The mean abundance cover of macroalgae decreased from 27% at the shallowest sites (54-107 m), to 13% at middle sites (110-156 m), to 5% at the deepest sites (160-260 m; Fig. 3-5). *Halimeda* (kept as a separate category for analyses) was also most prominent in shallower sites (10% mean abundance cover) and was found to a maximum depth of 150 m. Soft corals were seen down to 155 m. Sponges had greatest representation in the Middle sites (4-16% average cover). The encrusting community (coralline algae and bryozoans) was most abundant at Shallow sites (~22% mean abundance cover) decreased with increasing depth. Overall, the average percent cover of the total epibenthic community decreased from Shallow (72%), Middle (43%) to Deep sites (11%), with deeper sites having noticeably more 'bare' coverage (89%). Structural complexity also decreased with greater depth, largely due to the declining abundance of calcified reef (mean 45%, 54-107 m; 8%, 160-260 m). However, other hard substratum categories, such as bedrock and boulder, had limited but relatively consistent average abundance cover (1-4%). Rubble and sand became more common with increasing depth, while mud only appeared in the middle and deeper sites.

There was some notable habitat variation among locations surveyed and also at the level of sites within locations (e.g. Fig. 3-4). Overall, epibenthic composition was more similar between Myrmidon Reef and Northern Submerged Shoals than Viper Reef (Appendix Fig. A2). While coral was observed at shallow Viper Reef sites, it was absent from other locations (Viper included some shallower sampling depths). In addition, while the abundance of sponges was consistent between Myrmidon Reef and Northern Submerged Shoals, they were absent from Viper Reef. Interestingly, macroalgae was abundant at deeper sites of the Northern Submerged Shoals, occurring at three of the four sampling sites and down to 194 m. There were no major differences in substratum by location (Appendix Fig. A3), but what was visible in the FOV were coarse qualifications of substratum. The number of replicate sites per reef and depth varied (e.g. for inter-reefal sites there was only one site per depth category), and therefore, due to low replication at some locations (these results were not analysed by parametric tests by location).

Investigating habitats and fish assemblages within depth strata

There was great variation in species composition both among locations and sites nested within locations. The differences among locations were greatest at shallow depths, but there was still overlap between sites among locations (Fig. 3-6). Of the environmental variables responsible for differences among sites, only a few were significant by depth strata. Slope and the presence of filter-feeding organisms among Shallow sites were significant ($p < 0.05$), while Middle sites

had the significant separation based on longitude, latitude and the proportion of sand. The presence of boulder substratum differentiated among sites at Deep sites.

Variation within depth strata show some overall patterns between fish assemblages by location (Appendix Fig. A4-A6). Many species are shared among multiple locations, such as *Lethrinus rubrioperculatus*, *Aprion virescens*, *Gymnocranius euanus*, and *Carcharhinus albimarginatus*, indicated by the close clustering of species towards the middle of the ordination (Appendix Fig. A4). Among Middle sites, the species composition at Northern Submerged Shoals overlapped with Myrmidon sites, and Viper was most different in species composition (Appendix Fig. A5). For the within-location similarity between sites, SIMPER analysis showed the species that contributed to each location's assemblage were varied and there were also high levels of unexplained variation within depth strata among locations (Table 3-2). The species showing greater similarities within a location were often representatives of the Lutjanidae, Lethrinidae, Carcharhinidae and Carangidae families. At Shallow sites, locations sampled were dissimilar in species assemblages because of high species diversity, with the greatest dissimilarities between the inter-reefal transect and the other reefs sampled. Among sites at middle depths, Myrmidon and Northern Submerged Shoals were most similar.

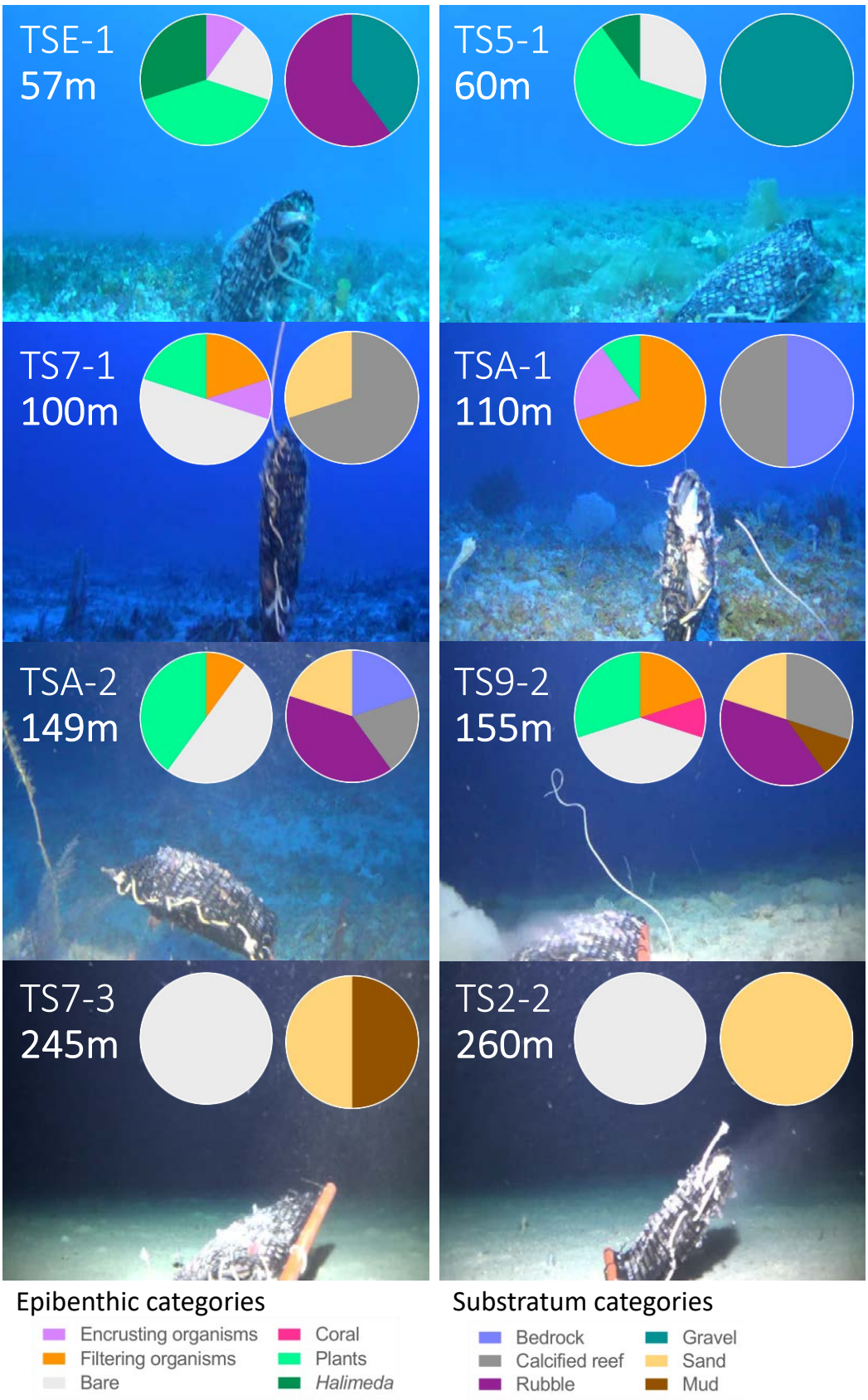


Figure 3-4: Examples of deep-reef habitats from the field-of-view of Baited Remote Underwater Video Stations. The bait arm extension is visible in the video frame. A unique BRUVS operation code (TS_ removed observer bias) and depth are noted for each site with the relative proportion of epibenthic and substratum categories.

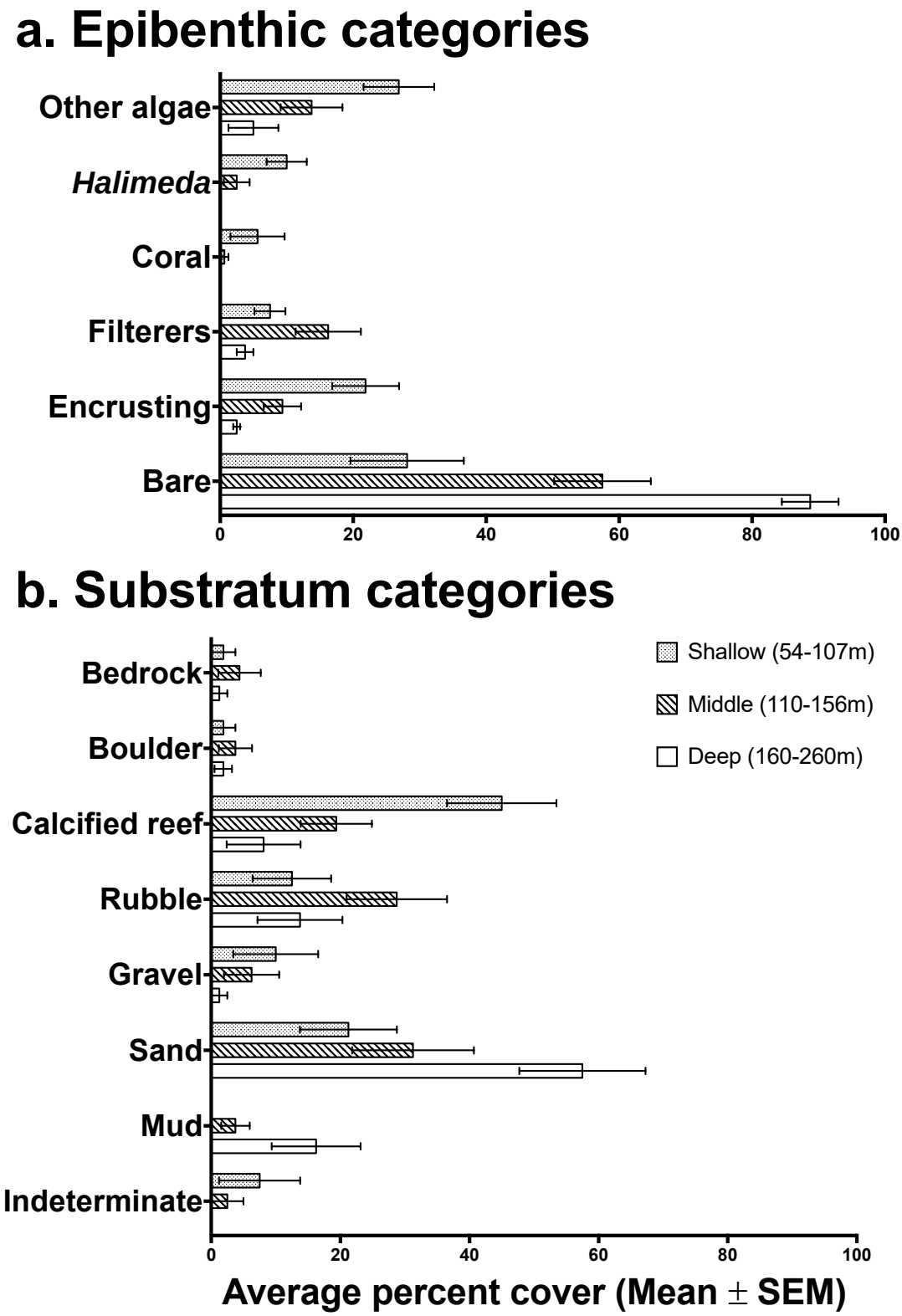


Figure 3-5: Deep-reef habitats varied by depth, measured by epibenthic and substratum cover in the field-of-view of the camera. Sites were divided into three depth strata: Shallow (54-107 m), Middle (110-156 m), and Deep (160-260 m) represented by three sequentially stacked bars (each n = 16 sites).

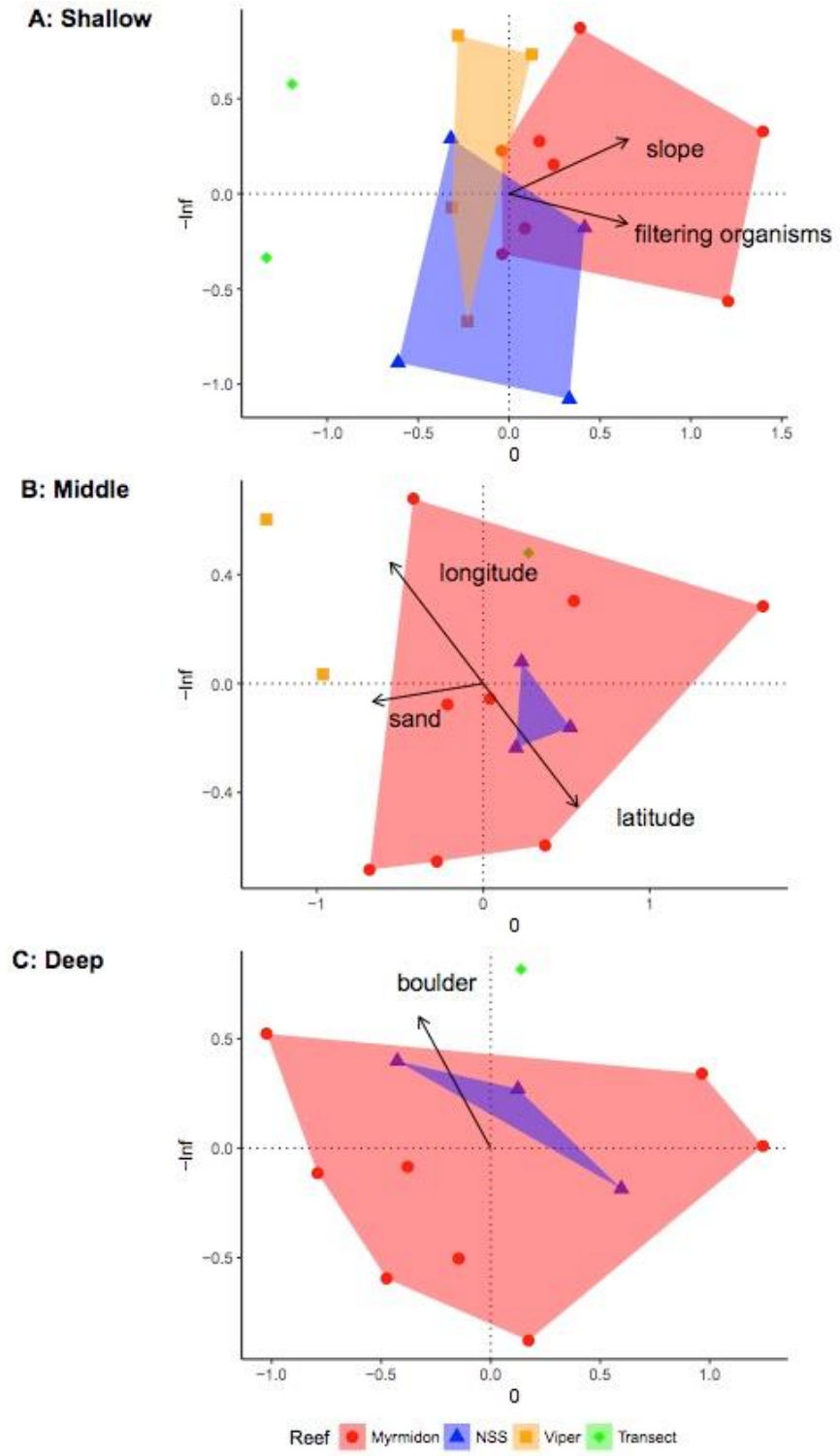


Figure 3-6: Nonmetric Multidimensional Scaling (nMDS) showed patterns between fish assemblage composition and environmental variables, including epibenthic and substratum measured in the underwater camera field-of-view and multibeam echo sounder measured variables. Sites were separated into shallow (54-115 m, nMDS non-metric fit, $R^2 = 0.967$, linear fit, $R^2 = 0.827$, stress = 0.21, top), middle (128-160 m, nMDS non-metric fit, $R^2 = 0981$, linear fit, $R^2 = 0.913$, stress = 0.15, middle), and deep (179-260 m, nMDS non-metric fit, $R^2 = 0.989$, linear fit, $R^2 = 0.924$, stress = 0.15, bottom) based of depth. Ordination from Bray-Curtis dissimilarities in species abundance data, transformed using fourth-root transformation and standardized using Wisconsin-double standardization. Coloured hulls show the affiliation of each site to a location. Environmental variables that are significant within these depth strata are depicted as vectors on the nMDS ordination ($p < 0.05$, 999 permutations).

Table 3-2: Similarity percentages (SIMPER) analysis on deep-reef fish assemblage data described the relative contributions of specific species to the dissimilarities between sites (among locations) with percent contribution of individual species to those differences. Species abundances were presence/absence-transformed, and Bray-Curtis similarity measures used. Species contributing to ~70% combined are listed.

Location	Myrmidon Reef	Northern Submerged Shoals	Viper Reef	Inter-reefal Transect
Shallow (54 – 115 m)	***n* sites= 8** **Average similarity: 28.0%** **Individual species contributions:** *Carangoides caeruleopinnatus*, (15.3%) *Lutjanus bohar* (13.6%) *Carcharhinus amblyrhynchos* (9.9%) *Aphareus rutilans* (8.9%) *Gymnocranius euanus* (8.9%) *Cirrhilabrus roseafascia* (6.0%) *Pristipomoides filamentosus* (5.3%) *Lethrinus miniatus* (5.0%).	***n* sites= 4** **Average similarity: 15.9%** **Individual species contributions:** *Carangoides caeruleopinnatus* (21.7%) *Gymnocranius grandoculis* (13.1%) *Carcharhinus albimarginatus* (10.0%) *Lethrinus rubrioperculatus* (9.1%) *Carcharhinus amblyrhynchos* (7.2%) *Pomacanthus imperator* (7.2%) *Plectropomus leopardus* (7.2%)	***n* sites= 4** **Average similarity: 25.6%** **Individual species contributions:** *Carangoides dinema* (23.6%) *Echeneis naucrates* (11.4%) *Lethrinus olivaceus* (9.5%) *Aphareus rutilans* (4.7%) *Carcharhinus albimarginatus* (4.7%) *Carangoides fulvoguttatus* (4.7%) *Lutjanus bohar* (4.7%) *Parapercis* sp. (4.7%) *Epinephelus cyanopodus* (4.7%)	***n* sites= 2** **Individual species contributions:** All similarities are zero
Middle (128 – 160 m)	***n* sites= 8** **Average similarity: 29.5%** **Individual species contributions:** *Aphareus rutilans* (31.2%) *Pristipomoides typus* (14.3%) *Pristipomoides filamentosus* (13.1%) *Parapercis nebulosa* (10.3%) *Pristipomoides multidens* (9.4%)	***n* sites= 3** **Average similarity: 58.3%** **Individual species contributions:** *Bodianus* sp. (10.4%) *Wattsia mossambica* (10.4%) *Aphareus rutilans* (10.4%) *Pristipomoides filamentosus* (10.4%) *Pristipomoides multidens* (10.4%) *Pristipomoides typus* (10.4%) *Gymnosarda unicolor* (10.4%)	***n* sites= 2** **Average similarity: 28.57** **Individual species contributions:** *Carcharhinus albimarginatus* (100%)	***n* sites= 1**
Deep (179 – 260 m)	***n* sites= 8** **Average similarity: 17.0%** **Individual species contributions:** *Pristipomoides argyrogrammicus* (39.0%) *Pristipomoides multidens* (31.2%)	***n* sites= 3** **Average similarity: 31.7%** **Individual species contributions:** *Gymnosarda unicolor* (48.9%) *Seriola dumerili* (13.2%) *Pristipomoides argyrogrammicus* (13.2%)	***n* sites= 0**	***n* sites= 1**

Relationships among fish species

The distribution of fishes among habitats may be both positively and negatively influenced by inter-species interactions. Of the 28 species present at five or more sites, many correlated species were identified (Fig. 3-7 and 3-8, correlation values with a significance of $p < 0.05$). *L. bohar* abundance was highly correlated with the abundance of *L. ravus* (0.71) and *L. olivaceus* (0.67), and weakly correlated to *Parapercis* sp. (0.50, Family Pinguipedidae). Deeper reefs often had mixed groups of lethrinid species: *L. olivaceus* was often found with *L. ravus* (0.59) and *L. miniatus* (0.57); *L. miniatus* was associated with *L. rubrioperculatus* (0.68); *G. euanus* was often frequented seen with species *L. rubrioperculatus* (0.55) and *L. miniatus* (0.60). Lethrinid and other family co-occurrences were common: *L. rubrioperculatus* and *C. caeruleopinnatus* (0.77); *G. euanus* with *C. caeruleopinnatus* (0.58) or the grey reef shark, *C. amblyrhynchos* (0.62), which also was frequently seen with *L. rubrioperculatus* (0.54) and *L. miniatus* (0.57). The silvertip shark, *C. albimarginatus,* was often seen with an attached sharksucker, *E. naucrates* (0.57). The deep-reef serranid *Epinephelus morrhua* and *P. typus* were frequently observed at the same sites (0.67). *W. mossambica* was weakly correlated in abundance to deepwater lutjanids *P. typus* (0.51) and *P. filamentosus* (0.57), as well as *E. morrhua* (0.67), and *G. unicolor* (0.54, Scombridae). Deep reefs commonly featured *Parapercis* species; *P. nebulosa* and the labrid, *Terelabrus rubrovittatus,* were often seen on the same videos, and *Parapercis* sp. abundance was weakly correlated with *L. ravus* abundance (0.51). *T. rubrovittatus* was also frequently seen with an unknown *Selenanthias* sp. (a potential new species for the GBR, 0.59).

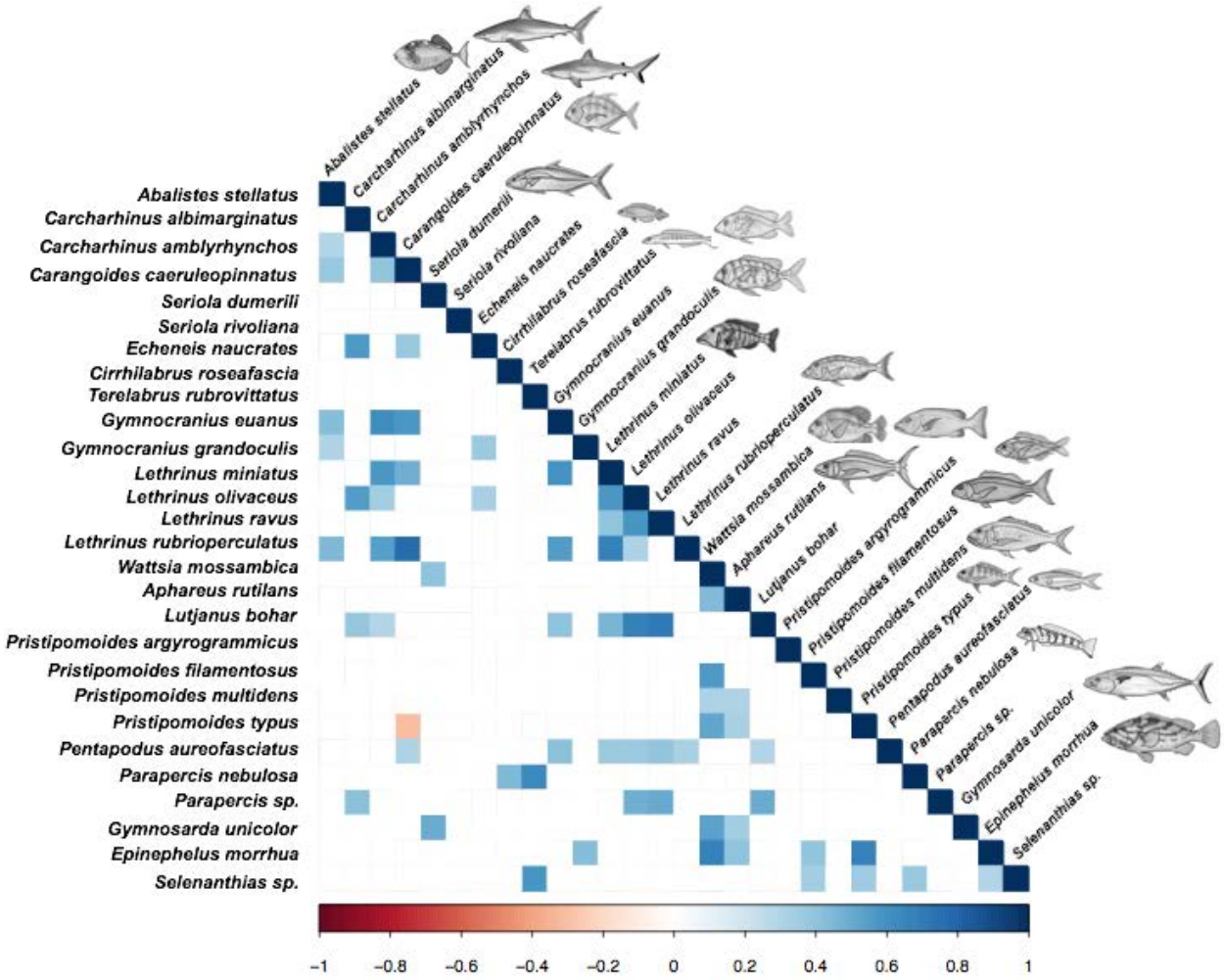


Figure 3-7: Species correlations of most frequently occurring 28 fish species from Baited Remote Underwater Video Station deployments on shelf-break reefs. Positive Pearson correlation values are depicted in blue and negative correlations in red (only significant correlations where $p < 0.05$ are depicted).

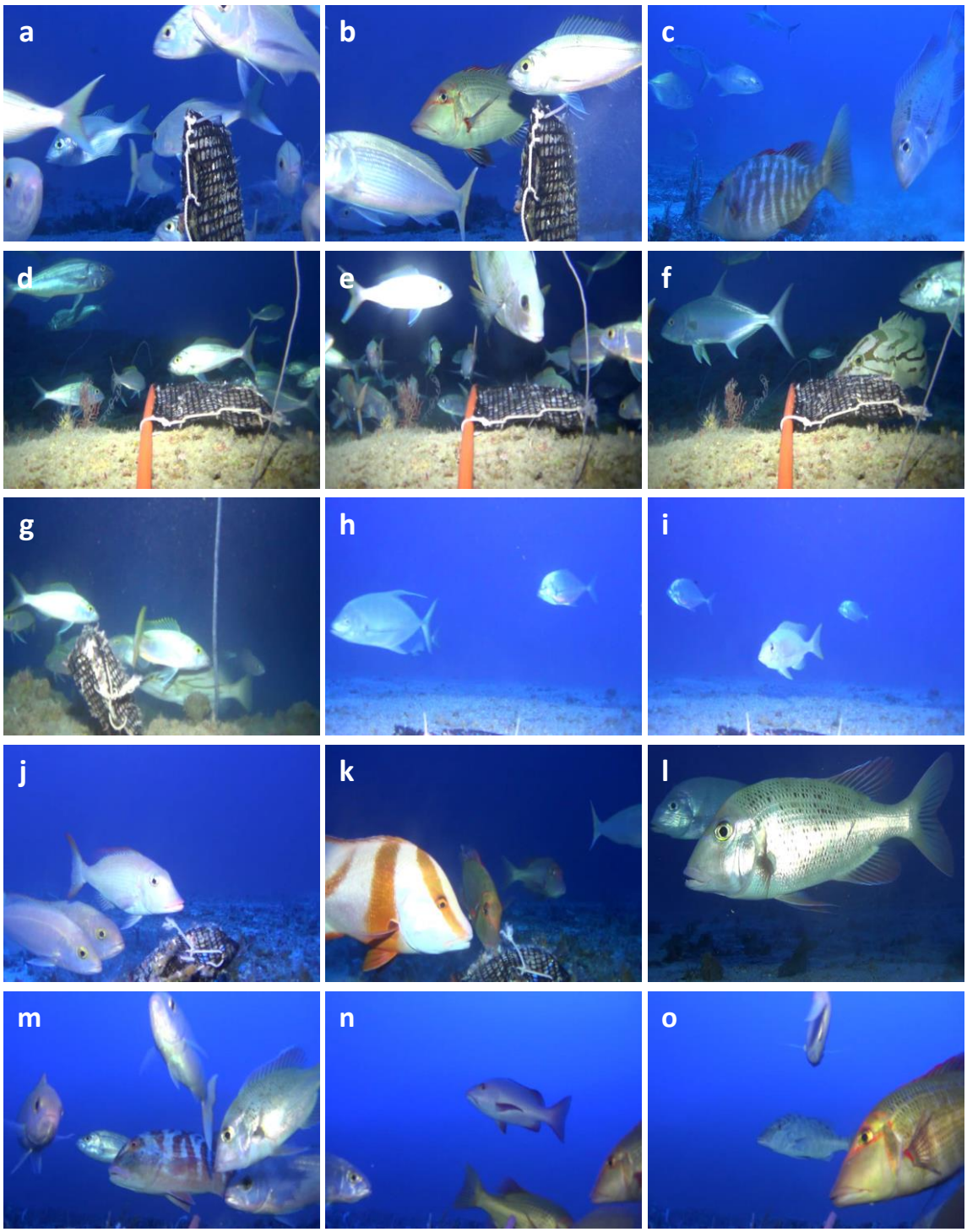


Figure 3-8: Examples of fish co-occurrences on deep-reefs of the Great Barrier Reef shelf-break: a-b) West Myrmidon 128 m; c) North Myrmidon 100 m; d-f) Northern Submerged Shoals (NSS) 155 m; g) NSS 160 m; h-i) West Myrmidon 129 m; j-k) North Myrmidon 103 m; l) North Myrmidon 107 m; and m-o) North Myrmidon 105 m.

Deep-reef fish trophic assemblages

The reef fishes detected in this study were ecologically diverse. Of the 98 fishes identified to species-level, piscivores (10 species), planktivores (7 species), benthic-associated carnivores (26 species), generalist carnivores (41 species) and four species of combined diets (e.g. planktivorous and piscivorous fishes) were represented, based on membership of known trophic guilds (Appendix Table A1). Twenty species recorded had no published trophic information (according to Fishbase); however, half of these were assigned to a trophic group based on other family members occupying that same trophic group. Only one species was herbivorous (*Acanthurus xanthopterus*), likely due to the decreased availability of edible algae with depth, or the amount of feeding activity around the BRUVS. PC1 and PC2 accounted for a combined 52.5% of the variation among sites, with the presence of general carnivores against the other trophic guilds accounting for the greatest separation and approximately 30% of the total variation (Fig. 3-9). Shallower sites tended to have a greater variety of feeding modes and less overlap with the other depth categories, however, overall there was a great degree of trophic overlap, especially between the middle and deeper sites (110-260 m).

Several environmental variables were found to have influence on trophic diversity across PC1 and PC2 (Appendix Table A2). Depth, aspect (orientation), planar curvature and surface ratio dimensions contribute toward the differences in assemblages along PC1; fish assemblages were affected by the local topography and habitat position, presumably because some habitats will be cliff-like features facing the prevailing currents. Proportional measures of bare, plants, bedrock, calcified reef, and presence of sand also correlated with differences along PC1. Slope and standard deviation of the bathymetry were found to significantly vary with PC2.

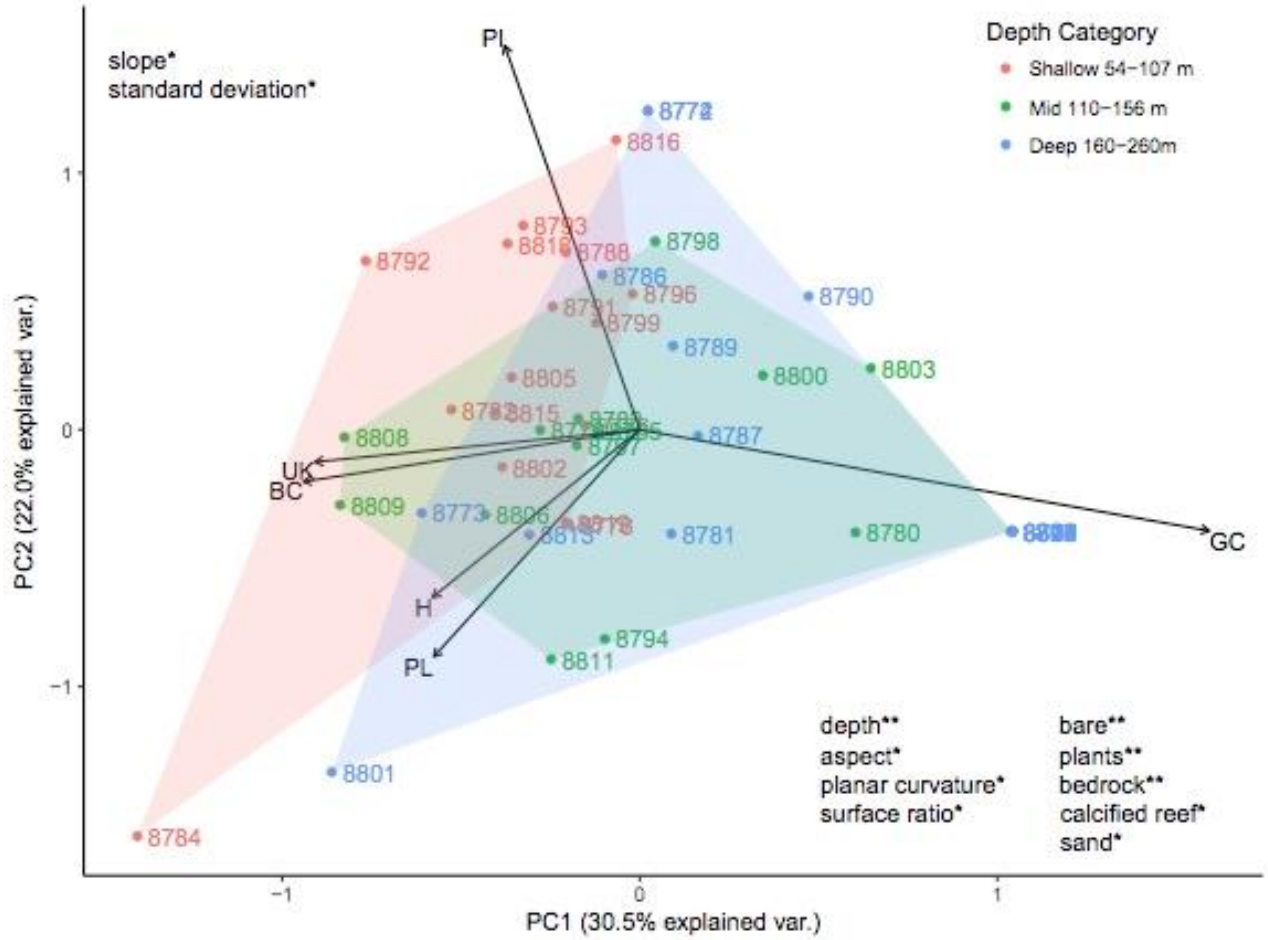


Figure 3-9: Principal Component Analysis show trophic assemblage differences of fishes between sites sampled on shelf-break reefs. The first two principal components explain 52.5% of the variation in trophic diversity between sites. Sites are grouped by depth category and each has a unique number. Vectors depicting the principal feeding strategies (H = herbivore, PI = piscivore, PL = planktivore, BC = benthic carnivore, GC = general carnivore, UK = unknown) show some of the key differences between sites. Environmental variables found to significantly contribute to the differences along PC1 and PC2 are summarized next to the corresponding axis (** $p < 0.01$ and * $p < 0.05$).

Discussion

Habitat type varied with depth and within depth strata, these differences in reef architecture and benthic cover affected both assemblage and trophic composition of fish assemblages. While the shelf-break sites sampled all exhibited a steep vertical gradient, individual habitats were highly heterogenous, varying in both biotic and abiotic characteristics. These factors influenced the distribution and abundance of many fish taxa, as well as broad trophic groups. Many habitat differences corresponded with increasing depth, likely driven by vertical variation in temperature, light, and pressure; however, habitats also varied within depth strata in regards to benthic community composition and underlying substrate type. Interestingly, most multibeam variables did not correlate with changes in overall fish assemblage composition, though a few (slope, aspect, planar curvature and surface ratio) could distinguish sites with different trophic assemblages. This may be because the measures of habitat from different spatial scales, from relatively small with BRUVS (<10 m^2) and multibeam derivatives describe broader spatial information (~10-100s m^2). Topographical features of habitat, such as slope angle, aspect (i.e. sites facing prevailing currents), rugosity, and planar curvature (e.g. local ridges or valleys) may contribute to the local availability of food and shelter. Among the shallow and middle-depth sites sampled, the fish assemblage composition at Viper Reef was clearly distinct from other locations. Viper was located on a shallower portion of the shelf-break, where the reef bottoms out to a maximum depth of 150 m and the slope was less steep. The maximum extent (i.e. deepest depth) of the reef may account for some of the variability in fish assemblages (Andradi-Brown et al. 2016b).

Trophic group composition and structure varied with depth, with a greater trophic diversity at upper mesophotic depths and increasing reliance on general carnivory at the deepest depths. This suggested that the ecology of deeper reef fish assemblages is fundamentally different from those found at shallower depths. Some previous studies have noted a greater abundance of certain trophic groups, such as piscivores, on outer-shelf reefs along the GBR (Newman et al. 1997); however, this is the first assessment of depth-related changes in trophic structure below 50 m. Worldwide, many mesophotic habitats are characterized by low herbivore abundances and high planktivore abundances (e.g. the Red Sea, Puerto Rico, Northwest Hawaiian Islands, Brazil, Main Hawaiian Islands; (Brokovich et al. 2010, Bejarano et al. 2014, Bridge et al. 2016b, Fukunaga et al. 2016, Pinheiro et al. 2016, Pyle et al. 2016a, Asher et al. 2017). While this study identified low numbers of planktivorous and piscivorous species compared to other feeding strategies (7-10%), this is largely due to the lack of trophic specificity available (some of the species observed had 'unknown' feeding modes). Depth-related trophic variation indicates a dramatic shift from shallow reef food-web dynamics to strategies that rely more on plankton and other mobile resources. It has been postulated that mobile invertivores (Fukunaga et al. 2016,

Asher et al. 2017) and anthiine fishes (Weaver et al. 2001, Bryan et al. 2013) are key links within other mesophotic food webs, and the high proportion of carnivores and piscivores found at mesophotic depths within the GBR suggests similar strategies are operating there. Even within the same species, deeper habitat-associated subpopulations of *Stegastes partitus* had broader diet niches than those in shallower depths (Goldstein et al. 2017). Future trophic comparisons should include relative measures of trophic-level hierarchy, mobility and prey size (e.g. Asher et al. 2017), as well as quantifying how reliant these predators are on food sources that originate at shallower depths and use vertical diel movements to target benthic prey (e.g. Papastamatiou et al. 2015) or if there are 'trophic subsidies' in operation where oceanic planktonic and nektonic resources make up the deficit for dwindling primary productivity at deeper depths (Weaver & Sedberry 2001).

Identifying where species co-occur is an important consideration in ecosystem-based fisheries management, used to predict the degree that species interact. Species distributions that are highly correlated will also affect fishing mortality estimates in multispecies fisheries (Pope 1979). More connected species are thought to have a higher vulnerability to combined anthropogenic threats as well as detrimental changes to the assemblage structure (Tulloch et al. 2018). The species co-occurrences identified in this study suggest the presence of both inter- and intra-family interactions, similar habitat needs or greater food availability. However, as the majority of overlapping species fishes are upper-level predators these are likely examples of competition or niche partitioning rather than predator-prey interactions. In addition to differences in trophic groups with depth, there was substantial variation in overall fish assemblage composition both between and within-depths, with this information on variability critical for future management plans. Previous surveys of mesophotic and sub-mesophotic shelf-break reefs suggested species composition is often highly heterogeneous (Hill et al. 2014) with potentially high proportions of both rare species (Bacheler et al. 2016) and endemism (Kane et al. 2014, Kosaki et al. 2016). New and highly unique fish assemblages are being frequently described as mesophotic research effort increases (Pyle et al. 2016a, Baldwin et al. 2018, Rocha et al. 2018); indeed, these surveys here identified a number of new potential species as well as new species location records for the GBR.

Variation in fish assemblage structure among and within depths likely reflects differences in the biotic and abiotic components of shelf-break reefs, with these habitats also distinctive from shallower reefs along the continental shelf. A greater proportion of sponges and macroalgae within the benthic community, and the presence of boulders, distinguish shelf-break environments from shallower habitats, as well as differences among shelf-break reef habitats. Not only were significant differences in assemblage composition found between the sampled reefs, but also between reefs and inter-reefal areas; especially at the shallower depths where a steep slope angle

and a high abundance of filter-feeding invertebrates were characterizing features. Sponges and filter feeders are an important habitat-forming component of the upper mesophotic zone along the central GBR (Fitzpatrick et al. 2012, Wahab et al. 2018), compared to shallow reefs where coral is the primary ecosystem engineer. Dominant benthic taxa shift from photosynthetic to obligate heterotrophic in deeper, mesophotic Indo-Pacific environments (Bridge et al. 2011b, Kahng et al. 2014). The central GBR shelf-break has similar benthic habitats to other clear, tropical mesophotic regions, with *Halimeda* and corals are observed down to >150 m (Kahng et al. 2010, Bridge & Guinotte 2012, Kahng et al. 2017). While the lower mesophotic zone is dominated by depth-specialist benthic communities that are distinct from shallower areas (Bongaerts et al. 2015), coral communities have been documented in transitional depths of 60-75 m at multiple sites (Webster et al. 2008, Bridge et al. 2011a). The lower depth-limits of corals vary, with isolated coral colonies documented to at least 125 m in some locations in the GBR and neighbouring Coral Sea (Hopley et al. 2007, Englebert et al. 2014, this study). *Halimeda* bioherms, while not explicitly studied here, are common macroalgal components of deep reef systems and provide important deposits of calcium carbonate that promotes deep-reef growth. In this study, I observed photosynthetic algae at deeper depths than reported in other MCEs worldwide, which is likely due to the well-documented nutrient upwelling. New mesophotic-specific algae species have been found in macroalgal communities in other mesophotic locations (Spalding 2012, Wagner et al. 2016). At the deepest depths surveyed, boulders replaced reef-building organisms in creating structural complexity. It is clear that in the GBR, the shelf-edge should be considered an ecologically unique ecosystem and fundamentally different from shallow reefs, similar to other MCEs (Olavo et al. 2011, Bacheler et al. 2016, Rocha et al. 2018), often narrow off the shelf and narrow parallel to the shelf-break.

Shelf-break reefs are likely critical habitats for key ecological processes and it is not yet known to what extent these habitats are necessary for certain species to thrive. Anecdotally, several of the BRUVS deployments observed juvenile fishes at mesophotic depths. While it was not always possible to identify juvenile fish to species-level (and single BRUVS only allow an estimated size), some fish appear to complete most of the life cycle in solely deep habitats, such as the grouper *Epinephelus morrhua* (Fig. 3-10). In general, the juvenile habitats of the deep-reef species I observed are not well-documented. For instance, juvenile habitats of *Pristipomoides* sp. were only accidentally discovered over deep (65-100 m), flat, soft habitats in Hawaii (Moffitt & Parrish 1996). Dogtooth tuna, *Gymnosarda unicolor*, were observed in groups of 1-3 in all BRUVS deployments except one (Fig. 3-10d). This unusual behaviour could be a spawning aggregation, to increase safety from predators, or to increase hunting success. Certain Lutjanidae and Serranidae spawning aggregations are reliable and infamous worldwide (Smith 1972, Heyman & Kjerfve 2008, Mourier et al. 2016). Many of these species' use of different habitats to

complete their life cycle is not known for the GBR, and future research should attempt to describe and quantify how deep reefs are important for spawning, ontogenetic shifts and life history cycles.

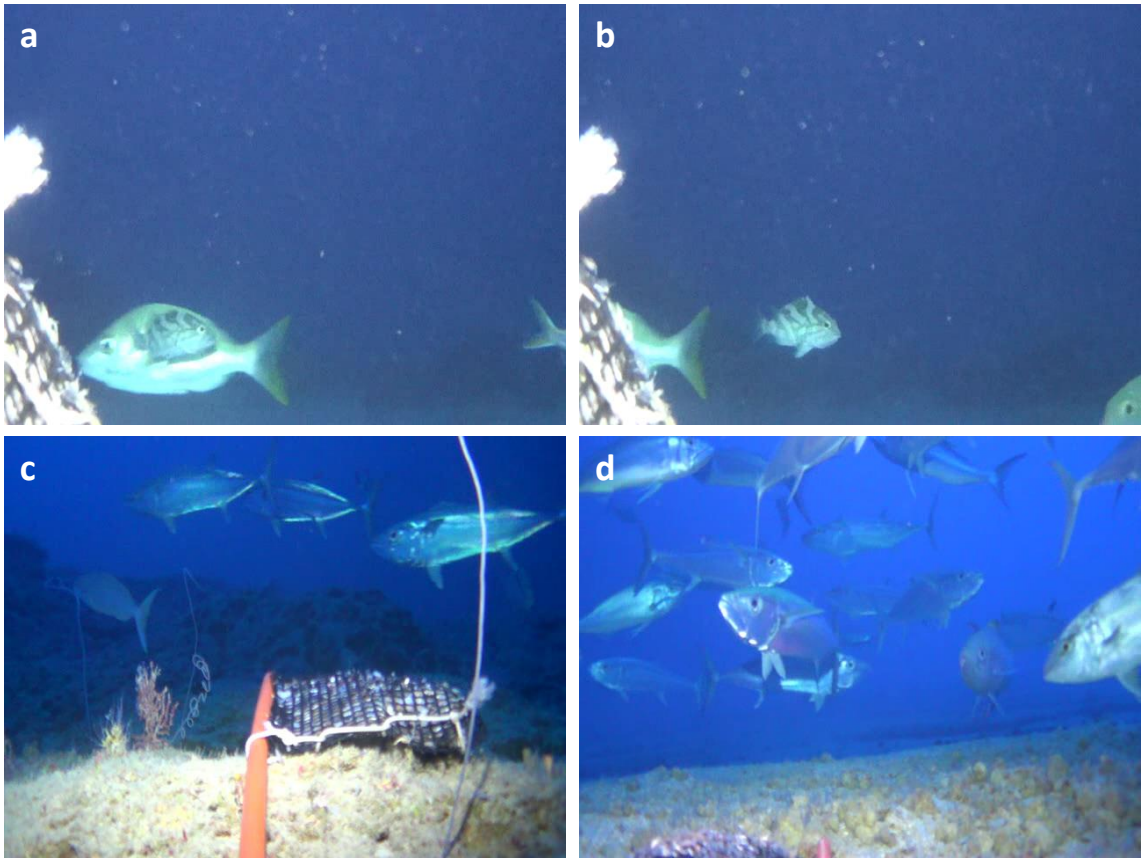


Figure 3-10: Some deep Baited Remote Underwater Video Stations captured juvenile fishes, including this *Epinephelus morrhua* at 194 m (a-b). Another deployment captured an unusually large aggregation of *Gymnosarda unicolor* and other species (d), which would indicate extremely favourable feeding conditions or group behaviour like spawning. Most often *G. unicolor* were found in small groups of one to three individuals (c).

This study has shown that benthic composition can influence the distribution and abundance of mesophotic fish assemblages, therefore, further research on the distribution and composition of deep reef habitat structure and epibenthic communities is critical to better characterize habitats necessary to preserve mesophotic biodiversity. Greater sampling effort of the GBR shelf-break along its latitudinal extent would better describe these deeper marine biomes for future conservation strategies. When GBRMP protection and mixed-use zonation was determined a decade ago only coarse environmental data was available for the deeper habitats within the GBRMP (Bridge et al. 2016a). The strategy of the conservation zones allows for some uncertainty and was designed to protect unknown habitats (Fernandes et al. 2005), and incidentally ~30% of submerged banks are within no-take areas and 88% of banks are protected from bottom-trawling (Harris et al. 2013, Bridge et al. 2016a). Of the locations sampled, Myrmidon and Viper Reef are afforded greater protection as 'no-take' areas. This research showed assemblage differences between reefs and also between reefal and inter-reefal sites. In the future, it will be important to compare species richness and abundance of areas over different

protection levels and to include inter-reefal areas for habitat protection. More detailed benthic habitat mapping and biotic surveys have improved the representative distributions of habitats and fishes in other marine conservation parks in Australia (Malcolm et al. 2016, Moore et al. 2016b), and increasing the understanding of GBR shelf-break habitats should be a priority. The species composition of fishes varied greatly among habitats. Although depth was important, habitat preferences clearly had a role in determining the distribution of species and trophic groups. Potential predictors of fish distributions on the shelf-break are depth, reef architecture and benthic cover. The narrow spatial extent of the mesophotic areas on the GBR and other locations makes them vulnerable to fisheries.

Chapter 4 Environmental predictors of species richness and abundance for deep-reef fish assemblages of the Great Barrier Reef (Australia)

Tiffany Sih, Tom Bridge, James Daniell, Rob Beaman, Andrew Chin, Michael Kingsford

Abstract

Deep-reef fish assemblages are ecologically and economically important; however, understanding patterns of species distribution is logistically difficult. Therefore, remote sensing techniques are the only option to provide essential information on fish assemblage structure over broad spatial scales. I combined remotely-sensed multibeam data, Baited Remote Underwater Video Station observations, and empirical modelling techniques to evaluate fish species richness and abundance patterns across an environmental mosaic of deep marine habitats of the Great Barrier Reef shelf-break (Australia). I explored the importance of habitat variables on fish species richness and abundance using boosted regression tree analysis and topographic, substratum and epibenthic measures on a range of spatial scales. The representation of encrusting organisms, amount of calcified reef substratum, depth range and average backscatter were important predictors of species richness and relative abundance on deep reefs. It was clear that complex spatial and environmental relationships between fish diversity, abundance and habitat exist and these patterns were robust in comparisons of spatial scale (10s-100s m^2). Some patterns of species abundance did vary with depth and not just habitat. However, variation in habitat types that included reef architecture within depth strata was an important predictor of assemblage composition of fishes. Neighbourhood information from multibeam improved our understanding of underwater features that contribute to higher local biodiversity. The inclusion of more spatial, rugosity, biotic and substratum measurements explained differences in species richness and abundance better than simpler models. Therefore, incorporating a more continuous view of the seafloor and benthic habitats combined with a knowledge of preferred depth of residency would improve fish diversity and abundance estimates for conservation and planning purposes.

Introduction

Worldwide, tropical deep-reef habitats (i.e. those below ~50 m in depth) and associated fish assemblages are under increasing stress (Andradi-Brown et al. 2016a, Rocha et al. 2018) due to anthropogenic factors such as greater fishing pressure and pollution (Andradi-Brown et al. 2016a, Rocha et al. 2018). Deep-reef habitats, often referred to as Mesophotic Coral Ecosystems (MCEs), form critical components of overall reef systems, increasing habitat availability and associated biodiversity. For instance, deep reefs along Australia's Great Barrier Reef (GBR) are home to unique fish assemblages that vary with both depth and habitat type (Chapters 2 and 3). While these communities form an important ecological and economic resource, global research on MCEs suggests fish assemblages vary widely in composition both within and between the Atlantic, Pacific and Indian Oceans. For this reason, there is a critical need for spatially disparate but methodologically comparable datasets on these environments that include explicit information on fish-habitat associations and assemblage structure.

For this to occur, the use of standard quantitative measurements of biodiversity are required, such as species richness (the number of different species in an assemblage) and species abundance (species evenness or how many individuals of a species are in a given area). For individual species, abundance and distribution is often mediated by a combination of biotic and abiotic environmental variables that form its 'multidimensional niche' (Brown 1984). Therefore, when coupled with habitat information, species richness and relative abundance can be used to identify relationships between the fish assemblage and underlying habitat characteristics (Fig. 4-1). For planning and management plans, strong relationships to spatial and environmental factors can allow researchers to predict spatial and temporal patterns of species richness and abundance within a seascape and vulnerability to exploitation, and estimate the effects of habitat change on fish assemblages (Grober-Dunsmore et al. 2008, Pittman et al. 2010, Moore et al. 2011).

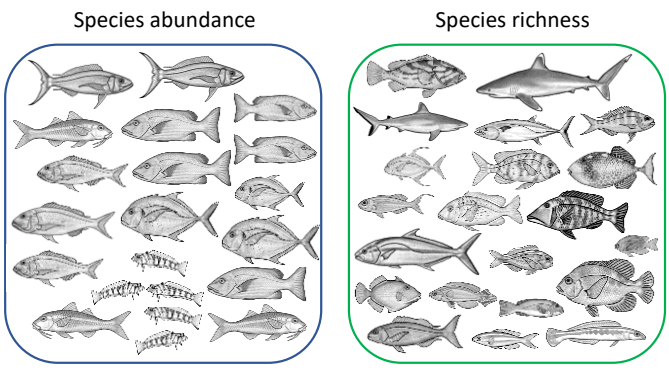


Figure 4-1: Species abundance compares the relative proportions of a species and species richness compares the number of different species. Both measurements show an aspect of a fish assemblage without relying on the species that explicitly comprise an assemblage and can be comparable for ecosystem-level assessments.

While understanding the relationships between deep-reef fishes and habitats is essential for effective management, gathering the required data has traditionally represented a significant logistical challenge. However, modern remote sensing techniques have proven to be a useful tool for evaluating large spatial areas and mapping mesoscale patterns of fishes and the habitats in which they live (Wilson et al. 2007, Brown et al. 2011). For instance, in the GBR various forms of remote sensing data have been used to map dominant benthic and substrate types (Pitcher 2004, Pitcher et al. 2007, Beaman et al. 2016), investigate geomorphology, and identify key bathymetric zones (Beaman et al. 2008, Abbey et al. 2011). Marine remote sensing techniques such as multibeam bathymetry have been especially useful in amassing biophysical information and providing critical insight into the patterns of marine biomes and deeper habitat variation (Monk et al. 2010, Moore et al. 2010, Monk et al. 2011, Moore et al. 2017). For deeper habitats, multibeam information may be useful in predicting areas of high species richness or abundance and can be used to effectively monitor deep-reef habitat change over time. Gathering information on associated fish assemblages also presents challenges not encountered at shallower depths. However, the modification and refinement of standard methods such as Baited Remote Underwater Video Stations (BRUVS) to suit deeper sampling has allowed the direct, high-resolution measurement of deep-reef fish assemblages to become a cost-effective, accessible option (Cappo 2010, Langlois et al. 2010, Cappo et al. 2012, Zintzen et al. 2012).

While depth is a major driver of fish distributions, studies using these or similar techniques have identified a number of deep-reef fishes where distribution and abundance is influenced by habitat variables such as depth (e.g. Chapter 2), aspect (e.g. Heyward et al. 2011), slope (e.g. Chapter 3, Moore et al. 2016a), substrate type (e.g. unconsolidated sediment variability, Schultz et al. 2015; coral cover, Espinoza et al. 2014), and topography measures (e.g. relative topography and range, Yates et al. 2016). As the list of potential environmental covariates that could influence species distribution and abundance can be extensive, managers require succinct and straightforward information on key factors of interest. However, this is complicated by the fact that the relative importance of factors that influence spatial distribution often varies among species (Moore et al. 2016a) and some factors perform inconsistently or poorly in species prediction models over large spatial scales (e.g. slope, Gomez et al. 2015). In addition, inadequate sampling may prevent the identification of critical fish-habitat associations if un-measured, but ecologically important, biotic and abiotic factors interact with measured covariates in unrecognized ways (Heyward et al. 2011). From Chapter 2, depth explained much of the pattern for species richness and abundance, however, ‘simpler’ models (e.g. depth and relative position along the shelf-break) may explain some additional unexplained variation. In Chapter 3, epibenthic and substratum information explained some differences in the fish assemblage composition, quantified from the BRUVS field-of-view, over a large depth gradient

on the GBR. From this work, key habitat components that explained the differences in fish assemblage were the presence of sponges and other filtering invertebrates, and substratum components including the proportion of sand and boulders. However, the factors that correlate with the presence of species over small scales may differ from the factors that influence abundance or richness over whole ecosystems. Therefore, the influence of environmental variation on these metrics should be evaluated at multiple spatial scales. Multibeam information can be detected remotely and may be more efficient in comparing fish assemblage responses over larger spatial scales. Therefore, complex models that include more habitat information may enhance our ability to predict how species richness and abundance vary over larger areas.

The objective of this research was to identify what broad-scale habitat characteristics (such as rugosity, depth, backscatter, percentage coverage by epibenthic or substrata) and types of habitat information (such as multibeam derivatives, epibenthic or substratum estimates) are best at predicting the species richness and abundance of associated deep reef fish assemblages. I focused on spatial changes to the overall deep-reef assemblage (species richness and total abundance) using analytical models of varying complexity and different types of environmental information on multiple spatial scales from multibeam bathymetry.

Methods

BRUVS and multibeam datasets

Baited Remote Underwater Video Stations (BRUVS), consisting of Sony high-definition cameras (HDR-CX110) in a waterproof camera-housing with a white spotlight and a plastic mesh bait bag extended into the camera's field-of-view were deployed (48 deployments in 2014 on three research cruises). All deployments occurred in daylight, targeting 50-300 m depths at multiple locations along the shelf-break. The abundance of each species was recorded separately for each video until MaxN was reached (highest number of individuals of a species per frame). Species abundance and species richness were standardized for time sampled (number of species per site for species richness and number of fish per species for relative abundance, each per hour). Number of species per hour and number of fishes per hour and are relative metrics to compare among the sites sampled and for simplicity I refer to as 'species richness' and 'species abundance'. Benthic habitat information was estimated from the BRUVS field-of-view in substratum categories (bedrock, boulder, calcareous reef, mud, gravel, rubble, sand and indeterminate, Table 4-1) and epibenthic categories (presence of filtering organisms, plants, *Halimeda*, encrusting organisms, coral and 'bare', no visible, epibenthos).

Multibeam sonar datasets depict the seafloor three-dimensional structure with bathymetry and backscatter layers, which are interpolated into multi-scale terrain analysis with neighbouring data points. A key component of this study was to examine multiple spatial scales, so multibeam

derivatives were extracted from four 'window' sizes for smaller or larger interpretations of nearby features (kernel size with radius values of 5, 10, 25, and 50 raster pixels). Some quantify shape characteristics (i.e. Easting, Northing, Slope, Topographic Position Index, Topographic Ruggedness Index, Surface Ratio, Total Curvature, Planar Curvature, and Profile Curvature) and have been used within marine habitat or seabed classifications (reviewed in Diesing et al. 2016).

Data Analysis

Univariate species richness and species abundance measures were compared to each possible habitat covariate to remove redundant variables that would not improve the explanatory power of the model through Pearson correlations of epibenthic, substratum and multibeam-derived metrics.

To further explore the relationship between fish assemblages and the relative importance of various habitat covariates, I used Boosted Regression Trees (BRTs) to identify the most explanatory habitat gradients affecting overall species richness and relative abundance. The advantages of analysing complex datasets using BRTs are the ability to fit many simple models to optimize overall prediction models (De'Ath 2007) and the flexibility to fit relationships with a mixture of predictor variable types with possible interactions between predictors (Elith et al. 2008). Gradient boosting optimizes the predictive performance by minimizing the deviance of each successive model (Friedman 2001, De'Ath 2007, Elith et al. 2008). Regression trees have outperformed other empirical modelling techniques such as multiple linear regression and neural networks in predicting fish species richness (Pittman et al. 2007, Knudby et al. 2010). BRTs indicate which variables are most relevant and have the lowest prediction error by their relative prediction estimates, which are averaged over the collection of trees (De'Ath 2007). Missing data, outliers, differing measurement scales of predictors, and irrelevant predictors are not issues as with some other statistical methods due to the sequential fitting and pruning techniques in BRTs (De'Ath 2007, Elith et al. 2008).

I used five types of models to compare the importance of four types of habitat characteristics: spatial, rugosity, substratum and biotic influences (Table 4-2). The first BRT model included fewer explanatory variables relating to spatial characteristics: depth, latitude and longitude (Type 1). The second BRT model included the explanatory variables from the first BRT model and added multiple measures of rugosity from multibeam derivatives (Type 2). The third and fourth BRT models included Type 2 predictors as well as either the estimated substratum data (including multibeam backscatter) or epibenthic data (Type 3 and 4). The fifth model included all environmental variables (Full Model). All models were run with multibeam information and each grid size was kept as a separate subset of multibeam information for the comparison of spatial scales (i.e. 5 x 5, 10 x 10, 25 x 25 and 50 x 50).

BRTs followed the advice of De'Ath (2007) and Elith et al. (2008) and examples of similar implementation in the recent literature include Yates et al. (2016), Oyafuso et al. (2017) and Moore et al. (2016). With BRTs it is important to reduce over-learning and over-fitting, so models must be built with a compromise of learning rate (shrinkage), tree complexity (possible interaction capacity of the model) and the number of trees used. Models were built using the maximum variables and trialled multiple shrinkage rates, bagging fractions (adding randomization), and interaction depths (functions gbm.step, package dismo, Hijmans et al. 2017; and gbm, Ridgeway 2006). Less influential (uninformative) predictor variables (<1%) can be removed, but the addition or removal of variables does not impact the relative influence of other variables, as non-informative predictors are ignored (Elith et al. 2008). However, efforts were taken to minimize highly correlated variables because bathymetry and backscatter datasets from multibeam typically have correlated variables. For example, Planar Curvature, Profile Curvature and Curvature are all calculated from the same bathymetry digital elevation model (Diesing et al. 2016). To reduce multicollinearity, highly correlated variables (>0.8) were removed (function ggpairs, package GGally; Emerson et al. 2012, Schloerke et al. 2018). This can vary with each spatial scale and correlations for each pair of predictors were evaluated separately for 5 x 5, 10 x 10, 25 x 25 and 50 x 50 kernel grid sizes.

For both species abundance and species richness, final models included up to 24 possible explanatory covariates. The BRTs used 75% of the dataset as a training set, included cross-validation (5 cross-validation folds), and a 'bag fraction' of 0.5, as adding stochasticity generally improves model performance (De'Ath 2007). Final models were built for interaction depths of 1-5, with shrinkage (learning rate) of 0.01-0.001 (generally, slow learning rates perform better) and the number of trees beginning at 1000 and increasing ten-fold for each decrease in shrinkage (De'Ath 2007). Multiple interactions depths were trialled to accommodate varying complexity up to 7 to evaluate the relative benefits of more complex models. Interaction depths of 1 test for only the main effects, while higher level interaction depths account for increasing complexity of interactions between covariates. Final trees used 'out-of-bag estimates', which are conservative. A Gaussian distribution was used for species richness and total species abundance data (Ridgeway 2006). Both richness and abundance were standardized among sites for sampling time but no other transformation to avoid complicated back-transformations, which affect model interpretation, and to compare the models with different spatial scales. Each BRT model was run for three iterations (because of stochasticity) and the range of R^2 values and relative influence of predictor variables was reported (if >5%). The amount of variance explained by the model divided by the total variance (R^2), referred to as the 'goodness-of-fit', was used to compare models.

The relative influence of each variable was based on the number of times that variable was important in splitting the data. Relative influences for the top ten covariates were scaled and

indicate stronger or weaker influences on the response expressed as a percentage. Partial dependence plots were plotted to visualize the effects of the eight most influential variables (De'Ath 2007). Interactions between variables were investigated using a modified BRT model (spatial scale 10 x 10, tree complexity = 5, learning rate = 0.0025, bag fraction = 0.5) that first reduced the number of variables (function gbm.simplify, package dismo) to 16, then investigated the strength of the interaction between each pair of variables using Friedman's H-statistic (function interact.gbm, package gbm).

Table 4-1: Explanatory covariates from multibeam bathymetry and backscatter and estimates from the Baited Remote Underwater Video Station. *Raw raster data. **Applied as a 3 x 3 kernel on bathymetry after it was averaged using kernels with a radius of 1, 5, 10, 25, 50 pixels. ***Applied kernels with a radius of 1, 5, 10, 25, 50 pixels.

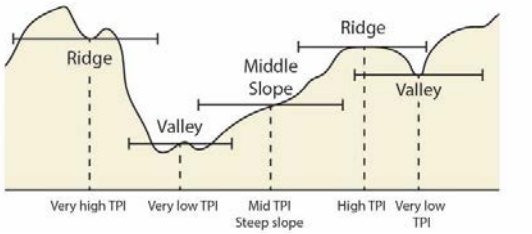
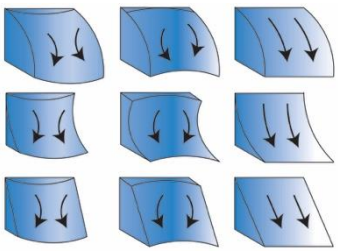
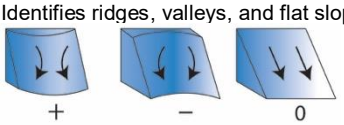
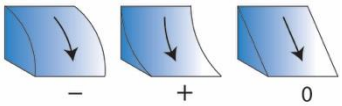
Covariate name (abbreviation)	**Definition**
Bedrock (bdrck)	Estimated % Bedrock
Boulder (bldr)	Estimated % Boulder
Calcified reef (calc.rf)	Estimated % Calcareous reef
Gravel (grvl)	Estimated % Gravel (2-64mm)
Indeterminate (ind)	Estimated % Indeterminate
Mud (mud)	Estimated % Mud/silt
Rubble (rbbl)	Estimated % Rubble
Sand (snd)	Estimated % Sand
Filter feeders (fltrs)	% combined Fans, Hydroids, Sponges, Whips
Encrusting organisms (encr)	Estimated % combined Bryozoans/encrusting animals, coralline algae
Coral (crl)	Estimated % combined Hard coral and Soft coral
Bare (bare)	Estimated % no epibenthic cover
Plants (plants)	Estimated % combined Macroalgae and Seagrass
Halimeda (hal)	Estimated % *Halimeda*
Name	**Possible ecological context**
Depth* (m)	Location relative to Photic Zone and thermoclines/haloclines
Easting**	Level of exposure or protection from oceanographic processes
Northing**	Level of exposure or protection from oceanographic processes
Slope** (in Degrees)	Relative substratum angle
Topographic Position Index** (TPI)	Relative topographic position: Positive TPI values are ridges and negative TPI values are valleys. TPI values near zero are flat areas. 
Terrain Ruggedness Index**	Structural complexity
Range***	Structural complexity
Surface Ratio**	Relative vertical relief indicating degree of structure complexity
Standard Deviation*** (m)	Index of surface roughness
Curvature** (Degrees/m)	Overall curvature within kernel 
Planar Curvature** (Degrees/m)	Identifies ridges, valleys, and flat slopes 
Profile Curvature** (Degrees/m)	Concave or convex slopes 
Acoustic Backscatter* (Decibels)	Proxy for seabed substratum
Average Backscatter*** (Decibels)	Proxy for seabed substratum
StdDev Backscatter*** (Decibels)	Variation in substratum within kernel

Table 4-2: Range of epibenthic, substratum and multibeam derivatives retained in Boosted Regression Tree models at multiple spatial scales (i.e. 5 x 5, to 50 x 50 grids). Highly correlated multibeam derivatives that were removed included average depth, surface ratio, Terrain Ruggedness Index, roughness, range, standard deviation of bathymetry, curvature and profile curvature.

	Predictor	Category	Range			
BRT Models			**5 x 5**	**10 x 10**	**25 x 25**	**50 x 50**
All models	Depth	**Spatial** (continuous)	54 – 260			
	Longitude	**Spatial** (continuous)	147.25 – 148.45			
	Latitude	**Spatial** (continuous)	-18.88 – -18.19			
	Easting	**Spatial** (continuous)	-0.99 – 1.00	-1.00 – 1.00	-1.00 – 1.00	-0.99 – 1.00
	Northing	**Spatial** (continuous)	-0.99 – 1.00	-0.95 – 1.00	-0.95 – 1.00	-0.94 – 1.00
Type 2-4 Full Model	Slope	**Rugosity** (continuous)	0.66 – 49.2	0.72 – 39.9	0.57 – 35.6	0.52 – 33.8
	Topographic Position Index	**Rugosity** (continuous)	-0.79 – 0.63	-0.12 – 0.18	-0.06 – 0.10	-0.06 – 0.08
	Planar curvature	**Rugosity** (continuous)	-0.007 – 0.009	-0.004 – 0.002	-0.003 – 0.001	-0.002 – 0.002
Type 3 Full Model	Backscatter average	**Substratum** (continuous)	-67.9 – -23.1	-67.1 – -23.8	-67.0 – -23.7	-56.3 – -25.3
	Backscatter standard deviation	**Substratum** (continuous)	0.01 – 11.2	0.02 – 14.8	0.42 – 14.1	0.62 – 15.8
	Bedrock	**Substratum** (proportional)	0-50%			
	Boulder	**Substratum** (proportional)	0-30%			
	Calcified Reef	**Substratum** (proportional)	0-80%			
	Gravel	**Substratum** (proportional)	0-100%			
	Indeterminate	**Substratum** (proportional)	0-100%			
	Mud	**Substratum** (proportional)	0-100%			
	Rubble	**Substratum** (proportional)	0-100%			
	Sand	**Substratum** (proportional)	0-100%			
Type 4 Full Model	Filter feeders	**Biotic** (proportional)	0-70%			
	Encrusting organisms	**Biotic** (proportional)	0-50%			
	Coral	**Biotic** (proportional)	0-60%			
	Bare	**Biotic** (proportional)	0-100%			
	Plants	**Biotic** (proportional)	0-70%			
	Halimeda	**Biotic** (proportional)	0-30%			

Results

Univariate fish assemblage and single habitat covariate correlations

No habitat variables fully explained patterns of species richness and abundance-habitat relationships. There were no strong (>0.7) correlations (Pearson, n = 48) between fish species richness or species abundance and any single multibeam, epibenthic or substratum measurements. However, there was a moderate positive correlation between encrusting organisms and richness ($r = 0.6$, $p < 0.001$ directional) and abundance ($r = 0.6$, $p < 0.001$) and the proportion of calcified reef and species richness ($r = 0.6$, $p < 0.001$) and abundance ($r = 0.5$, $p < 0.001$). Bare substratum (i.e. the absence of visible epibenthic cover) was moderately negatively correlated with species richness ($r = -0.5$, $p < 0.001$), abundance ($r = -0.5$, $p < 0.001$). Similarly, depth was negatively correlated with richness ($r = -0.4$, $p < 0.01$) and abundance ($r = -0.4$, $p < 0.001$). Single multibeam derivatives showed only weak correlations with species richness and abundance ($r < 0.4$) and this was true for all spatial scales (i.e. 5 x 5, 10 x 10, 25 x 25, 50 x 50 grids).

Model performance, complexity, and spatial scale

The combination of multibeam-derived information with estimated biotic and abiotic components from the BRUVS field-of-view enhanced BRT models describing relative species abundance and species richness of deeper fish assemblages (Appendix Table A1, Fig. 4-2). The increase in explanatory value of models increased more significantly when substratum/backscatter and epibenthic information was included in the model (Type 3, 4 and Full Model) than simpler models (Type 1 and 2). However, while the addition of biotic estimates increased the goodness-of-fit for species abundance (Type 4 and Full model, Fig. 2), it was the addition of substratum or biotic estimates (Type 3 and Type 4), and to a lesser extent the addition of rugosity metrics (Type 2), which made the more measurable increase in model performance for species richness. Models that included spatial, rugosity, and biotic/epibenthic measurements (Type 4 and Full Model) performed best for overall species abundance (e.g. R^2 = 39.8-43.9, interaction level = 5, spatial scale 10 x 10). Species richness had higher R^2 values when substratum measures were included along with spatial, rugosity and biotic measures (Full Model, e.g. R^2 = 50.2-57.3**,** interaction level = 5, spatial scale 10 x 10).

There are complex relationships among habitat factors that are responsible for differences in species richness and abundance; models with interaction levels of at least 3, which include main and interactive effects, performed better. Overall the increase in complexity between interaction depths of 1 and 3 demonstrated larger changes in the model's fit (>10% improvement for more complex model types). The interaction levels of >5 only gave marginal (<10%) improvement, therefore, models with interaction depths of 3-5 were sufficiently complex.

Spatial scale only subtly affected model performance. For simpler models, smaller scale information such as 5 x 5 and 10 x 10 performed best, especially for species richness. These scales may be more relevant to whole fish assemblages rather than larger scales, which may overlook important nearby habitat features. For more complex models, spatial scale did not affect the model performance for either species richness or species abundance.

Relative influence of environmental covariates

Multiple environmental variables influenced the relative species abundance and species richness of fish assemblages, with depth, proportion of encrusting organisms and calcified reef substratum and average backscatter having the most explanatory value (combined relative influence 45-50%, Appendix Table A1, Fig. 4-3). Within each model, various predictors held greater influence in 'splitting the data', reported as its relative influence (out of 100). Depth consistently held great influence over species richness and abundance and was one of the top variables for all complexities of models (Appendix Table A1), however, depth demonstrated slight differences for species abundance and species richness patterns. From partial dependency plots, where other factors are held at mean values, species abundance showed a slow rise and plateau from 50-170 m depths, then a steep decrease from 170 m to the maximum depths sampled (Fig. 4-4). Species richness followed a similar pattern but began to decrease from a shallower depth of 150 m (Fig. 4-5). Species abundance showed a general (but not uniform) increase with greater proportions of encrusting organisms and calcified reef. Species abundance increases dramatically with average backscatter of approximately the higher values (-20), indicating harder substrates, lower values (-40 to -60, softer sediments) had low abundance. Similar to abundance, higher species richness corresponded with higher proportions of Calcified reef and Encrusting organisms and harder substrates (average backscatter).

Patterns were slightly different in the ranking of important variables but the relative effects of these factors for both species abundance and species richness were similar. For fish abundance, Topographic Position Index was more influential than Slope or Planar Curvature. This may be interpreted as nearby features, such as ridges and valleys, were more important than overall slope steepness or the way the terrain was angled. For species richness, the range of relative influence and rank of rugosity measures (Slope, Topographic Position Index and Planar Curvature) fluctuated among iterations within multiple models, suggesting a more equivocal importance of these variables. Negative TPI values, which indicate the site was more of a 'valley' than a 'ridge', corresponded with higher values for both metrics. This was also similar to the effect of planar curvature on species abundance, which may reflect that fish may be more likely to congregate in areas where the local topography offers greater protection and less exposure. Of the substratum measures, proportion of Calcified reef and Average Backscatter were also the most

influential for species richness and abundance, but steeper slopes and higher proportions of boulder were more important for richness than abundance. Interestingly, spatial variables for Easting (a component of aspect) and Latitude affected fish abundance and richness over a relatively small spatial area (less than one degree of latitudinal change). Species abundance and richness were higher in the northern sites, but mostly uniform throughout the central GBR area studied. Many biotic and substratum measures such as proportion of Coral, Gravel, Bedrock, Indeterminate substratum, *Halimeda* and Plants consistently had very little or no influence on either species richness or species abundance (<5%).

There were no significant two-way interactions among variables for both species richness and abundance BRT models (Friedman's statistic, Appendix Table A2). Friedman's H-statistic values range between 0 and 1 and is a relative effect scale of non-linear interactions. Models were reduced to 16 variables (gbm.simplify recommended 4-8 variables for removal for species abundance and 7-8 variables for species richness) with proportions of mud, indeterminate, gravel, boulder, bedrock, *Halimeda*, coral and rubble removed. Some of these features may have greater importance in determining the composition of the assemblage, but for relative metrics of richness and abundance the specific species do not matter, and these differences might reflect species-specific preferences. Friedman's values were all less than 0.2, with slightly higher values, indicating stronger relationships, for species richness. These small values likely reflect more highly complex relationships between variables, the need for greater sampling to tease out more patterns, or variation from unaccounted sources.

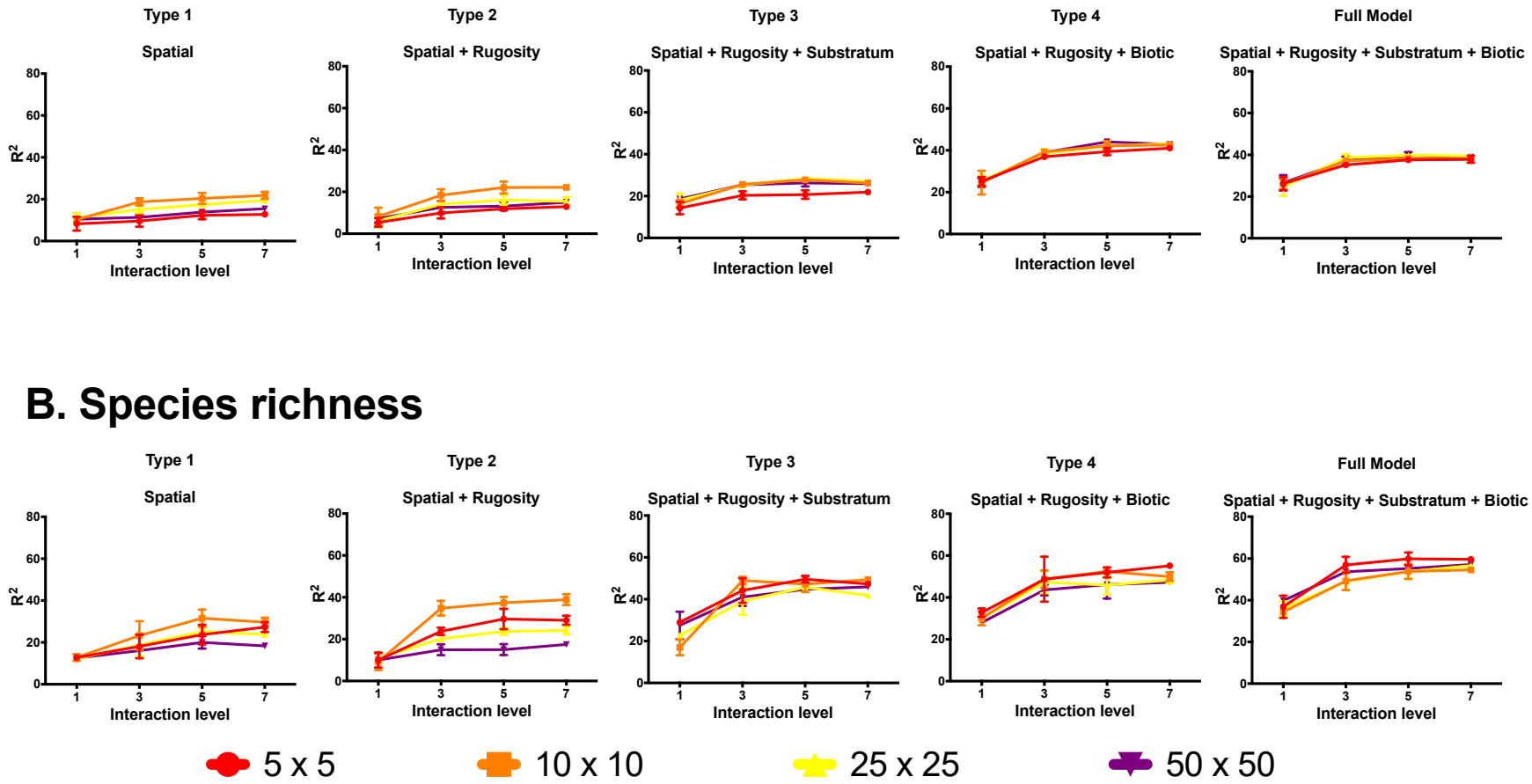


Figure 4-2: Multiple Boosted Regression Tree models explaining (A) species abundance and (B) species richness including spatial, rugosity, substratum, and biotic variables and multiple spatial scales (5 x 5, 10 x 10, 25 x 25 and 50 x 50) and with increasing complexity levels (i.e. greater interactions, 1-7). Each model was run for three iterations and R^2 values are reported as average values with standard deviations).

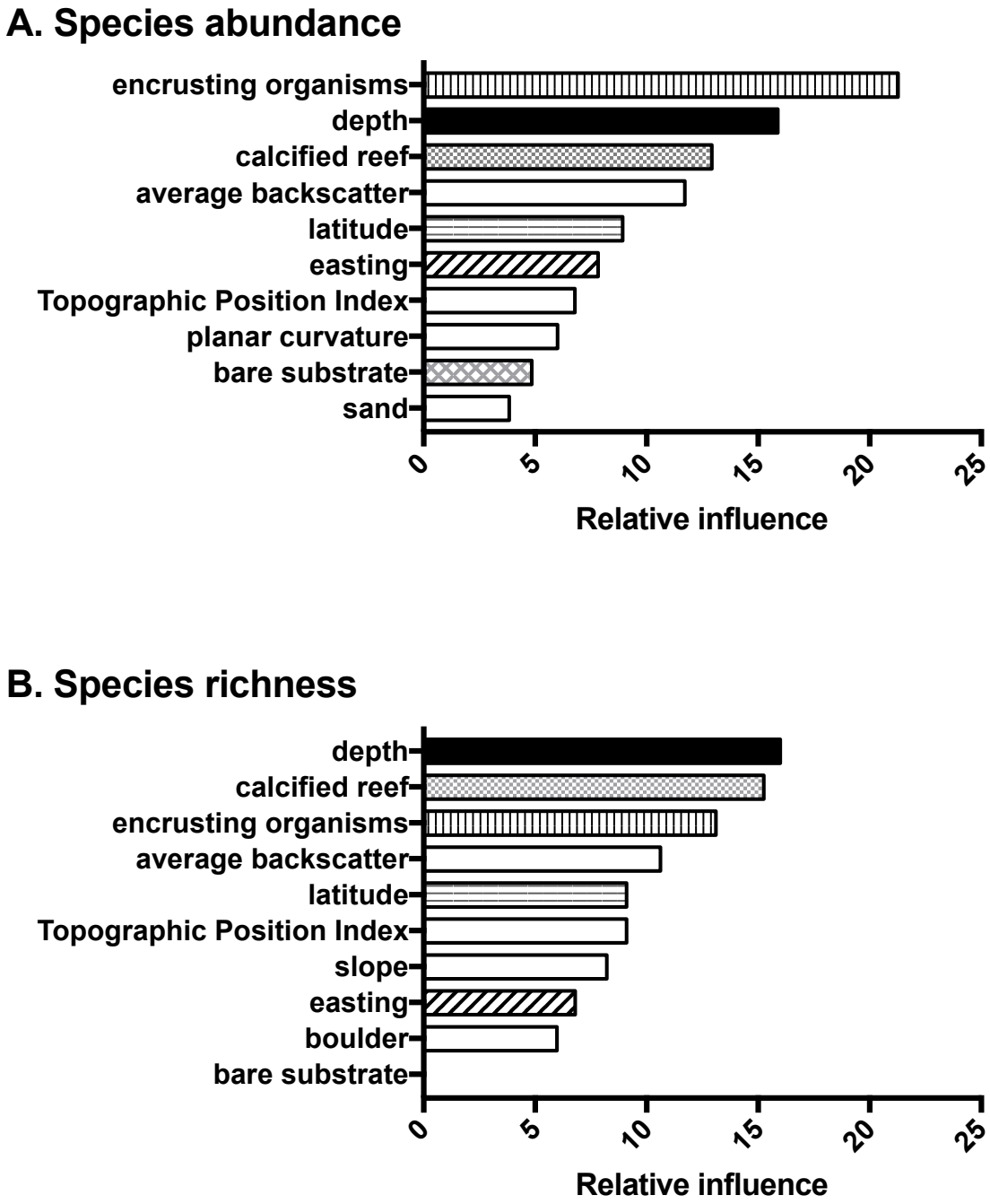


Figure 4-3: Relative influence of the ten most important environmental variables affecting (A) species abundance and (B) species richness on deep Great Barrier Reef shelf-break habitats. Environmental variables are from the 10 x 10 spatial scale, but other spatial scales have shown similar variable influence levels and rankings.

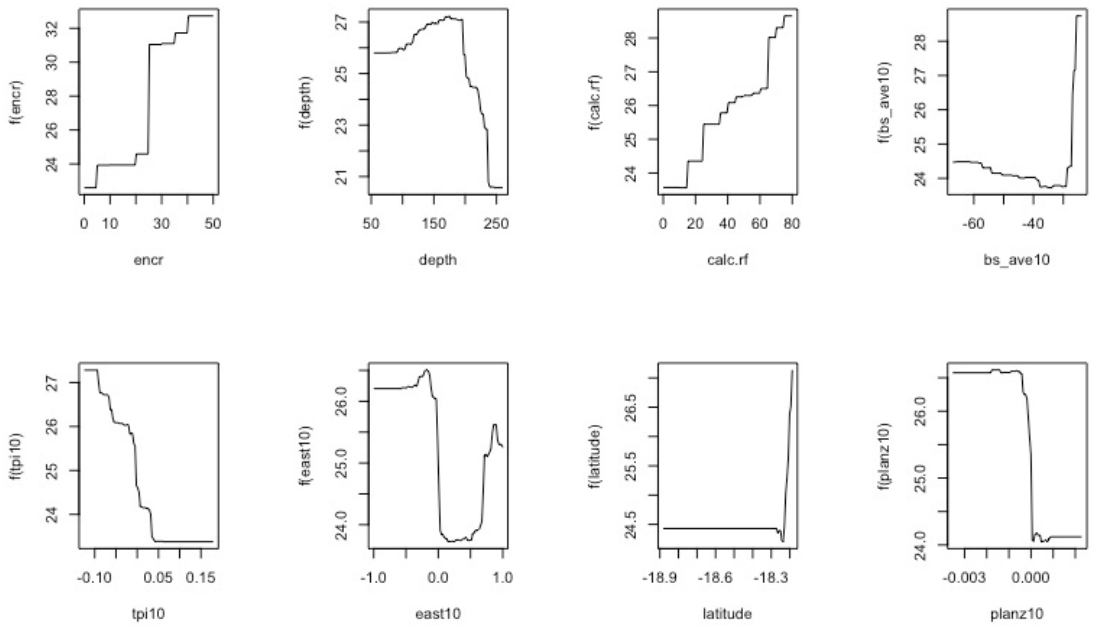


Figure 4-4: Partial dependency plots showing the average effects of select environmental variables (if other variables are held constant) on species abundance. The most influential variables for species abundance were proportion of encrusting organisms, depth, proportion of calcified reef, average backscatter, Topographic Position Index, easting (a component of aspect), latitude and planar curvature. All multibeam derivatives were taken at the 10 x 10 spatial scale.

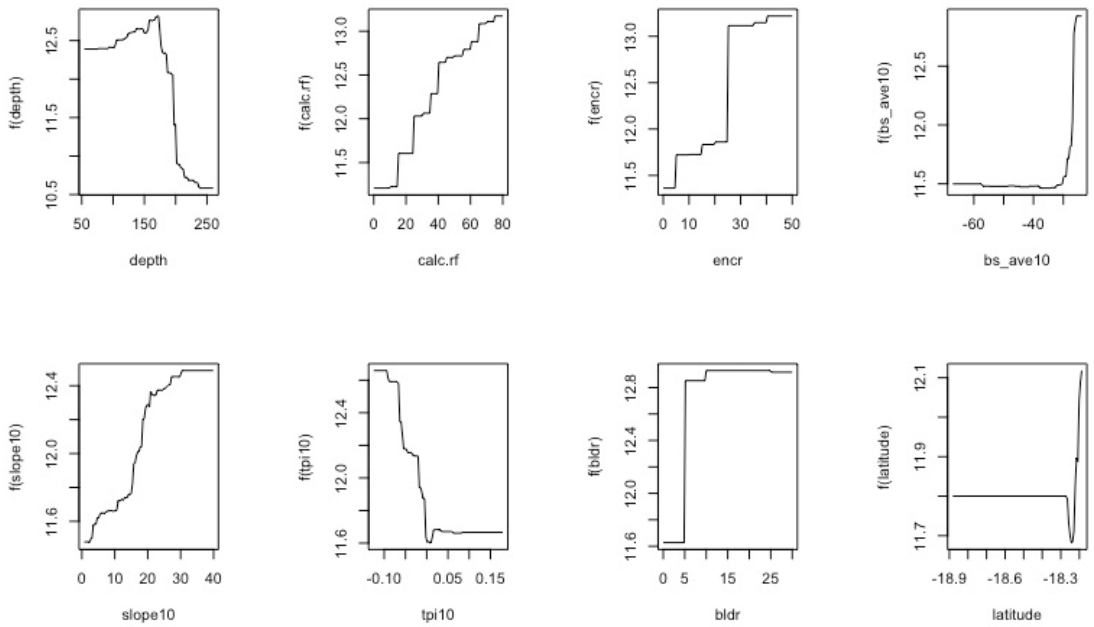


Figure 4-5: Partial dependency plots showing the average effects of select environmental variables (if other variables are held constant) on species richness. The most influential variables for species richness are depth, proportion of calcified reef, encrusting organisms, average backscatter, slope, Topographic Position Index, proportion of boulders, and latitude. All multibeam derivatives were taken at the 10 x 10 spatial scale.

Discussion

Deeper marine reefs are vastly understudied compared to their shallow water counterparts, but the advancement of remote sensing technologies may be critical to filling in the necessary knowledge gaps. Incorporating greater habitat information improved species richness and abundance model performance with the most valuable information coming from depth, the proportion of encrusting organisms and calcified reef structure. Topographic information such as the Topographic Position Index (a relative measure of rugosity) and average backscatter (an indication of underlying substratum), both derived from multibeam bathymetry and backscatter layers, can improve future predictions of deep-reef fish biodiversity. There were some slight differences between models for species richness and abundance, so the types of environmental variables considered are important for accurately modelling fish assemblages. For instance, models that included spatial, rugosity, biotic and substratum information were better fits for overall species richness, while models that included spatial, rugosity and biotic information more accurately portrayed species abundance. Some of the model subtleties indicate that the drivers of species richness and abundance are more similar than different, which means that management strategies to conserve species of interest or overall biodiversity would have positive outcomes for both.

It is therefore imperative to gather more information on the benthic communities of deeper reefs and to ground-truth the information derived from multibeam layers. While multibeam can greatly enhance the current information of deeper environments, I found direct observations of epibenthic and substratum components (from the BRUVS field-of-view) increased the explanatory power of BRT models than models with only multibeam data measures. This was similar to other studies using regression tree models for species richness and abundance (e.g. Yates et al. 2016) and patterns on whole fish assemblages described in Chapter 3. Higher proportions of encrusting organisms and calcified reef structure were positively correlated with higher relative species richness and abundance.

The habitat components that will be important in predicting fish biodiversity will depend on the range of depths incorporated; thus we need more comprehensive information on the benthic community. While the use of multibeam can provide high-resolution information relatively quickly, the predictive ability of models is only as good as the ecological understanding of how fish associate with the epibenthos and substrata on deep reefs. For instance, multibeam data cannot capture some ecologically meaningful features, such as the presence of macroalgae, which can be present on both hard and soft substrates at mesophotic depths (Kahng et al. 2017). In shallower MCE studies, the proportions of epibenthic cover generally decreased with depth. However, in those shallower depths encrusting coralline and calcareous algae were more important components of the benthic community (Kahng et al. 2010, Rooney et al. 2010). Species

richness was correlated with coral cover in studies of upper mesophotic depths (Garcia-Sais 2010, Kahng et al. 2014, Kane & Tissot 2017). Coral cover had very little effect in these BRT models, but this is not surprising since the depth gradient was very large. In shallower habitats (<100 m), coral cover would likely have had a larger measured role. In this study coral cover had limited influence but calcified reef, or habitat formed, was more important. The influence of encrusting organisms, calcified reef and depth in the best fitting models was similar to habitat components that better predict demersal fish patterns in other geographic locations (e.g. Moore et al. 2009, Moore et al. 2011, Malcolm et al. 2016). Many habitat measures may be direct or proximal variables that suggest certain ecological or physiological limiting factors such as food, shelter, orientation to currents or competition (Moore et al. 2009, Monk et al. 2010), and the subtle differences in species richness versus species abundance responses indicated this is true among the reefs studied here. More immediate habitat features affected species richness and abundance, which indicated that the variance in fish biodiversity is influenced on the scale of habitat patches within a mosaic rather than broader environmental drivers.

A key advantage of using remote technologies is that the measurements and methods used are easily replicable for a larger sampling design and future research can extend these models of fish biodiversity for other parts of the GBR as well as for comparing global MCE biodiversity patterns. Despite differences in sampling approaches, the overall patterns of fish biodiversity over similar spatial scales may not preclude broader comparisons on the regional or possibly global scale. Previously, Monk et al. (2010) postulated that better models to describe species-habitat relationships may be achieved if variables were generated at multiple spatial scales. However, I found that the use of multibeam derivatives showed only marginal differences over the spatial scales compared. This means that broader comparisons with differing resolutions of information may still be useful for comparing simpler univariate assemblage metrics. Similar to this study, studies comparing various prediction models (i.e. GAMs, GLMs, BRTs, MAXENT) found that increased complexity (i.e. more explanatory variables) and the ability to account for non-linear relationships among key habitat variables generally improved predictive performance for demersal fishes (Moore et al. 2009, Monk et al. 2010, Oyafuso et al. 2017). Further, the model comparisons show an adequate level of predicting species richness and abundance can be achieved with at least key spatial and rugosity measurements. This opens up the possibilities of supplementing bioregion maps with layers of scientific multibeam datasets, spatial information from advanced commercial fishing software such as WASSP multibeam, and fishing-log information for identifying potentially vulnerable habitats and fish assemblages.

There are alarming potential environmental and anthropogenic threats to deeper marine environments as often the ‘out of sight, out of mind’ mentality prevails for much of marine research. The amount of research effort to habitats >30 m deep is glaringly insufficient

considering the amount of basic ecological information still unknown. Despite the expansive research efforts on the shallower GBR, there is very little scientific information on how deeper GBR habitats may be affected by anthropogenic and environmental stressors. There are no baseline data to support if these deeper habitats are already subjected to some of these threats. In this study, I assumed that the deeper habitats sampled were under minimal fishing pressure, with no anthropogenic damage to the benthos (most of the reefal sites are in zones closed to fishing and >100 km offshore) and sampling occurred prior to the widespread coral bleaching of the GBR (Hughes et al. 2017). However, future studies should determine how the degradation of deep-reef seascapes will impact the spatial distribution of fish. This topic is particularly foreboding, with mining operations adjacent to the GBR expected to increase, and the dumping of dredge-spoil in deep waters had been proposed in 2015 as a solution for dealing with associated waste. The consequences of such action are uncharted territory, but the effects of the physical alteration to benthic habitats could be profound. Dredge-spoil may produce a 'blanket of sediment' that smothers benthic communities (Beaman et al. 2016), reducing topographic complexity and habitat availability (Pittman et al. 2010). Similar influxes of sedimentation have been observed after hurricanes in mesophotic depths (Rocha et al. 2018). Previous cyclone reef damage had been documented at Myrmidon Reef in the GBR at mesophotic depths (50-65 m) on outer-shelf reefs (Bongaerts et al. 2013) and climate change projections expect the frequency and intensities of tropical cyclones may increase and thus increase the risk of physical cyclone damage. Deep-reef habitats may also be vulnerable to other effects of climate change, as climate modelling generally predicts the thermocline may deepen and upwelling may become less frequent and weaker (Pitcher et al. 2007), consequently affecting deeper ecosystems shaped by these subtler oceanographic influences. Increased research on deep-reef fish assemblages is required for the GBR and similar tropical environments.

I have demonstrated that remote methods can provide important baseline information of deep-reef fish assemblages, could contribute to the identification of conservation hot spots, but also be used for forecasting how these assemblages may change in response to anthropogenic and environmental threats, including climate change. I suggest increasing research efforts of fish and benthic environments along the entire Great Barrier Reef shelf-break, including the spatial replication of reef and inter-reefal habitats. The geomorphology of the GBR is quite distinct in the northern, central and southern sections, and there is now evidence that there is substantial variation in deeper fish assemblages among reefs and habitat types. As a minimum, multibeam bathymetry information should cover the latitudinal extent as it could be an important measure of changes to the rugosity of deepwater habitats over time and identify conservation priority areas that may be hotspots of biodiversity. More often and more widespread use of multibeam

bathymetry and BRUVS could greatly enhance our knowledge of shelf-break habitats., providing vital information on these critically unique ecosystems.

Chapter 5 Deep-reef fishes and the importance of habitat for deepwater fisheries

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Abstract

With deep-reef ecosystems facing increasing fishing pressure, there is a critical need to understand the importance of habitat for associated fishes. Worldwide, reefs in mesophotic and sub-mesophotic depths (>50 m) support mixed-species fisheries of tropical snappers, emperors, jacks and groupers. For the majority of these species little information exists on species-specific fish-habitat associations. In this study, I assessed each species' habitat associations using presence-absence data from Baited Remote Underwater Video Stations (BRUVS) and habitat information from the BRUVS field-of-view and derived from multibeam bathymetry and backscatter for sites from the Great Barrier Reef (GBR) shelf-break, Australia, in 54-260 m depths. While habitats do vary with depth, fish species showed strong depth and habitat-related preferences, and the variation in habitat was a good predictor of where many species would be found. Several deep-reef fish species had moderate to strong habitat associations, including the deepwater snappers (*Pristipomoides typus*, *P. argyrogrammicus*, *P. filamentosus*, *P. multidens, Lutjanus bohar*), emperors (*Lethrinus rubrioperculatus*, *L. miniatus, Gymnocranius euanus*), onion trevally (*Carangoides caerulepinnatus*), grey reef shark (*Carcharhinus amblyrhynchos*), and smaller species including the yellow-stripe threadfin bream (*Pentapodus aureofasciatus*), rose-banded fairy wrasse (*Cirrhilabrus roseafascia*) and starry triggerfish (*Abalistes stellatus*). Smaller species unique to deep habitats, including *Cirrhilabrus* spp. and *Terelabrus rubrovittatus*, are frequently observed in deeper depths but these species have only recently been described and habitat preferences not been well-established. Here I review the existing information on depth and habitat associations and use local GBR distributions for empirical data. Many species of deep-reef fishes were limited to deeper habitats or were offshore and semi-pelagic species. Further, many species have not been found in shallow (<80 m) BRUVS studies on the GBR continental shelf. The inherent vulnerability of these species is a two-fold jeopardy of restricted depth distributions and specific habitat requirements that relate to reef architecture and habitat-forming biota. It is critical that conservation strategies to protect slope environments are implemented quickly to avoid localised extirpations.

Introduction

While the ecological and economic importance of tropical deep-reef fishes is becoming increasingly apparent, recent technological advancements have made deep fishing both easier and more efficient (Sumpton et al. 2013), and in many locations deeper fishing is now occurring at an industrial level (Roberts 2002, Norse et al. 2012), over greater spatial scales, and in previously unexploited environments such as deeper slope habitats (Grandcourt 2003, Morato et al. 2006). The removal of individuals by fishing can alter both the population and assemblage structure of exploited fishes, impacting their resilience to anthropogenic and environmental disturbances, while some fishing techniques can damage benthic habitats, reducing their complexity and diversity (Jennings & Kaiser 1998, Auster & Langton 1999). Deepwater demersal and benthopelagic fish assemblages are vulnerable to fishing pressure (Morato et al. 2006, Cheung et al. 2007, Williams et al. 2013) and the rate of information gathered on deepwater fish stocks does not keep pace with the intensity of fisheries exploitation (Haedrich et al. 2001).

In the Indo-Pacific, deepwater fisheries generally target multiple species, focusing on commercially-valuable deepwater snappers (Lutjanidae), emperors (Lethrinidae), groupers (Serranidae), and jacks (Carangidae; Ralston & Polovina 1982, Ralston & Williams 1988, Williams & Russ 1994). Many of the fishes have life history traits that make them vulnerable to fishing, including long lifespans, late maturation, and slow growth (Fry et al. 2006, Andrews et al. 2011, Andrews et al. 2012, Williams et al. 2013, Newman et al. 2016). Despite the widespread fishing effort in Indo-Pacific countries and territories, critical ecological information is missing and the amount of deep-reef habitat may limit the production of these fisheries (Ralston et al. 1986). Within a multispecies fishery, species will have varying life history characteristics (Heupel et al. 2010b, Newman et al. 2016) with some life history stages requiring specific habitat conditions. This difference in species' relative habitat needs may make some species more vulnerable to habitat declines. Therefore, incorporating habitat information into fisheries management may better capture whole assemblage or ecosystem-level dynamics (Sainsbury 1988, Leslie et al. 2003).

Identifying how habitat determines the distribution and abundance of deeper fishes is essential for ecosystem-based fisheries management. Research on Mesophotic Coral Ecosystems (MCEs) demonstrate these deeper environments are mosaics of topographical and biological diversity with underwater ridges, valleys, deep macroalgal beds, coral and sponge gardens (Abbey et al. 2011, Slattery et al. 2011, Bridge et al. 2012b, Spalding et al. 2013, Beaman et al. 2016). Shallower reef fish ecology studies indicate that fish distributions often reflect complex patterns of habitat use of multiple habitat types, such as reef and inter-reefal areas (Cappo et al. 2007, Grober-Dunsmore et al. 2007), seagrass and mangrove nurseries (Shibuno et al. 2008), and shallower and deeper habitats (Cappo & Kelley 2010, Cappo et al. 2012). However, the role of

deep habitats in sustaining fish diversity and abundance is poorly understood and it is imperative we increase the current understanding of these habitat connections. For instance, investigating the relationship between topography and fish assemblage structure can identify critical habitats that support ecologically important species throughout their life cycles (Fitzpatrick et al. 2012). Few juveniles of deepwater snapper species have been observed but juveniles often require specific nursery environments and shift to different habitats as adults (Ellis & DeMartini 1995, Moffitt & Parrish 1996, Parrish et al. 1997, Misa 2013). Not knowing where and when juvenile fish preferentially settle and grow is a substantial roadblock to effective fisheries management. There is some evidence that deep fish-habitat relationships can have important demographic consequences. For instance, older, larger and more fecund individuals of damselfish *Stegastes partitus* were found in mesophotic (60-70 m) habitats than shallower sub-populations (Goldstein et al. 2016a). Larvae of the reef-associated but oceanodromous amberjack (*Seriola dumerili*) peaked in abundance at 150 m depths but were also found much deeper in 250 m (Raya & Sabatés 2015). The hapuku, *Polyprion oxygeneios,* and the Atlantic wreckfish, *Polyprion americanus,* are deepwater fishes with long pelagic juvenile stages, remaining in the oceanic waters for up to four years (Roberts 1996, Francis et al. 1999, Sedberry et al. 1999, Machias et al. 2003), while the another polyprionid, the giant sea bass, *Stereolepis gigas,* has a much shorter pelagic larval duration of a month (Gaffney et al. 2007). Deeper habitats may be an important refuge for fishery species as larger individuals are frequently observed in deeper, mesophotic depths (Williams & Russ 1994, Lindfield et al. 2016).

The Great Barrier Reef Marine Park (GBRMP) is one of the largest marine reserve networks and presents an opportunity to investigate species-specific habitat associations where there is presumed lower levels of fishing activity on the deep-reefs of the continental shelf-break. There is currently insufficient information on the extent of deepwater fishing activities in Queensland, especially accurate information on catch composition in the tropical regions (Sumpton et al. 2013). The width of the GBR varies and is narrower in the northern section and widens towards the southern section. In the central section the shelf-break is approximately 120 km offshore. While the benthic environment of the shallower GBR has been studied (e.g. Pitcher et al. 2007), the available information on deep-reef environments (>100 m depths) is limited. While there is incidental representation of deeper environments included in the GBRMP network (Bridge et al. 2016a), more work is necessary to determine if this level of protection is sufficient to safeguard marine resources. Marine reserve networks may not sufficiently protect more mobile species; and knowledge gaps in the life history of these species may expose them to greater levels of vulnerability during larval, juvenile or adult stages.

Baited Remote Underwater Video Stations (BRUVS) have been widely used in Australia and the GBR to survey fish and benthic habitats (Speare et al. 2004, Cappo et al. 2007, Fitzpatrick

et al. 2012, Harvey et al. 2013, Monk et al. 2017); however, they have not been explicitly used to sample areas deeper than 100 m in the GBR. Research sampling of deepwater fishes has often occurred via fishery-dependent methods (e.g. trawls, traps; Williams et al. 1995, Newman & Williams 1996, Last et al. 2014) and with limited means to observe fish and habitats *in situ*. Fishery-dependent methods also selectively sample depending on the types of fishing gear used (e.g. hook or mesh size) and are often restricted to economically important species because of time and cost (i.e. fisheries development and single-species stock assessments; Cappo et al. 2004, Cappo 2010). BRUVS can be used safely in mesophotic (light-limited) and deeper environments and provides information on the benthic environment. Multibeam bathymetry provides information on the three-dimensional structure of marine environments and provides useful information on spatial differences at deep depths. Combining multibeam bathymetry with similar video survey techniques has proved useful for species-habitat investigations (e.g. Moore et al. 2016a, Oyafuso et al. 2017). Species-habitat information can be used to map habitat suitability or species distributions, providing managers with important information to justify marine protected areas.

I hypothesized that fishes would have different levels of habitat association and that these levels were due to differences in ecological niche (i.e. trophic group, mobility). The degree of habitat specialization between fishes can differ between closely related species and individuals of the same species (Wilson et al. 2008, Heupel et al. 2010b, Babcock et al. 2017), and often this information can complement whole assemblage measures such as relative species richness and abundance. In this chapter I reviewed the available species-habitat information and local GBR distribution information for fishes frequently seen in deep-reef BRUVS. I analysed multibeam and BRUVS information using Boosted Regression Trees (BRTs) to determine what habitat factors influenced the presence or absence of fish of the central GBR shelf-break reefs. This research provides preliminary empirical data on local species distributions.

Methods

BRUVS and multibeam data

Fish in deeper habitats were surveyed using deep Baited Remote Underwater Video Stations (BRUVS) deployed from 54-250 m depths along the GBR shelf-break. Deep BRUVS were similar in premise to BRUVS used for previous studies in shallower reef and inter-reefal locations of the GBR, but required a few modifications. The single Sony video camera (HDR-CX110) was illuminated with a white spotlight because of diminishing light with depth. The camera housing was encased in a purpose-built aluminium housing rated to withstand pressures up to 300 m depths. A bait bag extended into the field-of-view made of plastic mesh filled with crushed *Sardinops sagax*. All 48 BRUVS deployments in 2014 occurred during the daytime

(0700-1700) with limited prior information other than approximate bathymetry and depth but were depth-stratified to survey a range of depths and possible habitats.

Each video was analysed for the MaxN of each fish species identified to lowest taxonomic designation where possible. MaxN is the highest number of individuals of a species per frame per deployment and is a conservative metric to estimate the relative abundance of species per deployment (Cappo 2010). Substratum and epibenthic habitat information was estimated (to the nearest 10%) from what was visible in the BRUVS field-of-view into multiple categories. Substratum categories included bedrock, boulder, calcareous reef, mud, gravel, rubble, sand and indeterminate habitat. Epibenthic categories were presence of filtering organisms (e.g. sponges, hydroids, sea-fans and whips), plants, *Halimeda*, encrusting organisms (e.g. bryozoans and coralline algae), coral and bare epibenthos (i.e. no visible epibenthic cover).

Habitat information was also derived from multibeam sonar bathymetry and backscatter layers interpolated from multi-scale terrain analysis. Multibeam derivatives were extracted from a small 'window' size (kernel size with radius values of 5 raster pixels). Multibeam yields three-dimensional habitat information on the relative topography of marine habitats. For instance, Topographic Position Index (TPI) indicates relative ridges and valleys. Slope values indicate the relative steepness of a site. Easting and Northing are components of orientation or aspect (what direction the site faces). Topographic Ruggedness Index and Surface Ratio indicate how rough or smooth the neighbourhood is. Total Curvature, Planar Curvature and Profile Curvature all indicate how convex or concave a site is, indicating how water might run off down a surface.

The effect of bait should not be ignored and is an important caveat to fish surveys with baited methods. Bait is an attractant and some fishes are drawn into the field of view because of either bait, light or activity associated with the BRUVS (Harvey et al. 2007, Merritt et al. 2011, Dorman et al. 2012, Harvey et al. 2013). BRUVS have been shown to have a great capacity to sample whole fish assemblages compared to many fishery-dependent methods, such as trawls (Priede & Merrett 1996, Cappo et al. 2004) and fish traps (Harvey et al. 2012, Bacheler et al. 2013). Due to logistical constraints of sampling deep environments, stationary BRUVS may have better success than more 'mobile' methods such as diver-operated video surveys (e.g. Andradi-Brown et al. 2016c), ROVS and drop cameras (e.g. Easton et al. 2017, McLean et al. 2017) to sample whole fish assemblages linked to a particular habitat type in deeper environments. Each sampling method has some inherent bias; however, these have been reviewed for BRUVS and the occurrence of fishes sampled is considered to be sufficiently representative of the fishes across families within the neighbourhood of the BRUVS (Mallet & Pelletier 2014, Whitmarsh et al. 2017).

Review of deep-reef fish-habitat associations

I reviewed the information on fish-habitat associations through multiple search platforms including the topical research databases FishBase.org (Froese & Pauly 2018), FishesofAustralia.net.au (Bray & Gomon 2018), and Mesophotic.org (2018), the general research databases IUCNredlist.org (IUCN 2018), Web of Science (2018), and the broader academic search engine GoogleScholar (2018). Search criteria varied by platform. For Fishes of Australia, FishBase.org, IUCNredlist.org, and Mesophotic.org, which is a newer platform still compiling reference literature, only the species name was used. For Web of Science and GoogleScholar the search included specific mentions of 'habitat' (species scientific name AND habitat*). Multiple potential spellings of some species were included in the search criteria (e.g. *Carangoides caeruleopinnatus* is sometimes *C. coeruleopinnatus*). Known synonyms were not used as often this information may refer to one or more species. There were advantages of using multiple platforms, for instance, Web of Science in general had the fewest results while GoogleScholar provided references from the 'grey' literature, including technical reports from government and non-governmental agencies. Information from artificial habitats (e.g. gas/oil pipelines, Fish Aggregation Devices, marinas/piers) was not considered for this review. Information on fishery potential was based on FishBase information. Searches were restricted to the species of interest in this chapter that more frequently appeared in BRUVS (> 5 sites) and may have had sufficient information to quantify habitat associations. Some particular species had a larger representation in the available body of literature, while others had scant habitat information. Habitat information was not investigated for the two species not identified to species-level (i.e. did not match any known described species), but the *Selenanthias* sp. may be *Selenanthias barroi* (pers. comm. Tony Gill), which has little-known ecological information but found in depths to 300 m in the Western Pacific. *Parapercis* sp. is either one or more species and often was not able to be taxonomically distinguished for confident species identification from video.

Species-specific habitat associations

To test the strength of habitat associations for each fish species, each individual species' abundance (MaxN) was converted to presence-absence data and compared using Boosted Regression Trees (BRTs) to identify the most explanatory habitat gradients affecting a species' distribution. Of the 130 species identified in the BRUVS deployments, only 28 species occurred at five or more sites (10% of deployments). A separate BRT for each species was run to identify the specific habitat variables driving its distribution. BRTs fit many simple models to find an optimal solution (De'Ath 2007), are able to fit relationships with various predictor types, and can allow for complex interactions between predictors (Elith et al. 2008). Gradient boosting models

optimize prediction by calculating the deviance of each model and minimizing it the next iteration (Friedman 2001, De'Ath 2007, Elith et al. 2008). Regression trees are successful modelling techniques for predicting fish species richness (e.g. Chapter 4, Cappo et al. 2007, Pittman et al. 2007, Knudby et al. 2010) and can determine the most important variables averaged over many trees (De'Ath 2007). Sometimes 'messy' statistical problems like outliers, predictors measured on different scales and other issues associated with other methods are not as problematic because of the building and pruning steps in analysing BRTs (De'Ath 2007, Elith et al. 2008), which make BRTs an excellent quantitative diagnostic tool for preliminary investigations.

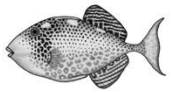
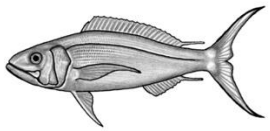
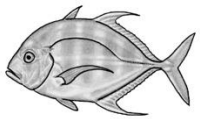
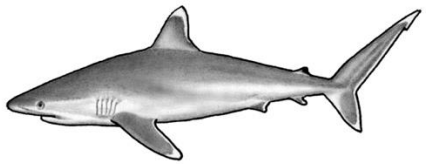
The first step of building BRT models included 24 explanatory variables (multibeam derivatives and epibenthic and substratum proportional measurements) with highly correlated variables removed. Then, gbm.step was used to evaluate models of interaction depth 3 (main effects and interactions) to identify the optimal learning rate and number of trees. BRT models with all 24 variables were run five times each to see if there was evidence of habitat associations, from these initial models only variables with a relative influence >5 were included in final models. The amount of variance explained divided by the total variance (R^2 or 'goodness-of-fit') was used to compare the strength of the BRT models. Only species with evidence of a moderate or strong habitat association ($R^2 > 0.5$) were then fitted with final BRT models that included 4-8 explanatory predictors. The number of variables does not impact the influence of other variables and less informative predictors are lower in 'relative importance' or the number of decision trees for which that variable was important in splitting the data (Elith et al. 2008). BRTs used individual species presence-absence data with a Bernoulli distribution, training fraction of 75%, an interaction depth of 3 (for moderate model complexity), learning rate of 0.001, bag fraction of 50% (added stochasticity), 5 cross-validation folds, 10,000 trees and conservative 'out-of-bag' estimates. Each BRT model was run for five iterations and the range of R^2 values and explanatory variables relative influence were summarised. For these purposes, I used R^2 as a measure of the relative predictability of a species presence-absence based on the measured environmental variables, with moderate associations (R^2 = 30-60) and strong associations ($R^2 > 60$). Partial dependency plots were plotted for each habitat covariate, which demonstrate the effect of that particular variable when all other covariates are held constant.

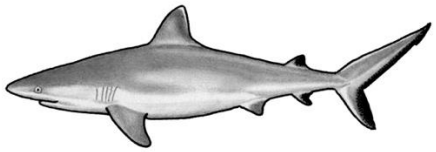
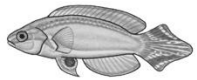
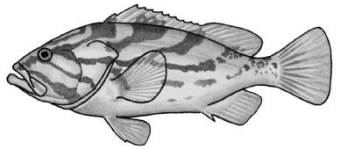
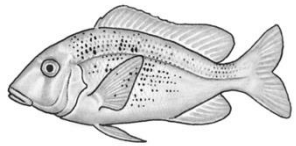
Results

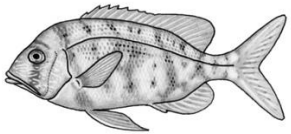
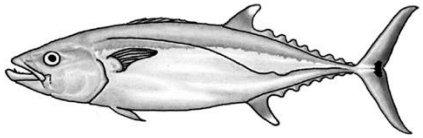
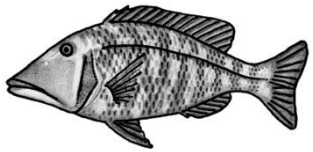
Review of fish-habitat associations for deepwater snappers

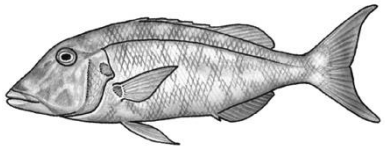
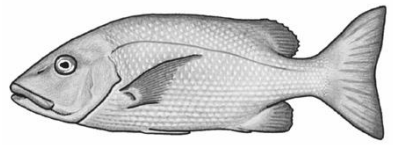
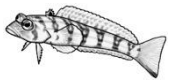
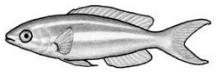
Of the 130 species that were identified from BRUVS, 26 were investigated for available habitat information. Many of the fishes are listed as 'reef-associated' by Bray & Gomon (2018), but in general there was limited species-specific quantitative habitat information and most information referred to broad habitat or depth categories without specific information on the physical and biological components of the local environment that may affect a species' distribution (Table 5-1). Some species had explicit information on habitat use and measurements of relative mobility/residency in the GBR (e.g. Currey et al. 2014b, Espinoza et al. 2015b). Often habitat use is categorized into shallower or deeper categories with slightly different depth definitions and this is reflected in the summary information. Some habitat information was related to fishing effort, so species *G. grandoculis* and *L. rubrioperculatus* were described as inhabiting 'trawling grounds', which are presumably the areas of lower complexity. Species that inhabit softer sediments are at higher risk from trawling, where it is allowed (Stobutzki et al. 2001). Most references to depth were repeated from other sources, and as depth ranges were discussed in Chapter 2, I have excluded reporting most depth ranges here as they may differ between ontogenetic stages or if they were likely carried over from other sources.

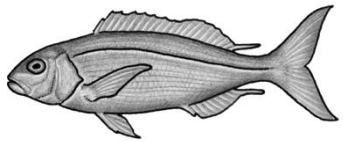
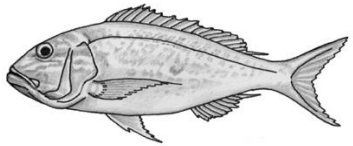
Table 5-1: Published information on fish-habitat associations of fishes commonly inhabiting deep reefs. Information is compiled from original sources where possible but some amalgamated information from databases is also included.

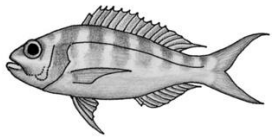
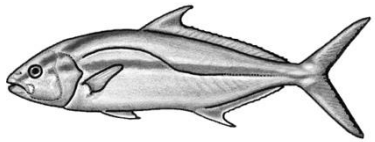
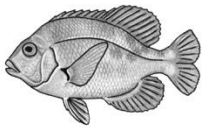
Species	Fishery	Published habitat information
Abalistes stellatus 	No	Reef-associated (Bray & Gomon 2018). Mud, silt or sand (Kuiter & Tonozuka 2001), deep slopes (FishBase). Sand, sponge, weed habitat (Hutchins 1984). Sandy habitats (Stowar et al. 2008, Wahab et al. 2018). Juveniles found among rubble/debris on open substrates (Kuiter & Tonozuka 2001).
Aphareus rutilans 	Yes	Reef-associated (Bray & Gomon 2018). Coral reef/rocky-bottom areas (Fishbase). Midwater (Chave & Mundy 1994). Occurrence associated with lower (shallower) slopes (Oyafuso et al. 2017). Rocky areas (Sumpton et al. 2013) Rare on shallow outer-shelf reefs (0-15 m) but occasionally found on deep outer-shelf reefs (15-100 m) and deep-reef areas (>100 m, Newman & Williams 1996).
Carangoides caeruleopinnatus 	Yes	Reef-associated, sand (Bray & Gomon 2018). Deep coastal reefs (Fishbase). Habitat generalist (Wahab et al. 2018). Inshore GBR (Cappo et al. 2007)
Carcharhinus albimarginatus 	Yes	Pelagic, oceanic (Bray & Gomon 2018). Benthopelagic (Fishbase). Inside lagoons, near drop-offs and also offshore (Compagno et al. 2005). Habitat generalist (Tickler et al. 2017). Resident at coral reefs for long periods but complex movement patterns; roamed between multiple reefs and often deeper in the water column during the day than at night (Espinoza et al. 2015a). Offshore habitats near reefs, may be using inter-reefal areas (Espinoza et al. 2014). Prevalent in deep offshore habitats (Cappo et al. 2007, Ceccarelli et al. 2014). Occurrence greater in the outer-shelf, absent from inshore GBR, higher probability in southern GBR among sites with higher algae and coral cover (Espinoza et al. 2014).

Carcharhinus amblyrhynchos 	Yes	Reef-associated (Bray & Gomon 2018); Midwater (Chave & Mundy 1994). Occur on reef slopes, not reef flats (Rizzari et al. 2014). Aggregate on outer reef slopes/crests/drop-offs with strong current flow (McKibben & Nelson 1986). Habitat generalist (Tickler et al. 2017). Size-related changes in habitats and movements (Heupel et al. 2010a). Structurally complex habitats in close proximity to hard substratum; offshore areas (Espinoza et al. 2014). Most tagged sharks stayed at the same reef for long periods of time; males disperse more frequently, 6-45 km distances (Espinoza et al. 2015b).
Cirrhilabrus roseafascia 	Aquarium	Reef-associated (Bray & Gomon 2018); Reefs, rock or rubble substrates (Fishbase). Rubble and coral 30-90 m, steep patches near clearings, often in proximity to epibenthos (e.g. gorgonians, sponges, black coral and Tubastraea; Tea 2015).
Echeneis naucrates	Yes	Pelagic, oceanic, reef-associated (Bray & Gomon 2018); Oceanic-pelagic (Gasparini & Floeter 2001). Free-swimming around coral reefs (IUCN).
Epinephelus morrhua 	Yes	Slopes of islands, seamounts or continental shelves (IUCN). Epinephelus spp. found near caves and overhangs (Sink et al. 2006). Coral reefs and rocky areas (Sumpton et al. 2013)
Gymnocranius euanus 	Yes	Reef-associated (Bray & Gomon 2018). Sand/rubble adjacent to reefs (Fishbase). Occasionally on (0-15 m) outer-shelf reefs and deep reefs (>100m) but most frequent in deeper outer-shelf reefs (15-100 m; Newman & Williams 1996).

Gymnocranius grandoculis 	Yes	Reef-associated (Bray & Gomon 2018). Rocky substrates, juveniles on muddy substrates (Fishbase). Trawling grounds and offshore rocky substrates (IUCN). Habitat generalist (Wahab et al. 2018). Occasional in mid-shelf and outer-shelf reefs (Newman & Williams 1996), particularly the lagoon and back-reef of shallower outer-shelf habitats (Newman et al. 1997). Northern GBR deep reefs (Cappo et al. 2007)
Gymnosarda unicolor 	Yes	Reef-associated, offshore (Bray & Gomon 2018); offshore around coral reefs (Fishbase).
Lethrinus miniatus 	Yes	Reef-associated (Bray & Gomon 2018). Shoal and rubble habitats between reefs (Leigh et al. 2006). Ontogenetic migration: adults in sand/rubble, migrate at night to forage (Carpenter and Allen 1989 from Fishbase); juveniles in shallow, inshore seagrass/mangrove areas (Fishbase). More abundant in the southern GBR, and diminishes in abundance towards Cairns/Cooktown where it is rarely encountered and absent north of Cooktown and in subtropical waters around Norfolk Island (Williams & Russ 1994). Southern GBR shallower reefs (Cappo et al. 2007). No information on larval, settlement or juvenile (less than 20 cm) stages, nor known juvenile habitat in GBR (Currey et al. 2014a). Very site-attached (Williams & Russ 1994, Currey et al. 2014b) Adults may move to shallower reefs with advection of deepwater during cyclones (Tobin et al. 2010). Northward or cross-shelf ontogenetic migration hypothesized with individuals moving up to 200 km (Williams et al. 2010a, Currey et al. 2014a).
Lethrinus olivaceus	No	Reef-associated (Bray & Gomon 2018); sandy lagoons and reef slopes; juveniles in shallow sand (Fishbase). Deep, rugose sponge reefs (Wahab et al. 2018). 'More open' gorgonian/seawhip habitats, rubble and sandy substratum adjacent to 20-50 m deep shoals (Stowar et al. 2008). Juvenile *Lethrinus* spp. on seagrass beds during the day and night (Nakamura & Tsuchiya 2008). Juveniles all on seagrass habitat (n=6, 6-9 cm; Shibuno et al. 2008). In the GBR occur from Cairns to Bundaberg (Walker 1975 in Williams & Russ 1994)
Lethrinus ravus	No	On/near reefs (Carpenter & Randall 2003). Rubble fields (Stowar et al. 2008)

Lethrinus rubrioperculatus 	No	Sand and rubble (Fishbase); Deep, rugose sponge reefs (Wahab et al. 2018). Coral reefs and trawling grounds (Allen and Swainston in Williams and Russ 1994). Rubble fields (Stowar et al. 2008)
Lutjanus bohar 	Yes	Reef-associated (Bray & Gomon 2018); steep outer reef slopes (Fishbase); Shallow hard coral reefs. In the GBR most common on outer-shelf and Coral Sea reefs, and also found on mid-shelf reefs. An aggregation of 500 individuals was recorded off Myrmidon in November 1989 in 23 m depth (Williams & Russ 1994). Northern GBR deep reefs (Cappo et al. 2007). Juveniles only found on tabular coral habitats (Japan, n=5, 7 cm; Shibuno et al. 2008)
Parapercis nebulosa 	Aquarium	Reef-associated, silt, sand, rubble (Bray & Gomon 2018); Silt, sand and rubble substrates (Fishbase); unvegetated bottoms (6-12 m depths, Travers & Potter 2002) "open sedimentary or rubble bottoms" (FAO). Larger grain-size substratum (Schultz et al. 2015).
Pentapodus aureofasciatus 	No, but used as bait for squid fishery in Japan (Motomura & Harazaki 2007)	Reef-associated (Bray & Gomon 2018); coral reefs, rubble (Fishbase). Widely distributed: Ryukyu, Indonesia to Tonga; Australian distribution includes Queensland and New South Wales (Russell 2001). Juveniles are epibenthic on rocky reef slopes and transition to 'mid-water' schools of hundreds of adults and sub-adults (Motomura & Harazaki 2007). Offshore, shallow areas (Cappo et al. 2007). Sandy habitats (Stowar et al. 2008).

Pristipomoides argyrogrammicus 	Yes	Reef-associated (Bray & Gomon 2018); rocky substrates (Fishbase). Rocky areas (Sumpton et al. 2013). Occasional in deep-reefs (>100 m) of GBR (Newman & Williams 1996).
Pristipomoides filamentosus 	Yes	Reef-associated (Bray & Gomon 2018); rocky substrates, migrate at night to feed (Fishbase); Above the bottom, near cliffs (Chave & Mundy 1994). Aggregate in large schools up-current (Ralston et al. 1986, Mees 1993). Coral reefs and rocky areas (Sumpton et al. 2013). Found on both high and low profile reefs (Moore et al. 2013) at shallower depths (125-225 m) with flatter slopes and unconsolidated sediments (Moore et al. 2016a) but not a strong preference for a particular bottom substrate (Merritt et al. 2011). Juveniles prefer featureless, silty or sandy habitat at shallower depths (60-100 m) than adults, perhaps for decreased predation by other species (Allen 1985, Ellis & DeMartini 1995, Parrish et al. 1997) but observed in BRUVS on *Halimeda* meadows (54 m depth), perhaps for foraging or refuge (Asher et al. 2017). Juveniles diurnally active, more actively moving in shallower areas during the day then deeper areas at night within ~300 m and ~10m depth difference (Parrish et al. 2015). In Hawaii, juveniles preferred sloped areas of 'coastal drainage' with uniform sediments as nurseries, such as reef channels (Parrish et al. 1997). Recruitment is variable from year to year, but juveniles were observed in nurseries from 7-10 cm to 20-30 cm, approximately 6 months beginning in the autumn (Moffitt & Parrish 1996). Believed to shift from soft-low to hard-low to hard-high habitats with increasing size (Misa 2013, Sackett et al. 2014) around 2-3 years old to offshore, deeper habitats (Haight et al. 1993a), due to diet shifts (Haight et al. 1993b). Large schools of juveniles occur around an area off SE QLD known as "Hardline" (a possible spawning/nursery area, Sumpton et al. 2013).
Pristipomoides multidens 	Yes	Hard, rocky, uneven, steep slopes (Parrish 1987, Fishbase). Offshore reefs, shoals and areas of flat, hard bottom with 'occasional epibenthos' and vertical relief (Newman et al. 2000a). Steep, hard, rocky and rugose habitats like drop-offs (Ovenden et al. 2004). In NW Australia juveniles inhabit deepwater sand and adults deepwater reefs (Newman et al. 2002). Juveniles on uniform sedimentary habitats, no relief 95-119 m (Newman 2006). In the southern GBR, deep lagoons (Cappo et al. 2007) Rocky areas (Sumpton et al. 2013). Caught in large abundance in Swains region (Brooks 2000 in Sumpton et al. 2013).

Pristipomoides typus 	Yes	Hard, rocky, uneven (Fishbase). Offshore reefs in association with P. multidens (Newman et al. 2000a). Northern GBR deep reefs (Cappo et al. 2007). Also caught in large numbers in the Swains region (Sumpton 2013) and present from northwest Australia to the border of QLD-NSW (Kailola *et al*. 1993 in Sumpton et al. 2013).
Seriola dumerili 	Yes	Reef-associated, oceanodromous (Bray & Gomon 2018); deep reefs, coastal bays, juveniles sometimes with floating plants/debris (Fishbase). Fore-reef habitats within Northwestern Hawaiian atolls (Holzwarth et al. 2006) Larval distribution deeper than 150 m, with abundance peak at 250 m and preference for warmer waters (24-25°C, Raya & Sabatés 2015)
Seriola rivoliana	Yes	Reef-associated (Bray & Gomon 2018). Offshore banks and outer reef slopes (Fishbase). Low flow environments (midwater BRUVS, Heagney et al. 2007). Acoustically tagged adults resident mostly year-round in N Atlantic seamounts at shallow depths (Fontes et al. 2014). Juvenile (5 cm) documented in mangrove estuary (Shibuno et al. 2008).
Terelabrus rubrovittatus 	Aquarium	Deep coastal and outer reef habitats (Kuiter and Tonozuka).
Wattsia mossambica 	Yes	Outer continental shelf-edge (Allen and Carpenter 1989 in Fishbase). Caught in smaller quantities in Queensland (Sumpton et al. 2013).

Fish-habitat associations

Differences in habitat type had a great influence on the distribution of deep reef-associated fishes. Specific habitat variables that may influence occurrence and distribution were identified for 14 species (Table 5-2). Depth had great influence on the total species richness and abundance of fishes (Chapter 4) and was identified as the most important predictor variable for eight of the species with moderate to strong habitat associations. Average backscatter (expressed in negative values of backscatter intensity) is an estimate of substratum hardness (higher backscatter means acoustically hard, and smaller absolute values) or softness (lower backscatter intensity means lower 'acoustic return' and larger absolute values; Siwabessy et al. 2013), was also highly influential for at least five of the species. Relative rugosity (i.e. Topographic Position Index) and planar curvature, which indicated the local geomorphology, did not influence many individual species' distributions. However, slope and relative position (easting, northing, longitude and latitude) were important habitat characteristics. The relative influence of epibenthic and substratum proportional measures varied by species, with encrusting organisms, filtering organisms, plants and bare epibenthos important for a species' presence-absence. The proportion of calcified reef and rubble were the only substratum measurement singled out as important in BRTs. While many of these BRT models resulted from 5-23 sites per species, these results are a preliminary description of habitat associations for deep-reef fishes in the GBR. For my purposes of identifying fish-habitat associations, some of the interpretations of multibeam-derived information are a simplified. However, 'average backscatter' and 'standard deviation of backscatter' of the seafloor are more complex functions of the roughness, epibenthos, and substratum grain size in addition to some technical factors of multibeam signal frequency and reverberation (Daniell et al. 2015).

Many deep-reef species demonstrated strong fish-habitat associations; however the degree and types of habitat association varied, even between closely related species. For instance, within the deepwater snapper *Pristipomoides* genus, *P. argyrogrammicus* and *P. typus* had slightly higher R^2 values than *P. filamentosus* and *P. multidens,* which may indicate a better fit among the habitat variables included in the models, or more generally speaking, allude to differences in habitat-partitioning, or differences in generalist or specific niche requirements among species. The first two smaller species may have more specialized habitat requirements, as *P. filamentosus* and *P. multidens* occurred across broader habitat types and depths. Depth was the most important for three of these species, but for rosy snapper *P. filamentosus* depth held lower relative influence among habitat variables. BRTs and partial dependency plots indicate positive occurrences for *P. filamentosus* for depths ~120-170 m. Instead, slope, planar curvature, the relative proportion of filtering organisms, and easting (a component of aspect) had greater influence on whether *P. filamentosus* was present or absent at a site. There was a greater chance

of rosy snappers occurring where there were sponges, fans, hydroids and whips (i.e. filtering organisms) visible nearby, at steeper ridge sites where slopes were >15 on surfaces more exposed to the currents from the easterly direction (Figure 5-1). Oblique-banded snapper (*P. argyrogrammicus*) only appeared below 160 m in almost flat, soft-bottomed environments. Goldband snapper (*P. multidens*) were more likely in depths of 110-180 m, on softer, but rougher (>SD of backscatter) environments with a more moderate effect of slopes >10. Sharptooth jobfish (*P. typus*) frequented depths 110-200 m and favoured valley-like sites (negative planar curvature values) with softer and more rugose substratum.

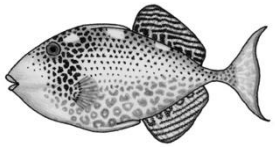
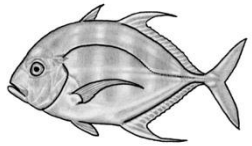
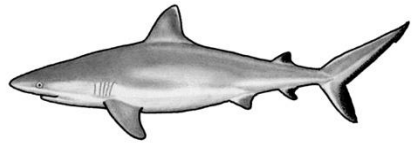
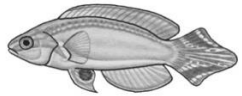
Within lethrinid species, for *L. rubrioperculatus*, *L. miniatus*, and *G. euanus* there was greater evidence of moderate or strong habitat associations, but for *G. grandoculis*, *W. mossambica*, *L. ravus* and *L. olivaceus* there was either insufficient evidence or these species did not display preferences based on the habitat variables investigated. The proportion of encrusting organisms was important for those species with habitat associations, perhaps due to prey availability. Emperor species *L. rubrioperculatus* and *L. miniatus* occurrence relied on proportions of encrusting organisms such as bryozoans and coralline algae comprising >40% of the epibenthos. Spotcheek emperors (*L. rubrioperculatus*) also heavily relied on calcified reef as substratum (>60%) and occurrence severely declined when bare epibenthos was greater than 10%. The redthroat emperor (*L. miniatus*) appeared in mesophotic depths down to 130 m around sites with lots of rubble in areas like valleys (negative TPI values). The paddletail seabream (*G. euanus*) is less likely to be present below 120 m, and preferred steeper valleys with slopes >20 and encrusting organisms and harder substratum.

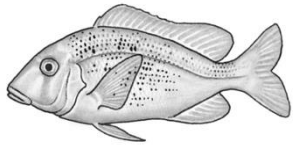
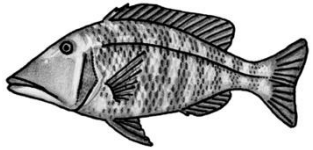
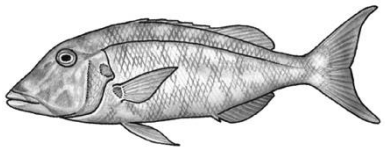
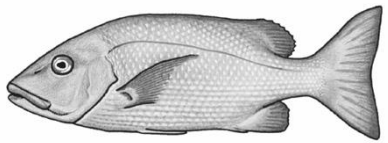
The red bass (*Lutjanus bohar*) occurred in mesophotic depths to 130 m, at the sites in the south of the central GBR. The onion trevally (*Carangoides caeruleopinnatus*) preferred harder substratum with greater proportions of encrusting organisms and calcified reef, occurrence declined with increased bare epibenthos and this species did not occur below 140 m at any of these sites. The grey reef shark was more likely to occur in shallower depths (<120 m) in steep locations (slope > 20) near ridges (positive TPI values). The starry triggerfish (*Abalistes stellatus*) preferred sites with abundant plant cover (>35%) and harder substratum in mesophotic depths <100 m. Habitat was important for smaller species; the highly abundant schooling yellowstripe threadfin bream (*Pentapodus aureofasciatus*; MaxN = 1-28, mean MaxN = 13.8) were observed only at sites where bare epibenthos was not greater than 20% and were more likely to occur where there were encrusting organisms and calcified reef was >40%. The *Parapercis* sp. observed preferred hard (average backscatter < -30), flat areas (slope < 20) facing east.

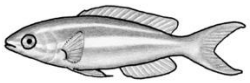
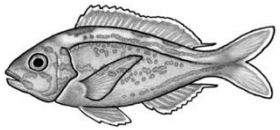
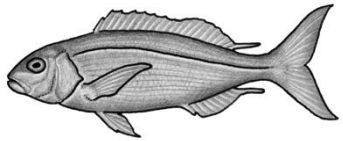
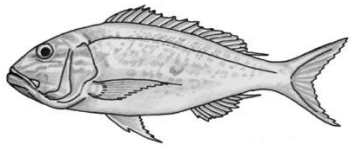
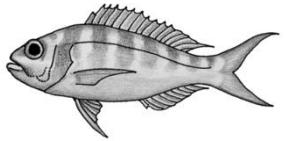
For locations where strikingly noticeable species like *Cirrhilabrus roseafascia* were found, the immediate epibenthos and substrate information was a better predictor than the

multibeam data. The occurrence of *C. roseafascia* was correlated with the presence of filtering and encrusting organisms, as well as greater proportions of calcified reef, and occurrence declined as the proportion of bare epibenthos increased (Fig. 5-2).

Table 5-2: Summary of most influential environmental habitat predictors from Boosted Regression Tree models on presence-absence of deep-reef fishes (n = number of sites observed). Model strength was evaluated by R^2 for each species and the R^2 range is provided (each model was run five times to account for stochasticity). Of the explanatory variables, only variables with >5% relative influence in initial BRT models were used for final models. All multibeam-derived habitat measures came from a 5 x 5 kernel.

Species	R^2	Best predictor variables		Evidence of habitat association?
Abalistes stellatus, n=6 	0.50-0.57	average backscatter depth plants longitude northing latitude SD of backscatter	21.96-24.44 21.50-24.39 19.98-23.33 12.21-14.51 6.96-7.63 5.70-6.80 4.84-6.43	Moderate
Carangoides caeruleopinnatus, n=12 	0.70-0.72	depth average backscatter calcified reef northing bare encrusting organisms	35.50-36.44 16.36-17.73 12.38-13.96 11.63-12.40 10.66-11.94 10.39-11.17	Strong
Carcharhinus amblyrhynchos, n=10 	0.33-0.38	depth slope TPI planar curvature northing easting filtering organisms	32.69-35.95 11.51-13.95 11.02-13.79 11.20-11.98 10.44-11.70 9.08-11.22 6.70-8.42	Moderate
Cirrhilabrus roseafascia, n=8 	0.49-0.56	filtering organisms calcified reef longitude bare epibenthos encrusting organisms slope	24.62-26.93 20.31-22.04 14.48-16.42 13.98-17.04 11.16-12.51 8.63-10.96	Moderate

Species	R^2	Best predictor variables		Evidence of habitat association?
Gymnocranius euanus, n=10 	0.66-0.71	depth encrusting organisms plants average backscatter planar curvature bare epibenthos slope	25.41-27.52 14.36-15.20 12.35-12.84 11.86-12.33 11.73-13.06 10.78-11.97 10.09-11.02	Strong
Lethrinus miniatus, n=8 	0.59-0.63	encrusting organisms depth TPI rubble planar curvature average backscatter calcified reef	37.50-39.32 18.17-20.49 8.92-10.32 8.85-10.23 7.60-8.48 7.46-8.30 6.91-7.74	Strong
Lethrinus rubrioperculatus, n=8 	0.74-0.75	encrusting organisms bare epibenthos calcified reef depth average backscatter	27.73-28.27 20.27-22.95 18.11-20.19 17.21-18.39 12.86-13.47	Strong
Lutjanus bohar, n=10 	0.65-0.66	depth latitude encrusting organisms filtering organisms average backscatter bare epibenthos calcified reef	34.48-36.25 16.47-17.05 10.28-11.99 8.94-10.12 8.34-9.67 8.39-9.51 8.62-9.29	Strong
Parapercis sp. n=10	0.50-0.52	average backscatter slope easting longitude latitude depth northing	40.15-41.03 12.81-13.66 11.70-12.27 9.19-10.63 8.60-9.74 7.73-8.78 6.50-7.71	Moderate

Species	R^2	Best predictor variables		Evidence of habitat association?
Pentapodus aureofasciatus, n=7 	0.66-0.70	bare epibenthos encrusting organisms calcified reef depth	37.89-40.01 25.11-26.64 19.18-21.09 14.38-15.66	Strong
Pristipomoides argyrogrammicus, n=6 	0.87-0.90	depth average backscatter SD of backscatter slope longitude	36.17-38.50 22.17-23.09 19.80-21.67 9.54-10.18 8.87-9.82	Strong
Pristipomoides filamentosus, n=16 	0.60-0.62	slope planar curvature filtering organisms easting longitude average backscatter depth SD of backscatter	25.70-26.89 14.78-15.74 13.06-14.03 10.87-11.71 8.74-9.94 8.27-9.39 7.65-8.84 6.56-7.21	Strong
Pristipomoides multidens, n=14 	0.59-0.64	depth average backscatter slope SD of backscatter TPI northing	24.00-26.00 18.63-19.74 17.26-18.83 13.99-16.37 11.31-12.87 10.87-11.11	Strong
Pristipomoides typus, n=18 	0.74-0.75	depth SD of backscatter planar curvature average backscatter rubble	33.37-34.94 23.79-26.39 20.76-21.78 11.71-13.43 6.59-7.27	Strong
Aphareus rutilans, n=23	No evidence of habitat association			
Carcharhinus albimarginatus, n=13	No evidence of habitat association			
Gymnocranius grandoculis, n=9	No evidence of habitat association			

Species	R^2	Best predictor variables	Evidence of habitat association?
Gymnosarda unicolor, n=17		No evidence of habitat association	
Parapercis nebulosa, n=11		No evidence of habitat association	
Echeneis naucrates, n=8		No evidence of habitat association	
Epinephelus morrhua, n=6		No evidence of habitat association	
Lethrinus olivaceus, n=5		No evidence of habitat association	
Lethrinus ravus n=5		No evidence of habitat association	
Selenanthias sp., n=6		No evidence of habitat association	
Seriola dumerili, n=11		No evidence of habitat association	
Seriola rivoliana, n=10		No evidence of habitat association	
Terelabrus rubrovittatus, n=8		No evidence of habitat association	
Wattsia mossambica, n=8		No evidence of habitat association	

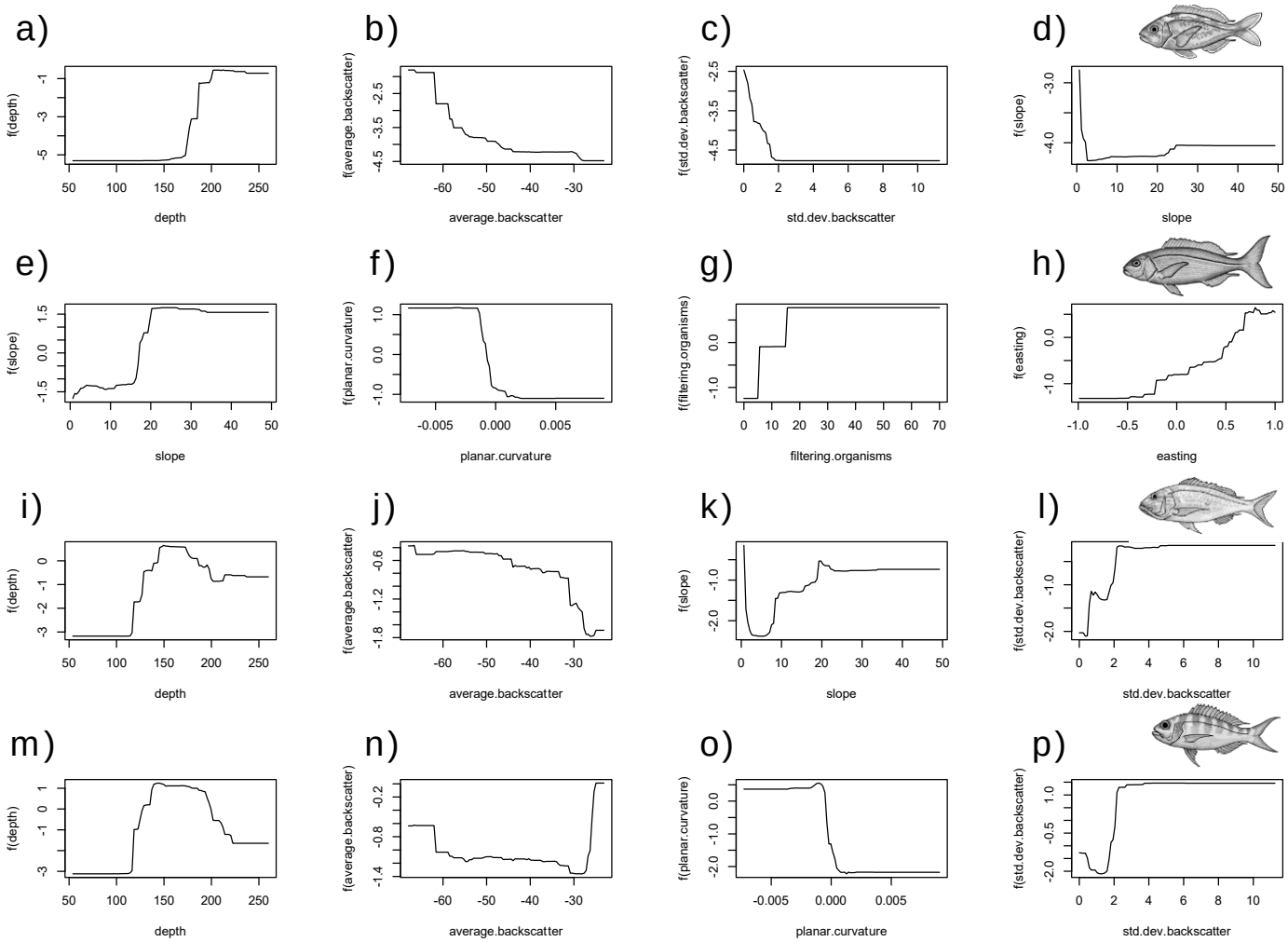


Figure 5-1: Examples of the influence of habitat for species occurrence for deep-reef fishes. Boosted Regression Tree models were run for four species of the deepwater snapper genus *Pristipomoides*. Partial dependency plots show the effect of each covariate when the effect of other covariates is kept constant for the best fitting BRT. The four most influential variables for *Pristipomoides argyrogrammicus* (a-d), *P. filamentosus* (e-h), *P. multidens* (i-l), and *P. typus* (m-p) varied by species.

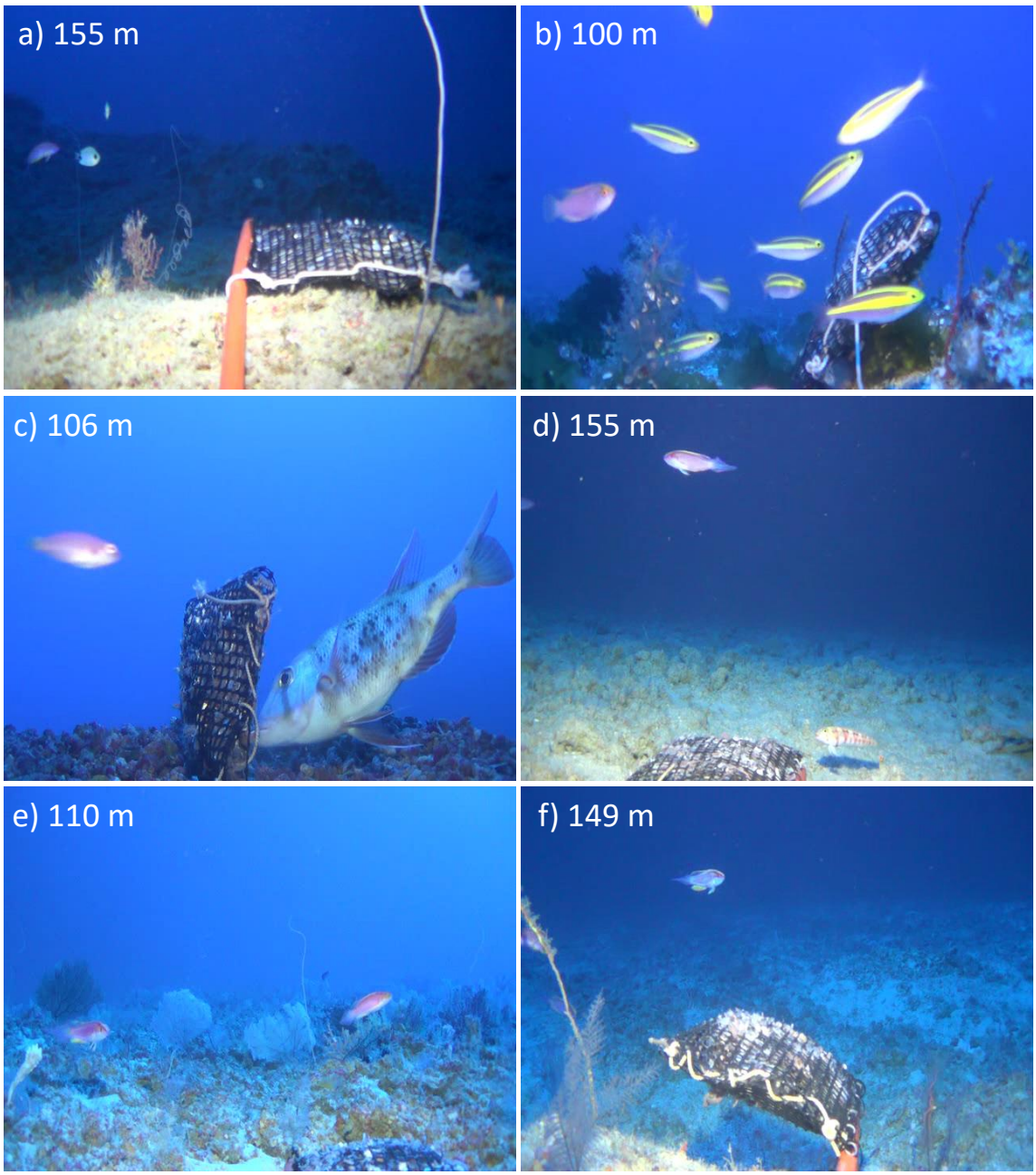


Figure 5-2: The rose-banded fairy wrasse, *Cirrhilabrus roseafascia*, observed in Baited Remote Underwater Video Stations on central Great Barrier Reef deep reefs. *Cirrhilabrus* spp. are sexually dimorphic and colors vary between males and females. Image b) may be a female *Cirrhilabrus lineatus* (pers. comm. Y.K. Tea).

Discussion

Effective management of deep-reef fisheries and ecosystems will require accurate, quantitative and spatially explicit information on the patterns of fish assemblages at spatial scales relevant to the management process. While much of the information gathered is preliminary, this study highlights several important criteria necessary for maintaining deep-reef fish populations. First, depth is a central feature delineating many species distributions; however, the specific qualities of those habitats determine species compositions. For instance, slope, topographic position index and planar curvature were important features of the three-dimensional environment, with some species preferring habitats of steeper slope (e.g. *P. filamentosus*) and others preferring flatter areas (e.g. *P. argyrogrammicus*). The physical aspects of the substratum were important, with average and standard deviation of backscatter components of the multibeam information featured as relatively important for the majority of species with moderate or strong habitat associations (10/14 species). Many species were also shown to prefer habitats with high epibenthic cover with presence more likely in areas with abundant filtering organisms (e.g. *P. filamentosus*, *C. roseafascia*) or abundant encrusting organisms (e.g. *G. euanus*, *L. miniatus, L. rubrioperculatus*). The relative importance of ridge or valley features demonstrates the complexity of deep-reef geomorphology, which may act as effective barriers to fish movements, creating isolated habitats and further sub-dividing suitable habitat. These results can be used to map deep-reef species distributions, but it is important to determine how the occurrence of species is affected by multiple habitat factors, and information on these habitat requirements is essential to understand a species' ecological role.

Certain groups such as the fairy wrasses (*Cirrhilabrus* spp.) exhibit habitat specificity but this is a group rapidly growing with the innovation of technological diving for specimen collection (e.g. Pyle 2000, Pyle et al. 2016a, Tea et al. 2016, Tea & Gill 2017, Tea et al. 2018a). The preferred habitat of the closely related *Cirrhilabrus shutmani* (Tea & Gill 2017) and *Cirrhilabrus sanguineus* (Cornic 1987) consists of low relief rubble slopes with limited to no structure (Tea & Gill 2017, Tea et al. 2018b). Given that both species are closely related to *C. roseafascia*, and that both species are found at similar depth ranges (Tea & Gill 2017, Tea et al. 2018b), the habitat of *C. roseafascia* is likely to be similar. I can confirm this with my observations; however, this record of *C. roseafascia* included some deeper records than the known distribution (Sih et al. 2017, Tea et al. 2018b). This is possibly due to the physiological limits of deep rebreather diving or because of differences in local topography. Depths of 155 m may be where unstructured, low relief, rubble slopes exist in that portion of the central GBR.

As technological diving has enhanced the ability to collect specimens from deep-reefs (Pyle 2000), it has also revealed new depth-specialist genera of fishes, including *Terelabrus* (Randall & Fourmanoir 1998, Fukui & Motomura 2015, 2016), *Bodianus* (Gomon 2001, Gomon

2006, Gomon & Walsh 2016) and *Cirrhilabrus* species. My observations of *T. rubrovittatus* indicate there may be some preference for higher epibenthic cover (Fig. 5-3), but this was not resolved in the BRT models. It is possible that this species' distribution relies more on specific depths than habitat features. From recent communication with Y. K. Tea, the species previously identified as *T. rubrovittatus* may also include *Terelabrus dewapyle* and these observations would extend this species' known range.

Other species also showed little evidence of habitat association, but many of these species are highly mobile and often described as semi-pelagic and oceanodromous. Many of these species are consistently abundant in deeper reefs (e.g. *S. dumerili*, *S. rivoliana*, *G. unicolor* and *A. rutilans*). Further investigation is warranted as additional sampling at more sites will likely strengthen the habitat models and increase the accuracy of species distribution predictions for all species.

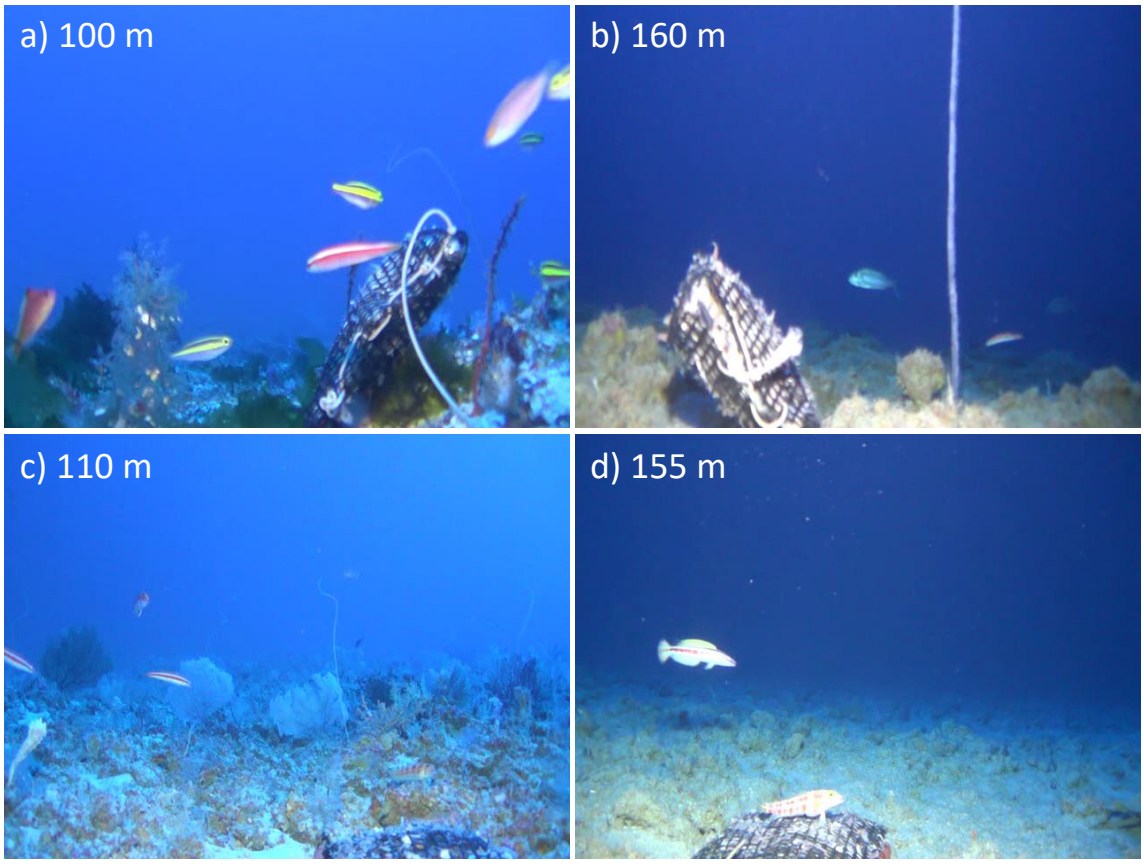


Figure 5-3: *Terelabrus "rubrovittatus*" observed in deep Baited Remote Underwater Video Stations in the central Great Barrier Reef. Images a) and c) may be a newly described *Terelabrus dewapyle* (pers. comm. Y.K. Tea).

Limited habitat = vulnerability

Where species occurrences may have strong correlations with benthic habitat suggests that if habitats are damaged or altered, these species would be susceptible to decline. Intense fishing effort of deepwater fishes can rapidly diminish the available stock (Grandcourt 2003, Fry et al. 2006) and because deep habitats are limited, this further intensifies the risks of overfishing. The loss of the three-dimensional habitat structure can decrease overall fisheries production (Rogers et al. 2014). For deeper reefs this can occur either by sedimentation, storm damage, or fishing, and will likely have large ramifications to the species composition on deeper reefs (Rocha et al. 2018). Similar to shallower reefs, the physical and biological components contributing to the structural complexity of deeper reefs may offer shelter niches and refuge from predators (Hixon & Beets 1993), resulting in higher biodiversity (Graham 2014). However, if habitats are altered and lose structural complexity, this may result in smaller fish and reductions in fisheries yield (Graham et al. 2007). These patches of suitable habitat may be resilient to localised changes (such as oil spills, increased sedimentation, or overfishing), or these changes may increase the vulnerability of these deep communities, creating lasting changes to species biodiversity (Hobbs et al. 2014). At mesophotic depths, sponges are an important biogenic component of deep-reef habitat (Lesser et al. 2009) and these results and other studies indicate filtering and encrusting organisms are linked to the occurrence of many species of *Pristipomoides*, *Epinephelus*, *Lutjanus* and *Carangoides* (Fitzpatrick et al. 2012, Wahab et al. 2018).

Our fish-habitat analysis results are similar to other locations, which is evidence that these habitat associations are not only species-specific, but ubiquitous throughout a species' global distribution. Factors such as the spatial arrangement of habitat types and architectural complexity can determine species distributions and diversity across a seascape (Pittman et al. 2011). While overall fish abundance decreased with increasing mesophotic depths in the Western Atlantic, the abundance of some smaller species was related to the availability of crevices rather than depth (Kahng et al. 2010). More habitat information is available for deepwater snapper species (*Pristipomoides* and *Etelis* spp.); they are valuable fisheries targets and well-represented in deeper BRUVS. Overall, deep 'bottomfish' have strong habitat associations with some size-related preferences; larger species are observed in aggregations near high-relief features, smaller species prefer high structural complexity and harder substratum (Ralston et al. 1986, Kelley et al. 2006, Parke 2007, Merritt et al. 2011). Among deep snapper species, depth is a major predictor of species distributions (Misa 2013, Gomez et al. 2015, Sih et al. 2017) but there were differences in habitat preferences. *Etelis coruscans* prefer high profile reef and sediment habitats (Moore et al. 2013) in deeper depths (200-300 m) also with less flat, unconsolidated sediment (Moore et al. 2016a). *Etelis carbunculus* preferences in habitat were similar to *E. coruscans*, only at slightly deeper depths (250-300 m, Moore et al. 2016a), and both species prefer greater bottom hardness

(Oyafuso et al. 2017). Oyafuso et al. (2017) found rugosity and slope were likely to influence *E. coruscans* and *P. filamentosus* distributions and both species prefer 'ridge-like' structures. Similarly *Aphareus rutilans* and *Pristipomoides zonatus* were primarily influenced by depth, rugosity and slope (Oyafuso et al. 2017). Some species have not been found to have habitat preferences (e.g. *Pristipomoides sieboldii*, Misa 2013). A lower profile seafloor generally translated to lower mean abundance for deep snappers (Moore et al. 2013). Habitat slope and substratum hardness related to size-related ontogenetic habitat shifts for some deepwater snapper species (Misa 2013).

Many of the commercially-valuable species or species of conservation concern were also observed at deep depths on BRUVS. The data collected were too few observations to detect habitat preferences that may exist. Some of these species may be naturally rare, and this may be a result of limited deepwater habitat. Further investigation may reveal some of these species may have strong and perhaps even more limiting habitat requirements.

Increase the level of protection for species of fishery concern

We need to increase the level of protection for deeper reefs, more closely monitor fishing effort, and identify which species would be good indicator species to base conservation and fisheries targets. At least 76 of the species encountered during this research (58% of 130 total species) are exploited by commercial and recreational fisheries, or the aquarium trade (Table 5-1; Appendix Table A1). This is likely an underestimate due to poorly-documented 'mixed fisheries' in nearby locations such as Indonesia (Newman et al. 2017). While some of these species are not currently targeted species in Australia and are considered by-catch (Appendix 2 in Cappo et al. 2010), these *Lethrinus* spp., *Epinephelus* spp., *Caranx* spp., *Gymnosarda unicolor*, and *Lutjanus bohar* are fished in many of countries in the Indo-Pacific: New Caledonia, Papua New Guinea, Fiji, Maldives, Palau and Tuvalu (Blaber 2009). Two of the species in this study are 'species of highest indicator value' in Western Australian commercial fisheries: *P. multidens* in the North Coast Bioregion and *L. miniatus* in the West Coast Bioregion (Newman et al. 2018). The goldband snapper *P. multidens* is the most common species in the deepwater trap and dropline fisheries in western and northern Australian waters (Newman et al. 2000b), but closely-related species are also marketed as 'goldband'.

Many of deep fish stocks are more vulnerable to overexploitation due to advancements in fishing technology and gear, as well as inherent life history characteristics that reduce production potential and slow population recovery times (Fry et al. 2006, Cheung et al. 2007, Sumpton et al. 2013, Williams et al. 2013). Furthermore, as fishing is inherently selective, preferentially removing larger individuals and selecting for larger specimens, it may reduce functional and phenotypic diversity (Martins et al. 2012, Brooker et al. 2016) and change species composition

and abundance for targeted and non-targeted fishes (Watson et al. 2007) even at very modest fishing pressure (DeMartini et al. 2008). As smaller species are not often targeted by commercial operations (e.g. small benthic invertivores), their abundance may increase and skew local predator-prey ratios, with possible ecosystem-level impacts (Martins et al. 2012). Shallow coral reef fisheries also target habitats with high species diversity and a range of life history strategies (Choat & Robertson 2002), but deeper fish populations have lower survival rates from barotrauma when brought up from depth. Besides the targeted species, deepwater fisheries will also impact a number of elasmobranch species; *C. albimarginatus* and *C. amblyrhynchos* are mentioned in this study, but a number of shark species are captured in fisheries and some deepwater (>200 m) chondricthyan species are highly vulnerable to fishing (Rigby & Simpfendorfer 2013, Rigby et al. 2015). For some deepwater fisheries, more specific knowledge on the depths and diel behaviours of fishes can increase fishing selectivity – creating a 'win-win' solution for fishers. For instance, fishers targeting Blue-eye trevalla (*Hyperoglyphe antarctica*) can avoid by-catch of Harrisson's dogfish (*Centrophorus harrissoni*) by targeting 280-550 m depths during the day, since the shark ascends to shallower depths at night to feed (Williams et al. 2016).

In Queensland, all fishing groups (recreational, charter and commercial) have recently increased fishing effort in deeper waters (Sumpton et al. 2013) but a substantial obstacle is the information available for management. In the GBRMP there are several layers of conservation management, including multiple fishery controls such as bag limits for specific species, size limits, spatial and temporal (spawning) closures, gear and effort controls and a limited licensing system. While there are some reporting requirements, generally the information is of limited use because of problematic species identification, difficulties in recording catch locations, and broad logbook categorizations ('mixed cod', 'mixed jobfish' and 'mixed fish'), which result in underestimates of catch (Sumpton et al. 2013). Compliance with fishery controls is uncertain due to deep reefs being in large, remote areas that have limited surveillance and unknown levels of illegal fishing and poaching. Anecdotally, fishers say the number of hooks per line gear restrictions are often ignored and transgressions are easily covered up. Deepwater fisheries have been fished in Queensland since the 1980s. Deeper fishing was proposed to lessen fishing pressure on shallower GBR fisheries (e.g. Coral Reef Fin Fishery) and by 1999 up to 40 deepwater L8 licenses were granted before a freeze was placed (Sumpton et al. 2013). Sumpton et al. (2013) believed that several deepwater fish stocks were subject to damaging levels of fishing pressure, including targeting spawning aggregations, in some areas of Queensland, including rosy and goldband jobfish, large-mouth nannygai (*Lutjanus malabaricus*) and bar rock cod (*Epinephelus septemfasciatus* and *E. ergastularius*). For instance, while *P. filamentosus* only comprises ~5% of the offshore recreational catch, there may be signs that there is already some localized

depletions as fishers have reported catch declines in the Fraser Island-North Reef area beginning in the 1970s (Sumpton et al. 2013). Serial, localized depletion can occur when fishers fish an area to the point of declining catches, wait for some recovery (within months or years), and then return before the fishery is 'productive' (Sumpton et al. 2013). Sumpton et al. (2013) quite comprehensively summarized many of the traits that make this species (and others) very vulnerable: highly marketable, caught under multiple fisheries' jurisdictions, long-lived, aggregate in large schools, occur in predictable locations and exhibit aggressive feeding behavior (and to bait).

Protecting important deep habitats is likely the most effective fishery management strategy and identifying fish-habitat associations is a critical first step to understanding the role habitat plays in species distributions, and this information can be incorporated into spatial management strategies. Studies on the effects of protected 'zones' in deep (20-50 m) shoals of the southern GBR indicate strong positive effects of spatial management, with greater abundance of fishery-targeted species in protected areas (Stowar et al. 2008). Given the distribution and abundance of many demersal fishes are constrained by various abiotic and biotic components of the seafloor (e.g. Friedlander & Parrish 1998, Jones & Syms 1998, Yoklavich et al. 2000, Anderson & Yoklavich 2007, Tissot et al. 2007, Anderson et al. 2009), damage to benthic habitats could irreversibly alter fish assemblages. Therefore, the permitted fishing methods and the amount of area set aside for protection are important. Past assessment of fish-habitat associations over the northwest Australian continental shelf resulted in spatial closures for the commercial trap and trawl fisheries. This tropical multispecies fishery is perhaps the best example of why the precautionary principle should be applied to deep-reef fisheries. The trawl fishery originally targeted *Lethrinus* and *Lutjanus* species, which were found to associate with 'large epibenthos' (sponges and gorgonians >25 cm). The effects of many years of trawling caused the catch composition to shift from commercially-valuable species to less-valuable species and the epibenthic cover was slow to recover (Sainsbury 1987, Sainsbury 1988, Sainsbury et al. 1993). The effects of fishing are unfortunately often only measured retrospectively and benthic trawling is one of the most destructive and lasting fishing methods due to the damage to benthic habitats (Turner et al. 1999, Thrush & Dayton 2002). It is important to bear in mind that the Australian continental shelf has experienced relatively low levels of trawling, estimated to be less than 5%, far below the trawl fishing 'footprint' of similar depths of other continents (Amoroso et al 2018).

Fish-habitat information can be used to refine specific targets for ecosystem-based fisheries management. For instance, Hawaiian fisheries management for deepwater snapper, grouper and jack species established Bottomfish Restricted Fishing Areas (BRFAs) to protect 'essential fish habitat' (Rosenberg et al. 2000) in the 1990s. When more information became available on species-preferred habitat, which for some species included steep, hard substratum, these BRFAs

were refined to include more of this type of habitat (Kelley et al. 2006, Parke 2007). Protected fishing areas will benefit some species more comprehensively than others, depending on a combination of factors, including fish-habitat associations, ontogenetic habitat shifts, trophic group, mobility, connectivity and life history (Palumbi 2004) and it is important to remember that 'no-take areas' without other fishery controls may be less effective (Newman et al. 2002) and still may not be sufficient to meet conservation needs (Moore et al. 2016b). Effective protection will require explicit information on species distributions across the mosaic of deep-reef habitats.

Species-specific information

I demonstrated great differences in depth and habitat to the overall fish assemblage and trophic groups (Chapters 3 and 4), but a substantial roadblock to effective management of deeper fisheries is the limited resolution of species-specific information available to managers. In the GBR and elsewhere, both recreational and commercial fisheries tally tens of species into broad categories, such as 'tropical snapper' and 'tropical grouper', for reporting and fishery controls. This may help with compliance but is inadequate for long-term management objectives. Tropical fisheries are increasingly targeting stocks along continental slopes and other deep bathymetric features, which are critical ecological areas of concentrated resources over a relatively narrow area (Olavo et al. 2011, Costa et al. 2014). Response to habitat damage will likely be species-specific, with changes in abundance reflecting both habitat-use and the degree of specialization. Research that identifies species- or population-specific parameters and trophic information can have important management implications. It is important to account for local-scale variations and to conduct broad regional comparisons throughout a species' entire range. For instance, eteline snappers are an important fishery throughout the Indian and Pacific Oceans and *Etelis carbunculus* is fished commercially in Hawaii, Tonga, Indonesia, and Australia, but its growth varies over this latitudinal and oceanic gradient (Williams et al. 2017). Much of the biological information on deepwater snappers come from only a few locations and throughout this distribution some areas have greater diversity within the *Pristipomoides* and *Etelis* genera. Where these species spatially overlap, there is evidence of both diet (trophic) and habitat-niche partitioning. For instance, while *Pristipomoides zonatus* feeds on benthic organisms, *Pristipomoides auricilla* consumes pelagic invertebrates and fishes (Seki & Callahan 1988). *Etelis coruscans* and *E. carbunculus* are found at deeper depths, feeding on squid (Haight et al. 1993b) while *Pristipomoides sieboldii* and *P. filamentosus* move with the diel vertical distribution of zooplankton. In general, *Pristipomoides* snappers are found near escarpments (Seki & Callahan 1988) and are most abundant on slopes with upcurrent exposure (Ralston et al. 1986). Fishing effort is concentrated close to the benthic habitat, where species *P. multidens* and *P. filamentosus* form aggregations (Allen 1985, Newman et al. 2008). However, there may spatial variation in the species distribution, for instance, among habitats where these species do not overlap. Species

distribution models of deepwater snappers in Hawaii indicated *P. filamentosus* occupied different depths and habitats to *Etelis* spp. so the proportion of species-specific habitat within the research area was only ~10% per species (Moore et al. 2016a). Anecdotal evidence from fishers in the goldband snapper fishery (western and northern Australia) have observed other species of *Pristipomoides* on the shelf-break, outside of the main *P. multidens* fishing area (Gastauer et al. 2017). Similar evidence of habitat partitioning has also been seen in other deepwater families (Balistidae, Lethrinidae, Lutjanidae, Carangidae, and Serranidae; Fitzpatrick et al. 2012). Accounting for these specific differences will take a conscientious effort to ensure adequate protection levels for the most vulnerable species and habitats.

Understand the life history

It is also not yet known how critical deeper habitats are for completing the life cycle of many commercially-valuable fishes and additional work is needed to identify how habitat-use may change with ontogeny. Understanding how life histories might differ among multiple species subject to the same fishing pressure may help to develop better management strategies. In shallower lethrinid fisheries, species had some similar early-life demographics but varied most in lifespan, maximum growth and spawning season (Currey et al. 2013). In shallower lutjanid comparisons, there was an even greater difference in age and growth among species (Heupel et al. 2010b). Diversity in life histories among closely-related species should make fishery management more cautious to account for some of these differences when setting fishery regulations like spawning closures, bag limits and spatial management zones.

Identifying juvenile and spawning habitats for deepwater species is strategic for fishery management. Shelf-break reefs may be key locations for spawning aggregations (Domeier & Colin 1997, Olavo et al. 2011). Juveniles may use different habitats and depend on different environmental processes; therefore, it is important to determine habitat requirements for all life-stages, especially for long-lived fishery species. Potential recruitment overfishing will affect stock sizes, cause assemblage composition shifts (Richards & Lindeman 1987), with unknown ramifications to multispecies fisheries. Species with deeper and more remote juvenile habitats are likely not adequately considered in fishery management strategies (Parrish et al. 1997) and the few deepwater nurseries identified appear distinct from those of other juvenile fishes (Moffitt & Parrish 1996). In the literature, few deep-reef species had explicit juvenile habitat information (e.g. *L. miniatus*, *L. olivaceus*, *P. filamentosus* and *P. multidens*), but juveniles of these species are found in habitats different to adults of the same species. For deepwater *Pristipomoides* species, few juvenile nursery grounds have been documented. Adults form large schools close to the bottom during the day (Allen 1985), which are easy to find with fish-finders. However, even with broad BRUVS sampling throughout the GBR, few juvenile eteline snapper recruitment habitats

have been identified. Greater sampling of the inter-reefal spaces may reveal more recruitment habitats for many deeper species. In Australia, many juvenile lethrinid species use seagrass nurseries (Wilson 1998 in Nakamura et al. 2008, Evans et al. 2014), therefore, deepwater macroalgae beds may be good candidates for recruitment spaces. Additionally, soft corals and sponges may provide juvenile habitat for some species (Garcia-Sais 2010). Migration to offshore and deeper areas may be a common life history strategy, as multiple lutjanid and lethrinid species exhibit ontogenetic cross-shelf movements, migrating as adults to mid- and outer-shelf reefs, such as *Lutjanus erythropterus*, *L. russellii* and *L. malabaricus* among others in the GBR (Williams & Russ 1994, Newman & Williams 1996, Mapleston et al. 2006), and *L. campechanus* in the Gulf of Mexico (Bradley & Bryan 1975). Not much is known about larval movements for deepwater snappers in the GBR, but generally, fish larvae use physical features like oceanographic fronts and internal waves for transport and successful recruitment (Richards & Lindeman 1987) and these may be critical dispersal mechanisms for deepwater snappers with long pelagic stages. Eteline larvae and pelagic juveniles are planktonic until 5-6 cm fork-lengths (Leis 1987, Leis & Lee 1994).

Investigations using length estimates will be necessary in the future to determine the importance of deep-reef habitats as a refuge for fishery targets, including 'shallower' and deep lutjanids. Information collated by Williams and Russ (1994) suggested that the red snappers (*L. malabaricus* and *L. sebae*) had different habitat utilization patterns more representative of 'coral reef' species and may have a greater presence in deeper (60-280 m), inter-reefal waters, with larger specimens found in deeper waters during the summer. Multiple families (e.g. Balistidae, Lethrinidae, Lutjanidae, Carangidae and Serranidae) exhibit increasing length with depth (Brouard & Grandperrin 1985, Kulbicki et al. 1987, Fitzpatrick et al. 2012), however, this may be a product of size-selective mortality as there tends to be increased fishing pressure in shallower habitats. Fitzpatrick et al. (2012) also suggested that competitive interactions may have led to habitat partitioning between species within the same family, with smaller species of Lutjanidae, Balistidae, Lethrinidae, Serranidae and Carangidae present inshore and larger species offshore.

If deepwater species require different habitats and depths during their lifespan, fishery management should plan to account for this movement, such as networks of connected habitat for fish migration and diel movements. For example, more mobile deep fishes may exhibit diel movements between habitats to access different resources (Weng 2013), as well as less-common larger scale movements between reefs and islands (Kobayashi 2008, Weng 2013). In addition, some species also exhibit variability in day and night catch rates by depth, potentially linked to plankton food resources (Haight et al. 1993b, Williams & Russ 1994). Understanding these movements is especially pertinent when establishing marine reserve networks, to ensure they are of sufficient size and have an optimal spatial distribution.

The use of multibeam bathymetry to forecast species distributions would greatly amplify the information available to fishery and conservation managers. The potential for rapid data collection using modern seabed mapping technology and using BRUVS to sample the fish assemblage would allow the Great Barrier Reef Marine Park Authority (GBRMPA) to implement ecosystem-based management plans more efficiently. The results of this study show that the distribution and composition of GBR deeper fish assemblages are closely tied to depth and the distribution of specific benthic habitats. We are beginning to identify deeper fish-habitat associations, which can then be translated into spatially explicit bioregion maps with predicted fish assemblage composition. This information will assist the GBRMPA to identify high-priority, critical habitats for greater protection. However, the 'predictability' of benthic habitat data to indicate species distributions may also increase vulnerability to exploitation if identified high-value areas are not sufficiently safeguarded (Weng 2013, Gomez et al. 2015). The sophistication of these techniques relies on a substantial sampling effort and more replications through space and time. Therefore, the establishment and monitoring of deepwater marine protected areas, with the goal of fisheries management, is a prudent measure. This concept has been enacted elsewhere with some success. In Hawaii BRFAs have had a positive effect on several deep-reef snapper and grouper species, increasing the size, relative abundance, and number of mature fish inside the BRFA, while species richness also increased outside (Sackett et al. 2014). However, marine protected areas can have different results depending on the length and level of protection (Babcock et al. 2010, Sackett et al. 2014). Some non-target species such as *P. sieboldii* did not seem to benefit from BRFA protection (Sackett et al. 2014) but this species did not exhibit strong habitat preferences (Misa 2013). Regardless, marine protected areas are believed to benefit the majority of species by leaving more intact habitat, rather than the absence of fishing pressure alone.

A note about the Coral Sea

The neighboring Coral Sea is regarded as one of the few remaining places where fish stocks are 'untouched' by substantial fishing pressure (Ceccarelli et al. 2013), however, deepwater fishes and their habitats are identified as substantial knowledge gap in this diverse ecosystem for fishery and conservation management (Young et al. 2011). The area boasts high levels of new species discovery and localized endemicity in deep fish assemblages (Last et al. 2014) and the Coral Sea Marine Park covers ~990,000 km^2, including an estimated 15,000 km^2 of reef. The history of fishing in the Coral Sea is not well-documented, however, recreational spearfishing trophy captures tend to be larger than in the GBR (Young et al. 2016) and commercial fishing efforts have also brought notably larger size classes of deepwater fish (Fig. 5-4). Greater efforts should be made to protect these fish stocks and non-destructive methods of sampling should be used to investigate these last vestiges of intact marine ecosystems.

Figure 5-4: Substantial deep-reef resources exist in the Coral Sea, within the Australian Exclusive Economic zone. To date there is depauperate biological information on deepwater fishery stocks but great interest by commercial, recreational and charter fishing operations. Photos by T. Sih and M. Cunningham (used with permission).

Conclusion

Worldwide few locations have the foresight to establish marine reserves before they are necessary. However, fishing is one of the oldest anthropogenic impacts on marine environments (Jackson et al. 2001) and most fisheries are fully exploited or overfished (Watson & Pauly 2013). While the GBR may not currently be experiencing heavy fishing pressure on deep reefs, the time to collect data is now, before it becomes necessary. With 'shifting baselines' in mind, Australia has the scientific capacity to monitor fishing effort, changes to species composition, and to use multibeam to assess changes in topographic complexity over time. We need 'ecological baselines', knowledge of the structure and functioning of ecosystems before human disturbance, and few large and 'intact' ecosystems remain (DeMartini et al. 2008, Friedlander et al. 2010). Further, understanding of depth and fish-habitat associations will greatly contribute to more focused conservation and fishery management strategies. Protection should extend to a wide range of deep habitats and future studies should consider the effects of zoning, especially if deeper environments receive greater fishing pressure throughout the GBR and similar MCEs worldwide. While there is incidental representation of deepwater habitats that fall within GBRMP 'no-fishing' management zones (Bridge et al. 2016a), it would be wise to assess whether current spatial management is adequate and representative for long-term protection of fishery resources, such as the ability to 'complete the life cycle' of fishery species, understanding and quantifying the importance of deep habitats, and safeguarding deep-reefs in perpetuity.

Chapter 6 High-resolution otolith elemental signatures in eteline snappers from valuable Pacific fisheries

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Abstract

Marine resources are often shared among countries, with Exclusive Economic Zones (EEZs) dividing fish stocks among nations. Therefore, understanding the spatial structure of populations is important for the management of fish stocks. Multiple complementary techniques can be used to identify non-mixing populations, including otolith chemical analyses, which discriminate among populations based on differences in chemical composition of otoliths. I used otoliths from two deep-reef snappers from high-value fisheries in Tonga, Vanuatu, Fiji, New Caledonia, Papua New Guinea, and Wallis and Futuna to compare methods of trace element otolith analyses using solution-based inductively coupled plasma mass spectrometry (ICP-MS) and laser ablation ICP-MS (LA-ICP-MS). For both species, the two methods demonstrated spatial separation among the EEZs sampled, implying multiple non-mixing populations, with high classification accuracy. Smaller laser ablation size gave detectable measures for some elements and also gave greater temporal resolution of the life history transect. Comparing the early life history section of the otoliths (i.e. the core) suggested that young fish experienced more uniform environments than adults, as the elemental fingerprints had greater overlap among multiple locations. LA-ICP-MS methods had some advantages over solution-based ICP-MS and generally better spatial discrimination (differences among EEZs) for the trace elements investigated. There were substantial between-species differences; however, both methods were able to discriminate among non-mixing populations at the regional scale. Otolith chemistry was an effective tool in discriminating region-wide spatial variation for deep-reef marine species in multispecies fisheries and edge measurements from LA-ICP-MS provided the greatest resolution. Otolith chemistry suggested that there are multiple stocks of deepwater snappers in the Pacific. Separate units at the spatial scales described should be considered for future fishery management plans until more data on stock discrimination is obtained.

Introduction

The management of global fish catch is of critical importance for human societies. Various conventions and policies define the rights and obligations of nations and societies to extract marine resources. One important mandate, the United Nations Convention on the Law of the Seas (UNCLOS), allows nations to have jurisdiction over a 200-nautical mile Exclusive Economic Zone (EEZ), which includes all fishing rights in these territorial waters. Pacific island EEZs are allocated according to UNCLOS agreement, but closely neighbouring countries likely have overlapping fish stocks and unequal allocations of productive fishing grounds. Regional organizations such as the Secretariat of the Pacific Community (SPC, New Caledonia) and Western Pacific Fisheries Management Council (WPFMC) can provide countries with information on which to base fisheries management decisions. However, fisheries research in this region is often limited by funding and resources (Newman et al. 2015, Williams et al. 2015). In practice, fisheries management often defines stock management units and the spatial separation of stocks based on units of convenience (i.e. EEZs) rather than ecological evidence on the spatial separation of stocks (Begg et al. 1999).

Greater fishing effort has been directed toward deepwater fisheries in recent decades (Morato et al. 2006), placing greater urgency on determining stock structure so that accurate assessments of stocks can be made (Newman et al. 2016). Some Pacific countries, including Tonga and Vanuatu, have established deep-reef fisheries, with eteline snappers among the most economically valuable and potentially vulnerable fishes (Williams et al. 2013, Newman et al. 2015). Although knowledge of deep-reef fish spatial ecology is limited (Kobayashi 2008, Weng 2013), there is growing evidence for spatial variation in demography (Williams et al. 2017) suggesting the existence of non-mixing populations and/or separate fish stocks. Previous genetic studies have revealed panmictic populations of some deepwater snapper species in the Indo-Pacific, suggesting widespread stock-mixing and highly connected populations (Gaither et al. 2011, Andrews et al. 2014, Andrews et al. 2016, Goldstein et al. 2016b), although there is some genetic evidence for population structure at spatial scales of 100s km (Ovenden et al. 2002, Ovenden et al. 2004, Gaither et al. 2011). However, only low levels of gene flow are needed to maintain population connectivity (Andrews et al. 2016), and there likely are ecologically-relevant population structure at scales more relevant to fisheries management.

Analysis of the chemical composition of otoliths may provide an alternative method for discriminating among populations and sub-populations that constitute ‘stocks’ (Campana 2005, Hammer & Zimmermann 2005, Cadrin & Secor 2009). Deepwater snappers live in heterogeneous seascapes and multiple species may use habitat differently, leading to the spatial structuring of metapopulations within a multispecies fishery (Chapters 2 and 3). Otolith chemistry has the

potential to assess the connectivity among multiple locations (Jones et al. 2016). Differences in water chemistry or diet may result in differences in the trace elemental composition of the otolith, which can delineate ecological sub-populations or manageable stock units (Campana 2005, Walther et al. 2017). Otolith microchemistry can also give insight into possible movements or ontogenetic shifts, through comparisons of otolith composition from point of origin (core) versus the catch-location (edge) chemistries (Elsdon et al. 2008). Stock structure, as it applies to fisheries management, strives to spatially delineate parts of a fishery into biological units of low connectivity that can be fished with little or no immediate consequences for sustainable yield from subpopulations within the metapopulation on ecologically-relevant temporal scales (i.e. 5-10 years; Thresher & Proctor 2007).

Chemical analyses of fish otoliths have been useful as natural tags of the environments fish have been exposed to over their lifespan (Campana et al. 2000). Concentric layers of calcium-based materials are layered as the fish ages, providing a chronological record of the environmental history of the fish (Campana 1999). Otolith chemical composition includes metals in trace amounts that, when measured against an internal standard such as calcium, can discriminate between environments or locations where the fish has been (Campana et al. 2000). These methods complement information from other methods such as morphometrics (e.g. Haddon & Willis 1995), parasite markers (e.g. Lester & Moore 2015), genetic analyses, and catch record comparisons to provide insights upon which fisheries managers can base decisions. Where there may be gaps or uncertainty in data collection, the combination of multiple techniques has been especially useful where decisions need to be made based on incomplete assessments (Brodziak et al. 2011) and may provide a more holistic view of the fishery (Begg et al. 1999, Begg & Waldman 1999); yet advanced techniques have not been used to look at region-wide stock discrimination for deep-reef species.

There are multiple techniques that could help delineate stocks based on trace element otolith chemistry. The primary techniques used are solution-based inductively coupled plasma mass spectrometry (ICP-MS) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Both techniques measure trace element concentrations, but they have different resolution capabilities and each technique has strengths and weaknesses. Given the challenges of researching deep-reef fisheries, methods are needed that deliver good spatial separation and maximize information on the structure of deep-reef fish populations for the region. The separation of stocks from otoliths relies on the assumptions that otolith material, once deposited is metabolically inert (Campana 1999), elements taken into the otolith reflect the ambient environment experienced by the fish (Bath et al. 2000, Campana et al. 2000), and there is sufficient geographic variation in water or other factors to influence the chemistry of the otolith

(Campana 2005, Elsdon et al. 2008). Solution-based ICP-MS is relatively faster in terms of time and efficiency for laboratory protocols. This technique is also faster (Kingsford et al. 2009), because there is less post-processing of data, but may be limited in interpretation because the whole otolith is dissolved in solution. This results in a 'whole-structure fingerprint' (Kerr & Campana 2014) that integrates the entire lifetime of the fish and can only distinguish among groups of fish that have experienced different overall environments (Thorrold et al. 1998, Campana 1999). However, there can be some resolution of life history stages; for instance, by isolating the core (e.g. Dove et al. 1996) it is possible to infer nursery origin for groups of fish (Gillanders & Kingsford 2000, Campana 2005). LA-ICP-MS has greater fine-scale spatial resolution, as specific areas of the otolith are selected for comparison. Selecting a 'life history transect' from the core to the edge of the otolith can be a useful to investigate how the elemental signatures change over the lifespan of an individual fish. This allows the discrimination of groups within a specific time-frame when matched with specific portions of the otolith or specific annuli in the otoliths. This method may be useful for species whose ecology is lesser known and where variations in distributions with growth may potentially be inferred from environmental information.

Both otolith analyses have been used successfully to delineate stocks of shallow-water demersal species (e.g. LA-ICP-MS of Western Australian dhufish and snapper, ~1000 km, Fairclough et al. 2013; solution-based ICP-MS of snapper, ~400 km, Gillanders 2002) and even deepwater species (e.g. solution-based ICP-MS and electron probe microanalysis of orange roughy, ~1300 km and ~5000 km, Edmonds et al. 1991, Thresher & Proctor 2007), over varying spatial scales. However, it is not known if the environmental variation is sufficiently different among locations (hundreds to thousands of kilometres apart) to discriminate stocks of deep-reef fish, which are further from coastal influences in a deepwater environment with limited biological, physical and chemical information over this spatial scale. There is some evidence that these species are highly site-attached with limited adult mobility (Weng 2013), and therefore, otolith chemical analyses have the potential to show successful discrimination between effective stocks. There are some studies that have compared trace elemental composition across similarly broad regions on more mobile species (e.g. pelagic tuna populations, Proctor et al. 1995, Rooker et al. 2016), but there are few studies that have examined otolith trace elemental composition for more site-attached reef species at large spatial scales.

The objective of this study was to evaluate the utility of solution-based ICP-MS and LA-ICP-MS for discriminating among populations of two closely related species of deep-reef snapper *Etelis coruscans* and *Etelis sp.* from multiple locations in the Pacific Island region. In the previous literature, *E. sp.* has been referenced as *Etelis carbunculus* in some locations. In the South Pacific,

this species often co-occurs with *E. carbunculus*, which is a cryptic sister species (Smith 1992, Loeun et al. 2014, Wakefield et al. 2014, Andrews et al. 2016). Both species are fully marine fishes, demonstrating high site-attachment as adults (Weng et al. 2013). Both species generally inhabit depths of 250 m or more, which makes telemetry studies and mark-recapture studies more difficult (Kobayashi 2008). These species are caught at similar depths and locations so if the otolith microchemistry is similar between species, it is likely due to environmental differences. My specific aims were: 1) to determine which elements and which technique yielded greatest spatial separation of elemental fingerprints for inferring stock structure; 2) to elucidate the 'temporal' differences between early and late life history by comparing the resolution of dissolved core and whole otoliths (solution-based ICP-MS) and core ablation and edge ablation spots (LA-ICP-MS) from transect measurements. This study provides useful information to inform the future application of elemental chemistry for discriminating among tropical deepwater fish stocks.

Methods

Sampling design

Otoliths for this study were collected from 2012-2015 by fisheries researchers at the Secretariat of the Pacific Community (SPC) in New Caledonia, Papua New Guinea and Tonga. In the Indo-Pacific region, concurrent sampling trips collected otoliths for ageing (Williams et al. 2015) and chemical analyses for stock identification (this study) between 2012-2015. Otoliths were selected from EEZs representing fishing Pacific countries spanning Papua New Guinea to Tonga and a distance of over 4500 km (Table 6-1). Otoliths from two deep-reef snappers, *E. coruscans* and *E. sp.*, were used in this study from six and five EEZs respectively.

Solution-based ICP-MS protocol

Elemental signatures were obtained for juvenile (core) and whole-life integrated with solution-based ICP-MS. Sixty-six otoliths from the two species from multiple EEZs were selected for solution-based analyses. Otolith cores were isolated using a handheld rotating diamond-blade saw (similar to Dove et al. 1996). Prior to dissolution, otolith cores and whole otoliths were weighed to the nearest 0.001 g, washed three times in Milli-Q Ultra-Pure (Type 1) water, placed in an ultrasonic bath for two minutes and then rinsed three times in Milli-Q water. Otoliths were placed in acid-washed vials and dried for 48 hours in a laminar-flow hood. For solution-based samples, 33 cores and 33 whole otoliths were dissolved into 20% HNO_3 solution based on otolith weight, diluted to a solution of 2% acidity and concentration of 1 g/L of otolith material. Elements ^{138}Ba, ^{88}Sr, ^{44}Ca, ^{24}Mg, ^{55}Mn, ^{65}Cu, ^{66}Zn and ^{57}Fe were measured against blank solutions and certified reference material (CRM) #22 from *Lutjanus sebae* otoliths from Western Australia (National Institute for Environmental Studies, Japan) and each line was tested five times. CRM is used as a quality control for ICP-MS analyses, and a *L. sebae* CRM calibration standard was representative of the Lutjanidae family (Yoshinaga et al. 2000). Elemental concentrations were measured in ppm and expressed as a ratio to calcium concentrations (metal:calcium, abbreviated as Me:Ca).

LA-ICP-MS protocol

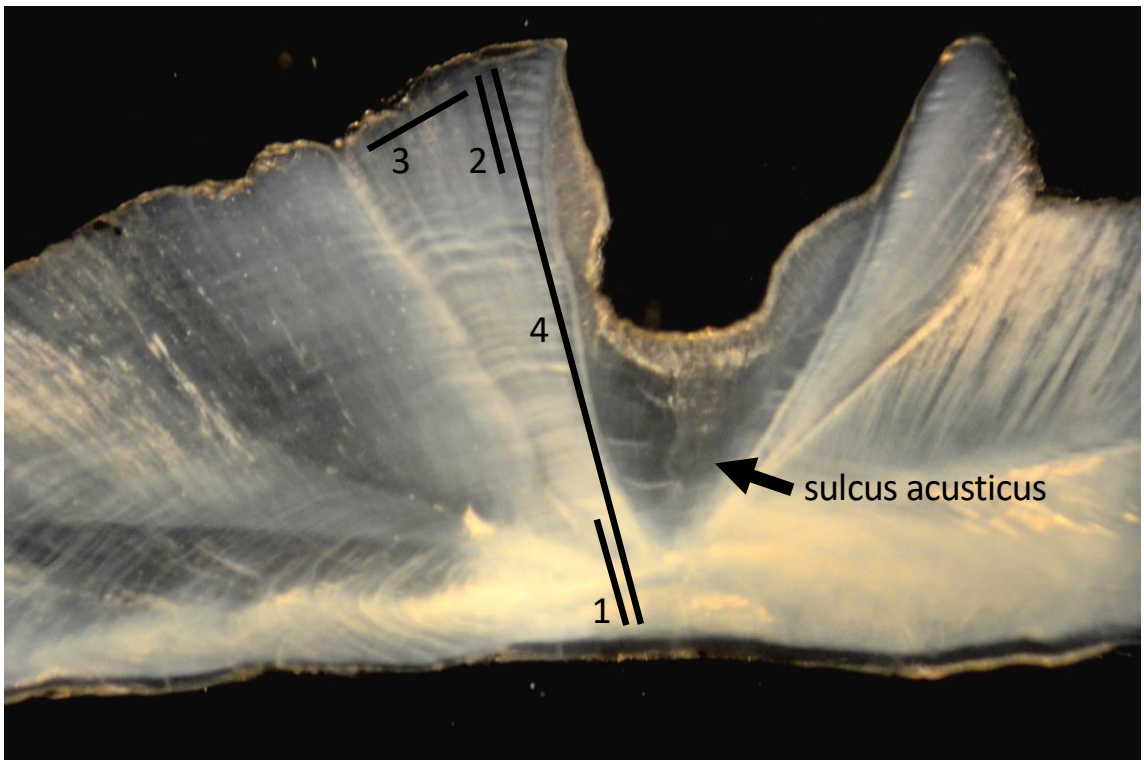
Spatial and temporal resolution elemental fingerprints were obtained from the time fish hatched (core) to the time of collection (edge). Further, the results were compared for two different ablation spot sizes that would integrate different amounts of the otolith chronology elemental deposition. Thirty-three otoliths from two species were selected for laser-based analyses. Otoliths were transverse-sectioned, embedded in CrystalBond 509 Amber resin to maintain an even ablation surface, using a combination of 600, 1200 and 3000-grit grinding wheels and 3-µm lapping film and Milli-Q water for polishing. For all LA-ICP-MS

measurements, the area was pre-ablated to remove potential contamination using a larger ablation spot-size. Each LA-ICP-MS transect consisted of a 20-sec background scan followed by a continuous ablation scan of 10-Hz pulses with a 395-nm Geolas Pro laser paired with a Varian mass spectrometer. The elements measured with LA-ICP-MS included: ^{7}Li, ^{24}Mg, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{88}Sr, and ^{138}Ba. For each otolith, LA-ICP-MS samples were taken in the following areas of each otolith (Fig. 6-1): a) a 'core-to-edge' transect with a 24-µm ablation mask, b) an adjacent 'core-to-edge' transect with a 32-µm ablation mask, and c) an edge measurement from the sulcus acusticus along the proximal surface-edge (approximately 200-µm long, using a 24-µm ablation mask). NIST610 and NIST612 readings were taken at the start, mid-point, and end of each sample chamber (16-18 otoliths); NIST readings are considered reliable for determining accuracy of measurements for a calcium carbonate matrix (Craig et al. 2000). LA-ICP-MS spectral data was analysed using IGOR PRO 6.37 software with Iolite v.2.2 interface with a mean and three standard deviation outlier rejection scheme. Calcium readings were checked for consistency across the otolith and elements were expressed as a ratio to calcium as an internal standard (Me:Ca).

Table 6-3: Geographic locations of otolith samples used for solution-based inductively coupled plasma mass spectrometry (ICP-MS) and laser-ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Eteline snapper otoliths were collected in multiple Exclusive Economic Zones. Latitude and longitude are expressed in decimal degrees.

Method	*Species*	*Etelis coruscans*				*Etelis sp.*			
	Exclusive Economic Zone	Latitude (°S)	Longitude (°E)	*n*	Mean age (years)	Latitude (°S)	Longitude (°E)	*n*	Mean age (years)
Solution-based ICP-MS	Papua New Guinea	2.35-2.57	150.40-150.80	3 cores 3 whole	15.7 14.7	2.35-2.50	150.40-150.60	3 cores 3 whole	12.7 13.7
	Vanuatu	15.55	167.33	3 cores 3 whole	12.7 10.3	15.55	167.33	3 cores 3 whole	13 13.3
	New Caledonia	20.94	165.59	3 cores 3 whole	12.3 12	20.54-21.13	164.99-165.76	3 cores 3 whole	13.3 12
	Fiji	22.36	181.03	3 cores 3 whole	9.7 9.7				
	Wallis and Futuna	13.42-13.59	180.77	3 cores 3 whole	15.3 15.3	13.42	180.77	3 cores 3 whole	17 20.3
	Tonga	22.98-23.52	183.75-184	3 cores 3 whole	9.3 6.7	18.35-19.78	185.25-185.70	3 cores 3 whole	11.7 11
Laser-ablation ICP-MS	Papua New Guinea	2.35-2.57	150.40-150.80	3	13.7	2.35-2.50	150.40-150.60	3	10
	Vanuatu	15.55	167.33	3	9.7	15.55	167.33	3	13
	New Caledonia	20.94	165.59	3	10.3	20.61-21.12	164.99-165.76	3	14.7
	Fiji	22.36	181.03-181.04	3	13.3				
	Wallis and Futuna	13.42	180.77	3	15.3	13.40-13.59	180.75-180.77	3	19.3
	Tonga	22.98-23.52	183.78-184	3	11	19.05-22.98	184-185.70	3	11.7

If calcium varied across the otolith, this could confound an estimate of average Me:Ca. All elements were expressed as µm/mol or mm/mol (depending on quantity) and then expressed as a ratio to calcium. Four locations on the otolith were compared using averaged LA-ICP-MS data points: 1) the 'early life' period, which was defined as the average of the first 50 Me:Ca data points of the transect ('average core', both 24 and 32-µm), 2) the 'late life prior to capture' encompassed an average of the last 50 data points of the transect ('average edge', both 24 and 32-µm), 3) average of separate edge ablation with 24-µm ('total edge load', only 24-µm), and 4) an average of 150 data points of the entire transect ('total load', both 24 and 32-µm). This method ensured no unequal weighting of points among samples. For each EEZ and each method there were three replicate otoliths. The average core measurement would have included the first several years including the larval and juvenile portions of the lifespan, the average edge would have included several years before capture, presumably in the environment of the EEZ it was captured in. The justification for using averaged values was to broadly compare how regions of the otolith may assist in the detection of spatial differences, and to understand how location on the otolith may change estimates, perhaps averaging to environmental differences with respect to age.



right and the approximate areas of the LA-ICP-MS transects (24 µm and 32 µm ablation mask sizes) and the edge measurement (24-µm) are indicated. The approximate locations of calculated averages are depicted with 1) the average of the first 50 data points of the transect (average core), 2) the average of the last 50 data points of the transect (average edge), 3) average of the separate edge measurement (total edge load), and 4) an average of 150 data points of the entire transect (total load).

Statistical treatment of data

To investigate the relative variation for each species, it was necessary to assess the natural variation among individual otolith samples and pooled variance. Averages for all groups of solution-based and LA-ICP-MS data were evaluated by a coefficient of variation (CV) based on single element concentration ratios, where the standard deviation over the mean was expressed as a percentage for untransformed data. Further, specific groups of otolith elemental ratios were evaluated by a linear regression to see if proportional variance trends were similar between methods for core vs whole (solution-based) and average core and average total (LA-ICP-MS) samples. Data was Box-Cox transformed, centred and scaled (package caret, Kuhn 2017) and a coefficient of determination (R^2) indicated the proportion of explained variance among measurements.

It is important for both univariate regression analyses and multivariate analyses such as multivariate analysis of variance (MANOVA) and linear discriminant function analysis (LDFA) that data were transformed, scaled and centred to meet assumptions of normality and homogeneity of variance. A Box-Cox power transformation was sufficient to transform most elements to conform with multivariate normality when a log(x+1)-transformation proved to be inadequate for some elemental distributions. Otolith chemistry data can be highly variable and specific elemental ratios are often non-normal and positively skewed (right-tailed). A Box-Cox transformation was optimal for otolith chemistry data and has been recommended in other otolith studies (Walther et al. 2017). It is stringent and resolves some positively-skewed distributions to better adhere to assumptions of normality. Pairs of elemental concentrations were also compared within a group of measurements (e.g. core, whole, average core, average edge) and for correlations greater than 0.7, one or both elements were removed from subsequent multivariate analysis. When select elements were not multivariate normal, they were removed. Elements were considered separate and independent for univariate analyses. Data were tested using Shapiro-Wilk's tests for normality, Mardia's test for multivariate normality (package MVN, (Korkmaz et al. 2014), and visually investigated with QQ-plots and histograms. For some regressions, specific data points were removed and analyses re-tested, and overall there were few statistical outliers; however, they were kept for the benefit of equal sample sizes (for parametric tests) and all assumptions were considered reasonably met.

Investigating age effects

Specific elements may be differentially incorporated into the otolith over time and may be correlated with the age of the individual fish. To evaluate if age posed any significant correlation with elements in the otolith, the age of each individual fish was included in a linear regression with the elemental ratios for each group of measurements. Age was independently

estimated from annual increment counts using the individual's other otolith (Williams et al. 2015). The distribution of age within each group was significantly different from normal for *E. sp.* samples only and this was corrected by a square-root transformation for LA-ICP-MS data (both measured from 32 and 24-µm mask sizes) and by a Tukey's Ladder of Powers transformation for solution-based whole otolith samples (rcompanion package, Mangiafico 2017) when a square-root transformation was insufficient to meet assumptions. Fish were all adults at capture, but differences in age among samples were due to the selection of individuals based on fork-length comparisons and not age, which was not known at the time of selection. Each elemental ratio from each group of measurements was plotted in a linear model against the variable age (or transformed age) to detect possibly significant relationships. Some stock structure investigations have found element-otolith weight relationships (Campana 2005), but due to the moderate sample size, as well as the fact some otoliths were chipped, otolith weight was determined to not be a reliable measurement, and element-otolith weight relationships were not investigated.

Single-element otolith variation among multiple EEZs

To evaluate whether single-elements were responsible for some of the variation between EEZs, solution-based ICP-MS samples were analysed using a linear model with the factors Species (a=2), EEZ (b=5) and Measurement (core vs. whole) for averaged elemental ratio for both species combined (5 EEZs for balanced design), and follow-up models for each species individually with the factors EEZ and Measurement (6 and 5 EEZs depending on the species). Since each of the dissolved otoliths came from separate fish, samples were treated as independent and data were Box-Cox transformed, centred and scaled. Normality was assessed by Shapiro-Wilk's test and homogeneity of variance by Levene's test.

LA-ICP-MS data were treated similarly, but as separate measurements (core, edge) were not from independent fish, there were two key differences. First, I used a regression between core and edge measurements to determine the coefficient of determination (R^2) between samples. Second, instead of a linear model a linear mixed-effects model (analogous to a repeated-measures ANOVA) was tested to include the variance of the individual fish. Data were similarly Box-Cox transformed, centred and scaled, then tested for block within-block interactions with a Tukey test (residualPlots, car package, Fox & Weisberg 2011; none of which were significant and, therefore, there was no evidence of such an interaction), assumptions of normality (Shapiro-Wilk's) and homogeneity of variance (Levene). For each Me:Ca, two models were compared using crossed factors EEZ, Species and Measurement, and then for each species separately, with only factors EEZ and Measurement. Models were compared using AICc values and this procedure was repeated for 24 and 32-µm LA-ICP-MS averaged data. To evaluate the attributes of the other types of averaged measurements, I ran similar linear mixed effects models to compare 'total edge'

and 'average edge' (both 24-µm). For the final comparison I looked for spatial variation across the averaged data from the entire transect ('total load', 24 and 32-µm) for variation at the EEZ level only.

Classification to EEZs of multiple stocks for two species

To assess how well the combined elemental concentrations were able to successfully classify membership to the correct EEZ, average concentrations of multiple elements were analysed using Linear Discriminant Function Analysis (LDFA) and multivariate analysis of variance (MANOVA). Discriminant function analysis maximizes the differences between groups using the standardized predictors (in this case average Me:Ca values), then predicted data were compared to the original discriminant function assignments to show where and if there are any misclassifications or commonly mistaken groups. In this study, classic discriminant function was preferable to the jack-knife cross-validation, which can be less accurate in calculating the re-substitution error with relatively small datasets (Moran 1975, Zollanvari et al. 2009). LDFA outperforms machine-learning methods as long as parametric assumptions are met (Jones et al. 2016). For all LDFA analyses, elemental concentrations that were multivariate normal and indicated no collinearity between pairs of elements were used as covariates (4-9 elements) with equal prior probabilities of class membership for all EEZs. Separate LDFAs were run for each group of samples (i.e. core and whole solution-based ICP-MS; average core and average edge LA-ICP-MS samples for both 24 and 32-µm measurements; function lda in package MASS, (Venables & Ripley 2002) and for each group the predicted values were graphed by the first two linear discriminants and the between-group variance (proportion explained) is reported.

MANOVA tests the differences between linear combinations of multiple measured variables based on a variance-covariance matrix. MANOVA determines where there are significant differences between the main effects and interactions of the independent variables (univariate analyses) as well as the importance of the dependent variable. Individual MANOVAs were run according to measurement type, with the same number of covariates (4-9 elements) as the corresponding LDFA. For MANOVA, Pillai's test statistic is considered the most robust and powerful to detect multivariate differences and provides a highly conservative F-statistic (Olson 1974).

Results

There were clear differences in variation among all samples for both methods (solution-based ICP-MS and LA-ICP-MS) between species, but importantly, among-sample variability was similar across all methods (Table 6-2). *E. coruscans* had greater variability among otolith samples for the following elements (Fe:Ca, Zn:Ca, Cu:Ca, Li:Ca), while some elements showed little variation among samples (Ba:Ca, Sr:Ca). In contrast, *E. sp.*, had lower variability across all samples and elements, but the elements with the highest among-sample variability were Ba:Ca, Mn:Ca, Fe:Ca and Zn:Ca.

Between methods, greater variability among samples can aid discrimination or add additional noise at the EEZ-level. The differences between methods were smaller than the differences between species and spatial patterns within each method, but there were very few notable differences. For some elements such as Mn:Ca and Fe:Ca, solution-based analyses had lower core and whole elemental concentrations than LA-ICP-MS measurements. For *E. sp.*, Mg:Ca and Ni:Ca had greater variability in solution-based measurements. Core measurements for both solution-based and LA-ICP-MS measurements were more variable than average edge or total edge measurements for some elements, but not consistently for both species, and these differences are explored in subsequent analyses.

Table 6-2: Coefficient of variation for trace elements from solution-based and LA-ICP-MS methods for two species to compare the variability between measurements (samples from multiple EEZs are pooled by method). CV values are shaded according to high values of variation (>80%, dark green), moderate (40-80%, medium green), and low (<40%, light green).

	***Etelis coruscans* (*n*=18)**									***Etelis sp.* (*n*=15)**								
	Solution-based ICP-MS		**LA-ICP-MS (24-µm)**				**LA-ICP-MS (32-µm)**			**Solution-based ICP-MS**		**LA-ICP-MS (24-µm)**				**LA-ICP-MS (32-µm)**		
	Core	Whole	Average core	Average edge	Total edge	Total load	Core	Edge	Total load	Core	Whole	Core	Edge	Total edge	Total load	Core	Edge	Total load
Ba:Ca	52.3	15.3	44.5	26.1	34.4	24.2	27.3	24.0	20.4	19.6	43.2	91.9	40.7	43.4	35.8	61.4	29.3	26.9
Sr:Ca	9.9	14.7	16.6	22.4	25.6	8.6	13.5	22.0	6.08	10.5	21.9	11.9	24.1	22.4	17.2	11.5	19.9	18.1
Mg:Ca	58.6	48.2	78.5	56.8	50.7	56.9	47.7	50.4	39.5	40.0	50.1	25.7	27.7	22.0	17.1	31.7	44.8	23.8
Mn:Ca	22.4	17.5	56.6	38.2	80.3	35.8	37.7	29.9	28.5	12.7	38.6	66.9	59.3	66.2	61.8	54.7	74.0	55.7
Li:Ca			137.2	197.8	167.3	153.7	100.5	178.7	135.5			26.7	30.0	29.1	22.0	49.7	33.9	33.0
Fe:Ca	4.6	1.1	113.5	59.8	41.4	55.1	103.5	30.1	46.4	2.7	1.3	71.1	55.2	59.0	58.7	44.4	66.5	56.8
Cu:Ca	66.2	25.8	118.4	138.0	69.5	74.3	84.5	49.4	66.4	88.1	20.9	28.0	26.5	46.5	21.3	34.8	37.2	30.1
Ni:Ca	60.5	41.9	52.0	51.1	66.3	47.1	40.8	37.7	40.4	47.2	54.5	19.6	39.9	25.4	18.6	31.8	34.3	24.2
Zn:Ca			144.8	76.1	59.1	101.8	180.3	78.5	95.5			31.8	54.7	108.5	34.7	64.2	49.1	51.3

Investigating the effect of age

Few elements showed consistent evidence of a relationship with age, and the relationship was not consistent between species. Significant relationships were plotted (Appendix Fig. B1-2); however, R^2 values were low and ranged between 0.2 and 0.44 for univariate elements. For solution-based samples, Sr:Ca showed a slight positive relation with age in dissolved whole otolith measurements for both species ($p < 0.01$ for *E. coruscans* and *E. sp.*) with older individuals having higher concentration ratios. While this trend was consistent in LA-ICP-MS samples, the variation was also greater. Age effects may also be confounded by the collection of fish from multiple locations.

Between-species variation and spatial variation: solution-based ICP-MS

Variation in Me:Ca ratios was detected among EEZs for both species and differences in spatial discrimination were found between otolith core and whole otolith measurements analysed by solution-based ICP-MS. Both species showed some patterns of spatial variation of trace element ratios (Table 6-3, Fig. 6-2), but rank abundance of ratio varied by species for each element. There were some significant differences in Ba:Ca, Sr:Ca, Mn:Ca and Zn:Ca among EEZs (two-way ANOVA). For instance, core samples from Vanuatu were significantly lower in Ba:Ca than New Caledonia (Tukey's HSD, $p_{adj} = 0.007$) and Papua New Guinea ($p_{adj} = 0.03$); samples from Papua New Guinea and Wallis and Futuna had significantly higher Sr:Ca than Tongan samples ($p_{adj} = 0.006$, $p_{adj} = 0.004$); while Vanuatu had lower Mn:Ca than Tonga ($p_{adj} = 0.04$).

Trace element concentrations of Mn:Ca and Fe:Ca were significantly higher in whole dissolved otoliths than core samples from individuals collected from the same EEZ. No single elements varied significantly for the interaction of EEZ*Measurement when samples from both species were combined, a significant interaction was detected when species were analysed separately. The two-way fixed-factor ANOVA (EEZ*Measurement) demonstrated greater congruency between species for the elements Ba:Ca, Mg:Ca, Cu:Ca and Zn:Ca. Interestingly, some elements (Sr:Ca and Fe:Ca) may be incorporated differently by species. For these elements, the three-factor model (EEZ*Species*Measurement, not reported here) had the lowest AICc values and the difference between models was highly significant.

For both species, there was significant variation between EEZs for most elements, and many elements had higher concentrations in the whole dissolved otolith than in dissolved cores. Where significant interactions existed, these were often caused by the rank of EEZ relative concentrations switching among core and whole samples.

Table 6-3: Spatial variation at the Exclusive Economic Zone (EEZ) level for two deepwater snapper species otolith chemistry by solution-based inductively coupled plasma mass spectrometry. Combined univariate elemental concentrations for two species and also separate species elemental concentrations were analysed with a two-factor analysis of variance (ANOVA). Prior to ANOVA, data was Box-Cox transformed, centred and scaled.

		Both species				*Etelis coruscans*				*Etelis sp.*			
Element	Source of Variation	Df	MS	F	p-value	Df	MS	F	p-value	Df	MS	F	p-value
Ba:Ca	EEZ	4	3.78	4.60	<0.01**	5	1.72	1.85	0.14	4	3.13	5.38	p < 0.01**
	Core vs whole	1	0.15	0.19	0.67	1	0.50	0.54	0.47	1	3.03	5.18	p < 0.05*
	Interaction	4	0.66	0.81	0.53	5	0.74	0.80	0.56	4	0.42	0.72	0.59
	Residual	50	0.82			24	0.93			20	0.58		
Sr:Ca	EEZ	4	3.79	5.38	<0.01**	5	3.18	7.66	<0.001***	4	3.67	8.20	<0.001***
	Core vs whole	1	3.34	4.74	0.03	1	0.15	0.36	0.55	1	4.60	10.29	<0.01**
	Interaction	4	1.34	1.90	0.12	5	1.80	4.34	<0.01**	4	0.19	0.43	0.79
	Residual	50	0.70			24	0.42			20	0.45		
Mg:Ca	EEZ	4	1.21	1.21	0.32	5	0.88	0.86	0.52	4	0.72	1.09	0.39
	Core vs whole	1	1.86	1.86	0.18	1	0.63	0.61	0.44	1	9.37	14.13	<0.01**
	Interaction	4	0.56	0.55	0.70	5	1.05	1.02	0.43	4	0.87	1.32	0.30
	Residual	50	1.00			24	1.03			20	0.66		
Mn:Ca	EEZ	4	2.49	3.33	<0.05*	5	2.41	7.85	<0.001***	4	1.94	3.22	<0.05*
	Core vs whole	1	8.87	11.87	<0.01**	1	10.61	34.52	<0.001***	1	7.30	12.11	<0.01**
	Interaction	4	0.70	0.94	0.45	5	0.99	3.21	<0.05*	4	0.47	0.78	0.55
	Residual	50	0.75			24	0.31			20	0.60		
Cu:Ca	EEZ	4	1.05	1.04	0.40	5	0.83	1.01	0.44	4	0.49	0.37	0.83
	Core vs whole	1	0.53	0.52	0.47	1	4.75	5.75	<0.05*	1	0.46	0.35	0.56
	Interaction	4	0.88	0.87	0.49	5	1.25	1.52	0.22	4	0.02	0.01	1.00
	Residual	50	1.01			24	0.83			20	1.33		
Fe:Ca	EEZ	4	1.24	1.82	0.14	5	1.36	25.71	<0.001***	4	1.11	12.09	<0.001***
	Core vs whole	1	16.60	24.27	<0.001***	1	22.14	417.34	<0.001***	1	17.92	195.75	<0.001***
	Interaction	4	0.81	1.18	0.33	5	0.95	17.99	<0.001***	4	1.21	13.16	<0.001***
	Residual	50	0.68			24	0.05			20	0.09		
Zn:Ca	EEZ	4	4.03	5.42	<0.01**	5	2.23	5.01	<0.01**	4	1.37	1.19	0.34
	Core vs whole	1	0.61	0.83	0.37	1	4.96	11.17	<0.01**	1	0.41	0.36	0.56
	Interaction	4	1.29	1.74	0.16	5	1.65	3.71	<0.05*	4	0.05	0.04	1.00
	Residual	50	0.74			24	0.44			20	1.15		

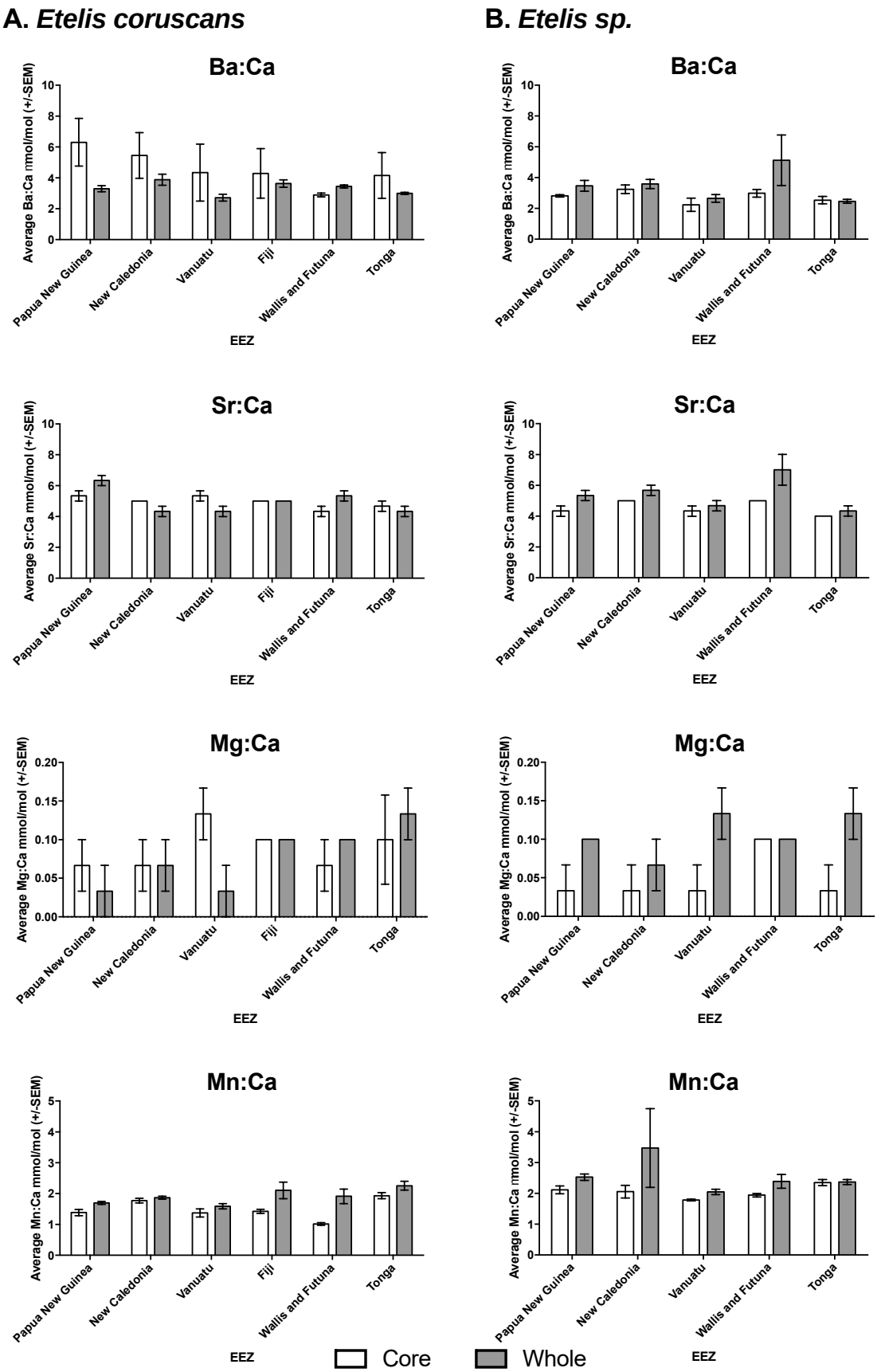


Figure 6-2: Variation in trace metal concentrations for (A) *Etelis coruscans* (*left*) and (B) *Etelis sp.* (*right*) among multiple locations (six and five Exclusive Economic Zones respectively) for selected elements Ba:Ca, Sr:Ca, Mg:Ca and Mn:Ca (mean concentration ± standard error of the mean) in solution-based ICP-MS otolith chemical analyses. There are no error bars where all three replicates had the same value.

Ablation spot size and LA-ICP-MS discrimination

LA-ICP-MS transects for both species followed the same general pattern across locations for both ablation spot sizes; however, there were differences in detection levels and magnitude. The smaller ablation spot size (24-µm) had slightly higher average concentrations than 32-µm measurements. For most elements, the differences between locations on the otolith (core vs. edge) were consistent between the measurements. For some elements (e.g. Mn:Ca) the differences between core and edge were significantly different in magnitude for the smaller ablation spot size (Appendix Fig. B3-4). Ablation profiles were longer for smaller ablation sizes resulting in a wider profile (i.e. more data points) than the larger laser ablation spot. This may increase the detection of elemental variation spatially on the otolith.

Between-species variation and spatial variation: LA-ICP-MS

Average core and edge LA-ICP-MS measurements showed clear differences among multiple elements, but these differed for the two species sampled. Overwhelmingly, LA-ICP-MS showed the differences within the life history transect (i.e. the differences between core and edge) were greater than the spatial variation per se for the majority of univariate analyses (Table 6-4, Fig. 6-3). Overall, Ba:Ca and Mg:Ca showed consistently higher magnitude in the earlier life history, while more Sr:Ca was incorporated in the later life history for both species (Fig. 6-3). Mg:Ca and Mn:Ca had higher concentration ratios for both species compared to solution-based ICP-MS samples (Fig. 6-2 and 6-3), and *E. sp.* had higher Mn:Ca edge concentrations than *E. coruscans*. Several elements (Ba:Ca, Sr:Ca, Li:Ca, Mn:Ca, Fe:Ca) had significant interactions at the level of Measurement*Species, indicating that the differences in concentrations of these elements between the otolith core and edge were not consistent between species. The differences between the levels evaluated here (EEZ, averaged Measurements and Species) were mostly consistent between both ablation sizes. Coefficient of determination (or the proportion of the variance between core and edge measurements) assessed the independence of the measurements and revealed few strong or consistent correlations between 24 and 32 µm measurements (Appendix Table B1, Appendix Fig. B5). High coefficients may indicate that high or low core measurements produce corresponding high or low edge measurements.

Although the otolith chemistry along the edge of the otolith may show different spatial patterns, few differences in the placement of laser-ablated measurements for either species were observed (i.e. Fe:Ca for *E. coruscans*, Fe:Ca and Mn:Ca for *E. sp.*; Table 6-5) when comparing the average edge measurement to the total edge (Fig 6-1; measurement 2 vs 3) showing overall congruency among the EEZ differences (Fig. 6-4 and 6-5). Most differences between edge measurements were not significant and much smaller in magnitude to the differences between average core and average edge measurements. Average edge measurements presumably sampled

the last few years of life prior to capture and there may be inconsistent otolith growth around the edge. By testing if where the edge measurements were taken affected comparisons, there can be greater confidence that temporal differences such as the year of capture or growth inconsistencies are not masking the spatial resolution. These results indicate that the edge measurement differences are not consequential to the interpretation of edge otolith chemistry for spatial discrimination at this scale.

The differences within the life history transects were better for spatial separation than the average of the entire transect ('total load'), which showed no significant separation for most elements among the EEZs investigated (Appendix Table B2, Appendix Fig. B6). Similar to the dissolution of the whole otolith in solution-based ICP-MS, the effect of averaging 150 data points may diminish the ability to detect differences, and variation in the life history may be better spatially resolved by separate measurements.

Table 6-4: Spatial variation at the Exclusive Economic Zone (EEZ) level for *Etelis coruscans* and *Etelis sp.* otolith chemistry by LA-ICP-MS. Combined univariate elemental concentrations for two species and also separate species elemental concentration ratios were analysed with linear mixed effects models for two otolith locations sampled from the LA-ICP-MS transect (average core, average edge). Data was Box-Cox transformed, centred, scaled and includes Type III with estimated Kenward-Roger approximations for degrees of freedom. Values reported here are for 24-µm and values in bold were significant for 32-µm data.

	Both species					*Etelis coruscans*					*Etelis sp.*			
Element	Source of Variation	Df	MS	F-value	p	Source of Variation	Df	MS	F-value	p	Df	MS	F-value	p
Ba:Ca	EEZ	4,20	0.28	0.68	0.61	EEZ	5,12	0.14	0.52	0.75	4,10	0.23	0.40	0.80
	Measurement	1,20	27.68	66.47	**<0.001*** **	Measurement	1,12	26.72	96.59	**<0.001*** **	1,10	6.47	11.20	<0.01**
	Species	1,20	0.32	0.77	0.39	Interaction	5,12	0.18	0.66	0.66	4,10	2.51	4.34	<0.05*
	EEZ*Measurement	4,20	0.61	1.46	0.25									
	EEZ*Species	4,20	0.15	0.37	0.83									
	Measurement*Species	1,20	4.13	9.92	**<0.01***									
	EEZ*Measurement*Species	4,20	1.51	3.63	<0.05*									
Sr:Ca	EEZ	4,20	2.06	6.42	**<0.01****	EEZ	5,12	1.11	2.34	**0.11**	4,10	1.29	6.46	<0.01**
	Measurement	1,20	31.16	97.19	**<0.001*** **	Measurement	1,12	14.02	29.52	**<0.001*** **	1,10	19.26	96.24	**<0.001*** **
	Species	1,20	0.00	0.00	0.97	Interaction	5,12	0.80	1.69	0.21	4,10	0.14	0.71	**0.60**
	EEZ*Measurement	4,20	0.15	0.45	0.77									
	EEZ*Species	4,20	0.48	1.51	0.24									
	Measurement*Species	1,20	1.43	4.46	**<0.05***									
	EEZ*Measurement*Species	4,20	0.71	2.22	0.10									
Li:Ca	EEZ	4,20	0.01	0.20	0.94	EEZ	5,12	0.02	0.06	1.00	4,10	0.08	0.19	0.94
	Measurement	1,20	0.31	5.92	<0.05*	Measurement	1,12	1.96	7.60	**<0.05***	1,10	9.58	22.02	**<0.001*** **
	Species	1,20	2.51	48.02	**<0.001*** **	Interaction	5,12	0.54	2.09	0.14	4,10	0.39	0.90	0.50
	EEZ*Measurement	4,20	0.02	0.42	0.79									
	EEZ*Species	4,20	0.01	0.20	0.93									
	Measurement*Species	1,20	1.07	20.47	**<0.001*** **									
	EEZ*Measurement*Species	4,20	0.15	2.93	<0.05*									

	Both species					*Etelis coruscans*					*Etelis sp.*			
Element	Source of Variation	Df	MS	F-value	p	Source of Variation	Df	MS	F-value	p	Df	MS	F-value	p
Mg:Ca	EEZ	4,20	1.13	2.58	0.07	EEZ	5,12	1.44	2.66	0.08	4,10	0.97	1.21	0.36
	Measurement	1,20	3.00	6.86	**<0.05***	Measurement	1,12	2.98	5.49	**<0.05***	1,10	0.55	0.69	0.42
	Species	1,20	6.22	14.21	**<0.01****	Interaction	5,12	0.37	0.67	0.65	4,10	0.62	0.77	**0.57**
	EEZ*Measurement	4,20	0.16	0.37	**0.82**									
	EEZ*Species	4,20	0.77	1.76	0.18									
	Measurement*Species	1,20	1.35	3.08	**0.09**									
	EEZ*Measurement*Species	4,20	0.25	0.57	0.69									
Mn:Ca	EEZ	4,20	0.10	0.60	0.67	EEZ	5,12	0.82	1.30	0.33	4,10	0.03	0.49	0.74
	Measurement	1,20	0.51	3.20	**0.09**	Measurement	1,12	14.18	22.59	**<0.001*****	1,10	9.99	161.90	<0.001***
	Species	1,20	4.27	26.66	<0.001***	Interaction	5,12	0.33	0.53	0.75	4,10	0.11	1.83	0.20
	EEZ*Measurement	4,20	0.13	0.82	0.53									
	EEZ*Species	4,20	0.14	0.86	0.51									
	Measurement*Species	1,20	12.29	76.63	**<0.001*****									
	EEZ*Measurement*Species	4,20	0.13	0.81	0.53									
Cu:Ca	EEZ	4,20	0.17	0.35	0.84	EEZ	5,12	0.15	0.31	0.90	4,10	0.58	0.61	0.66
	Measurement	1,20	0.24	0.50	0.49	Measurement	1,12	0.20	0.43	0.52	1,10	0.00	0.00	**0.95**
	Species	1,20	0.28	0.57	0.46	Interaction	5,12	0.35	0.73	0.62	4,10	0.47	0.50	0.74
	EEZ*Measurement	4,20	0.56	1.16	0.36									
	EEZ*Species	4,20	0.34	0.70	0.60									
	Measurement*Species	1,20	0.21	0.43	0.52									
	EEZ*Measurement*Species	4,20	0.23	0.47	0.75									
Fe:Ca	EEZ	4,20	0.08	0.36	0.83	EEZ	5,12	0.55	0.66	0.66	4,10	0.02	0.26	0.90
	Measurement	1,20	9.42	43.14	**<0.001*****	Measurement	1,12	2.01	4.86	**<0.05***	1,10	17.20	192.71	**<0.001*****
	Species	1,20	12.19	55.85	**<0.001*****	Interaction	5,12	0.69	0.83	0.55	4,10	0.09	0.97	0.46
	EEZ*Measurement	4,20	0.28	1.30	0.31									
	EEZ*Species	4,20	0.18	0.84	0.52									
	Measurement*Species	1,20	1.63	7.45	**<0.05***									
	EEZ*Measurement*Species	4,20	0.18	0.81	0.54									

	Both species					*Etelis coruscans*					*Etelis sp.*			
Element	Source of Variation	Df	MS	F-value	p	Source of Variation	Df	MS	F-value	p	Df	MS	F-value	p
Ni:Ca	EEZ	4,20	0.04	0.19	0.94	EEZ	5,12	0.06	0.14	0.98	4,10	0.50	0.61	0.67
	Measurement	1,20	0.01	0.04	0.85	Measurement	1,12	0.06	0.13	0.72	1,10	0.00	0.00	**0.95**
	Species	1,20	9.54	42.91	**<0.001*** **	Interaction	5,12	0.32	0.74	0.61	4,10	0.68	0.83	0.54
	EEZ*Measurement	4,20	0.07	0.34	0.85									
	EEZ*Species	4,20	0.07	0.33	0.85									
	Measurement*Species	1,20	0.05	0.24	0.63									
	EEZ*Measurement*Species	4,20	0.38	1.73	0.18									
Zn:Ca	EEZ	4,20	0.90	1.25	0.32	EEZ	5,12	0.73	0.79	0.58	4,10	0.23	0.40	0.81
	Measurement	1,20	5.55	7.72	**<0.05***	Measurement	1,12	2.51	2.73	**0.12**	1,10	2.71	4.73	**0.05**
	Species	1,20	0.77	1.08	**0.31**	Interaction	5,12	0.82	0.89	0.52	4,10	0.23	0.40	**0.80**
	EEZ*Measurement	4,20	0.82	1.15	0.36									
	EEZ*Species	4,20	0.35	0.48	0.75									
	Measurement*Species	1,20	0.45	0.62	0.44									
	EEZ*Measurement*Species	4,20	0.74	1.03	0.42									

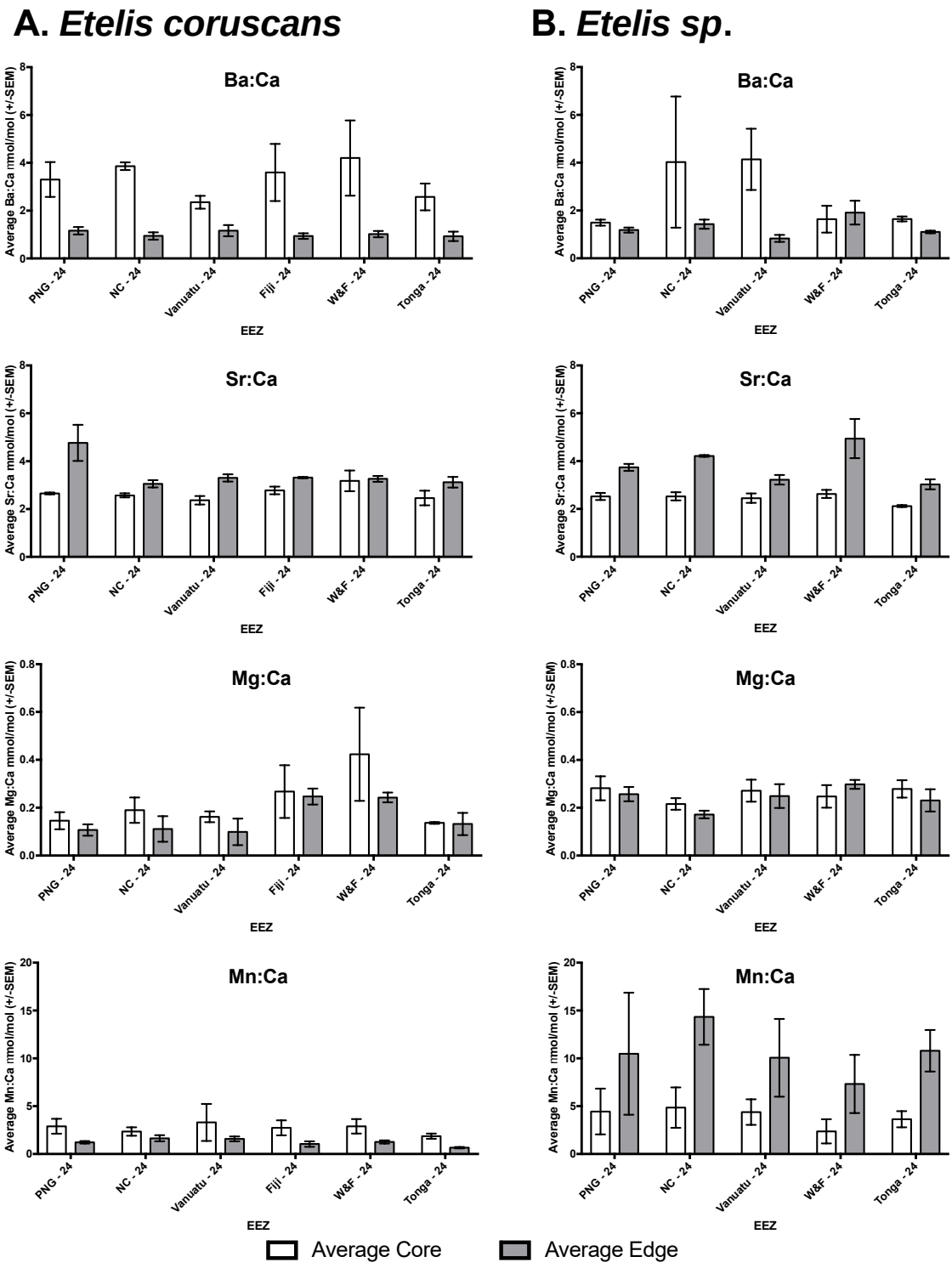


Figure 6-3: Sampling across the otolith (core-to-edge) showed distinct differences between species and capture location and magnitude of elemental concentration between average core and edge measurements LA-ICP-MS (24-µm) measurements for two species of deepwater snapper.

Table 6-5: Comparison of LA-ICP-MS measurements of total edge and average edge for spatial separation among Exclusive Economic Zones (EEZ). Linear mixed effects models to account for the variation within individuals on Box-Cox transformed, centred and scaled univariate 24-µm LA-ICP-MS measurements.

		Etelis coruscans				***Etelis sp.***			
Element	**Source of Variation**	**Df**	**MS**	**F**	**p-value**	**Df**	**MS**	**F**	**p-value**
Ba:Ca	EEZ	5,12	0.63	1.07	0.42	4,10	2.25	6.45	<0.01**
	Type of edge measurement	1,12	0.00	0.01	0.94	1,10	0.00	0.01	0.92
	Interaction	5,12	0.80	1.37	0.30	4,10	0.17	0.49	0.74
Sr:Ca	EEZ	5,12	0.90	5.85	<0.01**	4,10	1.62	9.14	<0.01**
	Type of edge measurement	1,12	0.87	5.68	<0.05*	1,10	0.26	1.45	0.26
	Interaction	5,12	0.29	1.90	0.17	4,10	0.10	0.57	0.69
Li:Ca	EEZ	5,12	0.20	0.72	0.62	4,10	0.79	0.73	0.59
	Type of edge measurement	1,12	0.35	1.27	0.28	1,10	1.15	1.06	0.33
	Interaction	5,12	0.41	1.46	0.27	4,10	0.69	0.64	0.65
Mg:Ca	EEZ	5,12	0.63	2.07	0.14	4,10	0.38	0.79	0.56
	Type of edge measurement	1,12	0.29	0.97	0.34	1,10	0.28	0.57	0.47
	Interaction	5,12	0.32	1.05	0.43	4,10	1.30	2.68	0.10
Mn:Ca	EEZ	5,12	1.67	1.87	0.17	4,10	0.17	0.89	0.50
	Type of edge measurement	1,12	0.29	0.32	0.58	1,10	9.66	51.64	<0.001***
	Interaction	5,12	0.97	1.09	0.42	4,10	0.17	0.93	0.48
Cu:Ca	EEZ	5,12	0.61	0.77	0.59	4,10	1.03	1.82	0.20
	Type of edge measurement	1,12	1.98	2.51	0.14	1,10	0.24	0.43	0.53
	Interaction	5,12	1.00	1.27	0.34	4,10	0.77	1.36	0.32
Fe:Ca	EEZ	5,12	1.82	2.30	0.11	4,10	0.23	0.84	0.53
	Type of edge measurement	1,12	4.34	5.51	<0.05*	1,10	10.26	36.92	<0.001***
	Interaction	5,12	0.53	0.67	0.65	4,10	0.07	0.24	0.91
Ni:Ca	EEZ	5,12	0.21	0.44	0.81	4,10	0.34	0.43	0.78
	Type of edge measurement	1,12	0.00	0.00	0.99	1,10	0.04	0.05	0.82
	Interaction	5,12	0.58	1.24	0.35	4,10	1.98	2.52	0.11
Zn:Ca	EEZ	5,12	0.70	1.13	0.39	4,10	0.78	0.66	0.64
	Type of edge measurement	1,12	2.43	3.92	0.07	1,10	0.03	0.02	0.88
	Interaction	5,12	0.93	1.50	0.26	4,10	0.53	0.44	0.78

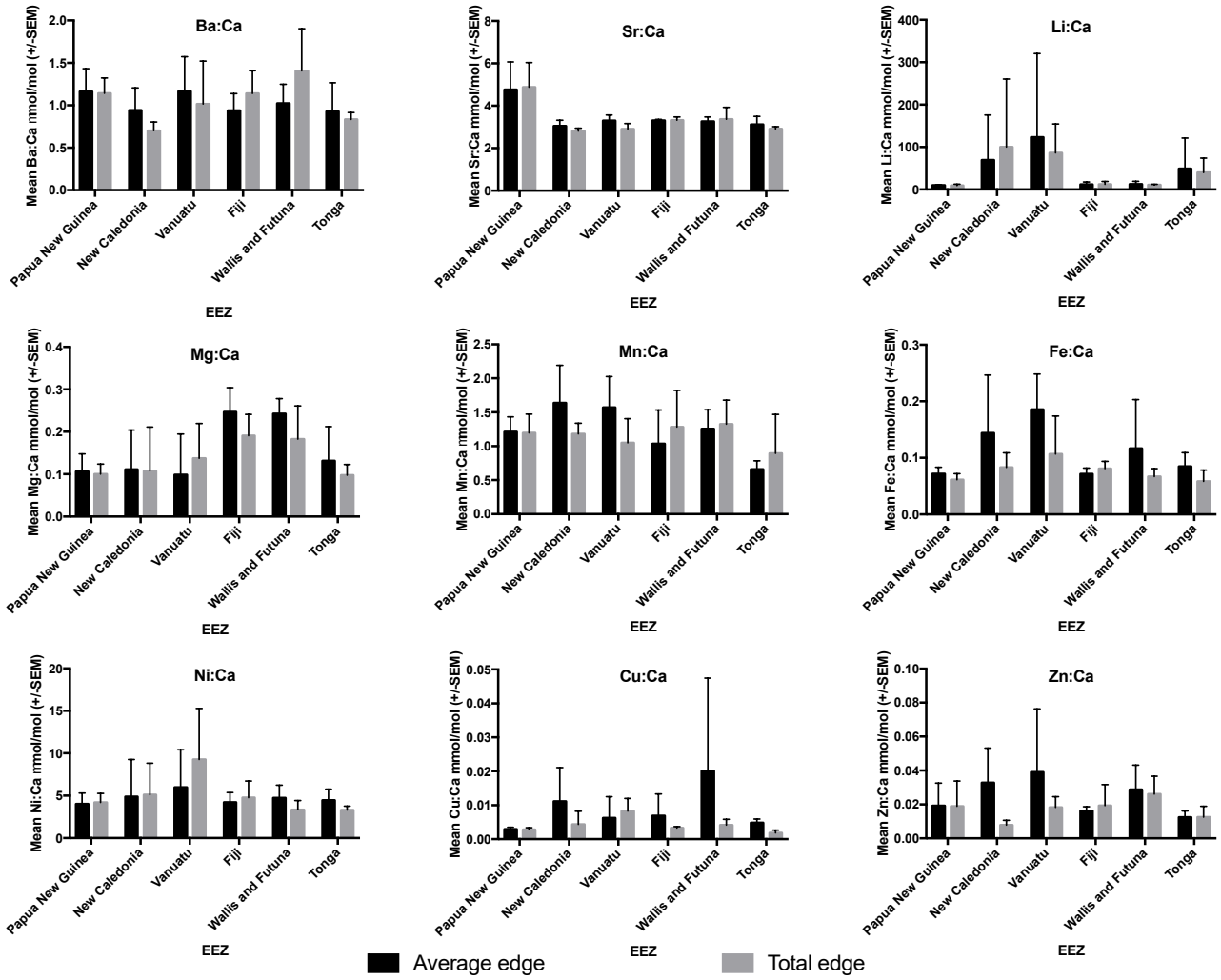


Figure 6-4: Edge comparisons of *Etelis coruscans* for nine elements sampled using LA-ICP-MS (24-µm ablation mask). Both measurements were averages of 50 data points. Average edge comprised the last few years before capture and the Total edge measurement sampled an area of the otolith edge to investigate the congruency of edge measurements for multiple elements.

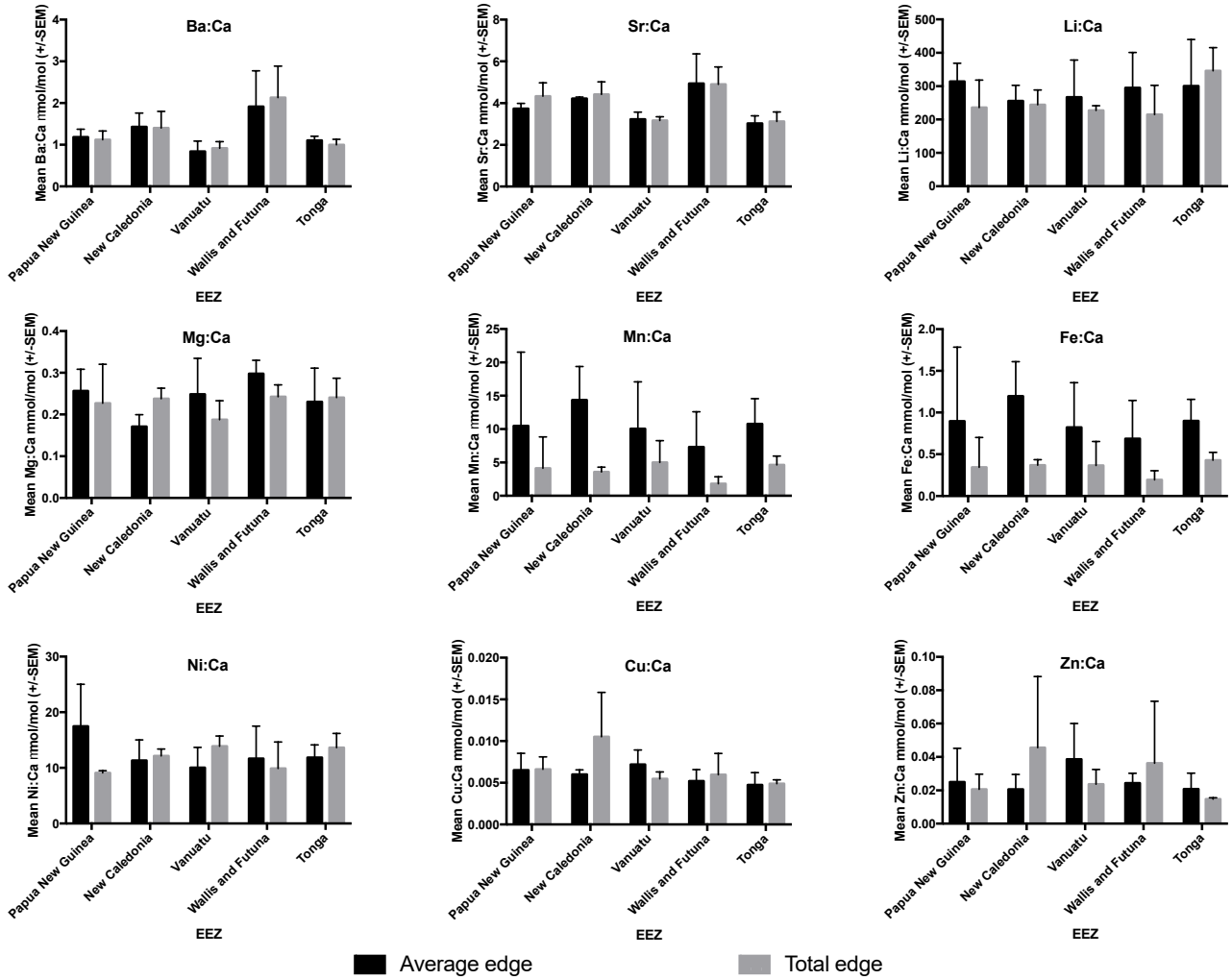


Figure 6-5: Edge comparisons of *Etelis sp.* for nine elements sampled using LA-ICP-MS (24 µm ablation mask). Both measurements were averages of 50 data points. Average edge comprised the last few years before capture and the Total edge measurement sampled an area of the otolith edge to investigate the congruency of edge measurements for multiple elements.

Elemental fingerprints by EEZ

All methods detected variation in elemental fingerprints, but the patterns were not consistent between species or methods. Solution-based ICP-MS showed more overlap between EEZs for core samples than whole otoliths for *E. sp.* than for *E. coruscans* (Fig. 6-6) with linear discriminants 1 and 2 combined describing 72.8-91.9% of the multivariate variance. For *E. coruscans*, whole otolith samples indicated that Vanuatu was separate from other locations, and core measurements indicated that Tonga and New Caledonia samples were separate from other groups. Whole otolith samples of *E. sp.* indicated two separate groups, with Tonga and Vanuatu sharing greater similarities in otolith chemistry than Papua New Guinea, New Caledonia and Wallis and Futuna, which shared some overlap in chemical composition. In contrast, the elemental compositions of the otolith cores did not differ among EEZ locations for *E. sp*.

LA-ICP-MS methods generally yielded similar results to solution-based ICP-MS with considerably more overlap in average core samples than average edge samples, and the first two linear discriminants accounting for 78.9-96.4% of the information for *E. coruscans* (Fig. 6-7) and 79.1-96.2% for *E. sp.* (Fig. 6-8). There were few consistent differences in LDFAs comparing 24 and 32 ablation sizes, but there was clearer separation along LD1 for *E. coruscans* evident in these small sample sizes for both ablation sizes. Tonga and Fiji may have more distinct stocks for *E. coruscans*, and Wallis and Futuna more clearly separated from other EEZs for *E. sp*.

Greater classification accuracy was achieved with LA-ICP-MS, but both solution-based and LA-ICP-MS analyses yielded high classification accuracy (Table 6-6), with classification success ranging from 67-100%. In general, LA-ICP-MS models included more elements, and performed slightly better than solution-based comparisons. Models that incorporated age as a covariate had marginal improvement on the model's predictive ability, often not changing classification accuracy. The average edge LA-ICP-MS measurements had the greatest classification accuracy 88.9-100%, while average core had the overall lowest 66.7-100%. There were some minor differences with ablation size, but these were smaller differences in accuracy than between models of different measurements.

MANOVA results indicated few significant differences among the measurements sampled. Both core and whole samples for *E. sp*. and for *E. coruscans* only core solution-based samples were significantly different. For almost all LA-ICP-MS samples, MANOVA results proved to be poor in resolving differences among EEZs, only average total load measurements were significantly different among EEZs for the smaller ablation size for one species.

Table 6-6: Linear Discriminant Function Analyses (LDFA) show classification accuracy by multiple-element ICP-MS models. Two sampling methods were compared for spatial separation and resolution: solution-based ICP-MS and laser ablation ICP-MS (LA-ICP-MS). Further comparisons included: core or whole (solution-based ICP-MS); and aperture of the laser ablation mask and the location of the measurement from the otolith transect (LA-ICP-MS). Both solution-based and LA-ICP-MS measurements for two deepwater snapper species show the classification percentage to the correct Exclusive Economic Zone (EEZ). Age of the specimen was included as a co-variate for some of the LDFA models to see if classification accuracy changed. Elemental measurements were Box-Cox transformed, scaled and centred. Elements included in the models conformed with multivariate normality, elemental ratios were assumed independent, and certain elements were removed if highly correlated (Pearson's $r > 0.7$). †Mardia's test for multivariate normality was adjusted for small samples ($n < 20$), non-significant values showed data was multivariate normal.

Solution-based ICP-MS			**Mardia's test†**	**MANOVA**					**LDFA**	
Species	**Sampling method**	**Elements included (#)**	**p**	**Source of variation**	**Df**	**Pillai**	**Approx. F (num Df/ den DF**	**p**	**Elements**	**Elements with age**
Etelis coruscans	Core	Ba, Mg, Mn, Zn (**4**)	0.43	EEZ	5,12	2.03	2.48 (20/48)	**0.005	77.8%	
	Whole	Ba, Sr, Mg, Mn, Fe, Cu, Zn (**7**)	0.45	EEZ	5,12	2.68	1.65 (35/50)	0.05	83.3%	88.9%
Etelis sp.	Core	Ba, Mg, Mn, Cu, Zn (**5**)	0.86	EEZ	4,10	2.17	2.13 (20/36)	*0.02	93.3%	
	Whole	Ba, Mg, Mn, Fe, Cu, Zn (**6**)	0.86	EEZ	410	2.46	2.13 (24/32)	*0.02	100%	100%
LA-ICP-MS										
Etelis coruscans	24µm – Total	Ba, Sr, Li, Mg, Mn, Fe, Zn (**7**)	0.08	EEZ	5,12	2.12	1.08 (35/50)	0.40	83.3%	83.3%
	24µm – Core	Ba, Li, Mg, Mn, Fe, Ni (**6**)	0.36	EEZ	5,12	1.77	1.00 (30/55)	0.49	72.2%	
	24µm – Edge	Ba, Sr, Li, Mg, Mn, Fe, Ni, Cu, Zn (**9**)	0.23	EEZ	5,12	2.91	1.24 (45/40)	0.25	88.9%	100%
	32µm – Total	Ba, Sr, Li, Mg, Mn, Fe, Ni, Cu, Zn (**9**)	0.39	EEZ	5,12	2.66	1.01 (45/40)	0.49	88.9%	88.9%
	32µm – Core	Ba, Li, Mg, Mn, Fe, Ni, Cu, Zn (**8**)	0.65	EEZ	5,12	1.96	0.72 (40/45)	0.85	66.7%	
	32µm – Edge	Ba, Li, Mg, Mn, Fe, Ni, Cu, Zn (**8**)	0.07	EEZ	5,12	2.56	1.18 (40/45)	0.29	94.4%	94.4%
Etelis sp.	24µm – Total	Ba, Sr, Li, Mg, Mn, Ni, Zn (**7**)	0.82	EEZ	4,10	2.68	2.03 (28/28)	*0.03	100%	100%
	24µm – Core	Ba, Sr, Mg, Mn, Ni, Cu, Zn (**7**)	0.94	EEZ	4,10	2.48	1.63 (28/28)	0.10	100%	
	24µm – Edge	Ba, Li, Mg, Mn, Ni, Cu, Zn (**7**)	0.27	EEZ	4,10	2.45	1.58 (28/28)	0.12	100%	100%
	32µm – Total	Ba, Sr, Mg, Mn, Zn (**5**)	0.57	EEZ	4,10	1.79	1.46 (20/36)	0.16	80.0%	80.0%
	32µm – Core	Ba, Sr, Li, Mg, Mn, Fe, Ni, Zn (**8**)	0.56	EEZ	4,10	2.40	1.12 (32/24)	0.39	93.3%	
	32µm – Edge	Ba, Sr, Mg, Mn, Cu, Zn (**6**)	0.12	EEZ	4,10	2.13	1.52 (24/32)	0.13	93.3%	93.3%

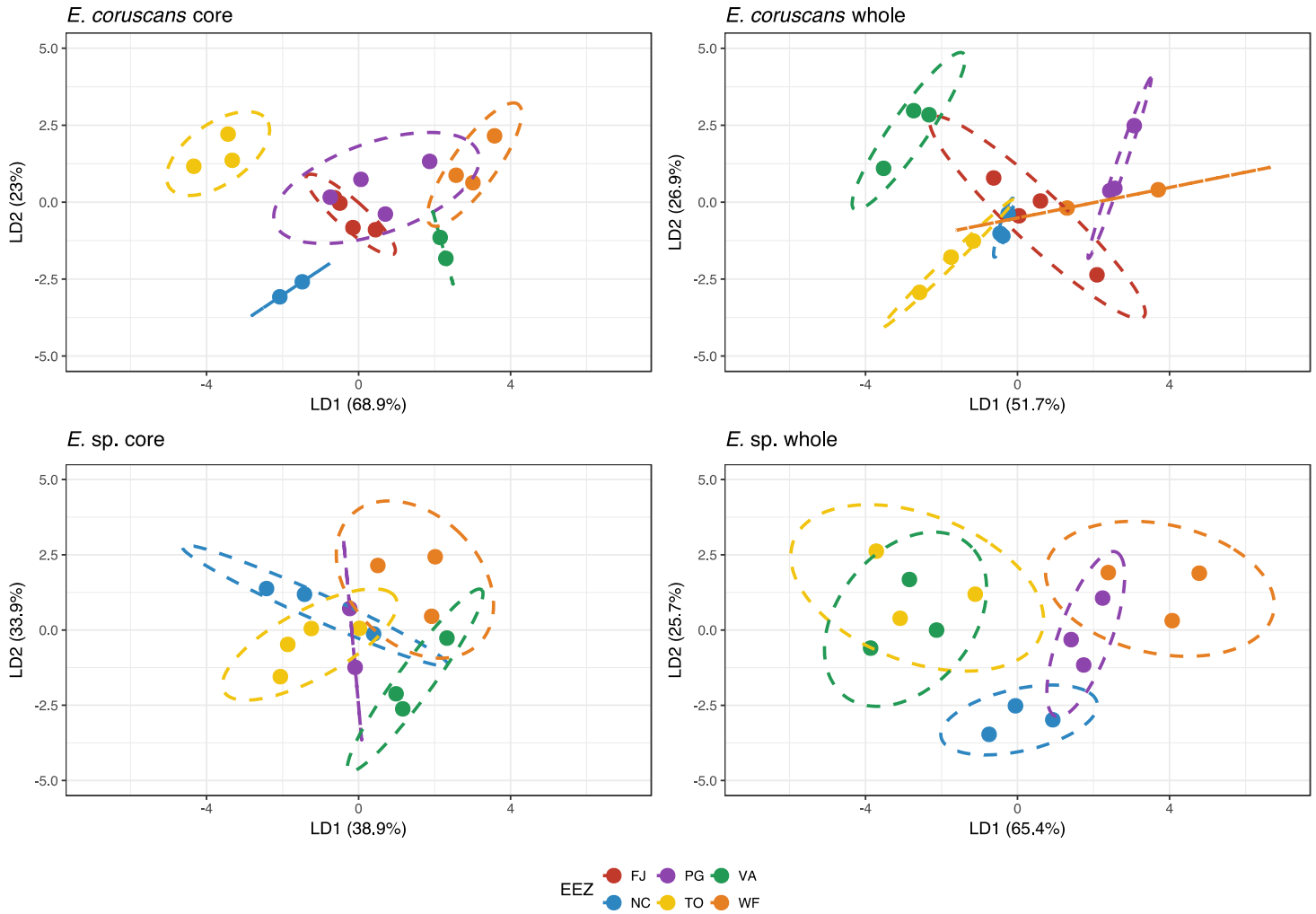


Figure 6-6: Spatial separation of core (*left*) versus whole (*right*) otoliths resolved by solution-based ICP-MS for two species of eteline snappers. Each plot shows predicted individual linear discriminant function scores incorporating seven trace elemental ratios, with separate Exclusive Economic Zone (EEZ) samples classified and 95% confidence ellipses showing the degree of overlap in elemental fingerprints.

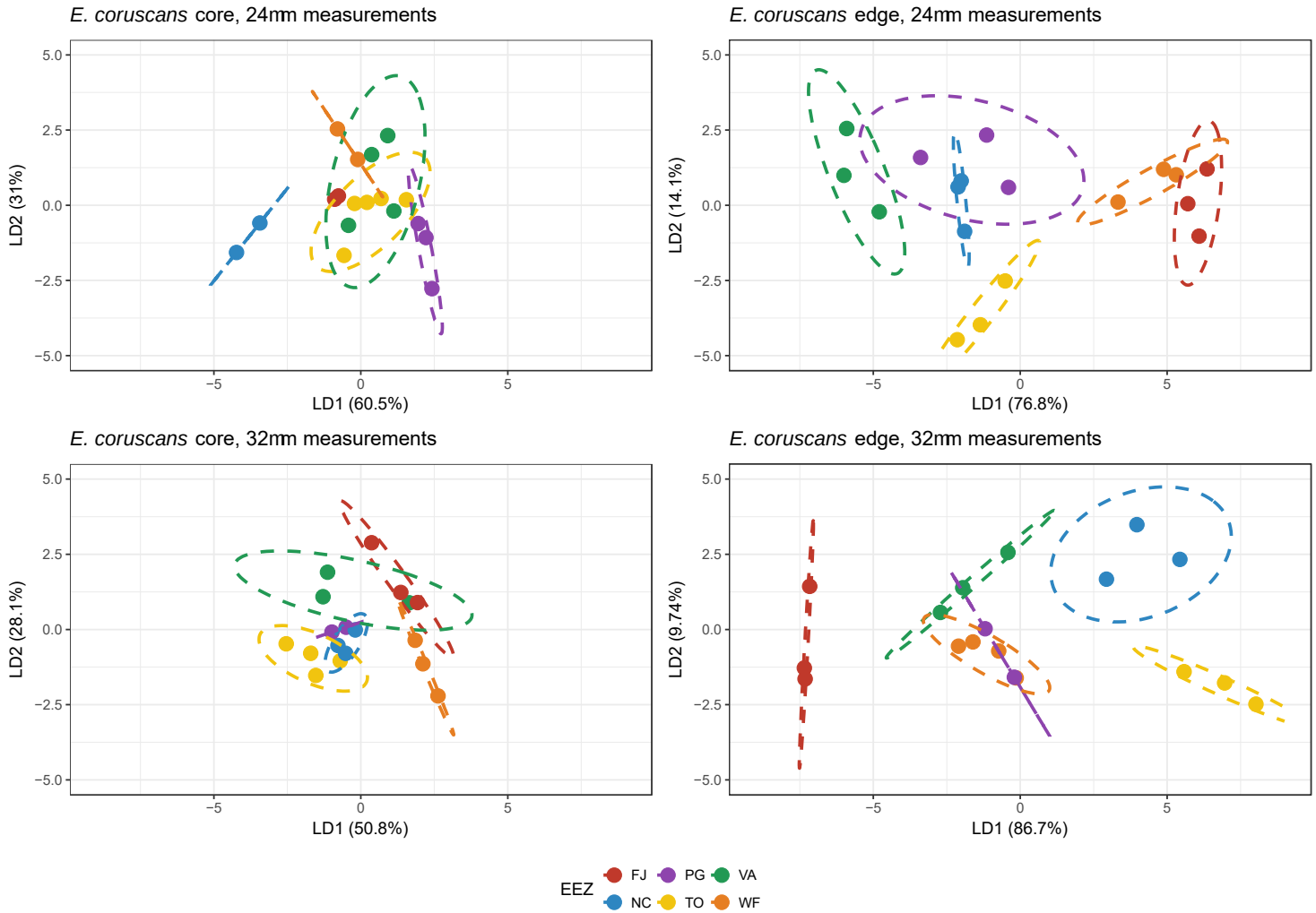


Figure 6-7: Spatial separation of juvenile-core (*left*) versus capture location-edge (*right*) otoliths resolved by LA-ICP-MS for *Etelis coruscans*. Each plot shows separate linear discriminant function analyses incorporating trace elemental ratios of predicted group membership with separate Exclusive Economic Zone (EEZ) samples classified and 95% confidence ellipses showing the degree of overlap in elemental fingerprints.

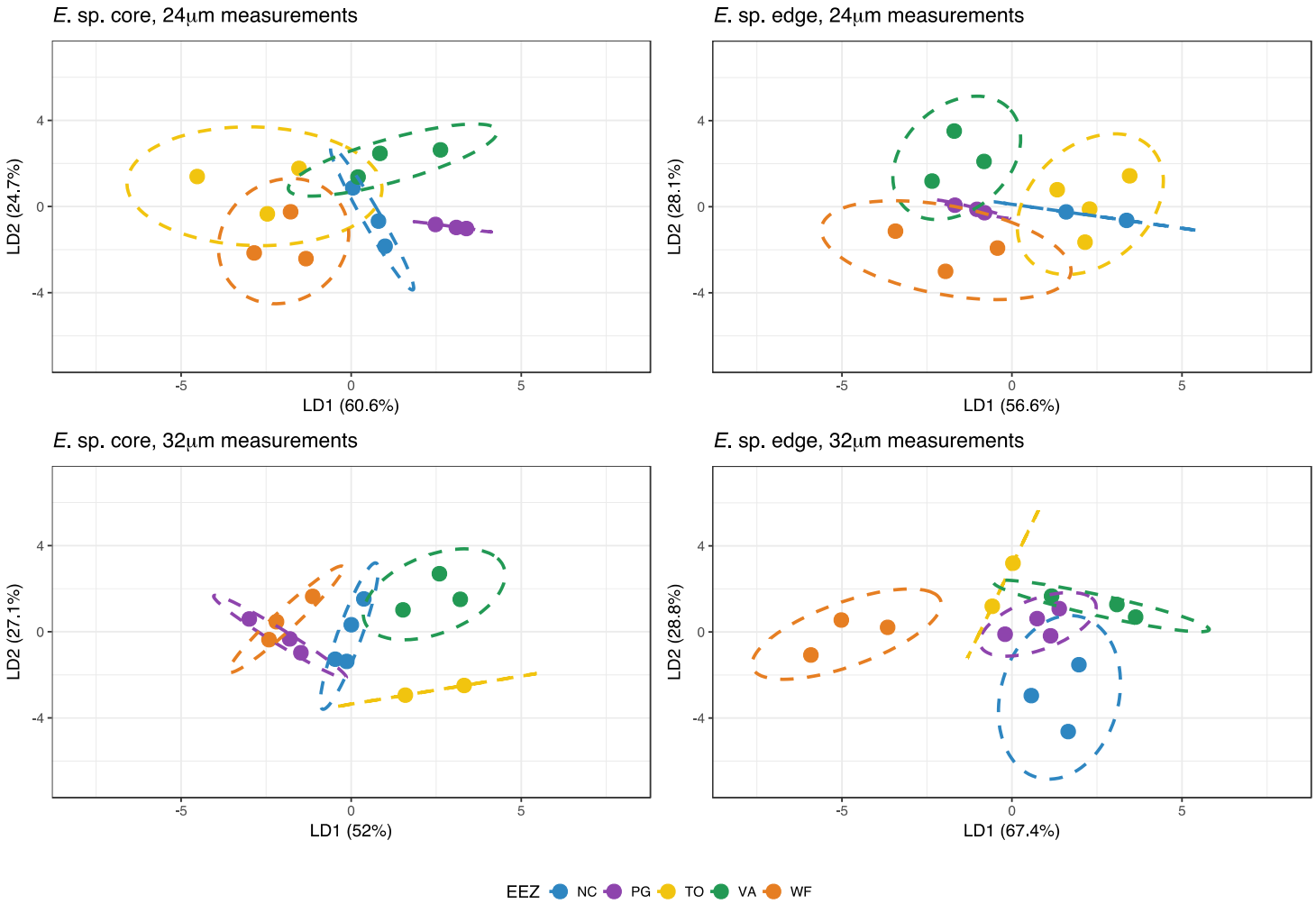


Figure 6-8: Spatial discrimination of juvenile-core (*left*) versus capture location-edge (*right*) otoliths resolved by LA-ICP-MS for *Etelis sp*. Each plot shows separate linear discriminant function analyses incorporating trace elemental ratios of predicted group membership with separate Exclusive Economic Zone (EEZ) samples classified and 95% confidence ellipses showing the degree of overlap in elemental fingerprints.

Discussion

The focus of the study was to determine the method that would give the best resolution of differences in elemental chemistry over multiple spatial scales that could assist with the stock discrimination of two species of deepwater snappers. There were significant differences in the otolith chemistry between species and among EEZs. This is the first evidence that geochemical signatures can successfully be used to distinguish the spatial structure within metapopulations for deep-reef fish species over a broad region in the Pacific. The important finding that otolith chemistry varies between closely-related species in the same environment emphasizes the importance of accounting for species-specific variability in metapopulation structure when evaluating stock structure for multiple species within a single fishery. Further, the differences between areas sampled on the otolith, representing various life history stages, varied significantly within an individual, so care must be taken to further resolve how these differences in life history are reflected when using otolith chemistry to delineate stock boundaries. For regional stock identification of deep-reef snapper, multivariate fingerprints for both solution and laser-based ICP-MS methods were discriminatory between fish caught among the six Pacific Island nations. Clearly microhabitat differences between species (benthic vs. nektonic for adult *E. coruscans* and *E. sp.* respectively) might importantly influence diet and growth.

There are relative advantages and disadvantages to using solution or laser-based ICP-MS methods, which should be carefully considered when designing studies for stock discrimination. Solution-based methods may be faster for large sample sizes (e.g. Kingsford et al. 2009) and between locations where chemical signatures have clear differences, but the results may be coarser. This may limit the degree of interpretation and the questions solution-based methods can answer. Dissolving the whole or part of the otolith may conceal subtle differences and some trace elements (e.g. Fe for solution-based samples) that are in low concentrations and limited to elements measured in the certified reference material. An assumption of whole otolith analyses is that larval dispersal or seasonal adult migration (i.e. stock-mixing) will not confound the signatures of discrete stocks (Thorrold & Swearer 2009). Solution-based methods are considerably less demanding in post-processing time but requires fastidious laboratory preparation and protocols. The advantages of LA-ICP-MS include the ability to look at the patterns across the otolith transect, which when sampled from the core to the edge corresponds to the fish's lifespan. Transects are useful as otoliths are 'superior chronological records' (Kerr and Campana 2014), with detailed and spatially-explicit information that can be applied over a spectrum of spatial scales. Post-processing LA-ICP-MS data is time-consuming, but transect patterns can confirm groups with different life histories (e.g. Secor et al. 2001), strengthening the evidence that groups form different metapopulations.

While a wholly marine fish may not have the same magnitude of differences as fishes experiencing riverine or estuarine influences, average core and edge samples were sufficient to reveal some clear separation between locations. Here, I controlled for the sampling timeframe (three years), and relative fish size (fork length). It is important to remember that otolith chemistry has limited interpretation on the temporal stability of stock structure, as even occasional movements into different environments may potentially introduce detectable differences into the otolith chemistry (Campana 2005). However, we can infer that individuals with overlapping chemical signatures (e.g. core signatures) come from more similar environments, which cannot definitively state, nor rule out, a common source population, or different location origins with similar water chemistry (Campana 2005). Otolith morphological studies of *E. sp.* have demonstrated the otolith does not grow at a constant rate along all dimensions (Smith 1992). It is important to maintain the same transect or sampling location for otolith chemical analyses, which was done in this study. Since fishery sampling can be limited year-to-year by funding and time, the edge comparison showed that the differences in edge measurements were less significant, meaning if multiple year-classes are sampled it would not affect the regional discrimination. The visualization of the transect from the core to the edge revealed how stable edge measurements are over time; therefore the 'edge' exhibits stable elemental ratios over several years before capture and is a useful area of the otolith for spatial resolution (Campana 2005, Tanner et al. 2011, Avigliano et al. 2017). The implication for broad-range studies is that these methods can potentially be used over longer time-spans and multiple-year classes. In this study I used a limited sampling window (2012-2015) as variability over inter-annual time scales is an important consideration in otolith chemistry analyses (Walther and Thorrold 2009). Resolution and classification accuracy may be improved with larger sample sizes and less coarse data reduction techniques (i.e. averaging). Comparing differences in the ablation spot sizes was useful to know as the 'stretch' of data points is wider, therefore accentuating the temporal differences better, while also slightly increasing the magnitude of these measurements. This can help in minimizing errors in assigning life history stages with specific places along the otolith elemental transect, ideal for combining otolith chemistry and microstructure analyses (e.g. Sih and Kingsford 2015).

The magnitude of change between the 'core' and the rest of the otolith indicates the early life physiology or environment is different than later life stages for both of the species investigated. This may be useful in future studies to assess natal origin, to estimate larval dispersal distances, and to generalize connectivity patterns. Deepwater snappers exhibit long pelagic larval stages (Leis 1987), which may explain the similarity in core signatures. As larvae and pelagic juveniles, deepwater snappers could be encountering more uniform conditions as they travel large distances with the currents for multiple months, resulting in highly overlapping elemental fingerprints.

I investigated the effects of age on otolith chemistry because age can affect the time of exposure to different water chemistry (Kerr & Campana 2014) such that elemental concentrations vary with fish size (Edmonds et al. 1989). I found limited evidence for significant correlations between fish age and trace element concentrations in the otolith. This may be due to small sample sizes and the confounding effects of pooling multiple locations where age, growth, size and environmental variation may occur. Otolith chemistry can vary at spatial scales of tens to hundreds of kilometres (Gillanders & Kingsford 2000, Dorval et al. 2005, Thorrold & Swearer 2009) and temporal scales of seasons to years (Campana et al. 2000, Gillanders 2001) so it is important to design the study to avoid confounding spatial and temporal factors that can influence otolith chemistry. Future studies should investigate if size-related effects on elemental signatures within stocks are important so that they could be statistically removed (Campana 2005). Recent studies have found sex-specific and regional growth differences for *E. carbunculus* (Williams et al. 2017) which may affect some elements' incorporation. Differences in growth and reproduction should be included as an additional layer of information in stock separation estimates as differences in demographics are important for metapopulation-based models. For instance, differences in growth may translate to differences in otolith chemistry. Also, for species where known spawning migrations occur (e.g. eels, groupers), these movements may confound elemental signatures for individuals that have reached spawning age.

Overall, the between-species differences were smaller than the location differences in the multivariate fingerprints, meaning the patterns were similar over the same spatial scale for both species. Investigating the trace element composition of otoliths has broad implications for using otolith chemistry as 'natural tags' over regional spatial scales (~1000s km) and mixed-species fisheries. Otolith chemistry has successfully been used to discriminate stocks of shallow-water and pelagic species over broad spatial scales, over varying physical, chemical, latitudinal and longitudinal gradients. The results from this study indicate that otolith chemistry can discriminate among stocks of eteline snappers (or similar deepwater species), for which the data on movements and migrations are limited, and life history transitions still remain key knowledge gaps. Variability in otolith chemistry across the otolith, among EEZs and between species suggests that there may be physiological differences between the species (i.e. differential diet and growth), which may mask some of the environmental effects (i.e. due to geography or oceanography).

Determining which elements offer the most discriminatory power is also important, as all elements can contribute to the whole elemental signature to resolve population structure, but individual elements incorporate differently into the otolith and the mechanisms behind this are still not well-understood. Thresher and Proctor (2007) hypothesized that the ontogenetic variability in Sr would be due to behavioural and ecological factors, since it provided clear

differences in spatial structure despite the presumed homogeneity in the deep marine environment. Differences in growth rates may also influence Mg and Ba concentrations in fish otoliths (see Kerr and Campana 2014 for some examples). Similarly, reproduction may influence elemental composition of otoliths (Fuiman & Hoff 1995). This study indicates that elemental inclusion varies across the otolith but is not uniform in pattern for all the elements studied here. From LA-ICP-MS transects, Ba:Ca was often higher in earlier stages and Sr:Ca was higher in later stages. Where these changes occur along the transect may also point to important environmental or demographic changes in the life history of the fish. These important distinctions were not evident in dissolved otoliths, because otolith material across all life stages is pooled into a single sample for analysis. Inter-specific variation was also observed for Mn:Ca measurements, with *E. sp.* exhibiting higher concentrations than *E. coruscans*.

Future otolith chemistry studies for eteline snappers would benefit from incorporating some of the potential sources of variation affecting either water chemistry or physiology. A major assumption of this study was that factors driving the changes in otolith chemistry (e.g. water chemistry, diet or the environmental history) would be sufficiently different spatially and relatively temporally stable for the period of capture locations analysed. Some elemental differences are expected to be species-specific, due to diet or physiology (Sturrock et al. 2014). If spatial effects are greater, then latitudinal, longitudinal or oceanographic mechanisms may be more important. It was assumed that these species would be exposed to similar water chemistry and environmental conditions. However, it was not possible to collect water samples at the times and locations fish were collected to test this hypothesis. Further, to be representative of the environment these fishes inhabit, water samples would have to be collected at great depths (>200 m for capture depths). Not much is known about variability in water chemistry at these depths and at spatial scales of 100s-1000s of kilometres in the Pacific, though it is presumed that local oceanographic processes (e.g. nutrient upwelling) could be operating that may produce differences in water chemistry that are sufficient for discrimination. Diet may influence elemental signals (i.e. Sanchez-Jerez 2002, Doubleday et al. 2013) and variation in food sources among EEZs may contribute to spatial variation in signatures, though in experiments diet often has less influence than water chemistry on element uptake (Walther & Thorrold 2006). The information on species-specific diet of deepwater fish species is often summarized from limited samples at disparate locations, and not throughout the species' distribution (Parrish 1987, Haight et al. 1993b), and deepwater snappers are known to feed on a wide range of pelagic and benthic fish and invertebrate groups. Feeding studies in Hawaii indicate that *E. coruscans* and *E. carbunculus* are mainly piscivorous, while other deepwater species from the *Pristipomoides* genus primarily eat zooplankton (Haight et al. 1993b) and there is some evidence of diet-partitioning among *Pristipomoides* species in the Mariana Archipelago (Seki & Callahan 1988). However, only

recently has *E. sp.* been distinguished from *E. carbunculus* (Andrews et al. 2014, Andrews et al. 2016). In Hawaii, where some of the trophic comparisons have been made, only *E. carbunculus* occurs, whereas *E. sp.* and *E. carbunculus* co-occur throughout the remainder of the Indo-Pacific distribution. There are considerable biological differences between these species (Williams et al. 2017), so it is likely that there are physiological and dietary differences reflected in the otoliths between *E. coruscans* and *E. sp.* as well. Diet-based influences are expected to influence Ba and Sr in the otolith and are less likely to affect elements Mg, Mn, Ca, and Cu (Kerr & Campana 2014).

I have demonstrated that the otolith elemental chemistry can discriminate between populations of deepwater fishes from multiple EEZs. Both solution-based and laser ablation methods were capable of resolving spatial differences in elemental finger prints of two species of *Etelis* with a high level of classification accuracy. However, LA-ICP-MS methods had the added advantage of analysing multiple life history stages along a single transect, allowing more detailed temporal resolution of changes in elemental fingerprints within individuals and multiple comparisons for classification to EEZ. This study provides initial evidence that there may be shared stocks among some EEZs, suggesting that collaboration among countries may provide the basis for improved management of eteline snapper fisheries in the Pacific. To facilitate future research on stock structure of eteline snappers, the results from this study provide a protocol of methodology that can have broader applicability for investigating the stock structure of deepwater fishes.

Chapter 7 Indo-Pacific stock structure of deepwater snapper

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Abstract

A major challenge in fishery management is differentiating stocks when species distributions extend across multiple sovereign jurisdictions. As fish otoliths incorporate chemical elements from the surrounding water as they grow, analysis of otolith microchemistry offers a well-established method for inferring stock structure over multiple spatial scales. This study used otolith microchemistry to evaluate evidence for stock structure in three deep-reef eteline snappers over their Indo-Pacific range. Otoliths were sampled in different regions from Western Australia and Indonesia in the Indian Ocean, to Samoa and Tonga in the central Pacific. Two areas of the core-to-edge laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) transect were used to compare the otolith chemistry of early life history and just prior to capture. Significant differences in otolith microchemistry were detected between fish from different locations and multivariate elemental fingerprints were able to discriminate between potential regional stocks. While some intraspecific variations were observed, otolith chemistry revealed clear separation between Indonesian and Western Australian populations for all three species. In the western Pacific, chemical fingerprints from samples collected in Vanuatu and New Caledonia were similar. In the central Pacific, the otolith fingerprints from Fiji and Wallis and Futuna were similar, and the otolith fingerprints from Tonga with Monowai Seamount (in international waters) were similar. These findings illustrate how otolith microchemistry can be used as a tool for identifying potential connectivity among stocks, and the value of this information for managers. For example, where similarities in elemental fingerprints exist between adjacent jurisdictions, indicating a straddling stock, international co-management may be required to maintain sustainable fisheries. In contrast, local management would be more appropriate in jurisdictions where elemental fingerprints are unique and indicate non-mixing stocks.

Introduction

Maritime boundaries rarely align with resource distributions, leading to frequent international disputes over ownership and exploitation rights (Miles & Burke 1989, Spijkers et al. 2018). Exclusive Economic Zones (EEZs) were formally established to resolve such political disputes over mining and fishing resources in 1982, granting each country economic sovereignty over a 200-nautical mile radius from its coastline (United Nations Convention on the Law of the Sea). However, many fisheries species are considered transboundary or 'straddling' stocks and are distributed across two or more EEZs. These cases present challenges for management and require the development and implementation of complex management strategies, such as the creation of intergovernmental regional authorities, to ensure the conservation and long-term value of these resources (Kawasaki 1984). Identifying fishery stocks is a central component of resource assessment and management, with the stock unit used as the basis for stock assessments (Begg et al. 1999). However, an incomplete understanding of stock structure is common and has impeded the ability to differentiate between stock units. Throughout its distribution, a species may comprise several spatially separated stocks that arise from the fragmentation of suitable habitats (Levins 1969); it is assumed that immigration and emigration among stocks will be zero to minimal. While historically thought of as single stocks, fish metapopulations are in fact often comprised of several smaller mesopopulations, or stocks, with significant differences in recruitment, reproduction, connectivity, and growth that affect responses to fishing pressure and exploitation (Cadrin et al. 2005). However, information on rates of movement, migration (i.e. immigration and emigration), and habitat availability are often limited, making it difficult to measure where, or how fluid, the effective boundaries between stocks are, and hindering our ability to effectively manage them.

Otolith microchemistry can provide information to infer fish movements and geographic stock boundaries. Fish otoliths constantly grow during an individual's lifetime, with ossified layers created on a frequent, regular basis (Pannella 1971). As these layers are formed, they incorporate chemical elements from the surrounding water, creating a chemical signature of that water body and providing a record of geographic, physiological, and demographic variation through time (Kalish 1989, Campana 1999, Campana & Thorrold 2001, Elsdon et al. 2008, Lawton et al. 2010, Fairclough et al. 2013, Sturrock et al. 2014). Therefore, the application of otolith chemistry analysis to fisheries species has the potential to increase our understanding of metapopulation structure, complementing genetic studies with additional information on the inferred spatial dynamics of stocks. Otolith microchemistry will have a different resolution than genetic studies, often able to detect patterns not evident from genetic data taken from similar spatial scales (Campana & Thorrold 2001, Palumbi 2004). For instance, combined otolith and genetic comparisons have demonstrated that elemental fingerprints identify spatial structure

within metapopulations where genetic data has implied a panmictic population (e.g. weakfish *Cynoscion regalis* along the US Atlantic coast (Thorrold et al. 2001). Therefore, while information from otoliths does not contradict genetic information, it could provide additional information on stock structure at on an ecological scale directly relevant to fishery management (Thorrold et al. 2001). Newer genetic analyses can help to reveal metapopulation substructure, in particular the use of microsatellite markers and comparisons of neutral versus adaptive genes; however, many of these methods are cost-prohibitive (Swain et al. 2005, Allendorf et al. 2010, Funk et al. 2012). Similarly, the use of otolith chemistry will be less time and cost-intensive than tag-recapture or telemetry studies to study species' movement. Where possible, the combination of multiple methods (i.e. otolith and genetic analyses) can maximize the information on spatially explicit stock structure (e.g. Izzo et al. 2017, Barton et al. 2018).

Otolith microchemistry could be particularly useful for discriminating among stocks of the mixed-species deepwater fisheries that exist across the tropical Indian and Pacific Oceans. These fisheries represent valuable socioeconomic resources and are increasingly important for future food security as shallow-water fisheries become depleted (Dalzell et al. 1996, Pauly et al. 2005, Fry et al. 2006, Hospital & Pan 2009, Williams et al. 2013). These fisheries are comprised of over a dozen snapper, grouper and jack species, many of which are vulnerable to fishing pressure (Dalzell et al. 1996, Fry et al. 2006). These fisheries experience a gradient of exploitative pressure, from artisanal fisheries to well-established export fisheries; however, global fishery production is still currently limited, most likely due to the relatively low productivity of the harvested species (Newman et al. 2016). Of the included species, the eteline snappers are considered the most commercially-valuable (Hospital & Pan 2009, Williams et al. 2013). As deepwater fishes are long-lived, slow-growing, experience late maturation and low natural mortality, they are at risk of overexploitation (Coleman et al. 2000, Andrews et al. 2011, Andrews et al. 2012, Wakefield et al. 2013, Williams et al. 2015). For mixed-species fisheries, differences in demographics can result in differential vulnerability to exploitation (Heupel et al. 2010b, Williams et al. 2013) and critical knowledge gaps on broad-scale distribution and life history characteristics of many deepwater species still remain.

Genetic and spatial movement studies on deepwater species indicate further investigation of spatially explicit information for deepwater snappers is warranted. Among the deepwater snapper species, *P. filamentosus* is highly dispersive, with capabilities of traversing deepwater channels ~400 km (Kobayashi 2008), while the congener *P. multidens* is highly constrained, with no evidence of the ability to cross the deepwater Timor Trench (3000 m), a channel between Australia and Indonesia over a distance of ~200 km (Ovenden et al. 2002). Genetic analyses of these two species indicate across a large expanse of Indo-Pacific locations (~14,000 km) *P. filamentosus* has little or no significant population structure (Gaither et al. 2011) but among a

smaller spatial comparison between Indonesian and Australian locations (~1500 km) there is substantial genetic heterogeneity for *P. multidens* over distances 191-491 km (Ovenden et al. 2002, Ovenden et al. 2004). Otolith stable-isotope data indicate adult *P. multidens* are sedentary (Newman et al. 2000b), which supports the hypothesis of limited movement and significant metapopulation structure of *P. multidens* in northwest Australia. Some studies suggest deepwater fish may be considered a single resource, with panmictic populations over broad geographic scales (Andrews et al. 2014, Andrews et al. 2016, Goldstein et al. 2016b) with no evidence of reproductive or genetic barriers (either through adult movement or larval dispersal). If this is indeed the case, managing this shared resource over an area as extensive as the Indo-Pacific may be challenging for political and economic reasons, as the health of the stock will depend on recruitment and dispersal from yet unidentified source populations. There are concerns in treating deepwater fish as a single resource, as some areas like Hawaii (USA) and the Kimberley (Australia) appear more isolated, with low genetic diversity and limited influx from possible source populations (Ovenden et al. 2002, Gaither et al. 2011). Heavy fishing pressure of more isolated stocks could lead to localised extirpations that may not be sufficiently replenished by other stocks.

Whether stocks are highly connected (e.g. *P. filamentosus*) or highly disconnected (e.g. *P. multidens*), much of what we understand of deepwater fishes comes from limited sample sizes at a few locations across the broader biogeographic range of these species, and more work is necessary to define spatial boundaries of potential stocks within larger metapopulations. For deepwater species such as eteline snappers, distribution is often linked to the availability of suitable habitat, which is often patchily distributed among islands, seamounts, and submerged shoals (Gomez et al. 2015). For species with strong habitat associations, populations may be effectively linked or isolated by deepwater habitat availability, and there is limited information on these scales of movement (e.g. Kobayashi 2008, Weng 2013).

My research aimed to elucidate the stock structure of three deepwater eteline snapper populations over a longitudinal range of 70º in the Indo-Pacific using otolith chemistry. In Chapter 6, I established that the overall elemental fingerprint using LA-ICP-MS was sufficient as a 'natural tag' to discriminate among groups of fish experiencing different environments in the Pacific. The specific aims of the present chapter were as follows: 1) determine the structure of stocks for three sister species of eteline snappers using elemental fingerprints analyzed by a hierarchical sampling design across three regions that encompassed the territorial waters of multiple countries, and multiple locations within each region; 2) compare the spatial separation suggested by the trace element composition of the otoliths among species to determine if territorial boundaries were congruent with sensible management of a mixed fishery.

Methods

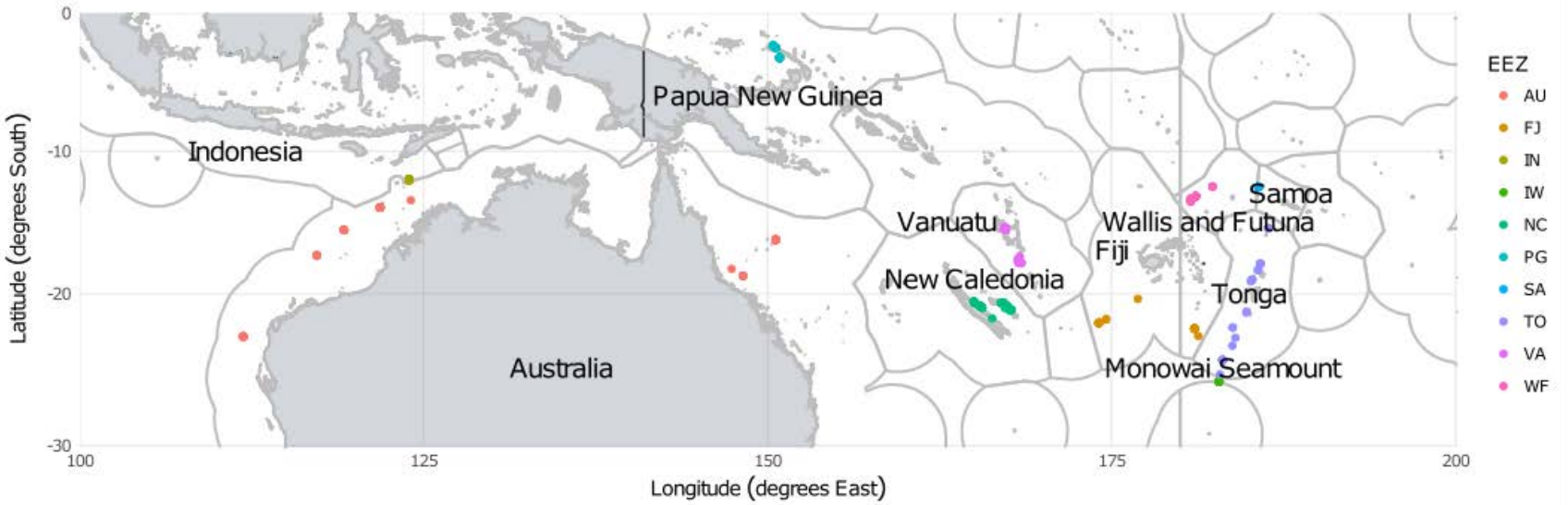
Otoliths from three species of deep-reef, eteline snappers (*Etelis coruscans*, *Etelis carbunculus* and *Etelis sp.*) sampled from nine countries: Australia, Indonesia, Papua New Guinea, New Caledonia, Vanuatu, Fiji, Wallis and Futuna, Samoa, and Tonga, and the Monowai Seamount in international waters (Fig. 7-1) were sourced from collections archived by Fisheries Western Australia and the Pacific Community. Otoliths were collected in the Great Barrier Reef (GBR), Coral Sea and Indonesia by local recreational and commercial fishermen. Otolith sample sizes varied by location and species, and not all the fish were aged (from the other otolith). Initial power analyses from the LA-ICP-MS samples from Chapter 6 indicated a sample size of 9-10 would be sufficient for the minimizing the variation between samples for select elements (standard error as a proportion of the mean for Ba:Ca, Sr:Ca, Mn:Ca, Mg:Ca). To minimise biases in comparisons among individuals, samples were selected based on fork-length (Table 7-1), date and location of capture, and all species were sampled from a similar depth range (120-473 m) and over much of their biogeographic range.

High-resolution sampling of otolith chemistry was obtained using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). Transverse sections of sagittal otoliths were prepared for LA-ICP-MS using the protocol in the previous study (Chapter 6). Element concentrations were measured using a 24-µm ablation mask and transects from the core to the edge of the otolith section (ablation speed 24 µm/sec, 10 Hz pulses). Elemental information was converted from time to distance using a constant scanning speed, and univariate concentrations were related to an internal calcium standard, NIST 610 and 612 standards. Two sections of the transect were selected to represent the life history of the individual fish. The 'core' signature represented the early life history and the 'edge' signature represented the stage of life just before capture; both measurement sections averaged 50 data points. Nine elements were analysed for average otolith edge and core signatures and expressed as a ratio to calcium (^{7}Li, ^{24}Mg, ^{43}Ca, ^{44}Ca, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{88}Sr, and ^{138}Ba).

Otolith core and edge signatures were classified by EEZ or location of capture for spatial comparisons and built on the results from the previous study. From the pilot study of two species' otolith chemistry among 5-6 EEZs (Chapter 6), I knew that there were significant differences between average core and edge measurements for spatial discrimination, and this may reflect differences between early life and later life history stages. I investigated the effects of age and measurement location (core and edge) on spatial discrimination in the previous chapter (Chapter 6); however, there was limited evidence of a substantial confounding effect. In multivariate comparisons, classification accuracy was higher for average edge concentrations than the averages of the total transect, lending more evidence that spatial discrimination would be clearer by dividing the otolith signature into two separate measurements. And lastly from the

pilot study results, there were differences in elemental incorporation that varied significantly among species. Therefore, each species was considered separately in both multivariate and univariate analyses in this study.

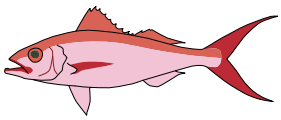
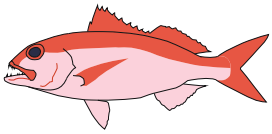
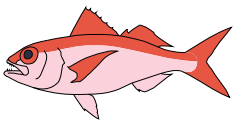
Otolith chemistry data was evaluated on multiple spatial scales: 1) on the broadest level, I investigated metapopulation structure over the entire sampling area, including samples from 10-12 EEZs for each species; 2) the next level nested EEZ locations within smaller regions. I formally tested for differences among regions, but in many respects, it was biologically and geomorphologically highly unlikely that they were connected. Accordingly, I carried out analyses for variation among locations separately for each region. For example, it was highly unlikely that fish would disperse from Tonga to Papua New Guinea (> 4500 km), or past shallow natural barriers such as Torres Strait that could connect eastern and western stocks on each side of Australia). This sampling design included three broad Regions (Indian Ocean, Western Pacific and Central Pacific) with multiple Locations nested within the Region-level. I used Location to represent a likely scale of possible stock discrimination as well as a distinction from the EEZ-level of classification (i.e. a large EEZ may contain multiple stocks). Therefore, a Region may contain one or more stocks. The Australian EEZ was divided into multiple Locations reflecting local management jurisdictions: two Western Australian fishing zones and the GBR/Coral Sea. This reflected current management strategies and addressed some of the natural dichotomy between NW and NE Australia. The Gascoyne/Pilbara region and the Kimberley region are two jurisdictions governed by Fisheries Western Australia. The GBR management falls under the Great Barrier Reef Marine Park Authority (GBRMPA) jurisdiction, with the Coral Sea and parts outside of the GBR World Heritage Area considered the deepwater L8 fishery, managed as Commonwealth resources by the Australian Fisheries Management Authority (AFMA), with some management overlap with state-run Queensland (QLD) Fisheries.



Exclusive Economic Zone	Australia (AU)		Indonesia	Australia (AU)	Papua New Guinea	New Caledonia	Vanuatu	International waters (IW)	Fiji	Wallis and Futuna	Tonga	Samoa
Region	Region 1: Indian Ocean			Region 2: Western Pacific				Region 3: Central Pacific				
Location code	Gascoyne/Pilbara (WA1)	Kimberley (WA2)	(IN)	GBR/Coral Sea (GBR)	(PG)	(NC)	(VA)	Monowai Seamount (MS)	(FJ)	(WF)	(TO)	(SA)
Etelis coruscans (n= 126)	12	12	12	6	12	12	12	10	12	12	12	2
Etelis sp. (n=114)	12	12	12	12	12	12	12	NA	3	12	12	3
Etelis carbunculus (n= 91)	NA	NA	5	4	12	12	9	10	12	12	12	3

Figure 7-1: Number and location of otolith chemistry specimens from three deepwater snappers across multiple spatial scales in the Indo-Pacific. Samples were divided into three Regions based on broad geographic separation. The map was created in R with Exclusive Economic Zone boundaries from marineregions.org (VLIZ 2014).

Table 7-1: Statistics describing three deepwater snappers specimens used for otolith chemistry studies investigating stock structure.

Species	Fork-length range (cm)	Fork-length mean (cm)	Weight range (kg)	Weight mean (kg)	Age range (years)	Age mean (years)
 *Etelis coruscans* Flame snapper	28.5-83.5	62.1	0.4-9.0	4.1	7-27	14.2
 *Etelis sp.* Ruby snapper	39.5-102.0	65.9	1.3-20.2	6.2	8-46	18.0
 *Etelis carbunculus* Pygmy ruby snapper	21.0-61.0	41.0	0.2-4.6	1.5	4-25	12.8

Single-element otolith variation among locations

The natural elemental variation among all locations was evaluated by the coefficient of variation (CV) on untransformed averages of LA-ICP-MS data. The values are presented as the percentage of the standard deviation over the mean for each elemental ratio for each species pooled across all locations. Variation at different spatial scales for LA-ICP-MS core and edge measurements were analysed using a fully hierarchical nested ANOVA where Regions (a) were compared and Locations were nested within Region a(b). The ANOVA was a mixed effects model with Region treated as fixed and Location as random (package nlme, Pinheiro et al. 2018b). Locations with too few replicates were removed for balance: *E. coruscans* a=3, b=3, n=12 fish; *E. sp.* a=3, b=2, n=12; and *E. carbunculus* a=2, b=2, n=12. Samoa, Monowai Seamount and the GBR samples were removed Samoa, Monowai Seamount the GBR samples were removed for the *E. coruscans* model. Fiji and Samoa replicates were removed for the *E. sp.* model. Samoa, Monowai, Vanuatu, Indonesia and GBR samples were removed for the *E. carbunculus* model. Independent samples were Box-Cox transformed, centred and scaled (package caret, Kuhn 2017) and visually tested for assumptions of normality and homogeneity of variance. Significant differences among Regions were investigated by post-hoc tests for differences among all means using a Tukey test (function glht, package multcomp, Hothorn et al. 2008; package lsmeans, Lenth 2016).

Classification of multiple species' stock structure

Multiple trace element concentrations were used to investigate spatial heterogeneity of otolith fingerprints using Linear Discriminant Function Analysis (LDFA) and multivariate analysis of variance (MANOVA) on two spatial scales. On the first level, all Locations were classified independently by species with separate LDFAs and MANOVAs by species across 10-12 Locations. On the second level, three broad regions were compared: 1) Indonesia and two Western Australian fishing locations; 2) GBR/Coral Sea, Papua New Guinea, New Caledonia and Vanuatu; and 3) Fiji, Wallis and Futuna, Samoa, Tonga, and Monowai Seamount. MANOVA tested the hypothesis that differences are due to differences among Regions or Locations. MANOVA determined whether there were significant differences among the main effects and interactions between the independent variables. Pillai's criterion was used as a conservative F-statistic to detect multivariate differences, and is robust despite unbalanced group sizes for some comparisons (Olson 1974). Assumptions for multivariate tests were tested for 1) homogeneity of covariance using Box's M (package biotools, da Silva et al. 2017) or Levene's test for homogeneity of variance for each element, 2) Mardia's test for multivariate normality and 3) visually investigated with QQ-plots and histograms (package MVN, Korkmaz et al. 2014). Elemental concentrations were also compared for correlations greater than 0.7. Similar to the previous chapter, specific elements that were not normal or homogeneous were removed, resulting in 7-9 elements per MANOVA or LDFA regional analysis (Table 7-2).

Separate discriminant function analyses used the averaged elemental concentrations of the otolith edge and core to show whether predicted samples were correctly allocated to the right group for each species. An advantage of LDFA is the ability for prediction and cross-validation and both classic discriminant function and jack-knife cross-validation were used to compare classification estimates (function lda in package MASS, Venables & Ripley 2002). For each group the predicted values were graphed by the first two linear discriminants and the between-group variance (proportion explained) is reported. Samples from all regions were included (even those with fewer replicates) as unbalanced data may not negatively affect LDFA results (Xue & Titterington 2008).

Table 7-2: List of select element concentrations that were removed from multivariate analyses if not normally distributed or not homogeneous in covariance after measurements were Box-Cox transformed, centred and scaled.

Species	Region 1	Region 2	Region 3	All Locations
Multivariate analyses of otolith *edge* chemistry				
Etelis coruscans		Mn:Ca removed	Mn:Ca removed	Mn:Ca removed
Etelis sp.	Mg:Ca removed	Mg:Ca, Mn:Ca removed	Mn:Ca removed	Mn:Ca removed
Etelis carbunculus		Cu:Ca, Fe:Ca removed	Li:Ca removed	
Multivariate analyses of otolith *core* chemistry				
Etelis coruscans			Mn:Ca removed	
Etelis sp.	Mg:Ca removed		Ni:Ca removed	
Etelis carbunculus		Zn:Ca removed	Mn:Ca removed	

Results

There was more variation in edge measurements among all samples than core measurements for most elements, though the difference in coefficients of variation magnitude varied by species (Table 7-3). Cu:Ca was the only element that had higher variation in the core than at the edge for all three species.

Table 7-3: Coefficients of Variation for otolith core and edge trace element measurements, all Locations pooled. Calculated coefficients are shaded for low (<40%, light green), medium (40-80%, medium green) and high variation (>80%, dark green).

	Etelis coruscans		*Etelis sp.*		*Etelis carbunculus*	
	Core	Edge	Core	Edge	Core	Edge
Ba:Ca	89.14	70.19	95.36	132.17	45.17	45.86
Sr:Ca	14.32	24.34	20.22	30.17	11.17	20.38
Mg:Ca	34.97	65.45	256.63	265.48	46.42	53.06
Mn:Ca	56.88	122.54	92.88	128.32	48.19	67.88
Li:Ca	47.48	56.02	97.41	70.23	101.23	94.10
Fe:Ca	38.92	81.82	34.33	84.49	54.30	72.49
Cu:Ca	207.17	173.63	293.05	142.51	132.66	102.63
Ni:Ca	42.30	111.86	44.18	88.80	43.44	55.54
Zn:Ca	91.77	136.99	84.92	81.79	57.42	135.00

Ba:Ca from Region 1 was slightly higher than Regions 2 and 3 (Figure 7-2). Only Ba:Ca mean otolith edge ratios were significantly different between Locations nested in Region for one species (nested ANOVA, *E. coruscans* $F_{(2,6)} = 7.47$, $p < 0.05$). No other elements were significantly different for univariate edge or core measurements among the three species.

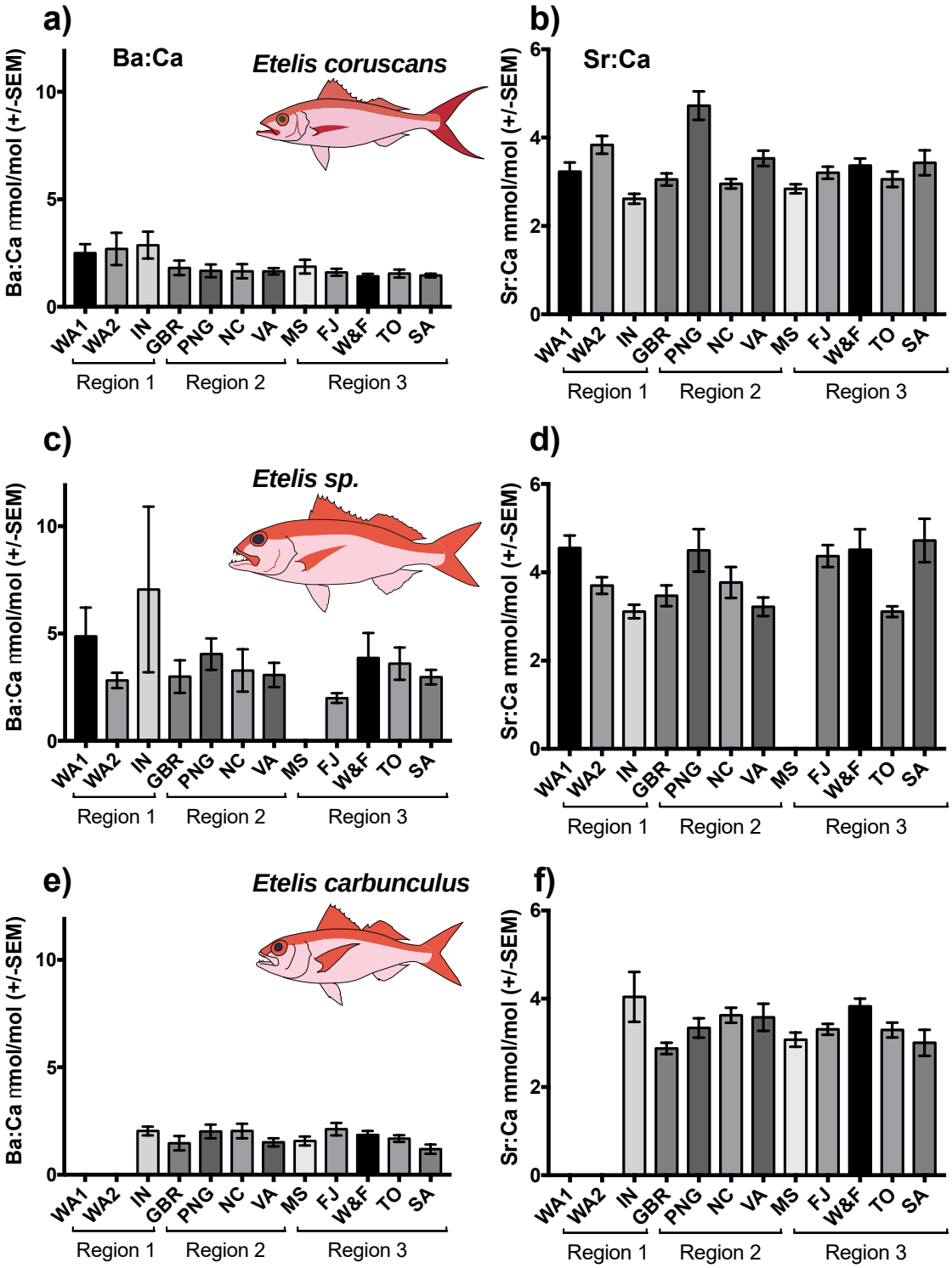


Figure 7-2: Spatial variation in otolith edge concentrations of Ba:Ca and Sr:Ca from three eteline snapper species: a/b) *Etelis coruscans*, c/d) *E. sp.*, and e/f) *E. carbunculus*. Ba:Ca differed significantly for *E. coruscans* with higher concentrations in Region 1. Replicates varied by Location and species but for most locations n=12.

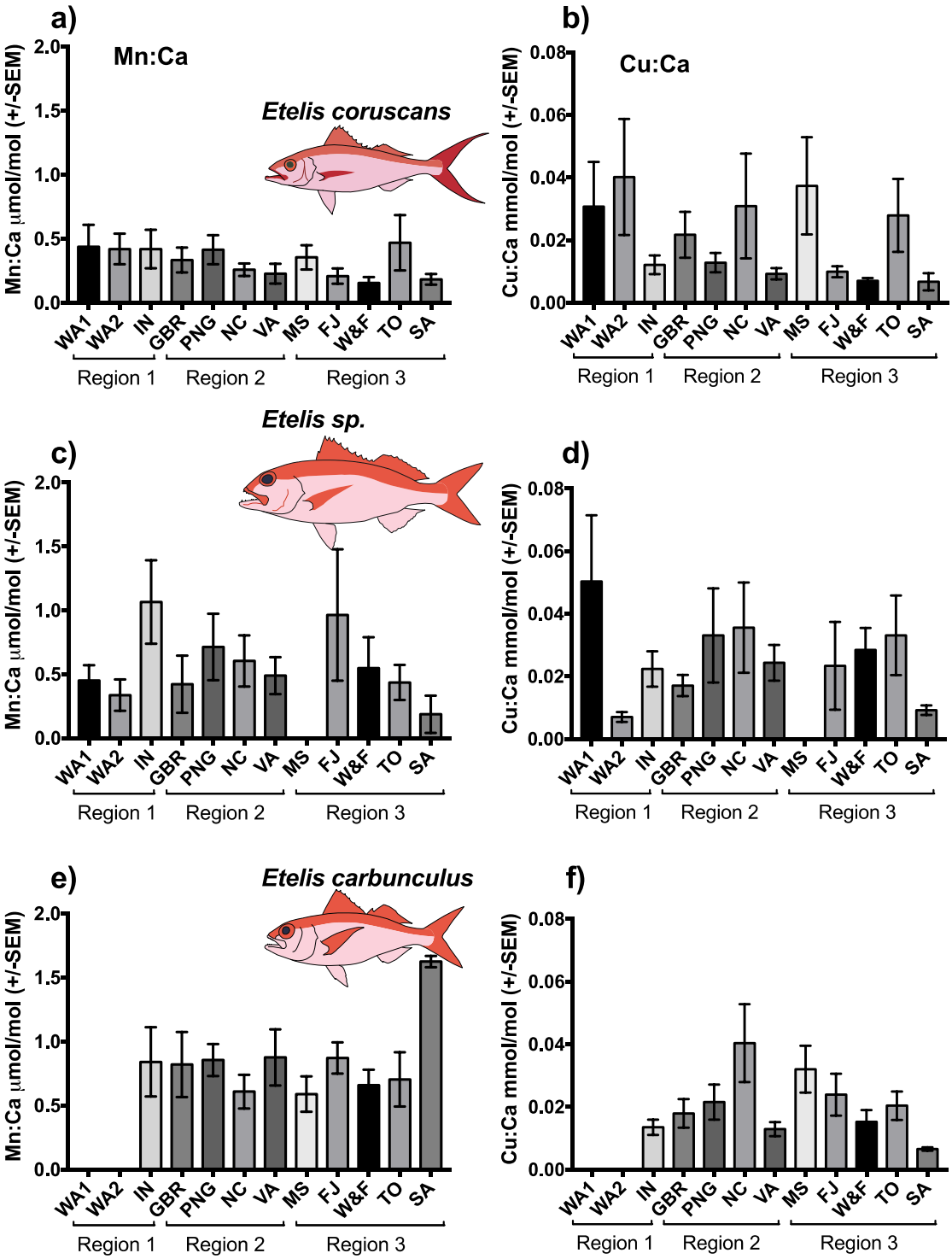


Figure 7-3: Spatial variation in otolith edge concentrations of Mn:Ca and Cu:Ca from three eteline snapper species: a/b) *Etelis coruscans*, c/d) *E. sp.*, and e/f) *E. carbunculus*. Replicates varied by Location and species but for most locations n=12.

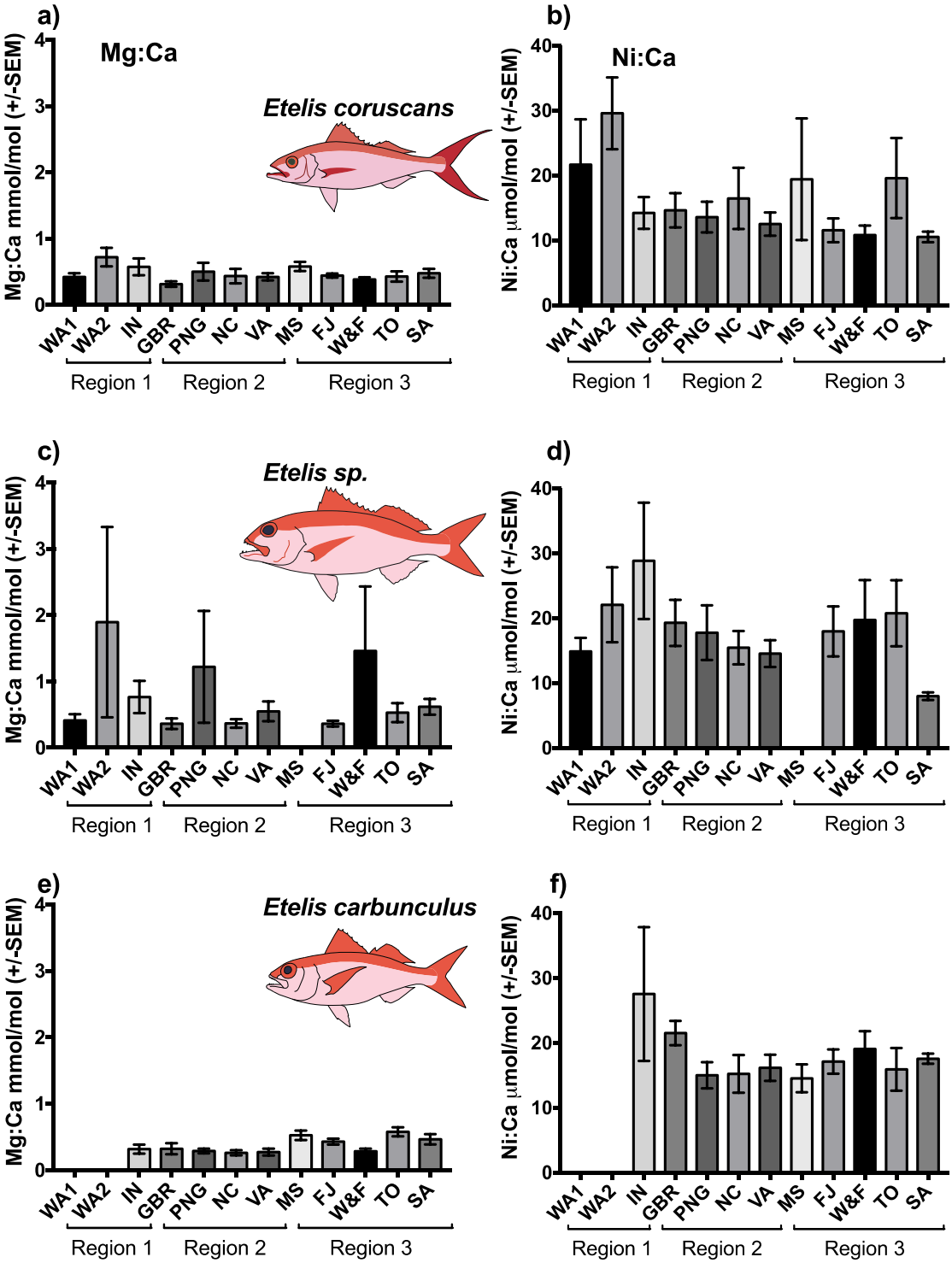


Figure 7-4: Spatial variation in otolith edge concentrations of Mg:Ca and Ni:Ca from three eteline snapper species: a/b) *Etelis coruscans*, c/d) *E. sp.*, and e/f) *E. carbunculus*. Replicates varied by Location and species but for most locations n=12.

Multivariate spatial separation for stock structure

On the broader level, there was some metapopulation discrimination based on edge signatures (Table 7-4); however, these patterns were less clear to interpret as each species had some Locations that separated more distinctly, and these varied by species and not across clear geographical gradients. For instance, *E. coruscans* from Indonesian and Papua New Guinean stocks were distinct from one another (Fig. 7-5), and these two Locations also had the highest proportion of correctly classified samples (75-83.3%). Samples from Fiji for *E. sp*. were distinct from GBR, Tonga and Indonesian stocks. Otoliths from the Kimberley had the most correct allocation (75%). *E. carbunculus* had clear separation between Locations as close as Tonga and Samoa. Classification accuracy was similar for all species, and most of the difference between Locations resulted from significant differences in Sr:Ca for all species and additionally Cu:Ca for *E. sp*. and Mg:Ca for *E. carbunculus*. Core measurements demonstrated no significant differences in multivariate elemental signatures among Locations and overall very low classification accuracy.

There were clearer differences between Locations nested in the Region level for edge measurements. Multivariate fingerprints had significant differences among the Western Australian fishing locations and Indonesia for two species (Region 1, Table 7-5, Fig. 7-6). The higher classification accuracy (69-83%) for both *E. coruscans* and *E. sp.* by Location indicated that there may be three independent stocks in Region 1. These differences were mostly due to spatial variation in Sr:Ca and Ni:Ca (*E. coruscans*) and Sr:Ca, Mn:Ca and Cu:Ca (*E. sp.*). Averaged core measurements showed very little differentiation among Locations within the Regions, with lower classification success for almost all comparisons (Table 7-6, Fig. 7-7). The decrease in classification success was substantial for Region 1, with LDFA accuracies ranging from 33-69%. Overall cross-validation showed an 8-27% decrease in classification accuracy compared to classic LDFA (no cross-validation) models.

Stocks were not well discriminated in Region 2 with lower LDFA classification accuracy (18-62%) for all species. MANOVA results indicated otolith edge fingerprints were not significantly different. Predictions from LDFA show there is greater overlap between New Caledonia and Vanuatu than the Great Barrier Reef/Coral Sea and Papua New Guinea samples for all three species. Only one species (*E. carbunculus* in Region 2) showed significant separation in one MANOVA core comparison, but follow-up analyses indicated this was not due to any single element. The core sample predictions from Vanuatu and New Caledonia again showed overlapping signatures.

There was also some separation between the far eastern Locations in Region 3 (e.g. Tonga, Samoa and Fiji) for two species, attributed to Sr:Ca and Mg:Ca variation (*E. carbunculus*)

and Sr:Ca (*E. sp.*) among these countries and territories. Tonga and Monowai Seamount may have more connected populations as the otolith edge fingerprints showed high overlap for *E. carbunculus*, and also *E. sp.* which did not have any Monowai samples, demonstrated higher classification success. Fiji and Wallis and Futuna also had similar otolith fingerprints for *E. sp.*. Core samples in Region 3 show minimal discrimination based on otolith chemistry with high overlap and low classification success.

Table 7-4: Spatial analyses (MANOVA/LDFA) of multivariate elemental fingerprints for otolith core and edge measurements for three species across all Locations. Trace element concentrations were Box-Cox transformed, centred, scaled and select elements were removed if assumptions were not met.

		MANOVA						LDFA	
	Measurement	**Df**	**Pillai**	**Approx. F**	**Num Df**	**Den Df**	**p**	**No CV**	**Cross-validation**
E. coruscans	Core	11 114	0.83	1.05	99	1026	0.37	27.8%	10.3%
	Edge	11 114	1.20	1.83	88	912	< 0.001***	37.3%	18.3%
E. sp.	Core	10 103	0.65	0.80	90	927	0.91	28.1%	6.1%
	Edge	10 103	1.23	1.63	90	927	< 0.001***	40.4%	18.4%
E. carbunculus	Core	9 81	1.05	1.19	81	729	0.13	37.4%	13.2%
	Edge	9 81	1.20	1.58	72	648	< 0.01**	48.4%	22.0%

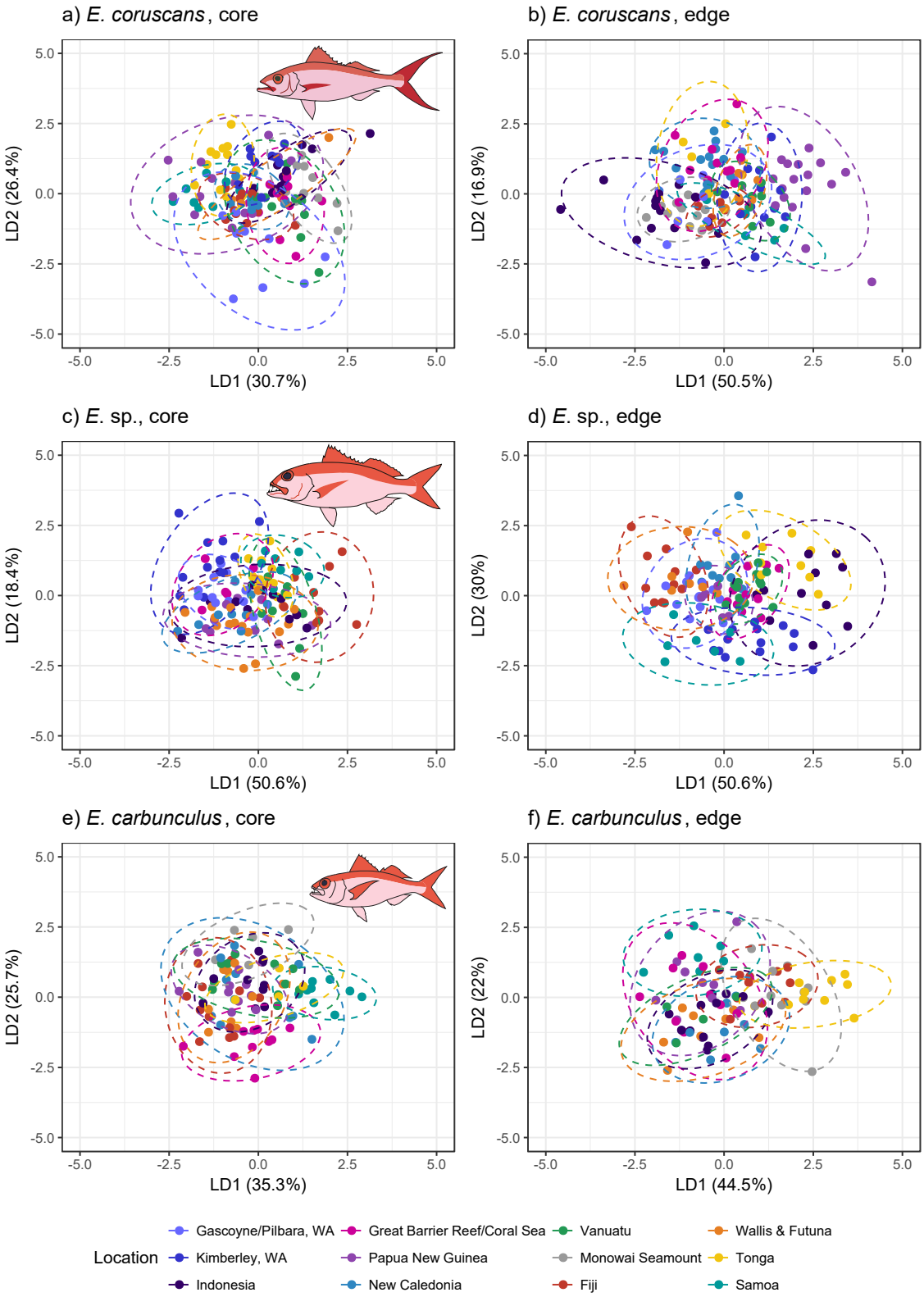


Figure 7-5: Otolith multivariate elemental signatures for three deepwater snapper species analysed with linear discriminant function analysis (LDFA) for core (left) and edge (right) measurements across 10-12 locations in the Indo-Pacific. Trace element concentrations were Box-Cox transformed, centred, scaled and select elements were removed if assumptions were not met.

Table 7-5: Spatial analyses (MANOVA/LDFA) of multivariate elemental fingerprints for otolith *edge* measurements for three species across three Regions. Trace element concentrations were Box-Cox transformed, centred, scaled and select elements were removed if assumptions were not met. †indicates balanced replicates

		MANOVA						LDFA	
	Regions	**Df**	**Pillai**	**Approx. F**	**Num Df**	**Den Df**	**p**	**No CV**	**Cross-validation**
E. coruscans	Region 1: WA1, WA2, IN†	2 33	1.03	3.04	18	52	< 0.001***	83.3%	69.4%
	Region 2: GBR, PG, VA, NC	3 38	0.81	1.53	24	99	0.08	61.9%	40.5%
	Region 3: FJ, WF, SA, IW, TO	4 43	0.74	1.10	32	156	0.34	54.2%	27.1%
E. sp.	Region 1: WA1, WA2, IN†	2 33	1.18	4.85	16	54	< 0.001***	80.6%	72.2%
	Region 2: GBR, PG, VA, NC†	3 44	0.53	1.23	21	120	0.24	47.9%	22.9%
	Region 3: FJ, WF, SA, TO	3 26	1.31	2.04	24	63	< 0.05*	86.7%	60.0%
E. carbunculus	Region 2: GBR, PG, VA, NC	3 33	0.46	0.75	21	87	0.77	56.8%	18.9%
	Region 3: FJ, WF, SA, IW, TO	4 44	1.09	1.87	32	160	< 0.01**	57.1%	44.9%

Table 7-6: Spatial analyses (MANOVA/LDFA) of multivariate elemental fingerprints for otolith *core* measurements for three species across three Regions. Trace element concentrations were Box-Cox transformed, centred, scaled and select elements were removed if assumptions were not met. †indicates balanced replicates

		MANOVA						LDFA	
	Regions	**Df**	**Pillai**	**Approx. F**	**Num Df**	**Den Df**	**p**	**No CV**	**Cross-validation**
E. coruscans	Region 1: WA1, WA2, IN†	2 33	0.60	1.24	18	52	0.26	69.4%	36.1%
	Region 2: GBR, PG, VA, NC	3 38	0.64	0.96	27	96	0.53	52.4%	21.4%
	Region 3: FJ, WF, SA, IW, TO	4 43	0.59	0.84	32	156	0.71	47.9%	18.8%
E. sp.	Region 1: WA1, WA2, IN†	2 33	0.46	1.00	16	54	0.47	66.7%	33.3%
	Region 2: GBR, PG, VA, NC†	3 44	0.33	0.52	27	114	0.97	47.9%	14.6%
	Region 3: FJ, WF, SA, TO	3 26	0.50	0.53	24	63	0.96	66.7%	20.0%
E. carbunculus	Region 2: GBR, PG, VA, NC	3 33	0.98	1.69	24	84	< 0.05*	62.2%	40.5%
	Region 3: FJ, WF, SA, IW, TO	4 44	0.72	1.09	32	160	0.35	46.9%	22.4%

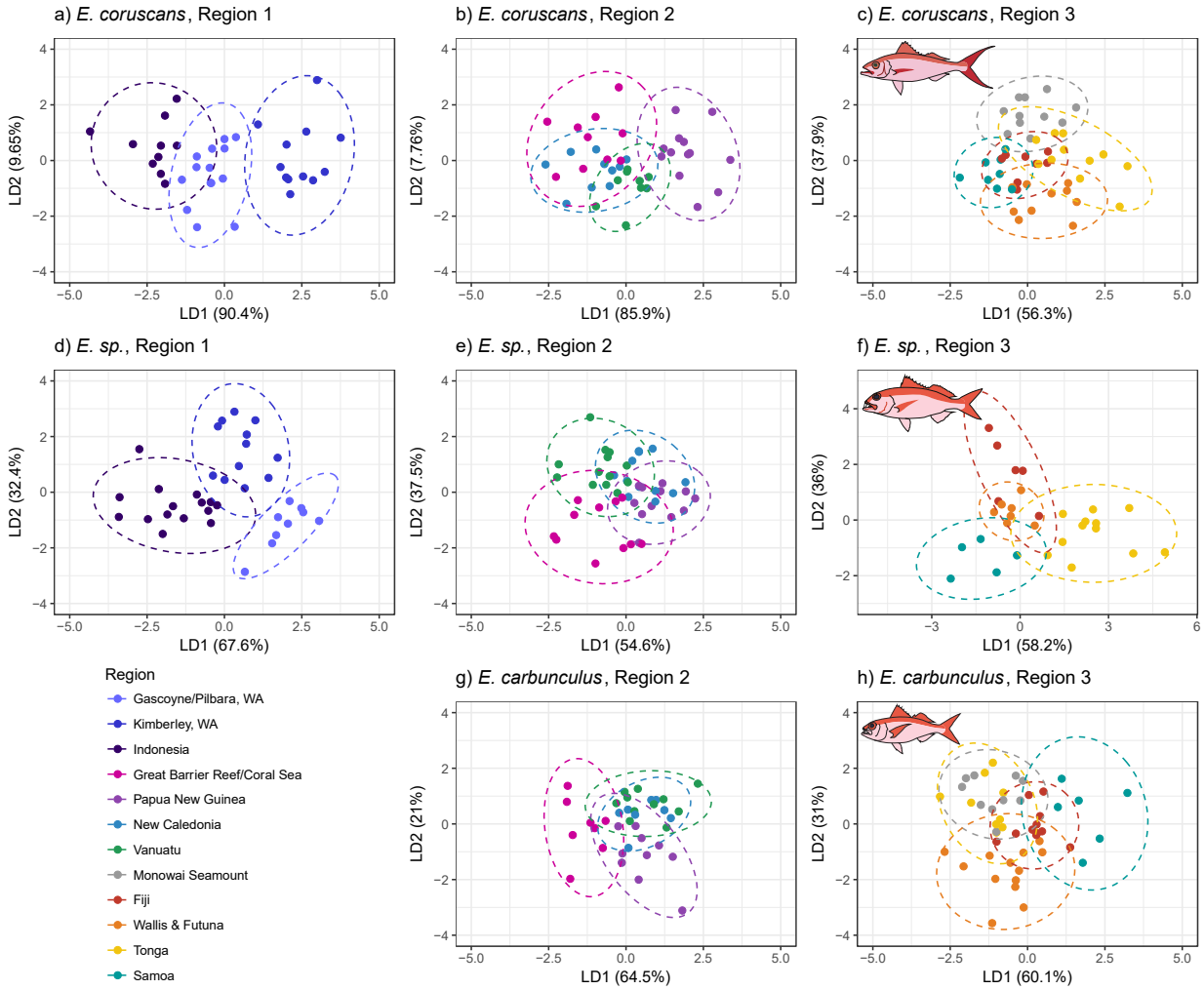


Figure 7-6: Spatial stock structure of three eteline species from linear discriminant analysis (LDFA) of otolith *edge* measurements. Samples were divided among three Regions with 3-5 Locations nested within Region. Trace element concentrations were Box-Cox transformed, centred, scaled and select elements were removed if assumptions were not met.

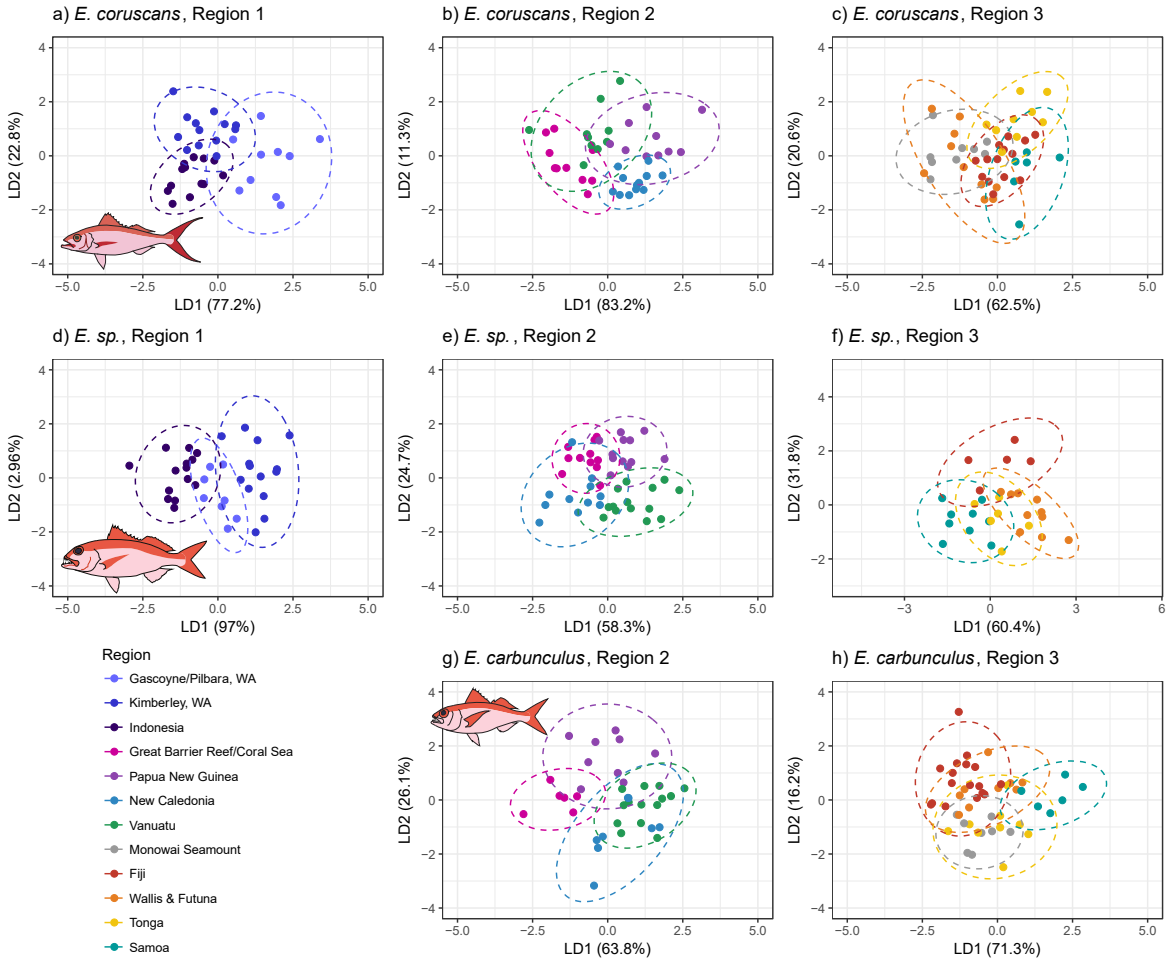


Figure 7-7: Spatial stock structure of three eteline species from linear discriminant analysis (LDFA) of otolith *core* measurements. Samples were divided among three Regions with 3-5 Locations nested within Region. Trace element concentrations were Box-Cox transformed, centred, scaled and select elements were removed if assumptions were not met.

Discussion

Differences in the multivariate otolith trace element signatures indicated clear separation in the broader metapopulation distributions of three deepwater eteline snapper species, which provide evidence for stock structure among the locations sampled across a longitudinal study area of over 7500 km. On two levels of spatial analyses, there were significant differences among edge measurements that clearly separated samples from some locations, which implied that there were some distinct stocks. On the Indian Ocean side of the biogeographic distribution, there was clearer separation between samples collected from Indonesia and Western Australian fishing jurisdictions for *E. coruscans* and *E. sp.* for both core and edge measurements. On the Pacific side, there was some overlap between samples collected from neighbouring EEZs, indicating that the scales of potential movement by deepwater snapper may be larger in the western and central Pacific. While some of these differences aligned with designated EEZ boundaries, otolith chemistry suggested deepwater snapper stocks likely cross EEZ boundaries in some regions. Alternatively, similarities in water chemistry may also account for some of the overlap in otolith measurements. This information is useful for fishery management, as some international cooperation will be required for effective management of this mixed stock fishery and the current status of many deepwater fish stocks are uncertain with limited biological and fisheries data (Newman et al. 2017).

Otolith chemistry indicated heterogeneous spatial structure, but despite some small differences in otolith chemistry between species, there is evidence of overall congruent stock structure among sympatric species. These results add another layer to what has been shown from genetic and otolith studies of eteline snappers. Genetic studies have revealed some genetic population structure and concluded there was overall genetic congruency between *Etelis* species in the Indo-Pacific (Loeun et al. 2014, Andrews et al. 2016, Goldstein et al. 2016b); I found congruent patterns in multivariate otolith fingerprints among the locations sampled, despite some intraspecies variation in elemental concentrations within regions. The differences among locations were stronger than among species, with fingerprints showing similar levels of separation in Region 1 for two species and similar overlap in Regions 2 and 3 for three species. Studies of less closely-related species that share similar ecological niches (i.e. same depths and habitats) have also demonstrated similarities in multivariate fingerprints over broader scales (e.g. *Pagrus auratus* and *Platycephalus bassensis* along three bays in southeast Australia, Hamer & Jenkins 2007).

There were few differences in otolith chemistry at the scale of region; however, there was substantial variation among locations within regions and among individuals. Some locations (e.g. Indonesia and Western Australia) I predict have little biological connectivity with adjacent

locations and constitute separate stocks, and these more isolated stocks may be more vulnerable to overfishing. The differences in otolith chemistry in Region 1 between Indonesia and Western Australian fishing locations demonstrated the greatest separation for both edge and core fingerprints for both species. This may be similar to the smaller spatial-scale stock structure Newman et al. (2000) and Ovenden et al. (2002, 2004) found for *P. multidens* in otolith carbonate and genetic analyses. This discrimination may reflect local oceanographic or habitat-based differences that create effective boundaries between Australian and Indonesian stocks. While this simplifies stock units for fisheries managers, it may also be a symptom of greater fishing pressure in this region. Indonesia is the second-largest producer of marine capture fisheries and many Indonesian fisheries are over or fully exploited (FAO 2018). Stocks of shallow and deepwater snappers are fished close to the EEZ border between Australia and Indonesia. Fishery scientists had recognized the potential of fishing shared resources and established a collaborative research project in the 1990s on shallower lutjanid species, which also included *Pristipomoides* spp. (Blaber et al. 2005, Dichmont & Blaber 2006). On the Australian side of the maritime border, the North West Slope Trawl Fishery and Western Deepwater Trawl Fishery (WDTF) operate in NW Australian waters (200 m isobath to 200-nm EEZ boundary). These fisheries rely on limited entry and the WDTF operates as a diversification of the Northern Prawn Fishery, hence, gear used are bottom trawl fishing methods. In 2000-2001, 51-53 tonnes of eteline snappers were caught, catches declined over a short period, with only 1.5 tonne harvested in 2006. Current WDTF annual catch (2010-2014) is less than one tonne of eteline snappers (AFMA 2018), and overall, there had been a declining catch per unit effort (from a peak of 334 tonnes for combined deepwater finfish and crustaceans in 2001); however, there have been gear modifications and shifts in target species with little information on the status of Australian stocks (Moore et al. 2007, Rodgers et al. 2010). Estimates from 2015-2016 have determined fishery catches are not currently being overfished but biomass levels are uncertain (ABARES 2018). Limited entry to the fishery, careful monitoring of catch composition, size and maturity of individuals of species of greater concern, and frequent assessments are some of the precautions undertaken by Australian fisheries scientists.

Among locations in the western and central Pacific (Regions 2 and 3), fishery management would benefit from cooperation where Pacific island stocks may be divided among multiple fishing jurisdictions. Several multivariate signatures overlapped among the island nations and classification to a particular location was poor, suggesting there may be greater connectivity among island stocks. Some of the lower discrimination success may be because of high overlap between adjoining Locations (i.e. New Caledonia and Vanuatu), which may indicate that these neighbours share deepwater fish resources. Demersal species are presumed less mobile and transitory than pelagic species (e.g. tuna), but several groundfish are demersal transboundary species (e.g. pollock, hake and Greenland halibut; Palsson et al. 2004, Mayo et al. 2009). This

study provides evidence that deepwater snapper are transboundary species, and Pacific countries should invest in regional cooperative fisheries management. Of the fishery information available, Tonga's deepwater resources are considered overfished (Hill et al. 2016), but it is not known if this impacts neighbouring fisheries.

Otolith chemistry relies on understanding a fish's environmental history and life history, which include the distribution of suitable habitats and species' movements during all life history stages (Carlson et al. 2017), but these are key knowledge gaps for deepwater snapper populations (Chapter 5, Misa 2013, Moore et al. 2013, Moore et al. 2016a). Deepwater snappers are believed to be generally sedentary, however, previous tagging studies have shown adult deepwater snapper *P. filamentosus* mostly have restricted movements (0-22 km) and, in rare cases, can travel greater distances (>400 km) and across deepwater channels (Kobayashi 2008). *Etelis* spp. tagging studies have shown smaller movements (<10 km; Weng 2013), in a smaller tagging pilot study. The dispersal of a species, whether through adult or larval movement, is important to mitigate potentially detrimental effects of fishing. For example, the deepwater roundnose grenadier *Corphaenoides rupestris* is a benthopelagic fish with limited adult movement (in 500-2000 m depths). Trace element analysis of otoliths revealed some individuals are restricted to specific seamounts (Régnier et al. 2017), and targeting more isolated stocks can lead to localised depletions with only moderate levels of fishing if there is no replenishment from surrounding areas.

Otolith chemistry analyses may be able to provide insight on the complex spatial dynamics that differ between stocks but also over the lifetime of an individual. There were differences in spatial overlap with core and edge areas of the otolith. Core measurements did not clearly discriminate between locations and this may be due to similarities in the early life history of the fishes, such as shared nursery environments, long pelagic residencies, or more similar physiological influences. The early life history of many deepwater fishes is still largely an unknown black box; however, otolith chemistry suggested that there are more shared histories among young deepwater snappers.

There is still insufficient information on deep-reef metapopulations; further broad-scale biological studies would add substantial value to fishery management. For instance, there were differences in growth between locations for *E. carbunculus* (Williams et al. 2017), meaning future stock assessments should incorporate information on size, age, gender and maturity. Similarly, otolith chemistry is influenced by fish physiology, reproduction and growth, directly or indirectly reflecting to changes in the ambient environment (Walther et al. 2010, Sturrock et al. 2014, Sturrock et al. 2015). Where and how these differences are reflected in the otolith is an important consideration for metapopulation analysis, as well as expanding to larger scale studies. A few

examples of large spatial or temporal scale studies are: there were different environmental drivers affecting growth of *Lutjanus bohar* in NW and NE Australia (Ong et al. 2017) and stock structure analysis also found a similar east-west subdivision between *Sardinops sagax* consistent over a 60-year period (Izzo et al. 2017). Understanding how scale and the relative importance of intrinsic (physiological) and extrinsic (environmental) influences on otolith elemental concentrations (Grammer et al. 2017) does not affect our interpretation of stock structure, but ranks potentially important drivers of population structure.

I found greater evidence for intrinsic drivers of population structure among locations for multiple elemental concentrations. Otolith Sr:Ca increases with older fish and was found to vary significantly among locations for *E. coruscans* and *E. sp*. In the otolith, Sr:Ca is controlled by physiological processes in marine fish, in particular reproduction (Kalish 1991, Sturrock et al. 2015), but uptake is also affected by environmental factors like temperature and freshwater/estuarine salinity (Bath et al. 2000, Macdonald & Crook 2010, Walther et al. 2010). Otolith Mg:Ca and Mn:Ca in marine fish are also under greater physiological control. Interestingly, Mg:Ca and Mn:Ca was not useful for discriminating regional differences in this study (as it has been for other marine species, e.g. Pracheil et al. 2014); however, it was clear that there were differences among species. Differences in Mg:Ca can correlate with fish growth rate (Sturrock et al. 2015), this is most likely because magnesium is required for multiple metabolic pathways (Kaim et al. 2013). Otolith Mg:Ca does not reflect water temperature or salinity (Elsdon & Gillanders 2002, Sturrock et al. 2012) and so differences in Mg:Ca otolith uptake over this study area would likely be due to biological variation among populations.

While I did not find much evidence to support environmental drivers affecting population structure, some differences in elemental concentrations of Ba:Ca among regions may be correlated with environmental factors. Correlations among physiologically-regulated elements may be stronger than links with environmentally-influenced elements (Grammer et al. 2017). Otolith Ba:Ca has been linked to water chemistry (Bath et al. 2000, Walther & Thorrold 2006), dietary sources (Sanchez-Jerez 2002, Izzo et al. 2015), growth rate (Miller 2011, Sturrock et al. 2015) and upwelling (Kingsford et al. 2009, Grammer et al. 2017). Primary productivity is linked to localised upwelling, where deep watermasses are enriched with barium, leading to spikes in concentrated Ba:Ca (Grammer et al. 2017). Differences in upwelling, water mass chemistry, and diet may be more subtly influencing otolith chemistry over the large spatial area of this study.

Chemical analyses of deepwater snapper otoliths revealed some stock structure through the Indo-Pacific. There was generally poor discrimination among geographically well-separated Locations, and the regional differences in otolith chemistry that were detected were likely driven by mega to macroscale (*sensu* Haury et al. 1978) environmental differences. There were

significant differences among Locations nested within Regions. I predict that these differences constitute separate stocks, which overlap with existing EEZ boundaries. Because of their unselective nature, mixed species handline fisheries of deepwater etelines that differ in their transboundary geographic stock structures will present the most challenging management problems. The resolution of these problems requires more than biological data justifying management by separate stocks; also needed is careful consideration of social and cultural factors. The higher levels of variation among stocks and among individuals are probably due to a combination of environmental and physiological factors. This study demonstrates that elemental chemistry can help to predict stock structure, which can be further tested with genetic, morphometric and demographic studies. I suggest employing a precautionary principle, whereby the chemical discrimination of stocks revealed in this study is used to assist the management of these highly vulnerable snappers until proven uninformative.

Chapter 8 General Discussion

Deep-reef habitats are unique and critical habitats, with a diverse fish assemblage comprised of both fishes with expansive depth ranges and fishes that are exclusive to mesophotic depths. My thesis has provided an important 'baseline' of the biodiversity of deep reefs of the Great Barrier Reef (GBR) shelf-break and demonstrates that deep reefs worldwide risk a double jeopardy of narrow depth distributions and, for many species, often narrow habitat requirements that indicate an inherent vulnerability to deep-reef fisheries. My objectives were to investigate these fish assemblages on the local scale in Chapters 2-5 and on the broader scale in Chapters 6 and 7.

On the GBR, I found a diverse assemblage of fishes to depths of 260 m that have been poorly described. My findings align with other studies on deeper reefs worldwide. Often referred to as Mesophotic Coral Ecosystems (MCEs) in the tropics, they support diverse benthic and pelagic communities, very different to those of shallower reefs. In the few locations where there have been comprehensive fish assessments, mesophotic depths often have high proportions of 'rare' or endemic species (Kane et al. 2014, Last et al. 2014, Fukunaga et al. 2016, Kosaki et al. 2016, Pyle et al. 2016a). However, we are in the 'Age of Discovery' for MCEs, with currently high rates of new species discoveries and description (e.g. Okamoto & Motomura 2012, Uiblein & McGrouther 2012, Allen & Walsh 2015, Baldwin & Robertson 2015, Fukui & Motomura 2015, Gomon & Walsh 2016, Pyle et al. 2016b, Tornabene et al. 2016a, Tea & Gill 2017, Pinheiro et al. 2018a, Shepherd et al. 2018a). As we gain more comprehensive views of species distributions worldwide, it is becoming more apparent that mesophotic and 'rariphotic' fish species' distributions are geographically broad, and depths below 100 m yield the highest undiscovered diversity (Chapter 2, Baldwin et al. 2018). Molecular techniques also indicate that there are cryptic species 'hiding in plain sight' and genetic analyses are being used to clarify taxonomic uncertainty, including for instance, fishes caught in deep-reef fisheries (e.g. genus *Etelis*, Andrews et al. 2016; and *Gymnocranius,* Borsa et al. 2010, Borsa et al. 2013, Chen et al. 2017).

Depth range was a strong predictor of species occurrence and abundance in my study. Going forward, it will be critical to properly document distribution of fishes and habitats with depth, as the typical depths that define MCEs (30-150 m) are comprised of several communities and transition zones between fish and benthic assemblages that may vary by location (Semmler et al. 2017, Baldwin et al. 2018, Rocha et al. 2018). Depth zonation is still the prevailing determining feature, with high turnover between transition zones, but some species are shared between depth strata and others have very narrow depth distributions (Chapter 2, Rocha et al. 2018). There is stronger evidence of connectivity between the upper mesophotic and shallower

reefs (Colin 1976, Garcia-Sais 2010, Tenggardjaja et al. 2014, Papastamatiou et al. 2015, Kane & Tissot 2017) than lower depth strata, which may have stronger links to deeper benthic and offshore, pelagic ecosystems, which are still largely unknown. The 'Deep Reef Refugia Hypothesis' (Glynn 1996) where deep reefs may increase resilience of shallow-water reefs (Riegl & Piller 2003, Lesser et al. 2009, Tittensor et al. 2010, van Oppen et al. 2011, Holstein et al. 2015) will have limited benefit to fish assemblages that are more unique in composition. More importantly, for deep reefs threatened from fishing pressure, will be to assess the role of deep reefs as fishing refuges (Lindfield et al. 2014, Lindfield et al. 2016), to monitor changes to these fish assemblages, and to include these depths and stock estimates in future resource management plans (Asher et al. 2017).

Benthic habitats influenced the distribution of many deep-reef fishes. It is important to define these linkages between fish and the benthic environment as habitat specialists may be more susceptible to habitat changes or loss (Munday 2004). I found the shelf-break reefs exhibited high variability within depth strata and this spatial heterogeneity was reflected in fish assemblage composition and the nature of habitats, such as the slope, epibenthic or substratum components (Chapter 3). The presence of filtering organisms was important in shallower mesophotic depths while sand and boulders (abiotic structural differences) were more important in deeper habitats. Shelf-break fish assemblages were diverse, and individual reefs had generally low percentages of overlap of assemblages among sites within depth strata. Some of the similarities could be explained by species interactions (Chapter 3) and trophic groups of deeper fish indicate structured feeding groups with depth, in general higher proportions of piscivores, planktivores, invertivores, and fewer herbivores (Chapter 3, Thresher & Colin 1986, Feitoza et al. 2005, Brokovich et al. 2010, Garcia-Sais 2010, Bryan et al. 2013, Bejarano et al. 2014, Andradi-Brown et al. 2016b, Fukunaga et al. 2016, Pinheiro et al. 2016, Pyle et al. 2016a, Asher et al. 2017, Moore et al. 2017). It was clear that there were complex spatial and environmental relationships that affected fish diversity and abundance, and more complex reef architecture may create a greater availability of niches (Chapter 4). Presence-absence data of individual species indicate that there are species-specific habitat preferences that may explain similarities and disparities in the deep-reef fish assemblages (Chapter 5).

The trophodynamics of shelf-edge environments are poorly known, but the pathways of energy flow on deep reefs warrant further investigation. The high variation among fish assemblages is partly explained by the benthic community and topography, however, the overlying water column may offer a 'third dimension' that affects nutrient availability, prey abundance, and recruitment processes (Brown et al. 2011). Many deep-reef fish make diel horizontal and vertical movements (Papastamatiou et al. 2015), and patterns between day and

night fish assemblages may be differentially influenced by day/photic and night/deeper influences. At night mesopelagic myctophid and gonostomatids migrate far up the water column to mesophotic depths, and their biomass is related to the underlying topography (Suthers et al. 2006), which suggests they may play a large role in deep-reef foodwebs. Anthiine species may also transfer energy from the water column to deep reefs as 'trophic subsidies' (Weaver & Sedberry 2001). To quantify trophic pathways, it will be vital to understand the local physical and biological oceanography in order to balance the 'energy budget'. One practical reason for collecting information on natural foodwebs is to mitigate potential cascading effects if some trophic links are removed. The Atlantic cod (*Gadus morhua*) fishery is one example where removing top predatory fishes caused multiple levels of trophic change and far-reaching ecosystem collapses (Scheffer et al. 2005), and deep fisheries may remove key trophic links by overfishing higher order predators with unintended ecosystem consequences.

Deep reefs are regions of confluence with many dynamic oceanographic processes. Some of these factors may explain the variation I observed among fish assemblages if we fully understood the underlying forces between local oceanography and topography. Along the shelf-edge are steep vertical gradients in light and temperature; however, there is substantial horizontal influx from currents, mixing from internal waves and upwelling, and up-current/down-current differences in fish assemblages related to the neighbourhood topography. There is a steep thermocline (Chapter 2) and rugose environment revealed from multibeam bathymetry (Chapters 3-5), which affect how fishes are distributed, but future studies should explore how fish distributions are linked to the biological, chemical and physical oceanography. Depth, topographical relief and substratum influenced the species abundance and richness of deep reefs (Chapter 4), and these species-environment relationships are useful to understand the distribution of species across the seascape (Guisan & Zimmermann 2000, Grober-Dunsmore et al. 2007). These spatial patterns in species biodiversity are whole assemblage responses used to identify significant factors that define the physical and ecological limits to species' distribution through heterogeneous and complex habitats (Choat & Ayling 1987, Ault & Johnson 1998, Friedlander & Parrish 1998, Cappo et al. 2007). Greater knowledge of the surrounding environment would help to identify priority management areas.

The GBR shelf-edge has substantial changes in rugosity over a large latitudinal gradient and the geomorphology and habitat-forming organisms require more research. In the GBR there is active accretion at mesophotic depths (Abbey et al. 2013) and there is evidence that deep reefs are actively being 'built-up' worldwide with calcium carbonate from crustose coralline algae (Gal Eyal pers. comm.) and macroalgae (Spalding 2012), in addition to more conspicuous habitat-forming sponges and corals (Lesser et al. 2009). This study added detailed multibeam bathymetry

to the central GBR, which will improve oceanographic models, such as jet-driven nutrient upwelling that determines *Halimeda* distribution in the GBR (Wolanski et al. 1988) and potentially help to strengthen hypotheses of larval recruitment in deeper environments. *Halimeda* requires nitrate and phosphate to prosper, so mapping their distribution indicates where upwelling occurs, since shelf and surface waters have low concentrations of inorganic nutrients (Wolanski et al. 1988). The shelf-edge is a dynamic area of biological, chemical and physical spatiotemporal change. We still need to understand the role of sediments, and particularly suspended sediments. The GBR shelf-edge multibeam indicated significant topographical relief and these relative 'ridges' and 'valleys' may be conduits in sediment accumulation, downslope shifts, and longshore drift in deepwater. Sediment fluxes may be highly variable on deep reefs but sediment loads may provide important ecosystem services as they do in the deepsea (e.g. in the microbial loop and nutrient regeneration, Danovaro et al. 2008). The bathymetry and oceanography are interlinked and deep reefs may be analogous to 'islands' that entrain and modify sediment transport, creating eddies that affect species distributions: patches of nutrients and plankton (Hamner et al. 1988, Suthers et al. 2004, Gove et al. 2016), as well as fish dispersal (Kingsford et al. 1991). Moore et al. (2017) also found abrupt topography corresponded with higher fish abundance. Therefore, 'better oceanic characterisation' would improve explanatory models for dynamic environments (e.g. for species like *P. filamentosus*, whose presence-absence was explained by the presence of vertical relief expressed in terms of slope and planar curvature in Chapter 5).

Deeper reefs face many threats and there are potentially high levels of anthropogenic disturbance. Worldwide, most impacts have been documented by opportunistic studies, but there have been important lessons learned that deeper environments are vulnerable and do not recover quickly. Deep reefs are unique communities but are susceptible to many of the same detrimental environmental and anthropogenic impacts as shallower ecosystems (Andradi-Brown et al. 2016a, Rocha et al. 2018). The impacts of fishing and the double jeopardy of narrow depth ranges and specific habitat requirements were mostly discussed in Chapter 5; however, some of the impacts of fishing were not discussed. Many targeted deep-reef fish worldwide are larger predators whose removal releases top-down controls. This magnifies the potential of cascading effects, including indirect effects that may negatively affect fish recruitment and diversity (i.e. Jennings & Polunin 1996, Stallings 2008, 2009). Some fishing gears create long-lasting impacts through ghost fishing and changes to the benthic architecture. While many studies lack previously collected data to compare changes over time, remote oceanic shoals in NW Australia (20-80 m depths) had higher species richness and abundance (1.4 and 2 times, respectively) than similar depths in the GBR (Moore et al. 2017), which suggests that the GBR shelf-break has been affected by fishing pressure to some extent. Where natural baseline data is not available, regional comparisons of fish assemblage structure (species richness, abundance and composition) along a gradient of

fishing histories may be useful to determine the ability of deeper fish assemblages to respond to different levels of fishing pressure. Very few locations would have completely unaffected fish assemblages.

Other anthropogenic impacts include the introduction of invasive species, including fish and algae. In the Caribbean, the invasive lionfish (*Pterois* spp.) can be dense in deep reefs (Lesser & Slattery 2011), voracious predators of juvenile fishes of ecological and commercial importance (Albins & Hixon 2008, Eddy et al. 2016), and these effects may be combined with other environmental and anthropogenic stressors to create a 'worst-case scenario' (Albins & Hixon 2013). Similar effects have been documented by the introduction of non-native species to reef fish assemblages in Hawaii, for example *Lutjanus kasmira* and *Cephalopholis argus* (Randall 1987). Deep reefs are not immune to coral bleaching (Frade et al. 2018) and other impacts on deeper reefs include subsea pipelines, dredging, and many types of marine pollution. The single most destructive event to deeper environments to-date was the *Deepwater Horizon* oil spill in the Gulf of Mexico, which caused catastrophic and long-term declines to fish, coral and benthic assemblages (Incardona et al. 2014, Etnoyer et al. 2016, Girard & Fisher 2018). While the oil spill originated at ~1500 m depths, deep reefs (60-90 m depths) were in the large area affected by the prolonged exposure (Etnoyer et al. 2016). A few of the many lessons learned were that much of the epibenthic and demersal fish assemblage was sensitive to damage from both physical and chemical impacts. Having some baseline data on the health and condition of deeper communities and habitats was a necessary 'insurance policy' to assess damages. In this case, pre-existing ROV footage was used to gauge the 'before' condition, but for many deep environments there would be insufficient data to estimate a baseline. Deep reefs near high-risk factors should be prioritized, mapped and surveyed, such as biota and habitats near shipping lanes, ports, and oil rigs. As environmental impact assessments are often required, emergency action plans should also be required and response procedures regularly practiced in order to anticipate and mitigate environmental disasters.

I have discussed how many deep-reef fishes have limited available data and are considered 'data deficient' based on IUCN criteria. This includes regular stock assessments and monitoring of catch composition in order to document potential declines in vulnerable species. A number of species show strong habitat associations, which is promising for resource management strategies (Chapter 5). Future research should also investigate the importance of deepwater macroalgal *Ulva* or *Halimeda* beds for fish assemblages and, specifically, if they are important to complete the lifecycle of deepwater fishes. Further, the shelf-break may be important for localised spawning aggregations for many species, and with the extent of BRUVS studies (and other sampling methods) over the breadth of the GBR shallower shelf, it is unlikely that many deep-

specialist species (e.g. *Pristipomoides* and *Etelis* genera) use shallower reefal habitats for recruitment. Therefore, deep macroalgal beds or inter-reefal areas are more likely choices for nursery habitat. It is important to remember that many fish species use a mosaic of habitats daily and any further information on habitat associations helps to estimate connectivity of deeper environments. This is necessary to gauge if spatial protections are sufficient to protect deep-reef communities. Recent research on fish and benthic habitats (e.g. this study, Beaman & Harris 2007, Beaman et al. 2008, Williams et al. 2010b, Bongaerts et al. 2011, Bridge et al. 2011a, Bridge et al. 2011b, Bridge et al. 2012a, Bridge et al. 2012b, Harris et al. 2013, Puga-Bernabéu et al. 2013, Englebert et al. 2014, Englebert et al. 2017, Frade et al. 2018) have all occurred since the last GBRMP re-zoning. In the next re-evaluation of the GBRMP zonation, deeper environments should be more carefully assessed with this new information, until then, the precautionary approach should be used for activities with a greater likelihood of impacting deep reefs, such as fishing, pollution and dredging.

I used a hierarchical approach to elucidate the structure of fish metapopulations. I considered multiple spatial scales from hundreds to thousands of kilometres to understand how connected deeper fish populations are. Using otolith chemistry, I provided evidence that multiple possible stocks exist within the Indo-Pacific regions, but overall, there is great connectivity between the Pacific island populations (Chapters 6-7). For many reef fish stocks, externally supplied recruits may be necessary to re-supply existing stocks (James et al. 2002), and if so then fishery stocks should be regionally managed. Gomez et al. (2015) mapped deepwater snapper distributions and found habitat types were a major driver of species occurrence. For example, *Etelis* spp. had the lowest proportion of predicted habitat of the *Aphareus*, *Pristipomoides* and *Etelis* group, and this habitat was not equally divided among EEZs. It is very likely that some countries are exploiting the same stock and based on the available information for genetic and otolith studies, these stocks should be cooperatively managed. From the otolith chemistry, Pacific island neighbours such as New Caledonia-Vanuatu or Fiji-Wallis and Futuna should co-manage deeper fishery resources at the very least, and broader regional management is the safest choice. I conclude that the precautionary approach should be employed and deep-reef fisheries should be managed with the regional benefit in mind.

The research presented in this thesis advances the current understanding of deep reefs with a comprehensive look at local fish assemblages over a large depth gradient and explicit information on these depths and habitats. This double jeopardy of narrow depth ranges and habitat availability is often overlooked, but it is clear that these unique deep reefs are vulnerable to many anthropogenic and environmental stressors. In the future, fishery managers will require additional information on population genetics, the quality and availability of deepwater habitats, and the

spatial variation in demographics such as growth and reproduction, in order to layer this information with the otolith chemistry data in order to improve the management of deepwater fishery resources. By evaluating potential connectivity using a metapopulation theory and a variety of approaches, robust stocks can be identified so that potential fishing quotas can be determined. I believe cooperatively managing fisheries in the Indo-Pacific to identify local risks and within a metapopulation structure would be the best and safest approach.

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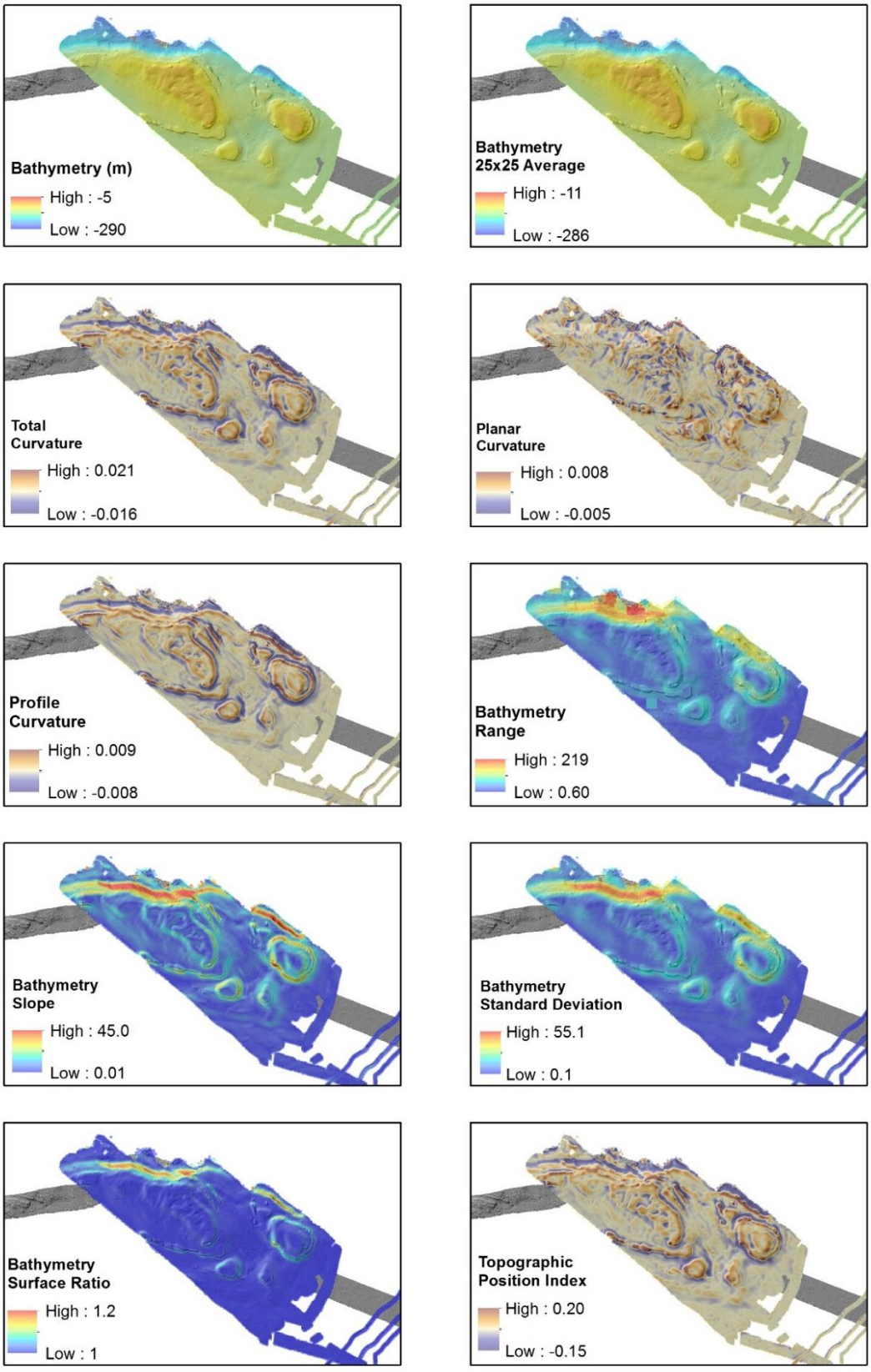
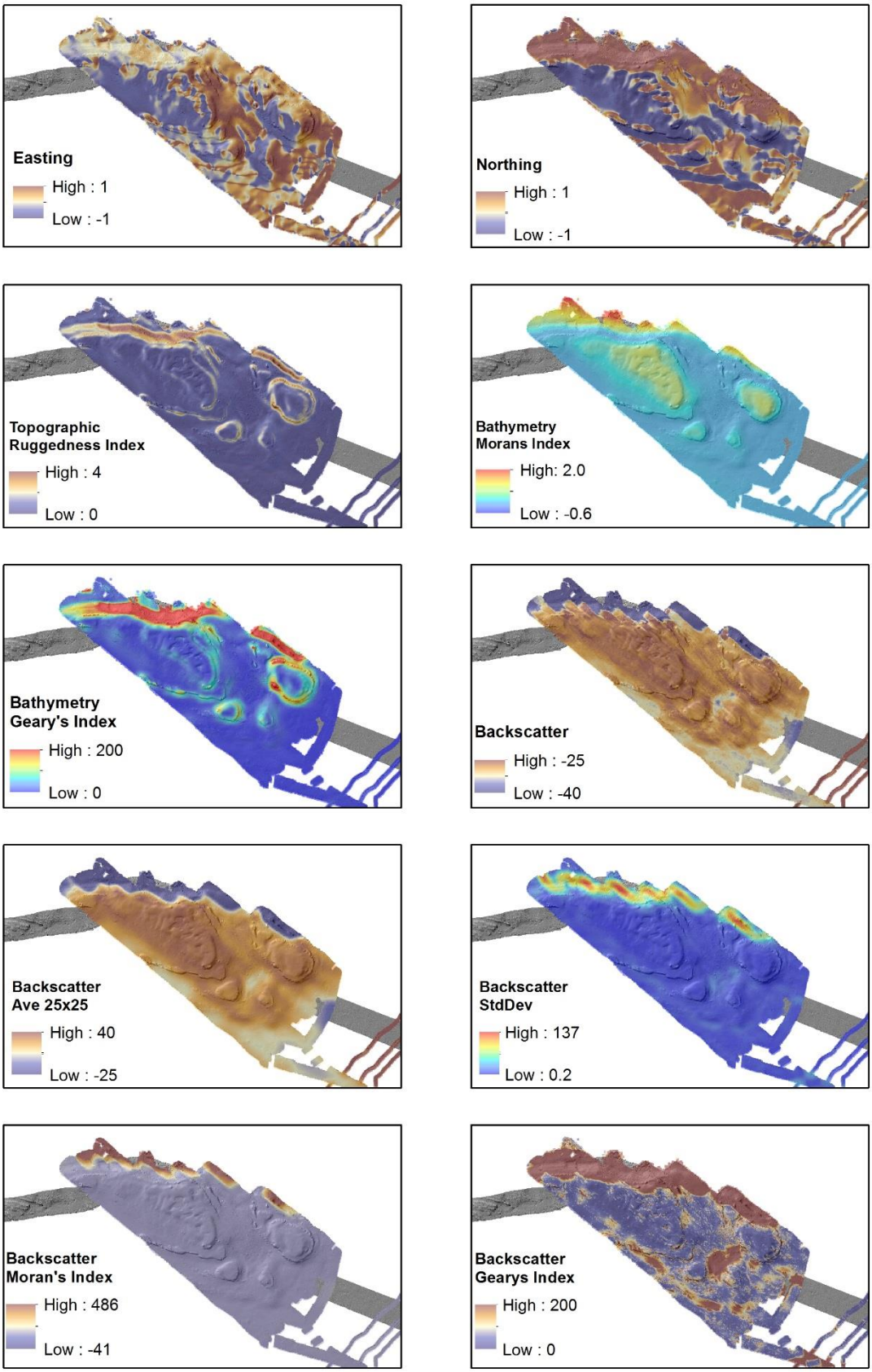
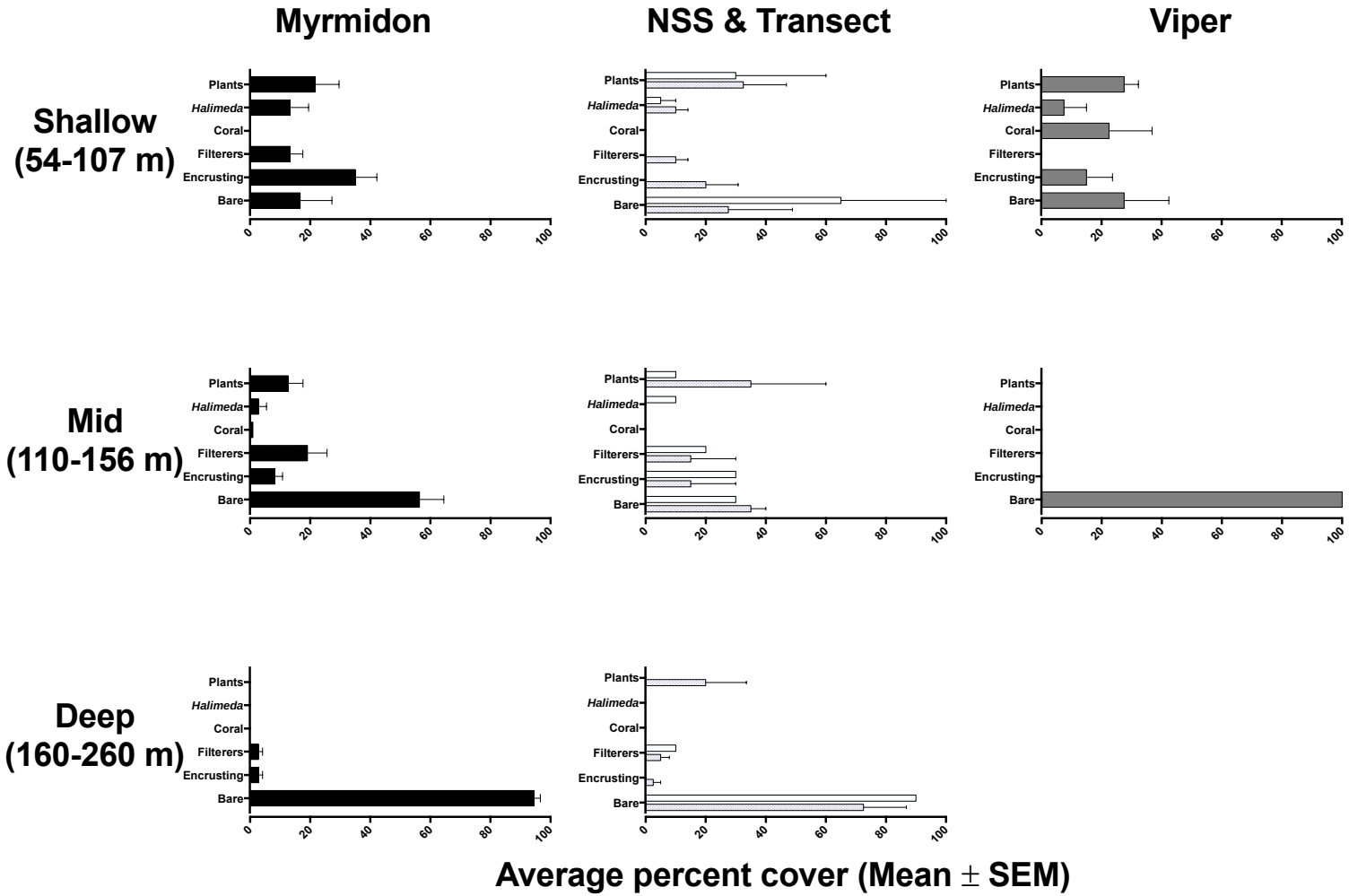
Appendix A: Supplementary Figures and Tables for Chapter 3

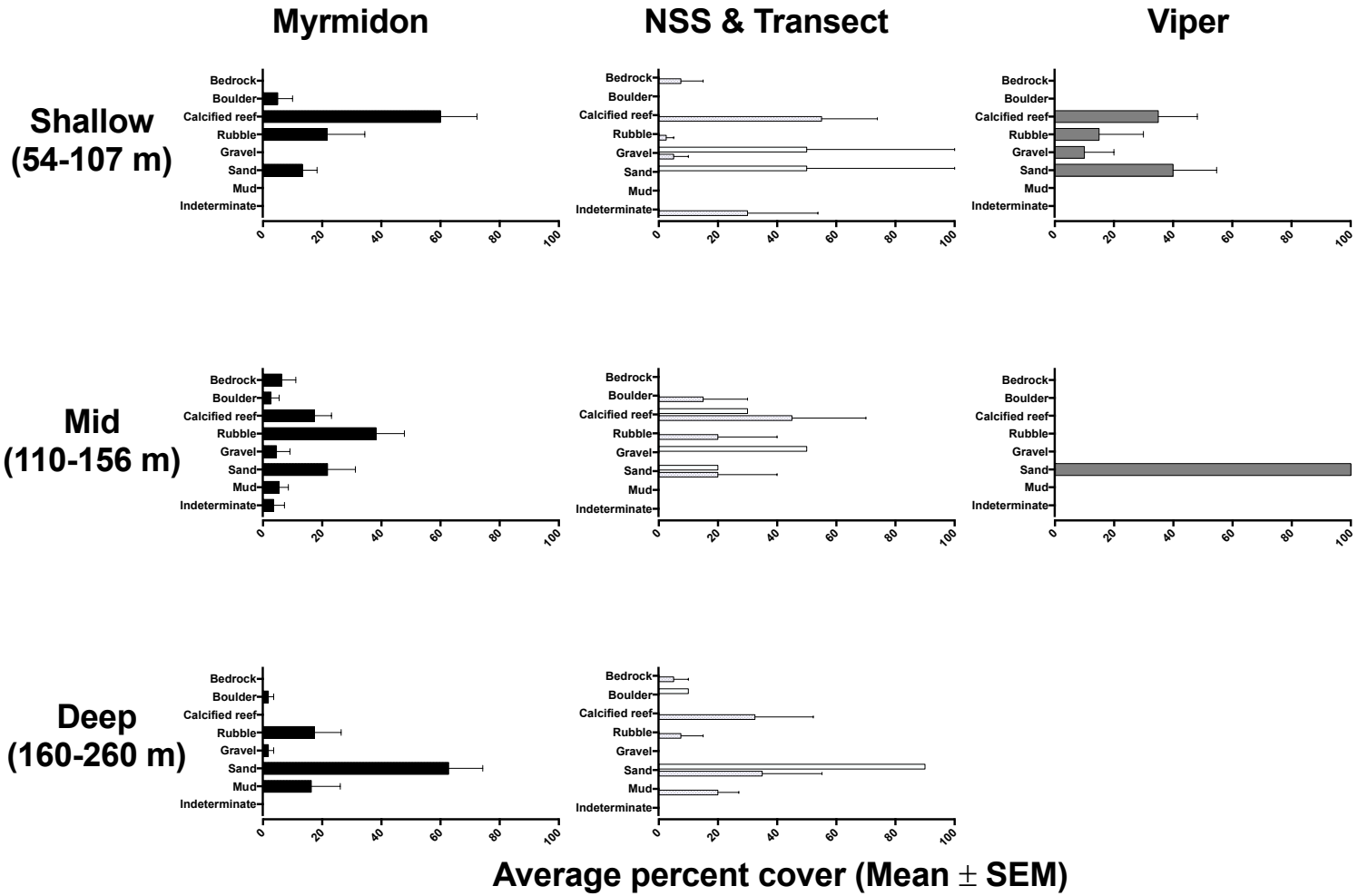




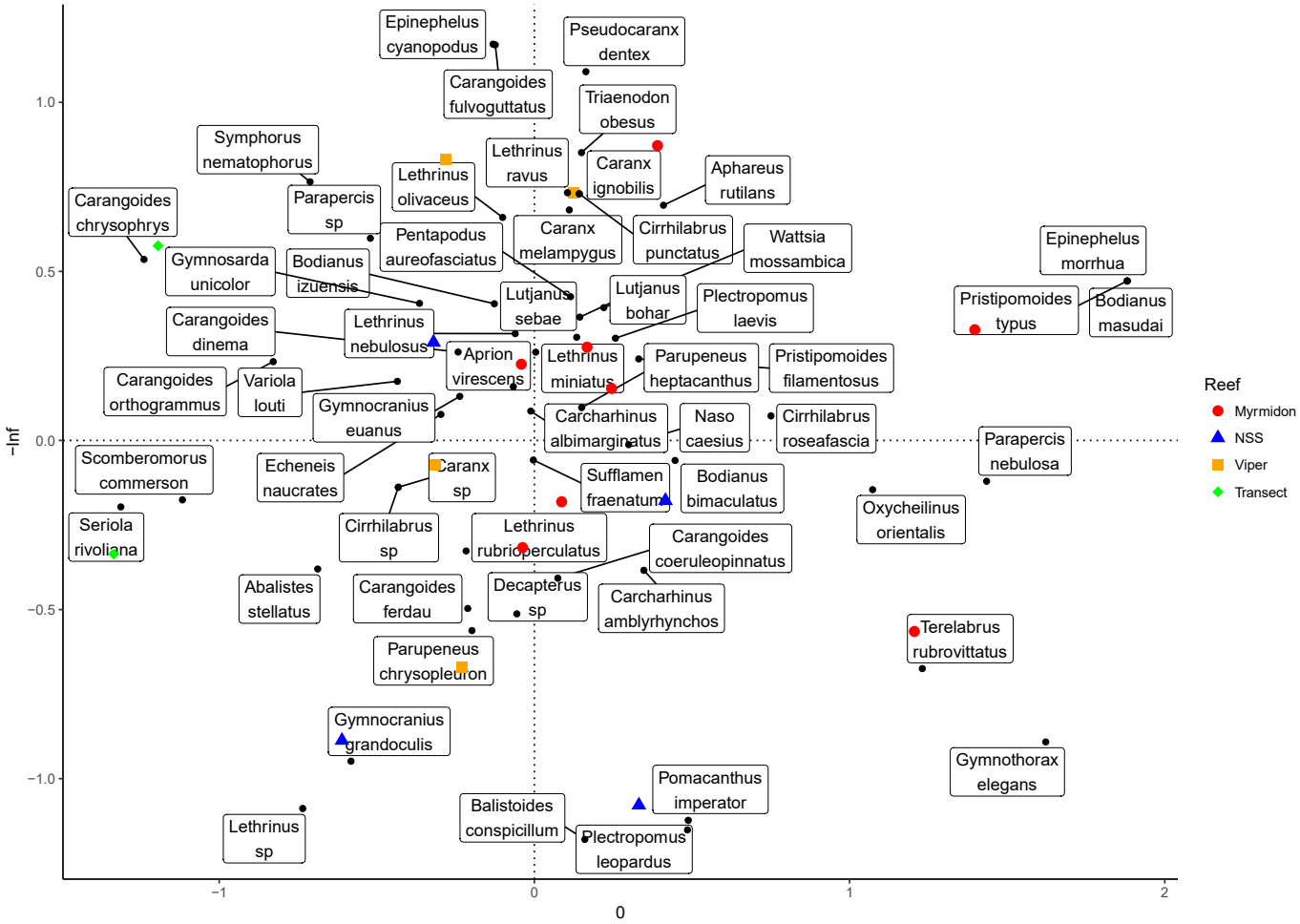
Appendix Figure A1: Examples of raw multibeam bathymetry and backscatter raster datasets and their derivatives for the Northern Submerged Shoals indicate the range in bathymetry, backscatter and derivative values (see Table 1 for list of derivatives and their ecological contexts).



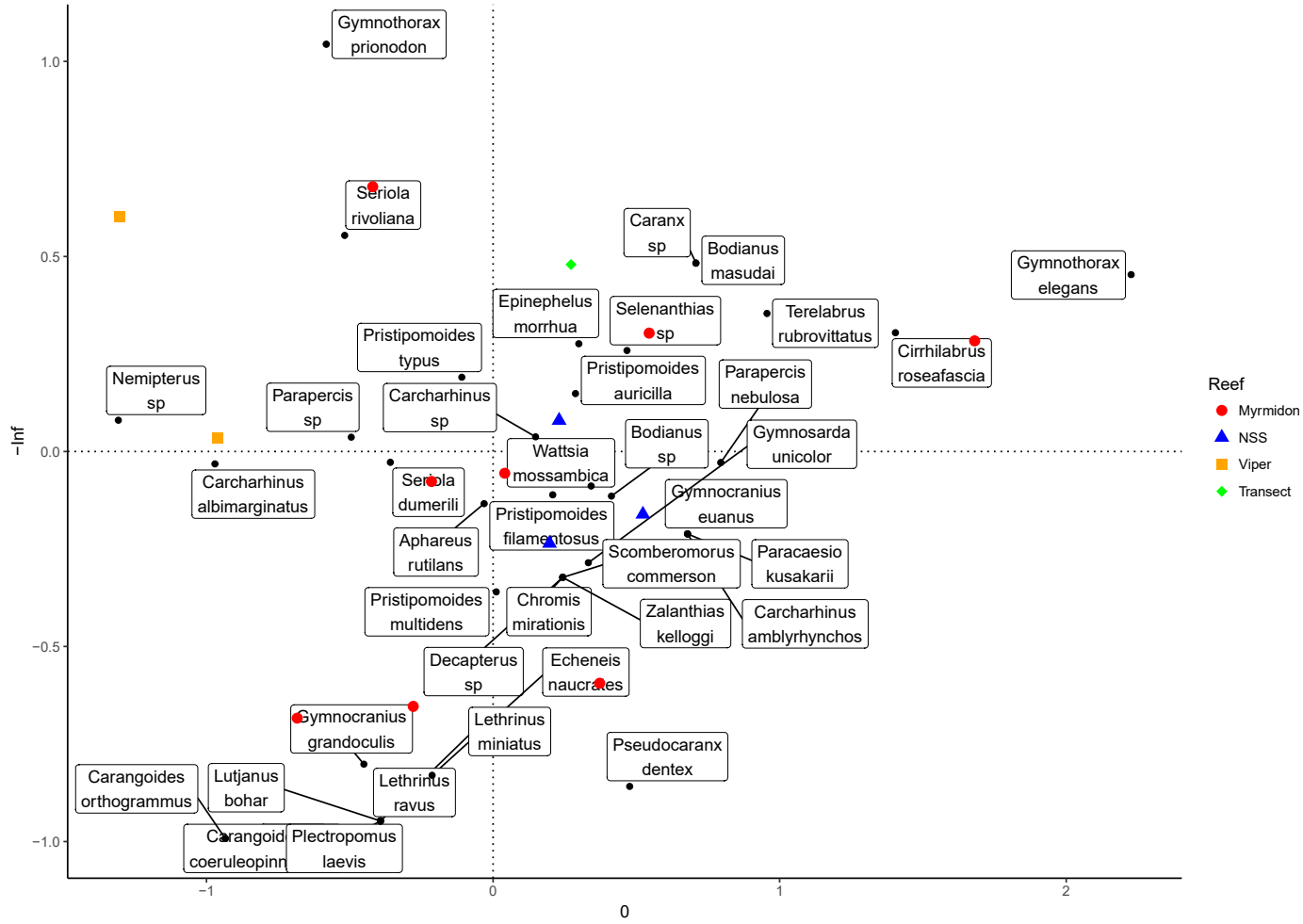
Appendix Figure A2: Comparing average epibenthic habitat measures in the field of view by reef. Percent cover of each category is estimated out of a total sum of 100 for each site. Four locations (Myrmidon Reef, Northern Submerged Shoals, Viper Reef and an inter-reefal transect) are included in this comparison, but there were no 'Deep' sites at Viper Reef. Note: n varies per bar, 1-11 sites.



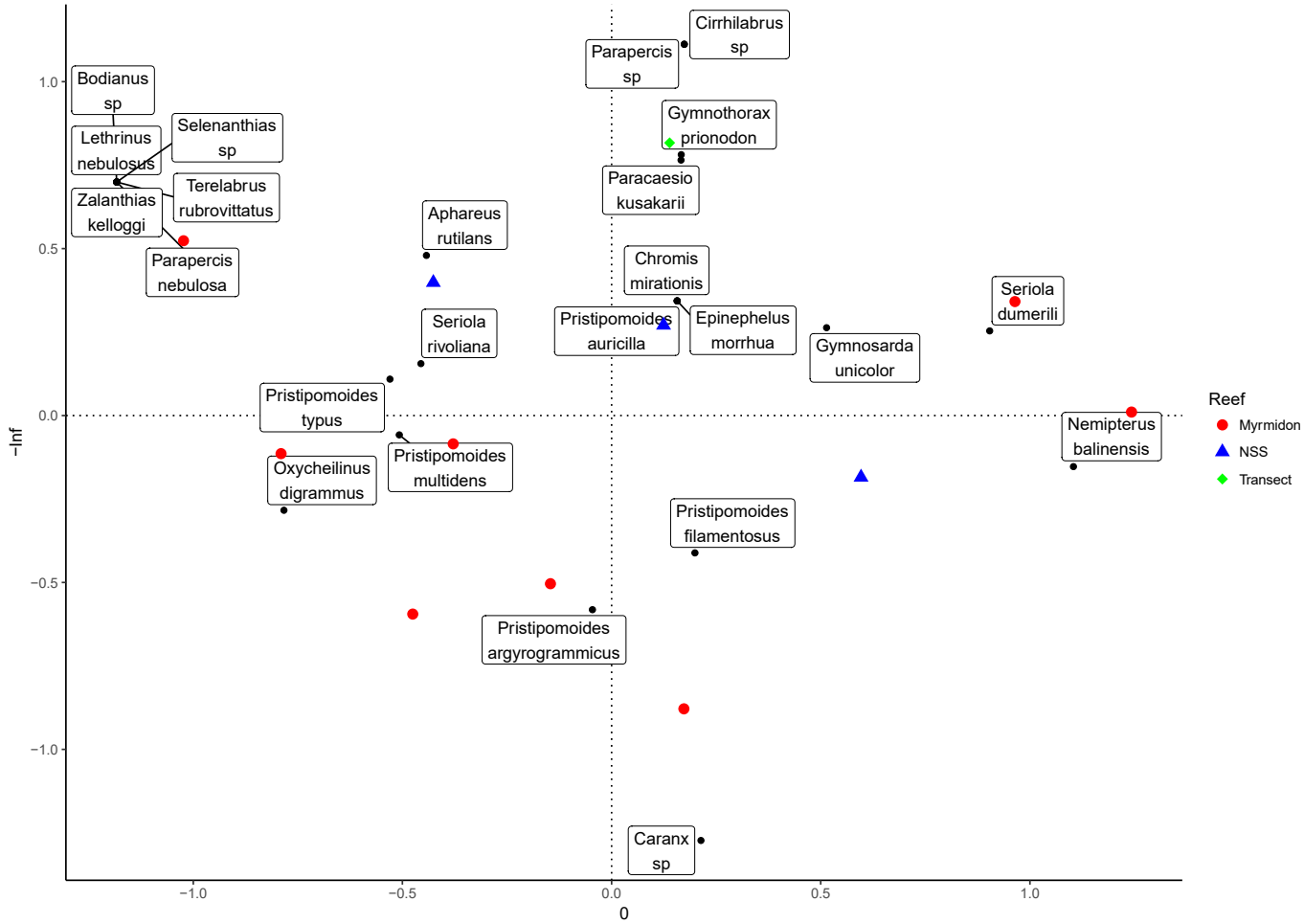
Appendix Figure A3: Comparing average substratum habitat measures in the field-of-view by reef. Percent cover of each category is estimated out of a total sum of 100 for each site. Four locations (Myrmidon Reef, Northern Submerged Shoals, Viper Reef and an inter-reefal transect) are included in this comparison, but there were no 'Deep' sites at Viper Reef. Note: n varies per bar, 1-11 sites.



Appendix Figure A4: Nonmetric Multidimensional Scaling (nMDS) shows patterns between fish assemblage composition and environmental variables, including epibenthic and substratum measured in the underwater camera field-of-view and multibeam echo sounder measured variables for four locations along the Great Barrier Reef shelf-edge with the fish species responsible for the variation among locations. Shallower sites nMDS (54-115 m) with ordination from Bray-Curtis dissimilarities in species abundance data, transformed using fourth-root transformation and standardized using Wisconsin-double standardization.



Appendix Figure A5: Nonmetric Multidimensional Scaling (nMDS) shows patterns between fish assemblage composition and environmental variables, including epibenthic and substratum measured in the underwater camera field-of-view and multibeam echo sounder measured variables for four locations along the Great Barrier Reef shelf-edge with the fish species responsible for the variation among locations. Middle sites nMDS (128-160 m) with ordination from Bray-Curtis dissimilarities in species abundance data, transformed using fourth-root transformation and standardized using Wisconsin-double standardization.



Appendix Figure A6: Nonmetric Multidimensional Scaling (nMDS) shows patterns between fish assemblage composition and environmental variables, including epibenthic and substratum measured in the underwater camera field-of-view and multibeam echo sounder measured variables for four locations along the Great Barrier Reef shelf-edge with the fish species responsible for the variation among locations. Deeper sites nMDS (179-260 m) with ordination from Bray-Curtis dissimilarities in species abundance data, transformed using fourth-root transformation and standardized using Wisconsin-double standardization.

Appendix Table A1: Ecology of deep-reef fishes from Baited Remote Underwater Video Station videos. Only species identified to species-level are included, listed by family. CAAB codes refer to the Codes for Australian Aquatic Biota *(Rees et al. 1999)*. Australian Standard Names are provided, where there is no Australian Standard Name, the Fishbase common name is provided and noted with *. Trophic information links from Fishbase database *(Froese & Pauly 2018)*. Trophic groups are divided as follows: BC = benthic-associated carnivores (e.g. benthic crustaceans and infauna, small fish may be a portion of diet), PL = planktivore, H=herbivore, PI= Piscivore, GC = Generalist carnivore (i.e. larger range of diet items, may include fish and benthic crustaceans). For some species, the trophic group of a species is inferred based on the known diet of close family members**. Fisheries designation is also from Fishbase. Fisheries status includes major or minor commercial, recreational or aquarium trade fisheries as these may pose a threat to general or local populations.

Species	CAAB code	Australian standard name	Trophic group	Fishbase trophic and habitat information	Fisheries
Carcharhinidae					
***Carcharhinus albimarginatus* (Rüppell, 1837)**	37018027	Silvertip Shark	GC	Fishbase/carcharhinus-albimarginatus	Yes
***Carcharhinus amblyrhynchos* (Bleeker, 1856)**	37018030	Grey Reef Shark	GC	Fishbase/Carcharhinus-amblyrhynchos	Yes
***Carcharhinus plumbeus* (Nardo, 1827)**	37018007	Sandbar Shark	GC	Fishbase/Carcharhinus-plumbeus	Yes
***Loxodon macrorhinus* Müller & Henle, 1839**	37018005	Sliteye Shark	GC	Fishbase/loxodon-macrorhinus	Yes
***Triaenodon obesus* (Rüppell, 1837)**	37018038	Whitetip Reef Shark	GC	Fishbase/triaenodon-obesus	Yes
Sphyrnidae					
***Sphyrna lewini* (Griffith & Smith, 1834)**	37019001	Scalloped Hammerhead	GC	Fishbase/Sphyrna-lewini	Yes
Dasyatidae					
***Taeniurops meyeni* (Müller & Henle, 1841)**	37035017	Blotched Fantail Ray	BC	Fishbase/taeniurops-meyeni	Yes
Muraenidae					
***Gymnothorax berndti* Snyder, 1904**	37060089	Y-Patterned Moray*	GC**	Fishbase/gymnothorax-berndti	
***Gymnothorax elegans* Bliss, 1883**	37060090	Elegant Moray*	GC**	Fishbase/gymnothorax-elegans	
***Gymnothorax intesi* (Fourmanoir & Rivaton, 1979)**	37060076	Whitetip Moray	GC**	Fishbase/gymnothorax-intesi	

Species	CAAB code	Australian standard name	Trophic group	Fishbase trophic and habitat information	Fisheries
Gymnothorax prionodon **Ogilby, 1895**	37060049	Sawtooth Moray	GC**	Fishbase/gymnothorax-prionodon	
Fistulariidae					
Fistularia commersonii **Rüppell, 1838**	37278001	Smooth Flutemouth	BC	Fishbase/fistularia-commersonii	Yes
Serranidae					
Epinephelus cyanopodus **(Richardson, 1846)**	37311145	Purple Rockcod	GC	Fishbase/epinephelus-cyanopodus	Yes
Epinephelus morrhua **(Valenciennes, 1833)**	37311151	Comet Grouper	GC	Fishbase/epinephelus-morrhua	Yes
Plectranthias kelloggi **Jordan & Evermann, 1903**	37311210	Eastern Flower Porgy*	Unknown	Fishbase/plectranthias-kelloggi	
Plectropomus leopardus **(Lacépède, 1802)**	37311078	Common Coral Trout	PI	Fishbase/plectropomus-leopardus	Yes
Plectropomus laevis **(Lacépède, 1801)**	37311079	Bluespotted Coral Trout	PI	Fishbase/plectropomus-laevis	Yes
Pseudanthias engelhardi **(Allen & Starck, 1982)**	37311115	Barrier Reef Basslet	Unknown	Fishbase/pseudanthias-engelhardi	
Variola louti **(Forsskål, 1775)**	37311166	Yellowedge Coronation Trout	GC	Fishbase/variola-louti	Yes
Malacanthidae					
Hoplolatilus marcosi **Burgess, 1978**	37331012	Redback Sand Tilefish*	BC**	Fishbase/hoplolatilus-marcosi	
Echeneidae					
Echeneis naucrates **Linnaeus, 1758**	37336001	Sharksucker	PI	Fishbase/echeneis-naucrates	Yes
Carangidae					
Carangoides caeruleopinnatus **(Rüppell, 1830)**	37337021	Onion Trevally	PI	Fishbase/carangoides-caeruleopinnatus	Yes
Carangoides chrysophrys **(Cuvier, 1833)**	37337011	Longnose Trevally	PI	Fishbase/carangoides-chrysophrys	Yes

Species	CAAB code	Australian standard name	Trophic group	Fishbase trophic and habitat information	Fisheries
Carangoides dinema **Bleeker 1851**	37337078	Shadow Trevally	PI	Fishbase/carangoides-dinema	Yes
Carangoides ferdau **(Forsskål, 1775)**	37337068	Blue Trevally	BC	Fishbase/carangoides-ferdau	Yes
Carangoides fulvoguttatus **(Forsskål, 1775)**	37337037	Turrum	BC	Fishbase/carangoides-fulvoguttatus	Yes
Carangoides orthogrammus **(Jordan & Gilbert, 1882)**	37337057	Thicklip Trevally	BC	Fishbase/carangoides-orthogrammus	Yes
Carangoides plagiotaenia **Bleeker, 1857**	37337070	Barcheek Trevally	GC	Fishbase/carangoides-plagiotaenia	Yes
Caranx ignobilis **(Forsskål, 1775)**	37337027	Giant Trevally	GC	Fishbase/caranx-ignobilis	Yes
Caranx melampygus **Cuvier, 1833**	37337050	Bluefin Trevally	GC	Fishbase/caranx-melampygus	Yes
Gnathanodon speciosus **(Forsskål, 1775)**	37337012	Golden Trevally	PI	Fishbase/gnathanodon-speciosus	Yes
Pseudocaranx dentex **(Bloch & Schneider, 1801)**	37337062	Silver Trevally	PL, BC	Fishbase/pseudocaranx-dentex	Yes
Seriola dumerili **(Risso, 1810)**	37337025	Amberjack	GC	Fishbase/seriola-dumerili	Yes
Seriola rivoliana **Valenciennes, 1833**	37337052	Highfin Amberjack	GC	Fishbase/seriola-rivoliana	Yes
Lutjanidae					
Aphareus rutilans **Cuvier, 1830**	37346001	Rusty Jobfish	GC	Fishbase/aphareus-rutilans	Yes
Aprion virescens **Valenciennes, 1830**	37346027	Green Jobfish	GC	Fishbase/aprion-virescens	Yes
Etelis carbunculus **Cuvier, 1828**	37346014	Ruby Snapper	GC	Fishbase/etelis-carbunculus	Yes
Lipocheilus carnolabrum **(Chan, 1970)**	37346031	Tang's Snapper	GC	Fishbase/lipocheilus-carnolabrum	Yes

Species	CAAB code	Australian standard name	Trophic group	Fishbase trophic and habitat information	Fisheries
Lutjanus bohar **(Forsskål, 1775)**	37346029	Red Bass	GC	Fishbase/lutjanus-bohar	Yes
Lutjanus sebae **(Cuvier, 1816)**	37346004	Red Emperor	GC	Fishbase/lutjanus-sebae	Yes
Paracaesio kusakarii **Abe, 1960**	37346060	Saddleback Snapper	GC	Fishbase/paracaesio-kusakarii	Yes
Pristipomoides argyrogrammicus **(Valenciennes, 1831)**	37346054	Ornate Jobfish	GC	Fishbase/pristipomoides-argyrogrammicus	Yes
Pristipomoides auricilla **(Jordan, Evermann & Tanaka, 1927)**	37346059	Goldflag Snapper	PI, PL	Fishbase/pristipomoides-auricilla	Yes
Pristipomoides filamentosus **(Valenciennes, 1830)**	37346032	Rosy Snapper	GC	Fishbase/pristipomoides-filamentosus	Yes
Pristipomoides multidens **(Day, 1870)**	37346002	Goldbanded Snapper	GC	Fishbase/pristipomoides-multidens	Yes
Pristipomoides sieboldii **(Bleeker, 1857)**	37346064	Lavender Snapper	GC	Fishbase/pristipomoides-sieboldii	Yes
Pristipomoides typus **Bleeker, 1852**	37346019	Sharptooth Snapper	GC	Fishbase/pristipomoides-typus	Yes
Symphorus nematophorus **(Bleeker, 1860)**	37346017	Chinamanfish	PI	Fishbase/symphorus-nematophorus	Yes
Caesionidae					
Pterocaesio marri **Schultz, 1953**	37346068	Bigtail Fusilier	PL	Fishbase/pterocaesio-marri	Yes
Nemipteridae					
Nemipterus balinensis **(Bleeker, 1859)**	37347039	Bali Threadfin Bream	BC**	Fishbase/nemipterus-balinensis	Yes
Pentapodus aureofasciatus **Russell, 2001**	37347029	Yellowstripe Threadfin Bream	BC**	Fishbase/pentapodus-aureofasciatus	
Pentapodus nagasakiensis **(Tanaka, 1915)**	37347012	Japanese Threadfin Bream	BC	Fishbase/pentapodus-nagasakiensis	Yes

Species	CAAB code	Australian standard name	Trophic group	Fishbase trophic and habitat information	Fisheries
Lethrinidae					
Gymnocranius euanus **(Günther, 1879)**	37351022	Paddletail Seabream	BC	Fishbase/gymnocranius-euanus	Yes
Gymnocranius grandoculis **(Valenciennes, 1830)**	37351005	Robinson's Seabream	GC	Fishbase/gymnocranius-grandoculis	Yes
Lethrinus laticaudis **Alleyne & Macleay, 1877**	37351006	Grass Emperor	GC	Fishbase/lethrinus-laticaudis	Yes
Lethrinus miniatus **(Forster, 1801)**	37351009	Redthroat Emperor	GC	Fishbase/lethrinus-miniatus	Yes
Lethrinus nebulosus **(Forsskål, 1775)**	37351008	Spangled Emperor	GC	Fishbase/lethrinus-nebulosus	Yes
Lethrinus olivaceus **Valenciennes, 1830**	37351004	Longnose Emperor	GC	Fishbase/lethrinus-olivaceus	
Lethrinus ravus **Carpenter & Randall, 2003**	37351031	Drab Emperor	Unknown	Fishbase/lethrinus-ravus	
Lethrinus rubrioperculatus **Sato, 1978**	37351012	Spotcheek Emperor	GC	Fishbase/lethrinus-rubrioperculatus	
Lethrinus semicinctus **Valenciennes, 1830**	37351016	Blackblotch Emperor	GC	Fishbase/lethrinus-semicinctus	Yes
Wattsia mossambica **(Smith, 1957)**	37351027	Mozambique Seabream	GC	Fishbase/wattsia-mossambica	Yes
Mullidae					
Mulloidichthys pfluegeri **(Steindachner, 1900)**	37355040	Orange Goatfish	BC	Fishbase/mulloidichthys-pfluegeri	Yes
Parupeneus heptacantha **(Lacépède, 1802)**	37355004	Cinnabar Goatfish	BC	Fishbase/parupeneus-heptacantha	Yes
Parupeneus multifasciatus **(Quoy & Gaimard, 1825)**	37355026	Banded Goatfish	BC	Fishbase/parupeneus-multifasciatus	Yes
Parupeneus pleurostigma **(Bennett, 1831)**	37355027	Sidespot Goatfish	BC	Fishbase/parupeneus-pleurostigma	Yes

Species	CAAB code	Australian standard name	Trophic group	Fishbase trophic and habitat information	Fisheries
Chaetodontidae					
Heniochus diphreutes **Jordan, 1903**	37365005	Schooling Bannerfish	PL	Fishbase/heniochus-diphreutes	Aquarium
Pomacanthidae					
Pomacanthus imperator **(Bloch, 1787)**	37365014	Emperor Angelfish	BC	Fishbase/pomacanthus-imperator	Yes & Aquarium
Pomacanthus semicirculatus **(Cuvier, 1831)**	37365080	Blue Angelfish	BC	Fishbase/pomacanthus-semicirculatus	Yes & Aquarium
Cirrhitidae					
Cyprinocirrhites polyactis **(Bleeker, 1875)**	37374006	Lyretail Hawkfish	PL	Fishbase/cyprinocirrhites-polyactis	Aquarium
Pomacentridae					
Chromis circumaurea **Pyle, Earle & Greene, 2008**	37372153	Gold-rim Chromis*	PL**	Fishbase/chromis-circumaurea	
Chromis mirationis **Tanaka 1917**	37372048	Japanese Puller	PL	Fishbase/chromis-mirationis	
Chromis okamurai **Yamakawa & Randall, 1989**	37372154	Okinawa Chromis*	PL**	Fishbase/chromis-okamurai	
Labridae					
Bodianus anthioides **(Bennett, 1832)**	37384052	Lyretail Pigfish	BC	Fishbase/bodianus-anthioides	Aquarium
Bodianus bennetti **Gomon and Walsh, 2016**	37384219	Lemon-striped Pygmy Hogfish	BC**	Fishbase/bodianus-bennetti	
Bodianus bimaculatus **Allen, 1973**	37384055	Twospot Pigfish	BC	Fishbase/bodianus-bimaculatus	Aquarium
Bodianus izuensis **Araga & Yoshino, 1975**	37384058	Striped Pigfish	BC	Fishbase/bodianus-izuensis	Aquarium
Bodianus masudai **Araga & Yoshino, 1975**	37384221		BC	Fishbase/bodianus-masudai	
Cheilinus undulatus **Rüppell, 1835**	37384038	Humphead Maori Wrasse	GC	Fishbase/cheilinus-undulatus	Yes & Aquarium

Species	CAAB code	Australian standard name	Trophic group	Fishbase trophic and habitat information	Fisheries
Choerodon venustus **(De Vis, 1884)**	37384042	Venus Tuskfish	Unknown	Fishbase/choerodon-venustus	
Cirrhilabrus punctatus **Randall & Kuiter, 1989**	37384083	Finespot Wrasse	Unknown	Fishbase/cirrhilabrus-punctatus	Aquarium
Cirrhilabrus roseafascia **Randall & Lubbock, 1982**	37384218	Pink-Banded Fairy Wrasse*	Unknown	Fishbase/cirrhilabrus-roseafascia	Aquarium
Coris dorsomacula **Fowler, 1908**	37384093	Pinklined Wrasse	Unknown	Fishbase/coris-dorsomacula	Aquarium
Labroides dimidiatus **(Valenciennes, 1839)**	37384028	Common Cleanerfish	GC	Fishbase/labroides-dimidiatus	Aquarium
Oxycheilinus digrammus **(Lacépède, 1801)**	37384065	Violetline Maori Wrasse	BC	Fishbase/oxycheilinus-digrammus	Yes & Aquarium
Oxycheilinus orientalis **Günther, 1862**	37384030	Oriental Maori Wrasse	GC	Fishbase/oxycheilinus-orientalis	Yes & Aquarium
Terelabrus rubrovittatus **Randall & Fourmanoir, 1998**	37384210	Yellowbar Hogfish*	Unknown	Fishbase/terelabrus-rubrovittatus	
Pinguipedidae					
Parapercis nebulosa **(Quoy & Gaimard, 1825)**	37390005	Pinkbanded Grubfish	BC	Fishbase/parapercis-nebulosa	Aquarium
Blenniidae					
Meiacanthus luteus **Smith-Vaniz, 1987**	37408054	Yellow Fangblenny	Unknown	Fishbase/meiacanthus-luteus	
Acanthuridae					
Acanthurus xanthopterus **Valenciennes, 1835**	37437020	Yellowmask Surgeonfish	H, PL	Fishbase/acanthurus-xanthopterus	Yes & Aquarium
Naso caesius **Randall & Bell, 1992**	37437046	Silverblotched Unicornfish	PL	Fishbase/naso-caesius	
Scombridae					
Gymnosarda unicolor **(Rüppell, 1836)**	37441029	Dogtooth Tuna	PI	Fishbase/gymnosarda-unicolor	Yes

Species	CAAB code	Australian standard name	Trophic group	Fishbase trophic and habitat information	Fisheries
Scomberomorus commerson **(Lacépède, 1800)**	37441007	Spanish Mackerel	PI	Fishbase/scomberomorus-commerson	Yes
Balistidae					
Abalistes stellatus **(Anonymous, 1798)**	37465011	Starry Triggerfish	Unknown	Fishbase/abalistes-stellatus	
Balistoides conspicillum **(Bloch & Schneider, 1801)**	37465031	Clown Triggerfish	BC	Fishbase/balistoides-conspicillum	Yes
Sufflamen bursa **(Bloch & Schneider, 1801)**	37465078	Pallid Triggerfish	BC	Fishbase/sufflamen-bursa	Yes
Sufflamen fraenatum **(Latreille, 1804)**	37465014	Bridled Triggerfish	BC	Fishbase/sufflamen-fraenatum	Yes
Tetraodontidae					
Trionodon macropterus **Lesson, 1831**	37991885	Threetooth Puffer*	PL, BC	Fishbase/trionodon-macropterus	

Appendix Table A2: Each environmental variable was compared in a simple linear model to principal components to see how different habitat gradients could explain the variation in the two major components (PC1 and PC2) between the trophic assemblages. Residual standard error is a measure of the quality of a linear regression fit and reported for a model with 45 degrees of freedom. For planar curvature, absolute values (denoted "abs") were compared. Significance values are expressed as * (** $p < 0.01$ and * $p < 0.05$). Non-significant variables (not listed in table) included curvature, range, topographic position index, epibenthic categories (filter feeders, encrusting organisms, coral, Halimeda) and substratum categories (boulders, gravel, indeterminate substratum, mud, rubble).

Environmental variable		Residual Standard Error	R^2	p-value	Residual Standard Error	R^2	p-value
depth (category)	Deep	0.55	0.20	NS			
	Middle			NS			
	Shallow			**			
reef (category)	Myrmidon	0.61	0.04	NS	0.61	0.02	NS
	Transect			NS			NS
	Northern Submerged Shoals			NS			NS
	Viper			NS			NS
		PC1			**PC2**		
depth		0.54	0.20	**	0.60	0.04	NS
aspect50		0.58	0.09	*	0.56	0.01	NS
ave50		0.57	0.13	*	0.54	0.08	NS
plancabs		0.58	0.09	*	0.56	0.02	NS
slope50		0.60	0.03	NS	0.52	0.13	*
std50		0.60	0.07	NS	0.53	0.09	*
surfrat50		0.57	0.10	*	0.54	0.09	NS
bare		0.55	0.19	**	0.61	0.00	NS
plants		0.55	0.18	**	0.60	0.03	NS
bdrck		0.56	0.15	**	0.59	0.05	NS
calc.rf		0.58	0.09	*	0.61	0.00	NS
snd		0.57	0.12	*	0.60	0.03	NS

Appendix B: Supplementary Figures and Tables for Chapter 4

Appendix Table B1: Boosted Regression Tree parameters, goodness-of-fit (R^2), and the relative influence of environmental variables on standardized species abundance and species richness. Each model was run three times and the range of values is reported. Relative influence of a variable is the percentage of trees where that variable was influential in splits (only variables with a relative influence of >5% are reported). Four spatial scales were analysed separately (5x5, 10x10, 25x25, 50x50) with multiple types of environmental information (spatial, rugosity, substratum and biotic) and levels of increasing complexity (only interaction depths=1-5 are reported, cross-validation folds=5). Abbreviations of variables follow Table 1.

Response	Spatial scale	Interaction depth	Learning rate	# of trees	Type 1 Spatial		Type 2 Spatial + Rugosity		Type 3 Spatial + Rugosity + Substratum		Type 4 Spatial + Rugosity + Biotic		Full model Spatial + Rugosity + Substratum + Biotic	
					R^2	Relative influence of variables	R^2	Relative influence of variables	R^2	Relative influence of variables	R^2	Relative influence of variables	R^2	Relative influence of variables
Species Abundance	5 x 5	Int=1	0.01	1000	5.6-12.0	depth 50.6-66.4 latitude 15.7-26.1 east5 6.1-17.8 north5 2.9-5.5 longitude 0.0-8.9	3.2-7.3	depth 50.5-60.1 latitude 12.6-20.3 longitude 0.0-13.9 tpi5 3.6-10.9 slope5 5.0-6.4	11.0-16.8	calc.rf 27.5-32.7 depth 16.5-20.6 bs_ave5 9.0-13.1 snd 7.7-12.4 latitude 2.6-9.8 bldr 4.2-6.4 slope5 1.3-6.2	23.0-27.1	encr 37.4-41.8 depth 15.7-20.5 bare 12.0-13.5 latitude 8.1-11.2 tpi5 5.9-6.8	23.9-29.7	encr 25.8-29.4 calc.rf 11.3-17.9 depth 8.3-11.6 bs_ave5 2.6-8.9 bare 5.0-8.6 snd 4.4-7.4 bldr 2.6-7.3 tpi5 2.2-7.0 latitude 3.0-6.3 fltrs 1.5-5.5
		Int=3	0.005	2000	6.5-11.6	depth 39.9-51.4 latitude 20.5-23.5 north5 13.4-15.3 east5 8.5-11.6 longitude 4.4-9.8	6.9-12.0	depth 31.7-36.6 latitude 11.6-15.5 slope5 9.2-13.8 tpi5 8.4-15.6 north5 5.8-11.0 east5 4.1-10.1 planz5 7.2-9.2 longitude 2.4-5.4	18.9-22.5	calc.rf 18.8-23.5 depth 18.0-18.5 bs_ave5 9.6-11.5 latitude 7.4-10.6 tpi5 5.4-8.2 snd 4.5-8.2 planz5 4.1-6.0	36.3-37.2	encr 27.3-28.7 depth 14.3-16.6 tpi5 9.8-11.1 latitude 8.1-8.7 bare 6.2-7.5 longitude 5.7-7.5 east5 4.9-5.9 fltrs 4.3-5.2 north5 4.2-5.7 slope5 3.7-5.8	33.8-36.3	encr 18.7-20.9 depth 11.5-12.7 calc.rf 10.7-13.9 tpi5 6.1-9.3 bs_ave5 6.0-7.9 latitude 4.7-6.6

		Int=5	0.0025	5000	10.6-14.2	depth 40.6-43.8 latitude 18.0-20.9 east5 11.8-14.5 longitude 11.3-13.9 north5 11.9-13.0	10.2-12.9	depth 33.3-34.4 latitude 11.6-14.4 planz5 9.2-11.4 tpi5 9.3-11.3 east5 6.9-9.2 slope5 7.6-11.1 north5 7.6-8.6 longitude 6.0-7.3	19.3-23.0	Calc.rf 19.4-21.7 depth 15.4-18.9 bs_ave5 9.8-12.6 latitude 6.7-8.1 planz5 5.2-6.7 tpi5 6.2-6.5 snd 4.4-5.6 bs_sd5 3.3-5.1	38.0-41.3	encr 27.2-28.6 depth 13.5-14.6 tpi5 9.3-10.6 latitude 7.8-9.8 longitude 6.3-7.6 bare 5.9-6.8 north5 5.5-6.2 east5 5.2-5.9 planz5 4.3-5.5	36.9-38.6	encr 18.0-20.5 depth 10.6-11.5 calc.rf 9.6-12.3 tpi5 6.4-7.5 bs_ave5 6.7-7.2 latitude 4.8-5.8
	10 x 10	Int=1	0.01	1000	9.3-11.2	depth 47.6-51.0 east10 17.1-21.9 latitude 12.9-25.3 longitude 2.8-9.7 north10 4.4-8.1	4.7-12.9	depth 37.8-51.0 latitude 15.6-18.6 east10 7.2-14.0 tpi10 0.0-13.8 longitude 2.6-7.1	14.2-17.8	calc.rf 20.8-33.7 depth 16.7-20.4 bs_ave10 10.8-16.1 snd 8.5-13.9 slope10 5.8-9.3 east10 1.6-5.8 tpi10 0.5-7.9	18.9-30.3	encr 40.4-41.3 depth 14.7-19.2 bare 8.0-13.3 slope10 2.7-7.4 tpi10 4.8-8.2 latitude 4.1-10.4 east10 2.5-6.2 longitude 0.7-5.7	23.1-27.7	encr 21.8-22.6 calc.rf 16.7-18.7 depth 7.2-12.8 bs_ave10 10.1-13.0 latitude 4.6-8.8 east10 4.6-7.1 bare 4.6-6.3
		Int=3	0.005	2000	16.8-20.0	depth 35.9-39.5 east10 22.6-25.1 latitude 15.6-16.5 north10 10.3-13.4 longitude 8.1-10.3	16.0-21.5	depth 27.3-33.1 east10 9.9-16.4 latitude 12.8-13.8 tpi10 11.8-12.4 slope10 10.8-11.7 planz10 8.1-11.5 north10 6.1-7.3 longitude 4.2-5.1	24.3-26.3	calc.rf 16.0-20.5 depth 13.8-15.4 bs_ave10 11.4-14.6 tpi10 6.5-8.0 east10 6.2-7.4 planz10 5.5-7.9 latitude 6.3-6.9 slope10 4.9-8.8 snd 4.6-8.1	38.9-39.8	encr 23.9-27.1 depth 15.1-16.3 tpi10 9.2-9.7 latitude 8.4-9.0 east10 6.9-8.2 bare 6.1-7.5 planz10 5.7-6.6 slope10 5.8-6.2 longitude 5.6-6.1	36.0-38.6	encr 16.4-18.4 calc.rf 11.5-12.4 depth 10.2-12.4 bs_ave10 8.6-9.9 tpi10 4.8-6.7 east10 4.5-6.3 latitude 3.9-6.4 planz10 4.0-5.3 bare 3.1-5.3
		Int=5	0.0025	5000	17.6-22.9	depth 33.2-39.5 east10 20.6-23.7 latitude 17.0-19.3 north10 12.4-14.0 longitude 9.7-11.1	19.2-24.9	depth 27.4-29.6 east10 12.0-13.7 slope10 11.6-12.8 tpi10 11.6-12.7 latitude 10.9-12.2 planz10 9.0-10.6 north10 6.6-7.3 longitude 6.0-6.6	27.0-28.2	calc.rf 18.9-20.0 depth 14.0-16.2 bs_ave10 12.0-12.9 east10 6.5-8.0 latitude 6.5-7.0 tpi10 6.3-7.1 planz10 5.4-6.9 slope10 5.0-5.8 snd 5.2-5.6	39.8-43.9	encr 24.4-26.9 depth 13.6-15.2 east10 8.2-9.8 tpi10 7.8-9.0 latitude 7.2-8.8 longitude 6.2-7.7 planz10 5.8-6.6 bare 5.5-7.9 slope10 5.5-6.6	37.4-39.6	encr 18.1-20.4 depth 12.0-12.3 calc.rf 10.1-10.3 bs_ave10 8.8-9.4 east10 5.4-6.2 latitude 5.2-6.4 tpi10 5.0-5.7 planz10 4.4-5.1
	25 x 25	Int=1	0.01	1000	8.7-12.5	depth 50.8-55.8 latitude 14.6-17.3 north25 5.1-14.4 east25 10.7-16.3 longitude 1.3-9.7	4.2-9.9	depth 38.0-72.8 latitude 10.4-18.2 north25 4.0-12.0 east25 3.3-10.9 slope25 5.5-17.2 planz25 2.1-6.4 tpi25 0.6-6.0	14.5-20.5	calc.rf 34.4-46.8 depth 14.8-18.1 snd 7.6-13.6 latitude 3.8-6.3 bs_ave25 1.5-7.2 slope25 2.8-6.9 tpi25 1.2-5.2 planz25 2.1-6.7	22.7-27.2	encr 35.9-41.4 depth 15.4-19.0 latitude 8.0-9.8 bare 7.3-14.2 east25 2.6-7.8 slope25 3.0-6.0 tpi25 2.0-6.7 fltrs 2.2-6.4	19.7-27.7	encr 28.4-32.6 calc.rf 15.8-16.6 depth 10.9-14.8 bare 5.2-11.9 bs_ave25 2.5-6.2 snd 5.4-8.8 latitude 2.6-6.2 planz25 0.0-6.3

		Int=3	0.005	2000	13.0-16.4	depth 38.5-44.6 east25 10.2-20.2 latitude 19.7-20.1 north25 12.6-15.7 longitude 8.3-11.9	12.6-15.7	depth 32.5-36.6 latitude 11.9-15.7 planz25 9.9-12.7 tpi25 9.5-10.4 north25 9.3-10.9 slope25 7.3-10.3 east25 7.2-9.4 longitude 2.9-6.2	24.2-26.4	calc.rf 21.9-23.2 depth 14.0-17.6 bs_ave25 10.9-13.1 planz25 7.3-9.8 latitude 6.0-7.8 snd 5.1-6.9 tpi25 4.2-5.8 north25 4.0-5.6	37.0-40.8	encr 27.0-29.1 depth 14.1-15.4 latitude 8.6-10.1 planz25 7.7-8.6 bare 7.1-7.8 tpi25 6.0-7.8 longitude 5.2-6.7 east25 4.9-5.9 slope25 3.6-5.1	36.8-39.7	encr 19.2-20.1 depth 11.7-13.4 calc.rf 10.9-11.8 latitude 5.2-8.3 bs_ave25 6.8-7.9 planz25 6.4-6.5
		Int=5	0.0025	5000	16.4-19.0	depth 37.0-39.7 east25 15.8-19.3 latitude 18.1-21.0 north25 12.0-15.1 longitude 9.9-11.5	14.2-18.7	depth 31.5-34.6 latitude 11.3-13.7 planz25 10.2-12.5 east25 8.9-11.6 slope25 7.6-11.4 tpi25 9.1-10.5 north25 6.7-8.6 longitude 5.4-6.6	27.1-29.1	calc.rf 20.0-20.4 depth 15.2-17.9 bs_ave25 10.4-11.1 planz25 7.3-8.1 latitude 7.3-8.0 tpi25 5.1-5.8 slope25 4.5-5.7 east25 4.7-5.1 north25 3.9-5.2 snd 3.0-5.1	41.2-43.0	encr 25.6-27.2 depth 14.6-15.5 latitude 7.8-9.0 planz25 7.7-8.8 bare 6.7-8.2 longitude 6.8-7.5 tpi25 6.7-7.5 east25 5.4-6.7 north25 4.6-6.4 slope25 4.3-5.6	38.9-40.6	encr 17.5-19.1 depth 11.9-12.9 calc.rf 10.3-12.2 bs_ave25 7.1-7.6 planz25 6.6-6.7 latitude 5.4-6.0 bare 3.9-5.1
	50 x 50	Int=1	0.01	1000	9.4-11.9	depth 46.6-65.1 latitude 8.3-29.8 north50 6.0-15.2 east50 5.3-11.3 longitude 3.2-9.5	5.4-9.4	depth 40.8-64.6 latitude 9.8-25.6 longitude 0.0-12.4 tpi50 0.0-10.5 planz50 0.0-8.4 north50 5.9-8.2	16.9-20.0	calc.rf 34.2-39.5 depth 16.3-20.2 snd 4.5-13.5 planz50 4.2-8.3 latitude 4.3-7.6 east50 1.4-5.1	22.9-26.9	encr 36.5-44.6 depth 14.4-16.6 bare 10.8-16.9 latitude 6.4-8.2 planz50 2.1-8.4 north50 4.4-6.4 tpi50 2.9-5.1 east50 1.6-5.7	23.9-30.8	encr 25.8-38.0 calc.rf 15.3-21.1 depth 10.9-12.8 snd 4.6-6.9 bare 3.1-6.0 east50 1.8-5.1
		Int=3	0.005	2000	10.2-13.3	depth 41.8-46.7 latitude 16.7-19.5 north50 14.0-15.5 east50 10.4-13.8 longitude 8.4-12.7	9.2-15.3	depth 32.9-40.6 latitude 12.4-18.3 planz50 9.1-14.7 tpi50 7.7-14.2 north50 7.6-11.1 slope50 5.2-8.8 east50 4.2-8.7 longitude 4.4-6.1	24.4-26.9	calc.rf 22.6-25.4 depth 15.3-18.7 planz50 10.4-11.2 latitude 6.5-10.1 snd 4.3-7.0 tpi50 4.8-6.2 north50 5.0-5.4	37.3-40.1	encr 27.1-28.6 depth 14.6-16.5 latitude 6.4-8.9 planz50 7.5-10.5 longitude 5.7-7.4 north50 5.9-6.8 bare 6.2-8.0 tpi50 6.1-7.3 east50 3.5-5.3	35.3-39.2	encr 19.4-21.7 calc.rf 12.2-16.0 depth 11.3-13.6 planz50 8.1-8.5 bare 2.9-6.1 north50 4.6-5.1 snd 3.9-5.5

		Int=5	0.0025	5000	12.9-14.9	depth 39.6-41.4 latitude 17.5-20.6 north50 14.7-16.7 longitude 12.0-14.3 east50 10.6-11.9	11.1-15.2	depth 33.0-36.7 latitude 10.8-14.6 planz50 9.9-14.4 tpi50 9.2-11.8 north50 7.4-10.7 slope50 5.3-9.5 east50 5.2-8.8 longitude 4.8-6.1	24.9-28.1	calc.rf 21.6-24.9 depth 15.8-18.1 planz50 10.0-11.8 tpi50 5.3-6.8 latitude 5.9-7.6 snd 4.4-6.5 north50 4.9-5.9 longitude 4.0-5.1	43.4-44.6	encr 26.6-28.1 depth 14.2-15.4 planz50 9.7-10.6 latitude 7.5-7.7 longitude 7.0-7.2 north50 5.9-6.8 tpi50 6.3-7.5 bare 6.1-6.9 east50 4.1-5.6	38.2-41.4	encr 19.1-20.9 calc.rf 11.2-13.0 depth 11.4-12.1 planz50 8.2-8.5 latitude 5.1-6.6 north50 4.5-5.5
Species Richness	5 x 5	Int=1	0.01	1000	11.9-14.1	depth 57.5-73.6 east5 7.8-22.0 latitude 1.0-13.2 north5 2.5-7.3 longitude 2.0-6.6	5.9-12.4	depth 57.5-64.5 slope5 0.0-16.3 planz5 1.5-11.5 tpi5 5.7-7.6 east5 3.7-12.9 latitude 2.9-8.4	28.1-30.4	calc.rf 28.0-31.4 depth 13.9-17.9 bs_ave5 11.6-13.9 bldr 7.8-11.2 slope5 3.5-13.4 tpi5 3.3-8.9 east5 3.5-7.3	30.7-34.7	encr 22.3-26.2 depth 15.6-21.5 bare 13.5-17.2 fltrs 8.6-10.4 slope5 7.3-11.5 tpi5 6.7-8.9 planz5 3.2-5.8 east5 1.8-6.2 latitude 1.8-5.3	30.7-40.1	encr 16.9-19.5 calc.rf 13.7-17.5 depth 11.0-14.9 bs_ave5 8.6-11.6 bldr 4.6-10.6 bare 4.1-8.5 tpi5 3.5-7.4 planz5 3.2-5.1 east5 2.6-6.3 fltrs 1.7-5.5 slope5 0.7-8.7 snd 0.0-5.6
		Int=3	0.005	2000	13.2-24.2	depth 46.9-52.5 east5 16.5-18.1 latitude 14.0-19.2 north5 7.0-10.4 longitude 6.2-8.6	21.8-25.3	depth 32.7-33.4 east5 16.2-17.4 slope5 11.5-14.4 latitude 10.3-11.4 tpi5 8.8-11.3 planz5 7.1-8.2	37.8-49.4	calc.rf 20.2-22.7 depth 16.2-18.6 bs_ave5 10.7-13.4 east5 7.8-8.7 bldr 5.7-7.7 latitude 5.8-7.5 slope5 5.7-6.2 planz5 4.1-6.4 tpi5 5.0-5.4	36.8-57.7	depth 19.6-21.5 encr 14.6-19.5 bare 7.5-13.6 east5 6.8-12.0 tpi5 7.3-10.6 slope5 6.8-8.4 fltrs 6.2-7.8 latitude 4.3-6.5 planz5 3.8-5.6	53.1-60.8	depth 11.6-15.3 encr 9.9-13.4 calc.rf 10.1-12.1 bs_ave5 7.4-10.0 bldr 6.2-7.5 east5 6.3-7.2 bare 4.4-6.7 tpi5 4.8-6.4 slope5 4.9-6.0 fltrs 3.5-5.1
		Int=5	0.0025	5000	20.2-29.1	depth 43.5-46.9 east5 18.7-20.8 latitude 15.2-16.9 longitude 7.8-10.0 north5 7.9-9.9	26.4-35.2	depth 29.1-31.4 east5 15.5-16.2 slope5 14.0-16.0 latitude 9.7-13.2 planz5 8.2-9.3 tpi5 6.9-8.6 north5 4.7-6.3 longitude 4.2-6.8	47.8-50.8	calc.rf 18.7-19.4 depth 14.9-17.3 east5 9.4-9.9 bs_ave5 9.5-9.8 slope5 6.8-8.0 latitude 6.8-7.0 planz5 5.6-6.7 bldr 5.2-6.6 tpi5 4.8-5.7	49.2-53.5	depth 19.1-21.3 encr 14.1-15.2 east5 9.6-13.0 bare 8.2-10.1 tpi5 7.7-9.0 slope5 7.5-8.7 latitude 6.3-7.9 fltrs 5.9-7.7 planz5 5.1-5.5	57.2-63.2	depth 12.9-13.1 calc.rf 11.2-11.3 encr 10.4-11.7 bs_ave5 5.9-7.9 bldr 5.9-6.9 tpi5 5.7-6.2 east5 6.1-7.5 latitude 5.6-5.9 slope5 5.3-6.2 planz5 4.1-5.2 bare 3.9-5.3

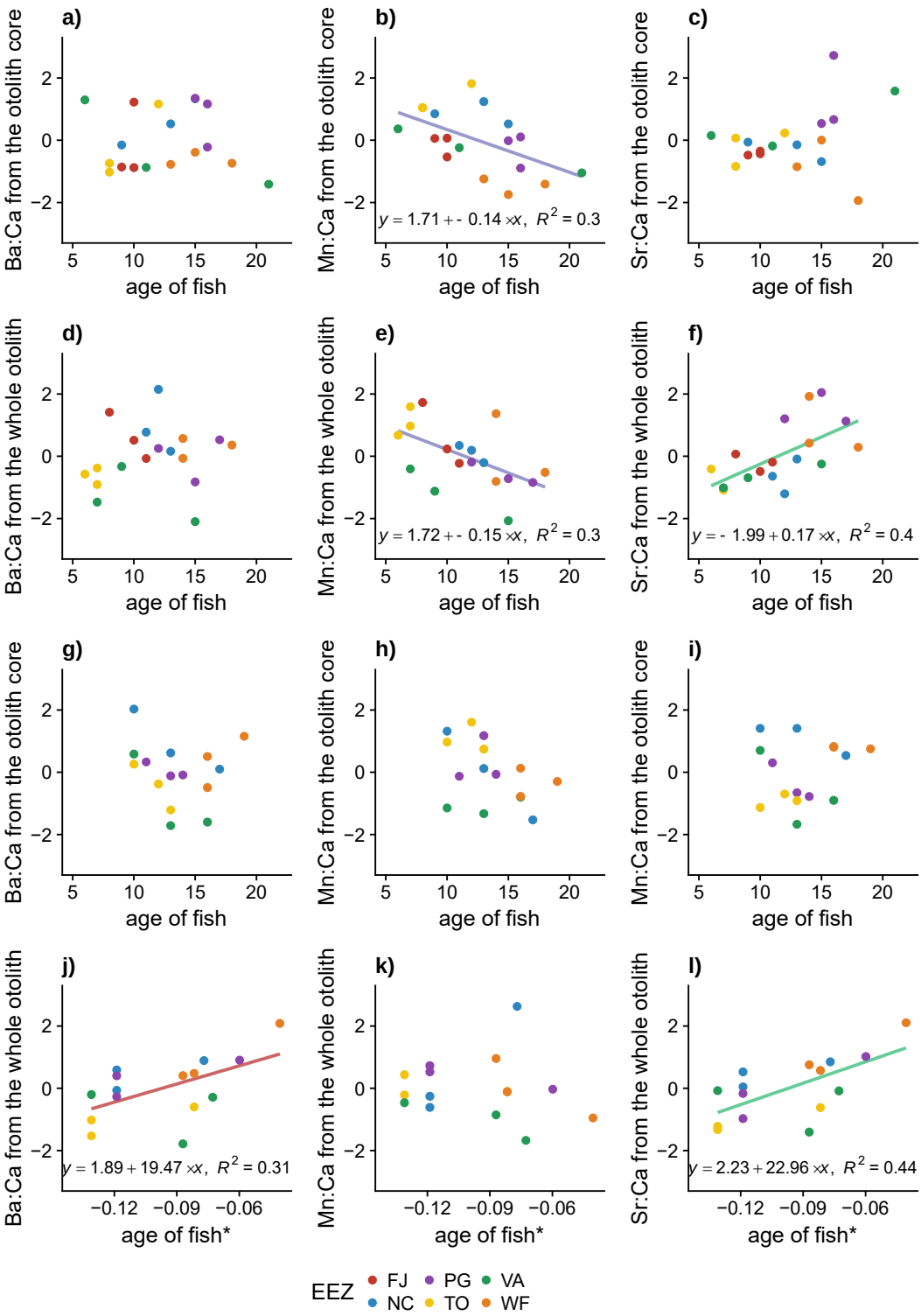
	10 x 10	Int=1	0.01	1000	10.9-13.9	depth 69.3-76.9 east10 9.4-19.1 north10 3.3-8.0 latitude 2.9-5.7	5.6-13.4	depth 60.3-83.5 tpi10 4.2-15.3 east10 0.0-12.9 latitude 0.0-8.7 slope10 0.0-6.1	12.7-19.1	calc.rf 32.3-43.4 depth 7.7-24.5 bs_ave10 15.9-20.0 tpi10 3.0-10.3 slope10 2.3-10.2 snd 3.1-7.7 planz10 0.0-7.4	26.6-33.4	depth 21.7-24.0 bare 12.5-22.1 encr 16.6-19.6 slope10 7.0-12.3 east10 9.2-10.4 tpi10 6.2-9.3 fltrs 4.6-8.4	32.8-35.9	encr 14.4-22.1 calc.rf 14.9-17.5 depth 13.0-16.5 bs_ave10 10.4-13.2 bare 5.9-11.1 bldr 5.3-7.9 fltrs 5.8-6.3 snd 2.9-5.1
		Int=3	0.005	2000	16.3-30.0	depth 40.8-46.6 east10 16.0-20.5 north10 11.2-15.9 latitude 11.9-14.3 longitude 8.8-13.6	32.3-38.8	depth 29.8-31.1 east10 13.2-15.5 tpi10 11.9-13.0 slope10 11.3-12.4 latitude 9.3-11.9 planz10 8.3-9.9 north10 5.3-8.2	47.3-50.9	calc.rf 19.7-22.4 depth 13.5-16.5 bs_ave10 10.6-11.9 tpi10 8.3-8.7 slope10 5.9-7.8 bldr 5.0-7.2 planz10 4.5-6.2 latitude 5.5-6.1 east10 5.0-5.9 bs_sd10 3.7-5.5	44.4-52.7	depth 21.5-22.1 encr 15.5-15.9 east10 10.3-11.7 bare 9.2-11.4 tpi10 7.0-9.9 latitude 5.4-7.1 slope10 6.9-7.8 fltrs 4.4-5.8 planz10 3.1-5.1	46.2-54.3	calc.rf 13.1-15.2 depth 13.9-14.4 encr 8.8-12.0 bs_ave10 8.3-9.5 slope10 5.5-6.3 bldr 4.3-6.0 bare 4.5-5.9 fltrs 3.1-5.7 latitude 4.3-5.5 tpi10 3.9-5.5 east10 4.4-5.2
		Int=5	0.0025	5000	26.9-34.5	depth 41.7-44.8 east10 17.0-21.0 latitude 13.6-15.1 north10 12.8-14.2 longitude 9.8-11.2	34.8-40.4	depth 29.2-30.4 tpi10 11.3-14.6 east10 11.7-14.1 slope10 12.4-13.4 planz10 9.8-11.3 latitude 8.2-10.0 north10 7.0-8.1	43.4-51.0	calc.rf 17.5-22.3 depth 14.8-16.4 bs_ave10 10.1-11.1 tpi10 7.9-8.9 slope10 7.0-7.9 east10 6.0-7.8 latitude 5.6-6.0 planz10 5.1-6.0 bldr 5.2-5.4	50.7-53.5	depth 20.3-22.1 encr 13.6-14.5 east10 10.2-11.3 bare 8.8-10.4 slope10 8.3-8.8 tpi10 8.2-8.8 latitude 6.7-8.5 planz10 4.8-5.3	50.2-57.3	depth 12.6-13.8 calc.rf 12.5-13.1 encr 9.3-12.3 bs_ave10 8.1-8.8 slope10 5.9-6.2 tpi10 5.0-6.0 bldr 5.4-5.9 latitude 5.0-5.9 east10 5.1-5.7 bare 4.2-5.1
	25 x 25	Int=1	0.01	1000	11.5-13.2	depth 72.7-86.0 east25 2.7-11.2 latitude 3.6-17.5	8.3-12.9	depth 59.6-60.9 planz25 4.3-15.0 slope25 4.0-9.7 longitude 3.2-9.1 tpi25 4.3-7.5 east25 3.8-6.8 north25 2.4-5.5	22.2-23.2	calc.rf 32.5-36.6 depth 20.7-25.6 bldr 5.1-8.4 planz25 1.4-8.1 tpi25 4.4-7.9 bs_ave25 3.9-7.6 snd 3.0-5.6	29.3-31.0	encr 19.8-33.7 depth 21.2-26.1 bare 7.7-19.2 fltrs 7.1-12.7 planz25 1.1-9.0 tpi25 2.9-7.0 east25 1.6-5.7 slope25 3.9-5.6	34.0-40.0	calc.rf 16.6-22.9 encr 16.3-20.6 depth 15.2-16.6 bldr 4.9-9.2 bare 3.7-8.8 tpi25 2.5-7.0 fltrs 5.4-6.5 bs_ave25 1.9-5.7

		Int=3	0.005	2000	13.2-24.1	depth 45.2-55.2 north25 11.2-16.4 latitude 14.4-16.1 east25 8.2-12.6 longitude 7.7-10.9	18.5-21.0	depth 37.1-41.0 tpi25 12.5-14.4 latitude 9.5-12.0 slope25 7.9-10.9 planz25 9.2-9.5 east25 8.1-8.9 north25 6.2-6.9	31.7-43.2	calc.rf 20.9-23.4 depth 16.0-23.4 bs_ave25 2.5-9.9 tpi25 5.7-9.7 planz25 6.6-8.1 slope25 3.6-7.7 latitude 6.0-7.0 bldr 4.5-6.4 bs_sd25 3.8-5.8	43.6-53.2	depth 21.8-22.2 encr 15.0-16.7 bare 9.8-11.6 planz25 8.1-9.7 slope25 7.1-9.0 east25 4.3-8.1 latitude 5.4-7.0 tpi25 5.2-6.6 fltrs 4.3-6.9	48.1-49.8	depth 15.7-19.0 calc.rf 11.9-14.3 encr 8.7-11.5 bldr 5.9-7.5 bare 4.4-6.4 latitude 4.8-5.9 planz25 5.8 fltrs 4.3-5.7 bs_sd25 4.1-4.9 bs_ave25 3.5-4.3 tpi25 3.7-5.5 slope25 3.7-5.2
		Int=5	0.0025	5000	24.0-27.2	depth 40.7-45.4 east25 14.6-18.1 north25 15.1-17.1 latitude 13.7-14.4 longitude 9.8-10.9	22.4-25.6	depth 33.0-37.2 planz25 11.8-12.1 slope25 9.6-13.1 tpi25 9.6-11.8 latitude 8.9-9.9 east25 7.4-9.6 north25 6.8-8.3	44.7-46.9	calc.rf 18.6-21.9 depth 16.0-17.8 planz25 8.4-8.6 latitude 6.2-8.0 tpi25 5.4-7.1 bs_ave25 4.7-7.0 bldr 6.2-6.8 slope25 5.5-6.3 bs_sd25 3.6-5.6 east25 5.0-5.1	41.0-49.6	depth 22.1-25.4 encr 13.8-14.8 bare 8.8-11.0 planz25 8.3-9.5 slope25 6.2-9.5 fltrs 5.0-7.4 tpi25 6.1-6.3 east25 5.3-6.3 latitude 5.7-5.9 longitude 4.0-5.3 north25 4.1-5.2	50.4-56.8	depth 14.6-15.7 encr 11.0-12.7 calc.rf 10.3-12.0 bldr 6.4-6.8 planz25 5.5-6.4 latitude 5.2-6.3 bare 4.0-5.8 slope25 5.0-5.5 bs_ave25 4.2-5.2 fltrs 4.1-5.1 tpi25 3.2-5.1
	50 x 50	Int=1	0.01	1000	11.3-13.8	depth 65.5-71.9 north50 8.4-21.9 latitude 5.3-6.7 longitude 4.6-5.3 east50 1.9-9.4	8.8-11.1	depth 52.3-62.9 tpi50 8.2-19.2 planz50 2.5-12.5 slope50 5.3-8.9 latitude 6.0-7.4 north50 2.9-5.9	22.6-35.0	calc.rf 34.7-44.3 depth 17.0-19.7 bldr 5.9-15.9 planz50 6.2-8.2 tpi50 1.6-6.5 east50 2.0-5.2 snd 1.4-5.1	26.3-29.7	depth 20.8-25.9 encr 22.6-25.6 bare 15.8-24.9 fltrs 3.7-11.1 planz50 5.6-8.1 latitude 1.6-6.6 tpi50 4.4-6.2	33.0-39.8	calc.rf 19.1-24.9 depth 13.8-16.2 encr 12.1-17.1 planz50 6.5-10.4 bare 4.9-10.4 bldr 5.3-9.0 fltrs 5.1-9.1 tpi50 4.8-8.9
		Int=3	0.005	2000	14.6-17.7	depth 46.0-53.5 north50 10.4-19.1 latitude 11.6-17.1 east50 7.9-16.0 longitude 6.8-12.7	12.1-17.2	depth 37.2-41.0 tpi50 14.6-16.0 planz50 10.5-12.6 slope50 7.9-9.9 north50 7.5-8.4 latitude 7.1-8.7 east50 4.2-7.9	36.3-44.4	calc.rf 20.7-25.5 depth 14.9-16.5 planz50 7.9-10.9 bldr 6.6-8.5 tpi50 6.9-8.1 latitude 4.3-6.1 bs_sd50 2.2-5.8	40.6-45.1	depth 21.9-25.0 encr 12.1-18.8 bare 10.8-12.0 planz50 10.3-11.0 slope50 6.7-8.1 tpi50 6.8-8.1 latitude 4.5-7.2 fltrs 3.7-7.1	49.8-53.6	depth 15.0-16.0 calc.rf 12.7-14.7 encr 8.8-11.9 planz50 7.8-8.8 bldr 5.6-7.6 tpi50 4.3-7.1 bare 5.2-7.0 slope50 3.4-6.4 latitude 3.9-5.2

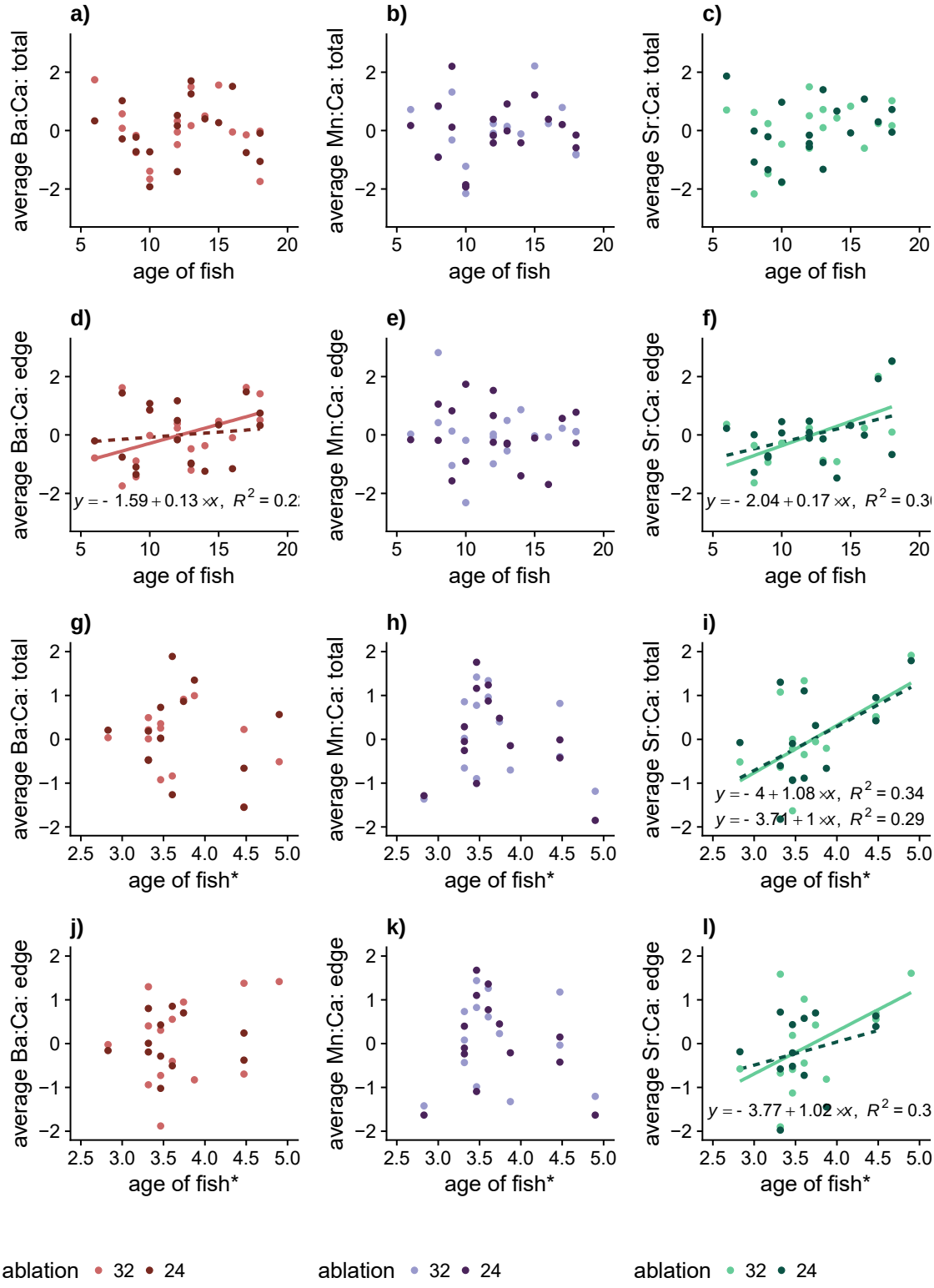
		Int=5	0.0025	5000	17.0-22.9	depth 44.6-45.3 north50 15.3-16.0 east50 12.5-14.0 latitude 12.6-13.9 longitude 11.8-13.6	12.0-16.9	depth 33.3-34.8 planz50 11.0-15.1 tpi50 12.1-14.4 slope50 9.8-10.4 north50 7.9-8.3 latitude 6.1-10.1 east50 5.2-10.6 longitude 4.7-6.6	43.0-45.9	calc.rf 20.5-22.4 depth 15.8-17.2 planz50 9.7-10.2 tpi50 8.5-9.4 latitude 5.1-6.9 bldr 5.0-6.9 bs_sd50 4.5-5.6	39.8-53.0	depth 20.7-23.8 encr 14.2-16.6 planz50 9.8-10.6 bare 8.5-11.5 tpi50 6.5-7.9 slope50 6.1-6.9 latitude 5.4-6.5 fltrs 5.5-5.8 north50 4.2-5.9 east50 4.1-5.4	55.2-59.0	depth 12.3-14.7 calc.rf 12.2-13.3 encr 11.2-11.9 planz50 8.0-8.4 bldr 6.2-8.1 bare 5.7-6.0 latitude 4.7-5.3 tpi50 4.6-5.4

Appendix Table B2: Relative strength of the two-way interactions among 16 habitat covariates for species richness (upper) and species abundance (lower). Friedman's H-statistic values range between 0 and 1 and are calculated from the best iteration of a Boosted Regression Tree model (spatial scale 10 x 10, training fraction = 0.75, interaction depth = 5, learning rate 0.0025, bag fraction = 0.5, cross-validation folds = 5). Values greater than or equal to 0.05 are highlighted.

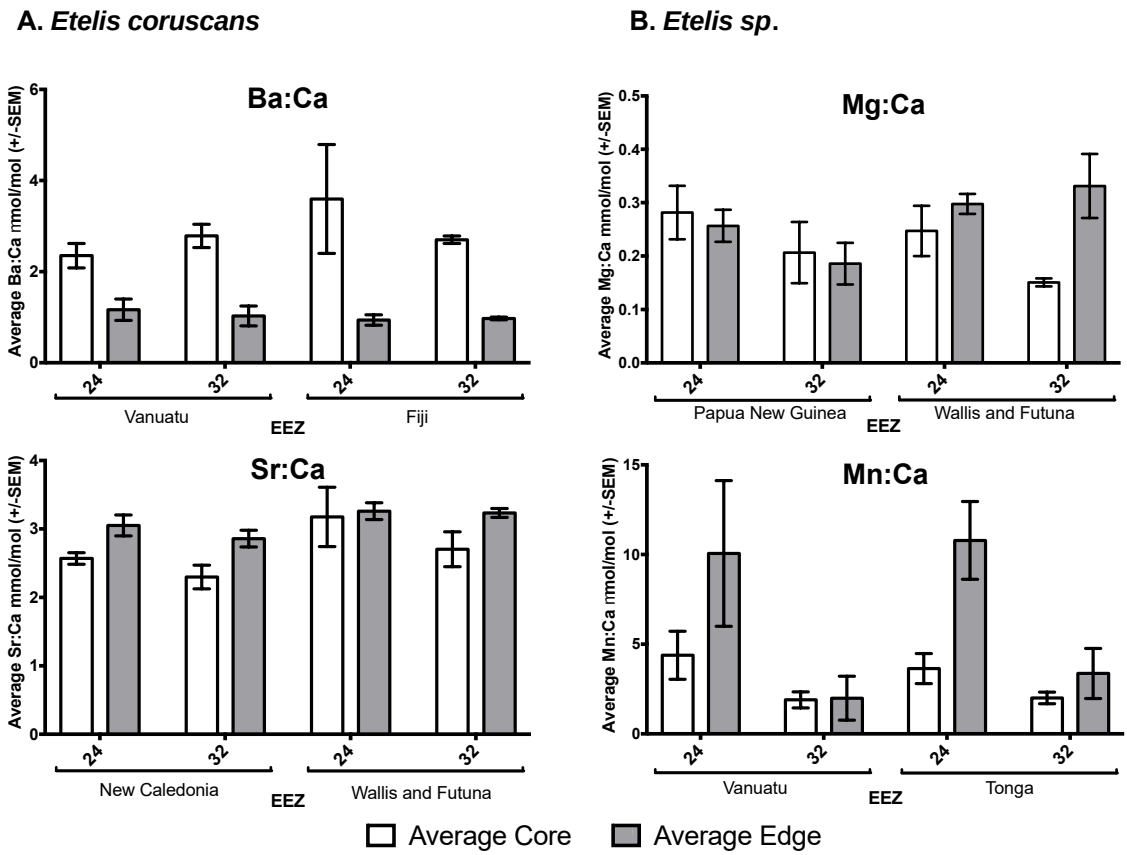
	Depth	Longitude	Latitude	Slope	Easting	Northing	Topographic Position Index	Planar curvature	Average backscatter	SD backscatter	Filter feeders	Encrusting Organisms	Bare epibenthos	Plants	Calcified Reef	Sand
Depth		0.039	0.038	0.038	0.046	0.020	0.038	0.024	0.067	0.048	0.028	0.033	0.042	0.006	0.067	0.038
Longitude	0.010		0.041	0.024	0.064	0.056	0.010	0.152	0.004	0.032	0.034	0.033	0.063	0.038	0.050	0.004
Latitude	0.012	0.030		0.062	0.094	0.062	0.060	0.043	0.096	0.064	0.054	0.046	0.019	0.063	0.076	0.090
Slope	0.013	0.030	0.015		0.132	0.022	0.047	0.035	0.091	0.012	0.012	0.063	0.039	0.016	0.027	0.018
Easting	0.023	0.037	0.071	0.049		0.028	0.032	0.012	0.013	0.031	0.017	0.036	0.019	0.029	0.036	0.003
Northing	0.015	0.011	0.023	0.038	0.014		0.020	0.031	0.004	0.014	0.039	0.003	0.003	0.043	0.011	0.048
Topographic Position Index	0.027	0.025	0.030	0.017	0.024	0.013		0.019	0.041	0.038	0.027	0.025	0.015	0.015	0.034	0.048
Planar curvature	0.052	0.071	0.022	0.054	0.042	0.026	0.049		0.012	0.018	0.059	0.033	0.013	0.018	0.048	0.026
Average backscatter	0.064	0.029	0.022	0.020	0.034	0.012	0.036	0.012		0.014	0.030	0.034	0.037	0.057	0.045	0.011
Standard deviation of backscatter	0.014	0.017	0.021	0.026	0.032	0.027	0.032	0.049	0.020		0.013	0.035	0.050	0.016	0.019	0.024
Filter feeders	0.012	0.075	0.010	0.058	0.007	0.042	0.049	0.030	0.013	0.026		0.006	0.002	0.021	0.009	0.005
Encrusting organisms	0.030	0.089	0.061	0.041	0.016	0.014	0.014	0.051	0.012	0.018	0.023		0.004	0.029	0.007	0.008
Bare epibenthos	0.019	0.037	0.031	0.033	0.019	0.010	0.028	0.033	0.030	0.046	0.004	0.005		0.056	0.020	0.006
Plants	0.005	0.007	0.000	0.008	0.012	0.024	0.004	0.008	0.016	0.020	0.002	0.003	0.002		0.048	0.023
Calcified reef	0.027	0.059	0.076	0.014	0.043	0.018	0.025	0.026	0.032	0.022	0.007	0.026	0.013	0.004		0.008
Sand	0.022	0.032	0.020	0.014	0.016	0.017	0.070	0.014	0.015	0.027	0.008	0.005	0.018	0.023	0.007	



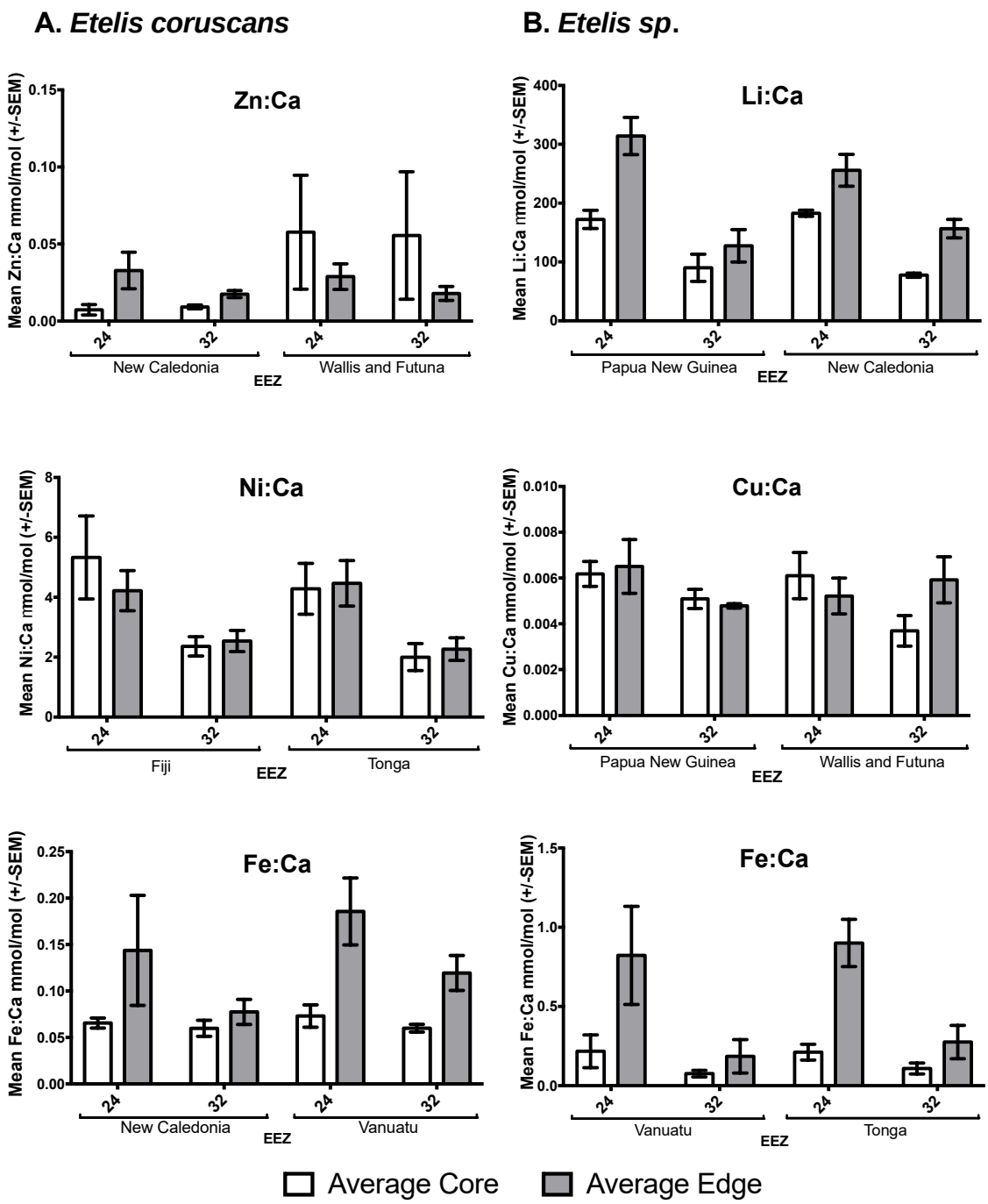
Appendix Figure C1: Correlations between age of fish and univariate elemental concentration ratios for *Etelis coruscans* (*a-f, n*=18) and *Etelis sp.* (*g-l,* n=15). Significant linear regressions are shown for solution-based ICP-MS measurements for both otolith cores (*a-c*, *g-i*) and whole otoliths (*d-f*, *j-l*) for three elemental concentrations (Ba:Ca, Mn:Ca and Sr:Ca). For *Etelis sp*. whole otolith samples (*j-l*), the independent variable age was transformed using a Tukey's Ladder of Power transformation. For both species, elemental measurements were Box-Cox transformed, centred, scaled and color-coded by Exclusive Economic Zone (EEZ) of capture.



Appendix Figure C2: Correlations between age and univariate elemental ratios for *Etelis coruscans* (*a-f*) and *Etelis sp.* (*g-l*). Linear regressions are shown for both averaged total transect (*a-c*, *g-i*) and edge laser ablation ICP-MS measurements (*d-f*, *j-l*), for three elemental concentrations (Ba:Ca, Mn:Ca and Sr:Ca) and two ablation spot sizes (24-µm and 32-µm).



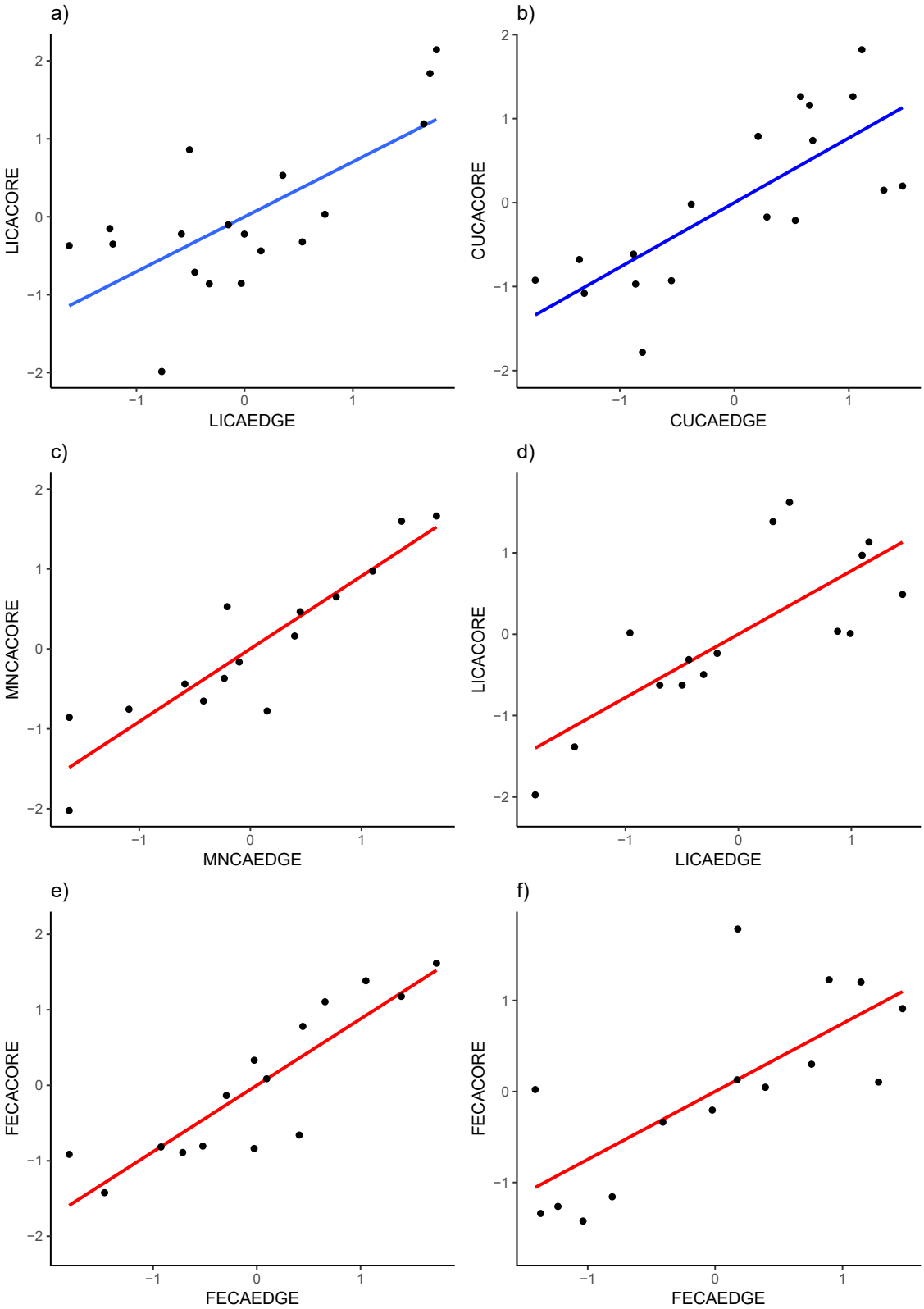
Appendix Figure C3: The effect of ablation spot size (24-µm and 32-µm) on LA-ICP-MS measurements for selected elements for two species of deepwater snapper. Each bar represents average data of the first 50 (average core) or last 50 (average edge) of a life history transect (n=3).



Appendix Figure C4: The effect of ablation spot size (24-µm and 32-µm) on LA-ICP-MS measurements for selected elements for two species of deepwater snapper. Each bar represents average data of the first 50 data points (average core) or last 50 data points (average edge) of a life history transect (n=3).

Appendix Table C1: Coefficient of determination (R^2) for regression models comparing core and edge (LA-ICP-MS) otolith samples for two eteline snapper species. Significant values of R^2 are highlighted in red.

	Etelis coruscans		*Etelis sp.*	
	LA-ICP-MS (24-µm)	LA-ICP-MS (32-µm)	LA-ICP-MS (24-µm)	LA-ICP-MS (32-µm)
Ba:Ca	0.25	0.10	0.20	0.02
Sr:Ca	0.02	0.02	0.17	0.16
Mg:Ca	0.30	0.21	0.07	0.04
Mn:Ca	0.05	0.00	0.83	0.29
Li:Ca	0.50	0.13	0.19	0.61
Fe:Ca	0.03	0.19	0.77	0.56
Ni:Ca	0.40	0.10	0.10	0.29
Cu:Ca	0.29	0.59	0.04	0.17
Zn:Ca	0.00	0.32	0.29	0.38

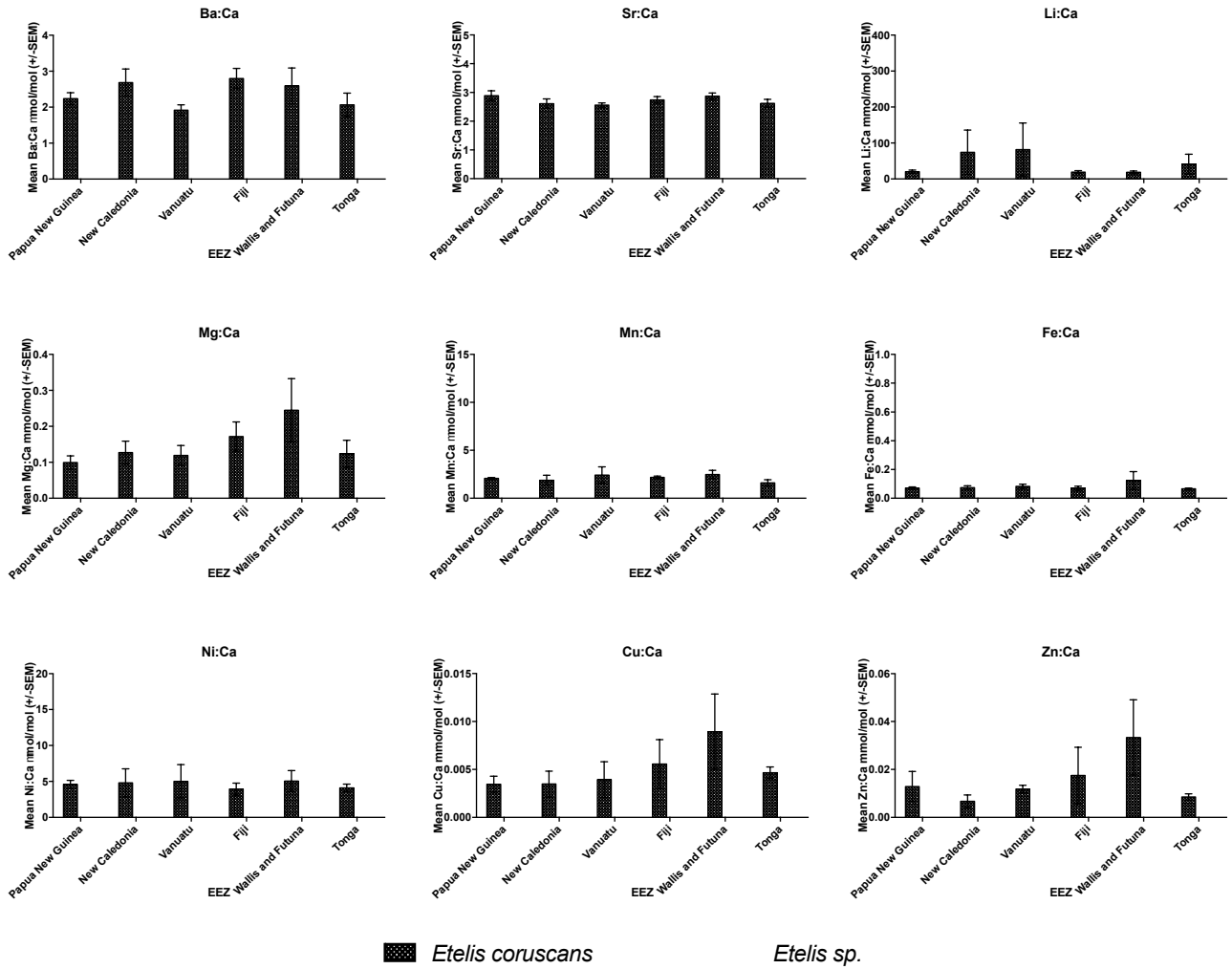


Appendix Figure C5: Regression of average core vs average edge samples for select elemental ratios. *Etelis coruscans* (blue) and *Etelis sp*. (red) are shown with selected measurements from two laser ablation inductively coupled plasma mass spectrometry mask sizes: 24-µm (left: a, c, e) and 32 µm (right: b, d, f). Samples were Box-Cox transformed, centred and scaled prior to regression and 95% confidence intervals are shown (lm smoothing function, package ggplot2).

Appendix Table C2: Comparison of LA-ICP-MS measurements of total load for two species among five Exclusive Economic Zones (EEZ). Total load was the average of 150 data points of the core-edge transect. ANOVA on Box-Cox transformed, centred and scaled univariate measurements. Values reported in the table are for 24-µm data, significance levels in bold for 32-µm data.

Both species						*Etelis coruscans*					*Etelis sp.*			
Element	**Source of Variation**	**Df**	**MS**	**F**	**p-value**	**Source of Variation**	**Df**	**MS**	**F**	**p-value**	**Df**	**MS**	**F**	**p-value**
Ba:Ca	EEZ	4	0.68	1.11	0.38	EEZ	5	1.15	1.22	0.36	4	0.97	0.96	0.47
	Species	1	12.29	19.99	**< 0.001*** **	Residual	12	0.94			10	1.01		
	Interaction	4	0.42	0.69	0.61									
	Residual	20	0.61											
Sr:Ca	EEZ	4	2.10	5.90	**< 0.01****	EEZ	5	1.08	1.12	0.40	4	2.92	12.61	**< 0.001*** **
	Species	1	9.16	25.73	**< 0.001*** **	Residual	12	0.97			10	0.23		
	Interaction	4	1.08	3.03	p < 0.05*									
	Residual	20	0.36											
Li:Ca	EEZ	4	0.09	0.23	0.92	EEZ	5	0.16	0.12	0.99	4	0.44	0.36	0.83
	Species	1	20.84	54.91	**< 0.001*** **	Residual	12	1.35			10	1.22		
	Interaction	4	0.06	0.15	0.96									
	Residual	20	0.38											
Mg:Ca	EEZ	4	0.82	1.46	0.25	EEZ	5	1.08	1.12	0.40	4	1.23	1.35	0.32
	Species	1	11.12	19.65	**< 0.001*** **	Residual	12	0.97			10	0.91		
	Interaction	4	0.81	1.44	0.26									
	Residual	20	0.57											
Mn:Ca	EEZ	4	0.10	0.16	0.96	EEZ	5	0.63	0.54	0.74	4	0.49	0.41	0.80
	Species	1	13.47	21.15	< 0.001***	Residual	12	1.16			10	1.20		
	Interaction	4	0.60	0.94	0.46									
	Residual	20	0.64											

Both species						*Etelis coruscans*					*Etelis sp.*			
Cu:Ca	EEZ	4	0.67	0.67	0.62	EEZ	5	0.50	0.42	0.83	4	0.88	0.84	0.53
	Species	1	2.78	2.75	**0.11**	Residual	12	1.21			10	1.05		
	Interaction	4	0.83	0.82	0.53									
	Residual	20	1.01											
Fe:Ca	EEZ	4	0.05	0.16	0.95	EEZ	5	0.24	0.19	0.96	4	0.45	0.37	0.82
	Species	1	21.97	73.43	**< 0.001*** **	Residual	12	1.31			10	1.22		
	Interaction	4	0.21	0.71	0.59									
	Residual	20	0.30											
Ni:Ca	EEZ	4	0.05	0.16	0.96	EEZ	5	0.09	0.07	1.00	4	0.52	0.43	0.78
	Species	1	21.62	62.36	**< 0.001*** **	Residual	12	1.38			10	1.19		
	Interaction	4	0.06	0.16	0.96									
	Residual	20	0.35											
Zn:Ca	EEZ	4	1.29	1.70	0.19	EEZ	5	1.00	0.99	0.46	4	0.61	0.52	0.72
	Species	1	5.41	7.15	**< 0.05***	Residual	12	1.00			10	1.16		
	Interaction	4	0.83	1.10	0.38									
	Residual	20	0.76											



Appendix Figure C6: Averaged otolith chemistry (total load) over the life history of two deepwater snapper species across 5-6 Exclusive Economic Zones (LA-ICP-MS 24-µm data). Each bar represents averaged elemental ratios for three samples per EEZ.