Multi-level responses of coral reef fishes to habitat degradation: An investigation with spatial, molecular and functional tools

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Multi-level responses of coral reef fishes to habitat degradation: An investigation with spatial, molecular and functional tools

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Statement of Contribution

This thesis includes collaborative work with my supervisor Richard Preziosi, my cosupervisor Tucker Gilman at The University of Manchester and my advisors at the Smithsonian Tropical Research Institute (STRI) in Panama, Matthieu Leray and Owen McMillan as well as other collaborators. During my thesis research I was responsible for developing research questions, experimental design, planning and carrying out field- and laboratory work, statistical analysis and preparing manuscripts for publication under the guidance of my supervisors. I conceived the initial ideas for each research chapter, except of Chapter four. Chapter four will be published as a shared first authorship with Jade Sourisse who had done previous but unpublished work on the same dataset but under a different scope. I analysed all data and wrote the chapter and manuscript.

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Abstract

Coral reef fishes are increasingly subjected to anthropogenic benthic change, habitat degradation and loss. This alters fish communities that rely in many different ways on coral reef habitat for resources and, in consequence, perils ecosystem functioning and human livelihoods. It is therefore vital to explore the underlying ecological interactions by which fish communities may shift, and to gain a detailed understanding of their functional properties in relation to spatial-environmental context. Yet, this requires a sufficient fine-scale resolution of empirical data, which has been difficult to achieve. This thesis investigates effects of spatial variation and habitat degradation on coral reef fish communities, diets, and gut microbiomes using spatial, functional, and molecular tools (DNA metabarcoding). **Chapter 2** investigates fish taxonomic and functional diversity patterns in relation to specific reef structures across three atolls within a high biodiversity system, the Mesoamerican Barrier Reef, Belize, and develops a visual analysis of relative functional trait-space occupancy. The results show a diversity gradient with highest levels at the largest and least isolated atoll contrasting relative protection levels among the three atolls at the time of sampling. This suggests that effects of biogeography and geomorphology may override protection status and highlight the need to integrate these factors into marine spatial planning for effective conservation. Furthermore, different levels of functional trait space occupancy among atolls may reflect variation in the dominant functional processes at play within each atoll ecosystem. Overall, the atoll fish communities featured low levels of redundancy suggesting a potential for functional vulnerability. **Chapter 3** investigates two benthic fish feeding strategies (browsing and active predation) with largely unknown levels of specialisation across a habitat gradient at Bocas del Toro, Panama. The results show that different feeding strategies exhibit variable responses in terms of resource use across reefs with varying levels of coral cover. DNA-based stomach and gut content analysis (metabarcoding) revealed that the diets of a facultative corallivore (*Chaetodon capistratus*) and a benthic crustaceans feeder (*Hypoplectrus puella*) were predicted by coral cover but to different degrees. Both species coped with low habitat quality at degraded reefs, but dietary adjustments appeared associated with subtle declines in physical condition. *H. puella* broadened its

diet where coral cover was low and increasingly consumed planktonic prey. *C. capistratus* switched its dominant diet item from cnidarians to annelids. These findings suggest that fish trophic roles may spatially vary and that such variation might be exacerbated with increasing coral decline. Building on Chapter 3, **Chapter 4** examines how the gut microbiome of a coral reef fish changes across Caribbean reefs that vary in coral cover. Using 16S high-throughput sequencing of the gut microbiome of *C. capistratus*, the results show an increase in gut microbiome variability for some components of the microbial assemblage at the most degraded reefs. This microbial pattern extended to closely host-associated, and presumably beneficial bacteria (i.e., the core microbiome) that were expected to remain stable. Altered fish-microbe associations in response to habitat degradation entail potential for acclimatisation, but on the other hand may bear consequences for fish health if stressors continue to intensify. This thesis provides a detailed spatial description of fish taxonomic and functional diversity patterns and contributes to understanding how coral reef habitat degradation affects fish trophic and microbial interactions via fish interrelations with the benthos. Using extensive visual surveys, DNA-Metabarcoding and spatial analysis, this thesis gives insight into spatial determinants of fish functional diversity and little explored processes of intraspecific variation and feeding strategy responses, influencing fish communities and ecosystem functioning.

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Chapter 1

General Introduction

1.1 Anthropogenic biodiversity change and underlying ecological mechanisms

Human-made global climate change and local modifications to the environment have become pervasive, causing biodiversity decline, widespread re-organisation of ecological communities and habitat degradation across terrestrial and aquatic ecosystems (França et al. 2019; Magurran et al. 2015; Dornelas et al. 2014; Pimm et al. 2014). Ecological communities (defined as assemblages formed by species occurrences overlapping in space and time, Ricklefs 2008) play important roles in ecosystem dynamics via the relationship between biodiversity and ecosystem functioning (this is, rates of energy flow and storage of materials such as carbon and organic matter, Hooper et al. 2005). Therefore, how communities respond to disturbances will shape ecosystem resilience (this is a system's capacity to recover from and absorb perturbations while maintaining functioning, Folke et al. 2004; Gunderson, Allen, and Holling 2009). On local scales, species assemblages appear to mainly change in terms of their relative abundances and due to species replacements, rather than declining species numbers (Dornelas et al. 2014). The underlying mechanisms causing this pattern are likely rooted in the variation and responses of species' behavioural traits, because animals respond to changes in their environment most immediately via their behaviour (Wong and Candolin 2015). Behavioural versatility forms a key mechanism by which species may cope with changing environmental conditions. Ecological versatility has been defined as 'the degree to

which organisms can fully exploit the resources in their local environment' (MacNally 1995; Berkstrom et al. 2012). This definition corresponds to the notion of exogenous, contextual plasticity (sensu Stamps 2016) describing variation in the behaviour of individuals in response to external factors. The relative ability of consumers to exploit dietary resources (specialisation to generalisation) influences population dynamics and community processes. In particular, relative levels of niche overlap and partitioning among closely related species mediate competitive interactions and facilitate co-existence (Schoener 1974). In response to changing environmental conditions, shifts in species' niches may translate into species assemblage structure and alter ecosystem productivity and stability. In this context, trophic interactions play an important role in modulating how biodiversity affects ecosystem functioning (Duffy et al. 2007). For example, reductions in the availability of prey caused by habitat fragmentation and/or land use change have the potential to cascade up through trophic levels and result in simplified food web structure (Layman et al. 2007; Price et al. 2019). Quantifying versatility (e.g., relative degrees of plasticity in feeding behaviour) within and among individuals informs the understanding of species' realised niches (this is, a species' niche space considering all limiting abiotic and biotic factors, Hutchinson 1957) and therefore their roles or functions within ecosystems towards the goals of (i) understanding species' susceptibilities to changing environments and their potential to adapt and use novel environments, and (ii) predicting ecosystem productivity and stability based on species' niche responses. While this type of information is urgently needed to better manage ecosystems under conditions of environmental change, the complexity of species behaviours and the scales and limited accessibility of natural environments—especially with marine ecosystems—prevent a comprehensive empirical assessment of species' realised niches across space and time (Donelson et al. 2019). In this context, recent technological advances such as high-throughput sequencing and DNA-Metabarcoding provide powerful tools to enhance our capability to assess and understand consumer-resource interactions (Parravicini et al. 2020) (see section DNA-based diet and microbiome analysis below).

Figure 1.1: Early depiction of sessile and mobile invertebrate and fish associates of a branching coral on an Indo-Pacific coral reef (Gerlach 1959 in Glynn and Enochs 2017).

A strategy to establish links between community and ecosystem levels is to consider the roles species play within ecosystems instead of treating species as comparable units with traditional taxonomic metrics (e.g., species richness) (Stuart-Smith et al. 2013; van der Plas 2019; Bellwood et al. 2018). Specifically, trait-based approaches represent a powerful tool in detecting community responses to environmental change (Mouillot et al. 2013) using simplified and thus manageable representations of ecosystem processes (Bellwood et al. 2018). Yet, these approaches require detailed empirical data from natural systems on species' niches for approximating the goal of precise trait assignments (Parravicini et al. 2020).

1.2 Fish trophic responses to coral reef degradation

The carbonate architecture and morphology of coral reefs provide complex habitat that supports a multitude of organisms (Gerlach 1959; Plaisance et al. 2009; Glynn and Enochs 2011; Coker, Wilson, and Pratchett 2014) (Fig. 1.1). This diversity underpins ecosystem functioning and thus enables important ecosystem services such as fisheries and coastal protection. Local stressors (e.g., overfishing, pollution) and global warming effects (e.g., mass coral bleaching events, increasing storms) alter biotic habitat assemblages towards more resilient coral species and non-reef building taxa such as macro-algae and sponges (Norström et al. 2009). This benthic community change and the associated deterioration of reef architecture and changing biogeochemical conditions in turn alter the composition and structure of associated fish- and non-habitat forming invertebrate communities (Wilson et al. 2006; Nelson, Stella et al. 2011; Kuempel, and Altieri 2016; Richardson et al. 2018). Because of their dependence on few particular resources (food and/or shelter), specialist species are usually the first to respond adversely to degrading conditions (Munday 2004; Sano 2004; Graham et al. 2009; Devictor et al. 2010, Clavel, Julliard, and Devictor 2011). In contrast, generalists have the ability to cope with changing environments owing to a broader behavioural spectrum (i.e.,

their versatility) that allows them to exploit alternative habitats and food resources (Graham 2007a; Berkstrom et al. 2012).

While coral feeding specialists tend decline rapidly if their preferred coral resources become scarce, facultative coral feeders and generalists of other trophic groups respond mainly to the subsequent loss of reef structure (Jones et al. 2004; Pratchett, Wilson, and Baird 2006; Graham et al. 2006; Graham et al. 2009). In addition, some species depend on life coral for recruitment and thus may experience population declines in the long-term (Feary 2007). Consequently, generalist species are expected to dominate ecosystems increasingly with decreasing habitat quality. However, this dichotomous view of generalists and specialists is being challenged by evidence showing that abundance of fishes across almost all trophic groups (including those considered generalists) decline in response to the loss of coral cover (Wilson et al. 2006; Pratchett et al. 2018). The underlying causes of these findings remain poorly understood but likely reflect complex fish responses to suboptimal habitat conditions associated with both coral cover decline and loss of reef structure inducing responses spanning direct and lagged abundance changes (Graham et al. 2007b). For example, altered resource use may compromise the physical condition of fishes and potentially their fitness (Pratchett et al. 2004; Berumen, Pratchett, and McCormick 2005; Hempson et al. 2018). Despite the immediacy of behavioural responses to disturbance (e.g., diet switches), associated sublethal effects may be hard to detect until ultimately fish populations decline (Hempson et al. 2018). These findings demonstrate that it is essential to study how diets vary intraspecifically over space and time to understand the effects of habitat change. For example, dietary plasticity allows species to adapt to habitat degradation on coral reefs (Graham 2007a; Karkarey et al. 2017), facilitate co-existence (Kingsbury et al. 2020) and potentially can compensate for functions if species are lost. In this context, a crucial knowledge gap regards how the functions provided by fish assemblages differ between healthy and degraded reefs and how this may influence ecosystem productivity and stability. On coral reefs, understanding degrees of resource use between specialisation and generalisation in response to habitat degradation forms an important starting

Figure 1.2: Images of study species *Hypoplectrus puella* (top) and *Chaetodon capistratus* (bottom). Photos: Friederike Clever

point to detect trophic relationships, which influence energy pathways and shape species' realised niches. Such knowledge informs the emerging conservation goal of protecting ecological processes as opposed to key species or species groups on coral reefs (Brandl and Bellwood 2014; Bellwood et al. 2018; Brandl et al. 2019).

1.3 Host associated microbes

Another crucial but little understood aspect that influences fish responses to changing environments regards host associated microbial communities. Symbiotic interactions between hosts and their microbiomes (this is, communities of host-associated microbes comprising bacteria, archaea, fungi, unicellular eukaryotes, protozoa, and viruses) influence ecological processes across organisational levels from individuals to ecosystems and thus play critical roles in ecosystem functioning in both marine (Wilkins 2019) and terrestrial systems (Coley, Endara, and Kursar 2018). Environmental stressors and disease, or compromised physical condition, may cause disruption of persistent host-microbe interactions (i.e., symbiosis) by decreasing microbial diversity (Zaneveld, McMinds, and Vega Thurber 2017; Ramsby et al. 2018), altering microbes towards becoming pathogenic (Alberdi et al. 2016; Oliver and Higashi 2021), or via stochastic assemblage shifts that increase microbial community variability among hosts (Zaneveld et al. 2016; Zaneveld, McMinds, and Vega Thurber 2017; Sepulveda and Moeller 2020). However, variation in microbial communities may also reflect non-detrimental processes related to environmental fluctuations or host trait variability and this microbial flexibility may facilitate host acclimatisation and adaptation (Webster and Reusch 2017; Apprill 2020). Despite the central role of host-associated microbes in responding to stressors and acclimatising and adapting to novel, anthropogenic environments, little is known on their spatial and temporal dynamics and the characteristics and causes of unfavourable community states (e.g., dysbiosis), especially within host-systems in the wild (Dethlefsen and Relman 2011; Voigt et al. 2015; Björk et al. 2019; Caporaso et al. 2011).

1.4 DNA-based diet and microbiome analysis

1.4.1 Metabarcoding

Traditionally, fish diet has been studied using visual, morphological approaches to gut and stomach content analysis and behavioural observations of foraging and bite rates (Hyslop 1980; Baker, Buckland, and Sheaves 2014). These approaches represent momentary snap-shots of dietary intake and can be combined with chemical approaches (i.e., stable isotopes and short-chain fatty acids) to detect longer term nutritional profiles (Nielsen et al. 2017; Traugott et al. 2013). Despite providing invaluable dietary information, these methods have the limitation of limited taxonomic resolution. DNA-Metabarcoding has opened up entirely new pathways to study diet (Ji et al. 2013) and outperforms morphological gut content analysis because of its ability to identify semi-digested and food items with unprecedented taxonomic resolution (Bohmann et al. 2011; Berry et al. 2015; Egeter, Bishop, and Robertson 2015; Albaina et al. 2016). By combining barcoding and high throughput sequencing (HTS) technology, metabarcoding allows to infer taxonomic information from DNA bulk samples such as environmental DNA (eDNA), community DNA from stomachs, guts or feces, or mass collections of organisms (Neigel, Domingo, and Stake 2007). The primary advantage of HTS technology over conventional Sanger sequencing is that it allows for rapid, simultaneous sequencing of hundreds of samples at low cost (Taberlet et al. 2012; Ji et al. 2013) and thus facilitates the processing of DNA mixtures (Hudson 2008). Although the field is fast moving, metabarcoding workflows are prone to biases with factors potentially affecting results regarding every step from sample collection to bioinformatics analyses of sequencing data (Alberdi et al. 2019; Corse et al. 2017; Zinger et al. 2019). Therefore, metabarcoding projects require study- and question-specific optimisation and the reliability of outcomes highly depends on careful consideration of steps and experience values. Yet, metabarcoding is a promising tool to study species dietary niches in relation to anthropogenic change in natural systems (Forin-Wiart et al. 2018), which can be especially challenging in

the marine realm. On coral reefs, metabarcoding has been recently employed to detect niche partitioning among species and its effects on ecosystem functioning (Leray, Meyer, and Mills 2015; Leray et al. 2019).

1.4.2 16S sequencing

High-throughput sequencing of the 16S rRNA gene is commonly applied to assess microbial communities (Armougom 2009) with more established workflows compared to metabarcoding of e.g., eDNA, gut contents, or bulk samples. However, this powerful tool is still in the beginning of revealing the extraordinary microbial diversity on coral reefs (Cleary et al. 2019; Neave et al. 2019; Frade et al. 2020) and associated with coral reef fishes (Neave et al. 2019; Chiarello et al. 2018; 2020; Miyake, Ngugi, and Stingl 2016; Nielsen et al. 2017). This is paramount given the current rate of biodiversity loss and the central roles of host-associated microbes in mediating host health and species acclimatisation and adaptation to environmental change.

1.5 Thesis aims and chapter objectives

This thesis aims to investigate little understood determinants of coral reef fish responses to habitat variability and human-induced habitat degradation across different ecological levels: i.e., communities, species, and host-associated microbes. Specifically, **Chapter 2** addressed how specific reef structures (atolls and reef zones) influence fish taxonomic and functional diversity patterns across spatial scales on the Mesoamerican Barrier Reef, Belize. The objective of this chapter was to assess the relative importance of geomorphological reef zones versus entire reef systems (atolls) in explaining coral reef fish taxonomic and functional diversity at spatial scales that apply to management. This chapter also developed a simple visual analysis of relative trait space occupancy in ecological communities. **Chapter 3** investigated the relative degrees at which fish feeders of sessile and/or mobile invertebrate prey rely on live coral using a gradient of coral cover at Bocas del Toro,

Panama. I capitalised on DNA-Metabarcoding to analyse the diets of invertebrate-feeding fishes that closely associate with the reef benthos at unprecedented high taxonomic resolution. This chapter's aim was to investigate fish dietary responses to habitat degradation for two different feeding strategies (browsing and active predation) with potentially different degrees of dietary versatility. Using the same habitat gradient, **Chapter 4** elucidated whether essential relationships among fish hosts and their gut microbiomes are vulnerable to coral reef degradation using 16S sequencing-based analysis. This chapter also improved statistical approaches for identifying ecologically relevant microbial community subsets and for teasing out responses in these subsets across the habitat gradient.

Chapter 2

Local and regional differences in fish community structure on the Mesoamerican Barrier Reef

2.1 Abstract

The importance of coral reefs as centres of biodiversity is well recognised, however, the scale at which specific structures relate to fish diversity patterns remains poorly explored. This is especially true for fish functional properties, despite their critical roles in buffering negative effects of global climate change. Here we investigated the relative importance of geomorphological reef zones versus entire reef systems in influencing fish communities across three Caribbean offshore atolls. We compared shallow water (<6 m) reef fish communities at 93 sites among and within atolls and across five geomorphological reef zones to assess their relative effects on both taxonomic and functional fish diversity. Overall, diversity levels varied more among reef zones than among atolls. Among atolls, fish richness and abundance were highest at the largest, least isolated and least protected atoll and lowest at a smaller, more isolated atoll that is partially protected from fishing but is periodically exposed to river plumes. This suggests that biogeographic effects of isolation and area, in tandem with environmental factors linked to geomorphology (e.g., wave exposure, water residence time), affect fish communities and have the potential to enhance or reduce the effectiveness of marine reserves. Taking into account functional traits revealed variation in fish niches among atolls, suggesting that some functional

pathways might differ among the atolls. Most functional trait groups comprised only a few fish species in our dataset. This suggested high vulnerability to functional loss in Caribbean coral reef fish communities, in agreement with previous findings from research conducted on larger spatial scales. Furthermore, we identify areas of high productivity, which are currently not accounted for in reserve protection schemes. Integrating geomorphological and functional metrics can aid in detecting spatial fish diversity patterns crucial to resilience-based conservation and fisheries management.

2.2 Introduction

Anthropogenic climate-related habitat degradation increasingly threatens the integrity of fish communities on coral reefs with far-reaching implications for ecosystem functioning and provisioning of essential resources to human livelihoods (Hughes et al. 2017; Robinson et al. 2019). Biodiversity and fisheries management can locally reduce negative effects, but successful interventions rely on detailed knowledge of spatial fish diversity patterns and underlying ecological mechanisms (Graham et al. 2011; Bates et al. 2019; Bellwood et al. 2018; Graham et al. 2020). Particularly promising strategies aim to manage resilience by promoting critical ecosystem functions (or processes) (Mcleod et al. 2019; Bellwood et al. 2018; Williams and Graham 2019). This requires data on the functional properties of fish assemblages and their spatial distributions. Integrating functional attributes of fishes with species-based diversity descriptors allows to spatially map critical ecological processes mediated by fishes and to assess their level of vulnerability to local stressors such as fishing (D'Agata et al. 2016b). Trait-based approaches often study the ecological robustness of different patches or site groups by identifying differences in the occupancy of functional trait space (Villéger, Mason, and Mouillot 2008; Mouillot et al. 2013). Several studies have investigated the distribution of functions within coral reef fish assemblages at large (Mouillot et al. 2014; Cinner et al. 2020; Mellin et al. 2016), regional (Micheli et al. 2014, D'Agata et al. 2016a; D'Agata et al. 2016b) and local spatial scales (Villéger et al. 2010; Brandl et al. 2016; Richardson et al. 2017). But little is known about spatial patterns of fish

functional diversity at meso- (10-100 km) and seascape-scales (0.1-10 km) (but see Guillemot et al. 2011, Elise et al. 2017, Rincón-Díaz et al. 2018).

The spatial distribution of reef organisms is governed by biophysical gradients (Graus and Macintyre 1989) that result in well established ecological zonation patterns occurring independently of geographic location (Done 1982; Galzin 1987; Lecchini et al. 2003; Depczynski and Bellwood 2005). Geomorphological reef zones (e.g., backreef, forereef, crest, lagoonal patch reef) are commonly considered in ecological studies as the most basic comparable spatial units (Beger, Jones, and Munday 2003) harbouring characteristic fish and coral assemblages (Geister 1977; Alevizon et al. 1985; Mejía and Garzón-Ferreira 2000, Letourneur et al. 2008; Friedlander et al. 2010; Harborne 2013) and modulate—together with specific seascape features (e.g., channels)—crucial ecological processes including spawning (Heyman and Kjerfve 2008; Ezer et al. 2011), larval dispersal (Pinsky et al. 2012) and food and shelter availability for fishes (Floeter et al. 2007; Noble et al. 2014). Yet, increasing anthropogenic influences may shift the composition of biotic communities away from what can be predicted by natural abiotic factors (Núñez-Lara, Arias-González, and Legendre 2005, Estrada-Saldívar et al. 2019; Williams et al. 2019) and potentially displace fish habitats (MacNeil et al. 2010; Brander 2010). While reef zone fish assemblages are taxonomically well described (e.g., Alevizon et al. 1985; Mejía and Garzón-Ferreira 2000; Harborne 2013), analyses considering functional traits have been focused on single zones (e.g., outer slopes, Richardson et al. 2017, Yeager et al. 2017), or have combined data from multiple zones (e.g., in temporal comparisons, Brandl et al. 2016; D'Agata et al. 2016a), while few exceptions took into account reef zonation (Rincón-Díaz et al. 2018, Elise et al. 2017). With on-going habitat degradation and associated biotic homogenization at play (Olden et al. 2004; van der Plas et al. 2016; Richardson et al. 2018) it is vital to revisit relationships among fish diversity and reef geomorphology to elucidate how physical-environmental factors in relation to reef structure and positioning influence diversity patterns.

We use taxonomy-based diversity metrics and a functional trait approach (based on Mouillot et al. 2013; 2014) to describe fish communities in relation to the relative influences of different spatial units (i.e., atolls and reef zones) across a latitudinal gradient spanning ~100 km off the Mesoamerian Barrier Reef. Our goal in this analysis was to represent trait parameter space in a way that captures both the magnitude of under- or over-occupancy and our statistical confidence in that measure, to answer the question whether different reef zones (or atolls) have relatively less occupancy of different functional groups. We studied spatial variation in fish community structure among and within three Caribbean atolls representing ecosystems of high biodiversity and endemism (McField, Hallock, and Jaap 2001; Smith et al. 2003). Being situated in relatively close proximity (<30 km apart), the atolls are comparable in terms of shared regional and geographic conditions, but differ regarding their geomorphological characteristics, local environmental conditions (Gischler and Hudson 1998; Stoddart 1962) and management schemes. Using three of the four atolls associated with the Mesoamerican barrier reef as a model system, we asked whether fish assemblages were more consistent across reef zones than among atolls at shallow depths (<6m). We quantified the relative contribution of reef-scale versus atoll-scale effects on fish community composition and investigated if observed patterns of species distributions hold when taking into account functional diversity.

2.3 Methods

2.3.1 Study area

The three atolls of Belize (i.e., Lighthouse Reef, Glover's Reef and Turneffe Atoll) are located on the Mesoamerican Barrier Reef (MBRS). Despite similar geological foundations (Purdy, Pusey, and Wantland 1975) and regional conditions, they exhibit local environmental and geomorphological differences. Most conspicuously, the atolls differ in lagoon size, depth, shape and coral development (e.g., number of patch reefs), island area, and mangrove cover. They also differ in permeability, determined

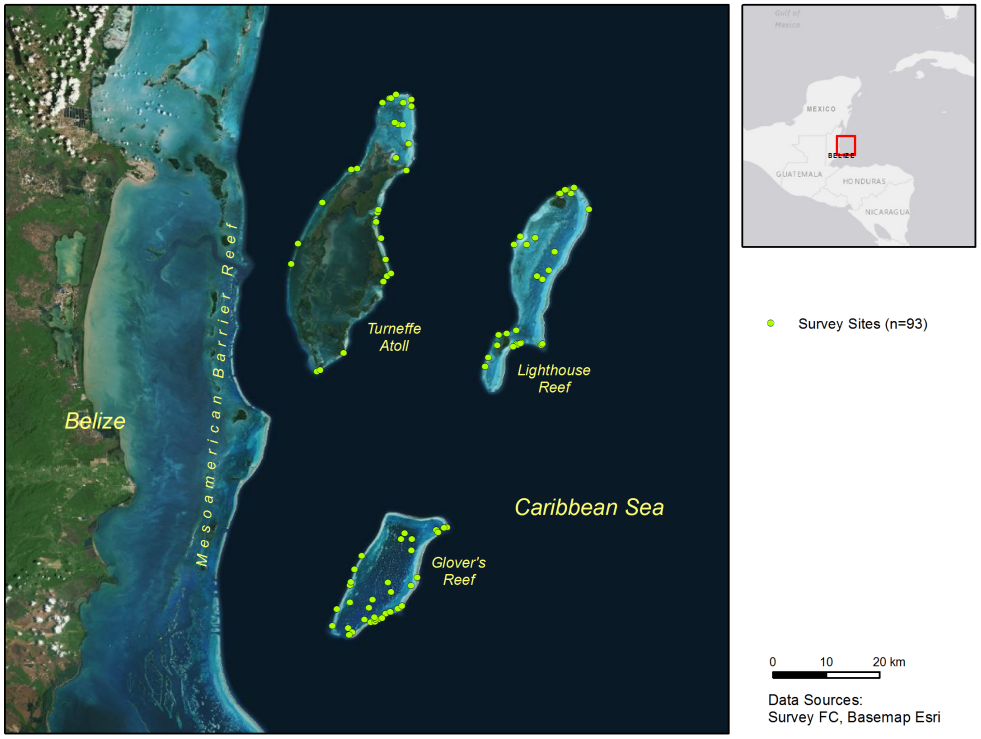

Figure 2.1: Study area with fish survey sites (n=93) across three adjacent offshore coral atolls at the Mesoamerican Barrier Reef, Belize. The satellite image depicts differences among the three atolls i.e., size, island area, and lagoon depths.

by the number and characteristics of passes (e.g. channels, swashes) within their outer rim as well as rim height regulating rates of lagoonal water exchange (Gischler and Hudson 1998). Furthermore, the atolls vary markedly in terms of protection level through conservation and fisheries regulations, historical fishing pressure, and disturbance histories (e.g. hurricanes, coral bleaching). Regional and local current regimes influence environmental conditions and larval recruitment as well as pollution rates (Soto et al. 2009) and generate gradients of exposure: Lighthouse Reef and Glover's Reef are fully exposed to the east, whereas Turneffe Atoll is partly sheltered by the Lighthouse Reef. This has allowed for development of an extensive mangrove system, which is unique among the three atolls and includes several lagoons with productive, sediment-rich waters and a multitude of creeks (Stoddart 1962).

2.3.2 Reef zone classification and site allocation

To generate meaningful spatial comparisons of atoll fish assemblages, and to explore the potential for geomorphological proxies of fish diversity based on satellite images, we defined five reef zones based on predominant geomorphological features using satellite images obtained from Google Earth (v. 7.1.2.2041) and personal communication with local fishermen and stakeholders: lagoonal patchreef, backreef, channel, windward forereef (in the following referred to as "forereef"), and leeward forereef (in the following referred to as "westreef"). Our distinction between forereef and westreef environments reflects the structure of atolls featuring exposed windward sides and relatively more sheltered leeward sides. Exposed forereefs mainly comprise high relief spurs and grooves structures dominated by *Agaricia* spp. and *Millepora complanata* and a few non-spur and groove sites dominated by *Orbicalla* spp. In contrast, sheltered westreefs consist of submerged, crest-like environments with rather fluent transition among crest and shallow forereef as well as the lagoon in most cases (e.g., Banco Chinchorro, Jordan and Martin 1987). We surveyed a total of 36 sites at Glover's Reef, of which four were protected from fishing; 28 sites at Lighthouse Reef, of which three were protected from fishing; and 29 sites at Turneffe Atoll, of which none were protected from fishing at the time.

2.3.3 Fish surveys

Fish species richness and abundance were assessed by visual census at all 93 sites (< 6 m). Fish surveys were conducted using a modified version of REEF Roving Diver Technique (RDT) (Pattengill-Semmens and Semmens 2003). Surveys were 45 min timed swims while snorkeling. One surveyor (F.C.) counted individual fish instead of assigning roving diver approximate abundance categories (as in the original description of the RDT). This method was chosen for being non-intrusive, having little diver effect on fishes, and being suitable for detecting high numbers of species (Holt et al. 2013; Schmitt and Sullivan 1996; Beck et al. 2014). All encountered fish individuals were counted and identified to the species level and life phase. During timed swims, the surveyor slowly moved in a circle (or in one

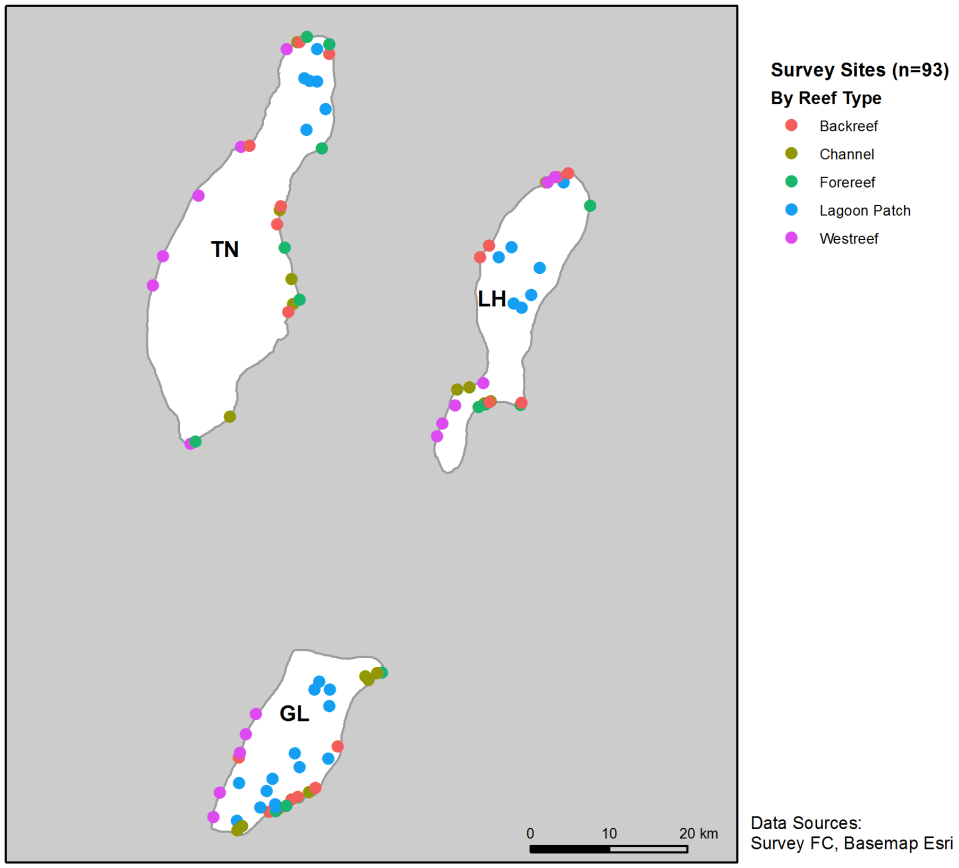

Figure 2.2: Distribution and classification of survey sites according to geomorphological reef zones at each atoll; TN = Turneffe Atoll, LH = Lighthouse Reef, GL = Glover's Reef

direction) across the reef without returning to the same location twice. The surveyor was accompanied by a snorkel buddy for safety where appropriate, staying a few meters behind with as little movement as possible avoiding diver effect on fishes.

2.3.4 Seasonality and Sampling Consistency

We controlled for seasonal variation in fish communities and consistency of our sampling method. To do so we re-surveyed fish communities at Turneffe Atoll (N=12) and compared the same sites among summer and winter using Analysis of Similarities (ANOSIM; Clarke and Warwick 2001).

2.3.5 Species-based analysis

Alpha diversity

We square root transformed the fish abundance data to control for extremely high abundance values. To account for differences in the numbers of surveyed sites between atolls, we calculated both mean richness and abundance across sites for each atoll (N=3). In addition, we calculated the unbiased Simpson's Index (Hurlbert 1971) using the raw fish abundance data (rarefy function, vegan package v. 2.5-6; Oksanen et al. 2012). The same metrics were also calculated for each geomorphological reef zone (N=5) (using pooled data from three atolls). Non-parametric Kruskal-Wallis tests were used with post-hoc Dunn tests and Benjamin Hochberg correction to compare alpha-diversity among three atolls and among the five geomorphological reef zones, respectively.

Beta diversity

We visualised distances among fish assemblages across five reef zones (i.e., lagoonal patchreef, backreef, windward forereef, leeward forereef) using Non-metric Multidimensional Scaling (NMDS; Clarke and Warwick 2001) with Bray-Curtis dissimilarity for both presence-absence and abundance data. To test differences in fish communities among the three atolls and five reef zones respectively, we used Permutational Analysis of Variance (PERMANOVA; Anderson 2001) in a 2-way ANOVA design accounting for potential interaction among atoll and reef zone (Adonis2 function, vegan package v. 2.5-6, Oksanen et al. 2012). To test the hypothesis that different reef zones support distinct fish communities within our study area, we tested for significant differences among five reef types pooling data from three atolls (N=93) (PERMANOVA; Anderson 2001). Pairwise tests were performed to explore variation in fish communities among individual reef zones (Adonis function; Oksanen et al. 2012). To infer whether fish communities at a specific reef zone differed or were consistent among atolls, we used PERMANOVA to test for significant differences within each individual reef zone among three atolls (e.g., backreef at Glovers vs. backreef at Lighthouse vs. backreef at Turneffe).

Pairwise tests were used to determine differences in fish communities at respective reef zones between atolls (Adonis function; Oksanen et al. 2012).

2.3.6 Functional analysis

We characterised six traits for each species in our data set (N=154) and including only adult individuals: trophic group, mobility, maximum size, gregariousness, water column position, and activity time. Traits were assigned based on ecological knowledge on fish species obtained from fishbase (www.fishbase.org), the literature (Randall 1967; Bohnsack et al. 1999; Claro, Lindeman, and Parenti 2001) and personal observations (F.C.) (Table A4-A9). These traits have been used to characterise species in previous studies due to their ecological relevance (D'Agata et al. 2016a; Mouillot et al. 2014; Richardson et al. 2017). Trophic group and mobility are categorical. All other traits are numerical, and we discretised them so that species could be classified ordinally. Groups of species with identical characterisations comprise functional entities (FEs) (Mouillot et al. 2014).

To build the functional space, we computed the Gower distance between each pair of FEs in our system. Gower distances allow us to combine categorical and ordinal variables into a single measure that weighs each variable equally (Legendre et al. 1998). Then, we conducted a Principal Coordinates Analysis (PCoA) based on the Gower distance matrix and plotted the results in two dimensions, these axes are interpreted as representing composites of trait categories. We then plotted points representing species sharing unique trait combinations (i.e., FEs). This functional space was based on the multidimensional functional trait space for the fish community of the three atolls combined, which served as a global reference space for comparisons of subcommunities (Mouillot et al. 2013; Villéger, Mason, and Mouillot 2008). To assess whether FE spaces were under- or over-occupied in different atolls or reef zones, we calculated the average number of individuals in each FE observed per visit in each atoll or reef zone, and divided this by the average number of individuals observed per visit in the same FE across all atolls and reef zones. We took the log of this ratio to standardise the measure for under-

and over-occupancy. We call the result the 'log relative occupancy', and indicate it by the colour (red to blue) and size of circles representing FEs in figures.

To assess our confidence that particular FEs were under- or over-occupied in particular atolls or reef zones, we compared each log relative occupancy to the null distribution of values that it might have taken if there were no relationship between the FE and the atoll or reef zone. We created permutations of the data in which there was no relationship between FEs and atolls by reassigning the observed numbers of individuals in each FE among all visits, subject to the constraints that i) observations made in a particular reef zone must be assigned to that reef zone, and ii) the number of visits per reef zone in each atoll could not change. We calculated the log relative occupancy of each FE in each atoll in the permutation, and repeated this 104 times to create null distributions for the log relative occupancy of each FE in each atoll. To create null distributions for the log relative occupancy of each FE in each reef zone, we repeated the same process, but replaced constraint i) so that observations made in a particular atoll must be assigned to visits to that atoll. In each case, constraints on permutations ensure that we do not confound effects of atolls and reef zones when the number of visits per reef zone differs among atolls. For each FE in each atoll or reef zone, we calculated the proportion of the null distribution in which the magnitude of the null was greater than the magnitude of the observed log relative occupancy. This is equivalent to assigning a p value to each FE within each atoll or reef zone. We indicate this by the darkness or lightness of the circles representing each FE in figures. This analysis does not account for multiple comparisons, and should not be interpreted as a test of statistical significance for the effect of atolls or reef zones on any particular FE. Nonetheless, areas of functional space that appear more red or blue in figures are likely to be numerically under- or over-occupied, respectively.

2.3.7 Indicator Analysis

We identified indicator species (i.e., species whose presence is indicative of a particular atoll or reef zone) following Dufrêne and Legendre (1997)) and using

the labdsv package in R (Roberts 2019). Indicator values (IndVal) were calculated for each fish species across two spatial scales i) among three atolls, ii) among five reef zones (three atolls combined), iii) among five reef zones within each atoll. We tested for significance of the relationships between each fish species and each sample group using 1000 permutations at a significance level of p value 0.05. All statistical analysis was done in R (version 3.6.1, R Core Team 2019) and Matlab (v. R2017b, Language and Computing 2004) except ANOSIM was run in Community Analysis Package 4 (Seaby and Henderson 2014).

2.4 Results

We recorded a total of 79,002 individual fish belonging to 156 species in 44 families at 93 reef sites across three atolls (Table A4-A9). The number of individuals per site ranged from 413 to 1921, while the number of species recorded per site ranged from 43 to 86.

2.4.1 Fish diversity

Fish species richness was significantly different among the three atolls (Kruskal Wallis Test; $\chi^2 = 18.03$, $p = 0.0001$); with higher levels at both Turneffe Atoll and Lighthouse Reef (mean species richness = 68.28 and 67.71 respectively) than Glover's Reef (mean species richness = 61) (Fig. 2.3A, Table 2.1). Posthoc testing confirmed that richness levels were significantly lower at Glover's Reef than at both other atolls (Table A.1). Fish abundance showed a similar pattern with highest levels at Turneffe Atoll (mean fish abundance = 886.17), intermediate levels at Lighthouse reefs (mean fish abundance = 851.21) and lowest levels at Glover's Reef (mean fish abundance = 819.14) (Kruskal Wallis Test; $\chi^2 = 14.88$, $p = 0.0006$) (Fig. 2.3B). Posthoc Dunn Test revealed significantly lower abundance levels at Glover's Reef than at the other two atolls (Table A.1). The unbiased Simpson index (Hurlbert 1971) also differed significantly between the three atolls (Kruskal Wallis Test; $\chi^2 = 7.1$, $p = 0.03$). In particular, Simpson's Index was significantly higher at Lighthouse Reef than Glover's Reef (Table A.1).

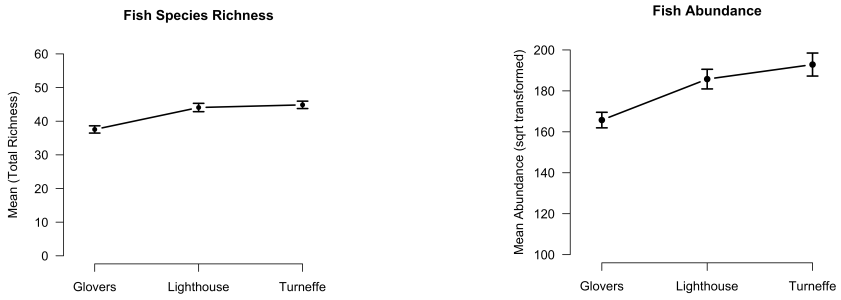

Figure 2.3: Mean (+/- SE) fish species richness (A) and abundance (square root transformed) (B) by coral atoll (n=93). Sampling period: August and September 2011 and August, September and October 2014.

Fish species richness differed significantly among five reef zones across three atolls (Kruskal Wallis Test; $\chi^2 = 22.27$, $p = 0.0002$) (Table 2.1); posthoc testing revealed significantly lower richness levels at lagoon patches (mean richness = 60.89) than both Westreefs (mean richness = 70.94) and channels (mean richness = 70.19) (Table A.1). Fish abundance levels were significantly different among the five reef zones (Kruskal Wallis Test; $\chi^2 = 18.69$, $p = 0.001$) and posthoc results were congruent with the pattern found for fish richness: channel was the zone with the highest fish abundance (mean abundance = 980.63), whereas lagoon patch (mean abundance = 814.70) showed significantly lower fish abundance levels than both westreef (mean abundance = 877.24) and channel; levels at backreefs (mean abundance = 717) were significantly lower than at channels (Table A.1). Fishes among reef zones also significantly differed in terms of the unbiased Simpson Index (Kruskal Wallis Test; $\chi^2 = 12.12$, $p = 0.016$); however, we found no significant differences in pairwise posthoc comparisons (Table A.1).

We tested for the temporal consistency of alpha diversity patterns. There were no significant differences in species richness (ANOSIM; $p = 1$, permutations >1000) (Fig. A.1) and abundance between seasons (ANOSIM; $p = 0.852$, permutations >1000) (Fig. A.1). Our results suggested that seasonality had no significant influence on fish communities in terms of species richness and relative abundance at our study site.

2.4.2 Fish community composition

Non-metric multidimensional scaling ordination (NMDS) based on pooled data from three atolls segregated sites of each reef zone with the majority of windward forereef sites furthest away from lagoonal patchreefs, whereas channel, back reef, and leeward forereefs largely spread in between (Fig. 2.4). We found a significant interaction between atolls and reef zones explaining 9% (Jaccard) and 8% (Bray Curtis) of variation in structuring fish communities (Table 2.2). Overall, the effect of reef zone (~20%) was stronger than the effect of atoll (< 5%). In total, our models explained ~36% of variation in fish species composition. Species composition differed significantly among the three atolls for both Jaccard similarity and Bray Curtis dissimilarity (Table 2.2). We tested the hypothesis that different reef zones (i.e., lagoonal patchreef, backreef, forereef, westreef) support distinct fish communities within our study area at the regional scale (using data from three atolls combined). There were significant differences in composition between the five reef zones with both distance metrics (Table 2.2). We compared fish communities at individual reef zones among the three atolls and found that the majority of comparisons differed significantly (Tables 2.3). However, there was no significant difference among atoll-specific forereefs (PERMANOVA; Jaccard $R^2 = 0.27$, $p = 0.16$; Bray Curtis; $R^2 = 0.16$, $p = 0.4$) and species presences at westreefs among the three atolls (PERMANOVA; Jaccard $R^2 = 0.16$, $p = 0.076$).

yet, fish assemblages at westreefs differed when taking into account abundances of common species (PERMANOVA; Bray Curtis $R^2 = 0.2$, $p = 0.002$). Backreefs communities significantly differed between Lighthouse Reef and Glover's Reef in terms of presence-absence (PERMANOVA; Jaccard $R^2 = 0.13$, $p = 0.044$) but they were similar between the other two atoll pairs i.e., Turneffe Atoll and Lighthouse Reef (PERMANOVA; Jaccard $R^2 = 0.11$, $p = 0.23$) and Turneffe Atoll and Glover's Reef (PERMANOVA; Jaccard $R^2 = 0.12$, $p = 0.071$) (Table A.2). This pattern was reversed when taking into account abundance (Table A.3). Communities at Lighthouse Reef and Glover's Reef did not significantly differ (PERMANOVA; Bray Curtis $R^2 = 0.11$, $p = 0.152$) but both other pairwise comparisons were significant

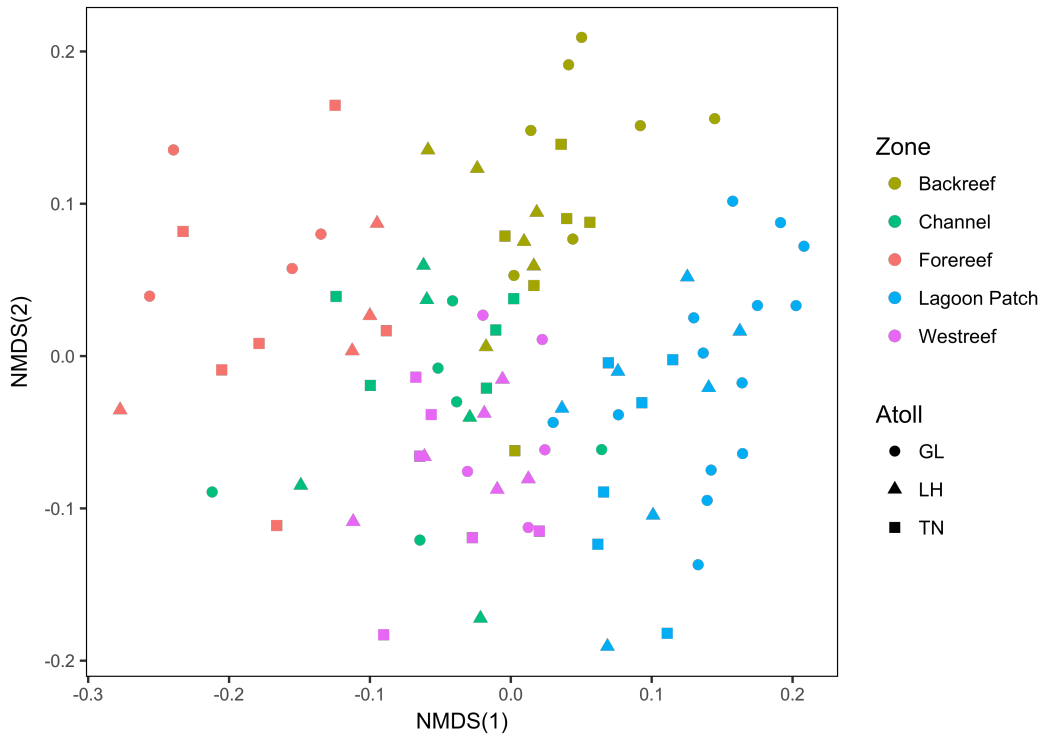

Figure 2.4: The relationship between fish community composition and five different reef zones at a total of 93 sites (n = 93) represented by points across three atolls based on Bray-Curtis dissimilarity using presence-absence data. Lagoonal patch reefs (left) appear clearly separated from all other reef types. Blue=lagoon patch; green=forereef; olive=channel; red=backreef; pink=westreef.

i.e., Turneffe Atoll and Lighthouse Reef (PERMANOVA; Bray Curtis $R^2 = 0.14$, $p = 0.019$) and Turneffe Atoll and Glover's Reef (PERMANOVA; Bray Curtis $R^2 = 0.14$, $p = 0.02$) (Table S2.1B). We found significant differences among zones within atolls for both Jaccard similarity and Bray-Curtis dissimilarity (Table A.2 and A.3).

2.4.3 Functional Analysis

Atolls

We identified a total of 89 functional entities (i.e., groups of species with identical sets of traits, FEs) among all fish species observed at our sites. Atoll communities differed in the degree to which they filled the global functional space (Fig 2.5; Fig. 2.6). Glover's Reef appeared most functionally depauperate with high levels of confidently under-occupied FEs. In contrast, over-occupied FEs had low levels of confidence across Glover's Reef. Lighthouse Reef was balanced between FE

Factor	Diversity	Kruskal-Wallis χ^2	DF	*P*-Value
Atoll	Mean richness	18.028	2	0.0001
	Mean abundance	14.884	2	0.0006
	Unbiased Simpsons	7.1028	2	0.03
Zone	Mean richness	22.274	4	0.0002
	Mean abundance	18.689	4	0.009
	Unbiased Simpsons	12.124	4	0.016

Table 2.1: Kruskal-Wallis test comparing alpha diversity levels among fish communities among 3 atolls and 5 reef zones, respectively.

Distance	Model	Factor	Df	SumsOfSqs	R2	F.Model	Pr(>F)	Significance
Jaccard	3 Atolls	Atoll*Zone	2	0.5704	0.04557	2.6824	0.0001	***
	5 Zones	Atoll*Zone	4	2.5537	0.20403	6.0045	0.0001	***
	Atoll:Zone	Atoll*Zone	8	1.0986	0.08777	1.2915	0.0076	**
	Residual		78	8.2935	0.66262			
	Total		92	12.5163	1			
Bray Curtis	3 Atolls	Atoll*Zone	2	0.3091	0.05255	3.3727	0.0001	***
	5 Zones	Atoll*Zone	4	1.5011	0.25524	8.1908	0.0001	***
	Atoll:Zone	Atoll*Zone	8	0.4973	0.08456	1.3569	0.0132	*
	Residual		78	3.5737	0.60765			
	Total		92	5.8812	1			

Table 2.2: Permutational Analysis of Variance among fish communities of 3 atolls and 5 reef zones and their interaction (data of 3 atolls combined) and testing the interaction among atoll and reef zone. The analyses were performed for using Jaccard similarity (species presence-absence) and Bray-Curtis dissimilarity (putting weight on common species) respectively. The fish abundance data was square root transformed to control for extremely high abundances of some fish species.

Distance	Model	Factor	Df	SumsOfSqs	R2	F.Model	Pr(>F)	Significance
Jaccard	Lagoon Patch	Atoll	2	0.2046	0.14392	2.0173	0.001	***
	Backreef	Atoll	2	0.13871	0.15741	1.4946	0.043	*
	Westreef	Atoll	2	0.25934	0.16379	1.3711	0.026	*
	Channel	Atoll	2	0.34319	0.20275	1.653	0.002	**
	Forereef	Atoll	2	0.19554	0.27209	1.246	0.16	
	5 Reef Zones	Glovers	4	0.88963	0.3625	4.4068	0.001	***
	5 Reef Zones	Lighthouse	4	0.52488	0.3457	3.038	0.001	***
	5 Reef Zones	Turneffe	4	0.59327	0.35628	3.3209	0.001	***
Bray Curtis	Lagoon Patch	Atoll	2	0.601	0.14463	2.029	0.003	**
	Backreef	Atoll	2	0.37627	0.15578	1.4762	0.033	*
	Westreef	Atoll	2	0.24054	0.20045	1.7549	0.002	**
	Channel	Atoll	2	0.339	0.233	1.9746	0.001	***
	Forereef	Atoll	2	0.18349	0.1609	1.0547	0.342	
	5 Reef Zones	Glovers	4	1.4664	0.36349	4.4257	0.001	***
	5 Reef Zones	Lighthouse	4	0.96936	0.35501	3.1648	0.001	***
	5 Reef Zones	Turneffe	4	1.168	0.39774	3.9625	0.001	***

Table 2.3: Permutational Analysis of Variance (PERMANOVA) results of atoll and reef zone models comparing individual reef zones among and within atolls using Jaccard Index and Bray-Curtis dissimilarity, respectively.

over- and under-occupation, whereas Turneffe Atoll showed a pattern of dominant over-occupation with only one trait entity appearing confidently under-occupied in our analysis. Turneffe Atoll also featured the highest number of entities that show no divergence from the global community, while Glover's Reefs showed the least. Overall, we found that the magnitude of FE occupancy across all three atolls increased towards traits related to smaller fish size classes and benthic feeding strategies (Fig. 2.6). Glover's Reef was dominated by planktivores and herbivores but appeared depauperate regarding larger commercially important groups (i.e., groupers and snappers) and to a lesser degree mobile, pelagic traits. The trait entity comprising surgeonfishes was more represented at Glover's Reef in comparison to the other two atolls. Smaller nocturnal invertivores were under-represented, while entities comprising rare, small, ectoparasite and invertebrate feeders were over-represented at Glover's Reef.

Lighthouse Reef uniquely featured entities comprising sessile invertivores (over-occupied) and mobile invertivores (over-occupied) such as the ecologically important

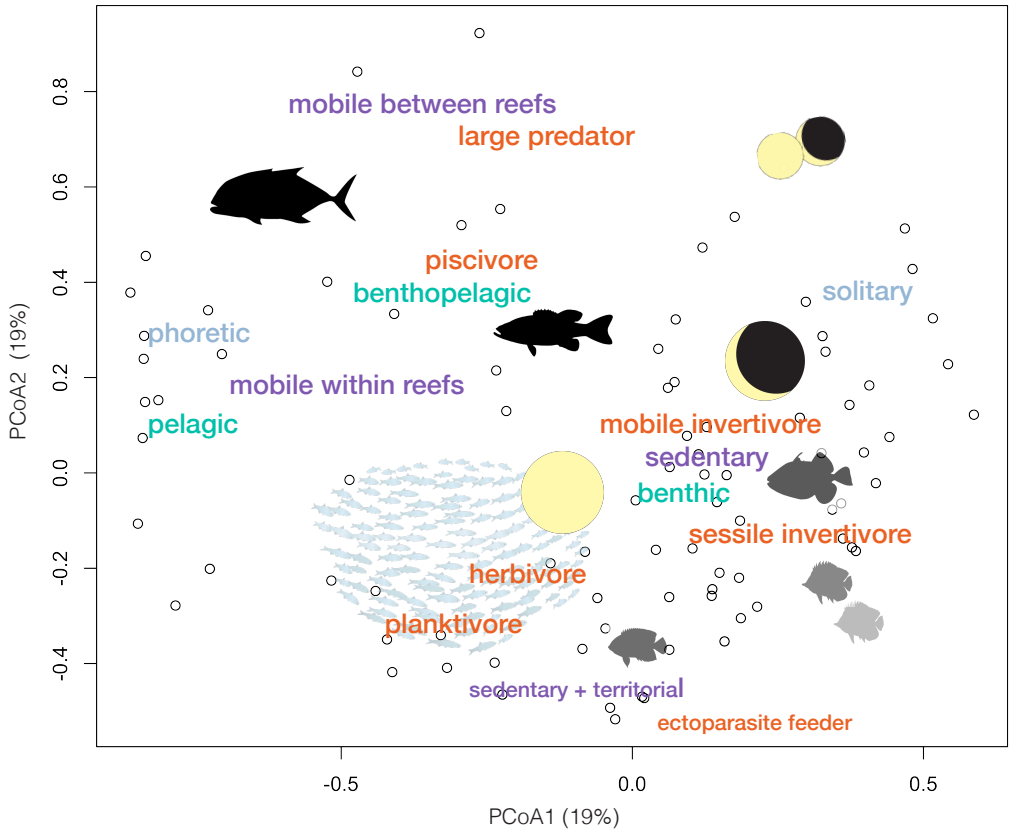

Figure 2.5: Functional trait niche space of the global community using data from three atolls combined. The PCoA is based on Gower distances between unique trait combinations (functional entities) in 152 species. Circles depict species in functional space, colours depict the respective mean trait levels of six trait categories: Trophic group, gregariousness, watercolumn position, activity time (depicted by planets), maximum size (gradient is reflected by relative fish shape sizes), mobility.

Queen Triggerfish *Balistes vetula*. Our analysis suggested an over-occupation of sharks (family Carcharhinidae) at Lighthouse Reef. Turneffe Atoll displayed the most complete trait space in comparison to all other atolls featuring the highest levels of over-occupation and lowest levels of under- combination with the highest proportion of neither over- nor under- occupation in occupation (neutral) FEs. In particular, Turneffe Atoll harboured over-occupied areas in trait space that appeared depauperate at the other two atolls and represented niches of commercially important

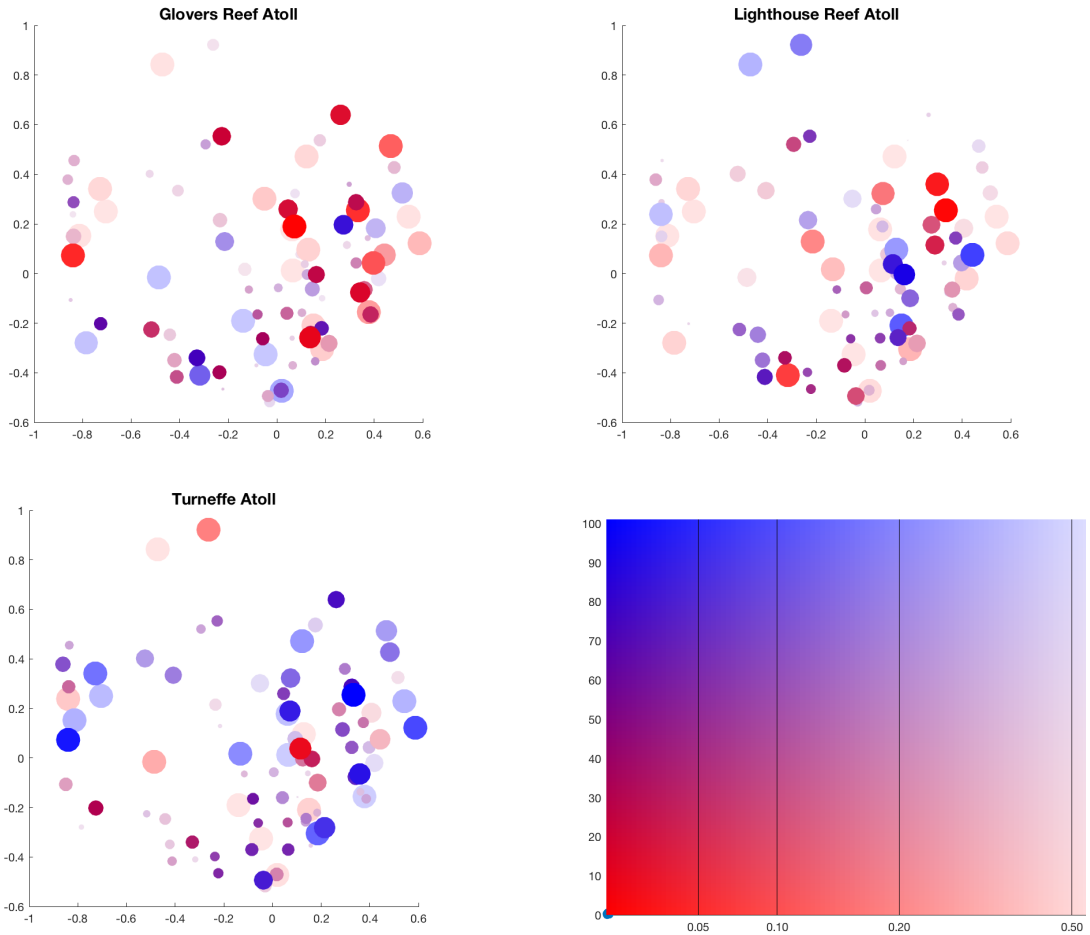

Figure 2.6: Functional niche trait space by atoll. Points represent functional entities and depict their relative over- and under-occupation at each atoll. Transparency indicates confidence. Red = under-occupied, blue = over-occupied. Colour chart represents p value.

large, highly mobile, piscivorous fishes. The distribution of species richness within functional trait entities appeared low for most entities (~2), whereas few entities were supported by several species (Fig. 2.8).

Reef zones

The functional niche space of the five reef zones for data from three atolls combined revealed functional differences among zones. Lagoonal patches and backreefs appeared functionally most similar (Fig. 2.7). Both zones shared large under-occupied areas in functional space comprising functional entities of both small and larger sized planktivores, herbivores as well as mobile, pelagic predators. Both zones were dominated by small to medium sized fisheries species (e.g., snappers, Lutjanidae) and non-commercial (e.g., wrasses, Labridae) invertivores. Backreefs featured two FEs comprising large predators i.e., sharks in the family Carcharhinidae. Channels showed a high proportion of over-occupied FEs. This zone was characterised by mobile, schooling, medium to large herbivores and small and mobile, medium sized planktivores as well as benthic feeding elasmobranchs. Under-occupied FEs at this zone included ~4 FEs of nocturnal, medium-sized invertivores and small mobile invertivores. Forereefs appeared occupied by herbivores and planktivores as well as phoretic behavioural niches. Westreef was over-occupied by pelagic, commercially important predators, whereas FEs featuring snapper species (Lutjanidae) were under-occupied. FEs composed of invertivorous meso-predators were over-occupied as well as FEs of small sedentary invertebrate-feeders and small planktivores.

2.4.4 Indicator Analysis

Indicator analysis among three atolls revealed three distinct sets of species but numbers of fish species identified of each atoll varied greatly with eleven and nine species respectively at Turneffe Atoll and Lighthouse Reef and only one indicator at Glover's Reef (i.e., intitial phase *Thalassoma bifasciatum* (indval = 0.493, p = 0.004, frequency = 93) (Table 2.4). Indicator species sets of reef zones were

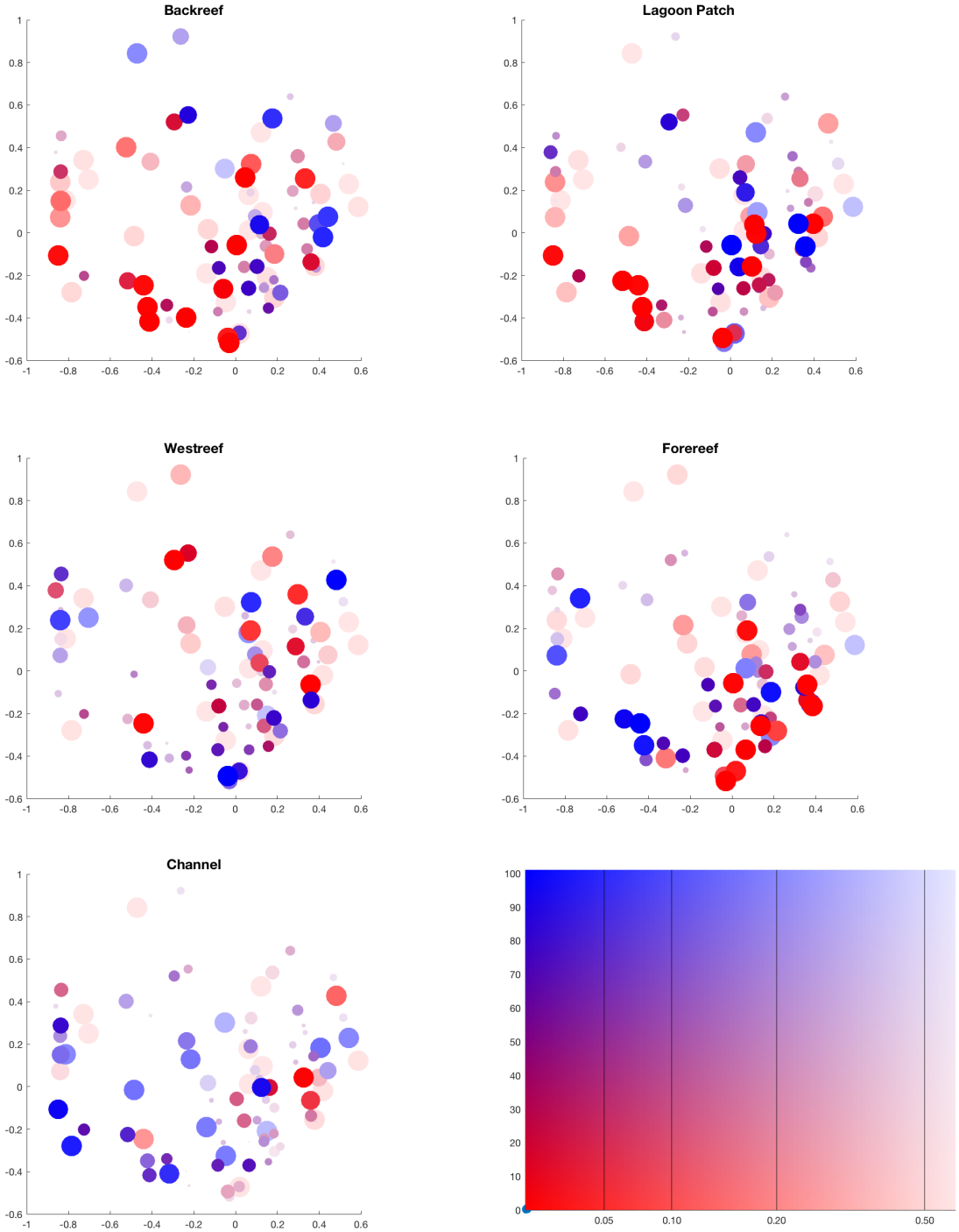

Figure 2.7: Functional niche trait space for each reef zone based on data from three atolls combined (regional scale). Points represent functional entities and depict their relative over- and under-occupation at each zone.Transparency indicates confidence. Red = under-occupied, blue = over-occupied. Colour chart represents p value.

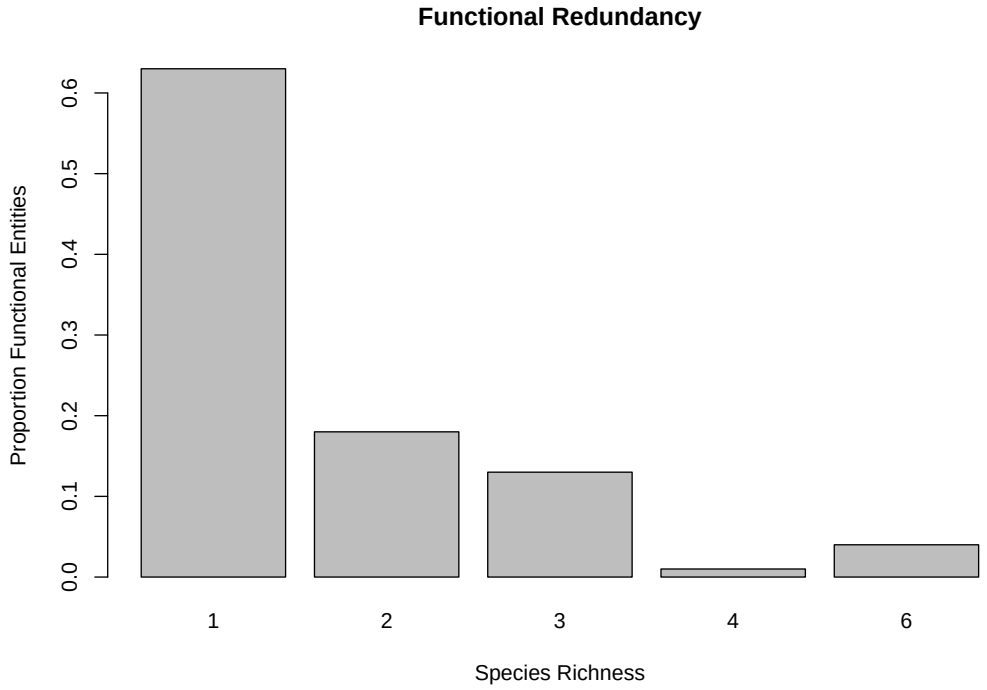

Figure 2.8: Number of fish species per functional trait entity. A high proportion of entities ($> 60\%$) is supported by only one species and few entities are supported by several species indicating a pattern of "over-redundancy" proposed by Mouillot et al. (2014).

overall numerically larger than those of atolls reflecting distinct assemblages. This was especially pronounced at both forereefs and westreefs with the largest number of indicator species among zones (19 and 18, respectively). In contrast, only eight relatively weak indicators characterised channels. Both lagoon patches and backreefs harboured the highest proportion of juvenile phase indicators (29.4% and 28.7%, respectively) (Table 2.5).

Reef zones among atolls

Zonal fish assemblages varied across atolls in terms of their most characteristic species. This reflected local influences on fish communities at each zone among the three atolls (Table A.5).

Species	Atoll	IndVal	*P* - value	Frequency
Thalassoma bifasciatum IP	Glovers	0.492506733	0.004	93
Holacanthus tricolor	Lighthouse	0.49569122	0.001	28
Chromis cyanea	Lighthouse	0.367442839	0.03	53
Chaetodon ocellatus	Lighthouse	0.355870467	0.023	59
Chromis multilienata	Lighthouse	0.294500838	0.028	27
Balistes vetula	Lighthouse	0.276743497	0.039	30
Hypoplectrus unicolor	Lighthouse	0.275481635	0.004	15
Scarus coelestinus TP	Lighthouse	0.239430767	0.002	13
Malacoctenus triangulatus	Lighthouse	0.229780491	0.008	16
Anchovie	Lighthouse	0.107142857	0.029	3
Halichoeres garnoti TP	Turneffe	0.441416113	0.003	58
Scarus iseri TP	Turneffe	0.439535419	0.009	78
Sparisoma rubripinne TP	Turneffe	0.377081012	0.011	46
Gramma loreto	Turneffe	0.336030686	0.036	39
Anisotremus virginicus	Turneffe	0.319120645	0.001	11
Stegastes adustus JV	Turneffe	0.287204362	0.038	35
Bodianus rufus JV	Turneffe	0.284770566	0.014	30
Pterois volitans/P. miles	Turneffe	0.261976284	0.026	25
Lutjanus griseus	Turneffe	0.212489113	0.022	18
Caranx crysos	Turneffe	0.137931034	0.015	4
Lutjanus jocu	Turneffe	0.128166005	0.04	7

Table 2.4: Indicator analysis comparing fish communities among three atolls. Indicator values reflect the degree to which a species is indicative of each atoll, respectively in terms of the specific conditions found there. P values reflect the significance of the relationships between each species and sample group and are based on 1000 permutations at a significance level of p value 0.05. JV = Juvenile, IP = Initial Phase, TP = Terminal Phase. Fish adult trophic groups are color coded, juvenile trophic groups are not assigned due to incomplete information.

Species	Reef Zone	Indval	*P* - value	Frequency
Stegastes leucostictus	BR	0.532537764	0.001	32
Halichoeres bivittatus JV	BR	0.464950856	0.001	59
Halichoeres bivittatus IP	BR	0.460406355	0.002	75
Sparisoma chrysopterum IP	BR	0.4187257	0.001	78
Lutjanus apodus	BR	0.401113936	0.005	93
Halichoeres radiatus JP	BR	0.38842892	0.003	59
Stegastes diencaeus JV	BR	0.370743231	0.002	80
Stegastes leucostictus JV	BR	0.357484305	0.004	61
Chaetodon ocellatus	BR	0.314286961	0.011	59
Stegastes diencaeus	BR	0.292444071	0.017	88
Chaetodon striatus	BR	0.291504288	0.026	70
Acanthurus tractus/chirurgus JV	BR	0.282653536	0.047	64
Halichoeres bivittatus TP	BR	0.251861966	0.015	28
Sparisoma chrysopterum JV	BR	0.157894737	0.021	3
Haemulon sciurus	LP	0.572518599	0.011	83
Haemulon plumierii	LP	0.56775784	0.001	90
Pomacanthus arcuatus	LP	0.500528939	0.001	39
Chaetodon capistratus JV	LP	0.444121072	0.001	18
Stegastes planifrons	LP	0.414431789	0.001	66
Scarus iserti JV	LP	0.393276437	0.001	90
Ocyurus chrysurus	LP	0.367802688	0.007	67
Sparisoma atomarium IP	LP	0.36667005	0.001	18
Holacanthus ciliaris	LP	0.349239968	0.001	35
Sparisoma aurofrenatum JV	LP	0.337031817	0.005	63
Sparisoma aurofrenatum IP	LP	0.305312296	0.002	86
Halichoeres garnoti IP	LP	0.299157525	0.015	91
Pomacanthus paru	LP	0.269427099	0.016	40
Thalassoma bifasciatum TP	LP	0.267770169	0.017	92
Sparisoma atomarium. JV	LP	0.254632709	0.006	15
Calamus SPP JV	LP	0.185185185	0.013	5
Hypoplectrus puella	LP	0.177483311	0.035	15
Gramma loreto	WR	0.721609393	0.001	39
Holacanthus tricolor	WR	0.450089614	0.001	28
Stegastes planifrons JV	WR	0.437126895	0.002	66
Scarus iserti TP	WR	0.350820819	0.001	78
Coryphopterus personatus/hyalinus	WR	0.338780558	0.043	36
Chromis cyanea	WR	0.334702167	0.006	53
Halichoeres garnoti TP	WR	0.331837625	0.005	58
Canthigaster rostrata	WR	0.32806271	0.004	64
Hypoplectrus indigo	WR	0.313525252	0.001	21
Clepticus parrae JV	WR	0.30969611	0.007	18
Sparisoma aurofrenatum TP	WR	0.290608912	0.014	75
Chromis cyanea JV	WR	0.290197455	0.03	35
Hypoplectrus nigricans	WR	0.287333645	0.008	33
Sphyraena barracuda	WR	0.286326478	0.037	49
Sparisoma viride IP	WR	0.285381282	0.022	90
Halichoeres pictus JV	WR	0.239100353	0.006	21
Holacanthus tricolor JV	WR	0.214176314	0.009	11
Epinephelus guttatus	WR	0.210435639	0.009	9
Lutjanus mahagony	CH	0.372198338	0.031	64
Pseudupeneus maculatus	CH	0.363242525	0.034	60
Caranx ruber	CH	0.36249084	0.031	87
Kyphosus sectatrix/incisor	CH	0.329920504	0.009	40
Haemulon chrysargyreum	CH	0.202276342	0.032	15
Haemulon carbonarium	CH	0.196071402	0.049	14
Lutjanus jocu	CH	0.155132193	0.03	7
Haemulon vittata	CH	0.123387097	0.043	3
Melichthys niger	FR	0.892883641	0.001	18
Ophioblennius macclurei	FR	0.70936519	0.001	32
Scarus vetula IP	FR	0.585561857	0.001	32
Microspathodon chrysurus JV	FR	0.577704355	0.001	57
Microspathodon chrysurus	FR	0.55762013	0.001	68
Cephalopholis fulva	FR	0.46806337	0.001	13
Scarus vetula TP	FR	0.458608272	0.001	20
Abudefduf sexatilis	FR	0.443895712	0.006	82
Sparisoma rubripinne TP	FR	0.428337958	0.002	46
Chromis multilienata	FR	0.415260799	0.004	27
Stegastes adustus	FR	0.408026639	0.001	83
Sparisoma rubripinne IP	FR	0.407901177	0.001	72
Acanthurus coeruleus	FR	0.352004201	0.005	93
Bodianus rufus	FR	0.316804722	0.006	47
Malacoctenus triangulatus	FR	0.311436305	0.001	16
Halichoeres radiatus IP	FR	0.293503847	0.011	46
Cantherhines pullus	FR	0.171250664	0.03	13
Malacanthus plumieri	FR	0.149342613	0.037	9
Echeneis naucrates	FR	0.142857143	0.02	2

Table 2.5: Fish indicator species at reef zones. Indicator values reflect the degree to which a species is indicative of individual reef zones across three atolls in terms of the specific conditions found at each reef zone, respectively. P values reflect the significance of the relationships between each species and sample group and are based on 1000 permutations at a significance level of p value 0.05. JV = Juvenile, IP = Initial Phase, TP = Terminal Phase. Fish adult trophic groups are color coded, juvenile trophic groups are not assigned due to incomplete information.

2.5 Discussion

Knowledge of appropriate biodiversity metrics and spatial scales forms a critical prerequisite when conservation relies on area prioritization and species diversity patterns (Beger, Jones, and Munday 2003; Mellin et al. 2011; Beier et al. 2015; Oliveira et al. 2017). Here we used a series of classic alpha and beta diversity descriptors to understand how reef fish communities vary within and across three atolls and among geomorphological reef zones of the MBRS across a north-south gradient of ~100 km. We then implemented a novel approach to describe differences in the functional diversity among the three atolls.

We found a conspicuous biodiversity gradient (taxonomic and functional) among the three atolls with the highest diversity levels at the largest, least isolated and least protected atoll, and the lowest levels at a smaller, more isolated atoll featuring a no-take fisheries closure implemented since 1995. Overall, our analysis showed that a high proportion of functional trait groups was supported by only one or few species suggesting that the atoll fish communities are highly partitioned and thus potentially vulnerable to functional loss. This corroborated previous findings from the tropical Western Atlantic at larger spatial scales (Micheli et al. 2014, Mouillot et al. 2014).

The three atolls differed in terms of their functional integrity; that is, the extent to which global niche space was filled at each atoll. The trait space at the largest atoll, Turneffe Atoll, was least depauperate, whereas the Glover's Reef appeared most depauperate. Similarly, species diversity levels were lowest at Glover's Reef and highest at Turneffe Atoll. It needs to be kept in mind that we censused fish assemblages across both fished and unfished areas at Glover's Reef, and that reserve benefits appear commonly most pronounced when considering the biomass of targeted species suites among no-take versus fished sites. Yet, it would have been still plausible to detect positive protection effects from our fish community data (Lester et al. 2009, especially since the small-scale fisheries at Glovers's reef targets a wide range of fish species (Babcock, Tewfik, and Burns-Perez 2018). Possible explanations for the reduced functional space and lower diversity at Glover's Reef

could be that local environmental factors such as low rates of lagoonal water exchange (McClanahan and Karnauskas 2011), lack of mangrove nursery habitat (Mumby et al. 2004), and insufficient enforcement of no-take zones override reserve effects at Glover's Reef. In contrast, diversity levels may have been highest in Turneffe Atoll due to larger atoll size, a largely open reef structure allowing water to circulate, greater vicinity to the Barrier Reef and coastal lagoons promoting larval connectivity, and the presence of an extensive mangrove system that serves as important nursery habitat for fishes (Mumby et al. 2004). The observed diversity gradient from highest levels at Turneffe Atoll to lowest levels at Glover's Reef may be consistent with species-area relationships (Lomolino 2000). Spatial isolation and reef area have previously shown to influence distributions of coral reef fishes in the Caribbean (Ault and Johnson 1998; Chittaro 2002; Sandin, Vermeij, and Hurlbert 2008). Within one atoll, Glover's Reef, Acosta and Robertson (2002) found larger patch reefs supported greater species abundances than smaller patch reefs. Furthermore, atoll fish diversity may be affected by a cross-shelf gradient typical for coral reefs (Williams and Hatcher 1983; McField, Hallock, and Jaap 2001) and latitudinal effects related to diverging fish and benthic distributions patterns observed between the northern and southern Belize Barrier Reef (McField, Hallock, and Jaap 2001).

In comparison to Turneffe Atoll, Glover's Reef lacked large, benthic feeding, reef-associated predators including important fished groups such as groupers (Serranidae) and snappers (Lutjanidae). However, Glover's Reef featured ecological important niches comprising medium and large sized benthic herbivores i.e., surgeonfishes (Acanthuridae) as well as large mobile solitary invertivores of several species groups. The presence of roving herbivores may relate to the predominance of macroalgae within the atoll's lagoon. Caribbean coral reefs have undergone a phase shift from coral towards macroalgae dominated states over the past fifty years (Gardner, 2003; Schutte, 2010) and the lagoonal patch reefs at Glover's Reef are among the most severely affected sites due to this regional habitat change and decline (McClanahan and Muthiga, 1998; Schutte, 2010). A recent study assessing effects of reserve protection since 1995 and a more recent parrot fish fishing ban implemented in Belize

found parrotfishes were decreasing on lagoonal patches at Glover's Reef (McClanahan and Muthiga 2020). Our findings corroborate this as we found functional entities capturing small and medium sized parrotfishes were under-represented at Glover's Reef. While Glover's Reef differed compositionally in terms of common species from the other two atolls, a unique fish assemblage featuring niches of more rare species characterised Lighthouse Reef. These were solitary, sedentary, sessile and to lesser extent mobile invertebrate-feeders. Moreover, Lighthouse Reef was the only atoll where niches representing large predators (i.e., sharks, family Carcharhinidae) were occupied. Fish abundance levels were similar to those of Turneffe Atoll, which may reflect a potential benefit from Lighthouse Reef's vicinity to Turneffe Atoll (~20 km) in terms of larval connectivity. Puebla et al. (2012) suggested reef fish dispersal encompasses distances of tens of km or even less on the Belize Barrier Reef.

Another factor influencing spatial patterns of fish diversity may be each atoll's vicinity to the mainland and associated levels of human access as has been recently demonstrated at other coral reef locations (Elise et al. 2017; D'Agata et al. 2016a; Maire et al. 2016). Fishermen traditionally fishing Glovers Reef originate from Northern Belize but are often seasonally based on the southern mainland for easier access to the atoll. In contrast, Lighthouse Reef being located further north may therefore be relatively less accessible and thus less frequented than Glover's Reef. Turneffe Atoll was still unprotected from fishing at the time of surveying apart from two spawning aggregation sites. Moreover, being closest to the mainland and the largest centre of human population, Belize City, it is the most accessible of the atolls and harbours the greatest emergent island area that allows for establishment of fishing camps. Thus, the largely over-occupied functional trait space at Turneffe Atoll with the presence of large, highly mobile, piscivorous fishes and functional entities relevant to fisheries exploitation may suggest that biogeographic and seascape effects were stronger than negative effects from human use. Human use (D'Agata et al. 2016a; Maire et al. 2016), biogeographic effects and the seascape (Sandin, Vermeij, and Hurlbert 2008, Karnauskas et al. 2012) previously have been found to influence fish communities.

Coral reef zonation patterns appear consistent among reefs at different biogeographic regions (Blanchon 2011), here we asked the question whether this effect was stronger in shaping fish communities than local influences at each atoll when considering species diversity. We found a significant interaction between atoll and reef zone explaining ~9% of variation in our model. Specifically, we found individual atoll characteristics influenced the way fish communities varied at particular reef zones across atolls, yet zones reflected distinct fish assemblages when pooling data from three atolls. Thus at the regional scale, our findings confirmed early ecological studies showing that fish assemblages vary across broad geomorphological reef zones such as lagoon, reef flat, and forereef (Goldman and Talbot 1976; Clarke 1977; Sale and Dybdahl 1978; Alevizon et al. 1985). Using similarly coarse zonal classes, our results confirmed distinct patterns of habitat use by fishes in relation to zonation. Our results were similar to those by Mejía et al. (2000) showing fish assemblages at the atolls of San Andrés and Providencia (Colombia) were stronger governed by reef zones than individual atolls. However, the significant interaction among atoll and reef zone in our data indicated local, atoll-specific influences contributed to variation in fish community composition. This was in line with studies from the near-by Mexican MBRS describing fish diversity as a function of variation in reef geomorphology among distinct sections along the barrier reef (Núñez-Lara, Arias-González, and Legendre 2005; Arias-González, Legendre, and Rodríguez-Zaragoza 2008).

Conspicuously, local atoll effects appeared least important in our study at the outer reef zones i.e., forereefs and westreefs, and most pronounced at lagoonal patches and channels. This may suggest higher homogeneity in habitat characteristics across each of the outer reef zones across atolls. Jordan and Martin (1987) suggested that westreefs ("leeward margins") exhibit the most consistent geomorphological features across the four atolls of the MBRS, including Banco Chinchorro located in Mexico. Furthermore, dominant physical forces such as high wave exposure commonly present across forereefs may have contributed to reduced influences of local effects, whereas environmental conditions among the other zones may appear less variable among different zones and less consistent across atolls. Interestingly,

fish species composition (considering presence/absence) between the two atolls with the most pronounced differences in terms of both species and functional diversity—Glover's Reef and Turneffe Atoll—only significantly differed in terms of their lagoons. This may have been caused by the marked geomorphological variation among atoll lagoons. Lagoon morphology may also influence local environmental conditions; for example, more enclosed lagoons will more likely retain sediments and pollutants than flushed areas (Fabricius 2011) and geomorphology influences salinity gradients among the three atolls (Gischler, 2007). Lagoonal environments appear more susceptible to habitat degradation than exposed reefs and were more impacted by human activities than other reef zones within the Mexican MBRS (Núñez-Lara, Arias-González, and Legendre 2005).

The distribution of fish functional niches varied among reef zones. Both lagoons and backreefs reflected their role as habitat for small to medium sized commercial Lutjanidae but otherwise were functionally more depauperate than the other four zones, which reflected spatial patterns of species diversity. In contrast, channels supported processes such as herbivory, planktivory and those linked to mobile life-styles. While functionally resembling channels, forereefs additionally supported species interactions associated with phoresy. Westreefs emerged as remarkably species-rich fish habitat in our study and showed to serve as nursery for planktivores and live coral associated juveniles. Westreefs supported the highest abundances of large predators in comparison to all other reef types at the limited depth gradient examined here. This was also reflected by the over-occupancy of functions associated with pelagic, commercially important predators and may be explained due to sheltered westreefs being potential *Orbicella* spp. habitats (Chollett and Mumby 2012), which have shown to support the most diverse fish communities in the Caribbean (Mumby et al. 2008). Our finding is relevant in the context that atoll westreefs are largely excluded from no-take conservation areas in current management plans in Belize. This also has previously been highlighted by Acosta et al. (2015) who reported similar observations for the west side at Glover's Reef; however, their study regarded deeper reefs than ours. Our findings underpin their

recommendation that atoll westreefs should be considered in fisheries protection. Furthermore, westreef locations in Turneffe Atoll appeared especially productive, possibly due to mangrove islands providing additional shelter to leeward reefs. These sites were among the most fish species rich and abundant in our study but are to date not protected from fishing in the relatively recently implemented Turneffe Marine Reserve management plan. We further suggest that the western reefs at Turneffe reflect a unique functional role of atoll ecosystems within the MBRS.

Our findings suggest that local processes at the scale of atolls mediate the influence of reef zones on fish communities, which are related to largely universal biophysical forces. This suggests that considering both reef zonation and locality as proxies of biophysical forces and local influences (e.g., fishing, pollution, geographic setting) respectively, may aid in discerning factors influencing fish communities that are in different ways caused by or subjected to human induced environmental change.

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Author contributions

F.C. conceived the study. F.C. and R.F.P. designed the study with input from R.T.G. F.C. conducted the fieldwork. F.C. analysed the data. R.T.G. and F.C. developed the functional analysis with input from R.F.P.; R.T.G. wrote the Matlab code. F.C. wrote the manuscript with input from R.F.P. and R.T.G.

Chapter 3

Metabarcoding reveals dietary versatility of coral reef fishes in response to habitat degradation

3.1 Abstract

The ability of consumers to behaviourally adjust to shifts in resources associated with habitat degradation is a key mechanism promoting population persistence since it determines a given species' adaptive capacity. While generalist feeders are expected to be less vulnerable than specialist feeders to changes in prey availability associated with habitat change, the extent to which species manage to expand or switch diet as a behavioural response to mitigate environmental changes is poorly known. To test how the degradation of coral reef habitat has the potential to directly affect food web linkages and trophic functions of benthic feeding fish, and ultimately their physical condition, we used a DNA-based approach (metabarcoding) to link variation in fish diet to differences in habitat quality across sites on the Caribbean coast of Panama. Furthermore, we studied how fish condition varied as a function of coral cover. Metabarcoding of gut contents of two invertebrate-feeding fish species representing different feeding strategies (*Chaetodon capistratus*, a browser and *Hypoplectrus puella*, an active predator) revealed dietary responses to habitat degradation (i.e., live coral cover) in both species. However, the response was much more pronounced for the browsing species showing a shift from an anthozoan to an annelid dominated diet. Our results indicate the potential for adaptive

capacity in the form of behavioural switches where diet is adjusted in degraded environments according to prey availability.

3.2 Introduction

Behavioural versatility, particularly as it pertains to diet, is important for species to persist despite habitat degradation (MacNally 1995; Wong and Candolin 2015). The ongoing severe decline of coral reefs has shown to affect fish communities beyond well-recognised negative effects on specialist species (Pratchett et al. 2018). Fish adaptive capacity to altered habitats depends on how fishes cope with depleted or alternative sets of resources (Munday 2004; Pratchett et al. 2004; Brooker, Brandl, and Dixson 2016). However, the degree to which species may adjust their diet in response to benthic change remains poorly understood.

Generalised feeding strategies are common among coral reef fishes and may allow fishes to adjust to changes in resource availability (Graham 2007a; Berkstrom et al. 2012). However, alternative prey choice in response to habitat change may entail lowered nutrition and thus reduce fish health condition (Pratchett et al. 2004; Berumen, Pratchett, and McCormick 2005; Hempson et al. 2017) with potential negative consequences for fitness and population persistence (Graham 2007a). The majority of fishes that directly forage within benthic reef habitats on sessile (e.g., corals, sponges) and mobile (e.g., crabs, worms) invertebrates (later referred to as invertivores) are generalist feeders and therefore largely assumed to be unaffected by habitat decline due to their ability to switch diet in response to food availability (Vázquez and Simberloff 2002). Recent research suggests that a more detailed knowledge of dietary resource use is required to understand potential responses to habitat and prey community change in coral reef fish (Harborne et al. 2017; Pratchett et al. 2015; Brandl, Robbins, and Bellwood 2015). First, the realised dietary niches of "generalist" species may be narrower than previously thought (Kramer et al. 2015; Leray, Meyer, and Mills 2015). Second, contrary to expectations, the response of generalist invertivores to habitat disturbance and decline appears

variable (McClanahan et al. 2000; Wilson et al. 2006; Roff et al. 2013), likely as a result of differences in their ability to switch prey or need for a broad diet.

Previous studies looking at responses in fish diet to habitat decline have mostly focused on butterflyfishes (Chaetontidae) that specialise to various degrees on live corals in the Indo-Pacific (Pratchett et al. 2004; Graham 2007a). However, with extreme feeding specialisation being rare (Fox and Morrow 1981) we need to examine the whole spectrum of resource use by fishes in order to better understand ecological processes. Furthermore, it is not known whether and to what degree species' versatility is sufficient to cope with rapid human-induced change. In addition, while generalist behaviour is part of ecosystem tropho-dynamics, extreme dietary switches may alter trophic pathways with unknown consequences for ecosystem functioning.

We here investigated the dietary versatility in two generalist feeding benthic fishes. We selected two common reef fish species that feed on benthic prey and represent two different feeding strategies: the barred hamlet *Hypoplectrus puella* (Cuvier), a small, reef-associated sea bass (Perciformes: Serranidae) that is a generalist, benthic predator (Holt et al. 2008) of mainly crustaceans and to a lesser extent fishes (Randall 1967; Whiteman, Côté, and Reynolds 2007) and forages within the reef structure within small foraging territories (Barlow 1975) (as opposed to other hamlet species, foraging in *H. puella* has not been associated with aggressive mimicry, Puebla 2009); the foureye butterflyfish *Chaetodon capistratus* (Linnaeus), a browser feeding primarily on anthozoans with a preference for scleractinian corals but has shown to complement its diet with other invertebrates such as polychaetes (Birkeland and Neudecker 1981; Liedke et al. 2018). *Chaetodon capistratus* exhibits differences in diet among geographical locations indicating dietary plasticity in response to prey availability or differences in dietary preferences (Lasker 1985). Geographical diet variation has also been observed in hamlets (*H. nigricans, H. chlorus*) although not specifically in *H. puella* (Whiteman, Côté, and Reynolds 2007). Because hamlets exhibit little morphological and ecological variation (Lobel 2011; Hench et al. 2017; Thresher 1978), *H. puella* likely possess comparable capabilities to adjust its diet.

We quantified links between diet (composition and breadth), fish condition, resource availability, and coral cover across a habitat gradient at the Bahia Almirante in Bocas del Toro, Panama. We capitalised on the bay's disturbance history of recent severe hypoxic events leading to the die-off of many benthic organisms including corals (Altieri et al. 2017) with increasing intensity towards the inner parts of the bay. To assess levels of dietary versatility and fine-scale differences in generalist diets with unprecedented taxonomic resolution, we employed DNA-metabarcoding. Metabarcoding of gut contents allows identifying semi-digested as well as soft bodied prey and small or cryptic organisms (meio- and microbiota) (Leray and Knowlton 2015; Chariton et al. 2015) that can remain undetected by conventional methods (Nagelkerken et al. 2009, Berry et al. 2015).

3.3 Methods

3.3.1 Study area

The Bahia Almirante is a large (450 m^2), semi-enclosed coastal lagoon forming part of the Bocas del Toro Archipelago on the Caribbean coast of Panama (Aronson et al. 2014). The bay harbours a diverse coral reef ecosystem characterised by strong environmental forcing that influences the distribution, abundance, and persistence of corals and associated benthic communities (Greb 1996; Seemann et al. 2014; Cortes 2003). Reefs form isolated shallow structures on the slopes of the numerous mangrove islets and larger islands, and to a lesser extent on shoals rising from the lagoon seafloor (Greb 1996). The bay is confined by the mainland and protected from ocean swell by several islands leading to restricted water-exchange with the open ocean. Together with local climatic conditions, this creates an environment with limited water flow, variable salinity (fluctuating locally from 30–34 PSS to 20 PSS) (Kaufmann and Thompson 2005; Collin et al. 2009) and elevated sea surface temperatures during calm weather periods (Altieri et al. 2017; Cramer 2013). While terrestrial run-off naturally elevates nutrients within the bay, both untreated wastewater from tourism development and agricultural discharges intensify eutrophic

levels (Guzmán et al. 2005; D'Croz, Rosario, and Gondola 2005; Cramer 2013; Altieri et al. 2017). Together these factors contribute to occasional reductions in dissolved oxygen levels. In 2010 an unprecedented hypoxic stress event led to drastic coral cover decline and die-off (Altieri et al. 2017), which resulted in a hypoxia-induced gradient of habitat degradation across the bay. Similarly, this gradient represents an exposure gradient from the outer bay to increased levels to land-based pollution at the inner bay (Cramer et al. 2012). We took advantage of this gradient of reef condition formed by hypoxia disturbance to test how reef condition affects diet and condition of two species of reef associated, benthic-feeding fishes. We selected nine discrete reefs that we assigned to one of three reef zones based coral cover data: "outer bay", "inner bay" and "inner bay disturbed". The outer bay zone featured highest coral cover and high topographic complexity, while reefs in the inner bay zone were characterised by intermediate levels of coral cover and coral morphologies of lower architectural profile, and reefs in the inner bay disturbed zone comprised recently dead reefs of very low live coral cover.

3.3.2 Benthic, fish and invertebrate surveys

Benthic cover and reef fishes were surveyed in May and June of 2016 at our nine study reefs. Three replicate transect lines (20 m) per reef were placed parallel to the shore at a depth of 2-4 m. To estimate benthic cover and community composition, ten quadrats (100 x 70 cm) were photograped at two meter increments along each transect (quadrats per site N = 30). We analysed photos using CoralNet (Beijbom et al. 2015) with a stratified random sampling approach consisting of ten rows by ten columns with one point per cell (100 points per photo). Mean cover per reef was quantified at the level of broad taxonomic groups (e.g., hard coral, soft coral, macroalgae, sponge, dead coral, zoanthids, rubble) to avoid potential identification errors arising from variation in image quality. Fish communities were surveyed along a 20 m belt (2.5 m at each transect side) by one experienced surveyor recording the abundance and identity of all non-cryptic fish species using scuba. Diversity and abundance of macro-invertebrates (> 2 millimetres; mm) was assessed using three

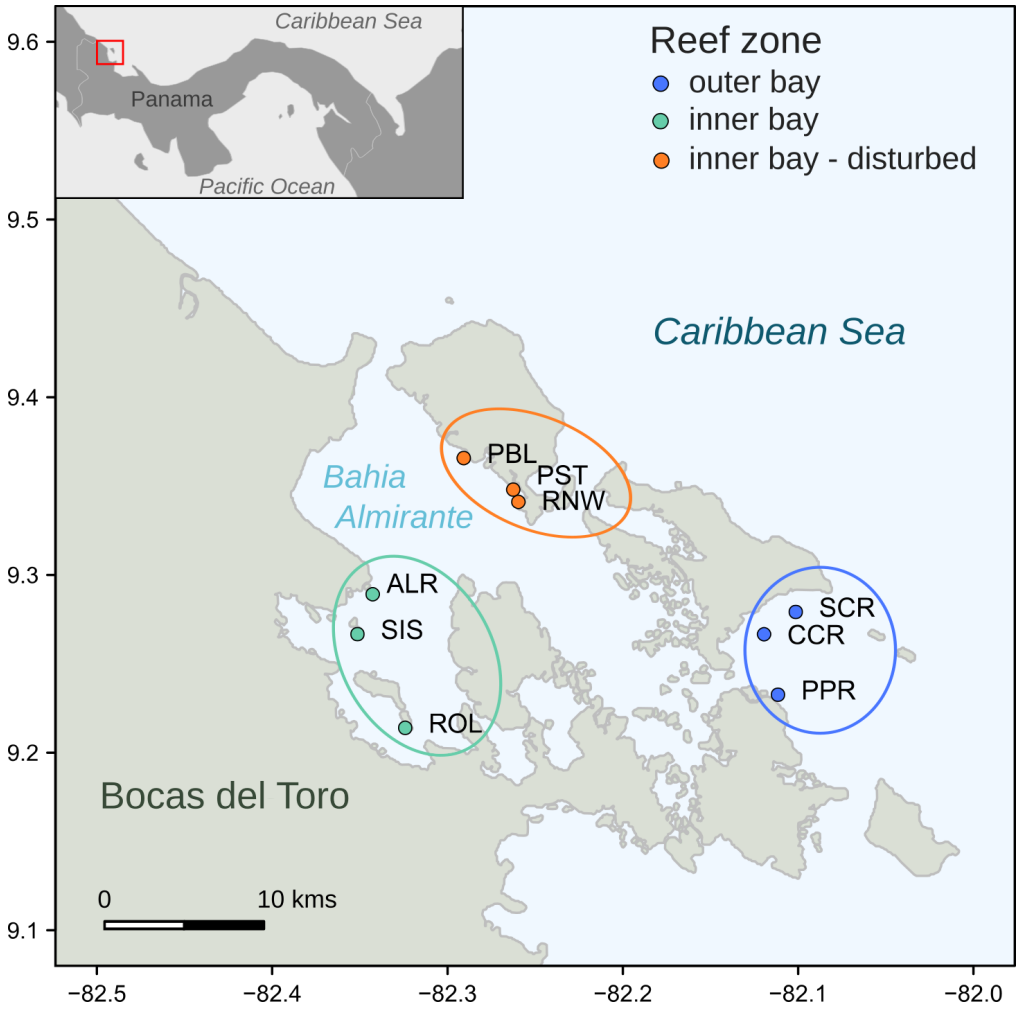

Figure 3.1: Study area at the Bahia Almirante, Bocas del Toro (Panama). Fish were collected at nine reefs across three zones of different levels of coral cover: outer bay (blue), inner bay (green) and inner bay disturbed (orange).

quadrats per reef (0.5 x 0.5 m). Quadrats were placed on continuous surfaces of dead coral (mainly Agaricia tenuifolia) and all coral rubble was carefully collected in bins and transported to the field station. At the laboratory, collected invertebrates were counted and identified to the lowest taxonomic level.

3.3.3 Fish collection

Both study species are common Caribbean reef fishes and their conservation status is of least concern (IUCN; Rocha et al. 2010; Anderson et al. 2015).Twenty adult fishes per species were collected by spearfishing at each of the nine reefs in February and March of 2018 (Fig. 3.1) following protocols approved by the Institutional

Animal Care and Use Committee of the Smithsonian Tropical Research Institute (IACUC). Immediately after capture, each fish was anesthetized on the boat in a sterile and labelled Whirl-Pak bag with seawater and clove oil and subsequently stored on ice. Upon return to the field station, fish weight (g wet weight), Standard Length (mm SL), and Total Length (mm TL) were measured using a digital calliper. At a later stage, further linear body size parameters were obtained after thawing fish [i.e., head length (mm), body depth (mm)]. Each fish was dissected under a laminar flow hood using sterile, DNA de-contaminated tools and gastrointestinal tracts were individually preserved in 96% ethanol and stored at $-20°C$ until DNA extraction. To prevent cross-contamination, sterile gloves were changed after processing each fish and the hood was DNA de-contaminated using 10% Sodium Hypochlorite followed by 90% EtOH. A new set of sterile, de-contaminated tools was used for each fish. To do so, scissors and forceps were put in a 10% Sodium Hypochlorite bath for 15 min, thoroughly rinsed with Milli-Q water and subsequently 70% ethanol and flamed to remove any remaining bleach, water contaminants or tissue.

3.3.4 Prey tissue preparation

Due to different degradation states of prey between stomach and gut potentially leading to amplification biases during PCR, the digestive tracts of both fish species were separated into stomach and gut. In addition, variation in size or biomass among prey remains may inhibit recovery of the complete prey community when extracting DNA in bulk samples of benthic marine communities (Aylagas et al. 2016; Leray and Knowlton 2015). To exclude as much predator tissue as possible from the metabarcoding analysis, the stomachs of *C. capistratus* and intestines of *C. capistratus* and *H.puella* were dissected longitudinally and stomach contents and digesta isolated respectively. In the case of *H. puella*, stomachs contained mainly morphologically identifiable, whole or partial prey organisms; thus, these were excluded from metabarcoding.

Prey tissue was removed from stomachs and digesta and mucosa isolated from guts using sterile and DNA-decontaminated forceps and disposable sterile surgical

blades. Gut mucosa was included here since samples were also used for analysis of bacterial communities with a 16S marker. Isolated stomach contents and digesta were then weighed (wet weight mg) individually on clean, sterile weighing boats on a digital scale. A dissection and extraction blank as negative control was introduced at this step by performing each preparation step with a sample consisting of nuclease-free water. One negative control was included in each set of extractions (~20 samples).

3.3.5 DNA extraction

DNA was extracted using the Qiagen Powersoil DNA isolation kit following the manufacturers instructions with minor modifications to increase the yield. Since the Powersoil kit is designed to counteract potential PCR inhibitors such as humic acids that can induce false negative results (Matheson et al. 2010; Thomsen and Willerslev 2015, Aylagas et al. 2016) it is well-suited for extracting DNA from stomach contents of mixed diet including invertebrates containing high levels of polysaccharides.

Between 0.05 and 0.25 g of prey tissue per sample from *C. capistratus*' stomach contents and between 0.05 and 0.25 g of *H.puella* digesta (gut contents) were added to individual eppendorf tubes containing power beads with bead solution, C1 solution (60 ul) and 20 ul proteinaseK (0.4 mg.mL-1) to enhance the lysis of animal tissue as well as algae. Because yield was low in an initial set of five extractions of gut content samples, we briefly vortexed the eppendorf tubes with digesta before an additional incubation step of 15 min at $60°C$ with 1000 rpm agitation to allow for beginning of tissue lysis by Proteinase K before mechanic disruption by vortexing with beads. Samples were then vortexed for 5 min on the Vortex Genie 2 with vortex adapter followed by a 1:45 hour incubation at $60°C$ with 1000 rpm agitation. The eppendorf tubes containing stomach content samples were vortexed for 5 min using a Vortex Genie 2 (Scientific Industries) with vortex adapter and subsequently incubated at $60°C$ for two hours with agitation (1000 rpm) on an Eppendorf™ Thermomixer™ R. The longer incubation step recommended in previous studies (Leray, Meyer, and Mills 2015; Wangensteen and Turon 2017)

helped lyse hard shelled invertebrates as well as coral and potentially algae. DNA was eluted in 100 ul buffer (C6 solution).

3.3.6 DNA extract quality assessment

DNA extracts were diluted 5 times in nuclease-free water and the molecular weight of the extracted genomic DNA was assessed with electrophoresis of GelRed™-stained DNA on an agarose gel of 1.5%. DNA concentration (ng/ul) was quantified with Quant-iT™ dsDNA High-Sensitivity Assay Kit using a Invitrogen Qubit® Fluorometer (Life Technologies), before storing DNA extracts at $-20°C$.

3.3.7 Metabarcoding

To enable identification of prey items at species levels, we targeted a 313bp fragment of the hyper variable mitochondrial Cytochrome c Oxidase subunit I (mtCOI) gene region with a versatile PCR primer set (mlCOIintF and jgHCO2198, Geller et al. 2013; Leray et al. 2013a) (Table B.1). This primer set was originally designed for the amplification of metazoan DNA, was tested on coral reef fish gut contests (Leray et al. 2013a), and has previously successfully amplified diverse bulk samples of marine benthic taxa as well as provided reliable abundance estimates (Leray and Knowlton 2015). In each PCR reaction, we included consumer-specific annealing blocking primers (Table B.2) (at 10x COI primers) since amplification of consumer DNA can overwhelm the recovery of prey (Vestheim and Jarman 2008). Blocking primer design and thermocycling parameters followed the methods described in Leray et al. (2013b).

Tagging approach

We used matching oligonucleotide indices (Coissac, Riaz, and Puillandre 2012; Binladen et al. 2007) on both forward and reverse primers of each sample to allow for multiplexed sequencing runs and subsequent identification of read-sample affiliations as well as to prevent tag-jumping—a process that may generate spurious assignments of sequence reads to samples (Schnell, Bohmann, and Gilbert 2015;

Alberdi et al. 2017; Caroe and Bohmann 2020). The first tag was introduced at the amplicon PCR stage using indexed primers and a second tag was added via the ligation of single indexed TruSeq adaptors (Y adaptors) during library preparation as detailed in Leray et al. (2016). This resulted in each sample being indexed following the scheme: sample1: Index1-mlCOIF/jgHCO-Adapter 1; sample 2: Index2-mlCOIF/jgHCO-Adapter 2. For each PCR primer, indices of 6bp nucleotide sequences were placed at the 5' end with a minimum of variation in 3bp among primers. Using the same index sequences has previously been shown not to significantly compromise the retrieval of OTUs (Leray and Knowlton 2017).

Amplicon PCRs

Polymerase Chain Reaction (PCR) was carried out for three replicates of each sample to enhance prey detection probability and account for variation in PCR amplifications caused by PCR drift (Leray and Knowlton 2015; De Barba et al. 2014; Alberdi et al. 2017). Per reaction, a total volume of 20 µl comprised of 2 x PCR buffer (Clonetech) with 1.8 mM MgCl2, 3% DMSO, 0.2 mM dNTP, 0.4 Advantage TAQ polymerase (Clonetech), 1 µM of forward and reverse primer respectively (mlCOIintF and jgHCO2198) and 1 ng/µl of DNA template. PCR blank (using 1 ng/µl nuclease free water instead of DNA template) was included in each PCR run and positive controls were included in PCRs of gut samples. PCR thermal cycling conditions consisted of an initial denaturation step of 5 minutes at $95°C$ followed by 38 cycles of $95°C$ (30 seconds), $48°C$ (30 seconds), $72°C$ (45 seconds), with a final 5 minute extension at $72°C$ and a final cooling step of $4°C$. Aliquots of PCR product were diluted five times in nuclease-free water and amplicon size assessed with electrophoresis on 1.5% agarose gel stained with GelRed™. DNA concentration (ng/ul) was quantified using a Qubit Fluorometer (dsDNA High-Sensitivity Assay Kit, Invitrogen, Life Technologies) and PCR product stored at $3°C$ for subsequent clean-up.

PCR clean-up and quantitation

To ensure optimal conditions for downstream preparation of libraries for sequencing, PCR reaction artefacts and impurities such as primer dimers and dntps need to be removed from the PCR product. PCR clean-up was performed using DNA Purification SPRI (Solid Phase Reversible Immobilization) Magnetic Beads (KAPA Pure Beads, Roche) with fragment size selection of desired clean-up targets, which was achieved by using a bead:DNA ratio of 1:1.6. The three PCR replicates generated for each sample were pooled and eluted in 30 µl nuclease-free water and 28 µl purified PCR product obtained per sample. An aliquot per sample of the cleaned PCR product was diluted 10 times (2 µl PCR product and 18 µl of nuclease-free water) and quantified using a Qubit R 2.0 Fluorometer with Qubit R dsDNA HS Assay Kit.

Library preparation

To achieve similar numbers of reads per sample after sequencing, amplicon DNA was normalized at 5ng/µl using nuclease-free water for equimolar concentration among cleaned PCR products for pooling. Equimolar amplicon DNA of samples with each unique tags were then pooled into the same adapter group respectively, for adapter ligation. Adapters consist of short sequences of few nucleotides that enable DNA fragments to bind to flow cells on the sequencing platform. Using adapter ligation prevents the need of additional PCR cycles, which would represent additional sources of bias. TruSeq DNA PCR-free LT library Prep Kit (Illumina) was used for the following library preparation steps. First, end repair was performed to convert damaged DNA fragments (e.g., resulting from PCR or repeated freezing and thawing) incompatible protruding ends (5L- and/or 3L-) to 5L-phosphorylated and 3L-hydroxyled, blunt-ended fragments. Then, an A homopolymeric nucleotide was added to the 3' end of the double stranded, blunt ended DNA molecule via enzymatic reaction to prepare for ligation of adapters with 3'dT overhangs. Y-adapters were ligated and adapter pools diluted to 10ng/µl using resuspension buffer. 4µl of each 10ng/µl adapter pool was pooled into the same tube for the the final library and paired end sequenced on an Illumina Miseq.

Sequence analysis

After demultiplexing, sequence reads were adapter-, primer- and quality-trimmed with Flexbar (version 3.0.3, Roehr, Dieterich, and Reinert 2017). Subsequently, sequences were filtered, chimera-checked, and processed into amplicon sequence variants (ASVs) with DADA2 (Callahan et al. 2016). ASVs were then clustered with VSEARCH (Rognes et al. 2016) at a 97% identity threshold into OTUs to approximate biological species. OTU were curated with the LULU algorithm (Frøslev et al. 2017) by reducing taxonomic redundancy and enhancing the richness estimate accuracy (LULU parameters: minimum ratio type = "min", minimum ratio = 1, minimum match 84, minimum relative co-occurrence = 0.95). OTUs were assigned taxonomy using the Bayesian Least Common Ancestor (BLCA) taxonomic classifier (Gao et al. 2017) against the Midori-Unique v20180221 database (Machida et al. 2017), which is a curated metazoan COI sequence library (available at www.reference-midori.info). We omitted all BLCA taxonomy assignments of less than 50% confidence. Unassigned OTUs were blasted (BLAST searches, word size = 7; max e-value = 5e-13) against the whole NCBI NT database (retrieved May 2018) and the lowest common ancestor of the top 100 hits was used to assign taxonomy. The taxonomy assignment results and LULU-curated OTU table were analysed in R (R Development Core Team, 2008).

3.3.8 Statistical analyses

Benthic and fish surveys

Differences in benthic composition among three reef zones were visually assessed using Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity (Bray and Curtis 1957). We plotted eigenvectors depicting the relative contribution of benthic groups to separation among zones. To test for significance in differences among mean percentage coral cover among zones, we used Kruskal Wallis tests (Kruskal and Wallis 1952). Fish and invertebrate communities were compared among zones using Nonmetric Multidimensional Scaling (NMDS) (Clarke and Warwick 2001) based on Jaccard similarity (Jaccard 1912) for presence absence

data and Bray-Curtis dissimilarity for abundances. Plots were generated of fish focal species abundance across zones using line graphs.

Diet composition

Dietary composition for both fish species was visualised with stacked bar charts of prey relative abundances using the phyloseq package version 1.30-0 (McMurdie and Holmes 2013). To examine differences in fish dietary composition among reef zones, we used NMDS ordination based on Bray Curtis dissimilarity (MASS package v7.3.51.6, Riplley et al. 2002).

Fish length-weight relationships and condition

We first modelled the length-weight relationship for the whole dataset using linear regression (lm function, FSA R package v 0.8.30, Ogle, Wheeler, and Dinno 2020)

$$logW \backsim logL$$

where L and W are the respective natural log transformed fish total length (mm) and weight (g). To assess whether fish length-weight relationships followed an allometric or isometric growths pattern, we tested the hypothesis that the slope of the fitted regression models was not equal to three (b 3) (hoConf function, FSA R package v 0.8.30). To compare fish condition (e.g., the relative 'plumpness' of a fish in relation to a given length—with plumper fish of a given length assumed to be in better condition, Tesch 1968; Froese 2006) among zones, we calculated the relative condition factor (Kn) (Cren 1951) by estimating the deviation between the observed weight to the predicted length-specific mean weight of the population (Blackwell 2000, Froese 2006)

$$K_n = \frac{W}{aL^{b\prime}}$$

where a and b are the species- and population specific length – weight parameters obtained from the length-weight regression, L is the natural log transformed observed total length (mm) and W the natural log transformed observed weight. Due to the local scope of our study focusing on small-scale spatial differences among fish

subpopulations within species, the relative condition measure was used as opposed to the relative weight (wr), the latter of which is based on standard weight developed across populations (Blackwell, Brown, and Willis 2000). To assess how fish condition varied across zones, we plotted relative fish condition b' against fish size classes grouped by zone. Lastly, we assessed whether the slopes of the regression differed among zones by modelling the interaction between fish total length and zone in affecting the length-weight relationship

$$logW \backsim logL * Zone$$

One-way Analysis of Variance (ANOVA) was used to determine whether slopes differed significantly (anova function, FSA R package v 0.8.30; Ogle, Wheeler, and Dinno 2020)

Statistical models

We used generalized linear mixed effects models (GLMMs) with a negative binominal distribution (function glmer.nb, lme4 package v1.1-21, Bates et al. 2015) to test for effects of percent coral cover and position (inside versus outside of the bay) on sequence relative read abundance of dominant diet categories as identified prior by metabarcoding for *C. capistratus* (annelids and hard corals) and *H. puella* (benthic and planktonic crustaceans). In addition, we tested whether coral cover predicted fish and sessile invertebrate prey as well as parasite load in *H. puella*. According to our study design, we included the random effect of zone, with reef nested within zone. The distribution of data was checked using histograms and transformed to optimise models i.e., hard coral was square root-, annelid fourth root-, and crustaceans log transformed. Akaike information criterion (AICc) (aictab function, AICcmodavg v2.2-2, Mazerolle 2019) was used to pre-select models. Final selection was based on likelihood ratio tests (function anova, lme4 package v1.1-21, Bates et al. 2015) testing the significance of the predictor variables against null-models. Data were visualised using q plots and model fit was examined with Q-Q plots.

Diet strategy

To characterise the feeding strategy of both fish species in terms of how specialised or generalised the diet appears on the population level, we used a graphical analysis proposed by Amundsen et al. (Amundsen, Gabler, and Staldvik 1996) modified from Costello (1990). To generate diagrams representing feeding strategy and prey importance at three reef zones, frequency of occurrence was calculated as the percentage of fish individuals in which a prey item is present against the total number of fish. Prey specific abundance was calculated as the percentage of the diet that a food item represents across only those fish individuals where it was present

$$P_i = (\sum S_i / \sum S_{it}) * 100$$

where P_i is the prey-specific abundance of prey i, S_i is the abundance prey i in the stomach (or gut) content and S the total prey abundance in only those consumers where prey i is present. Because a species generalist diet profile may arise from either broad individual diets and/or high variation in diet composition among individuals (Bolnick et al. 2003; Amundsen, Gabler, and Staldvik 1996), Amundsen's method includes an indirect measure of the contribution to niche width of both within individual variation (within phenotype component, WPC) and variation among individuals (between phenotype component, BPC).

3.4 Results

3.4.1 Benthic, fish, and invertebrate surveys

Benthic composition and amount of live coral cover differed among the nine study reefs and the three reef zones. Reefs located at the outer bay featured the highest levels of live coral cover (mean cover per transect: SCR 37.1%, PPR 33.0%, CCR 29.3%; Fig. 3.2) and coral diversity (Shannon diversity) and were dominated by stony coral species (i.e., *Acropora cervicornis*, *Agaricia tenuifolia*) and fire corals (i.e., *Millepora alcicornis*, *Millepora complanata*). Live coral cover was lower at inner bay reefs (mean cover per transect: ALR 21.21%, SIS 13.33%, ROL 9.4%;

Fig. 3.2) and dominated by lettuce coral *Agaricia tenuifolia* but cover of sponges was high (mean sponge cover per transect: ALR 23%, SIS 18.5%, ROL 34.23%). The inner bay disturbed zone showed the lowest levels of live coral cover (mean cover per transect: RNW 0.77%, PST 0.27%, PBL 0%; Fig. 3.2) with a high proportion of dead coral (mean cover per transect: RNW 45.3%, PST 21.4%, PBL 53.6%) and similar high levels of sponges to the inner bay (mean cover per transect: RNW 27.33%, PST 21.33% and PBL 21.93%). Nonmetric multidimensional scaling (NMDS) of fish communities showed no separation between the outer and inner bay zone but the inner bay disturbed zone appeared distinct (NMDS; Bray Curtis dissimilarity) (Fig. B.4A). Abundance levels of both fish species appeared overall similar among reef zones (<5 individuals per transects) with highest abundances at the inner bay zone for both species (Fig. B.2A and B.2B). Invertebrate communities (>2 mm) did not significantly differ significantly among the three reef zones (NMDS, Fig. B.4B). However, crustaceans in the family Mithracidae, which were important diet items of *H. puella*, showed significantly lower abundances at the inner bay disturbed zone (Fig. B.3B).

3.4.2 Fish length-weight relationship and condition

Chaetodon capistratus total length (TL) ranged from 53.49 to 98.19 mm (mean ± SD = 79.99 ± 10.65) and wet weight (W) ranged from 5.34 to 34.40 gr (mean ± SD = 17.93 ± 7.19). *Hypoplectrus puella* total length (TL) ranged from 56.73 to 125.23 mm (mean ± SD = 91.35 ± 8.96) and wet weight (W) ranged from 3.01 to 23.49 gr (mean ± SD = 14.67 ± 3.91). One-way ANOVA showed that fish length and weight differed significantly among zones for both species (ANOVA; *C. capistratus* $F = 3482.79$, $p <$ 2e-16; *H. puella* $F = 1736.52$, $p <$ 2e-16) with *C. capistratus* being of largest size and heaviest at the inner bay zone (TL mean ± SD = 87.00 ± 10.26; W mean ± SD = 23.04 ± 6.71) and smallest and lightest at the inner bay disturbed zone (mean ± SD = 73.17 ± 9.92; W mean ± SD = 13.57 ± 6.46) with intermediate values at the outer bay zone (mean ± SD = 80.33 ± 6.99; W mean ± SD = 17.59 ± 5.16). *Hypoplectrus puella* was largest and heaviest at the inner bay disturbed

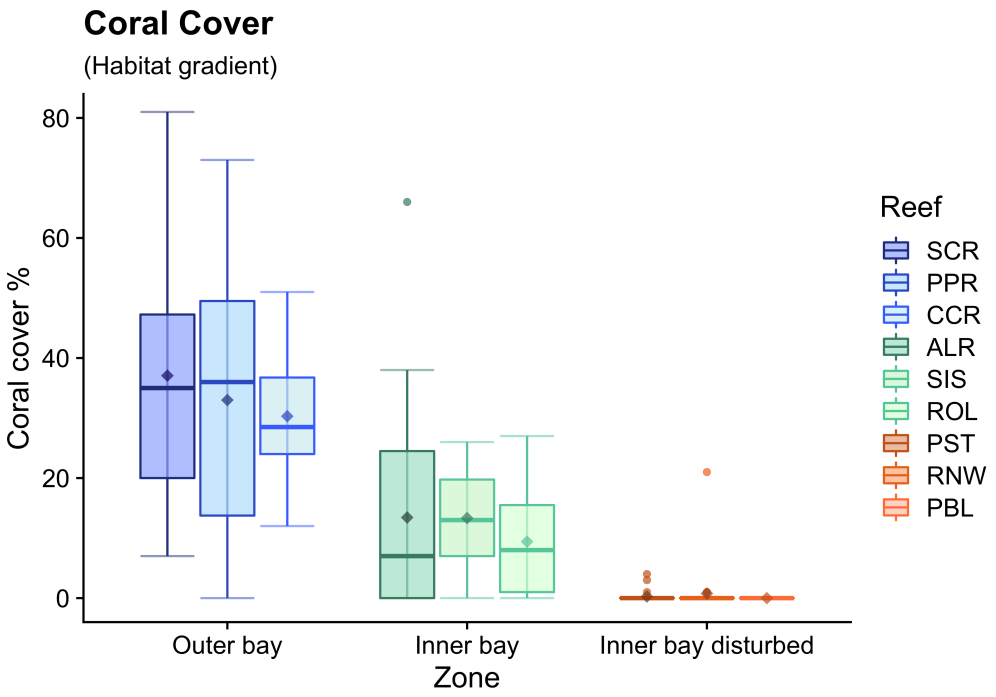

Figure 3.2: Percent live hard coral cover across the habitat gradient from high coral cover (outer bay zone) to low coral cover (inner bay disturbed zone). Diamonds depict means across transects per reef.

zone (TL mean ± SD = 94.07 ± 6.56; W mean ± SD = 15.98 ± 3.08), whereas individuals were slightly smaller and lighter at the inner bay (TL mean ± SD = 92.44 ± 9.01; W mean ± SD = 14.13 ± 4.97) and outer bay zone (TL mean ± SD = 93.91 ± 9.71; W mean ± SD = 13.23 ± 4.31). We found a significant interaction between fish total length and zone in affecting the length-weight relationship for both species (ANOVA; *C. capistratus* $F = 3.383$, $p = 0.037$; *H. puella* $F = 4.546$, $p = 0.012$). The relative fish condition factor (Kn) across fish size classes varied by zone (Fig. 3.3B, 3.4B). *Chaetodon capistratus* showed a greater variability in condition within and among size classes at the inner bay disturbed zone where it also featured the most size classes (class 1-9) compared to the other zones. At the inner bay zone, condition dropped sharply below the optimum value of 1 for the second largest size class (size class 8), contrasting smaller size classes (classes 3-5) with mean condition factors above 1, and the largest size class (9) was not observed at this zone. The outer bay zone showed the most consistent condition values across size classes in *C. capistratus* (Fig. 3.3B). Unlike *C. capistratus*, *H.*

puella featured the greatest spectrum of size classes at the outer bay (Fig. 3.4B). Fishes of largest size classes appeared in better condition at the outer bay than at both zones inside of the bay (Kn > 1). Fish condition was similar at inner bay and inner bay disturbed zones; however, only five of ten size classes were present at the inner bay disturbed zone (Fig. 3.4B).

3.4.3 Diet composition

Sequence Analysis

A total of 18,427,824 raw paired-end reads were recovered. After denoising, removing chimeras, and processing, retained high quality reads resulted in 1009 OTUs assigned to the kingdom of Metazoa, of which 166 (16.5%) were matched to species level in the Midori reference library and Genbank (>97% similarity). An additional 613 OTUs could be assigned to higher taxonomic levels. The extraction and PCR controls did not show contamination.

Chaetodon capistratus

Non-metric multidimensional scaling (NMDS; Clarke and Warwick 2001) of sequence read relative prey abundance data showed clear separation among reef zones of varying coral cover: high coral cover reefs at the outer bay grouped together and were clearly separated from the group of the most degraded lagoonal reefs (Fig. 3.5). Thus, the NMDS ordination of *C. capistratus*' diet composition reflected the habitat gradient (Fig. 3.5). Barplots exploring diet composition by phylum among sites across the habitat gradient showed diet was dominated by cnidarians at high coral cover sites whereas diet was dominated by annelids at the most degraded sites with less dietary preference apparent at intermediate sites (Fig. 3.7). The predominant dietary pattern across the habitat gradient described a switch between phyla from a cnidarian-dominated diet towards an annelid-dominated diet. *Chaetodon capistratus*' dietary composition of cnidarian taxa shifted across the habitat gradient: at the high coral cover zone *Porites* sp. together with soft corals were prevalent, while anemones and *Porites* sp. were dominant in the diet inside of the bay. There were

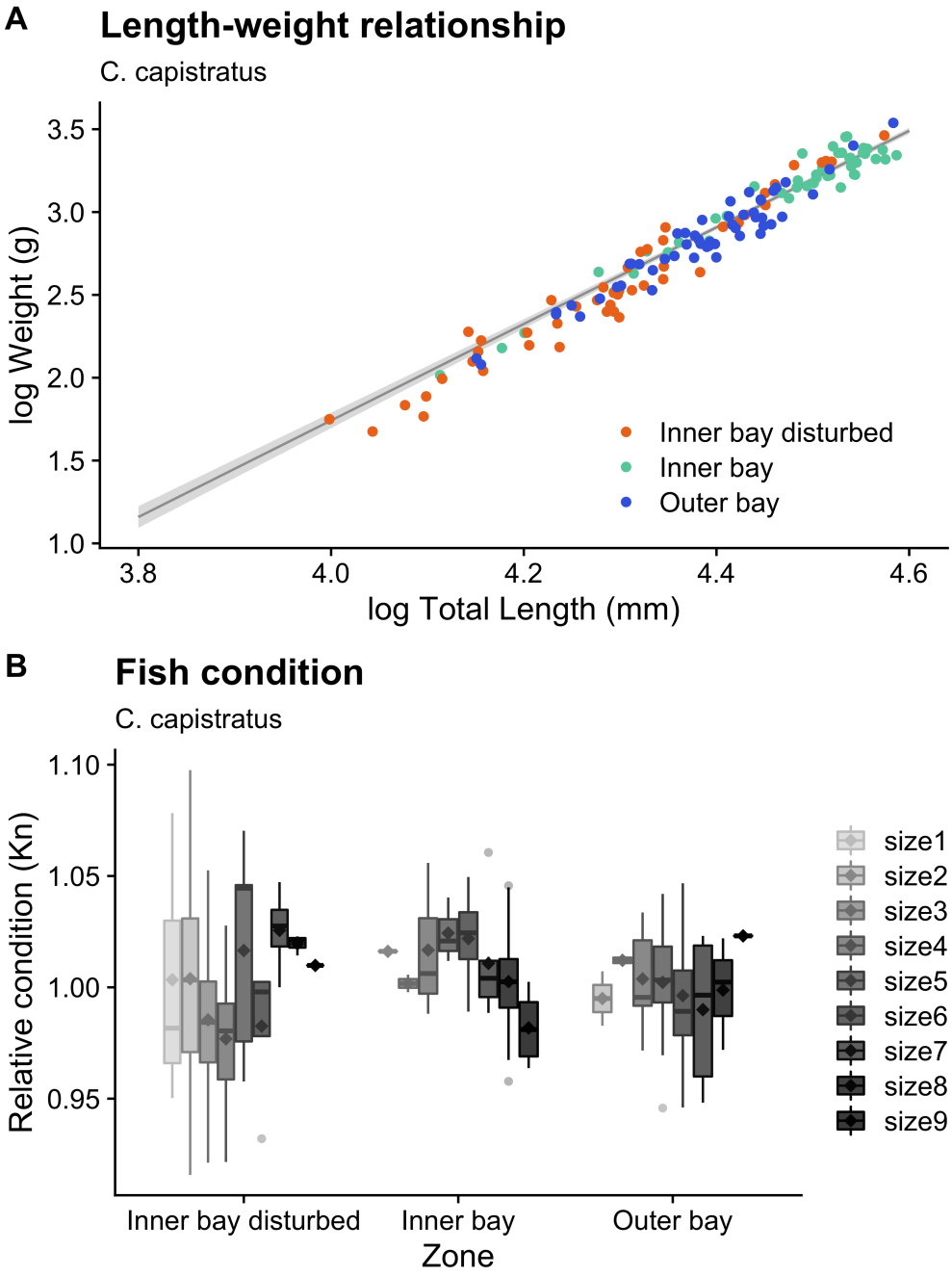

Figure 3.3: Length-Weight regression for *C. capistratus* (A) and Condition Factor (Kn) (B) across reef zones by fish size classes from smallest (1) to largest (9).

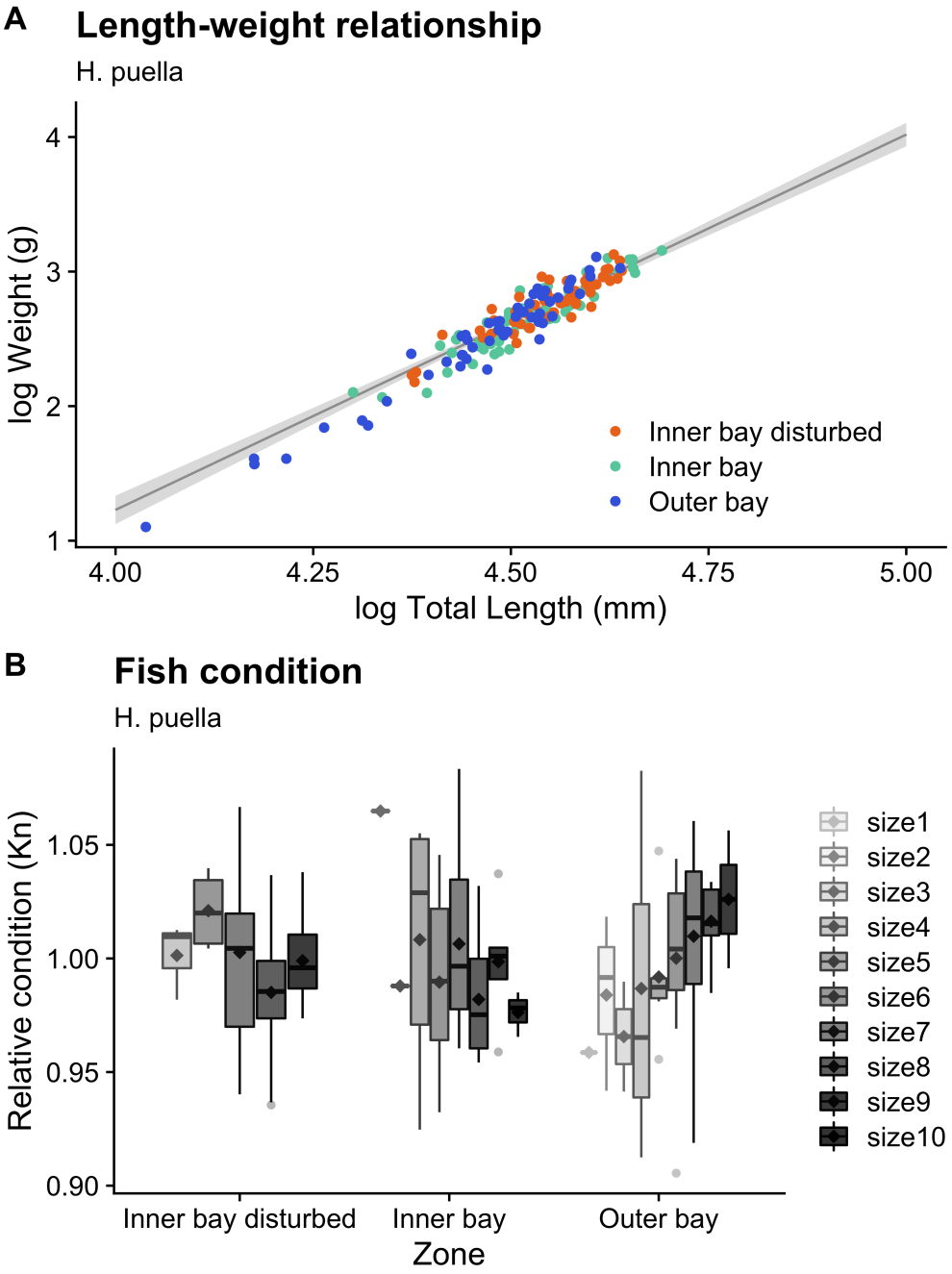

Figure 3.4: Length-Weight regression for *H. puella* (A) and Condition Factor (Kn) (B) across reef zones by fish size classes from smallest (1) to largest (9).

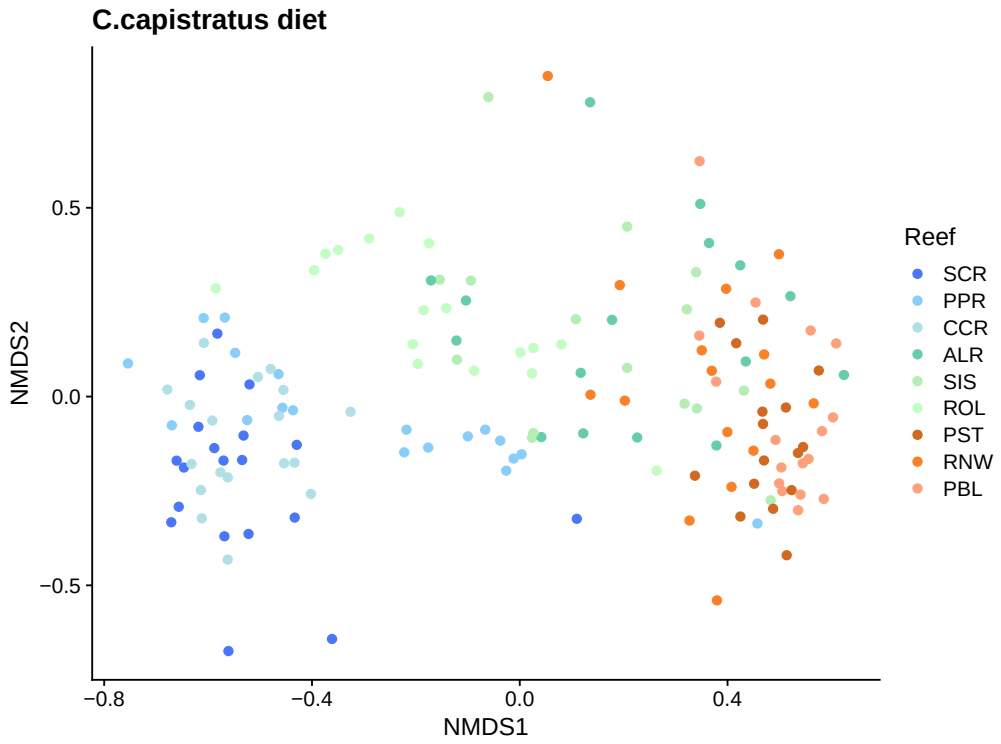

Figure 3.5: Differences in fish diet across sites for *C.capistratus*. Nonmetric multidimensional scaling plot (NMDS) is based on Bray-Curtis distance matrices between individual fish. Dots depict fish individuals; reef zones are coded by colour: blue = outer bay, green = inner bay, and orange = inner bay disturbed.

low proportions of reads belonging to coralimorpharia and zoantharia, and soft corals were in negligible proportions in diets of fish at the inner bay.

Hypoplectrus puella

The NMDS showed a similar but less pronounced separation of prey taxa relative abundances among reefs and reef zones for *Hypoplectrus puella* (Fig 3.6). Outer bay reefs separated from the inner bay disturbed and inner bay zone; however, there was no separation between two zones located inside of the bay. At the phylum level, diets were dominated by arthropods across all reefs and zones. However, differences in diet composition among reefs and zones emerged at lower taxonomic levels. At the order level, differentiation among reefs became apparent within Arthropoda, with more copepods being consumed inside of the bay and more decapods at the high coral cover outer bay reefs. When comparing arthropods in the diet of *H. puella* across reefs at the genus level, macro-crustacean dominated diet at outer bay reefs and a planktonic

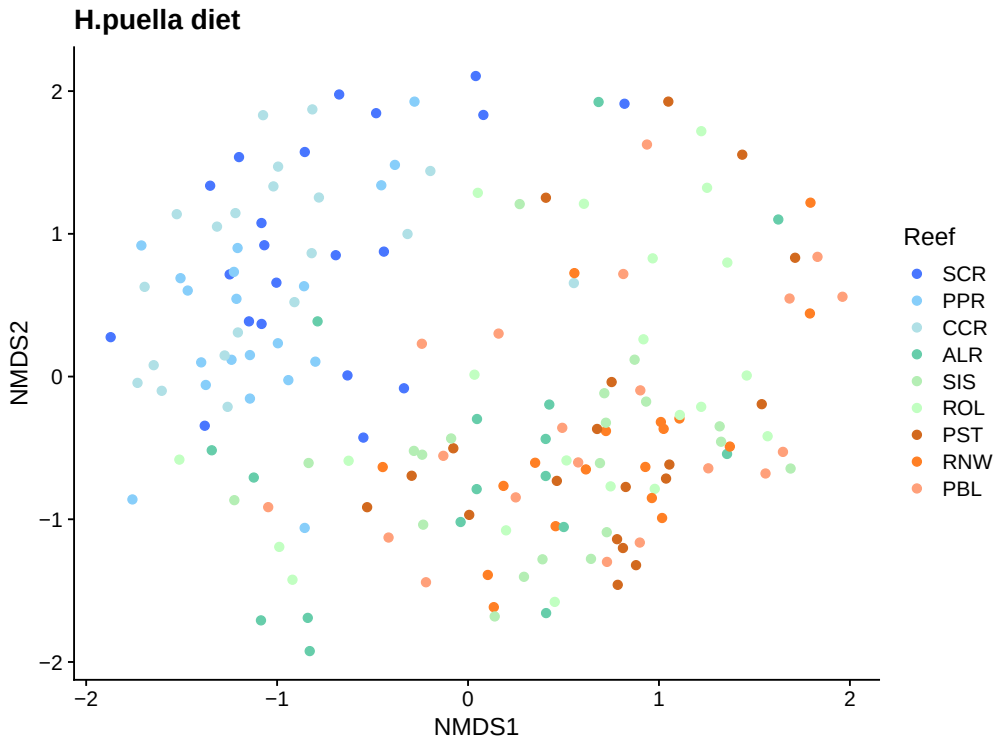

Figure 3.6: Differences in fish diet across sites for *H. puella*. Nonmetric multidimensional scaling plot (NMDS) is based on Bray-Curtis distance matrices between individual fish. Dots depict fish individuals; reef zones are coded by colour: blue = outer bay, green = inner bay, and orange = inner bay disturbed.

micro-crustacean dominated diet across the inner bay and inner bay disturbed zones (Fig. 3.8). Diets within the bay had a large proportion of the copepod *Temora stylifera*, whereas diets at outer bay reefs were dominated by the genus *Mithraculus* and to a lesser extent *Leptochelia*, and *Pseudosquilla* as well as smaller proportions of *Sicyonia*, *Panoplax*, *Synalpheus*, and *Neogonodactylus* (Fig. 3.8).

3.4.4 Effects of coral cover on fish diet

Coral cover had a significant effect on the relative abundance of hard corals ($\chi^2 = 8.58$, $p = 0.003$) and annelids in the diet of *C. capistratus* ($\chi^2 = 5.94$, $p = 0.015$) (Fig. 3.9A and 3.9B, Table 3.2). Reef position (outside versus inside of the bay) also affected the butterflyfish diet (hard coral, $\chi^2 = 4.46$, $p = 0.035$; annelid, $\chi^2 = 7.64$, $p = 0.006$). Likewise, the interaction between position and coral cover was significant for both corals and annelids in the diet of *C. capistratus*, indicating

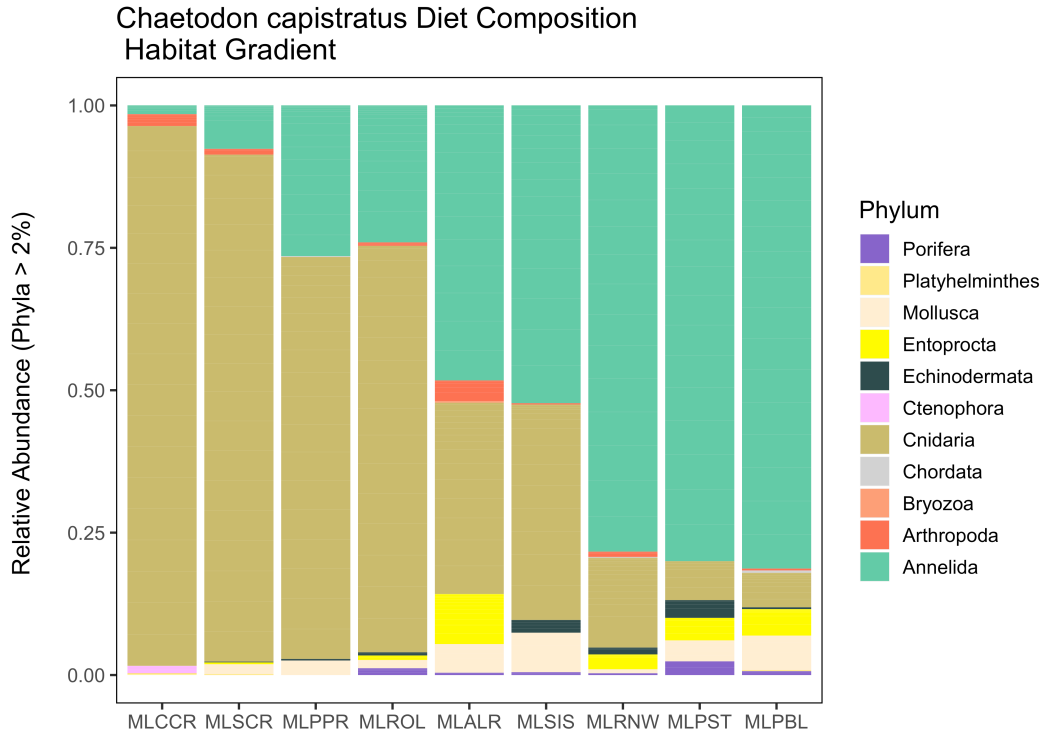

Figure 3.7: Variation in diet composition across the habitat gradient for *Chaetodon capistratus* by phylum. MLCCR = high coral cover – MLPBL = low coral cover

that the effect of coral cover differed if inside or outside the bay (hard coral, $\chi^2 = 9.87$, $p = 0.02$; annelid abundance, $\chi^2 = 8.56$, $p = 0.036$).

Coral cover did not predict benthic arthropods, the dominant diet item in the gut of *H. puella*, ($\chi^2 = 1.73$, $p = 0.188$) (Fig. 3.9C, Table 3.2). In contrast, coral cover predicted the consumption of planktonic arthropods ($\chi^2 = 4.62$, $p = 0.032$) (Fig. 3.9D, Table 3.2). Position across the bay had no effect on the consumption of benthic or planktonic arthropods but the amount of sessile invertebrates in the guts of *H. puella* was predicted by position ($\chi^2 = 3.89$, $p = 0.049$), whereas coral cover had no significant influence ($\chi^2 = 1.66$, $p = 0.297$) (Table 3.2). We found no significant relationship between the abundance of fishes consumed by *H. puella* with coral cover or position; this was also the case for parasite DNA detected within the guts of *H. puella* (Table 3.2).

Class	Order	Family	Genus	Species	Common name
Actinopteri	Acanthuriformes	Acanthuridae	Acanthurus	Acanthurus chirurgus	doctor fish
Actinopteri	Kurtiformes	Apogonidae	Phaeoptyx	Phaeoptyx xenus	sponge cardinalfish
Actinopteri	Kurtiformes	Apogonidae	Phaeoptyx	Phaeoptyx pigmentaria	dusky cardinalfish
Actinopteri	Blenniiformes	Blenniidae	Hypleurochilus	Hypleurochilus geminatus	crested blenny
Actinopteri	NA	Centropomidae	NA	NA	snook
Actinopteri	Blenniiformes	Chaenopsidae	Acanthemblemaria	Acanthemblemaria chaplini	papillose blenny
Actinopteri	Blenniiformes	Chaenopsidae	Emblemariopsis	Emblemariopsis arawak	araw glass blenny
Actinopteri	Gobiiformes	Gobiidae	Coryphopterus	Coryphopterus glaucofraenum	bridled goby
Actinopteri	Gobiiformes	Gobiidae	Elacatinus	Elacatinus illecebrosus	barsnout goby
Actinopteri	Gobiiformes	Gobiidae	Coryphopterus	Coryphopterus personatus	masked goby
Actinopteri	Gobiiformes	Gobiidae	Coryphopterus	Coryphopterus eidolon	pallid goby
Actinopteri	Gobiiformes	Gobiidae	Risor	Risor ruber	tusked goby
Actinopteri	Gobiiformes	Gobiidae	Gnatholepis	Gnatholepis thompsoni	goldspot goby
Actinopteri	Lutjaniformes	Haemulidae	Haemulon	Haemulon macrostomum	spanish grunt
Actinopteri	Lutjaniformes	Haemulidae	Haemulon	Haemulon steindachneri	latin grunt
Actinopteri	Gymnotiformes	Hypopomidae	Brachyhypopomus	Brachyhypopomus occidentalis	bluntnose knifefish
Actinopteri	Labriformes	Labridae	Sparisoma	Sparisoma chrysopterum	redtail parrotfish
Actinopteri	Blenniiformes	Labrisomidae	Starksia	Starksia occidentalis	occidental blenny
Actinopteri	NA	Sciaenidae	NA	NA	drum
Actinopteri	Perciformes	Serranidae	Serranus	Serranus flaviventris	Twinspot bass
Actinopteri	Blenniiformes	Tripterygiidae	Enneanectes	Enneanectes altivelis	lofty triplefin

Table 3.1: Fishes identified in the diet of *Hypoplectrus puella* (including only those OTUs that have been identified to at least family level, 41% of all 51 OTUs assigned to Actinopteri).

Species	Model	Response (diet)	Predictor	Random Effect	*X2*	*P*
Chaetodon capistratus	1	Annelid	Coral cover	Zone/Reef	5.94	**0.015**
	2	Annelid	Position	Zone/Reef	7.64	**0.006**
	3	Hard coral	Coral cover	Zone/Reef	2.39	0.122
	4	Hard coral	Coral cover	Reef	8.58	**0.003**
	5	Hard coral	Coral cover	Zone	5.38	*0.02*
	6	Hard coral	Position	Reef	4.46	**0.035**
Hypoplectrus puella	7	Benthic arthropod	Coral cover	Zone/Reef	1.73	0.188
	8	Benthic arthropod	Position	Zone/Reef	1.66	0.197
	9	Planktonic arthropod	Coral cover	Zone/Reef	4.62	**0.032**
	10	Planktonic arthropod	Position	Zone/Reef	1.55	0.214
	11	Sessile invertebrates	Coral cover	Zone/Reef	1.66	0.297
	12	Sessile invertebrate	Position	Zone	3.89	**0.049**
	13	Fish	Coral cover	Zone/Reef	0.77	0.38
	14	Parasite	Coral cover	Zone/Reef	0.02	0.885

Table 3.2: Results of general linear mixed effects models examining the effect of coral cover on different prey items in the diets of *Chaetodon capistratus* and *Hypoplectrus puella*. Coral cover accounted for variation in both the hard coral and annelid diet of *C. capistratus* and the consumption of pelagic arthropods in the diet of *H. puella*. Significant effects are depicted in bold.

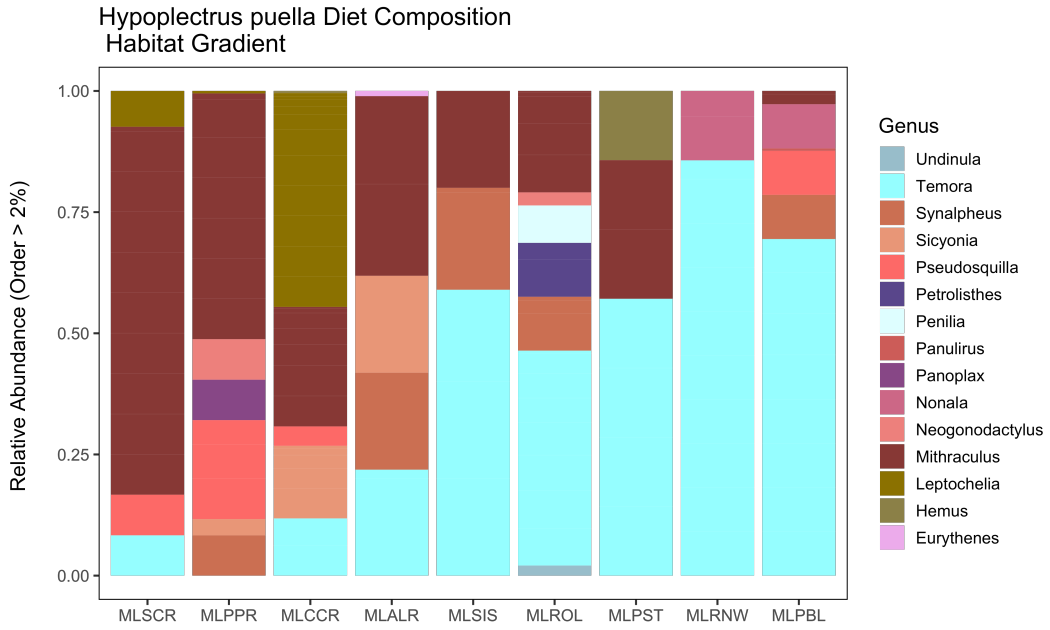

Figure 3.8: Variation in diet composition across the habitat gradient for *Hypoplectrus puella* at the genus level within arthropods, their main prey target group. MLCCR = high coral cover – MLPBL = low coral cover

3.4.5 Diet strategy

Chaetodon capistratus

Amundsen plots of fish diet strategy across zones indicated that the diet of *C. capistratus* was dominated by very few dominant prey items indicated by points located in the middle to upper right corner of the plots (Fig. 3.11A, 3.11B, 3.11C). However, this relatively specialised diet was complemented by a diverse array of occasional prey items that were consumed in low abundance (lower left corner of the plot). According to the Amundsen plots, *C. capistratus* appears as a facultative specialist. Across the habitat gradient, *C. capistratus* switched its main diet item from hard coral i.e., *Poritis* sp. (phylum Cnidaria) at the outer bay zone (Fig. 3.11A, 3.11B, 3.11C), to a mix of *Poritis* sp. and a sessile worm, *Loima medusa* (phylum Annelida) at the inner bay zone (Fig. 3.11B), towards a diet dominated by *Loima medusa* at the inner bay disturbed zone (Fig. 3.11C). The observed switch in specialisation entailed a decrease in the within

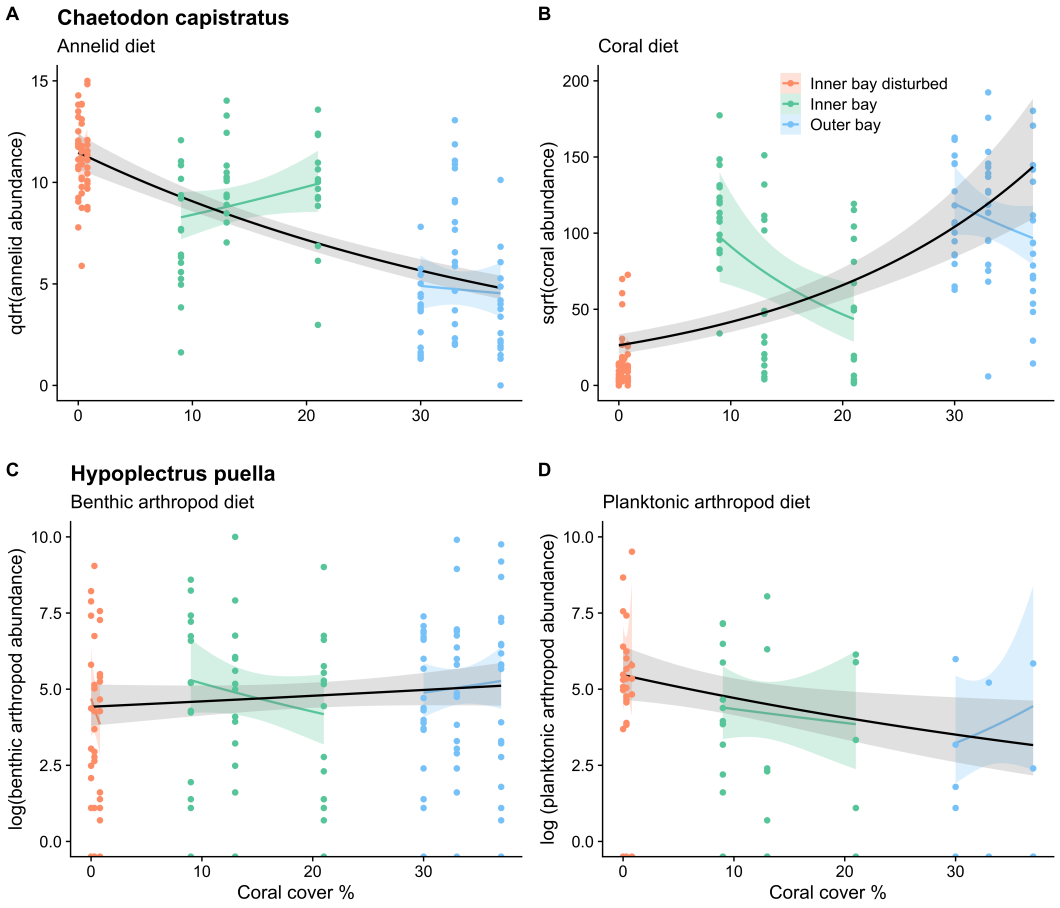

Figure 3.9: Negative binomial generalized linear mixed-effects models (GLMMs) were fitted to predict the effect of percent coral cover on the amount of dominant diet items consumed by two fish species; *C. capistratus* feeding on annelids (A) and hard coral (B) and *H. puella* feeding on benthic arthropods (C) and planktonic arthropods (D). Smoothed black lines depict the overall trends across the coral cover gradient while coloured lines represent variability within each reef zone; the contrasting patterns between black and coloured lines suggest the presence of scale-dependent trends in prey consumption. Annelid read abundance was fourth root transformed, hard coral read abundance was square root transformed and reads of both arthropod groups were log transformed to improve model fitting.

phenotype component (WPC component) indicating that the diet was less diverse within individuals at the disturbed zone and more specialised in comparison with both other zones (Fig. 3.11C).

Hypolectrus puella

H. puella displayed an arthropod dominated, generalist diet across zones (Fig. 3.11D,3.11E,3.11F). At the outer bay, some arthropods (e.g., crabs in the genus

Mithraculus) appeared to be consumed frequently and in large quantities (25-50%) relative to other prey items, but this was not as apparent at both zones located inside of the bay (<25%) (Fig. 3.11D,3.11E,3.11F). In contrast, the frequency of micro-crustaceans in the diet increased across the inner bay dominated by copepods in the genus *Temora* (Fig. 3.11E,3.11F). At both inner bay zones, we found an increase of the between phenotype component (BPC component) indicating an increase in individual specialisation and a broader diet on the population level at these reefs as opposed to the outer bay reefs. There was also slightly more fish consumed at reefs located inside of the bay (Fig. 3.11E,3.11F).

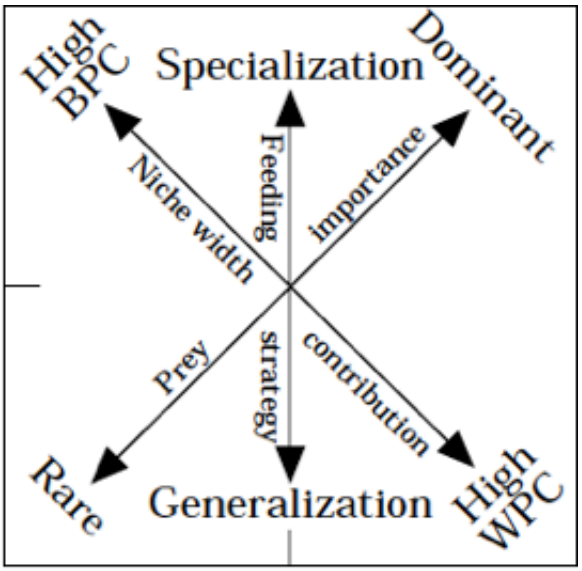

Figure 3.10: Schematic feeding strategy diagram obtained from Amundsen et al. 1996. The diagram illustrates how niche width contribution (feeding strategy) and prey importance is inferred from i) the vertical axes indicating specialisation (upper part) and generalisation (lower part) and ii) the diagonal axes representing the WPC (within phenotype component, lower right corner) and BPC (between phenotype component, upper left corner) indices. For example, if there are no prey points in the upper right of the diagram and all prey points are located along or below the diagonal from the upper left to the lower right, the predator population is considered to have a broad dietary niche width.

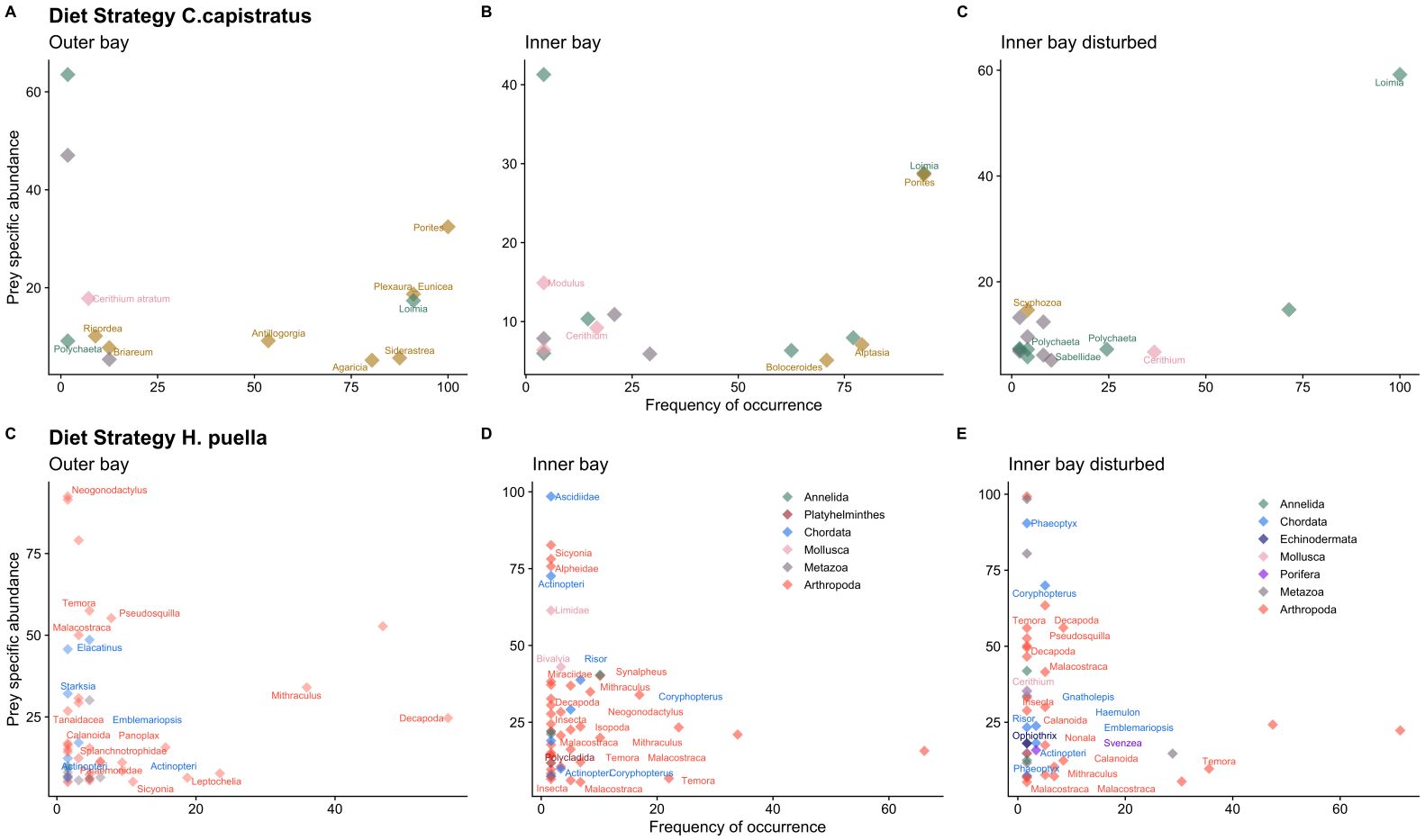

Figure 3.11: Diet strategy of *C. capistratus* (A) and *H.puella* (B) at three reef zones. Points represent prey OTUs. Points located in the far upper right indicate a specialised diet at the population level (abundant and frequent diet items). Points in the lower left corner indicate opportunistic, occasional diet items that are found rarely and in low abundance in the diet.

3.5 Discussion

Here we showed that the diet composition of two invertivorous coral reef fish species (*Chaetodon capistratus*, *Hypoplectrus puella*) is triggered by a change in the proportion of live coral cover despite their relatively generalised feeding strategies and tendency to feed on different components of the community. The browsing, sessile invertebrate feeder *C. capistratus* gradually switched from a diet dominated by cnidarians on the healthier reefs (~30% live coral cover) to a diet dominated by annelids on severely degraded reefs (~0% live coral cover). *Hypoplectrus puella*, a predator of predominantly mobile invertebrates, also showed dietary changes associated with the proportion of coral cover. It shifted from a diet primarily composed of macro-crustacean on outer bay reefs (~30% coral cover) to a diet dominated by pelagic micro-crustacean on inner bay reefs (~0-13% coral cover). Additionally, some *H. puella* individuals broadened their diet by including an increased proportion of fish prey at reefs located inside of the bay.

The dietary switch in *C. capistratus* was clearly driven by coral cover. On the population-level, its diet appeared to relate to availability of prey as suggested by a mixed diet of corals and annelids at intermediate levels of coral cover at the inner bay zone, whereas corals dominated the diet in the high coral cover outer bay zone and annelids dominated at the low coral cover disturbed zone. Prey switching is predicted to occur in relation to disproportional high versus low prey frequency or rate of encounter, defined as change in preference between two prey items as a function of their relative densities in the environment (Murdoch 1969; Rindorf, Gislason, and Lewy 2006). At degraded reefs, *C. capistratus* switched to the presumably less preferred diet item (*Loima medusa*). Given the high mobility of this species within reefs, and the observation that live hard corals are still present at the degraded zone (although scarce and scattered) along with other anthozoans such as anemones and zoanthids, we did not expect *C. capistratus* to entirely switch its dietary preference. When adjusting diet, species are predicted to seek maintaining a constant effort regarding energy expense (foraging) and

return (nutrition) (Uchida, Drossel, and Brose 2007). It is possible that prey needs to exceed a certain abundance threshold to represent a diet item worth exploiting to *C. capistratus* rendering scarce coral colonies inefficient at degraded sites. This observation may describe a type III functional response; this is, a predator disproportionally consumes a particular prey when abundant but excludes it prey when scarce (Holling 1959; Beukers-Stewart and Jones 2004; Nentwig, Bacher, and Brandl 2011). Interestingly, *C. capistratus* maintained its browsing mode on degraded reefs. This selectivity may reflect and efficiency that comes from predators seeking out resources that matches attributes of familiar prey, e.g., regarding morphology, chemical defences, or spatial positioning within the habitat matrix (Van Leeuwen et al. 2013), probably to maintain predictability of foraging energy budget (Uchida, Drossel, and Brose 2007).

Our findings contrast previous studies that found no correlation between the diet of *C. capistratus* and food availability (Birkeland and Neudecker 1981; Lasker 1985) describing it to feed selectively (Birkeland and Neudecker 1981) with high percentages of anthozoans in its diet (>80%) (Pitts 1991). It is important to bear in mind that these previous studies investigated less disturbed reefs during the early 1980's than our study of a habitat gradient nearly 40 years later. However, a recent study from Puerto Rico reported similar prey composition as early studies for *C. capistratus* (Liedke et al. 2018). Birkeland Neudecker (1981) described *C. capistratus* to forage for an even diet by complementing anthozoan prey with higher nutritional value items such as polychaete worms (Rotjan and Lewis 2008). Mixed diets have been proposed to enhance fitness (balanced diet hypothesis, Pulliam 1975); however; recent meta-analysis found the 'single optimal prey item' better promoted predator fitness (Lefcheck et al. 2013). The annelid diet at degraded reefs increased condition only for the largest size classes and exhibited most variability among and within classes suggesting suboptimal conditions for *C. capistratus* where coral cover was very low. In contrast, the coral dominated diet lead to the most stable levels of body condition among size classes.

In addition, we found the largest and heaviest individuals at the inner bay zone, notwithstanding decreasing body condition in the largest size class. This might be due to more sheltered conditions and potentially decreased predation pressure possibly due to overfishing (Guzmán et al. 2005; Cramer 2013; Seemann et al. 2018). Noble et al. (2014) found higher wave exposure led to greater foraging intensity on scleractinian coral by butterflyfishes on the Great Barrier Reef. In our study system in Bocas, browsing on hard corals at the outer bay zone might support higher metabolic energy costs associated with foraging at this exposed location. Additionally, there might be differences between time spent searching, travelling, and feeding among the inner and outer bay zones suggesting different energy costs associated with browsing at different exposure regimes (Noble et al. 2014)

The dietary switch was accompanied by a decrease in dietary variability among individuals (i.e., reduced individual specialisation) (sensu Bolnick et al. 2003) at the degraded zone, which potentially could lead to interspecific competition. Furthermore, closely related butterflyfishes have shown to partition their diets at fine taxonomic scales (Nagelkerken et al. 2009; Liedke et al. 2018). To this end, the observed dietary shift from cnidaria to annelids in our study might forecast increased competition among Caribbean butterflyfishes if reefs further degrade. For example, a closely related butterflyfish species, Chaetodon striatus, predominantly feeds on annelids (Liedke et al. 2016).

The diet of *Hypoplectrus puella* was largely based on crustaceans in agreement with previous findings (Randall 1967; Whiteman, Côté, and Reynolds 2007), but we found evidence of shifts within this prey type in association with coral cover. More specifically, our model of coral cover predicted the consumption of planktonic arthropods. The increase in micro-crustaceans (e.g., calanoid copepods) at the disturbed sites may be indicative of a decreased availability of preferred dietary items. *Mithraculus* crabs were a dominant diet item and most abundant in surveys and at high coral cover sites, whereas both consumption and abundance of this prey was low at disturbed reefs. This provides some evidence for *H. puella* preferring *Mithraculus*

to other crustacean prey at our study area given that overall macro-crustacean abundance did not vary among zones in our benthic surveys.

This was in line with predictions from optimal foraging theory: the predator seeks out the more profitable food item if present in high densities but less so if less readily available (Charnov 1976; Nentwig, Bacher, and Brandl 2011). Similarly, a recent study found no differences in zooplankton composition across reef zones at our study sites (Rodas et al. 2020). This suggests the observed dietary pattern was not driven by differences in plankton availability. Instead, the increased consumption of fishes across both inner bay zones might entail increased secondary consumption of planktonic prey. Whiteman et al. (2007) found hamlets prey upon fish recruits (e.g., Blenniidae, Gobiidae, Pomacentridae, Acanthuridae), which may feed upon planktonic prey. The higher taxonomic range of fishes (i.e., 13 families) detected in the gut of *H. puella* in our study (Table 3.1), could be either facilitated by a higher detection rate achieved with metabarcoding or due to geographic variation in the diet. Furthermore, digestion times vary between fishes and crustaceans, with fishes having shown to be digested four times faster than crustaceans in rock cod from the Great Barrier Reef (Beukers-Stewart and Jones 2004). This implies that previous dietary analyses based on visual inspections of digestive tracts might have underestimated both the proportion of fish and fish diversity in the diet of *H. puella*

The observed pattern in dietary variation across the habitat gradient in both study species may be due to versatile, behavioural responses to prey availability but could also be a sign of distinct subpopulations with differential adaptations to habitat condition. We lack information regarding to what extent subpopulations inside versus outside the bay rely on self-recruitment. However, dispersal distances can be surprisingly small; Puebla et al. (2009b) found a mean dispersal distance of only 10 km for *H. puella* along the Caribbean coast of Central America indicating recruitment remains within a range of 2-14 km from parents. This pattern may be exacerbated by seascape morphology at the Bahia Almirante where small, discontiguous reefs and an enclosed lagoon might promote relatively closed subpopulations (Pinsky et al. 2012).

Focal fish species abundances did not largely vary across reefs at our study area. While peaked abundances at the inner bay reefs for both species may reflect a preference for sheltered conditions, the local abundance of versatile feeders will not immediately respond to changes in prey availability. In Indo-Pacific butterflyfishes, the population size of only the most specialised species appeared limited by preferred resources (Lawton and Pratchett 2012). Thus, potential consequences of benthic change may remain unnoticed but potentially bear sublethal consequences for fish health. Specifically, diet change may imply different foraging costs and/or energy returns based on prey nutritional quality potentially compromising fish health (Pratchett et al. 2004; Berumen, Pratchett, and McCormick 2005; Hempson et al. 2017).

Here we found evidence of diet switching allowing fishes to persist in coral depauperate environments. While diet switching maintains species diversity in the environment, it might come at a cost for fish physical condition. Our findings suggest that the study species' functional roles differed with habitat state and/or wave exposure level (inside versus outside of the bay). This implies high spatial variability in trophic pathways and levels of species' functional redundancies with potential consequences for ecosystem functioning especially if reefs further decline.

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Author contributions

F.C. and M.L. conceived the study. M.L., F.C. and R.F.P. designed the study. F.C. and M.L. conducted the fieldwork. F.C. dissected the fish stomachs and guts. F.C. extracted the DNA. F.C. and M.L. prepared the DNA for sequencing B.N. processed the sequencing data. R.F.P., A.H.A., W.O.M. and M.L. contributed reagents and supplies. F.C. analysed the data and wrote the first draft of the manuscript with input from R.F.P., M.L. and A.H.A.

Chapter 4

The gut microbiome variability of a butterflyfish increases on severely degraded Caribbean reefs

4.1 Abstract

Environmental degradation has the potential to alter key mutualisms that underline the structure and function of ecological communities. While it is well recognised that the global loss of coral reefs alters fish communities, the effects of habitat degradation on microbial communities associated with fishes remain largely unknown despite their fundamental roles in host nutrition and immunity. Using a gradient of reef degradation, we show that the gut microbiome of a facultative, coral-feeding butterflyfish (*Chaetodon capistratus*) is significantly more variable among individuals at degraded reefs with very low live coral cover (~0%) than reefs with higher coral cover (~30%), mirroring a known pattern of microbial imbalance observed in immunodeficient humans and other stressed or diseased animals. We demonstrate that fish gut microbiomes on severely degraded reefs have a lower abundance of *Endozoicomonas* and a higher diversity of anaerobic fermentative bacteria, which suggests a broader and less coral dominated diet. The observed shifts in fish gut bacterial communities across the habitat gradient extend to a small set of potentially beneficial host associated bacteria (i.e., the core microbiome) suggesting essential fish-microbiome interactions are vulnerable to severe coral degradation.

4.2 Introduction

Environmental degradation associated with the Anthropocene is threatening the persistence of mutualistic relationships that are key to the stability of ecological functioning (Kiers et al. 2010). The increasingly severe degradation of coral reefs from both local and climatic stressors has led to novel habitat states with conspicuously altered fish and invertebrate communities, making them a model system for studying ecological responses to environmental change (Idjadi and Edmunds 2006; Wilson et al. 2006; Norström et al. 2009; Richardson et al. 2018). A potentially pervasive but largely overlooked response to habitat degradation is the change to host-associated microbiomes - the communities of bacteria, archaea, fungi, unicellular eukaryotes, protozoa and viruses that live on internal and external surfaces of reef organisms. It has been suggested that coral microbiomes respond faster than their hosts to changing environmental conditions and can promote acclimatisation processes as well as genetic adaptation (Webster and Reusch 2017). Microbial communities could play a key role in mediating a host's resilience and ability to adapt to environmental change. However, it remains to be explored whether mutualisms between fish hosts and gut microbiomes can shift to alternative beneficial relationships to provide a mechanism of resilience to habitat change, or whether the mutualism breaks down and simply reflects a cascading effect of degradation at all levels of ecological organisation.

The importance of gut microbial communities in maintaining host health is well recognised in mammals and other vertebrates (Ley, Hamady, et al. 2008; Ley, Lozupone, et al. 2008), including a wealth of research into the importance of microbes in fish in aquaculture (Llewellyn et al. 2014; Tarnecki et al. 2017; Egerton et al. 2018; Wang et al. 2018). In coral reef fishes, recent studies have revealed that intestinal microbiomes can perform key physiological functions associated with nutrient acquisition, metabolic homeostasis and immunity (Clements et al. 2014; Miyake, Ngugi, and Stingl 2015; Parris et al. 2016; Jones et al. 2018; Neave et al. 2019). For example, gut bacteria provide many herbivorous fish hosts with the ability

to digest complex algal polymers (Clements et al. 2014; Miyake, Ngugi, and Stingl 2015; Ngugi et al. 2017). The gut microbiome is also a major actor in the innate immune responses to a wide variety of pathogenic microorganisms and other stressors in the surrounding environment (Gómez and Balcázar 2008; Butt and Volkoff 2019). Given the rapid physical, chemical and biotic changes affecting coral reefs, it is essential to gain a predictive understanding of how fish gut microbiome assemblages and metabolic functions respond to environmental variation to assess how the response of these mutualisms govern host health and resilience to habitat change.

Fish harbour microbiomes that are unique from the microbial communities in their surrounding environment (Legrand et al. 2019; Rawls et al. 2006). The development of the gastrointestinal microbiome can start during hatching via acquisition of microorganisms from the egg's chorion (i.e., the acellular protective envelope encasing the oocyte) (Cotelli et al. 1988), and with both water and the first food source entering the gastrointestinal tract (Egerton et al. 2018; Romero and Navarrete 2006; Ghanbari, Kneifel, and Domig 2015; Llewellyn et al. 2014; Hansen and Olafsen 1999). Parental effects and host genotype likely mediate the early microbiome colonisation process from egg and environmental sources (Legrand et al. 2019; Llewellyn et al. 2014; Wilkins, Fumagalli, and Wedekind 2016). As the gut microbiome diversifies throughout the development of the fish host, a relatively stable gut microbiome is typically established within the first months of the fish's life and is influenced by a combination of host selection mechanisms and bacterial regulation of the fish host's gene expression (Egerton et al. 2018; Gómez and Balcázar 2008; Kim, Brunt, and Austin 2007). These resident (autochthonous) microbes, which are consistently found associated with the fish population across space and time and potentially provide critical functions for the host are referred to as the "core microbiome" (Shade and Handelsman 2012; Sullam et al. 2012; Tarnecki et al. 2017). In contrast, the numerous microbes occurring in the gastrointestinal tract after being ingested are transient (allochthonous) and may vary intraspecifically with developmental stage and potentially include opportunistic pathogens that may colonise in the case of impaired residential communities.

Because of their importance in maintaining host metabolic homeostasis, the degree of stability of the core microbiome across a range of environmental conditions is a key trait for predicting the resilience of the host population (e.g., Ainsworth et al. 2015; Hernandez-Agreda et al. 2016; Roeselers et al. 2011). The stability of the core gut community may be altered if the host experiences severe physiological stress. It may switch to an alternative stable state (i.e., a novel but stable community), or communities may become more variable between individuals (i.e., communities are destabilised as stressors reduce the ability of hosts or their microbiome to regulate community composition) (Zaneveld, McMinds, and Vega Thurber 2017).

The Chaetodontidae family (Butterflyfishes) is among the largest and most iconic families of coral reef-associated fishes (Bellwood et al. 2010) and an ideal group for studying microbiome responses to habitat degradation. Chaetodontidae species range from extreme diet specialists to facultative corallivores and generalists capable of consuming different types of prey such as corals, algae, polychaetes or crustaceans (Berumen, Pratchett, and McCormick 2005; Pratchett 2005; Nagelkerken et al. 2009). Due to their intimate link to the reef benthos, specialised coral feeding species of Indo-Pacific butterflyfishes were shown to be highly sensitive to reductions in coral cover (Bouchon and Harmelin-Vivien 1985; Graham 2007a; Pratchett et al. 2006). The foureye butterflyfish, *Chaetodon capistratus* (Linnaeus, 1758), is the only one of the four Western Atlantic *Chaetodon* species with a relatively high proportion of anthozoans in its diet (mainly hard and soft corals) (Birkeland and Neudecker 1981; Gore 1984; Liedke et al. 2018). Due to this relative specialisation, we chose it as a model species to study links between reef habitats, hosts and the gut microbiome.

Here, we examined how differences in benthic habitat composition and coral coverage influence the variability and composition of the gut microbiota of *Chaetodon capistratus* across a tropical coastal lagoon in Bocas del Toro on the Caribbean coast of Panama. The Bay of Almirante encompasses an inner bay of protected reefs subjected to seasonal high temperatures and a watershed delivering nutrients from agriculture and sewage. In 2010, the bay faced an unprecedented hypoxic event, which led to massive coral bleaching and mortality on some reefs while

others located near the bay's mouth remained unaffected (Altieri et al. 2017). We capitalised on this gradient of habitat states across the bay of Almirante to test the resilience of fish gut microbiomes to environmental degradation. We hypothesised that fish residing on more degraded reefs have a more diverse microbiome as a result of alternative feeding behaviours and potentially increased stress (Zaneveld, McMinds, and Vega Thurber 2017). On the other hand, we predicted that the core microbial community remains stable across the habitat gradient.

4.3 Methods

4.3.1 Study area

Bahia Almirante, located in the Bocas del Toro Archipelago on the Caribbean coast of Panama, is a coastal lagoonal system of approximately 450 km2 where numerous, relatively small and patchy fringing coral reefs occur (Greb 1996). Hydrographic and environmental conditions vary across the semi-enclosed bay but are generally characterised by limited water exchange with the open ocean (Altieri et al. 2017). Furthermore, areas of the bay are subjected to uncontrolled sewage and dredging due to increasing coastal development and agricultural runoffs from the adjacent mainland (D'Croz, Rosario, and Gondola 2005; Collin et al. 2009; Aronson et al 2014; Seemann et al. 2014). A total of nine discontiguous reefs distributed from the mouth towards the inner bay were selected for this study based on distinct hydrogeographical zones and disturbance history (Fig. 4.1A). Throughout the manuscript, we will refer to the three distinct reef zones as "outer bay", "inner bay", and "inner bay disturbed". Outer bay reefs [Salt Creek (SCR), Cayo Corales (CCR) and Popa (PPR)] are located at the mouth of the bay. These reefs represent typical Caribbean reef communities featuring both massive and branching coral colonies with higher benthic cover and diversity as compared to the inner bay (Fig. 4.1B). Inner bay reefs [Almirante (ALR), Cayo Hermanas (SIS), and Cayo Roldan (ROL)] are largely coral and sponge dominated reefs of lower coral diversity than the outer bay reefs (Fig. 4.1C). Inner bay disturbed reefs [Punta Puebla (PBL),

Punta STRI (PST) and Runway (RNW)] were heavily impacted by the 2010 hypoxic event (Altieri et al., 2017), which resulted in the current cover of largely dead coral comprised of formally prevalent Agaricia and Porites species (Fig. 4.1D).

4.3.2 Benthic habitat and fish communities

Visual surveys of benthic cover and fish communities were conducted between May and June 2016. At each of the nine reefs, three 20 m transects were placed parallel to the shore at 2-4 m depth. Benthic community cover was estimated from 100 x 70 cm photographic quadrats placed every two meters resulting in a total of 10 quadrats per transect. Photos were analysed on the CoralNet platform (Beijbom et al. 2015) using a stratified random sampling design (10 rows x 10 columns with 1 point per cell for a total of 100 points per image). Due to the difficulty involved with photo-based taxonomic identification, analyses were conducted at the level of broad functional groups. Mean cover of each benthic category was calculated per reef. Fish communities were characterised by one trained surveyor who recorded the identity and abundance of all reef fishes encountered along each 20 m belt (2.5 m on each side of the transect line) while swimming slowly using scuba (except at CCR).

4.3.3 Sample collection

The foureye butterflyfish, *Chaetodon capistratus*, is a common member of Caribbean coral reef fish communities (IUCN classified as least concern, Rocha et al., 2010) with a distribution that extends across the subtropical Western Atlantic (Froese, 2019; McBride and Able, 1996; Smith, 1997) (Fig. 4.1E). The following protocol of fish capture and euthanisation was approved by the Smithsonian Tropical Research Institute's Institutional Animal Care and Use Committee (IACUC). An average of 11 individual adult fish were collected at each of the nine reefs (min = 7; max = 16; total = 102) by spearfishing in February and March 2018 (Table D.1). Captured fish were immediately brought to the boat, anesthetised with clove oil and placed on ice in an individual and labelled sterile Whirl-Pak bag. Upon return to the research station, fish were weighed (g wet weight), and Standard Length (mm

SL) as well as Total Length (mm TL) were measured with a digital caliper. The intestinal tract of each fish was removed under a laminar flow hood using tools decontaminated with 10hypochlorite, preserved in 96% ethanol in individual 15 ml or 5 ml centrifuge tubes and stored at $-20°C$ until DNA extraction. To assess microbial communities present in the fish's environment, we also obtained samples of potential prey taxa and seawater. At each of nine reefs, a total of four liters of seawater was collected immediately above the reef substratum using sterile Whirl-Pak bags and filtered through a 0.22 µm nitrocellulose membrane (Millipore). Small pieces of hard coral (*Siderastrea siderea, Porites furcata, Agaricia tenuifolia*), soft coral (*A. bipinnata, Eunicea* spp.), sponges (*Amphimedon compressa, Amphimedon* sp., *Chondrilla caribensis, Mycale* sp., *Dysidea* sp., *Xestospongia* sp.), macroalgae (*Amphiroa* sp.), turf, and zoantharia (*Zoanthus pulchellus, Palythoa caribaoerum*) were collected and kept in sterile Whirl-Pak bags on ice on the boat. At the field station, samples were individually placed in 50 ml or 15 ml centrifuge tubes with 96% ethanol and stored at $-20°C$ until DNA extraction.

4.3.4 DNA analysis

The gastrointestinal tract of each fish was opened longitudinally to isolate the digesta and the mucosa tissue by lightly scraping the intestinal epithelium. Between 0.05 and 0.25 g of both tissue types combined was used for DNA extraction using the Qiagen Powersoil DNA isolation kit following the manufacturers instructions with minor modifications to increase yield (see Supplementary text). Tissues of all potential prey organisms (invertebrates and macroalgae) were homogenised per sample. Additionally, infaunal communities (small worms) were isolated from two sponges, *Amphimedon compressa* and *Dysidea* sp. and the tissue homogenised for each sponge separartely. DNA was extracted (0.25g per sample) following protocols described previously. Seawater DNA was isolated from nitrocellulose membranes filters using the Qiagen Powersoil Kit following a modified protocol described previously (Nguyen et al. 2019).

A dual Polymerase Chain Reaction (PCR) approach was used to amplify the V4 hypervariable region [primers 515F (Parada, Needham, and Fuhrman 2016) and 806R (Apprill et al. 2015)] of the 16S ribosomal rRNA gene of each sample and the product of all samples was sequenced by combining into a single Illumina MiSeq sequencing run. Our protocol followed the 16S Illumina Amplicon Protocol of the Earth Microbiome Project (Weber et al. 2018) using locus-specific primers to which Illumina "overhang" sequences were appended. These overhang sequences served as a template to add dual index Illumina sequencing adaptors in a second PCR reaction (see supplementary text for detailed PCR protocols). The final product was sequenced on the Illumina MiSeq sequencer (reagent kit version 2, 500 cycles) at the Smithsonian Tropical Research Institute with 20% PhiX. The absence of contaminants was confirmed with negative DNA extractions and negative PCR amplifications (see supplementary text for detailed DNA extraction and PCR protocols).

4.3.5 Analysis of sequence data

Illumina adapter and primer sequences were removed from forward and reverse reads using "cutadapt" (Martin 2011) with a maximum error rate of 0.12 (-e 0.12). Remaining reads were filtered and trimmed based on their quality profiles and potential chimeras removed using DADA2 1.12.1 (Callahan et al. 2016) in R environment version 3.6.1 (R Core Team 2019). Sequences were discarded if they had more than two expected errors (maxEE = 2), or at least one ambiguous nucleotide (maxN = 0) or at least one base with a high probability of erroneous assignment (truncQ = 2). Forward and reverse reads were trimmed to 220 bp and 180 bp respectively to remove lower quality bases while maintaining sufficient overlap between paired end reads. Sequences were kept when both the forward and reverse reads of a pair passed the filter. Quality filtered reads were dereplicated and Amplicon Sequence Variants (ASVs) inferred. Paired-end reads were merged and pairs of reads that did not match exactly were discarded. Taxonomy was assigned to each ASV using a DADA2 implementation of the naive Bayesian RDP

classifier (Wang et al. 2007) against the Silva reference database version 132 (Quast et al. 2013). ASVs identified as chloroplast, mitochondria, eukaryota, or that remained unidentified (i.e, "NA") at the kingdom level were removed from the dataset. Sequences of each ASV were aligned using the DECIPHER R package version 2.0 (Wright 2016). The phangorn R package version 2.5.5 (Schliep et al. 2017) was then used to construct a maximum likelihood phylogenetic tree (GTR+G+I model) using a neighbor-joining tree as a starting point. Fourteen samples containing few sequences were removed from the dataset (Fig. C.1). The remaining samples were rarefied to even sequencing depth (n = 10,369 sequences) for downstream analysis. Our approach followed the recommendation for normalisation of sequencing data (Weiss et al. 2017). Statistical analysis was conducted using phyloseq version 1.28.0 in R (McMurdie and Holmes 2013).

4.3.6 Delineation of the core gut microbiome

To identify the persistent and potentially beneficial bacteria associated with the fish gut [i.e., the "core gut microbiome" (Shade and Handelsman 2012; Astudillo-García et al. 2017)], we employed a statistical approach taking into account both relative abundance and relative frequency of occurrence of ASVs as opposed to the common procedure of using an arbitrary minimum frequency threshold based on presenceabsence data only (Astudillo-García et al. 2017). Indicator species analysis (Dufrêne and Legendre, 1997; labdsv package in R, Roberts, 2019) was used to identify which ASVs were relatively more abundant and predominantly found in fish guts and not in their surrounding environment. We calculated an Indicator Value (IndVal) Index between each ASV and two groups of samples: (1) all fish gut samples, and (2) all seawater and sessile invertebrate samples upon which the fish potentially feeds (for a schematic diagram of the analysis workflow see Fig. 4.2). The statistical significance of the association between ASVs and groups of samples was tested using 1000 permutations. ASVs were considered indicators of fish guts (i.e., components of the core) if P-value 0.001.

Sequences of ASVs identified as part of the core microbiome were compared to the non-redundant nucleotide (nr/nt) collection database of the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool for nucleotides (BLASTn) (Altschul et al. 1990). We extracted metadata associated with all sequences that matched each query at 100% similarity or the first five top hits to identify where each core ASVs and close relatives were previously found.

4.3.7 Diversity analyses

The workflow of our microbial community analysis is visualised in a diagram (Fig. 4.2). To account for presence of rare sequence variants caused by sequencing errors or other technical artifacts (Leray and Knowlton 2017), we used Hill numbers (Hill 1973) following the approach recommended by Alberdi and Gilbert (2019) for sequence data to compare alpha diversity between groups of samples. Hill numbers allow scaling the weight put on rare versus abundant sequence variants while providing intuitive comparisons of diversity levels using "effective number of ASVs" as a measuring unit (Hill 1973; Alberdi and Gilbert 2019; Jost 2006). This approach allowed for balancing the over representation of rare ASVs that might be inflated due to sequencing errors (Chiu and Chao 2016). We calculated three metrics that put more or less weight on common species: (1) observed richness, (2) Shannon exponential that weighs ASVs by their frequency, and (3) Simpson multiplicative inverse that overweighs abundant ASVs. Alpha diversity was calculated and visualised using boxplots for the whole and core fish microbiome. Because Shapiro-Wilk tests indicated that the data were not normally distributed, non-parametric Kruskal-Wallis tests were used to compare alpha diversity among reefs (N=9) and the three reef zones (outer bay, inner bay, inner bay – disturbed) with post-hoc Dunn tests.

To test the hypothesis that fish gut microbiome are more variable between individuals at disturbed sites, we calculated non-parametric Permutational Analysis of Multivariate Dispersion (PERMDISP2) [betadisper function, vegan package implemented in phyloseq (Oksanen et al. 2012; McMurdie and Holmes 2013)]. PERMDISP2 is a measure of the homogeneity of variance among groups and

compares the average distance to group the centroid between each predefined group of samples in multidimensional space. We used a range of phylogenetic and nonphylogenetic dissimilarity metrics that differentially weigh the relative abundance of ASVs to identify the effect of abundant ASVs (Anderson, Ellingsen, and McArdle 2006) [Phylogenetic: Unifrac, Generalized Unifrac and Weighted Unifrac (Lozupone et al. 2007) (R package GUniFrac, Chen et al., 2012); non-phylogenetic: Jaccard (Jaccard 1912), modified Gower with log base 10 (Anderson, Ellingsen, and McArdle 2006) and Bray Curtis (Bray and Curtis 1957)]. P-values were obtained by permuting model residuals of an ANOVA (Analysis of Variance) null-model 1000 times [betadisper function, vegan package implemented in phyloseq (McMurdie and Holmes 2013)]. Principal Coordinates Analysis (PCoA) plots were generated for each distance measure respectively to visually explore patterns of variance dispersion across the three reef zones.

Differences in microbial composition were tested using Permutational Multivariate Analysis of Variance (PERMANOVA) with the Adonis function in vegan (Anderson 2001) computed with 10,000 permutations. Comparisons were made (1) between fish gut microbiomes of the three reef zones ('zone model'), (2) between fish gut microbiomes of outer bay reefs versus inner bay reefs ('position model') and (3) between fish gut microbiomes of inner bay reefs and inner bay disturbed reefs which differed in coral cover ('cover model'). Permanova is robust to the effect of heterogeneity of multivariate dispersions in balanced or near balanced designs as in our study (Anderson and Walsh 2013). Pairwise Adonis with Bonferroni corrected p-values was computed using the pairwise Adonis function in R (Martinez Arbizu 2019).

Finally, we used the Prevalence Interval for Microbiome Evaluation (PIME) R package (Roesch et al. 2020) to identify sets of ASVs that are predominantly found (more frequent) in fish guts of each zone of the Bay of Almirante (outer bay, inner bay, inner bay – disturbed). PIME uses a supervised machine learning Random Forest algorithm (Breiman 2001) to reduce within-group variability by excluding low frequency sequences potentially confounding community comparisons of microbiome data (Roesch et al. 2020). PIME identifies the best model to predict

community differences between groups by defining a prevalence threshold that retains as many ASVs as possible in the resulting filtered communities (i.e., the random forest classifications), while minimising prediction error (out of bag error, OBB). To do so, the algorithm uses bootstrap aggregating (100 iterations) of each sample group at each filtering step (prevalence interval) by 5% increments. Random Forest calculates a global prediction from a multitude of decision trees based on the bootstrap aggregations and estimates the out of bag error rate (OBB) from omitted subsamples during aggregating (Breiman 2001). Validation was done by randomising the original dataset (100 permutations) and subsequently estimating Random Forest error to determine if group differences in the filtered dataset were due to chance (pime.error.prediction function, PIME R package, Roesch et al. 2020). A second function (pime.oob.replicate, PIME R package, Roesch et al. 2020) repeated the Random Forest analysis using the filtered dataset for each prevalence interval without randomising group identity. In a preliminary step, we assessed whether the OOB error for our unfiltered data was >0.1, which indicated that de-noising with PIME would improve model accuracy.

4.4 Results

4.4.1 Benthic habitat and fish communities

Reefs located in the three zones classified a priori as outer bay, inner bay and inner bay disturbed, differed both in terms of their benthic composition (PCoA; Fig. 4.3A) and level of live coral cover (Fig. 4.3B). Live coral cover (mean cover per site: SCR 37.1%, PPR 33%, CCR 29.3%; Fig. 4.3B) and coral diversity (Shannon diversity; Fig. C.2) were highest on reefs of the outer bay. Both stony coral species (i.e., *Acropora cervicornis*, *Agaricia tenuifolia*) and fire corals (i.e., *Millepora alcicornis*, *Millepora complanata*) dominated at outer bay reefs. At the inner bay zone, reefs displayed an intermediate level of live coral cover (mean cover per transect: ALR 21.2%, SIS 13.3%, ROL 9.4%; Fig. 4.3B), largely dominated by the lettuce coral *Agaricia tenuifolia*. Sponges represented more than a quarter of the benthic cover

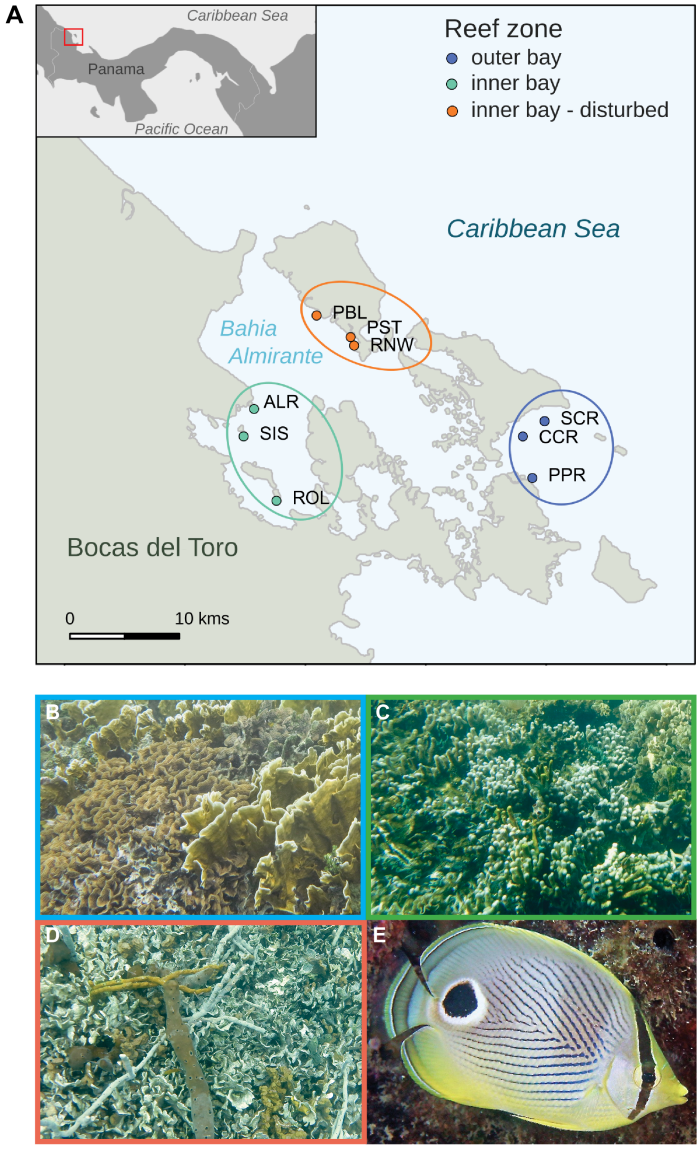

Figure 4.1: Study area and study species. (A) Map of the Bay of Almirante (Bocas del Toro, Panama) indicating the position of the nine reefs where samples were collected. (B) Outer bay reefs with highest levels of live coral cover, (C) inner bay reefs with intermediate levels of coral cover, (D) reefs located in the inner bay disturbed zone were highly impacted by a hypoxic event in 2010, (E) the study species foureye butterflyfish (*Chaetodon capistratus*).

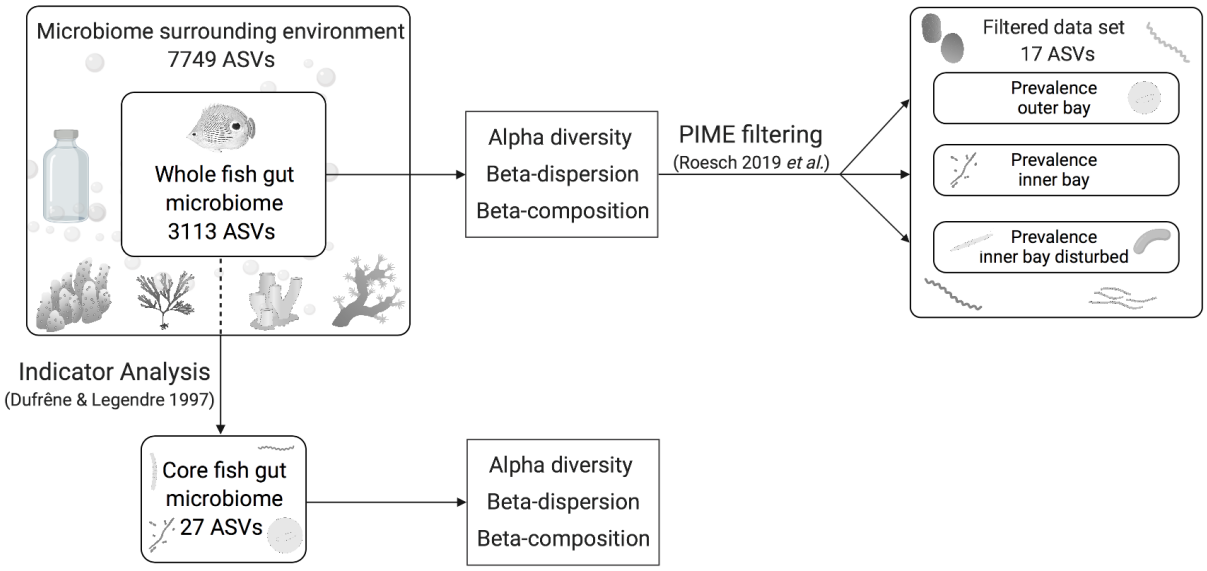

Figure 4.2: Microbial community analysis workflow illustrating how we subsetted the whole fish gut microbiome dataset to delineate the core microbiome and microbial zone communities, respectively. To identify the core microbiome, we used indicator analysis between the whole fish gut microbiome and the environmental sample fraction consisting of samples of potential fish prey taxa and the surrounding seawater. Diversity analysis was done for the whole and core fish gut microbiome, respectively. The whole fish gut microbiome was filtered for prevalence with a machine learning-based algorithm (PIME) to detect community differences among zones that reflect fish microbiome responses to the habitat gradient.

at these reefs (mean sponge cover per transect: ALR 23%, SIS 18.5%, ROL 34.2%; Table D.3). Live coral cover was lowest at the inner bay disturbed zone (mean cover per transect: RNW 0.8%, PST 0.3%, PBL 0%; Fig. 4.3B) where dead coral skeleton was prevalent (mean cover per transect: RNW 45.3%, PST 21.4%, PBL 53.6%) together with sponges (mean cover per transect: RNW 27.3%, PST 21.3% and PBL 21.9%; Table D.3). Principal Coordinates Analysis (PCoA; Bray Curtis dissimilarity) indicated distinct fish communities at the inner bay disturbed zone. In contrast, fish communities at the inner and outer bay zone appeared more

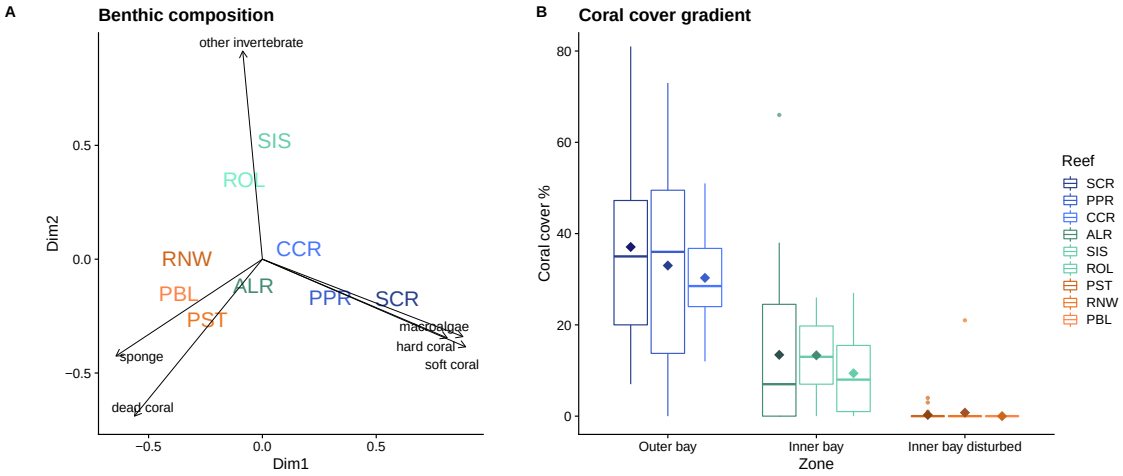

Figure 4.3: Composition and percent coral cover of benthic communities across nine reefs and three reef zones illustrating a habitat gradient: (A) PCoA representing dissimilarities in benthic community composition based on Bray-Curtis. Reefs are colour coded by reef zone, substrate groups are depicted in black; (B) percent live coral cover across reef zones from high coral cover at the outer bay to very low cover at disturbed reefs at the inner bay. Diamonds depict means.

similar (Fig. S4.3A). Our focal species *Chaetodon capistratus* was present at all surveyed reefs in similar abundance levels (1 - 5 individuals per 100 m2 transect) apart from Cayo Hermanas (SIS, inner bay zone) where up to 25 individuals were recorded in one of the transects (Fig. C.3B).

4.4.2 Composition of the whole gut microbiome

A total of 5,844,821 high quality reads were retained for subsequent analyses. The number of reads per sample ranged from 10,369 to 79,466, with a mean ± SD of 41,307 ± 10,990 reads. 10,711 different ASVs were identified in the total dataset. The number of ASVs per sample ranged from 13 to 1,281, with a mean ± SD of 179 ± 210 ASVs. This data set primarily comprised ASVs belonging to 15 bacterial phyla (abundance > 5% ; Fig. C.4A). As predicted, *C. capistratus*' gut microbiome composition was distinct from the microbiome in seawater and the microbiome of potential prey items (sessile invertebrates) (Fig. C.4A and S4.4B). *Chaetodon capistratus*' overall gut microbiome was dominated by Proteobacteria (68.6%) followed by Firmicutes (16.1%), Spirochaetes (9.27%), Cyanobacteria (3.98%) (Fig. C.4A). Bacteria in the phylum Proteobacteria were

dominated by a single genus (*Endozoicomonas*) in the gut of *C. capistratus* (93.9%) (Fig. C.4B). Firmicutes was abundant in fish guts (16.1% of fish gut bacteria) but representatives of this phylum were nearly absent from potential prey (0.005%, 0.06%, 0.47%, 0.26% and 0.07% in algae, hard corals, soft corals, sponges and zoanthids, respectively) and seawater (and 0.02%) (Fig. C.4A and C.4B).

4.4.3 Composition of the core gut microbiome

Indicator Analysis identified 27 ASVs in eight families (i.e., Endozoicomonadaceae, Brevinemataceae, Ruminococcaceae, Lachnospiraceae, Vibrionaceae, Peptostreptococcaceae, Clostridiaceae, Thermaceae) as part of the 'core' microbiome associated with the fish intestinal tract (IndVal; p 0.001) (Fig. C.5, Table C.7). The genus *Endozoicomonas* (phylum Proteobacteria, class Gammaproteobacteria), described as a symbiont of marine invertebrates (Naeve 2017), comprised 71.3% of the ASVs in the core followed by genus Brevinema (phylum Spirochaetes, class Spirochaetia) (10.7%) and anaerobic fermentative bacteria in the families Ruminococcaceae (9.7%), Lachnospiraceae (5.6%), and Clostridiaceae (1.7%) (phylum Firmicutes, class Clostridia).

Blastn searches against nr/nt NCBI database revealed that ASVs identified as part of the core gut microbiome were previously found in scleractinian and soft coral tissue (*Endozoicomonas* ASV1, ASV3, ASV5, ASV6, ASV11, ASV17) at our study area and in Curacao (ASV1, ASV3, ASV5, ASV17, ASV68) among other locations (ASV1, ASV5, ASV7, ASV11, ASV68) (Table 4.1). Some *Endozoicomonas* ASVs were closely related to sequences identified previously in sponges, clams, ascidians, tunicates, and coral mucus (ASV7, ASV59, ASV68, ASV 163) as well as the intestinal tract of a coral reef fish species (*Pomacanthus sexstriatus*) (ASV5). Sequences assigned to Ruminococcaceae closely resembled bacteria reported from herbivorous marine fishes (*Kyphosus sydneyanus, Naso tonganus, Acanthurus nigrofuscus, Siganus canaliculatus*) (ASV9, ASV14, ASV15, ASV25, ASV39), the omnivorous coral reef fish *Pomacanthus sexstriatus* (ASV25) and a freshwater fish (ASV18). An *Epulopiscium* ASV matched with 100% identity to a sequence

detected in the guts of two coral reef fishes, the omnivorous *Naso tonganus* and the carnivorous *Lutjanus bohar* (ASV27) and to sequences found in the coral *Orbicella faveolata* (ASV27). Other Lachnospiraceae bacteria found in this study resembled sequences known from cattle rumen, hot springs, farm waste, human and other animal feces (ASV10, ASV 24). Within Ruminococcaceae in Firmicutes, ASVs assigned to genus *Flavonifractor* closely resembled bacteria reported from the hind gut of the temperate herbivorous marine fish *Kyphosus sydneyanus* in New Zealand (ASV9). Brevinema sequences similar to ours have been previously isolated from the gut of the coral reef fish *Naso tonganus* as well as freshwater and intertidal fish intestinal tracts (ASV2). Retrieved Vibrionaceae (genus *Vibrio*) were similar to sequences found in a coral reef fish gut of *Zebrasoma desjardinii* (ASV95). An *Romboutsia* ASV (family Peptostreptococcaceae), a recently described genus of anaerobic, fermentative bacteria associated with the intestinal tract of animals including humans (Ricaboni et al. 2016; Zhang et al. 2020; Gerritsen et al. 2019) but which also occur in mangrove sediments (Fernández-Cadena et al. 2020) matched 100% a sequence found in tissue of the sea fan *Gorgonia ventalina* at our study site Bocas del Toro (ASV 30) (Table 4.1).

4.4.4 Alpha diversity of the whole gut microbiome

We estimated alpha diversity using Hill numbers of three different orders of diversity (Hill numbers, q = 0, 1, 2) that place more or less weight on the relative abundance of ASVs. This approach allowed for balancing the representation of rare ASVs that might be the result of sequencing errors. Diversity of the gut microbiome was lower in fishes of the outer bay zone than in fishes of the inner bay and inner bay disturbed zones [Observed ASV richness (Hill number q=0); 60.23, 85.49, 75.53; Shannon index (Hill number q=1); 4.77, 7.39, 10.1; Simpson index (Hill number q=2); 2.29, 2.96, 4.58; Table D.4] (Fig. 4.4A, 4.4B and 4.4C). Diversity differed significantly among the three zones when taking into account ASV frequency with the Shannon index (Kruskal-Wallis-Test, $p = 0.004$; Fig. 4.4B and Table D.4)

and when emphasising abundant ASVs with the Simpson index (Kruskal-Wallis-Test; $p = 0.013$, Fig. 4.4C and Table D.4). However, observed ASV richness did not significantly differ among zones (Kruskal-Wallis-Test; $p = 0.174$, Table D.4) (Fig. 4.4A). Benjamin Hochberg corrected posthoc tests showed significantly higher Shannon diversity in fish guts of the inner versus the outer bay zone (Dunn Test; $p = 0.033$, p 0.001, Table D.5). Fish of the inner bay disturbed zone had a higher microbial diversity than fishes of the outer bay zone based on both Shannon and Simpson (Dunn Test; $p = 0.004$, Table D.5). Pairwise comparisons of alpha diversity between reefs revealed that fishes resident on the reef with the highest level of coral cover overall (37.07%), Salt Creek (SCR, outer bay), had a significantly lower diversity of microbes in their guts than fishes from all three inner bay disturbed reefs (RNW, PST, PBL) for both Shannon (Dunn-Test; SCR-RNW $p = 0.013$, SCR-PBL $p = 0.024$) and Simpson diversity (Dunn-Test; SCR-RNW $p = 0.016$, SCR-PST $p = 0.04$ SCR-PBL $p = 0.026$, Table D.5).

4.4.5 Patterns of alpha diversity of the core gut microbiome

Diversity of ASVs in the core microbiome was lowest at the outer bay when comparing ASV richness among fishes of the outer bay, inner bay, and inner bay disturbed zones [(Hill number q=0); 11.57, 14.26, 14.05] and was highest in fishes at the inner bay disturbed zone with both the Shannon index [(Hill number q=1); 2.71, 3.27, 4.45] and Simpson index [(Hill number q=2) 1.8, 2.06, 2.83] (Fig. 4.4D, 4.3E and 4.3F; Table D.4). Alpha diversity differed significantly among the three zones [Kruskal-Wallis-Test; observed richness: $p = 0.025$; Shannon index: $p = 0.015$ and Simpson index: $p = 0.016$; Table D.4) and pairwise testing revealed that this was largely due to differences between fishes of the outer bay and inner bay disturbed zones (Dunn-Test with Benjamin Hochberg correction; Observed $p = 0.049$; Shannon $p = 0.012$; Simpson $p = 0.012$, Table D.5). When comparing by reef, lower core microbial diversity in fishes from Salt Creek (SCR, outer bay) than fishes from other reefs across all zones was responsible for most significant comparisons (Table D.6).

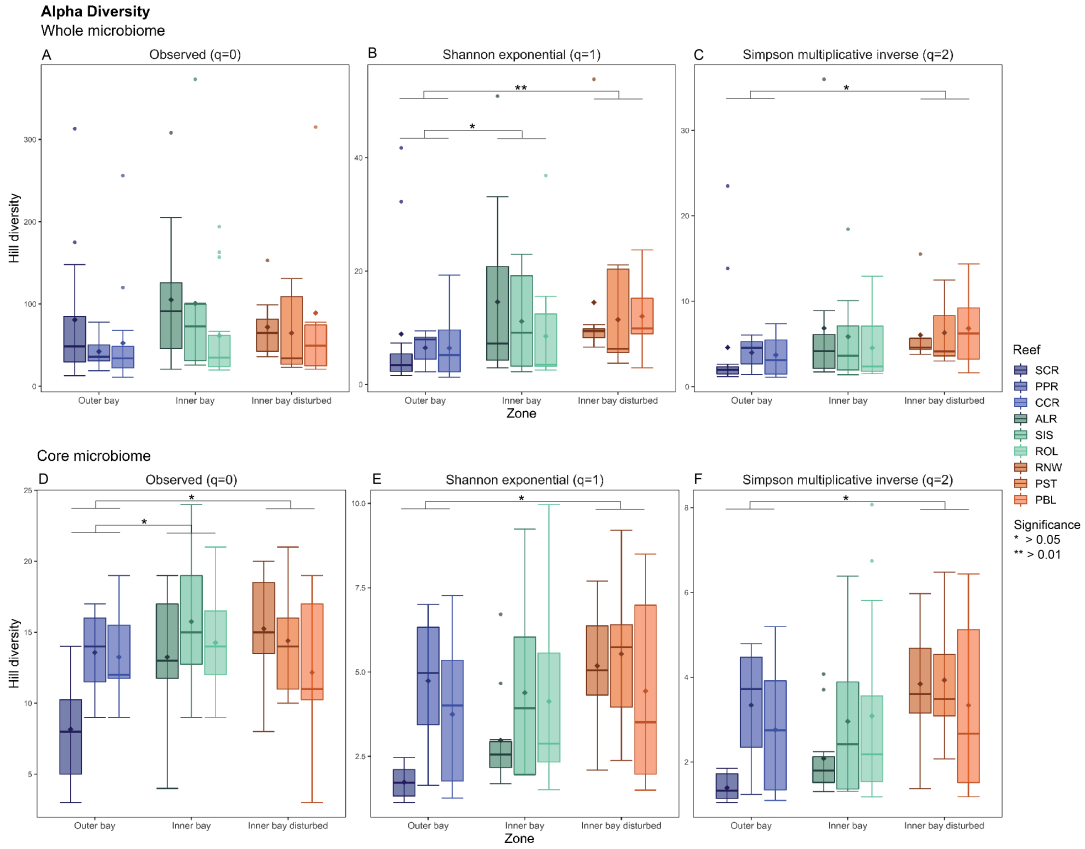

Figure 4.4: Differences in diversity of ASVs between the whole gut microbiome (A-C) and the core gut microbiome (D-F) of *Chaetodon capistratus* across reefs. Alpha diversity was measured based on Hill numbers using three metrics that put more or less weigh on common species. The observed richness (panels A and D) does not take into account relative abundances. Shannon exponential (panels B and E) weighs OTUs by their frequency. Simpson multiplicative inverse (panels C and F) overweighs abundant OTUs. Diamonds depict means.

4.4.6 Beta Diversity for the whole gut microbiome

Permutational Analysis of Multivariate Dispersion (PERMDISP2) indicated no difference in variability in the whole fish gut microbiome across zones and reefs using dissimilarity metrics that put limited weight on abundant ASVs (PERMDISP2; Jaccard: $p = 0.978$; modified Gower: $p = 0.182$; Fig. 4.5A and 4.5B, Table D.8). However, Bray-Curtis, which more heavily weights abundant ASVs, identified significantly higher multivariate dispersion for fishes from the inner bay disturbed

zone than for fishes from the outer bay zone (PERMDISP2, $p = 0.0007$, Fig. 4.5C). The same pattern was observed with phylogenetic dissimilarity metrics. Only the two metrics taking into account relative abundances (i.e., GUniFrac, WUniFrac) revealed significant differences in dispersion patterns among the three zones. Using GUniFrac, an index that adjusts the weight of abundant ASVs based on tree branch lengths, gut microbiomes of fishes from the inner bay disturbed zone were significantly more spread in multivariate space than gut microbiomes of fishes from the outer bay zone (PERMDISP2, $p = 0.021$, Fig. 4.5E). Gut microbial communities were significantly more variable in fishes from the inner bay zone than in fishes from the outer bay zone using both GUniFrac (PERMDISP2, $p = 0.038$, Fig. 4.5E) and WUniFrac (PERMDISP2, $p = 0.025$, Fig. 4.5F).

The three Permanova models explained a small portion of the variance in the composition of the whole gut microbiome using all metrics (2.29% - 9.22%; Fig.4. 6A, Table D.10). Nevertheless, gut microbiome composition was significantly different between fishes from all three zones (zone model), between fishes collected inside and outside the bay (position model) and between fishes collected at inner bay reefs that differ in coral cover (cover model) when using Jaccard (Permanova; $R^2 = 0.04$, $p = 0.0001$; $R^2 = 0.03$, $p = 0.0002$; R^2=0.03, P =0.002), modified Gower (Permanova; $R^2 = 0.06$, $p = 0.0001$; $R^2 = 0.04$, $p = 0.0002$; $R^2 = 0.04$, $p = 0.001$) and Bray Curtis (Permanova; $R^2 = 0.04$, $p = 0.0001$; $R^2 = 0.03$, $p = 0.0002$; $R^2 = 0.03$, $p = 0.002$) (Fig. 4.6A, Table D.10) distances. Whole gut microbiomes differed using phylogenetic metrics UniFrac (Permanova; $R^2 = 0.04$, $p = 0.0004$; $R^2 = 0.03$, $p = 0.0007$; $R^2 = 0.03$, $p = 0.047$) and GUniFrac (Permanova; $R^2 = 0.05$, $p = 0.008$; $R^2 = 0.03$, $p = 0.013$; $R^2 = 0.03$, $p = 0.115$) but not when emphasising microbial relative read abundance (WUniFrac) (Permanova; $R^2 = 0.04$, $p = 0.071$; $R^2 = 0.02$, $p = 0.091$; $R^2 = 0.03$, $p = 0.229$) (Fig. 4.6A, Table D.10). Pairwise Adonis with Bonferroni corrected P-values revealed significant differences among all pairs of zones using non-phylogenetic metrics (Table S4.7C). Pairwise tests were significant using the Unifrac distance except between gut microbiomes of fish from the inner

bay and inner bay disturbed zones. None of the pairwise tests using GUnifrac and WUnifrac were significantly different among zones (Table D.12).

Gut microbiomes of fishes from the inner bay disturbed zone had a lower proportion of microbial reads assigned to Endozoicomonadaceae (Proteobacteria), (48.0%, 67.0%, 69.4%) but a higher proportion of Vibrionaceae (6.5%, 0.8%, 0.8%), and Rhodobacteraceae (1.0%, 0.4%, 0.4%). In contrast, the relative contribution of Spirochaetes (12.8%, 8.9%, 7.7%) and Firmicutes (20.7%, 13.5%, 16.1%) was highest in guts of fishes at the inner bay disturbed zone (Fig. C.6). Within Spirochaetes, the relative abundance of Brevinemataceae was highest in gut microbiomes of fishes from the inner bay disturbed zone (13.8%, 9.1%, 7.8), while Clostrideaceae within Firmicutes contributed more to gut microbiomes of fishes at inner bay reefs but relatively little to the gut microbiomes of fishes of the outer bay zone (1.5%, 4.3%, 0.5%). Schewanellaceae (phylum Proteobacteria) represented a higher proportion of the gut microbiome of fishes at inner bay disturbed reefs (2.2%, 0.2%, 0.6%).

4.4.7 Beta Diversity for the core gut microbiome

Patterns in multivariate dispersion were largely consistent between whole and core gut microbiomes. Differences among the three reef zones were significant with metrics that place more weight on ASV relative abundance (PERMDISP2; Jaccard $p = 0.83$; modified Gower $p = 0.13$; Bray Curtis $p = 0.005$) (Fig. 4.5G, 4.5H, 4.5I, Table D.9). The variability of the core gut microbiome differed significantly between fishes from the inner bay and inner bay disturbed zones (PERMDISP2; modified Gower $p = 0.037$) and between fishes from the inner bay disturbed and outer bay zones (PERMDISP2; Bray Curtis P=0.001) with highest variability levels at the inner bay disturbed zone. However, none of the phylogenetic metrics showed significant differences in dispersion among zones (PERMDISP2; Unifrac $p = 0.12$; GUnifrac $p = 0.299$; WUnifrac $p = 0.301$) (Fig 4.5J, 4.5K, 4.5L, Table D.9).

As with the whole gut microbiome, the three Permanova models explained a limited amount of the variance in the composition of the core gut microbiome [0.6% (position model with weighted Unifrac); 10.1% (zone model with Jaccard);

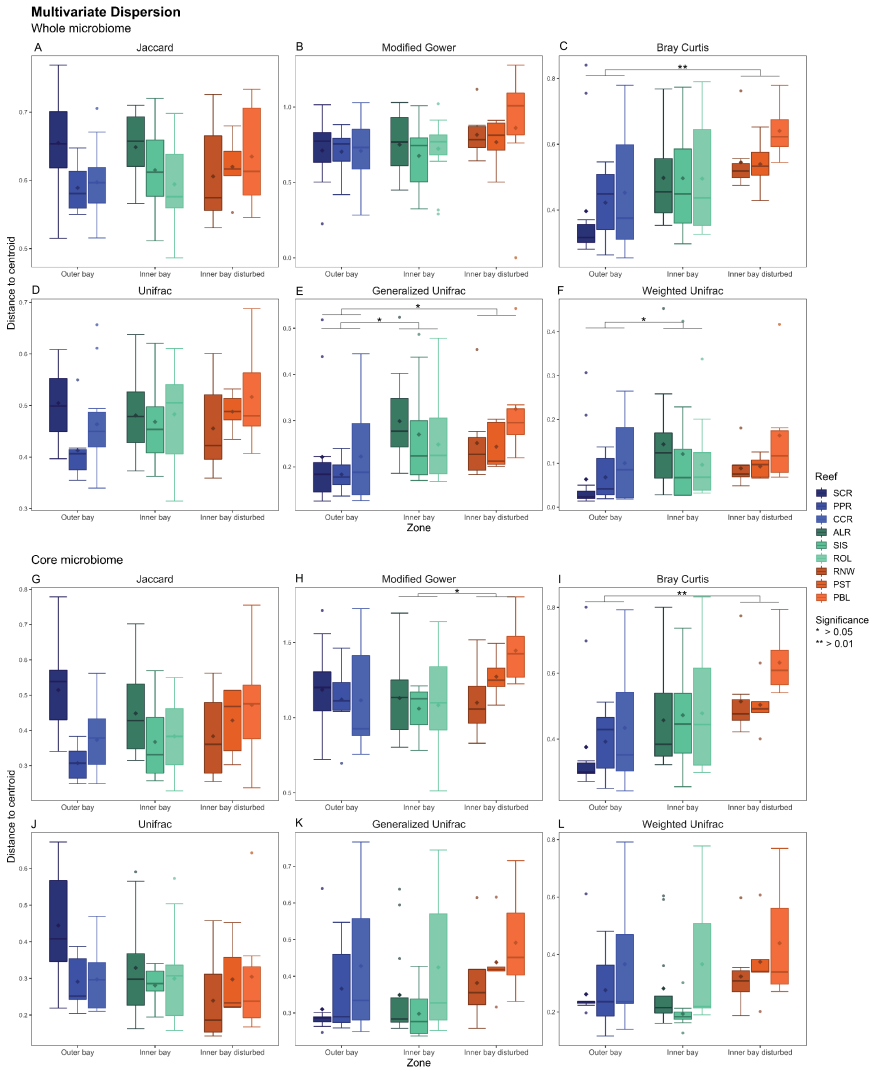

Figure 4.5: Compositional variability of the whole gut microbiome (A-F) and core gut microbiome (G-L) of *Chaetodon capistratus* across reefs. Compositional variability is measured as the distance to centroid of each group (fishes at each reef) in multivariate space. Multivariate analyses were computed with non-phylogenetic [Jaccard: panels A and G; Modified Gower: panels B and H; and Bray Curtis: panels C and I] and phylogenetic (Unifrac: panels D and J; Generalized Unifrac: E and K; Weighted Unifrac F and L) that differ in how much weigh they give to relative abundances. On one end of the spectrum, Jaccard and Unifrac only use presence-absence data, whereas on the end of the spectrum Bray Curtis and Weighted Unifrac give a lot of weigh to abundant ASVs in dissimilarity calculations. Significance depicts differences in multivariate dispersion between reef zones (ANOVA). Diamonds depict means.

Fig. 4.6B, Table D.11]. Yet, composition differed significantly among fish from the three zones (Permanova 'zone model'; Jaccard $R^2 = 0.1$, $p = 0.0001$; modified Gower $R^2 = 0.09$, $p = 0.0001$; Bray Curtis $R^2 = 0.09$, $p = 0.0001$) and between fish at inner bay and outer bay zones (Permanova'position model'; Jaccard $R^2 = 0.07$, $p = 0.0001$; modified Gower $R^2 = 0.06$, $p = 0.0001$; Bray Curtis $R^2 = 0.05$, $p = 0.0006$) as well as between zones of differential coral cover within the bay (Permanova 'cover model'; Jaccard $R^2 = 0.05$, $p = 0.003$; modified Gower $R^2 = 0.04$, $p = 0.012$; Bray Curtis $R^2 = 0.06$, $p = 0.006$) (Fig. 4.6B, Table D.11). The core gut microbiome appeared largely similar in composition using all phylogenetic metrics but Unifrac (Table D.11): (Permanova 'zone model'; Unifrac $R^2 = 0.07$, $p = 0.001$, 'position model' $R^2 = 0.06$, $p = 0.0001$, 'cover model' $R^2 = 0.02$, $p = 0.279$). Similar to the whole microbiome, Pairwise Adonis with Bonferroni corrected P-values showed significant differences among almost all pairs of zones when using taxonomic metrics (Table D.13). Of the phylogenetic metrics, the only significant differences were found between the inner bay versus outer, and inner bay disturbed versus outer bay zones, with Unifrac (Table D.13). Differences in the composition of the core microbiome among reef zones was largely driven by changes in the relative abundance of ASVs assigned to the genus *Endozoicomonas* (class Gammaproteobacteria) (Fig. C.5). For example, the most common *Endozoicomonas* ASV (ASV1) was much more represented in the guts of fishes of outer bay and inner bay zones than in the gut of fishes at inner bay disturbed zones (57.7%, 53.4%, 25.6%) while *Endozoicomonas* assemblages became more even towards the inner bay disturbed zone. In contrast, bacteria in the genus *Brevinema* (phylum Spirochaetes) were most abundant relative to other members of the core in fish of the inner bay disturbed zone (15.4%) and least abundant at the outer bay zones (9.6%). The giant bacterium *Epulopiscium* (family Lachnospiraceae, order Clostridia), which is known to aid the digestion of algae in surgeonfishes, contributed more to the core gut microbiome of fishes at reefs of the inner bay disturbed zone (3.5%) than the inner (1.0%) and outer bay zones (0.9%). Anaerobic, fermentative bacteria showed contrasting patterns: The relative abundance of the four Ruminococcaceae

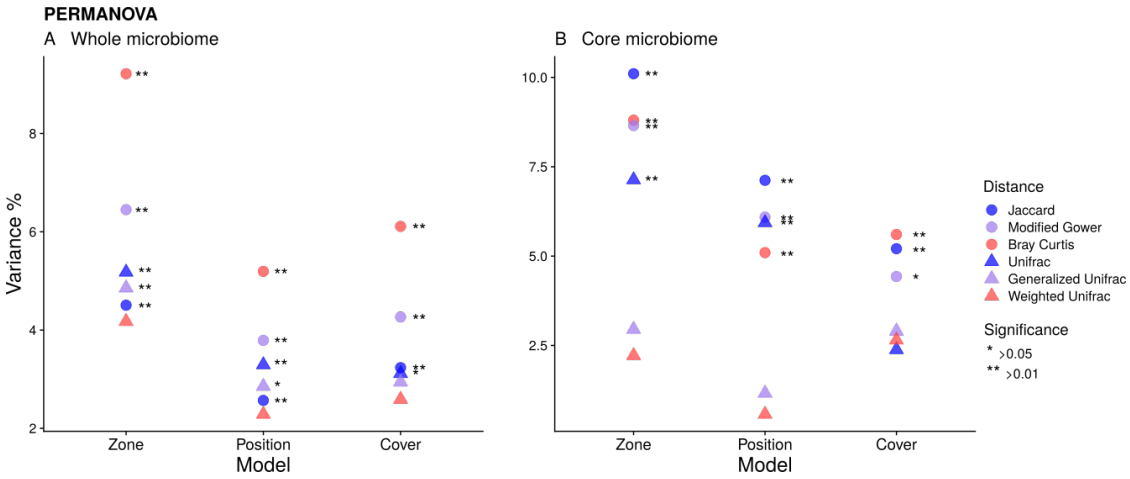

Figure 4.6: Proportion of the variance explained in Permutational Analysis of Variance (PERMANOVA) comparing the composition of the whole gut microbiome (A) and the core gut microbiome (B) of *Chaetodon capistratus*. Three independant PERMANOVA analysis were conducted. "Zone" compares gut microbiones of the three zones of the bay (inner bay, inner bay disturbed and outer bay). "Position" contrasts the composition of gut microbiones of fishes collected inside vs. outside the bay. "Cover" compares gut microbiomes of fishes on disturbed and undisturbed reefs inside the bay. Three non-phylogenetic (circles) and three phylogenetic (triangles) dissimilarity metrics were used. They place more (red) or less (blue) weigh on relative abundances

core ASVs respectively varied across reef zones (ASV15 outer 3.0%, inner 0.3%, inner disturbed 0.8%; ASV14 outer 1.9%, inner 1.2%, inner disturbed 2.2%; ASV19 outer 1.6%, inner 1.2%, inner disturbed 1.6%; ASV25 outer 0.1%, inner 1.5%, inner disturbed 1.8%), whereas Flavonifractor was slightly more abundant at outer reefs (outer 4.2%, inner 3.1%, inner disturbed 3.%) (Fig. C.5).

4.4.8 Prevalent ASVs in each reef zone

A machine learning-based, de-noising algorithm (PIME) was used to detect sets of ASVs in the whole gut microbiome that significantly contribute to differences between reef zones. The initial out of bag (OOB) error rate (i.e., the prediction error in a RandomForest model) for our unfiltered dataset was greater than 0.1 (PIME, OOB 0.27) indicating that PIME filtering would effectively remove noise. PIME identified a prevalence cut-off of 65% for the highest improved accuracy (OOB=2.25) indicating that the model was 97.75% accurate (Table D.14). The validation step found no significant differences between the randomised errors (Fig.

C.7B) and the predicted prevalence cut-off value of 0.65 indicating absence of false positives (Type I error).

The filtered dataset after selecting ASVs that were present in at least 65% of all fish guts comprised 17 ASVs in eight families; i.e., Endozoicomonadaceae, Ruminococcaceae, Pirellulaceae, Lachnospiraceae, Brevinemataceae, Cyanobiaceae, Rhodobacteraceae, Peptostreptococcaceae (Fig. 4.7, Table D.15 and D.16). Fish of the inner bay zone showed highest richness levels with 13 ASVs, compared to eight and nine ASVs in fish of outer bay and inner bay disturbed zones, respectively (Fig. 4.7). An *Endozoicomonas* ASV (ASV1), which was also a dominant component of the core, had a much higher relative abundance in fish of the outer bay zone (82.1%) than in fish of the inner bay disturbed zone (41.0%) (Fig. 4.7). Communities differed most in composition between fish of the outer bay and inner bay disturbed zone, whereas, fish of the inner bay zone reflected an intermediate community between these two and the highest richness of *Endozoicomonas* ASVs (N=5). Evenness among *Endozoicomonas* increased and richness decreased (3 ASVs) in fish of the inner bay disturbed zone, as observed with the core community. Bacteria in the genus *Flavonifractor* occurred in fish of both inner bay zones but not outside, whereas the outer bay zone uniquely featured Rhodobacteraceae, genus *Ruegeria*. Two distinct ASVs of the giant bacterium *Epulopiscium* (family Lachnospiraceae) were significantly prevalent in fish of the inner and inner bay disturbed zones, respectively but were more abundant at disturbed reefs (2.75%). Disturbed reefs uniquely featured anaerobic gut bacteria in the genus *Romboutsia* (family Peptostreptococcaceae) and a particular ASV in the family Lachnospiraceae (Fig. 4.7).

4.5 Discussion

Detecting how host associated microbial communities differ as a function of habitat state and how spatial turnover of microbiomes varies within and among host populations is essential to understanding and predicting host species responses to environmental change. We show that both the whole and the core gut microbiome of

ASV ID	Taxon	% Identity	Isolation source	Host group	Host species	Country	Ocean/River	Reference
ASV1	Endozoicomonas	100	coral tissue	scleractinian coral	*Porites astreoides*	Panama (Bocas del Toro)	Western Atlantic	Sunagawa 2010
		100	coral tissue	scleractinian coral	*Orbicella faveolata*	Panama (Bocas del Toro)	Western Atlantic	Sunagawa et al. 2009
		100	coral tissue	scleractinian coral	*Orbicella annularis*	Curacao	Western Atlantic	Klaus et al. 2007
ASV5	Endozoicomonas	99.6	GI tract	coral reef fish	*Pomacanthus sexstriatus*	NP	NP	Ward et al. 2009
		99.21	coral tissue	scleractinian coral	*Porites astreoides*	Panama (Bocas del Toro)	Western Atlantic	Sunagawa et al. 2010
ASV6	Endozoicomonas	100	coral tissue	scleractinian coral	*Porites astreoides*	Panama (Bocas del Toro)	Western Atlantic	Sunagawa et al. 2010
ASV11	Endozoicomonas	99.6	coral tissue	scleractinian coral	*Porites lutea*	South Africa	Western Indian Ocean	Sere et al. 2013
		99.6	coral tissue	scleractinian coral	NP	Thailand, Ko Tao	Western South China Sea	Roder et al. 2014
		99.6	coral tissue	scleractinian coral	*Porites sp.*	Panama (Bocas del Toro)	Western Atlantic	Roder 2014
ASV9	Flavonifractor	98.2	GI tract	Marine fish	*Kyphosus sydneyanus*	New Zealand	South-Western Pacific	Moran et al. 2005
ASV14	Ruminococcaceae	98.42	GI tract	coral reef fish	*Naso tonganus*	Australia (Great Barrier Reef)	Pacific	Mendell et al. 2010 Accession: HM630215
ASV7	Endozoicomonas	98.81	gill	bivalve mollusc (clam)	*Ctena orbiculata*	Florida, Sugarloaf Key	Western Atlantic	Lim et al. 2017 Accession: KY687505
		98.81	gill	bivalve mollusc (clam)	*Loripes lacteus*	Meditarranean	Meditarranean	Mausz et al. 2008
		98.81	sponge tissue	sponge	Theonella swinhoei	China	South China Sea	Feng 2015 Accession: KT121420
ASV2	Brevinema	93.7	GI tract	coral reef fish	*Naso tonganus*	Australia (Great Barrier Reef)	Pacific	Mendell et al. 2010 Accession: HM630215
		93.68	GI tract	marine and brakish fish	*Gillichthys mirabilis*	United States (California)	Pacific	Bano et al. 2007
ASV3	Endozoicomonas	100	coral mucus	scleractinian coral	NP	Curacao	Western Atlantic	Frade et al. 2016
		100	coral tissue	scleractinian coral	*Porites astreoides*	Panama (Bocas del Toro)	Western Atlantic	Sunagawa 2010
ASV17	Endozoicomonas	99.6	coral tissue	scleractinian coral	*Porites astreoides*	Panama (Bocas del Toro)	Western Atlantic	Sunagawa 2010
		99.6	coral mucus	scleractinian coral	NP	Curacao	Western Atlantic	Frade et al. 2016
ASV18	Ruminococcaceae	95.26	GI tract	freshwater fish	*Thymallus sp.*	Russia	Bol'shaya Tira River	Sukhanova et al. 2011 Accession:HE584732
		95.28	biogas reactor	reactor water	NP	Japan (Hokkaido)	NP	Nishioka et al. 2019 Accession: LC473933
ASV10	Lachnospiraceae	94.7	rumen	black beef cattle	NP	Japan	NP	Koike 2013 Accession:AB821803
		94.7	feces	human	*Homo sapiens*	NP	NP	Turnbaugh et al. 2009
		94.7	feces	human	*Homo sapiens*	United States	NP	Ley et al. 2006
ASV27	Epulopiscium	100	healthy coral tissue	scleractinian coral	*Orbicella faveolata*	Puerto Rico	Western Atlantic	Kimes et al. 2013
		100	GI tract	coral reef fish	*Naso tonganus*	Australia (Great Barrier Reef)	Pacific	Mendell et al. 2010 Accession:HM630230
		100	GI tract (distal intestine, feces)	coral reef fish	*Lutjanus bohar*	Palmyra Atoll	Pacific	Smriga et al. 2010
ASV15	Ruminococcaceae	98.41	GI tract	coral reef fish	Acanthurus nigrofuscus	Saudi Arabia	Red Sea	Miyake et al. 2015
		98.02	GI tract	coral reef fish	Siganus canaliculatus	China	NP	Zhang et al. 2018
		96.43	feces	kangaroo	*Macropus rufus*	Australia	NP	Ley et al. 2008
ASV68	Endozoicomonas	99,60	tissue	marine tunicates	NP	Malaysia	Western South China Sea	Danish-Daniel et al. 2018 Accession:MG896199
		99,60	tissue	ascidians	*Styela clava*	Denmark	NP	Schreiber et al. 2016 Accession: KU648381
		99,60	coral tissue	scleractinian coral	*Colpophyllia natans*	Curacao	NP	Klaus et al. 2011
ASV30	Romboutsia	100	soft coral tissue	soft coral	*Gorgonia ventalina*	Panama (Bocas del Toro)	Western Atlantic	Sunagawa 2010
ASV95	Vibrio	99,61	GI tract	coral reef fish	*Zebrasoma desjardinii*	Saudi Arabia	Red Sea	Miyake et al. 2016
		99,60	water	water	NP	Brazil	NP	Coutinho et al. 2012 Accession: JQ480694
		99,21	marine sediment	marine sediment	NP	India (Andaman Islands)	Indian Ocean	Cherian et al. 2019 Accession: MK975459
ASV94	Romboutsia	NP	NP	NP	NP	NP	NP	NP
ASV19	Clostridium_sensu_stricto_1	99.6	feces	goose	*Branta canadensis*	Canada	NA	Lu et al. 2009
	Clostridium_sensu_stricto_1	99.21	aquaponic biofilm	NP	NA	Mexico	NA	Munguia-Fragozo et al. 2016 Accession: KY125439
	Clostridium_sensu_stricto_1	98.81	feces	human child	*Homo sapiens*	Nigeria	NP	Tidjani Alou et al. 2016 Accession: LT161894
ASV24	Tyzzerella	97.23	suspended plant residue in a methanogenic reactor of cattle farm waste	NP	NP	NP	NA	Ueki et al. 2017
ASV25	Ruminococcaceae	98.02	fish gut	coral reef fish	*Acanthurus nigrofuscus*	Saudi Arabia	Red Sea	Miyake et al. 2016
	Ruminococcaceae	97.62	fish gut	coral reef fish	*Siganus canaliculatus*	China	South China Sea	Juan et al. Accession: HG970996
	Ruminococcaceae	96.03	feces	Red Kangaroo	*Macropus rufus*	USA, Saint Louis Zoological Park	NA	Ley et al. 2008
	Ruminococcaceae	95.28	GI tract	coral reef fish	*Pomacanthus sexstriatus*	NP	NP	Ward et al. Accession:EU885024
ASV39	Anaerofilum	97.62	fish gut	coral reef fish	*Acanthurus nigrofuscus*	Saudi Arabia	Red Sea	Miyake et al. 2016
	Anaerofilum	97.22	fish gut	coral reef fish	*Siganus canaliculatus*	China	South China Sea	Juan et al. Accession: HG970996
	Anaerofilum	96.83	GI tract	coral reef fish	*Naso tonganus*	Australia (Great Barrier Reef)	Pacific	Mendell et al. Accession: HM630257
ASV41	Epulopiscium	100	coral mucus	scleractinian coral	NP	Curacao	Western Atlantic	Frade et al. 2016
	Epulopiscium	100	freshwater microbialite	NA	NA	Mexico	NP	Corman et al. Accession:KP479649
ASV59	Endozoicomonas	99.21	bivalve gill	bivalve	Ctena orbiculata	USA,Florida	Atlantic	Lim et al. Accession: KY687505,
	Endozoicomonas	99.21	pharynx tissue	ascidian	Ascidia sp.	Sweden	North Sea	Schreiber et al. Accession: KU64822
	Endozoicomonas	99.21	gill	bivalve mollusc	*Loripes lacteus*	NP	Meditarranean	Mausz et al. Accession: GQ853556
ASV74	Clostridium_sensu_stricto_2	98.02	ontaminated groundwat	NA	NA	USA	NA	Bowman et al. 2008
ASV163	Endozoicomonas	100	marine tunicates	tunicate	NP	Malaysia	NP	Danish-Daniel et al. ACCESSION: MG896199
	Endozoicomonas	100	pharynx tissue	ascidian	*Ascidia sp.*	Sweden	North Sea	Schreiber et al. ACCESSION: KU648273
ASV589	Thermus	100	plant root	plant	NP	USA	NA	Bueno de Mesquita et al 2020

Table 4.1: Basic Local Alignment Search Tool for nucleotides (BLASTn) (Altschul et al. 1990) search results for ASVs identified as part of the core microbiome to infer where these ASVs or close sequences have been previously identified. Core ASVs were compared to the non-redundant nucleotide (nr/nt) collection database of the National Centre for Biotechnology Information (NCBI) with BLASTn. Metadata are recorded for sequences that matched each query at 100% similarity or the first five top hits. NP = Information not provided. NA = Not Applicable.

a facultative coral feeding fish are destabilised on the most coral-depauperate reefs across a habitat degradation gradient of reefs ranging from 0% to ~30% live corals. Shifts in the fish gut microbiome may reflect changes in diet in degraded habitats and/or suggest possible limits to the host's ability to regulate its microbiome with increasing severity of habitat degradation.

Whole gut microbial communities were significantly more diverse and variable in fish from inner bay disturbed reefs than from the outer bay zone. Conspicuously, the core microbiome, a small set of microbial strains that form sustained relationships with the fish host, also showed higher dispersion on degraded reefs, with greater variability of microbial assemblages among individual fish. Significant differences in diversity (Fig. 4.4B, 4.4C) and group dispersion were only observed with diversity metrics that place less weight on rare ASVs (Fig. 45C, 4.5E and 4.5F) indicating that changes in the relative abundance of the most common taxa are responsible for this pattern. Unstable host-associated microbial communities have been observed in humans with immunodeficiency syndromes (reviewed in Williams, Landay, and Presti 2016; Zaneveld, McMinds, and Vega Thurber 2017) and in marine animals such as scleractinian corals and anemones under acute stress (Zaneveld, McMinds, and Vega Thurber 2017; Zaneveld et al. 2016; Beatty et al. 2019; Ahmed et al. 2019). Zaneveld et al. (2017) referred to this pattern of variability as the "Anna Karenina principle" applied to host associated microbiomes (AKP). They argued that this is a common but often overlooked response of organisms that become unable to regulate their microbiome. Our results are consistent with patterns expected under the Anna Karenina principle suggesting that fish experience some level of stress in association with habitat degradation.

More variable gut microbial communities at disturbed reefs, where corals, the preferred food of *C. capistratus*, are nearly absent, could be a symptom of stress induced by reductions in resource availability including increased foraging costs if, for example, fish spent more energy to search, capture and handle their prey. Indeed, physiological stresses imposed by environmental conditions may cause immune signals that imbalance gut microbiota (Zaneveld, McMinds, and Vega

Thurber 2017; Ma et al. 2019; Pita et al. 2018; Butt and Volkoff 2019). Disturbance to the microbiome, in turn, can affect the brain and further alter behaviours related to movement such as the ability to forage (Johnson and Foster 2018; Butt and Volkoff 2019). The scarcity of resources may also increase stress through intra- and interspecific competition. For example, social stress in the form of aggressive interactions among conspecifics was shown to alter the behaviour and microbial assemblages associated with mice by setting off immune responses critical to host health (Werbner et al. 2019). In Indo-Pacific butterflyfishes, coral degradation was shown to decrease aggressive encounters among and within *Chaetodon* species (Keith et al. 2018) as well as change the frequency of pair formation (Thompson et al. 2019), and the way species responded to loss of the coral resource was dependent on their level of dietary specialisation (Thompson et al. 2019; Keith et al. 2018). Foraging on degraded reefs may also increase predation risk when architectural complexity is reduced (Almany 2004). Anxiety-like behaviours induced by exposure to predators can lead to sustained physiological stress in vertebrates (reviewed in Clinchy, Sheriff and Zanette, 2013).

Another possible explanation for more variable gut microbiomes at disturbed reefs could be increased behavioural heterogeneity among fish individuals (e.g., feeding behaviour). Where preferred food sources are scarce, foraging behaviour may become more diverse and lead to increased individual specialisation in various alternative food items (Bolnick et al. 2007; Svanbäck and Bolnick 2007) translating into more varied gut microbiomes. Higher alpha diversity at the inner bay disturbed zone supports this explanation (Fig. 4.4B, 4.C). Although *C. capistratus* is able to consume a broad range of diet items (Birkeland and Neudecker 1981; Neudecker 1985; Lasker 1985), deviations from its preferred coral prey may come with fitness consequences as shown for Indo-Pacific Butterflyfishes (Pratchett et al., 2004; Berumen, Pratchett and McCormick, 2005; Cole, Pratchett and Jones, 2008). For example, studies found that *Chaetodon* species have reduced energy reserves at reefs where they diversify or shift their diet in response to limited coral availability (Berumen, Pratchett, and McCormick 2005; Pratchett et al. 2004). To this end,

more variable gut bacterial communities at disturbed reefs in our study could be a symptom of weakened fish health due to altered nutrition.

Significant differences in the composition of the whole gut microbiome in nearly all comparisons (i.e., between all three zones, between inner and outer bay, and between inner bay disturbed and undisturbed; Fig 4.6A) may primarily reflect changes in diet. Microbial prevalence analysis (PIME, Roesch et al. 2020) identified sets of ASVs that suggested a more broad, likely omnivorous trophic profile for fish where coral cover was low. This included a distinct *Endozoicomonas* community in codominance with anaerobic fermentative bacteria (e.g., Flavonifractor and *Romboutsia* in Firmicutes, *Epulopiscium* as well as other Lachnospiraceae in Firmicutes) (Fig. 4.7). Prevalence of these fermentative microbes at disturbed reefs likely reflect the consumption of algae. *Epulopiscium*, often considered a hostspecific symbiont of herbivorous surgeonfishes (family: Acanthuridae) (Miyake, Ngugi, and Stingl 2015; Ngugi et al. 2017; Fishelson, Montgomery, and Myrberg 1985), was represented in the core microbiome and identified as distinct to the inner bay predominantly at disturbed reefs. This suggests that *C. capistratus* can assimilate nutrients from algae and that this metabolic function is enhanced on degraded reefs by the increase in key microbial functional groups. Alternatively, the fish in our study may take up these microbes while foraging for invertebrates on the epilithic algal matrix. Overall, levels of *Epulopiscium* here were approximately similar to those previously found in omnivores and detritivores in the Red Sea (Miyake, Ngugi, and Stingl 2015) with the two most prevalent ASVs matching (100%) to a strain previously isolated from the turf algal grazer Naso tonganus (Choat, Robbins, and Clements 2004). Additionally, the presence of Rhodobacteraceae, which are often found associated with algal biofilms (Pujalte et al. 2014; Elifantz et al. 2013), may suggest detritus feeding but might also be related to the consumption of mucus from stressed (Glasl, Herndl, and Frade 2016) and diseased corals (Sunagawa et al. 2009; Roder et al. 2014) where Rhodobacteraceae are also found. Lower relative abundance of a compositionally distinct *Endozoicomonas*community at disturbed

reefs could reflect different proportions of prey species featuring *Endozoicomonas* (Morrow et al. 2012) in the diet of *C. capistratus*.

In contrast, a single dominant *Endozoicomonas* ASV along with a few Firmicutes characterised the gut microbiome of *C. capistratus* on outer bay reefs (Fig. 4.7). The presence of some *Endozoicomonas* ASVs shared between fish guts and potential prey (i.e., hard corals, soft corals, zoanthids, sponges) including exact matches to microbial sequences previously detected in two coral species (*Orbicella faveolata, Poritis asteroides*) at our study area in Bocas del Toro (Sunagawa, Woodley, and Medina 2010; Sunagawa et al. 2009), suggests the horizontal acquisition of these microbes via feeding on corals. In addition, we identified an ASV in the genus Ruegeria as indicative of outer bay reefs, which matched (100%) a sequence previously retrieved from the soft coral species *Pterogorgia anceps* on the Caribbean coast of Panama (unpublished sequence, GenBank Accession: MG099582) and which was also present across samples of potential prey taxa including hard and soft corals and sponge-infauna. Even if *Endozoicomonas* originated from the food, they might nevertheless promote the assimilation of nutrients via interactions with resident bacteria (Zhang et al. 2016).

The core microbiome composition differed under similar environmental conditions across the inner bay between fish from disturbed and undisturbed reefs (Fig. 4.6B). This finding suggests that bacterial communities that are most likely to have intimate metabolic interactions with *C. capistratus* might fail to provide beneficial functions to hosts at severely degraded habitats. Distinct core assemblages at the more exposed outer bay could also reflect microbial plasticity mediated by diet, gut colonisation history (Uren Webster et al. 2020) and/or potential genetic differentiation between inner bay and outer bay fish subpopulations (Smith et al. 2015; Uren Webster et al. 2018; Fietz et al. 2018).

Our analysis identified ten *Endozoicomonas* ASVs as part of the core microbiome indicating potential true resident symbionts. Members of the genus *Endozoicomonas* spp. are known as bacterial symbionts of marine sessile and some mobile invertebrates and fishes (Neave et al. 2017; Pogoreutz et al. 2018; Fiore et al. 2015; Neave et

al. 2016). Reverter et al. (2017) found *Endozoicomonas* associated with butterflyfish gill mucus in *Chaetodon lunulatus* and Parris et al. (2016). found *Endozoicomonas* in the gut of damselfishes (Pomacentridae) and cardinalfishes (family: Apogonidae) pre- but not post-settlement on the reef. Corallivory in butterflyfish has been found to have evolved in close association with coral reefs (Bellwood et al. 2010; Reese 1977) and this likely involved adaptive mechanisms to metabolise defense compounds from corals and many other sessile invertebrates (e.g., polychaetes). Adapted gut microbial communities may help butterflyfish hosts cope with toxins or facilitate the digestion of complex prey tissues as in insects, mammalian herbivores and surgeonfish (Hammer and Bowers 2015). It is likely that the gut microbial profile of *C. capistratus* — featuring high abundances *Endozoicomonas* — facilitates the digestion of complex coral prey. More detailed knowledge will be required to understand whether the potential intake of *Endozoicomonas* via fish browsing on sessile invertebrates is essential to trophic strategies involving fish corallivory. Although the health of fishes is thought to be highly dependent on the state of their microbiome (Gómez and Balcázar 2008; Llewellyn et al. 2014), little is known about what constitutes a balanced versus imbalanced microbial assemblage. Thus, defining microbial homeostasis or dysbiosis remains challenging and these terms should be applied with caution (Hooks and O'Malley 2017; Levy et al. 2017; Brussow 2019). We found an increase in microbiome variability, diversity, and community turnover that extended to the core microbiome suggesting that the microbiome may be vulnerable to extremely low levels of live coral cover. Additional work should focus on linking changes in the gut microbiome to host health. Our results give insight into the poorly understood spatial fluctuations in host associated microbial communities across a natural system and in response to coral reef habitat decline. This work highlights intricate links between ecosystem-scale and microbial-scale processes, which have so far been mostly overlooked. We suggest there is an urgent need to integrate measurements of the role of microbes in the response of reef fishes to the global loss of coral reefs.

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Author contributions

F.C., M.L., and J.J.S. conceived the study. M.L., F.C. and R.F.P. designed the study with input from AHA. F.C., M.L., and J.J.S. conducted the fieldwork. E.C.R.G. and F.C. dissected the fish guts. F.C. extracted the DNA. J.S. and M.L. prepared the DNA for sequencing and processed the sequencing data. R.F.P., A.H.A., W.O.M. and M.L. contributed reagents and supplies. E.C.R.G. analysed the photographic benthic quadrats. F.C., J.S. and M.L. analysed the data and wrote the first draft of the manuscript with input from R.F.P. and L.G.E.W. All authors reviewed the manuscript and contributed to the final version.

Chapter 5

General discussion

The ongoing reorganisation of ecological communities in response to human pressures poses major challenges in detecting and interpreting community patterns and underlying ecological mechanisms such as species' trophic interactions. Knowledge of species-level mechanisms and how they scale up to communities is crucial for predicting the functioning and service provision of future ecosystems and to inform the development of resilience-based management strategies. This thesis generated in-depth descriptions of ecological communities at unprecedented taxonomic resolution and on different levels of biological organisation (i.e., coral reef fishes, prey within fish stomachs, host associated microbes) using traditional visual-census techniques, dietary DNA-Metabarcoding and 16S-based gut microbiome analysis. By investigating spatial variability in diversity patterns and ecological interactions, this thesis gained insight into fish functional (**Chapter 2**), dietary (**Chapter 3**) and gut microbial (**Chapter 4**) niches along habitat gradients (**Chapter 3 and 4**) and across spatial scales (**Chapter 2**).

At the community level, I confirmed that taxonomic species assemblages of Caribbean coral reef fishes are governed by the spatial axis of geomorphological reef zonation (Alevizon et al. 1985, Mejía et al. 2000, **Chapter 2**). Yet, the significant interaction among atolls and reef zones indicated that local environmental characteristics at each atoll contributed to fish diversity patterns (**Chapter 2**). Both taxonomic and functional diversity levels were highest at the largest, least isolated but also least protected atoll, whereas taxonomic diversity was significantly lower at the atoll that was most protected. All three atolls differed in terms of

the relative levels of occupancy of different functional trait entities. This may reflect differences in the dominant functional processes at play within each atoll ecosystem. Fish communities were vulnerable to losing functions at the regional scale, indicated by a pattern of functional over-redundancy (sensu Mouillot 2014) (**Chapter 2**). At the species level, I found that different benthic feeding strategies showed variable resource use patterns across a habitat gradient (**Chapter 3**). Coral cover predicted the diet of a facultative corallivore (*Chaetodon capistratus*), whereas, the diet of a benthic crustaceans feeder (*Hypoplectrus puella*) responded to exposure level rather than habitat. Interestingly, *C. capistratus* switched from a coral dominated diet at high coral cover reefs to an annelid dominated diet at low coral cover reefs (**Chapter 3**). This finding suggested that the trophic functions provided by this species differ between healthy and degraded reefs (**Chapter 3**) and highlighted variable responses to habitat degradation between different feeding strategies and dietary niches. Building on Chapter 3, **Chapter 4** found—at the level of host-associated microbes—that a microbial community pattern, which has been previously linked to microbiome instability (Zaneveld, McMinds, and Vega Thurber 2017) characterised the gut microbiome of *C. capistratus* at degraded reefs.

Species interactions underpin mechanisms potentially stabilising communities and ecosystem functioning under conditions of change, such as functional redundancy (i.e., the presence of species performing equivalent roles ensures ecosystem functioning in cases of diversity loss, Rosenfeld 2002) and trophic compensation (e.g., Ghedini, Russell, and Connell 2015). Whether and how such mechanisms actually stabilise ecological processes on coral reefs is being increasingly scrutinised. For example, dietary studies of high taxonomic resolution based on either expert knowledge or novel technologies (e.g., DNA-metabarcoding, compound specific isotopes) revealed that resource partitioning among closely related coral reef fish species appears more complex and differentiated than previously assumed, rendering communities potentially vulnerable to losing functions (Longenecker 2007; Leray, Meyer, and Mills 2015; Leray et al. 2019; Brandl and Bellwood 2014; Brandl, Casey, and Meyer 2020; Kramer et al. 2015; McMahon et al. 2016). Similarly, studies

analysing coarser resolutions of fish species' traits at the community level found that species were disproportionately distributed among functions, with a broad suite of functions represented by only one species lacking insurance against species loss (Guillemot et al. 2011, Micheli et al. 2014, Mouillot et al. 2014; D'Agata et al. 2016b, McLean et al. 2019, **Chapter 2**). This thesis showed that this globally consistent pattern of functional over-redundancy in fish assemblages, which was first established at the scale of tropical bioregions, persisted at spatial scales relevant to local conservation and resource management (10-100 km, Spalding 2000) in the Caribbean. Proposing a standardised measure of relative trait-space occupancy, this thesis generated novel insight by taking into account both the occurrence and abundance distribution among fish functions (**Chapter 2**).

Functionally vulnerable fish assemblages may be reflective of the high levels of niche complementarity detected among closely related species based on, for example, their diets, habitat use, swimming abilities, and gut microbiomes (Whiteman, Côté, and Reynolds 2007; Frédérich et al. 2009; Nagelkerken et al. 2009; Brandl and Bellwood 2014; Kramer et al. 2015; Leray, Meyer, and Mills 2015; Bejarano et al. 2017; Liedke et al. 2018; Leray et al. 2019; Scott et al. 2020; Brandl, Casey, and Meyer 2020). This thesis highlighted the role of within-species trait variation (e.g., dietary plasticity) in potentially mediating this pattern (Albert et al. 2010; **Chapter 3**). For example, species may influence levels of niche overlap within communities through dietary plasticity, which may allow them to maintain functions if richness declines. Yet, knowledge of coral reef fish intraspecific dietary variation across space, e.g., between geographic locations (Whiteman, Côté, and Reynolds 2007; Lawton and Pratchett 2012; Liedke et al. 2016) or on small spatial scales (Berumen, Pratchett, and McCormick 2005), is scarce but crucial to understanding potential niche shifts in relation to habitat change (Berumen, Pratchett, and McCormick 2005; Graham 2007a; Layman et al. 2007; Hempson, Graham, MacNeil, Williamson, et al. 2017; Kingsbury et al. 2020; Monaco et al. 2020, **Chapter 3**). Versatile reef fish predators have been little explored regarding their potential for feeding plasticity in response to altered prey resources on degraded

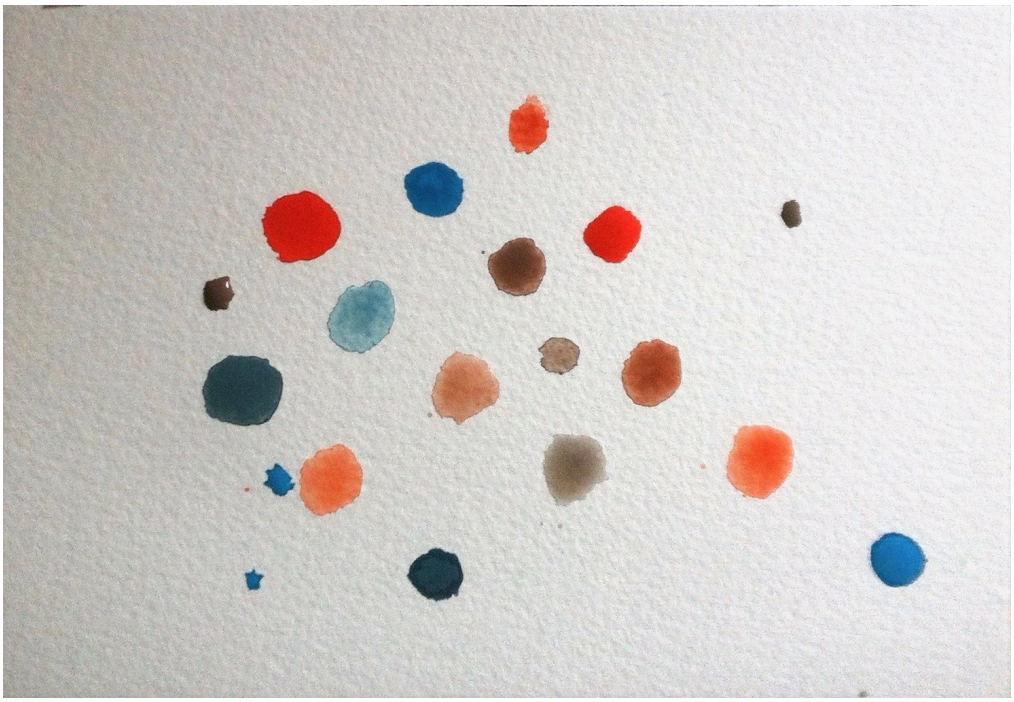

Figure 5.1: Sketch for the visual method developed in Chapter 2 for depicting relative levels of occupancy in functional trait space by fish assemblages. Drawing: Friederike Clever

reefs. Few examples document switches in foraging mode and/or prey (Hempson et al. 2017; Karkarey et al. 2017, **Chapter 3**) or dietary niche contraction (Layman et al. 2007). Prey switching (Murdoch 1969) was found in predators that feed on small fishes (Hempson, Graham, MacNeil, Williamson, et al. 2017), juvenile fishes (**Chapter 3**), and benthic mobile and sessile invertebrates (**Chapter 3**) at mid-trophic levels. These findings suggest that the trophic functions delivered by a given species may spatially vary, even at small spatial scales (**Chapter 3**). The spatial and/or temporal variation in feeding interactions may mediate whether and how trophic networks adjust to degraded habitat conditions (Nagelkerken et al. 2020) and suggests that patterns of niche overlap are dynamic and context dependent, which may influence the adaptive capacity of communities.

Feeding behaviour may be mediated by interactions between animal hosts and their gut microbiomes, the latter of which can constitute a crucial determinant of dietary niches in coral reef fish herbivores (Clements et al. 2014; Ngugi et al. 2017; Scott et al. 2020). Diet can also directly affect the composition of fish gut

microbiomes (Lyons et al. 2017). Thus, knowledge about gut microbiomes can aid in delineating fish trophic niches and vice versa (Clements et al. 2014; Colston and Jackson 2016; Escalas et al. 2021). Moreover, host associated microbes can adjust rapidly in response to changing environmental conditions or diets and, thus, may facilitate host acclimatisation processes (Alberdi et al. 2016) and underpin plastic feeding behaviour (Chapter 4). Vertebrate gut microbiomes have shown diverse responses to suboptimal habitat conditions. For example, a study of bird gut microbiomes in Costa Rica found that only few host species appeared vulnerable to land use change (San Juan et al. 2020). Yet, microbiomes often become less diverse as found in primates (Amato et al. 2013; Barelli et al. 2015), likely related to simplified diets and potentially associated with a loss of microbial functions (Amato et al. 2013; West et al. 2019; Borbón-García et al. 2017). The gut microbiome of a coral feeding butterflyfish (*Chaetodon capistratus*) increased in alpha diversity and became more variable among individuals at coral depauperate reefs in tandem with a change in main diet item (**Chapter 3 and 4**). Similar microbial patterns have been found to characterise responses to disturbance in invertebrate hosts such as sponges (reviewed in Pita et al. 2018) and corals (Zaneveld et al. 2016). These findings highlight the complexity and diversity in gut microbial responses among different taxa (West et al. 2019) and demonstrate that detailed information on within-species dietary and gut microbiome variation enhances our understanding of species' realised niches and can elucidate species' responses to changing conditions (**Chapter 3 and 4**).

5.0.1 Implications for management

The findings of this thesis suggested that the effectiveness of marine reserves for protecting fish diversity depends on biogeographic setting and the seascape characteristics at a given reef location (**Chapter 2**). Biogeography and seascape factors (e.g., reef area, spatial isolation, reef geomorphology) can mediate the relative strength of both, global climate effects and local human stressors on local scales and thus should guide decision making for area prioritisation, while ideally also

incorporating knowledge on reef recovery trajectories (Graham et al. 2015; Graham et al. 2020). I showed that biogeographic factors may counteract benefits from protection, while on the other hand they may mitigate detrimental local human influences (e.g., fishing, pollution) and potentially vice versa (Chapter 2). For example, Cinner et al. (2016) identified coral reefs of high fish biomass despite proximity to negative local impacts. Identifying and understanding the drivers behind these observations may guide choosing areas for conservation (Cinner et al. 2016; Graham et al. 2020).

A central goal in resilience-based management is to manage ecosystem processes (McLeod et al. 2019). To this end, groups of functional traits can serve as proxies for important ecological functions provided by fishes (**Chapter 2**) and should be increasingly integrated into management to maintain and rebuild ecosystem resilience (e.g., Micheli et al. 2014) and to better predict the impacts of fishing on ecosystem functioning (e.g., Allgeier et al. 2016; D'Agata et al. 2016b). This thesis showed that reef geomorphology provides simple proxies of fish diversity (taxonomic and functional), which could aid in mapping the spatial distributions of fish functions (**Chapter 2**). However, effectively managing ecological processes requires more research to better understand the nature fish functions within coral reef ecosystems (Bellwood et al. 2018, Brandl et al. 2018). For example, a potentially crucial but little understood aspect, which appears largely ignored by fisheries and conservation management, regards the role of intraspecific trait variability in influencing functioning (Allgeier et al. 2020, **Chapter 3 and 4**) (see 'Future outlook' below).

Furthermore, a holistic approach integrating different levels of ecological organisation could inform the development of effective tools for monitoring and targeted interventions. My findings suggested that species' trophic roles can vary across small spatial scales and are (to various degrees depending on the species in question) predictable by habitat characteristics (e.g., coral cover) (**Chapter 3**). Based on further research, such knowledge could be extended towards key fish functions to better predict ecosystem dynamics. For example, local management may incorporate

spatially explicit information on fish functions to improve trait-based, community-level assessments of functional parameters (e.g., functional redundancy) to better predict which functions may be particularly vulnerable to fishing (e.g., D'Agata et al. 2016b). Screenings of fish gut microbiomes (using non-lethal methods e.g., collecting fecal samples) could potentially aid in detecting fish population stress levels, overall health, and dietary states as well as acclimatisation and adaptation potential under changing conditions (Apprill 2020). However, establishing effective indicators will require more baseline knowledge on drivers of fish gut microbiome variability in natural systems.

5.0.2 Future outlook

By investigating different levels of ecological organisation from fish communities to fish diets to gut microbiomes, this thesis marks a first step towards a holistic view of key processes that influence coral reef fish responses to changing habitat conditions. This approach generated a number of novel questions and possible future research directions.

A useful next step would be experiments testing hypotheses regarding i) degrees of specialisation in relation to resource availability, habitat and physical condition; and ii) relationships between host gut microbiomes, habitat condition and diet with the aim to understand and discriminate stress versus plastic responses and to investigate variation in microbial functioning.

The findings of this thesis raised the question whether the two fish species studied across reefs at the Bahia Amirante at Bocas del Toro constitute one or several subpopulations. Population genetics tools (e.g., microsatellite or mitochondrial DNA markers) could elucidate potential influences of host genotypes on diet breadth and microbiome composition and potentially provide insight into whether the observed dietary differences reflect adapted traits or versatile behaviour. In this context, a further question would be whether versatile feeding behaviour reflects immediate plastic responses or was acquired during development. These questions could also potentially be addressed with field experiments using species replacements.

Chapter 3 and 4 could be expanded to sequencing the diets and gut microbiomes of larger species suites while ideally retaining robust sample sizes per species across different habitats, habitat conditions, and if feasible across time (using non-lethal sampling methods). For example, analyses spanning multiple trophic levels would allow to determine trophic interaction networks (e.g., Casey et al. 2019) and elucidate pathways by which individual-level processes (e.g., dietary plasticity) affect ecosystem functioning. Trophic networks could further be used to understand how habitat change may shift food-web structure and potentially ecosystem functioning on coral reefs (e.g., Nagelkerken et al. 2020).

This thesis showed that diets of versatile feeders can be highly context-dependent to the degree where species' trophic functions may vary across space within an ecosystem (**Chapter 3**). However, trait-based approaches studying coral reef fish communities commonly treat diet as a 'fixed' as opposed to a trait that is flexible across space and time (regardless of degree of dietary specialisation), due to a lack of empirical and high-resolution data. This corresponds to the notion that, as useful tools, 'traits should vary more between than within species' (McGill et al. 2006; discussed in Violle et al. 2012). Extracting trait values at the species level as opposed to population or individual level may mask niche variability and hinder the understanding of ecosystem processes (Bolnick et al. 2003; Violle et al. 2012; Hadj-Hammou, Mouillot, and Graham 2021). Integrating intraspecific trait variation is challenging in high diversity assemblages such as coral reef fishes. A way forward may be to combine dietary metabarcoding data obtained along habitat gradients (e.g., **Chapter 3**) and ideally across spatial scales with easy to measure but powerful traits such as size (total length) (proposed by Hadj-Hammou, Mouillot, and Graham 2021) or potentially a measure of the level of specialisation.

Fishes are important players in the functioning, maintenance and resilience of coral reef ecosystems through their roles in tropho-dynamics. This thesis contributed to identifying and understanding mechanisms by which trophic interactions and community assemblages may shift under increasingly severe conditions of change.

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Appendices

Appendix A

Supporting material for Chapter 2

Factor	Diversity	Groups	Z	*P* adjusted
Atoll	Mean richness	Lighthouse - Glovers	-3.522625	0.0004
		Turneffe - Glovers	-3.682877	0.0003
		Turneffe - Lighthouse	-0.118272	0.45
	Mean abundance	Lighthouse - Glovers	-3.111691	0.002
		Turneffe - Glovers	-3.420814	0.0009
		Turneffe - Lighthouse	-0.262282	0.4
	Unbiased Simpsons	Lighthouse - Glovers	-2.418518	0.023
		Turneffe - Glovers	-2.07294	0.04
		Turneffe - Lighthouse	0.347873	0.36
Zone	Mean richness	Backreef - Channel	-2.318789	0.05
		Backreef - Forereef	0.344821	0.73
		Backreef - Lagoon Patch	1.18844	0.47
		Backreef - Westreef	-2.429633	0.05
		Channel - Forereef	2.481791	0.05
		Channel - Lagoon Patch	3.621816	0.001
		Channel - Westreef	-0.069882	0.47
		Forereef - Lagoon Patch	0.711789	0.71
		Forereef -Westreef	-2.584018	0.04
		Lagoon Patch - Westreef	-3.769233	0.0008
	Mean abundances	Backreef - Channel	-2.938466	0.01
		Backreef - Forereef	-0.838741	0.4
		Backreef - Lagoon Patch	0.697502	0.24
		Backreef - Westreef	-1.991411	0.16
		Channel - Forereef	1.917216	0.17
		Channel - Lagoon Patch	3.822304	0.0007
		Channel - Westreef	0.953796	0.51
		Forereef - Lagoon Patch	1.531203	0.31
		Forereef -Westreef	-1.02356	0.61
		Lagoon Patch - Westreef	-2.821891	0.02
	Unbiased Simpsons	Backreef - Channel	1.900072	0.14
		Backreef - Forereef	2.309405	0.07
		Backreef - Lagoon Patch	2.328868	0.08
		Backreef - Westreef	-0.143961	0.44
		Channel - Forereef	0.460999	1
		Channel - Lagoon Patch	0.166908	0.87
		Channel - Westreef	-1.988933	0.14
		Forereef - Lagoon Patch	-0.35237	1
		Forereef -Westreef	-2.387014	0.08
		Lagoon Patch - Westreef	-2.407628	0.08

Table A.1: Comparison of fish community alpha diversity among atolls and reef zones, respectively showing posthoc Dunn test results with Benjamin Hochberg corrected *P-values.*

Model	Factor	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Lagoon Patch GL-TN	Atoll	1	0.11064	0.110641	2.1473	0.10658	0.01
Lagoon Patch GL-LH	Atoll	1	0.09944	0.099444	1.8598	0.08916	0.007
Lagoon Patch LH-TN	Atoll	1	0.08856	0.088561	2.0936	0.1599	0.007
Backreef LH-TN	Atoll	1	0.05258	0.052579	1.2486	0.111	0.23
Backreef LH-GL	Atoll	1	0.07978	0.079778	1.6599	0.13111	0.044
Backreef TN-GL	Atoll	1	0.07442	0.074423	1.5296	0.12208	0.071
Westreef LH-TN	Atoll	1	0.11669	0.116695	1.2094	0.10789	0.184
Westreef LH-GL	Atoll	1	0.13991	0.13991	1.5656	0.14818	0.046
Westreef TN-GL	Atoll	1	0.13359	0.133593	1.3679	0.13194	0.088
Channel LH-TN	Atoll	1	0.2007	0.200703	2.16	0.2126	0.009
Channel LH-GL	Atoll	1	0.19052	0.19052	1.6779	0.15713	0.006
Channel TN-GL	Atoll	1	0.1262	0.1262	1.2165	0.11907	0.238
LP-BR	Glovers	1	0.2506	0.250599	4.6385	0.19623	0.001
LP-WR	Glovers	1	0.16259	0.162591	3.2175	0.15914	0.001
LP-CH	Glovers	1	0.18976	0.18976	3.449	0.1608	0.001
LP-FR	Glovers	1	0.4092	0.4092	7.8235	0.32839	0.001
BR-WR	Glovers	1	0.17693	0.176931	3.8208	0.27645	0.001
BR-CH	Glovers	1	0.19237	0.192366	3.5929	0.24621	0.002
BR-FR	Glovers	1	0.32382	0.32382	6.7173	0.42738	0.001
WR-CH	Glovers	1	0.06563	0.065628	1.3945	0.13416	0.155
WR-FR	Glovers	1	0.2325	0.232496	5.9808	0.46074	0.01
CH-FR	Glovers	1	0.15435	0.154354	3.0971	0.27909	0.006
LP-BR	Lighthouse	1	0.1427	0.142697	3.2252	0.22672	0.001
LP-WR	Lighthouse	1	0.14197	0.141973	3.3734	0.2347	0.002
LP-CH	Lighthouse	1	0.18087	0.180866	3.9661	0.28398	0.001
LP-FR	Lighthouse	1	0.24028	0.24028	5.0767	0.36064	0.001
BR-WR	Lighthouse	1	0.1279	0.127899	3.2724	0.24656	0.001
BR-CH	Lighthouse	1	0.09781	0.097806	2.2928	0.20303	0.023
BR-FR	Lighthouse	1	0.11026	0.110261	2.4927	0.23756	0.009
WR-CH	Lighthouse	1	0.06031	0.06031	1.507	0.14343	0.073
WR-FR	Lighthouse	1	0.12328	0.123282	2.9875	0.2719	0.004
CH-FR	Lighthouse	1	0.06367	0.063673	1.379	0.16458	0.091
LP-BR	Turneffe	1	0.14852	0.148522	3.6274	0.26619	0.001
LP-WR	Turneffe	1	0.15259	0.152592	3.6522	0.26752	0.004
LP-CH	Turneffe	1	0.13725	0.13725	3.7769	0.2956	0.006
LP-FR	Turneffe	1	0.32871	0.32871	6.5158	0.39452	0.001
BR-WR	Turneffe	1	0.15302	0.15302	3.5097	0.25979	0.004
BR-CH	Turneffe	1	0.07731	0.077308	2.0154	0.18296	0.003
BR-FR	Turneffe	1	0.18037	0.180369	3.451	0.25656	0.001
WR-CH	Turneffe	1	0.08942	0.089415	2.2758	0.20183	0.001
WR-FR	Turneffe	1	0.11001	0.110011	2.0717	0.17161	0.004
CH-FR	Turneffe	1	0.0893	0.089301	1.8255	0.16863	0.052

Table A.2: Posthoc pairwise Permutational Analysis of Variance (PERMANOVA) results of atoll and reef zone models comparing reef zones among atolls within atolls using Jaccard Index.

Model	Factor	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Lagoon Patch GL-TN	Atoll	1	0.44211	0.44211	3.0014	0.14291	0.004
Lagoon Patch GL-LH	Atoll	1	0.1583	0.15827	0.98832	0.04944	0.45
Lagoon Patch LH-TN	Atoll	1	0.18233	0.18233	1.7804	0.13931	0.009
Backreef GL-TN	Atoll	1	0.12866	0.128664	1.7274	0.13572	0.02
Backreef GL-LH	Atoll	1	0.09737	0.097368	1.3336	0.10812	0.152
Backreef LH-TN	Atoll	1	0.11002	0.110019	1.593	0.13741	0.019
Channe GL-TN	Atoll	1	0.1533	0.1533	1.8266	0.16872	0.006
Channel GL-LH	Atoll	2	0.73974	0.36987	3.9515	0.46755	0.002
Channel LH-TN	Atoll	1	0.16666	0.16666	2.0963	0.20763	0.005
Westreef GL-TN	Atoll	1	0.11684	0.116844	1.595	0.15054	0.042
Westreef GL-LH	Atoll	1	0.12326	0.123256	1.9337	0.17686	0.001
Westreef LH-TN	Atoll	1	0.1194	0.119402	1.7152	0.14641	0.004
LP-BR	Glovers	1	0.354	0.354	4.1966	0.18092	0.001
LP-WR	Glovers	1	0.30157	0.30157	3.6351	0.17616	0.001
LP-CH	Glovers	1	0.41808	0.41808	4.652	0.20537	0.001
LP-FR	Glovers	1	0.58737	0.58737	6.9741	0.30356	0.001
BR-WR	Glovers	1	0.3311	0.3311	4.4777	0.30928	0.002
BR-CH	Glovers	1	0.37733	0.37733	4.3839	0.28497	0.003
BR-FR	Glovers	1	0.47833	0.47833	6.3618	0.41413	0.007
WR-CH	Glovers	1	0.1651	0.165099	1.9696	0.17955	0.015
WR-FR	Glovers	1	0.31605	0.316051	4.5682	0.39489	0.008
CH-FR	Glovers	1	0.18918	0.189178	2.1881	0.21477	0.022
LP-BR	Lighthouse	1	0.22641	0.226408	3.0349	0.21624	0.002
LP-WR	Lighthouse	1	0.26086	0.260859	3.6622	0.24977	0.003
LP-CH	Lighthouse	1	0.30365	0.303647	3.5978	0.26459	0.001
LP-FR	Lighthouse	1	0.35122	0.35122	4.1418	0.31516	0.003
BR-WR	Lighthouse	1	0.29169	0.291688	4.5764	0.31396	0.003
BR-CH	Lighthouse	1	0.19438	0.194379	2.5069	0.21786	0.005
BR-FR	Lighthouse	1	0.19797	0.197967	2.5667	0.2429	0.009
WR-CH	Lighthouse	1	0.18131	0.181313	2.4696	0.21532	0.005
WR-FR	Lighthouse	1	0.25764	0.257641	3.5539	0.30759	0.003
CH-FR	Lighthouse	1	0.12351	0.12351	1.35	0.16168	0.084
LP-BR	Turneffe	1	0.2843	0.284296	4.4282	0.30691	0.006
LP-WR	Turneffe	1	0.33215	0.33215	4.9251	0.32999	0.002
LP-CH	Turneffe	1	0.27268	0.272677	4.3585	0.32627	0.005
LP-FR	Turneffe	1	0.61498	0.61498	8.1463	0.44892	0.003
BR-WR	Turneffe	1	0.26984	0.269838	3.6504	0.26742	0.005
BR-CH	Turneffe	1	0.14501	0.145013	2.0787	0.18763	0.008
BR-FR	Turneffe	1	0.3891	0.3891	4.7467	0.32188	0.005
WR-CH	Turneffe	1	0.14325	0.143252	1.9527	0.17829	0.008
WR-FR	Turneffe	1	0.22693	0.226929	2.6631	0.21031	0.006
CH-FR	Turneffe	1	0.20859	0.208591	2.5343	0.21972	0.009

Table A.3: Posthoc pairwise Permutational Analysis of Variance (PERMANOVA) results of atoll and reef zone models comparing reef zones among atolls within atolls using Bray-Curtis dissimilarity.

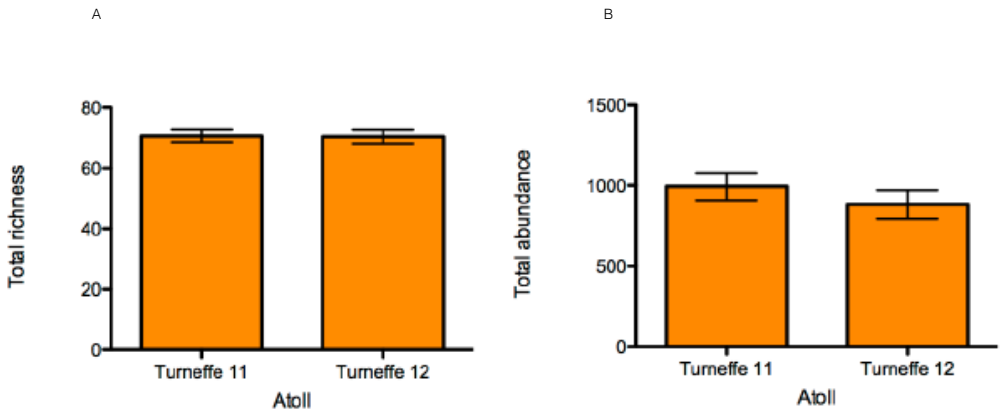

Figure A.1: Mean (+/- SE) seasonal comparison of fish species richness and abundance compared among September 2011 and January 2012 at 12 sites at Turneffe Atoll (n=12).

Family	Species	Species ID	Max. size	Trophic group	Mobility	Water column position	Activity time (feeding)	Gregariousness
ACANTHURIDAE	*Acanthurus coeruleus*	ACA COER	4	herbivore	mobile within reefs	benthopelagic	D	schooling
	Acanthurus tractus	ACA TRAC	4	herbivore	mobile within reefs	benthopelagic	D	schooling
	Acanthurus chirurgus	ACA CHIR	4	herbivore	mobile within reefs	benthopelagic	D	schooling
AULOSTOMIDAE	*Aulostomus maculatus*	AUL MACU	6	piscivore	sedentary	benthic	D	solitary
BALISTIDAE	*Balistes vetula*	BAL VETU	5	mobile invertivore	sedentary	benthic	D	solitary+schooling
	Melichthys niger	MEL NIGE	4	planktivore	sedentary	pelagic	D	schooling
	Canthidermis sufflamen	CAN SUFF	5	planktivore	mobile within reefs	pelagic	D	solitary+schooling
BELONIDAE	*Tylosurus crocodilus*	TYL CROC	6	piscivore	mobile within reefs	pelagic	D	solitary+schooling
	Belonidae spp	BEL SPP	5	piscivore	mobile within reefs	pelagic	D	schooling
BLENNIIDAE	*Ophioblennius macclurei*	OPH MACC	2	herbivore	sedentary	benthic	D	solitary
	Malacoctenus triangulatus	MAL TRIA	2	mobile invertivore	sedentary	benthic	D	solitary
	Malacoctenus macropus	MAL MACR	1	mobile invertivore	sedentary	benthic	D	solitary
	Malacoctenus aurolineatus	MAL AURO	1	mobile invertivore	sedentary	benthic	D	solitary
CARANGIDAE	*Caranx ruber*	CAR RUBE	5	piscivore	mobile within reefs	pelagic	D	solitary+schooling
	Caranx bartholomaei	CAR BART	6	piscivore	mobile within reefs	pelagic	D	solitary+schooling
	Caranx crysos	CAR CRYS	5	piscivore	mobile within reefs	pelagic	D	schooling
CARCHARHINIDAE	*Negaprion brevirostris*	NEG BREV	6	large predator	mobile between	benthopelagic	D+N	solitary+schooling
	Carcharhinus perezii	CAR PERE	6	large predator	mobile between	benthopelagic	D+N	solitary
	Carcharhinus spp	CAR SPP	6	large predator	mobile between	benthopelagic	D+N	solitary
CHAETODONTIDAE	*Chaetodon capistratus*	CHA CAPI	2	sessile invertivore	sedentary	benthic	D	solitary+pairing
	Chaetodon ocellatus	CHA OCEL	3	sessile invertivore	sedentary	benthic	D	pairing
	Chaetodon striatus	CHA STRI	3	sessile invertivore	sedentary	benthic	D	pairing
CIRRHITIDAE	*Amblycirrhitus pinos*	AMB PINO	2	planktivore	sedentary	benthic	D	solitary

Table A.4: Species list and trait assignment. Part 1/6.

DASYATIDAE	*Himantura schmardae*	HIM SCHM	6	mobile invertivore	mobile within reefs	benthic	D	solitary
	Dasyatis americana	DAS AMER	6	mobile invertivore	mobile within reefs	benthic	D	solitary+schooling
DIODONTIDAE	*Diodon hystrix*	DIO HYST	6	mobile invertivore	sedentary	benthic	D+N	solitary
	Chilomycterus antillarum	CHI ANTI	3	mobile invertivore	sedentary	benthic	D+N	solitary
	Chilomycterus antennatus	CHI ANTE	4	mobile invertivore	sedentary	benthic	D+N	solitary
ECHINEIDAE	*Echeneis naucrates*	ECH NAUC	6	piscivore	mobile between	pelagic	D	phoretic
	Remora remora	REM REMO	5	piscivore	mobile between	pelagic	D	phoretic
ELOPIDAE	*Megalops atlanticus*	MEG ATLA	6	large predator	mobile within reefs	pelagic	D	schooling
EXOCOETIDAE	*Hemiramphus brasiliensis*	HEM BRAS	5	piscivore	sedentary	pelagic	D	schooling
GINGLYMOSTOMATIDAE	*Ginglymostoma cirratum*	GIN CIRR	6	mobile invertivore	mobile within reefs	benthic	N	solitary
GOBIIDAE	*Coryphopterus* spp	COR SPP	2	herbivore	sedentary	benthic	D	solitary
	Coryphopterus personatus/hyalinus	COR PERS/HYAL	1	planktivore	sedentary	benthic	D	schooling
	Gnatholepis thompsoni	GNA THOM	2	herbivore	sedentary	benthic	D	solitary
	Elacatinus prochilos	ELA PROC	1	ectoparsite feeder	sedentary	benthic	D	schooling
	Elacatinus lobeli	ELA LOBE	1	ectoparsite feeder	sedentary	benthic	D	schooling
	Elacatinus colini	ELA COLI	1	sessile invertivore	sedentary	benthic	D	schooling
HAEMULIDAE	*Haemulon sciurus*	HAE SCIU	4	mobile invertivore	sedentary	benthic	N	schooling
	Haemulon flavonileatum	HAE FLAV	3	mobile invertivore	sedentary	benthic	N	schooling
	Haemulon plumierii	HAE PLUM	5	mobile invertivore	sedentary	benthic	N	schooling
	Haemulon carbonarium	HAE CARB	4	mobile invertivore	sedentary	benthic	N	schooling
	Haemulon chrysargyreum	HAE CHRY	3	mobile invertivore	sedentary	benthic	N	schooling
	Haemulon macrostomum	HAE MACR	4	mobile invertivore	sedentary	benthic	N	schooling
	Haemulon aurolineatum	HAE AURO	3	mobile invertivore	sedentary	benthic	N	schooling
	Haemulon melanurum	HAE MELA	4	mobile invertivore	sedentary	benthic	N	schooling
	Haemulon album	HAE ALBU	5	mobile invertivore	sedentary	benthic	N	pairing+schooling
	Haemulon parra	HAE PARR	4	mobile invertivore	sedentary	benthic	N	pairing+schooling
	Anisotremus virginicus	ANI VIRG	4	mobile invertivore	sedentary	benthic	D+N	solitary+schooling
	Haemulon vittata	HAE VITT	3	planktivore	mobile within reefs	pelagic	D	schooling
HOLOCENTRIDAE	*Holocentrus adscensionis*	HOL ADSC	4	mobile invertivore	sedentary	benthic	N	solitary+schooling
	Holocentrus rufus	HOL RUFU	4	mobile invertivore	sedentary	benthic	N	solitary+schooling
	Myripristis jacobus	MYR JACO						
	Sargocentron vexillarium	SAR VEXI	3	mobile invertivore	sedentary	benthic	N	solitary+schooling
	Sargocentron coruscum	SAR CORU	2	mobile invertivore	sedentary	benthic	N	solitary+schooling

Table A.5: Species list and trait assignment. Part 1/6.

KYPHOSIDAE	*Kyphosus sectatrix/incisor*	KYP SPP	5	herbivore	mobile within reefs	pelagic	D	schooling
LABRIDAE	*Thalassoma bifasciatum*	THA BIFA	3	planktivore	sedentary	benthopelagic	D	schooling
	Halichoeres garnoti	HAL GARN	3	mobile invertivore	sedentary	benthic	D	schooling
	Halichoeres bivittatus	HAL BIVI	3	mobile invertivore	sedentary	benthic	D	schooling
	Halichoeres maculipinna	HAL MACU	3	mobile invertivore	sedentary	benthic	D	schooling
	Halichoeres radiatus	HAL RADI	5	mobile invertivore	sedentary	benthic	D	schooling
	Halichoeres pictus	HAL PICT	2	planktivore	sedentary	benthopelagic	D	schooling
	Clepticus parrae	CLE PARR	3	planktivore	sedentary	pelagic	D	schooling
	Halichoeres poeyi	HAL POEY	3	mobile invertivore	sedentary	benthic	D	schooling
	Xyrichtys splendens	XYR SPLEN	3	mobile invertivore	sedentary	benthic	D	schooling
	Bodianus rufus	BOD RUFU	3	mobile invertivore	sedentary	benthic	D	solitary
	Lachnolaimus maximus	LAC MAXI	6	mobile invertivore	sedentary	benthic	D	solitary
LUTJANIDAE	*Lutjanus analis*	LUT ANAL	5	mobile invertivore	sedentary	benthic	D+N	solitary+schooling
	Lutjanus apodus	LUT APOD	5	piscivore	sedentary	benthopelagic	D+N	solitary+schooling
	Lutjanus mahagoni	LUT MAHA	4	piscivore	sedentary	benthic	N	schooling
	Ocyurus chrysurus	OCY CHRYS	6	piscivore	sedentary	pelagic	N	solitary+schooling
	Lutjanus synagris	LUT SYNA	5	mobile invertivore	sedentary	benthic	N	schooling
	Lutjanus griseus	LUT GRIS	6	piscivore	sedentary	benthic	N	schooling
	Lutjanus jocu	LUT JOCU	6	large predator	sedentary	benthic	D+N	solitary
	Lutjanus cyanopterus	LUT CYAN	6	large predator	sedentary	benthic	N	solitary+schooling
MALACANTHIDAE	*Malacanthus plumieri*	MAL PLUM	5	mobile invertivore	sedentary	benthic	D	solitary
	Aluterus scriptus	ALU SCRI	6	sessile invertivore	mobile within reefs	benthopelagic	D	solitary
	Aluterus schoepfii	ALU SCHO	5	herbivore	sedentary	benthic	D	solitary
	Cantherhines pullus	CAN PULL	3	sessile invertivore	sedentary	benthic	D	solitary
MULLIDAE	*Mulloidichthys martinicus*	MUL MART	4	mobile invertivore	sedentary	benthic	D	schooling
	Pseudupeneus maculatus	PSE MACU	3	mobile invertivore	sedentary	benthic	D	schooling
MURAENIDAE	*Gymnothorax funebris*	GYM FUNE	6	large predator	sedentary	benthic	D	solitary
	Gymnothorax moringa	GYM MORI	6	large predator	sedentary	benthic	D	solitary
	Gymnothorax miliaris	GYM MILI	5	piscivore	sedentary	benthic	D	solitary
	Echidna catenata	ECH CATE	6	mobile invertivore	sedentary	benthic	D	solitary

Table A.6: Species list and trait assignment. Part 3/6.

MYLIOBATIDAE	*Aetobatus narinari*	AET NARI	6	mobile invertivore	mobile between	benthopelagic	D	solitary+schooling
OPHICHTHIDAE	*Myrichthys breviceps*	MYR BREV	6	mobile invertivore	sedentary	benthic	D	solitary
OPISTOGNATHIDAE	*Opistognathus aurifrons*	OPI AURI	2	planktivore	sedentary	benthic	D	schooling
OSTRACIIDAE	*Lactophrys trigonus*	LAC TRIG	5	mobile invertivore	sedentary	benthic	D	solitary+schooling
	Lactophrys bicaudalis	LAC BICA	4	mobile invertivore	sedentary	benthic	D	solitary+schooling
	Lactophrys triqueter	LAC sp.	4	mobile invertivore	sedentary	benthic	D	solitary+schooling
	Lactophrys sp.	LAC TRIQ	4	mobile invertivore	sedentary	benthic	D	solitary+schooling
	Acanthostracion polygonia	ACA POLY	4	sessile invertivore	sedentary	benthic	D	solitary
	Acanthostracion quadricornis	ACA QUAD	5	sessile invertivore	sedentary	benthic	D	solitary
PEMPHERIDAE	*Pempheris schomburgki*	PEM SCHO	2	planktivore	sedentary	benthic	N	schooling
POMACANTHIDAE	*Pomacanthus arcuatus*	POM ARCU	5	sessile invertivore	sedentary	benthic	D	pairing
	Pomacanthus paru	POM PARU	4	sessile invertivore	sedentary	benthic	D	pairing
	Holacanthus ciliaris	HOL CILI	4	sessile invertivore	sedentary	benthic	D	solitary
	Holacanthus tricolor	HOL TRIC	4	sessile invertivore	sedentary	benthic	D	solitary
POMACENTRIDAE	*Abudefduf sexatilis*	ABU DEFD	3	planktivore	sedentary+territorial	benthopelagic	D	pairing+schooling
	Stegastes diencaeus	STE DIEN	2	herbivore	sedentary+territorial	benthic	D	solitary+pairing
	Stegastes adustus	STE ADUS	2	herbivore	sedentary+territorial	benthic	D	solitary+pairing
	Stegastes planifrons	STE PLAN	2	herbivore	sedentary+territorial	benthic	D	solitary+pairing
	Microspathodon chrysurus	MIC CHRY	3	herbivore	sedentary+territorial	benthic	D	solitary+pairing
	Stegastes Leucostictus	STE LEUC	2	herbivore	sedentary+territorial	benthic	D	solitary+pairing
	Stegastes variabilis	STE VARI	2	herbivore	sedentary+territorial	benthic	D	solitary+pairing
	Stegastes partitus	STE PART	2	herbivore	sedentary+territorial	benthic	D	solitary+pairing
	Chromis cyanea	CHR CYAN	2	planktivore	sedentary	pelagic	D	schooling
	Chromis multilienata	CHR MULT	3	planktivore	sedentary	pelagic	D	schooling
PRIACANTHIDAE	*Heteropriacanthus cruentatus*	HET CRUE	4	mobile invertivore	sedentary	benthic	N	solitary+schooling
SCARIDAE	*Scarus coeruleus*	SCA COER	6	herbivore	sedentary	benthic	D	schooling
	Scarus guacamaia	SCA GUAC	6	herbivore	sedentary	benthic	D	solitary+schooling
	Scarus coelestinus	SCA COEL	5	herbivore	sedentary	benthic	D	solitary+schooling
	Scarus vetula	SCA VETU	5	herbivore	sedentary	benthic	D	solitary+schooling
	Scarus taeniopterus	SCA TAEN	4	herbivore	sedentary	benthic	D	solitary+schooling
	Scarus iserti	SCA ISER	4	herbivore	sedentary	benthic	D	schooling

Table A.7: Species list and trait assignment. Part 4/6.

	Sparisoma viride	SPA VIRI	5	herbivore	sedentary	benthic	D	solitary+schooling
	Sparisoma aurofrenatum	SPA AURO	3	herbivore	sedentary	benthic	D	solitary+schooling
	Sparisoma rubripinne	SPA RUBR	4	herbivore	sedentary	benthic	D	solitary+schooling
	Sparisoma chrysopterum	SPA CHRY	4	herbivore	sedentary	benthic	D	solitary+schooling
	Sparisoma atomarium	SPA ATOM	3	herbivore	sedentary	benthic	D	solitary+schooling
	Sparisoma radians	SPA RAD	3	herbivore	sedentary	benthic	D	solitary+schooling
	Cryptotomus roseus	CRY ROSE	2	herbivore	sedentary	benthic	D	solitary+schooling
SCIAENIDAE	*Equetus punctatus*	EQU PUNC	3	mobile invertivore	sedentary	benthic	N	solitary
	Odontoscion dentex	ODO DENT						
SCOMBRIDAE	*Scomberomorus regalis*	SCO REGA	6	large predator	mobile within reefs	pelagic	D	solitary+schooling
SCORPAENIDAE	*Scorpaena plumieri*	SCO PLUM	4	piscivore	sedentary	benthic	D	solitary
	Pterois volitans/P. miles	PTE VOLI/MILE	4	piscivore	sedentary	benthic	N	solitary
SERRANIDAE	*Mycteroperca bonaci*	MYC BONA	6	large predator	sedentary	benthic	D+N	solitary
	Epinephelus striatus	EPI STRI	6	large predator	sedentary	benthic	D+N	solitary
	Epinephelus morio	EPI MORI	6	large predator	sedentary	benthic	D+N	solitary
	Mycteroperca tigris	MYC TIGR	6	large predator	sedentary	benthic	D+N	solitary
	Mycteroperca venenosa	MYC VENE	6	large predator	sedentary	benthic	D+N	solitary
	Epinephelus adscensionis	EPI ADSC	5	mobile invertivore	sedentary	benthic	D+N	solitary
	Epinephelus guttatus	EPI GUTT	5	mobile invertivore	sedentary	benthic	D+N	solitary
	Cephalopholis cruentatus	CEP CRUE	4	piscivore	sedentary	benthic	N	solitary
	Cephalopholis fulva	CEP FULV	4	piscivore	sedentary	benthic	N	solitary
	Serranus tigrinus	SER TIGR	3	mobile invertivore	sedentary	benthic	D	solitary+pairing
	Gramma loreto	GRA LORE	2	planktivore	sedentary	benthic	D	schooling
	Hypoplectrus nigricans	HYP NIGR	2	mobile invertivore	sedentary	benthic	D	solitary
	Hypoplectrus indigo	HYP INDI	2	mobile invertivore	sedentary	benthic	D	solitary
	Hypoplectrus puella	HYP PUEL	2	mobile invertivore	sedentary	benthic	D	solitary
	Hypoplectrus unicolor	HYP UNIC	2	mobile invertivore	sedentary	benthic	D	solitary
	Hypoplectrus sp.	HYP SP	2	mobile invertivore	sedentary	benthic	D	solitary

Table A.8: Species list and trait assignment. Part 5/6.

SPARIDAE	*Calamus bajonado*	CAL BAJO	5	mobile invertivore	sedentary	benthic	D	solitary
	Calamus calamus, C. pennatula, C. pen	CAL SPP	5	mobile invertivore	sedentary	benthic	D	solitary
	Gerres cinereus	GER CINE	4	mobile invertivore	sedentary	benthic	D	schooling
SPHYRANIIDAE	*Sphyraena barracuda*	SPH BARR	6	large predator	mobile within reefs	pelagic	D	solitary+schooling
	Sphyraena picudilla	SPH PICU	5	large predator	mobile within reefs	pelagic	D	schooling
SYNGNATHIDAE	Syngnathidae spp	COS SP	3	mobile invertivore	sedentary	benthic	D	solitary
SYNODONTIDAE	*Synodus intermedius*	SYN INTE	4	piscivore	sedentary	benthic	D	solitary
TETRAODONTIDAE	*Canthigaster rostrata*	CAN ROST	2	mobile invertivore	sedentary	benthic	D	solitary+schooling
TRIPTERYGILDAE	*Enneanectes* spp	ENN SPP	1	mobile invertivore	sedentary	benthic	D	solitary
UROLOPHIDAE	*Urolophus jamaicensis*	URO JAMA	5	mobile invertivore	mobile within reefs	benthic	D	solitary+schooling

Table A.9: Species list and trait assignment. Part 6/6.

Species	Zone within atoll	IndVal	*P* - value	Frequency
Stegastes leucostictus	Backreef Glovers	0.308324769	0.001	32
Halichoeres bivittatus JV		0.211908422	0.049	59
Lutjanus apodus	Backreef Lighthouse	0.213157303	0.019	93
Chaetodon ocellatus		0.161916132	0.046	59
Chaetodon striatus		0.153730012	0.044	70
Gnatholepis thompsoni	Backreef Turneffe	0.2734375	0.029	5
Stegastes diencaeus		0.146046602	0.034	88
Chaetodon capistratus JV	Lagoon patch Glovers	0.226510067	0.037	18
Sparisoma atomarium JV	Lagoon patch Lighthouse	0.277992278	0.009	15
Sparisoma atomarium IP		0.270880361	0.006	18
Holacanthus ciliaris		0.222565137	0.019	35
Stegastes leucostictus JV		0.213304366	0.02	61
Pomacanthus arcuatus		0.206270627	0.019	39
Halichoeres garnoti JV		0.15762263	0.03	81
Sparisoma aurofrenatum IP		0.136119141	0.017	86
Halichoeres garnoti IP		0.134966592	0.033	91
Haemulon sciurus	Lagoon patch Turneffe	0.45564328	0.002	83
Lutjanus griseus		0.41750167	0.003	18
Haemulon plumierii		0.295450255	0.011	90
Gerres cinereus		0.285990981	0.041	49
Epinephelus striatus		0.248667851	0.01	20
Aulostomus maculatus		0.216352908	0.01	35
Scarus iserti JV		0.162287481	0.012	90
Canthigaster rostrata	Westreef Glovers	0.203736974	0.005	64
Holacanthus tricolor JV		0.191271347	0.05	11
Holacanthus tricolor	Westreef Lighthouse	0.440998304	0.001	28
Halichoeres pictus JV		0.215179969	0.031	21
Clepticus parrae JV	Westreef Turneffe	0.459918749	0.005	18
Gramma loreto		0.385830642	0.002	39
Hypoplectrus indigo		0.329773869	0.001	21
Anisotremus virginicus		0.277044855	0.008	11
Stegastes planifrons JV		0.271441002	0.035	66
Epinephelus striatus		0.26119403	0.013	9
Hypoplectrus nigricans		0.19658013	0.038	33
Stegastes planifrons		0.189205295	0.035	66
Scarus iserti TP		0.158149392	0.029	78
Mulloidichthys martinicus	Channel Lighthouse	0.276437034	0.024	53
Lutjanus jocu	Channel Turneffe	0.336	0.005	7
Halichoeres garnoti TP		0.260285763	0.002	58
Scarus iserti IP		0.19537686	0.05	87
Chaetodon capistratus		0.180665397	0.019	84
Scarus vetula IP	Forereef Glovers	0.326169087	0.003	32
Scarus vetula TP		0.287593985	0.016	20
Acanthurus coeruleus		0.17756623	0.029	93
Thalassoma bifasciatum IP		0.173853087	0.035	93
Melichthys niger	Forereef Lighthouse	0.440740408	0.006	18
Chromis multilienata		0.423529412	0.014	27
Microspathodon chrysurus		0.234616176	0.022	68
Microspathodon chrysurus JV		0.224741647	0.017	57
Echeneis naucrates	Forereef Turneffe	0.333333333	0.023	2
Ophioblennius macclurei	Forereef Turneffe	0.300820419	0.004	32
Sparisoma rubripinne TP	Forereef Turneffe	0.219389887	0.018	46

Table A.10: Fish indicator species at reef zones within atolls. Indicator values reflect the degree to which a species is indicative of individual reef zones at a given atoll and in terms of the specific conditions found at each site group, respectively. *P*-values reflect the significance of the relationships between each species and sample group and are based on 1000 permutations at a significance level of *P*-value 0.05. JV = Juvenile, IP = Initial Phase, TP = Terminal Phase. Fish adult trophic groups are color coded, juvenile trophic

Appendix B

Supporting material for Chapter 3

primer name	primer sequence (5'-3')
Hpuella-Blocker	CAAAGAATCAGAATAGATGTTGGTAAAGA-C3
Ccapistratus-Blocker	CAAAGAATCAGAACAGGTGTTGGTAAAGA-C3
mlCOIintF	GGWACWGGWTGAACWGTWTAYCCYCC
jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA

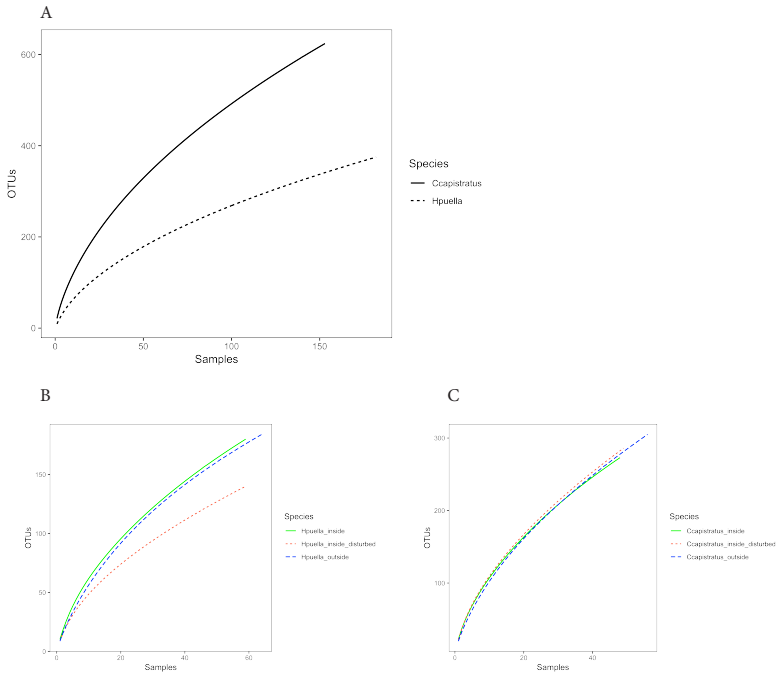

Figure B.1: Sample-based rarefaction curves for both study species across all samples (A) and for *Hypoplectrus puella* (B) and *Chaetodon capistratus* (C) by reef zone, respectively

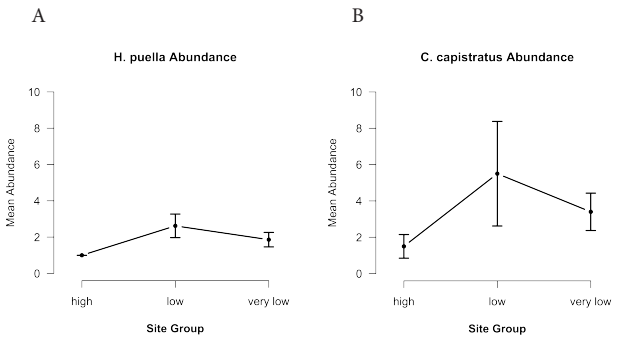

Figure B.2: Mean abundance of two fish species among three habitat zones (high= high coral cover, outer bay; low = intermediate coral cover, inner bay; very low = low coral cover, inner bay disturbed zone); *Hypoplectrus puella* (A) and *Chaetodon capistratus* (B)

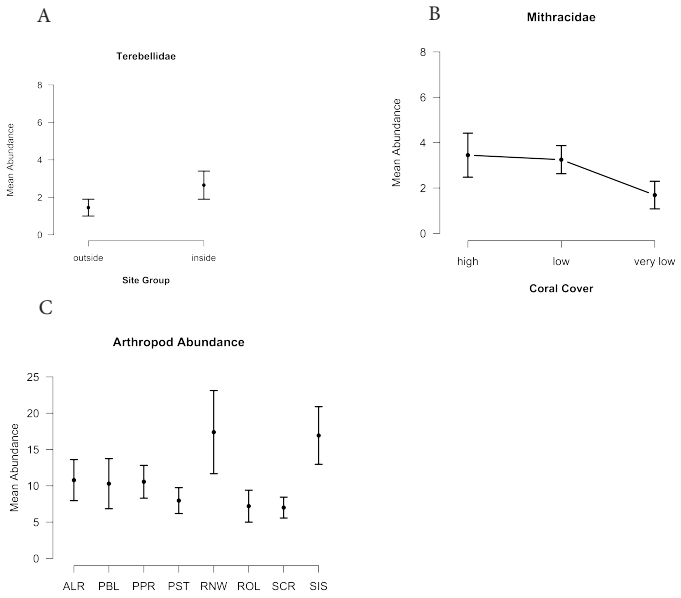

Figure B.3: Invertebrate prey taxa mean abundance for terrebelid worms (A) between inside and outside of the bay; mithracid crabs (B) across three zones of different levels of coral cover (high = outer bay, low = inner bay, very low = inner bay disturbed); and arthropod mean abundance across eight reefs (C).

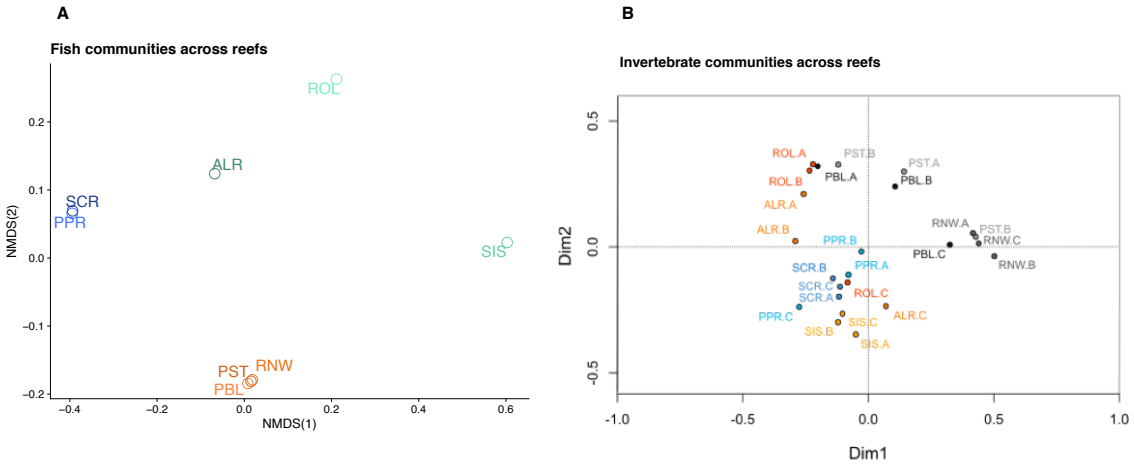

Figure B.4: Nonmetric multidimensional scaling (NMDS) using Bray Curtis dissimilarity of fish (A) and invertebrate (B) communities across reefs and zones (color coded). For A = blue = outer bay, green = inner bay, red = inner bay disturbed. For B = blue = outer bay, orange = inner bay, grey = inner bay disturbed.

Appendix C

Supporting material for Chapter 4 - Text and Figures

DNA extraction of gut contents

The gastrointestinal tract of each fish was opened longitudinally to isolate the digesta and the mucosa by lightly scraping the intestinal epithelium. Between 0.05 and 0.25 g of tissue was used for DNA extraction using the Qiagen Powersoil DNA isolation kit following the manufacturers instructions with minor modifications. To improve tissue lysis, 20 µL of Proteinase K (0.4 mg.mL-1) was added into eppendorf tubes containing power beads and solution. Samples were briefly vortexed and incubated in a shaking incubator (1000 rpm) at $60°C$ for 15 minutes (min). Samples were vortexed for 5 min using a Vortex Genie 2 (Scientific Industries) with vortex adapter and incubated at $60°C$ for 1 hour and 45 min (1000 rpm) for a total of 2 hours incubation time. DNA was eluted in 100 ul buffer (Qiagen PowerSoil kit C6 solution) and DNA concentration was quantified with a Qubit Fluorometer (dsDNA High-Sensitivity Assay Kit, Invitrogen, Life Technologies). DNA extractions of the invertebrate and macroalgal tissues (0.25g per sample) were conducted using the same modified protocol.

PCR protocols

The first PCR amplification was performed in a total volume of 12.5 µL with 0.2 µL of 10 millimolars (mM) forward primer 515F (5' GTGYCAGCMGCCGCGGTAA 3'; 9, 0.2 µL of 10 mM reverse primer 806R (5' GGACTACNVGGGTWTCTAAT 3'; 10), 5 µL of "5 PRIME Hot Master Mix (2.5x)" solution, 5.1 µL of nuclease-free water and 2µL of genomic DNA extract. This combination of primers has been recommended for marine microbial studies 11. Primers were phased with heterogeneity spacers to increase the per base variability during Illumina sequencing

12. PCR cycling conditions were $94°C$ for 3 min, followed by 35 cycles at $94°C$ for 45 s, $50°C$ for 1 min and $72°C$ for 1 min 30 sec and a final elongation step at $72°C$ for 10 min. Each sample was amplified three times independently and the product checked on 1.5% agarose gel. Triplicate PCRs were then pooled and purified using paramagnetic beads (KAPA Pure Beads) at a ratio sample:beads of 1:1.6. Purified PCR products were quantified using a Qubit Fluorometer and diluted to 5 nanograms per microlitre (ng.µL-1). These dilutions were used in a second PCR to add unique combinations of dual index Illumina sequencing adaptors to each sample. Each PCR amplification was performed in a total volume of 11.5 µL with 1µL of 2.5 mM Forward indexed Illumina primer, 1 µL of 2.5 mM Reverse indexed Illumina primer, 5 µL of "5 PRIMER Hot Master Mix (2.5x)" solution, 3.5 µL of nuclease-free water and 1µL of diluted DNA. Here, cycling conditions were: $94°C$, 3 min, followed by 6 cycles of $94°C$ for 45 s, $50°C$ for 1 min and $72°C$ for 1 min 30 s; and a final elongation step at $72°C$ for 10 min. Unique combinations of 16 unique Forward and 24 unique Reverse Illumina indexed primers were used in order to allow multiplexing of all samples (Table S2). Finally, an equal volume of indexed PCR product for each sample was mixed into a single tube. The pool was purified two successive times with paramagnetic beads at a ratio bead:sample of 1:1 to remove leftover primers and primer dimers.

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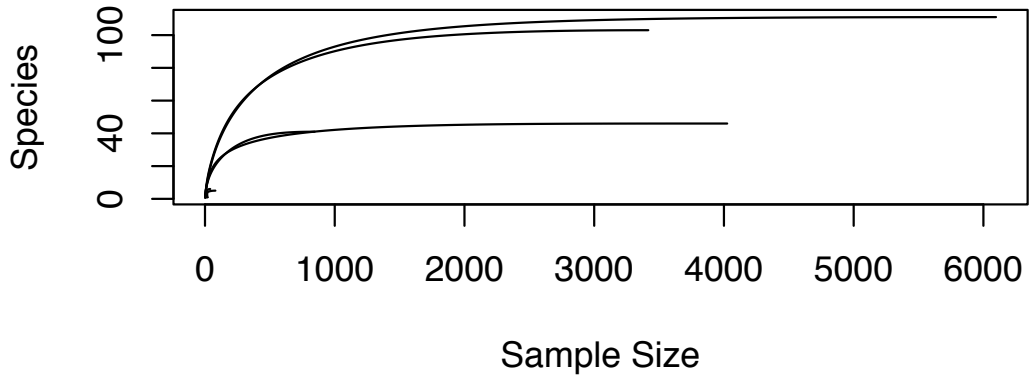

Figure C.1: Rarefaction curves for 14 samples with fewer than 10000 sequences. Curves plateau after a few thousand reads. To limit the variability in number of sequences between samples, these samples were removed from our dataset. We rarefied the remaining samples to even sequencing depth (n = 10,369 sequences).

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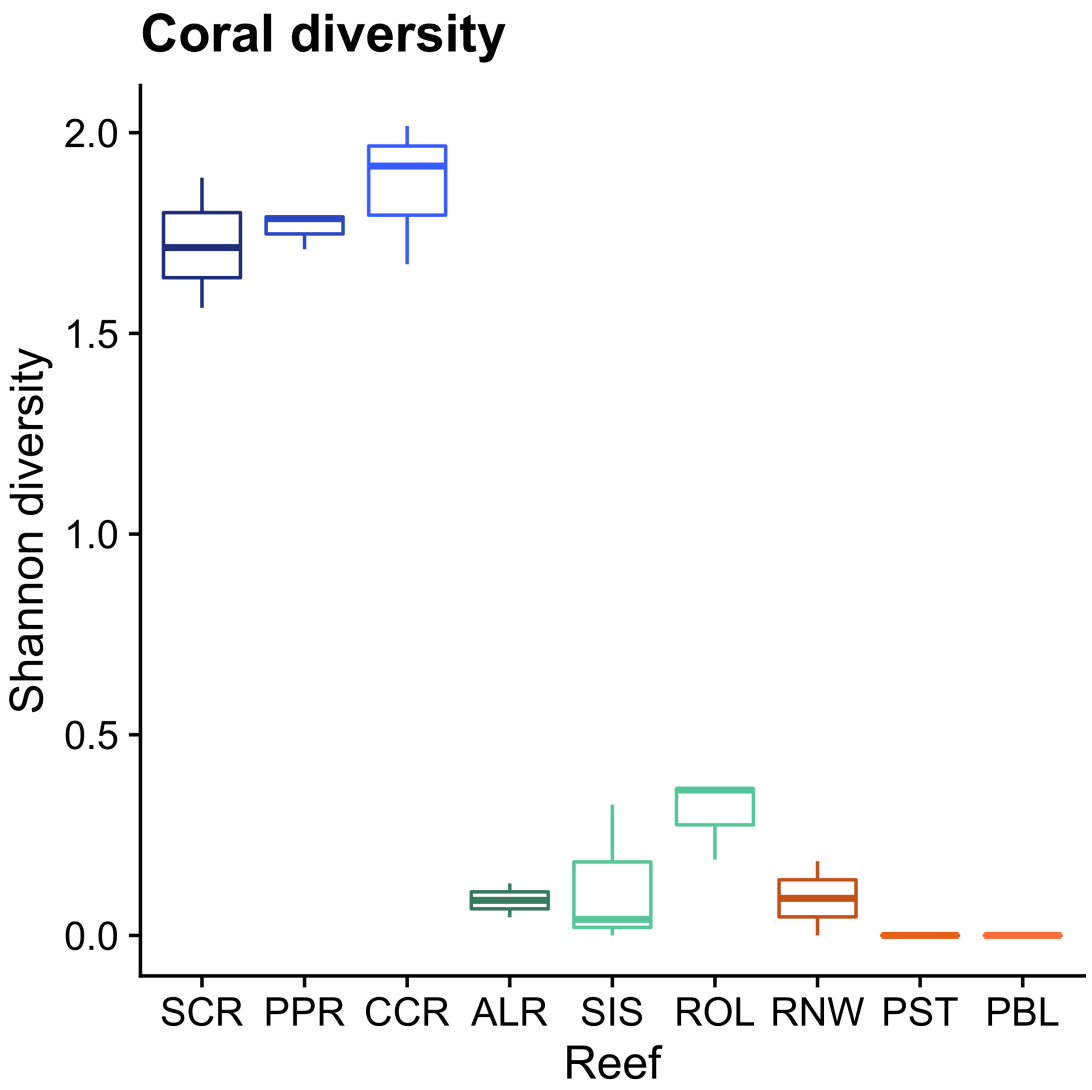

Figure C.2: Shannon diversity of the coral community at each of nine reefs inferred from transect data. The data reflect a gradient from high coral cover at the outer bay reefs (SCR, PPR, CCR) to the inner bay (ALR, SIS, ROL) and inner bay disturbed reefs (RNW, PST, PBL).

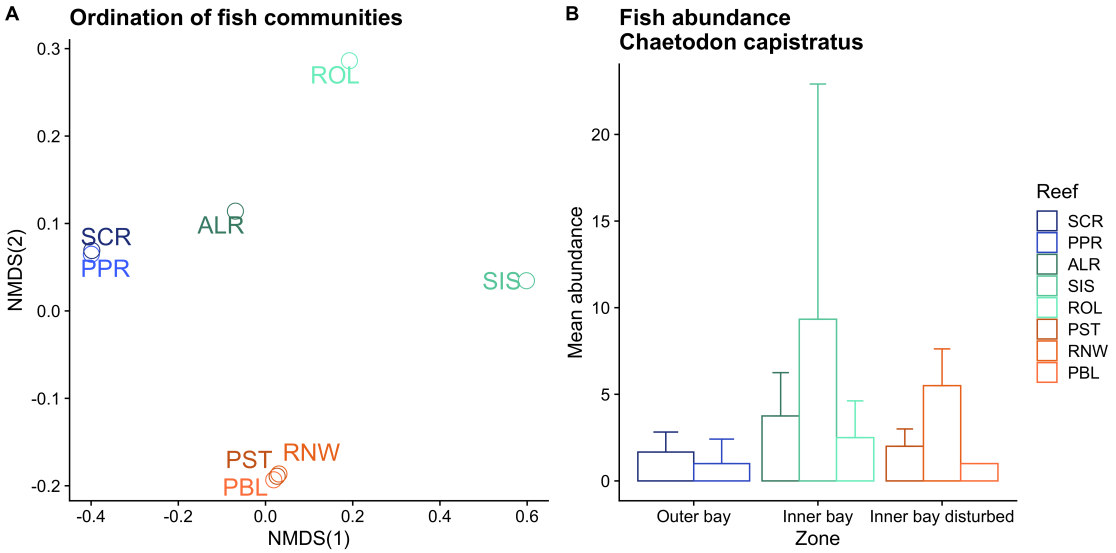

Figure C.3: (A) Differences in fish community composition among nine study reefs visualised using Nonmetric Multidimensional Scaling (NMDS) based on Bray Curtis dissimilarity. Reefs are colour-coded by reef zone: blue= outer bay, green=inner bay, orange=inner bay disturbed. (B) Study species (*Chaetodon capistratus*) abundances across nine study reefs.

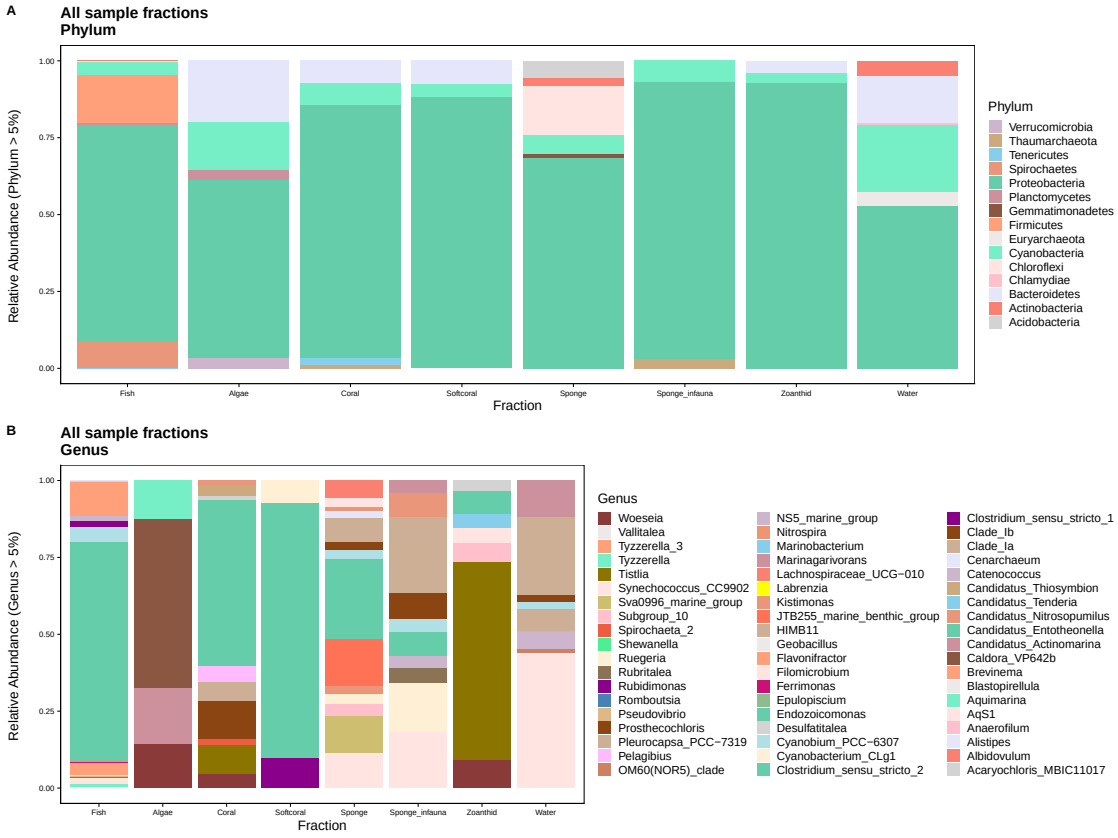

Figure C.4: Relative abundance of microbial taxa across the different sample fractions comparing fish gut microbiome to potential prey items (i.e., algae, hard coral, soft coral, sponge, sponge infauna, zoanthid, anemone) and the surrounding seawater by phylum (A) and family (B).

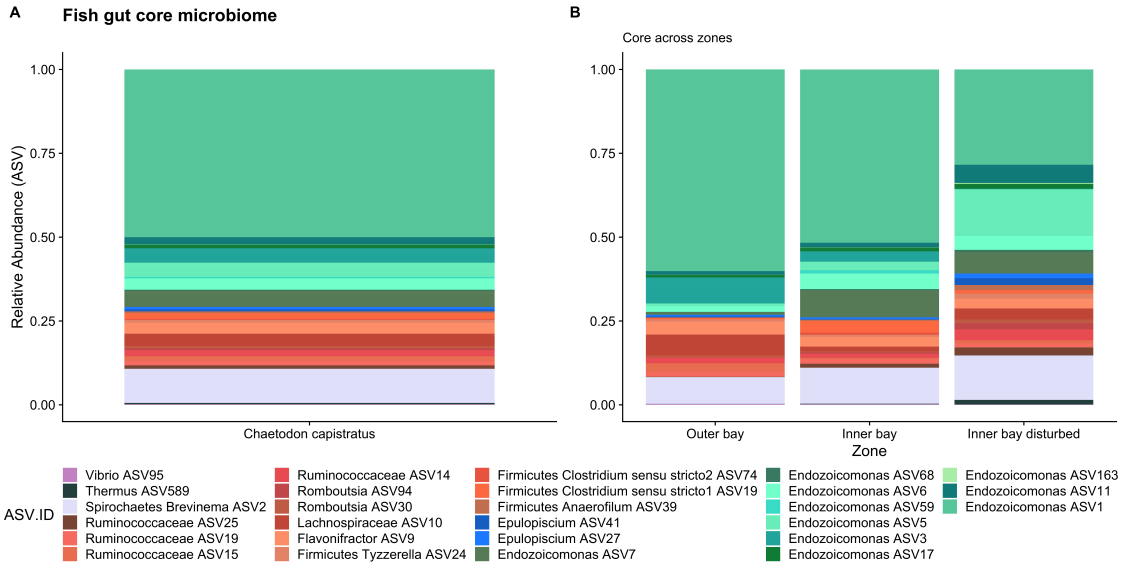

Figure C.5: Core bacterial community (relative read abundance on the level of ASVs) in the gut of *Chaetodon capistratus* identified with Indicator Analysis by comparing 16S sequences found in fish guts to all other sample fractions (seawater and potential prey taxa) combined (A); core microbiome community variation shown across three zones (B).

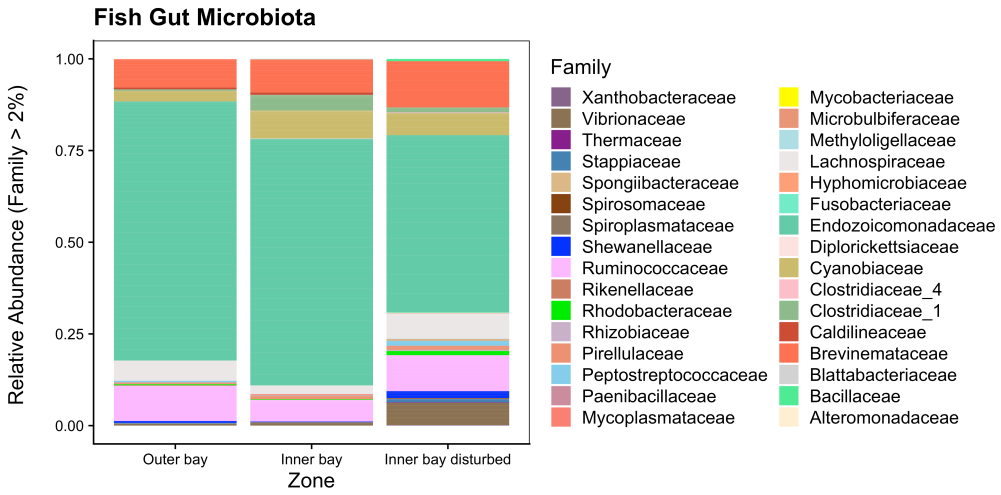

Figure C.6: Relative read abundance of bacteria in the whole fish gut microbiome across three reef zone by family

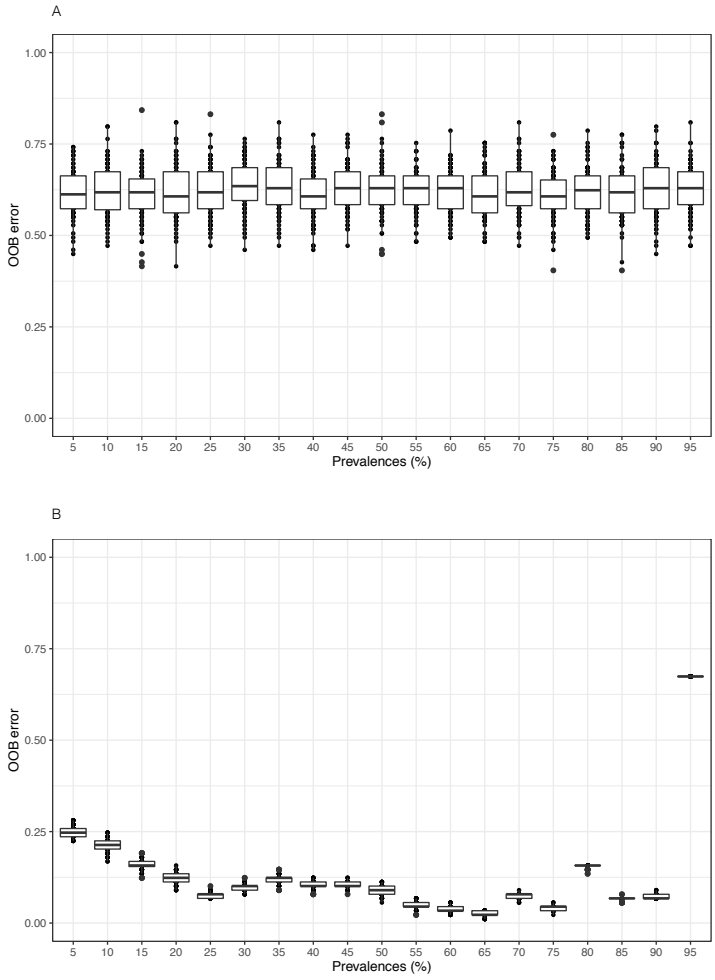

Figure C.7: To validate PIME results, the algorithm assessed the likelihood of bias by simulating OBB error predictions. This was done by (i) by randomising the group labels (i.e., zone identity) of the original dataset (17 ASVs) and using bootstrap aggregating (100 iterations) to perform a Monte Carlo simulation of Random Forest classifications on each filtering step (by 5 increments) generating boxplots for each prevalence interval at approximately the predicted best prevelance cut-off (65%) (A) and (ii) by repeating Random Forest OBB error estimations on the bootstrap aggregations of the filtered dataset at each prevalence interval (resulting in boxplots corresponding to the empirical data in Table 8A) (B).

Appendix D

Supporting material for Chapter 4 - Tables

Reef	Reef name	Latitude	Longitude	Reef Zone	Number of fishes sampled
ALR	Almirante	N 09.28998	W 82.34308	Inner Bay	14
ROL	Cayo Roldan	N 09.21478	W 82.32454	Inner Bay	16
SIS	Cayo Hermanas	N 09.26751	W 82.35178	Inner Bay	10
PBL	Punta Puebla	N 09.36665	W 82.29124	Inner Bay, dead	9
PST	Punta STRI	N 09.34885	W 82.26292	Inner Bay, dead	6
RNW	Runway	N 09.34193	W 82.25997	Inner Bay, dead	9
CCR	Coral Caye	N 09.26747	W 82.11983	Outer Bay, exposed	16
PPR	Popa Reef	N 09.23344	W 82.11189	Outer Bay, exposed	7
SCR	Salt Creek	N 09.28002	W 82.10175	Outer Bay, exposed	15

Table D.1: Number of fish individuals sampled per reef and reef site geographic coordinates. The reef zone category shows the assignment of individual reefs to zones.

Primer name	Illumina flow cell adapter sequence	Index sequence	Locus specific primer
SC501F	AATGATACGGCGACCACCGAGATCTACAC	ACGACGTG	ACACTCTTTCCCTACACGAC
SC502F	AATGATACGGCGACCACCGAGATCTACAC	ATATACAC	ACACTCTTTCCCTACACGAC
SC503F	AATGATACGGCGACCACCGAGATCTACAC	CGTCGCTA	ACACTCTTTCCCTACACGAC
SC504F	AATGATACGGCGACCACCGAGATCTACAC	CTAGAGCT	ACACTCTTTCCCTACACGAC
SC505F	AATGATACGGCGACCACCGAGATCTACAC	GCTCTAGT	ACACTCTTTCCCTACACGAC
SC506F	AATGATACGGCGACCACCGAGATCTACAC	GACACTGA	ACACTCTTTCCCTACACGAC
SC507F	AATGATACGGCGACCACCGAGATCTACAC	TGCGTACG	ACACTCTTTCCCTACACGAC
SC508F	AATGATACGGCGACCACCGAGATCTACAC	TAGTGTAG	ACACTCTTTCCCTACACGAC
SD501F	AATGATACGGCGACCACCGAGATCTACAC	AAGCAGCA	ACACTCTTTCCCTACACGAC
SD502F	AATGATACGGCGACCACCGAGATCTACAC	ACGCGTGA	ACACTCTTTCCCTACACGAC
SD503F	AATGATACGGCGACCACCGAGATCTACAC	CGATCTAC	ACACTCTTTCCCTACACGAC
SD504F	AATGATACGGCGACCACCGAGATCTACAC	TGCGTCAC	ACACTCTTTCCCTACACGAC
SD505F	AATGATACGGCGACCACCGAGATCTACAC	GTCTAGTG	ACACTCTTTCCCTACACGAC
SD506F	AATGATACGGCGACCACCGAGATCTACAC	CTAGTATG	ACACTCTTTCCCTACACGAC
SD507F	AATGATACGGCGACCACCGAGATCTACAC	GATAGCGT	ACACTCTTTCCCTACACGAC
SD508F	AATGATACGGCGACCACCGAGATCTACAC	TCTACACT	ACACTCTTTCCCTACACGAC
SC701R	CAAGCAGAAGACGGCATACGAGAT	ACCTACTG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC702R	CAAGCAGAAGACGGCATACGAGAT	AGCGCTAT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC703R	CAAGCAGAAGACGGCATACGAGAT	AGTCTAGA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC704R	CAAGCAGAAGACGGCATACGAGAT	CATGAGGA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC705R	CAAGCAGAAGACGGCATACGAGAT	CTAGCTCG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC706R	CAAGCAGAAGACGGCATACGAGAT	CTCTAGAG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC707R	CAAGCAGAAGACGGCATACGAGAT	GAGCTCAT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC708R	CAAGCAGAAGACGGCATACGAGAT	GGTATGCT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC709R	CAAGCAGAAGACGGCATACGAGAT	GTATGACG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC710R	CAAGCAGAAGACGGCATACGAGAT	TAGACTGA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC711R	CAAGCAGAAGACGGCATACGAGAT	TCACGATG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SC712R	CAAGCAGAAGACGGCATACGAGAT	TCGAGCTC	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
SD701R	CAAGCAGAAGACGGCATACGAGAT	ACCTAGTATGCTCTTC	GTGACTGGAGTTCAGACGTG
SD702R	CAAGCAGAAGACGGCATACGAGAT	ACGTACGTTGCTCTTC	GTGACTGGAGTTCAGACGTG
SD703R	CAAGCAGAAGACGGCATACGAGAT	ATATCGCGTGCTCTTC	GTGACTGGAGTTCAGACGTG
SD704R	CAAGCAGAAGACGGCATACGAGAT	CACGATAGTGCTCTTC	GTGACTGGAGTTCAGACGTG
SD705R	CAAGCAGAAGACGGCATACGAGAT	CGTATCGCTGCTCTTC	GTGACTGGAGTTCAGACGTG
SD706R	CAAGCAGAAGACGGCATACGAGAT	CTGCGACTTGCTCTTC	GTGACTGGAGTTCAGACGTG
SD707R	CAAGCAGAAGACGGCATACGAGAT	GCTGTAAC	GTGACTGGAGTTCAGACGTG
SD708R	CAAGCAGAAGACGGCATACGAGAT	GGACGTTA	GTGACTGGAGTTCAGACGTG
SD709R	CAAGCAGAAGACGGCATACGAGAT	GGTCGTAG	GTGACTGGAGTTCAGACGTG
SD710R	CAAGCAGAAGACGGCATACGAGAT	TAAGTCTC	GTGACTGGAGTTCAGACGTG
SD711R	CAAGCAGAAGACGGCATACGAGAT	TACACAGT	GTGACTGGAGTTCAGACGTG
SD712R	CAAGCAGAAGACGGCATACGAGAT	TTGACGCA	GTGACTGGAGTTCAGACGTG

Table D.2: Index PCR primers used in this study.

	Cover %		
Reef type	**Sponge**	**Dead hard coral**	**Live hard coral**
Outer bay	3.1	17.37	33.46
Inner bay	25.24	10.8	14.65
Inner bay - disturbed	23.53	40.1	0.35

Table D.3: Percent substrate cover across reef zones of the main substrate groups.

A By Zone

Microbiota	Factor	Diversity	Kruskal-Wallis χ^2	DF	*P*-Value
Whole	Zone	Observed q=0	3.494	2	0.174
		Shannon exponential q=1	10.996	2	0.004
		Simpsons multiplicative inverse q=2	8.634	2	0.013
Core	Zone	Observed	7.416	2	0.025
		Shannon exponential q=1	8.357	2	0.015
		Simpsons multiplicative inverse q=2	8.263	2	0.016

B By Reef

Microbiota	Factor	Diversity	Kruskal-Wallis χ^2	DF	*P*-Value
Whole	Reef	Observed q=0	9.916	8	0.271
		Shannon exponential q=1	13.543	8	0.094
		Simpsons multiplicative inverse q=2	10.529	8	0.23
Core	Reef	Observed	19.944	8	0.011
		Shannon exponential q=1	23.421	8	0.003
		Simpsons multiplicative inverse q=2	22.052	8	0.005

Table D.4: Kruskal Wallis Rank Sum Test of Hill diversity. Alpha diversity was measured using three metrics that put more or less weight on common species (ASVs) (Hill numbers, q = 0, 1, 2) and Kruskal Wallis tests were used to test for significant differences in alpha diversity levels for each metric among reef zones and reefs respectively.

C

Microbiota	Factor	Diversity	Zone	Z	*P*-Value	adjusted *P*-Value
Whole	Zone	Observed q=0	Inner bay-Inner bay disturbed	0.009	0.993	0.993
			Inner bay-Outer bay	1.701	0.089	0.267
			Inner bay disturbed-Outer bay	1.418	0.156	0.234
		Shannon exponential q=1	Inner bay-Inner bay disturbed	-1.416	0.157	0.157
			Inner bay-Outer bay	2.128	**0.033**	**0.05**
			Inner bay disturbed-Outer bay	3.201	**0.001**	**0.004**
		Simpsons multiplicative inverse q=2	Inner bay-Inner bay disturbed	-1.579	0.114	0.114
			Inner bay-Outer bay	1.587	0.113	0.169
			Inner bay disturbed-Outer bay	2.911	**0.004**	**0.011**
Core	Zone	Observed q=0	Inner bay-Inner bay disturbed	0.16	0.436	0.436
			Inner bay-Outer bay	2.535	**0.006**	**0.017**
			Inner bay disturbed-Outer bay	1.966	**0.025**	**0.049**
		Shannon exponential q=1	Inner bay-Inner bay disturbed	-1.633	0.102	0.154
			Inner bay-Outer bay	1.48	0.139	0.139
			Inner bay disturbed-Outer bay	2.875	**0.004**	**0.012**
		Simpsons multiplicative inverse q=2	Inner bay-Inner bay disturbed	-1.945	0.052	0.078
			Inner bay-Outer bay	1.106	0.269	0.269
			Inner bay disturbed-Outer bay	2.872	**0.004**	**0.012**

Table D.5: Posthoc Dunn Test by zone with Benjamin Hochberg correction for the whole and the core gut microbiome by zone.

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Microbiota	Factor	Diversity	Reef	Z	*P*-Value	adjusted *P*-Value
Core	Reef	Observed q=0	SCR-SIS	-3.525	**0.0002**	**0.008**
			SCR-ROL	3.441	**0.0003**	**0.01**
			SCR -PST	2.448	**0.007**	0.216
			SCR-RNW	3.493	**0.0002**	**0.008**
			SCR-PBL	1.758	**0.039**	1
			SCR-ALR	2.847	**0.002**	0.073
			SCR-CCR	2.837	**0.002**	0.073
			SCR-PPR	2.483	**0.007**	0.202
		Shannon exponential q=1	SCR-SIS	-2.83	**0.005**	**0.033**
			SCR-ROL	3.266	**0.001**	**0.01**
			SCR-ALR	2.252	**0.024**	0.11
			SCR-RNW	3.869	**0.0001**	**0.004**
			SCR-PBL	2.484	**0.013**	0.067
			PST-SCR	3.443	**0.001**	**0.01**
			SCR-CCR	2.794	**0.005**	**0.031**
			SCR-PPR	3.27	**0.001**	**0.013**
		Simpsons multiplicative inverse q=2	ALR-PST	-1.943	**0.052**	0.208
			ALR-RNW	-2.06	**0.039**	0.177
			SCR-SIS	-2.417	**0.016**	0.081
			SCR-ROL	3.046	**0.002**	**0.021**
			SCR-PBL	2.419	**0.016**	0.093
			SCR-PST	3.385	**0.001**	**0.013**
			SCR-RNW	3.742	**0.0002**	**0.007**
			SCR-CCR	2.667	**0.008**	0.055
			SCR-PPR	3.061	**0.002**	**0.027**

Table D.6: Posthoc Dunn Test by zone with Benjamin Hochberg correction for the core gut microbiome by reef.

ASV ID	Indicator Valu	*P*-Value	Frequency	Phylum	Class	Order	Family	Genus
ASV.1	0.996349277	0.001	111	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV.5	0.7413017	0.001	67	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV.6	0.730337079	0.001	65	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV.9	0.707730187	0.001	65	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Flavonifractor
ASV.7	0.696629213	0.001	62	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV.14	0.696629213	0.001	62	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	NA
ASV.11	0.674157303	0.001	60	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV.2	0.659837502	0.001	71	Spirochaetes	Spirochaetia	Brevinematales	Brevinemataceae	Brevinema
ASV.18	0.572730393	0.001	52	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	NA
ASV.10	0.550260717	0.001	53	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	NA
ASV.17	0.539325843	0.001	48	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV.3	0.538432561	0.001	68	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV.27	0.505419537	0.002	46	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Epulopiscium
ASV.68	0.447638938	0.001	41	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV.15	0.426966292	0.001	38	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	NA
ASV.30	0.370786517	0.003	33	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Romboutsia
ASV.95	0.348314607	0.002	31	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Vibrio
ASV.94	0.34551753	0.004	32	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Romboutsia
ASV.25	0.325842697	0.004	29	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	NA
ASV.19	0.314606742	0.003	28	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_1
ASV.24	0.314606742	0.001	28	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Tyzzerella
ASV.41	0.269662921	0.009	24	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Epulopiscium
ASV.74	0.258426966	0.008	23	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto_2
ASV.163	0.251085004	0.005	24	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV.59	0.235955056	0.006	21	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV.589	0.235955056	0.006	21	Deinococcus-Thermus	Deinococci	Thermales	Thermaceae	Thermus
ASV.39	0.213483146	0.009	19	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Anaerofilum

Table D.7: Core fish gut microbiome taxa identified with Indicator Analysis (Dufrêne and Legendre 1997) comparing all fish gut microbial data combined to all other fractions combined (i.e. hard coral, soft coral, sponge, sponge infauna, zoanthid, anemone, algae, seawater). ASVs are ordered from the highest to lowest indicator value.

Metric	Model	Source of Variation	Df	SumSq	MeanSq	F	N.Perm	Pr(>F)
Jaccard	Inner vs. Outer bay	Groups	1	0.00124	0.0012389	0.34	10000	0.5637
		Residuals	87	0.31702	0.0036439			
	Low vs. high coral	Groups	1	0.000012	0.0000116	0.0027	10000	0.9614
		Residuals	52	0.222844	0.0042855			
Modified Gower	Inner vs. Outer bay	Groups	1	0.051	0.050968	0.9796	10000	0.3213
		Residuals	87	4.5267	0.052031			
	Low vs. high coral	Groups	1	0.11257	0.112573	2.1817	10000	0.1467
		Residuals	52	2.68311	0.051598			
Bray-Curtis	Inner vs. Outer bay	Groups	1	0.27014	0.270138	13.215	10000	5e-04 ***
		Residuals	87	1.77841	0.020441			
	Low vs. high coral	Groups	1	0.09116	0.091155	2.7183	10000	0.1066
		Residuals	52	1.74376	0.033534			
Unifrac	Inner vs. Outer bay	Groups	1	0.0058	0.0058003	0.9247	10000	0.3446
		Residuals	87	0.69462	0.0079841			
	Low vs. high coral	Groups	1	0.00025	0.0002453	0.0353	10000	0.8512
		Residuals	52	0.36158	0.0069535			
Generalized Unifrac	Inner vs. Outer bay	Groups	1	0.07265	0.072651	7.6237	10000	0.006899 **
		Residuals	87	0.82908	0.00953			
	Low vs. high coral	Groups	1	0.00005	0.0000468	0.005	10000	0.9426
		Residuals	52	0.48636	0.0093531			
Weighted Unifrac	Inner vs. Outer bay	Groups	1	0.02471	0.024714	4.8833	10000	0.0268 *
		Residuals	87	0.4403	0.0050609			
	Low vs. high coral	Groups	1	0.00176	0.0017639	0.2856	10000	0.6054
		Residuals	52	0.32119	0.0061768			
Jaccard	3 habitat zones	Groups	2	0.00017	0.0000857	0.0208	10000	0.9775
		Residuals	86	0.35484	0.0041261			
Modified Gower	3 habitat zones	Groups	2	0.1586	0.07928	1.7449	10000	0.182
		Residuals	86	3.9074	0.045435			
Bray-Curtis	3 habitat zones	Groups	2	0.27062	0.13531	5.9346	10000	0.0033 **
		Residuals	86	1.96081	0.0228			
	Outside vs Inner disturbed							0.0007
Unifrac	3 habitat zones	Groups	2	0.0038	0.0018976	0.2856	10000	0.7483
		Residuals	86	0.57141	0.0066443			
Generalized Unifrac	3 habitat zones	Groups	2	0.06929	0.034647	3.6283	10000	0.0295 *
		Residuals	86	0.82122	0.009549			
	outside vs inner							0.0212
	outside vs inner							0.0381
Weighted Unifrac	3 habitat zones	Groups	2	0.02588	0.0129393	2.561	10000	**0.07729 .**
		Residuals	86	0.43451	0.0050524			
	outside vs inner							0.0251

Table D.8: Multivariate beta dispersion (Anderson 2006; Anderson, Ellingsen, and McArdle 2006) of the whole fish gut microbial communities compared among reef zones. We were interested whether the variability of individual fish gut microbiomes differed among the different zones.

Metric	Model		Df	SumSq	MeanSq	F	N.Perm	Pr(>F)
Jaccard	Inner vs. Outer bay	Groups	1	0.00212	0.0021159	0.1517	10000	0.7032
		Residuals	87	1.21375	0.0139511			
	Low vs. high coral cover	Groups	1	0.00561	0.0056089	0.3921	10000	0.5372
		Residuals	52	0.74391	0.014306			
mod Gower	Inner vs. Outer bay	Groups	1	0.0179	0.017936	0.2367	10000	0.6254
		Residuals	87	6.5933	0.075785			
	Low vs. high coral cover	Groups	1	0.3177	0.3177	4.5296	10000	**0.0387** *
		Residuals	52	3.6472	0.07014			
Bray-Curtis	Inner vs. Outer bay	Groups	1	0.23731	0.237309	10.907	10000	**0.0011** **
		Residuals	87	1.89296	0.021758			
	Low vs. high coral cover	Groups	1	0.07669	0.076693	3.5233	10000	**0.06939** .
		Residuals	52	1.1319	0.021767			
Unifrac	Inner vs. Outer bay	Groups	1	0.0547	0.054697	3.4941	10000	**0.06419** .
		Residuals	87	1.3619	0.015654			
	Low vs. high coral cover	Groups	1	0.01119	0.011193	0.7669	10000	0.3815
		Residuals	52	0.75896	0.014596			
Generalized Unifrac	Inner vs. Outer bay	Groups	1	0.0082	0.0082037	0.3851	10000	0.5394
		Residuals	87	1.8534	0.0213034			
	Low vs. high coral cover	Groups	1	0.04683	0.046834	2.3263	10000	0.1372
		Residuals	52	1.04689	0.020132			
Weighted Unifrac	Inner vs. Outer bay	Groups	1	0.00466	0.0046557	0.1563	10000	0.7019
		Residuals	87	2.59206	0.0297938			
	Low vs. high coral cover	Groups	1	0.07048	0.070476	2.4078	10000	0.1297
		Residuals	52	1.52204	0.02927			
Jaccard	3 habitat zones	Groups	2	0.00563	0.0028155	0.1903	10000	0.8311
		Residuals	86	1.27258	0.0147974			
mod Gower	3 habitat zones	Groups	2	0.3199	0.15996	2.0397	10000	0.1333
		Residuals	86	6.7443	0.078422			
	Inner disturbed vs inner							**0.036996**
Bray-Curtis	3 habitat zones	Groups	2	0.2554	0.127699	5.4236	10000	**0.005** **
		Residuals	86	2.0249	0.023545			
	Outside vs Inner disturbed							**0.0014**
	Inner disturbed vs inner							0.0675932
Unifrac	3 habitat zones	Groups	2	0.06817	0.034086	2.1679	10000	0.1203
		Residuals	86	1.3522	0.015723			
Generalized Unifrac	3 habitat zones	Groups	2	0.05237	0.026185	1.2036	10000	0.2994
		Residuals	86	1.87099	0.021756			
Weighted Unifrac	3 habitat zones	Groups	2	0.07351	0.036753	1.2255	10000	0.3012
		Residuals	86	2.5791	0.029989			

Table D.9: Multivariate beta dispersion (Anderson 2006; Anderson, Ellingsen, and McArdle 2006) of the core fish gut microbial communities compared among reef zones. We were interested whether the variability of individual fish gut microbiomes differed among the different zones.

Distance	Model	Factor	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Jaccard	Zone/Reef	Zone	2	1.561	0.78028	2.0715	0.04506	**1.00E-04**
		Zone:Reef	6	2.935	0.48922	1.2988	0.08476	1.00E-04
	Position/Reef	Position	1	0.889	0.88946	2.361	0.02568	**2.00E-04**
		Position:Reef	7	3.606	0.51521	1.3678	0.10414	1.00E-04
	Cover/Reef	Cover	1	0.6711	0.6711	1.7386	0.03235	**0.0019**
		Cover:Reef	4	2.0033	0.50082	1.3304	0.09657	0.0015
Modified Gower	Zone/Reef	Zone	2	3.479	1.73941	3.0818	0.06451	**1.00E-04**
		Zone:Reef	6	5.293	0.88213	1.5629	0.09815	2.00E-04
	Position/Reef	Position	1	2.044	2.0439	3.6213	0.0379	**2.00E-04**
		Position:Reef	7	6.728	0.9611	1.7028	0.12476	1.00E-04
	Cover/Reef	Cover	1	1.435	1.43492	2.4181	0.04267	**0.0009999**
		Cover:Reef	4	3.707	0.92679	1.5618	0.1102	0.0016998
Bray Curtis	Zone/Reef	Zone	2	2.2745	1.13725	4.4843	0.09217	**1.00E-04**
		Zone:Reef	6	2.1136	0.35226	1.389	0.08565	0.049
	Position/Reef	Position	1	1.2818	1.28176	5.0541	0.05194	**0.0003**
		Position:Reef	7	3.1063	0.44376	1.7498	0.12588	0.0029
	Cover/Reef	Cover	1	0.9927	0.99273	3.4134	0.0611	**0.0022**
		Cover:Reef	4	1.2951	0.32377	1.1133	0.07971	0.2915
Unifrac	Zone/Reef	Zone	2	1.0915	0.54576	2.3968	0.05179	**0.0004**
		Zone:Reef	6	1.7687	0.29479	1.2946	0.08392	0.0292
	Position/Reef	Position	1	0.6944	0.69435	3.0494	0.03294	**0.0006999**
		Position:Reef	7	2.1659	0.30941	1.3588	0.10276	0.0092991
	Cover/Reef	Cover	1	0.3972	0.39716	1.69	0.03116	**0.0472**
		Cover:Reef	4	1.0699	0.26748	1.1382	0.08393	0.1919
Generalized Unifrac	Zone/Reef	Zone	2	0.3157	0.157865	2.2534	0.04856	**0.008199**
		Zone:Reef	6	0.5813	0.096875	1.3828	0.0894	0.055794
	Position/Reef	Position	1	0.1855	0.185504	2.6479	0.02853	**0.0129**
		Position:Reef	7	0.7115	0.10164	1.4508	0.10943	0.0283
	Cover/Reef	Cover	1	0.1302	0.130226	1.5992	0.0294	0.1154
		Cover:Reef	4	0.3903	0.097563	1.1981	0.08811	0.1997
Weighted Unifrac	Zone/Reef	Zone	2	0.06542	0.032708	1.9084	0.04175	0.07089
		Zone:Reef	6	0.13032	0.02172	1.2673	0.08318	0.18758
	Position/Reef	Position	1	0.03581	0.035813	2.0896	0.02286	0.09119
		Position:Reef	7	0.15992	0.022846	1.333	0.10207	0.14389
	Cover/Reef	Cover	1	0.0296	0.029603	1.3917	0.0259	0.229
		Cover:Reef	4	0.09229	0.023073	1.0847	0.08075	0.3582

Table D.10: Permutational Analysis of Variance (PERMANOVA) (Anderson 2001) results for the whole fish gut microbiome. Differences among fish gut microbial communities were tested using three models: (1) among three zones ("Group" in table); (2) between reefs located inside versus outside of the bay (position model) and (3) between reefs of differential coral cover levels inside of the bay (cover model).

Distance	Model	Factor	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Jaccard	Zone/Reef	Zone	2	1.7548	0.87738	5.1227	0.10103	**1.00E-04**
		Zone:Reef	6	1.9124	0.31873	1.861	0.1101	0.0005999
	Position/Reef	Position	1	1.2367	1.23675	7.2209	0.0712	**1.00E-04**
		Position:Reef	7	2.4304	0.3472	2.0272	0.13993	2.00E-04
	Cover/Reef	Cover	1	0.518	0.518	2.9928	0.05207	**0.0031**
		Cover:Reef	4	1.1226	0.28065	1.6215	0.11284	0.0163
modified Gower	Zone/Reef	Zone	2	11.341	5.6703	4.4255	8.65E-02	**1.00E-04**
		Zone:Reef	6	17.216	2.8694	2.2395	1.31E-01	1.00E-04
	Position/Reef	Position	1	7.98	7.9798	6.228	0.06089	**1.00E-04**
		Position:Reef	7	20.577	2.9396	2.2943	0.15701	1.00E-04
	Cover/Reef	Cover	1	3.361	3.3608	2.5099	0.04429	**0.0115**
		Cover:Reef	4	8.244	2.0609	1.5391	0.10865	0.0302
Bray Curtis	Zone/Reef	Zone	2	1.9772	0.98858	4.2749	0.08802	**1.00E-04**
		Zone:Reef	6	1.9861	0.33102	1.4314	0.08841	0.05409
	Position/Reef	Position	1	1.1449	1.14491	4.9509	0.05097	**0.0005999**
		Position:Reef	7	2.8183	0.40262	1.741	0.12546	0.0053995
	Cover/Reef	Cover	1	0.8323	0.83225	3.1407	0.05604	**0.005599**
		Cover:Reef	4	1.2991	0.32478	1.2256	0.08748	0.184882
Unifrac	Zone/Reef	Zone	2	0.7624	0.3812	3.4988	0.07136	**0.0006999**
		Zone:Reef	6	1.2049	0.20082	1.8431	0.11278	0.0079992
	Position/Reef	Position	1	0.6341	0.63405	5.8195	0.05935	**1.00E-04**
		Position:Reef	7	1.3332	0.19046	1.7481	0.12479	0.0103
	Cover/Reef	Cover	1	0.1284	0.12835	1.2834	0.02381	0.2794
		Cover:Reef	4	0.462	0.11549	1.1548	0.08569	0.3073
Generalized Unifrac	Zone/Reef	Zone	2	0.4435	0.22176	1.3724	0.0295	0.1942
		Zone:Reef	6	1.6639	0.27732	1.7163	0.11068	0.0421
	Position/Reef	Position	1	0.1747	0.1747	1.0812	0.01162	0.3097
		Position:Reef	7	1.9327	0.27611	1.7087	0.12856	0.0346
	Cover/Reef	Cover	1	0.2688	0.26882	1.5667	0.02899	0.1618
		Cover:Reef	4	0.7682	0.19206	1.1193	0.08285	0.3236
Weighted Unifrac	Zone/Reef	Zone	2	0.2567	0.12835	1.0153	0.02215	0.38056
		Zone:Reef	6	1.2182	0.20304	1.6061	0.10513	0.09289
	Position/Reef	Position	1	0.0664	0.066447	0.52562	0.00573	0.60884
		Position:Reef	7	1.4085	0.201211	1.59165	0.12154	0.08489
	Cover/Reef	Cover	1	0.1903	0.19025	1.4289	0.0265	0.2238
		Cover:Reef	4	0.5972	0.14929	1.1212	0.08319	0.3352

Table D.11: Permutational Analysis of Variance (PERMANOVA) (Anderson 2001) results for the core fish gut microbiome. Differences among fish gut microbial communities were tested using three models: (1) among three zones ("Group" in table); (2) between reefs located inside versus outside of the bay (position model) and (3) between reefs of differential coral cover levels inside of the bay (cover model).

Distance	Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
Jaccard	Outer vs Inner bay	1	0.9179362	2.391052	0.03396813	0.001	**0.003**
Jaccard	Outer vs Inner dist.	1	0.7110085	1.852244	0.03439494	0.003	**0.009**
Jaccard	Inner vs Inner dist.	1	0.6710959	1.738583	0.03235261	**0.002**	**0.006**
modGower	Outer vs Inner bay	1	1.771492	3.2316	0.04536756	**0.001**	**0.003**
modGower	Outer vs Inner dist.	1	2.002315	3.31333	0.05990112	**0.001**	**0.003**
modGower	Inner vs Inner dist.	1	1.434917	2.3179	0.04267286	**0.002**	**0.006**
Bray Curtis	Outer vs Inner bay	1	0.7351019	3.061599	0.04308373	**0.012**	**0.036**
Bray Curtis	Outer vs Inner dist.	1	1.8030557	7.091213	0.12000453	**0.001**	**0.003**
Bray Curtis	Inner vs Inner dist.	1	0.9927345	3.38389	0.06109882	**0.004**	**0.012**
Unifrac	Outer vs Inner bay	1	0.7211911	3.130732	0.04401377	**0.001**	**0.003**
Unifrac	Outer vs Inner dist.	1	0.4669362	2.030862	0.03758708	**0.012**	**0.036**
Unifrac	Inner vs Inner dist.	1	0.397157	1.672233	0.03115639	0.056	0.168
GUnifrac	Outer vs Inner bay	1	0.1862079	2.693074	0.03809531	**0.021**	0.063
GUnifrac	Outer vs Inner dist.	1	0.1487629	2.294963	0.04226843	**0.019**	0.057
GUnifrac	Inner vs Inner dist.	1	0.1302265	1.575228	0.02940218	0.114	0.342
WUnifrac	Outer vs Inner bay	1	0.04234835	2.472368	0.0350828	0.063	0.189
WUnifrac	Outer vs Inner dist.	1	0.02331571	1.672923	0.03116885	0.136	0.408
WUnifrac	Inner vs Inner dist.	1	0.0296028	1.382646	0.02590066	0.227	0.681

Table D.12: Pairwise Permutational Analysis of Variance (PERMANOVA) (Anderson 2001) results for the whole fish gut microbiome. Posthoc pairwise PERMANOVA (Martinez Arbizu 2019) were calculated with Bonferroni corrected P-values.

Distance	Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
Jaccard	Outer vs Inner bay	1	1.0424578	5.865352	0.079406	0.001	**0.003**
Jaccard	Outer vs Inner dist.	1	1.0227554	5.475954	0.09527383	0.001	**0.003**
Jaccard	Inner vs Inner dist.	1	0.5180037	2.856284	0.05206849	0.007	**0.021**
modGower	Outer vs Inner bay	1	6.22975	4.689234	0.06451071	0.001	**0.003**
modGower	Outer vs Inner dist.	1	7.254523	4.926	0.0865334	**0.001**	**0.003**
modGower	Inner vs Inner dist.	1	3.360785	2.409937	0.04429222	**0.019**	0.057
Bray Curtis	Outer vs Inner bay	1	0.6038367	2.763475	0.03905227	**0.012**	**0.036**
Bray Curtis	Outer vs Inner dist.	1	1.6436521	7.066327	0.11963376	**0.001**	**0.003**
Bray Curtis	Inner vs Inner dist.	1	0.8322521	3.087106	0.05604045	**0.005**	**0.015**
Unifrac	Outer vs Inner bay	1	0.4691397	3.860933	0.05372784	**0.004**	**0.012**
Unifrac	Outer vs Inner dist.	1	0.5200624	4.280836	0.07606205	**0.001**	**0.003**
Unifrac	Inner vs Inner dist.	1	0.1283554	1.268345	0.02381048	0.243	0.729
GUnifrac	Outer vs Inner bay	1	0.1198879	0.7424537	0.01080051	0.497	1
GUnifrac	Outer vs Inner dist.	1	0.306756	1.734408	0.03227742	0.142	0.426
GUnifrac	Inner vs Inner dist.	1	0.2688158	1.5524443	0.02898923	0.164	0.492
WUnifrac	Outer vs Inner bay	1	0.05790827	0.4694963	0.006857015	0.664	1
WUnifrac	Outer vs Inner dist.	1	0.15775916	1.1256392	0.021188247	0.328	0.984
WUnifrac	Inner vs Inner dist.	1	0.1902502	1.4156865	0.026503198	0.213	0.639

Table D.13: Pairwise Permutational Analysis of Variance (PERMANOVA) (Anderson 2001) results for the core fish gut microbiome. Posthoc pairwise PERMANOVA (Martinez Arbizu 2019) were calculated with Bonferroni corrected P-values.

Prevalence Interval	OBB error rate %	ASVs	Nseqs
5%	22.47	1143	897208
10%	20.22	266	844546
15%	13.48	118	799278
20%	10.11	76	784277
25%	8.99	57	770883
30%	8.99	42	753340
35%	11.24	36	744032
40%	10.11	36	739985
45%	8.99	30	732452
50%	6.74	23	706945
55%	4.49	21	674387
60%	3.37	18	600083
65%	**2.25**	**17**	**561593**
70%	7.87	10	496649
75%	4.49	7	457736
80%	15.73	4	424993
85%	6.74	4	418457
90%	6.74	3	405206
95%	67.42	1	365907

Table D.14: Prevalence Interval for Microbiome Evaluation (PIME)(Roesch et al. 2019). Using RandomForest, the algorithm determined the prevalence level, which provided the best model to predict differences among the three reef zone microbial communities. The table lists the output of the best prevalence function (PIME R package, Roesch et al. 2019) with the out of bag (OBB) error rate for each prevalence interval and the AVSs retained in the dataset as well as the associated number of remaining sequences (Nseq). The prevalence bin chosen for the present study (65%) is depicted in bold.

ASV	Inner bay	Inner bay disturbed	Outer bay	Mean Decrease Accuracy	Mean Decrease Gini
ASV6	0.056103703	0.021597619	0.174855597	0.093632368	6.740867519
ASV7	0.08671229	0.088669841	0.099411424	0.09076883	7.98316477
ASV3	0.005125934	0.160498485	0.050850058	0.053926743	5.720833825
ASV9	0.001270734	0.011368326	0.096647221	0.040011572	3.573840343
ASV42	0.02385976	0.043803535	0.049101124	0.037602644	4.043649685
ASV27	0.024402986	0.03225267	0.055083597	0.036728284	3.320584212
ASV41	0.021518957	0.062058802	0.024365059	0.031886283	3.95981884
ASV2	0.023511911	0.029188745	0.038238022	0.030100204	3.120207217
ASV10	0.008987344	0.066901371	0.027017528	0.028201424	3.030194539
ASV4	0.018321354	0.030409235	0.037108942	0.02782255	2.688240385
ASV14	0.054866006	0.012504834	0.007273479	0.026189242	2.776662362
ASV38	0.019521715	0.029386797	0.018296985	0.020691546	2.628507801
ASV94	0.010840017	0.032298196	0.018855767	0.018416502	2.371929934
ASV1	0.00192246	0.018927056	0.011505233	0.008862312	1.360170402
ASV11	0.000392347	0.051716667	-0.003903	0.008319567	1.061015645
ASV8	0.00148597	9.00E-05	0.012271321	0.005533648	1.131796017
ASV5	3.85E-05	0.005655916	0.005317374	0.003572141	0.954048098

Table D.15: Prevalence Interval for Microbiome Evaluation (PIME)(Roesch et al. 2019). Results for the PIME filtered community comprising 17 ASVs that are responsible for discriminating the three reef zone communities. Based on RandomForest, the algorithm calculated (i) Mean Decrease Accuracy where higher values indicate the importance of taxa in causing differences among the three reef zones, while positive values indicate ASVs truly contributed to discern zones – the importance values are reported across all decision trees and broken up by respective reef zones and (ii) Mean Decrease Gini (or mean decrease impurity), a measure of how reliable a variable predicts the model and refers to node splitting in a decision tree, where the algorithm looks for the feature (here ASV) to split where the split results in the lowest node impurity for the most optimal model.

ASV	Kingdom	Phylum	Class	Order	Family	Genus
ASV1	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV2	Bacteria	Spirochaetes	Spirochaetia	Brevinematales	Brevinemataceae	Brevinema
ASV3	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV4	Bacteria	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Synechococcus_CC9902
ASV5	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV6	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV7	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV8	Bacteria	Cyanobacteria	Oxyphotobacteria	Synechococcales	Cyanobiaceae	Cyanobium_PCC-6307
ASV9	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Flavonifractor
ASV11	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Endozoicomonadaceae	Endozoicomonas
ASV27	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Epulopiscium
ASV42	Bacteria	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	NA
ASV10	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	NA
ASV14	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	NA
ASV41	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Epulopiscium
ASV94	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Romboutsia
ASV38	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Ruegeria

Table D.16: Prevalence Interval for Microbiome Evaluation (PIME)(Roesch et al. 2019). Taxonomy table of the PIME filtered community comprising 17 ASVs that are responsible for discriminating the three reef zone communities.

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