Drivers to ecosystem functions provided by grazing coral reef fishes in the Anthropocene

Thesis submitted by

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Statement of contribution of others

I declare that this thesis is my own work, and has not been submitted in the same form for a higher degree elsewhere. My thesis and associated published work include collaborations with my supervisors Nick Graham (NG) and Valeriano Paravicini, (VP) and my co-authors Jordan Casey (JC), James Robinson (JR), Anne Haguenauer (AH), Mayalen Zubia (MZ), Cassandra Benkwitt (CB), Jeneen Hadj-Hammou (JHH), Jan-Claas Dajka (JCD), Andrew Hoey (AH), Ines Lange (IL), Chris Perry (CP), Jamie M. McDevitt-Irwin (JMI), Alexia Graba-Landry (AGL), Kirsty Nash (KN) and Shaun Wilson (SW). I was responsible for the project's conceptual and experimental design, data collection, analysis, interpretation and synthesis into final form for publication. My supervisors provided intellectual guidance, equipment, funding support, and editorial assistance. Funding for this PhD project was provided through Valeriano Parravicini's Reef Services Grant from the BNP Paribas, and the Lancaster University Graduate School travel grants.

Details on contributions by chapters

Chapter 1- Temporal patterns of parrotfish functions during habitat disturbance

Samantha Howlett (SH) and NG conceived the ideas with support from VP and JR. Approaches to converting community data into functional rates was adapted from additional work published in collaboration with JHH, JCD, JMI, AGL and SW (Robinson et al. 2020). Data on bite rates and scar sizes was provided by AH, CP and IL. SH analysed the data, with NG and VP contributing critically to the drafts.

Chapter 2: Using stereo video cameras to identify drivers to functional rates

SH, conceived the ideas with support from NG and VP. Kyle Thomas assisted SH with data collection in the field, and Jennifer Meecham assisted with processing photo quads. Converting community data into functional rates was adapted from approaches used in the publication mentioned above (Robinson et al 2020). SH analysed the data, with NG and VP contributing critically to the drafts.

Chapter 3: Micro-habitat variation and bleaching response influences algal successional processes following widescale coral bleaching

The ideas were conceived by SH with support from NG, VP, JC and AH. Metabarcoding techniques were defined by SH, JC and AH. SH conducted the field work and processed the samples. SH led the stable isotope analysis. Extracted DNA was sent to Jonah Ventures for sequencing and bioinformatics. SH analysed the data. SH, NG and JC contributed critically to the drafts.

Chapter 4: Early stage successional turf communities following coral mortality and implications for juvenile reef fish grazing and fitness

The ideas were conceived by SH with support from NG, VP, JC and AH. Aquarium set up had input from Alexander Merciere (AM), Marc Besson and Camille Gache. SH collected the fish and AM ran the respirometry. Metabarcoding techniques were defined by SH, JC and AH. Extracted DNA was sent to Jonah Ventures for sequencing and bioinformatics. SH analysed the data. SH, NG and JC contributed critically to the drafts.

Abstract

As we move into the Anthropocene, a variety of local and global stressors are reorganising coral reef communities, that can push them away from coral dominated states, potentially reducing their capacity to provide ecosystem services. Quantifying ecosystem functions is becoming an increasingly popular tool in helping us understand ecosystem resilience. This thesis focuses on one of the primary ecosystem functions on coral reefs: the grazing of algal turf communities by roving herbivorous fishes. In **Chapter 1** and **Chapter 2**, I use a combination of long-term monitoring UVC data and stereo-cameras to estimate grazing and bioerosion rates, and look at a habitat and management features that influence these both temporally and spatially. In **Chapter 1** I show that two functions associated with parrotfish grazing respond differently following a large-scale disturbance, with scraping rates increasing initially and bioerosion rates lagging behind as fish require time to attain large body size. In **Chapter 2** I show that, other than greater species diversity, there are varying spatial drivers to grazing rates by functional groups. These chapters both show that converting community data into associated grazing functions sheds light on just how complex drivers to ecosystem functions can be.

The second half of the thesis focuses on characterising benthic changes that occur following mass coral bleaching events, and how these can act as bottom-up drivers to grazing rates by affecting the foraging landscape for grazing fishes over time. I use a range of approaches to track the successional stages of algae and microbial communities in the first three months post-bleaching, how these changes relate to turf algal features known to influence grazing rates. In **Chapter 3** I use an experimental approach to show how micro-habitat variations; such as coral life histories and morphology; can influence successional stages and associated features. In **Chapter 4**, I apply similar approaches to corals which suffered natural mortality during a bleaching event, showing similarities between natural and experimental approaches. Finally, I use a range of experiments to show how successional turf algal communities can vary through time in their attractiveness to grazing fishes, and even their ability to sustain them energetically.

Publications during the PhD

Robinson, J.P., McDevitt-Irwin, J.M., Dajka, J.C., Hadj-Hammou, J., Howlett, S., Graba-Landry, A., Hoey, A.S., Nash, K.L., Wilson, S.K. and Graham, N.A., 2020. Habitat and fishing control grazing potential on coral reefs. *Functional Ecology*, *34*(1), pp.240-251. https://doi.org/10.1111/1365-2435.13457

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General Introduction

The importance of grazing fishes

Coral reefs are one of the most biodiverse, and yet also threatened habitats on the planet. Gradual degradation to reefs from localised pressures such as fishing and terrestrial runoff can restructure reef communities over time, while large-scale disturbances linked to climate change can rapidly reduce live coral cover and structural complexity. As we progress into the Anthropocene, both global and localised impacts are set to increase, raising concerns over the future resilience of many reefs. One of the underlying questions is how will reef ecosystem functions respond as communities change? Functions are described by Bellwood et al. (2019) as the movement or storage of energy and matter, and grazing by reef fishes provides a number of key ecosystem processes which revolve around algal and sediment removal (Bonaldo & Bellwood 2011, Tebbett et al. 2017a, Tebbett et al. 2018). Algae can directly outcompete corals (McCook et al. 2001, Swierts & Vermeij 2016), and the accumulation of sediment can also inhibit reef processes, such as coral recruitment and herbivory (Birrell et al. 2005, Bellwood & Fulton 2008, Duran et al. 2018, Tebbett et al. 2018, Speare et al. 2019).

Typically, on coral reefs the majority of primary production is characterised by low standing mass with rapid turnover (Carpenter 1985, Russ 2003), and these algal communities are colloquially referred to as algal turfs. These algal turfs are multi-species assemblages containing material such as detritus, microorganisms and sediment, and are important feeding sites as their high rates of primary productivity support secondary productivity (Russ 2003, Kelly et al. 2017, Bellwood et al 2018). Furthermore, they provide protein rich resources besides algal material through detritus, microorganisms and micro-invertebrates (Wilson et al. 2003) which are lacking in the diets of fish that feed on established macro-algae (Crossman et al. 2005, Clements et al. 2017). Within these multi-species assemblages are propagules for macro-algae that can dominate under low grazing environments and shift habitats away from

coral dominated systems to alternative stable states (Bellwood et al. 2004, Puk et al. 2016). All this material combined is referred to as the epilithic algal matrix (EAM).

Given the importance of turf algal communities as trophic pathways and energy transfer within the ecosystem, their ability to shift away from this under certain conditions, and the fact that algal turfs are poised to become more prominent in the Anthropocene (Tebbett & Bellwood 2019), understanding what drives turf algal composition, and how this relates to fish communities and their associated functions is vital.

This thesis focusses on grazing fishes that primarily feed on or functionally remove turf algae and the EAM, namely those within the families Scaridae and Acanthuridae. Within these, fish can be divided into functional groups depending on their physical traits and how they remove and process material (Tebbett & Bellwood 2019). Grazing fish have been traditionally grouped within herbivores, however recent research has shown that only a few of these have the correct anatomy to breakdown and effectively draw nutrition from algae (Mountfort et al. 2002, Choat et al. 2004). Most parrotfishes are now considered microphages (Clements et al. 2017), with some Acanthurids, especially those within the genus *Ctenochaetus*, considered detritivores (Wilson et al. 2003). Surgeonfish that target the fine filamentous algae in turf communities have spatulate teeth which are used for nipping the ends of fronds without ingesting sediment or detritus, whereas detritivore surgeonfishes have long comb-like teeth that brush sediment and detritus from the substrate (Purcell & Bellwood 1993, Tebbett & Bellwood 2019). These surgeonfish are functionally considered as croppers as they remove the upper portions of standing plant biomass, sediment and detritus (Tebbett et al. 2017b). Parrotfish with their fused beaks and strong jaws are considered either scrapers or bioeroders as they remove the entire EAM and a portion of the upper layer of the substratum through their bites, or for larger bodied parrotfishes process large chunks of the substrate also (Bellwood et al 1990, Bonaldo et al. 2014).

Removal of algal material is important on reef ecosystems as algae can outcompete corals and cause coral tissue death by producing allelochemicals (Jompa & McCook 2003) or inhibit

recruitment through the trapping of sediment (Speare et al. 2019). Macro-algal beds have a number of positive feedback processes that reinforce and maintain algal dominance (Dajka et al. 2020). High grazing pressure and the maintenance of short productive algal turfs (SPATs) promotes secondary productivity (Carpenter 1986, Burkepile 2013), and also promotes the growth of crustose coralline algae, which has been shown to encourage coral recruitment (Arnold et al. 2010, Tebben et al. 2015). Maintenance of SPATs also keeps canopy heights down, and therefore reduces the ability of turfs to trap sediment (Purcell 2000, Bonaldo & Bellwood 2011), with greater sedimentation levels being shown to also inhibit coral recruitment (Birrel et al. 2005). Scraping by parrotfish is especially effective at maintaining all of the above due to the removal of all epilithic material. Bioerosion is the removal of reef substratum and the generation and reworking of sediment which contributes towards large-scale sediment regimes that affect island formation (Perry et al. 2015, Morgan & Kench 2016). It also affects localised habitat characteristics such as reef growth and complexity (Bozec et al. 2015, Glynn & Manzello 2015, Perry et al. 2018).

A widely applied approach to calculate the rate of ecosystem functions performed by various fish communities is to quantify bite metrics for a given species of a given size (Hoey & Bellwood 2008, Marshell & Mumby 2012, Yarlett et al. 2018, Lange et al. 2020). A growing body of research shows that there are strong allometric relationships for certain functions (Lokrantz et al. 2008, Lange et al 2020, Hoey & Bellwood 2008). However, while larger fish have been shown to provide a greater contribution to ecosystem functions on an individual basis, smaller fish have been shown to have higher bite rates (Lokrantz et al. 2008, Lange at al. 2020) and are typically found in higher densities, meaning that functions such as area grazed can be less tied to body size. Therefore, any changes to the number and types of species present, and the size structure of the community will affect functional rates in different ways.

The loss of large bodied parrotfish has been shown to result in a decline in bioerosion rates (Bellwood et al. 2003, Bellwood et al 2012), however such relationships are less defined for cropping and scraping rates, likely due to higher functional complementarity (Brandl &

Bellwood 2014). Furthermore, the effects of fishing pressure are less clear for other functional rates such as cropping. Fishing can be considered a top down pressure as it alters the diversity and size structure of communities, which can have cascading effects to lower trophic levels (Mumby et al. 2006, McClanahan & Muthiga 2016). Natural predation can also have a strong influence on structuring coral reef communities also (Mumby et al. 2007a, Boaden & Kingsford 2015, Rasher et al. 2017). However, such top-down affects have been shown to not be a golden rule in complex ecosystems such as coral reefs and can be context specific (Casey et al. 2017), with some studies arguing that bottom-up drivers such as habitat features can have a buffering effect in some situations (Desbiens et al. 2021). Monitoring predator density and habitat can be challenging and time intensive, requiring the deployment of Baited Remote Underwater Videos (Phenix et al. 2019), or tags (Stewart & Jones 2001). Therefore, predation is not addressed within this thesis, and top-down influence is only explored in terms of fishing pressure.

Bottom-up drivers to functions provided by grazing reef fishes have been identified as structural complexity (Graham et al. 2006, Nash et al. 2016), live coral cover versus available grazing space (Robinson et al. 2020), primary productivity (Russ 2003), nutrient input (Shantz et al. 2017) and sedimentation (Bellwood & Fulton 2008, Gordon et al. 2016a, Adam et al. 2018). However, the importance of these drivers can vary spatially, with few examining how these scale up to macro-ecological patterns (Robinson et al. 2019).

The relationships between features that affect grazing rates have not yet been explored temporally. Since reefs are dynamic ecosystems, and that anthropogenic pressures are set to increase, understanding how drivers interact through time is of the utmost importance if we are to understand and predict how functions provided by grazing fishes will exist in the future.

The fate of bleached corals

In terms of severity, impacts associated to climate are arguably one of the main threats facing coral reefs (Bellwood et al 2004, Hughes et al. 2017). Events such as cyclones and coral bleaching can rapidly convert huge swaths of benthic cover from live coral to bare substrate in

a matter of days (Adjeroud et al. 2005, Stobart et al. 2005). These substrates are swiftly colonised by algae, expanding grazing space and increasing resources for fishes, while potentially reducing competition. Following such events, populations of grazing fishes have been observed to increase in response (Han et al. 2016, Graham et al. 2020). Through this conversion of live coral cover to colonising turfs, it is believed that grazing fishes are no longer resource limited (Tootell & Steele 2016, **Chapter 1**), and it is simply the increase in grazing space that drives increases to secondary production. However, this view is far too simplistic as it ignores temporal changes that occur through colonisation. Colonisation processes start with fast growing species that can rapidly exploit available spaces, which then gradually become out-competed by slow-growing species that have a more competitive edge, for example resilience to grazing, shading tolerance etc. (Bruno et al. 2006). Environmental and biological factors interact to shape this process (Tilman 1994, McClanahan 1997, Wang et al. 2021), and the faunal species associated with these communities change through time. Therefore, our broad view of these new bare substrates following disturbances as simply 'available grazing space' ignores a lot of complexity, does not capture the temporal changes that will be occurring, and prevents us from making detailed connections between benthic changes and secondary productivity.

There is a growing body of evidence that indicates high spatial variation in turf algal communities, affecting their attractiveness as feeding sites and their ability to support various fish species. Grazing fish biomass has been linked with algal productivity over standing mass (Russ 2003), with factors such as ambient nutrient levels (Shantz et al. 2017), light availability (Nemeth & Appledorn 2009), hydrodynamics (Purcell 2000) and reef zone (Russ 1984) being shown to affect algal composition and productivity, and therefore in turn grazing rates. Since grazing fishes are diverse in how they utilise material within turfs and the EAM (Crossman et al. 2005, Clements et al. 2009, Tebbett & Bellwood 2019), understanding community composition is important as variation to the dominant primary producers; plant versus autotrophic micro-organisms; will influence grazer species. Furthermore, variation within plant

communities will drive grazing variations, as species within phaeophyceae typically contain greater proportions of tough fibrous materials which are challenging to breakdown and require hindgut fermentation by microorganisms in order to digest (Clements & Choat 1995, Clements et al. 2017), Some algae even produce herbivore deterring chemicals through secondary metabolites (Gache et al. 2019, Vieira et al. 2019).

Sedimentation within algal turfs has been shown to be a strong driver in reducing grazing rates (Bellwood & Fulton 2008, Goatley & Bellwood 2012) by restricting access to food (Adam et al. 2018) or through the dilution of organic material (Gordon et al. 2016a, Tebbett et al. 2017). Fine scale habitat features such a structural complexity have been shown to influence turf algal sediments (Duran et al. 2018, Tebbett et al. 2019), with sedimentation loads following typical patterns across reef zones, being lower in high energy environments such as reef crests and higher in low energy environments such as reef flats (Purcell 2000, Duran et al. 2018). This negative impact of sediments to grazing fishes varies by species and relates to their feeding styles. Detritivorous surgeonfishes have been shown to be more susceptible to increases to sediment loads than algal grazers due to their brushing feeding styles (Tebbett et al. 2017). Factors such as grain size and ratio of organic to inorganic material has been shown to influence the feeding behaviour of *Scarus rivulatus* (Gordon et al. 2016a).

To date, patterns of colonisation on reefs following large scale disturbances such as coral bleaching is currently described at broad, functional levels (Hixon & Brostoff 1996, McClanahan 1997, Diaz-polido & McCook 2002), with a few studies identifying taxa to low taxonomic levels (Cecarelli et al. 2011). Cyanobacteria have been acknowledged to be prominent during early stages of colonisation on coral reefs (Diaz-polido & McCook 2002, Davey et al. 2008), with brown algae within the class phaeophyceae comprising climax communities which shift towards macro-algal stands (Hughes et al. 2007, Norström et al. 2009). However, detailed knowledge of interim species and even species that dominate climax turf communities is still lacking. To date, the majority of studies exploring colonisation patterns use artificial substrates since it is easier to standardise surface area (Fricke et al. 2011,

Ceccarelli et al. 2011), however this also means that such substrates are biased towards flat, uniform and horizontal orientation. Studies show that variation to microstructure and porosity affect colonisation patterns (Davis 2009, Mallela et al. 2017), as does substrate orientation (Duran et al. 2018), indicating a need to quantify community features on natural substrates.

Identifying algae to high taxonomic resolution is typically done by identifying key features using microscopy (Zubia et al. 2018), which is reliant on high specialist knowledge and is a laborious and time-consuming process. The advent of metabarcoding bypasses these issues and gives access to data at high taxonomic resolution. It also allows us to track not only algal community composition, but changes to microbial ones too. Metabarcoding techniques have shown us that microbiomes are varied between corals (van Oppen & Blackall 2019), and this has been proven to influence coral resilience to thermal stress and diseases (Doering et al. 2021). Mass mortality of corals following bleaching has been shown to release a large amount of nutrients into the area, both through mucus production and tissue loss, and nitrogen fixation from colonising cyanobacteria (Davey et al. 2008, Haas et al. 2013). In terrestrial ecosystems, microbial decomposition of organic material is known as a vital part of nutrient recycling (Condron et al. 2010, Shahbaz et al. 2017). Sudden inputs of nutrients can influence microbial communities (Frade et al. 2020), which influences nutrient recycling, which in turn affects primary productivity (McCook 1990), which then drives secondary productivity (Russ 2003, Kelly et al. 2013). However, such connections have not yet been shown in coral reef ecosystems. Metabarcoding allows us to track community changes through colonisation following bleaching, and the use of stable isotopes allows us to see where nutrients are being sourced from as communities establish (Kolasinski et al. 2011). However, metabarcoding comes with its own associated drawbacks, such as high cost and gaps in barcode databases where species are missing. This puts restrictions on sample size and can hinder inferences from the data, however the results have the potential to be re-run in the future as barcode databases are updated.

Ecosystem processes provided by grazing fishes in the Anthropocene

The effects of large-scale bleaching events have been overlooked to date in terms of their role in nutrient recycling. Natural nutrient subsidies provided by sea birds have been shown to boost growth rates of the bioeroding parrotfish *Chlorurus sordidus*, therefore likely enhancing bioerosion rates in the process (Benkwitt et al. 2021). Taylor et al. (2019) have shown that parrotfish growth rates increase in response to bleaching, therefore suggesting that similar increases to secondary productivity occur with recycled naturally sourced autochthonous nutrients also. Besides a switch from food limited to food excess, to date there are few studies that explore how such nutrients affect algal community structure, EAM composition and productivity, which are all major drivers of grazing rates. Most studies looking at the effects of nutrients on primary productivity have explored this in the context of anthropogenic or terrigenous sourced nutrients (Sammarco 1999, Costa et al. 2003, Donovan et al. 2020), or teasing out the relative strengths of top-down pressures (grazing) against bottom-up forces (nutrients) on community composition (Miller et al. 1999, Burkepile & Hay 2006, Thacker et al. 2014). Such connections have been challenging to define since there is interaction of a multitude of factors, rendering many studies context specific.

Nutrient enrichment tends to enhance algal growth (Lapointe 1997, Blanchette et al. 2019, Adam et al. 2021), potentially pushing it past the point that current grazing levels can withstand. However, nutrient enrichment has also been shown to alter the elemental content of algal tissues, and thereby alter feeding behaviour of fishes. Algal tissues enriched with nitrogen and phosphorous have been shown to be fed upon more readily by young *Sparisoma* sp. parrotfishes (Shantz et al. 2017). Growth rates in fish are typically higher early on, especially in the first year of life, and decrease as fish age (Stallings et al. 2010). This means that fish have greater nutritional demands during their younger, juvenile stages, especially on limited nutrients such as phosphorous which is required for skeleton formation (Smedley et al. 2016). An increase to nutrient rich food sources has been shown to enhance metabolic rate and growth rates of juvenile fish (Auer et al. 2015, Zeng et al. 2018). However, while climatic disturbances

have been shown to have short-term, initial positive effects on grazing fish populations, these trends are responsive and are typically not maintained long-term. Graham et al. (2007) found that while fish size increased following bleaching, counts of fish in smaller categories were greatly reduced, meaning a rapid drop in recruitment was imminent. Though grazing populations show a responsive increase, the long-term projections of community composition, and therefore grazing functions, is less clear.

Benthic communities associated with coral loss shift towards some form of climax community, which can either be a return to coral dominance under a situation of high grazing and low nutrient input, macro-algal beds in low grazing and high nutrient scenarios, or long sediment-laden algal turfs in areas with high rates of sedimentation (Littler and Littler 1983, Steneck 1997, Miller et al. 1999, Adam et al. 2019). Benthic communities following coral mortality are not typically characterised as short productive algal turfs, and while these alternative states have been shown to produce high fish biomass (Robinson et al. 2019), species composition is changed, typically with less biodiversity and trophic complexity (Pratchett et al. 2018, Morais et al. 2020). Even in areas where reefs retain coral dominance, localised anthropogenic pressures can alter fish community structure, and therefore erode functional rates. This has been most notably shown through a reduction to mean fish size over time in response to fishing pressure and the loss of key-stone parrotfish species, resulting in certain areas seeing almost a complete loss of bioerosion (Bellwood et al. 2012, Hoey & Bellwood 2008). Fishing pressure has been shown to be less influential on cropping rates (Robinson et al. 2019).

Predicting how grazing fish communities will provide functions in the future is confounded by habitat changes. Coral dominated systems are also showing long-term gradual benthic shifts in response to climatic impacts. Around the world, fast-growing branching corals, especially within the family Acroporidae, are decreasing and being replaced by more thermo-tolerant genera (Adjeroud et al. 2008, Edmunds 2018). Corals which are more thermally tolerant are typically less structurally complex, providing fewer refugia for certain species (Li et al 2011, Roff et al. 2014). Furthermore, there is even evidence of coral predating parrotfish actively

avoiding feeding from *Montipora* substrates, a resilient coral genus that is becoming more common in response to climate change (Hoey & Bellwood 2008). The authors argue that one reason for this could be the concave nature of the surface, preventing parrotfish from taking substantial bites, something that could be applicable to a range of parrotfish species.

Aims and thesis outline

In this study I draw upon current research that calculates functional rates using bite metrics and apply it to novel data types, to explore to what extent top-down or bottom-up drivers affect functional rates performed by grazing fishes across space and time. This is the first study of its kind to model functional rates through time to see whether ecosystem functions performed by grazing fishes are being eroded, and explore temporal connections between shifts in habitat, community structure and anthropogenic pressures.

The second half of the thesis focuses on how bleaching events alter the feeding landscape for grazing fishes, and what changes to community features may mean for grazing fishes. I directly link specific habitat features; such as community composition, standing mass and sediment loads; to the ability of establishing turf communities to provide nutritious feeding sites for fishes. This begins to bridge our gap in knowledge of exactly how benthic changes drive changes to fish community composition, and therefore rates of functioning, following large scale climatic disturbances such as coral bleaching. I apply metabarcoding techniques to gain high taxonomic level detail on algal and microbial communities, and compare these changes through time to build some definition on colonisation processes, since such data is currently lacking.

Specifically, my thesis addresses the following overarching questions:

- How do ecosystem functions performed by parrotfish vary temporally, and can drivers to change be identified?
- Do spatial drivers to ecosystem functions for herbivorous reef fish vary by functional group?

- How do establishing turf communities on recently bleached corals change through time?
- Do recently established turf communities provide attractive and nutritious feeding sites for grazing fishes?

1. Temporal patterns of parrotfish functions in French Polynesia during habitat disturbance

Abstract

Grazing parrotfish primarily provide two important ecosystem functions through the act of feeding; scraping of algal turfs and bioerosion of dead reef matrix. These functions are spatially influenced by habitat condition and human pressures. To date, differences in scraping and bioerosion rates on reefs have mainly been assessed on spatial scales, while the temporal response of these functions to large scale disturbances has not been explored in detail. Functional rates were calculated and applied to a fifteen-year Underwater Visual Census (UVC) time series in French Polynesia spanning a period of large-scale benthic disturbance and recovery. Biomass of parrotfish increased in response to crown of thorns and cyclone disturbance. Scraping and bioerosion responded differently through the time series, with scraping being the first function to respond increasing alongside biomass, while there was a lag effect until peak bioerosion rates in the following years. Scraping was tied closely to abundance, whereas bioerosion was primarily driven by fish size. Scraping shows greater functional complementarity whereas bioerosion is driven by key species. Though estimated fishing pressure increased within the time period, and benthic communities of coral dominated periods changed, all functions were similar in recovered communities to pre-disturbed levels. While this may suggest long-term resilience in ecosystem functioning provided by parrotfish, a significant reduction to mean fish size was observed in recovered communities in comparison to pre-disturbance. Furthermore, mean counts of parrotfish nearly tripled in response to the disturbance period in 2006, however mean counts only increased by 1.5 times in response to the bleaching in 2019. Parrotfish are likely resource limited and driven by an increase in available substrate. High coral cover therefore limits parrotfish biomass and the provision of functions, regardless of coral community composition.

1.1 Introduction

Coral reefs are the most diverse marine ecosystem, but they are facing increasing threats from both global and local disturbances, which are increasing both in severity and frequency (Hughes et al. 2017). Impacts to coral reefs that are linked to climate are generally associated with mass mortality of corals, which results in a drastic loss of the three-dimensional structure of the habitat (Adjeroud et al. 2009, Magel et al. 2019) Alternatively, reefs may change more progressively and these changes are associated with a reshuffling of benthic communities toward a dominance of species that are either more resilient to impacts or are fast growing, opportunistic species (Edmunds 2017). Reefs tend to respond to disturbance in a relatively consistent way; from structurally complex multitrophic assemblages to flatter bare or algal dominated habitats (Pratchett et al. 2018, Morais et al. 2020). Millions of people worldwide rely on coral reefs for food, income and wellbeing (Moberg & Folke 1999, Cinner 2014, Woodhead et al. 2019), therefore the capacity of reefs to still maintain their functioning and services while they are subjected to escalating threats is a major concern. However, most of the existing research on the effect of climate-induced habitat loss focuses on changes in community composition (Pratchett et al. 2008, Lamy et al. 2015), while the long-term effects on ecosystem functions still remain to be determined.

Grazing behaviour by parrotfishes that primarily target the epilithic algal matrix is associated with two key ecosystem functions on coral reefs: i) the clearing of substratum through scraping and ii) the erosion of reef substrates and generation of sediments (Bellwood & Choat 1990, Bellwood 1996). Scraping is thought to be a key contributor to ecosystem recovery following a disturbance, as the clearing of substrate facilitates coral settlement and growth (Bellwood 2004). Bioerosion is not only important for contributing to large-scale sediment regimes that affect island formation (Perry et al. 2015, Morgan & Kench 2016), but it also affects localised habitat characteristics such as reef growth and complexity (Bozec et al. 2015, Glynn & Manzello 2015, Perry et al. 2018). Scraping pressure is commonly defined as the surface area cleared per bite (Hoey & Bellwood 2008, Robinson et al. 2019), whereas bioerosion is defined

as mass of carbonate reef material removed per year (Lange 2020, Yarlett et al 2020). These functions vary in that scraping is predominantly associated with clearing the surface of the substrate only, whereas bioerosion removes portions of the carbonate framework itself. The quantification of both scraping and bioerosion is dependent upon the bite size and bite rates of individuals. Each of these metrics vary by both species and size of the fish, with higher bite rates associated with smaller fish (Lange et al. 2020), whereas area and volume increase exponentially with size (Lokrantz et al. 2008). Therefore, compositional changes of parrotfish assemblages; considering both species present, size and abundance may severely affect these functions.

Parrotfish have been shown to increase in biomass following large-scale climate associated disturbances (Han et al. 2016, Graham et al. 2020), which is largely attributed to the increase in foraging space as a result of a conversion from coral dominance to grazeable turf algae (Pratchett et al. 2008), and increased food quality enhancing growth rates (Taylor et al. 2020). This increase in biomass of parrotfishes following disturbances is often assumed to relate to increases in reef processes such as bioerosion and scraping, however, biomass alone is not always a good indicator for functions performed by parrotfish. For example, on the Great Barrier Reef, high biomass of parrotfish can be associated with large bodied bioeroders, while scraping is maximised where abundance is higher (Hoey and Bellwood, 2008). Fine, patch scale habitat characteristics have also been shown to affect the spatial patterning of functioning performed by parrotfish. This is logical in that different species have different requirements through resource partitioning and are not typically distributed evenly across all reef habitats (Yarlett et al. 2020). Another habitat feature that has been found to affect the distribution of fish, and therefore influence functioning is reef complexity (Pratchett et al. 2008, Robinson et al. 2019). However, the contribution of benthic drivers of parrotfish functions may be diminished under fishing pressure (Robinson et al 2019). Fishing typically drives down the mean size of fish (Robinson et al. 2017), and since bioerosion is provided primarily by large bodied fishes, this metric is more sensitive to fishing pressure (Bellwood et al. 2012). However,

scraping rates may be maintained by high densities of smaller individuals (Bellwood et al. 2012, Robinson et al. 2019). Other top-down pressures such as natural predation can alter herbivore fish communities, and these two factors can also interact with one another. An increase to herbivores can be observed through 'prey release' with reduced predation in fished habitats (Boaden & Kingsford 2015), however, many studies have observed little to no change to herbivore communities under varying predation regimes (Rizzari et al. 2014, Casey et al. 2017). This is most likely explained through the hyper-diversity of coral reef food webs, and the likelihood that habitat features may provide a buffering effect (Desbiens et al. 2021).

The use of bite rate metrics to infer ecosystem functioning of parrotfish has primarily been applied to spatial datasets (Hoey & Bellwood 2008, Robinson et al. 2019, Yarlett et al. 2020). Since habitat influences the distribution and abundance of parrotfishes, and many reefs show not only short-term changes in benthic composition in response to disturbances, but also long-term shifts (Adjeroud et al. 2018), an understanding of temporal trends in parrotfish functions is necessary. Given that most anthropogenic impacts, such as climate change and fishing pressure, are increasing with time, there is a concern that many reef processes will not be maintained long term (Graham et al. 2011, Hughes et al. 2017). Therefore, understanding whether these functions are maintained after disturbance will require a temporal perspective and the analysis of a complete disturbance-recovery cycle.

The reefs of Mo'orea, French Polynesia, provide an opportunity to address these questions as there is consistent long-term monitoring of the area which covers a benthic cycle from coral dominance through a severe benthic disturbance and finally back to coral dominance again (Edmunds 2018). In response to these benthic changes, reef fish communities have been shown to change in beta diversity and functional composition (Lamy et al. 2015, Han et al. 2016). The reefs are widely considered resilient, due to the rapid recovery of live coral cover and structural complexity (Carlot et al. 2020). However, through time the coral community composition, have shifted away from *Acropora* species towards an increase in *Pocillopora* and *Montipora* (Adjeroud et al. 2008). Mo'orea currently faces a new cycle of disturbance and recovery.

French Polynesia underwent a severe bleaching event in 2019, with 50% coral mortality on some north shore reefs (Vaughan et al. 2021). The warm period in 2020 also saw further bleaching (pers. obs), supporting the fact that climate associated impacts are becoming more frequent. While data on catches is limited on Mo'orea due to the size of the island with no centralised market, studies on household surveys suggest that artisanal fishing pressure is increasing (Dubois et al. 2019). This could potentially alter the size structure and species composition of the parrotfish community, raising concerns that the capacity of ecosystem functions performed by these fish may be eroded through time. However, since catch data is limited to a few studies using household surveys that does not allow for comparison throughout time, data on fishing pressure was not explicitly modelled in this study. Any effects of fishing pressure are inferred through changes to the size structure of the community.

To understand temporal changes in parrotfish functions in Mo'orea, I ask the following questions: Are parrotfish functions maintained over a decade or more? Are functional rates lower in live coral cover recovered communities in comparison to pre-disturbance communities? How are bioerosion and scraping rates related to parrotfish biomass and how does this change through time? Are functions performed by parrotfish bottom-up driven, and if so by which benthic categories?

1.2 Materials and Methods

1.2.1 Study Area

The study took place on the island of Mo'orea (17°30′S, 149°50′W), located 17 km to the northwest of Tahiti in the Society Archipelago, which is part of the Windward Island group in French Polynesia. The island is roughly triangular with three coastlines that face to the north, southwest and southeast. Northern sites are affected by the northern swell which takes place during the austral summer (November to April), whereas western sites are exposed to the prevailing southwest swell which is of a higher amplitude and occurs throughout the year (Adjeroud et al. 2018).

The total data set spanned fifteen years from 2005 until 2020. This time period begins with reefs in a coral dominated state with high live coral cover in 2005. After the 2006 surveys until 2010 the outer reefs faced successive bouts of outbreaks of the coral predator, crown of thorns sea stars (*Acanthaster spp.*; COTS), followed by Cyclone Oli in February 2010 (Adjeroud et al. 2018). Both of these impacts had much greater affects to outer fore reefs than reefs within the lagoon, where coral cover dropped to 3% (Lamy et al 2016, Adjeroud et al. 2018). However, even with this, rapid recruitment of coral (primarily within the genus *Pocillopora*) resulted in live coral cover on the fore reef returning to pre-disturbance levels by 2016 (Edmunds 2016). This meant that within the data set, there were two periods of coral dominance; the first covering the 2005 and 2006 surveys, and the second spanning the 2018 – 2019 surveys. Due to the huge loss of coral and subsequent rapid recovery, I focussed the study on the outer reefs only.

1.2.2 Fish Benthic Surveys

Three randomly placed transects were conducted per site at approximately 10m depth, with a total of fourteen sites located around the island. Fish surveys consisted of 25m belt surveys with a corridor of 2m, giving a survey area per transect of $50m^2$. Parrotfish were identified to species level and size was estimated to the nearest cm. Benthic coverage was recorded using point intercept transect surveys recording every 50cm for 25m, and summarised as percentage coverage per transect. Corals were recorded to genus level. Grazing substrates consisted of carbonate reef covered in fine turfing algae and were recorded as either rubble fragments or with consolidated reef matrix recorded as pavement. For both fish and benthos, each site had three transects and the surveys took place annually around the full moon during the warm wet season from January to March.

1.2.3 Benthic Trends

Benthic coverage was summarised for each transect, with means calculated across transects per site. Data was grouped for visualisation within a principle component analysis (PCA) to

identify major drivers to benthic trends. Data within the PCA was grouped as pre-disturbance (2005, 2006), disturbance (2007 – 2010; including COTs and cyclone), post-disturbance (2011 – 2015), and recovered (2016 – 2019; live coral cover is either the same or greater than pre-disturbance levels). Mean coral cover was the same in 2016 as in 2006, and continued to increase to 2019. Benthic data was scaled and centred prior to running the PCA. PC1 and PC2 explained 39.6 and 17.5% of variation, respectively. Data collection in the field for fish and benthic transects followed protocols described in Lamy et al. (2015).

1.2.4 Size structure of the parrotfish community

To determine patterns to size structure and density, I calculated the average size and density of parrotfish across transects for each site per year. Annual trends presented were then means between sites within a year. Visually inspecting the data using boxplots, two values were removed for exceptionally high counts (n= 102 per transect) from Afaraeitu in 2007, one for *Scarus psittacus* and one for *Chlorurus spilurus*. Mean fish size through time was calculated as a weighted mean per transect per site per year (length weighted by abundance). Means were then calculated per site per year, with annual trends presented as means across sites within a year. Biomass was calculated using species specific length-weight relationship values from Fishbase (Froese & Pauly 2020). To determine how biomass of parrotfish communities was comprised of various fish sizes and abundance, fish were placed into 10 cm size classes to show how the proportion of fish within those size categories changed annually. All other analyses on fish size were done on size estimates to the nearest centimetre, only using size classes to visualise annual changes to size structure. I also calculated Large Fish Indicator (LFI) values as a measure of the size structure of the community (Robinson et al. 2017, Robinson et al. 2019), which was defined as the length at the 75% quantile of the size distribution of the full data set, giving relative abundance of fish greater than 26 cm. LFI was then compared with scraping and bioerosion rates to see how the size structure of the community varied at different levels of functioning.

1.2.5 Calculating scraping rates

Data used to calculate scraping was based on observations of individual fish collected in Indonesia, the Red Sea and the Great Barrier Reef (Bellwood et al. 2003, Hoey & Bellwood 2008, Hoey et al. 2016). Individual fish of target species were randomly selected and body size (total length) estimated to the nearest cm. After an acclimation period of 30 s the fish was followed for a minimum of 3 minutes during which the number of bites and type of feeding substrate was recorded. Scar size was measured in a separate data set whereby a diver followed a fish, estimated its size, and when a visible feeding scar was seen this was measured by length and width. Scraping was defined as area scraped for a fish of specific size multiplied by bite rate for that species at that size which gave a value of area scraped per total of all transects per site per minute (cm^2 /50m/min). Data on bites per minute were available for all species. However, due to a less extensive data set for scar sizes, an estimate for scar area increase with size was calculated and was the same across all species. For the species with data available this value was found to increase with size similarly for all species (Robinson et al. 2019), with similar trends found in other studies (Lange et al. 2020).

1.2.6 Calculating bioerosion rates

Bioerosion rates were derived from a variety of existing published studies quantifying bite rates, scar volume and proportion of bites leaving scars (Ong & Holland 2010, Bellwood & Choat 1990, Lokrantz et al. 2008, Hoey et al. 2016, Yarlett et al. 2017, Lange et al. 2020). Where data were not available for specific species, values were assigned for the closest relative (Choat et al. 2012). Studies were conducted on SCUBA or snorkelling. For bites leaving scars, this was derived from the number of visible scars left within a successive bout of feeding (foray). Scar volume was either estimated as scar area (length x width) multiplied by depth (mm^2, Bellwood & Choat 1990, Lokrantz et al. 2008, Hoey et al. 2016, Yarlett et al. 2017, Lange et al. 2020), or through direct volume measurement through wax casts (Ong & Holland

2010, Bellwood 1995). While the first method may lend itself to overestimation, no significant differences have been found in estimations from the two methods (Lange et al. 2020). Percentage of day spent feeding was estimated separately for large species of parrotfish (*Cetoscarus, C. microrhinos*) and for smaller parrotfish (*C. spilurus* and *scarus spp.*, Bellwood et al. 1995). Since life phase was not recorded in the UVC monitoring for Mo'orea, blanket values were assigned for terminal phase parrotfish. While Lange et al. (2020) found that life phase explained little variation for bite rates when compared to body size, this assumption does assume larger bite volume than for the presence of initial phase individuals, likely giving an overestimation and does not consider the effect of community composition ratio of males to females.

Bioerosion rates were visualised using boxplots and an observation of a high count of *C. spilurus* was excluded for the bioerosion analysis (n= 52). Two additional outliers were detected; however, these were due to the presence of large *C. microrhinos* (size > 30cm) that, due to allometric relationships between body size and function for this species, meant that they erode high levels of reef substrate. Since the presence of these species is important, we ran models with and without *C. microrhinos* values to see how the presence/absence of this species affected trends.

1.2.7 Comparing periods of coral dominance

To compare between the pre-disturbance coral dominated community (2015 and 2016) and the final two years of the following recovered coral dominated community (2018 and 2019), we grouped data and calculated means across all years for live coral cover, biomass, scraping, bioerosion, fish abundance and size. Data was summarised to the transect level with means calculated for each site for each year, then calculating means across sites and years. Data for all metrics excluding live coral cover was right skewed, therefore I used either log10 or square root transformations to account for normality assumptions. These were tested for using the Shapiro-Wilk test. All metrics excluding fish size met normality assumptions after transformations. T-

tests were used on normalised data, with Mann-Whitney U tests being on the untransformed mean size data.

1.2.8 Modelling functions with benthic features

To explore relationships between benthic drivers and parrotfish functioning, I aggregated the data to three levels of scale: i) the coarsest measure whereby benthic categories were grouped simply as live coral cover, available substrate (rubble and pavement combined) and macroalgae, and ii) the finest scale where we calculated annual means for each of the dominant benthic categories surveyed (individual coral genera, macroalgal type, bare substrate type). By taking such an approach, this would allow me to explore relationships between functions with changes to individual substrate types, but also allow me to see how interactions of changes across multiple substrate types may factor together to influence functioning. Once the data was aggregated, I applied generalised linear mixed effects models (GLMM) to both the coarse and fine scale data aggregations.

At the coarse level, data was grouped as either live coral, available substrate (rubble and pavement) or macroalgae (all types) and run as a generalised linear mixed-effects model, with year and site included as random effects. Exposure; whether the site was situated on the north, west or east of the island; was included as a model term to see whether this had an effect, and was used as a rough proxy for structural complexity (Carlot et al. 2020) and exposure/island hydrodynamics. I checked for collinearity between benthic variables, with live coral cover and available substrate being significantly negatively correlated ($t= -29.30$, $df= 191$, $p<0.01$, method = Pearson), therefore live coral cover and available substrate were run separately.

For the fine scale model, dominant coral genera within the data set were analysed individually (*Pocillopora*, *Acropora*, *Monitpora* and *Porites*), with all other coral genera grouped together into “other corals”. *Halimeda* was kept separate within the macroalgae group as it was dominant on the outer reefs, with others kept together as general ‘macroalgae’. Data was scaled and centred and then both forwards and backwards stepwise AIC was run to identify key

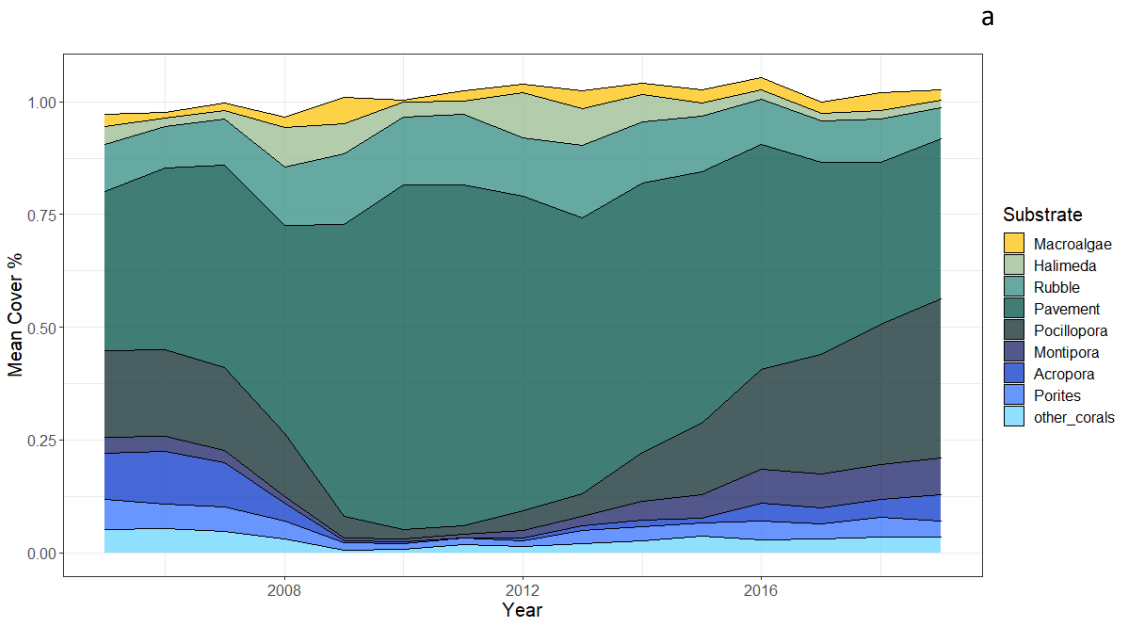
variables. These were then run in a generalised linear mixed-effects model, with year and site included as random effects and exposure as a fixed effect. I checked for collinearity, with pavement and *Pocillopora* being highly negatively correlated ($t = -16.9$, $df = 191$, $p = <0.05$), so these were run separately to avoid variance inflation. Following separating variables, VIF values were < 2 for all models. For both the coarse and fine scale models, year was only included as a random effect and not a fixed factor. Likelihood ratio tests were done by comparing the full models with null models using ANOVA, to look at the strength of these individual factors on the overall model. All analyses of benthic data and comparisons with functions was applied with data from 2005 until 2019, to assess one full cycle of coral dominance, disturbance and recovery. Data for 2020 was excluded due to coral bleaching in 2019, and therefore the start of a new cycle of disturbance.

1.3 Results

The benthic communities of the outer reefs of Mo'orea went through continuous changes throughout the fifteen-year data set (Figure 1.1a, 1.1b). The time series starts with benthic communities characterized by the dominance of corals and a greater cover of *Acropora* and *Porites* (Figure 1.1b). Live coral cover decreased and proportion of pavement continuously increased from the first year of data (2005) following the initial COTs outbreak in 2006 until eventually this trend began to reverse in 2011, only two years after cyclone Oli (Figure 1a). An increase in pavement was the primary driver of changes, however rubble was more prominent during the post disturbance period of 2011 to 2015 (Figure 1.1b). Pavement and rubble coverage gradually reduced from 2011 and 2013 respectively as coral cover recovered. By 2016 there was no significant difference between mean live coral cover compared to that of 2006 ($t= -2.0$, $df= 15.72$, $p=0.07$), indicating that from this time the community could be considered 'recovered' to a coral dominated state. Coral cover continued to rise, so that by the final two years of coral dominance prior to bleaching in 2019, live coral cover was higher than in pre-disturbance years ($t= 2.38$, $df= 36.36$, $p= 0.02$). Mean coral cover in the first period of coral dominance (2005 & 2006) was 41.32%, ± 0.076 se, with a mean live coral cover in the second

phase of 44.69% ± 0.079 se. During this second period of coral dominance, however, the communities were comprised of greater proportion of *Pocillopora* and *Monitpora* than the previous community, with *Pocillopora* gradually becoming more common as the period of coral dominance progressed (Figure 1.1b). *Halimeda* and macroalgae were more prominent during recovery years from 2011 – 2015 (Figure 1.1b).

Principal component analysis of benthic types revealed two key trends of benthic change throughout the time series (Figure 1.1b). The primary trend explained by PC1 was the loss of absolute coral cover and a shift towards pavement and rubble which occurred throughout the disturbance period and characterised some post disturbance also. The second trend shown by PC2 is a shift in coral communities between the pre-disturbance and those of recovered communities. Both periods are characterised by coral dominance, however with a loss of *Acropora* and other corals from pre-disturbance and a shift towards a greater abundance of *Pocillopora* and *Montipora* in recovered. Central values around 0 for PC2 are reflective of habitats with greater available substrate, with extreme values of PC2 relating to coral dominance.



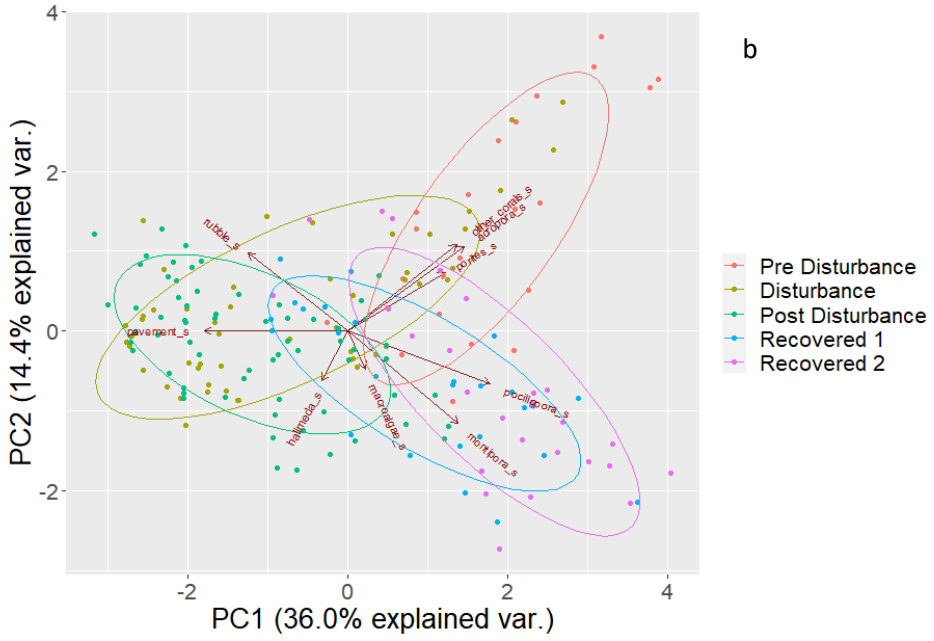
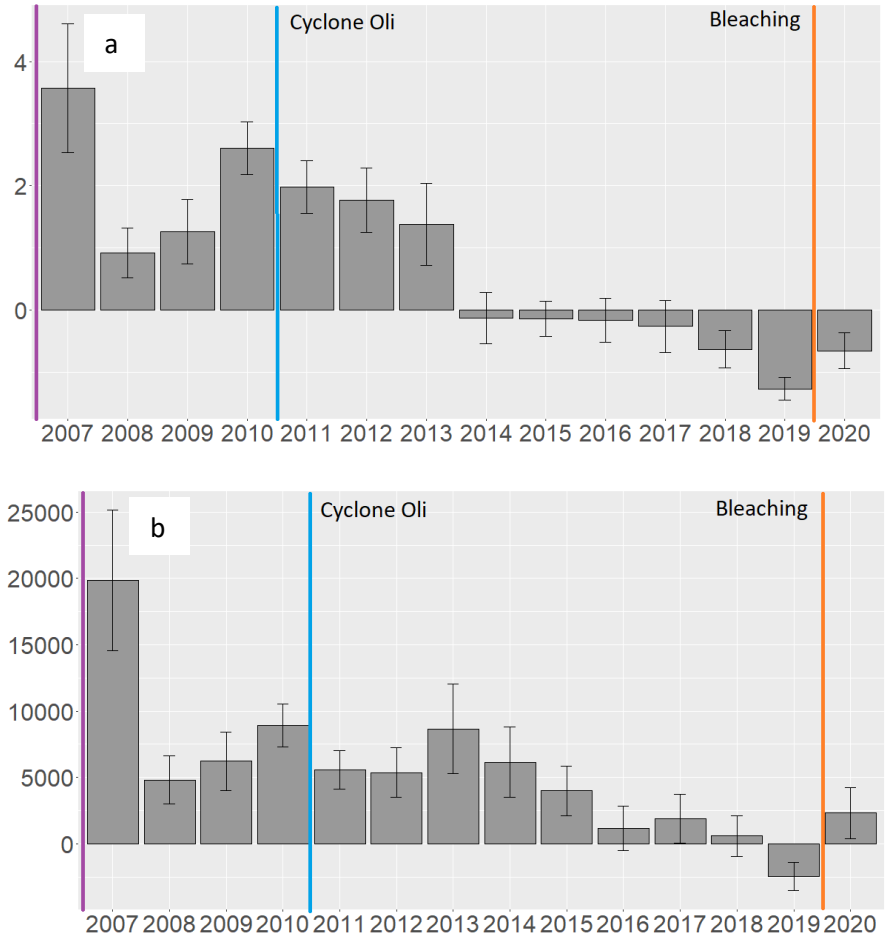


Figure 1.1a. Mean coverage of benthic types throughout the time series. Data was summed per transect and then means calculated per site. b) Principal component analysis of benthic communities throughout the time series (Pre-Disturbance 2005 – 2006, Disturbance 2007 – 2010, Post Disturbance 2011 - 2015. The period of recovered coral communities was split into two categories (2016, 2017 = recovered 1; 2018, 2019 = recovered 2) to show how these coral dominant communities are shifting through time. Benthic data was scaled and centred prior to analysis.

Biomass of parrotfish and both metrics of functions increased following the initial COTS outbreak in 2006 (Figure 1.2). This increase in biomass was driven by a large increase in the density of parrotfish in 2007, which was the highest in comparison to any other year within the data set (Figure 1.3a). Mean size of fish increased gradually throughout the years with COTs (2006 – 2010), and continued following Cyclone Oli in 2010 for a further two years (Figure 1.3b). The increase in biomass in 2007 was mainly within the size category of small fishes (10 – 20 cm), however by 2010 the dominant size class was medium fishes (20 – 30 cm, Figure 3c). Biomass and scraping declined following Cyclone Oli, as did mean counts, however mean fish size and bioerosion rates continued to increase the following year (Figure 1.2, Figure 1.3b). Biomass and scraping rates were both highest in 2007, which was the year which saw the highest counts of fish (Figure 1.2, Figure 1.3a), however bioerosion rates were highest in 2011, the year with one of the highest mean sizes (Figure 1.2, Figure 1.3c).

There was no significant difference in scraping rates (t= 0.62, df=37.6, p= 0.54), or for bioerosion (t= -1.44, df= 47.62 p= 0.16) or biomass (t= -1.75, df= 47.93, p= 0.09), showing similar levels of functioning between the two time periods of coral dominance. There was no significant difference between mean counts of fish between the two periods of coral dominance (t= 0.10, df= 43.64, p= 0.92), however mean fish size was higher in pre-disturbance years than in recovered years (Mann Whitney, W= 415, p= 0.002), dropping from a mean of 21.62 cm in pre-disturbance years to 18.28 cm in recovered years. Following the bleaching in 2019, counts of fish indicated a similar pattern of response to disturbance to biomass, by increasing the following year. However, the response of the community through increase in abundance was less following the 2019 bleaching in comparison to the first year of COTs in 2006. Mean density for 2006 was 13.65 fish per 50m, which increased by 2.8 times to 37.77 fish per 50m in 2007. Mean counts were 9.81 per 50m in 2019 which increased by 1.5 times to 14.54 per 50m in 2020.



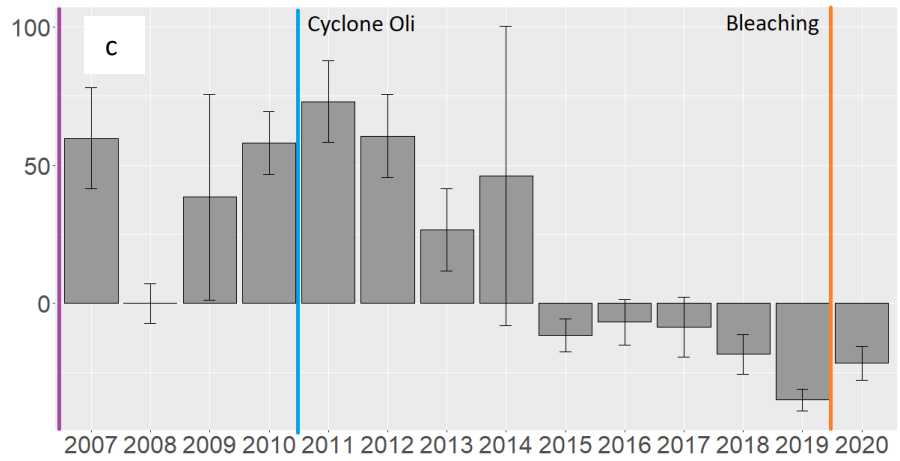
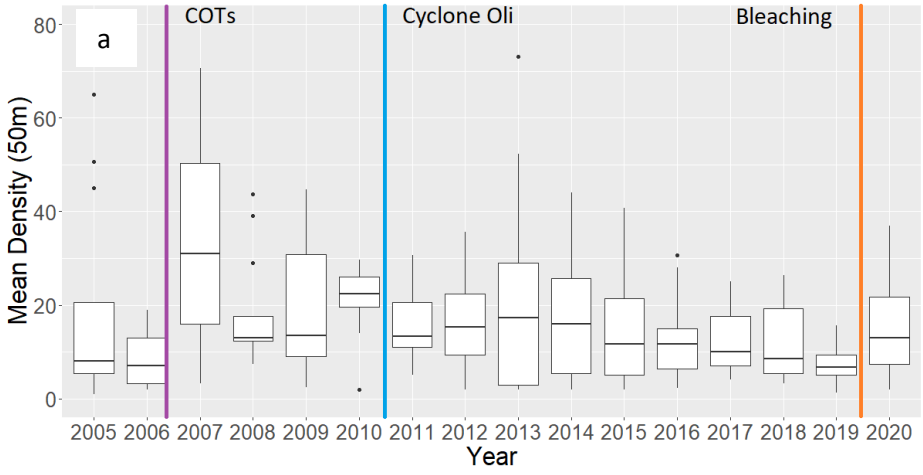


Figure 1.2 Mean values for a) biomass (kg/50m), b) scraping (cm^2 /min/50m), and c) bioerosion (kg/50m/year) for the time periods within the data set. Data were totalled per transect per site per year, then means calculated (per 50m). Values are mean centered on the mean of pre-disturbance levels (2005 – 2006) to act as a baseline, with error bars showing standard error.



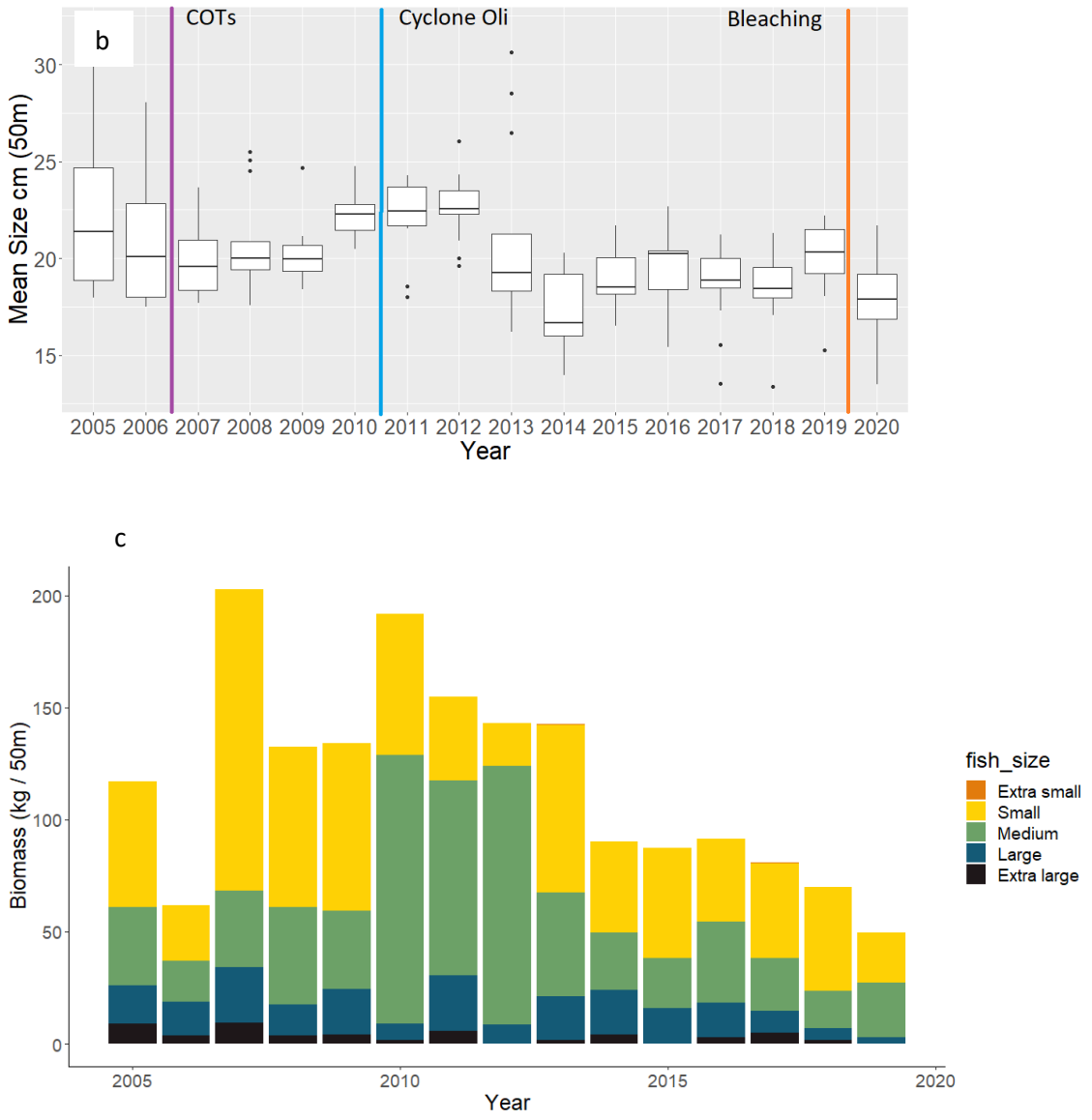


Figure 1.3. Trends in mean abundance a) and size b) of fish throughout the time series. Data was summarised to per transect per year per site and then means calculated. Distribution of biomass by fish size annually d). Extra small (0 – 10cm), small (11 – 20cm), medium (21 – 30cm), large (31 – 40cm) and Extra-large (41 cm +).

Size structure of the community had varying effects on the two functional metrics. Scraping rates were consistently higher for communities where they were mostly comprised of smaller fishes (25% of fish were large bodied fish ≥26cm), as opposed to large bodied communities (where 75% of individuals were large bodied fish ≥26cm Figure 1.4a). However, for bioerosion, communities of smaller bodied fish only provided a greater contribution towards ecosystem functioning at the lower end of the scale, rapidly becoming overtaken at the higher erosion levels by communities of large bodied fish (Figure 1.4b).

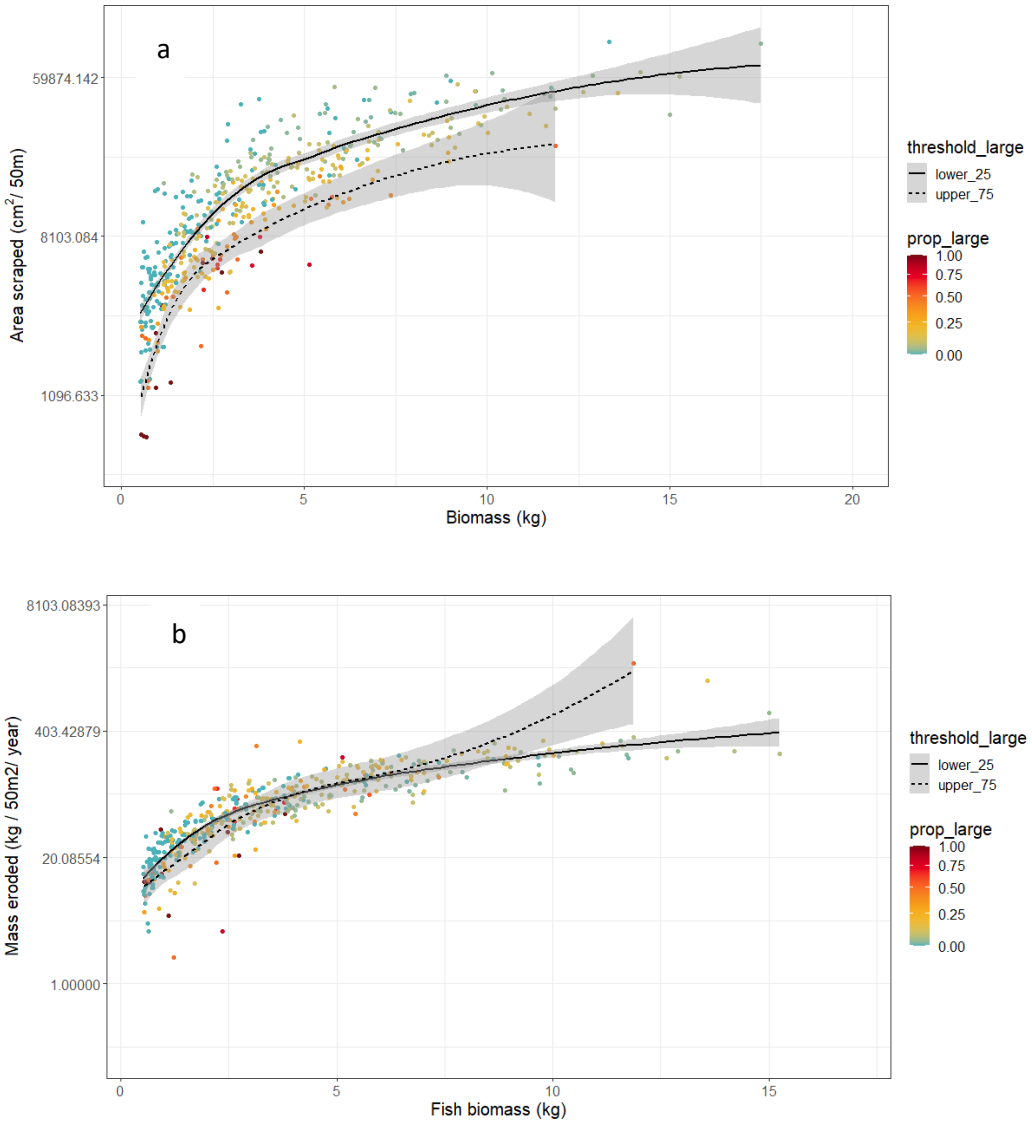


Figure 1.4. Contribution to scraping (a) and bioerosion (b) rates by assemblage size structure for a given biomass, coloured by the Large Fish Indicator. Solid line represents 25% of the community is large bodied fish (≥26cm) and dashed line represents 75% of the community is large bodied.

When the model was run at the coarse level with available substrate, this was associated with increased scraping rates (X^2 = 20.15 p< 0.01) by 0.34 cm^2 50m/ min ± 0.07. Macroalgae (X^2 = 3.60 p=0.06) and exposure (X^2 = 3.60 p=0.84) had no effect. When the coarse model was run with live coral cover, this was associated with reduced scraping rates (X^2 = 18.13 p< 0.01) by 0.33 cm^2 $25m^2$ min^{-1} ± 0.07. Macroalgae was associated with reducing scraping rates by 0.27 cm^2 $25m^2$ min^{-1} ± 0.06 (X^2 = 16.68 p< 0.01). Exposure had no effect (X^2 = 0.40 p=0.82). When the coarse model was run on bioerosion rates including *C. microrhinos* and available substrate, available substrate was associated with an increase bioerosion rates (X^2 = 22.23 p< 0.01) by

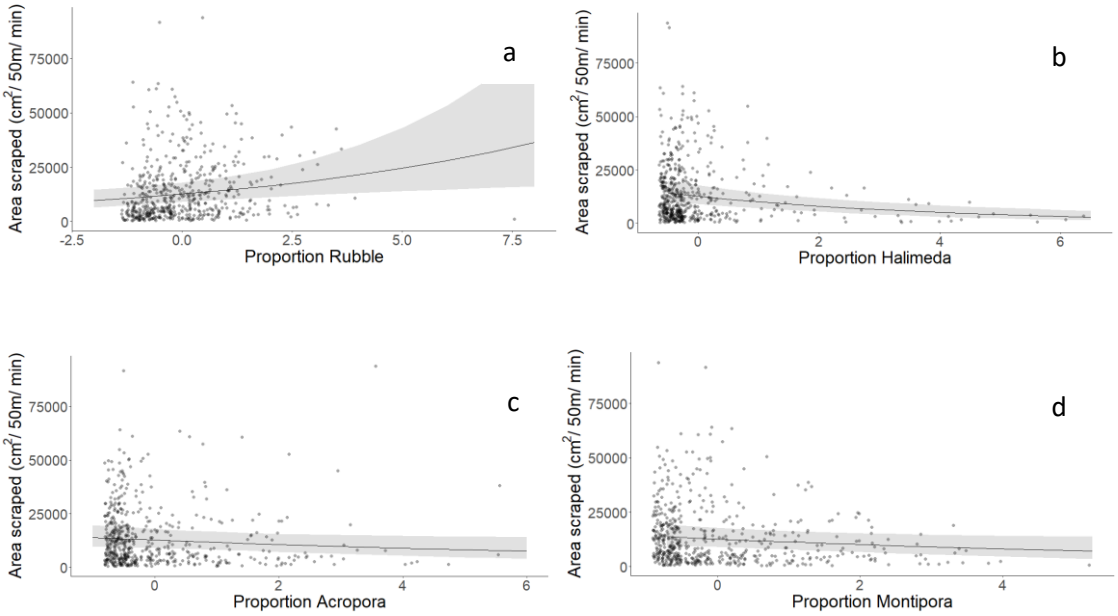
0.39 kg per 50m per year ± 0.07. Macroalgae also was associated with reduced bioerosion rates (X^2 = 4.10 p< 0.01) by 0.14 kg per 50m^2 per year ± 0.07. Exposure had no effect (X^2 = 0.87 p=0.65). When the coarse model with *C. microrhinos* and live coral cover was run, coral cover was associated with reduced bioerosion rates (X^2 = 20.33 p< 0.01) by 0.38 kg per 50m per year ± 0.07, as was macroalgae (X^2 = 20.07 p< 0.01) by 0.29 kg per 50m per year ± 0.06. Exposure had no effect (X^2 = 0.86 p=0.65). When *C. microrhinos* was excluded from the data set and the model was run with available substrate, available substrate was associated with an increase in bioerosion rates (X^2 = 23.33 p< 0.01) by 0.38 kg per 50m per year ± 0.003, however macroalgae had no effect (X^2 = 3.35 p= 0.06). When the data with *C. mirorhinos* was run at the coarse level with live coral cover, live coral cover was associated with reduced bioerosion rates (X^2 = 22.22 p< 0.01) by 0.38 kg per 50m per year ± 0.003 and macroalgae was also associated with reduced bioerosion rates (X^2 = 18.21 p< 0.01) by 0.27 kg per 50m per year ± 0.003. Partial plots for coarse models included as supplementary material in the Appendix.

Stepwise AIC selection identified that *Halimeda* and *Acropora* was negatively associated with all functions with all data sets (with and without *C. microrhinos*). *Porites* and other corals were not selected as influential for any models. For scraping, *Montipora, Halimeda, Acropora,* macroalgae, rubble and exposure were selected when run with either pavement or *pocillopora*, so one final model was run. For bioerosion with *C. microrhinos* included, *Pocillopora*, *Montipora, Halimeda* and macroalgae were selected when run with *Pocillopora*. Pavement was not selected, however the other variables remained the same, so one final model was run including *Pocillopora*. When bioerosion was run without *C. microrhinos*, *Montipora*, *Halimeda, Acropora,* macroalgae and exposure were included in both, however *Pocillopora* was dropped and pavement was retained, hence one final model was run including pavement and the other associated variables.

For scraping, *Montipora* had a negative effect (X^2 = 4.15 p=0.04) associated with reduced scraping rates by 0.16 cm^2 50m min ± 0.08. *Halimeda* also had a negative effect on scraping (4.15 p=0.04) reducing rates by 0.28 cm^2 50m min ± 0.07. Scraping rates were reduced with

greater coverage of acropora (X^2 = 10.04 p<0.01) by 0.22 cm^2 50m min ± 0.07. Scraping rates increased with rubble coverage (X^2 = 4.27 p=0.04) by 0.14 cm^2 50m min ± 0.07. Exposure (X^2 = 2.38 p=0.30) and macroalgae (X^2 = 0.70 p=0.40) were not significant (Figure 1.5).

For bioerosion with *C. microrhinos* included, *Pocillopora* was associated with reducing bioerosion rates (X^2 = 5.17 p=0.02) by 0.18 kg per 50m per year ± 0.08. *Acropora* was associated with reduced bioeorsion rates (X^2 = 11.74 p<0.01) by 0.24 kg per 50m per year ± 0.07. Bioerosion rates reduced with an increase in macroalgae (X^2 = 6.95 p<0.01) by 0.14 kg per 50m per year ± 0.05, as with *Halimeda* (X^2 = 15.29 p<0.01) by 0.28 kg per 50m per year ± 0.07. For bioerosion without *C. microrhinos* included, only *Halimeda* (X^2 = 10.61 p< 0.01) and *Acropora* (X^2 = 7.41 p< 0.01) had significant effects on bioerosion rates, associated with reduced rates by 0.25 kg per 50m per year ± 0.08 and 0.20 kg per 50m per year ± 0.08 respectively. Exposure (X^2 = 1.40 p=0.50), pavement (X^2 = 2.05 p=0.15) and macroalgae (X^2 = 0.47 p=0.49) were not significant (Figure 1.5).



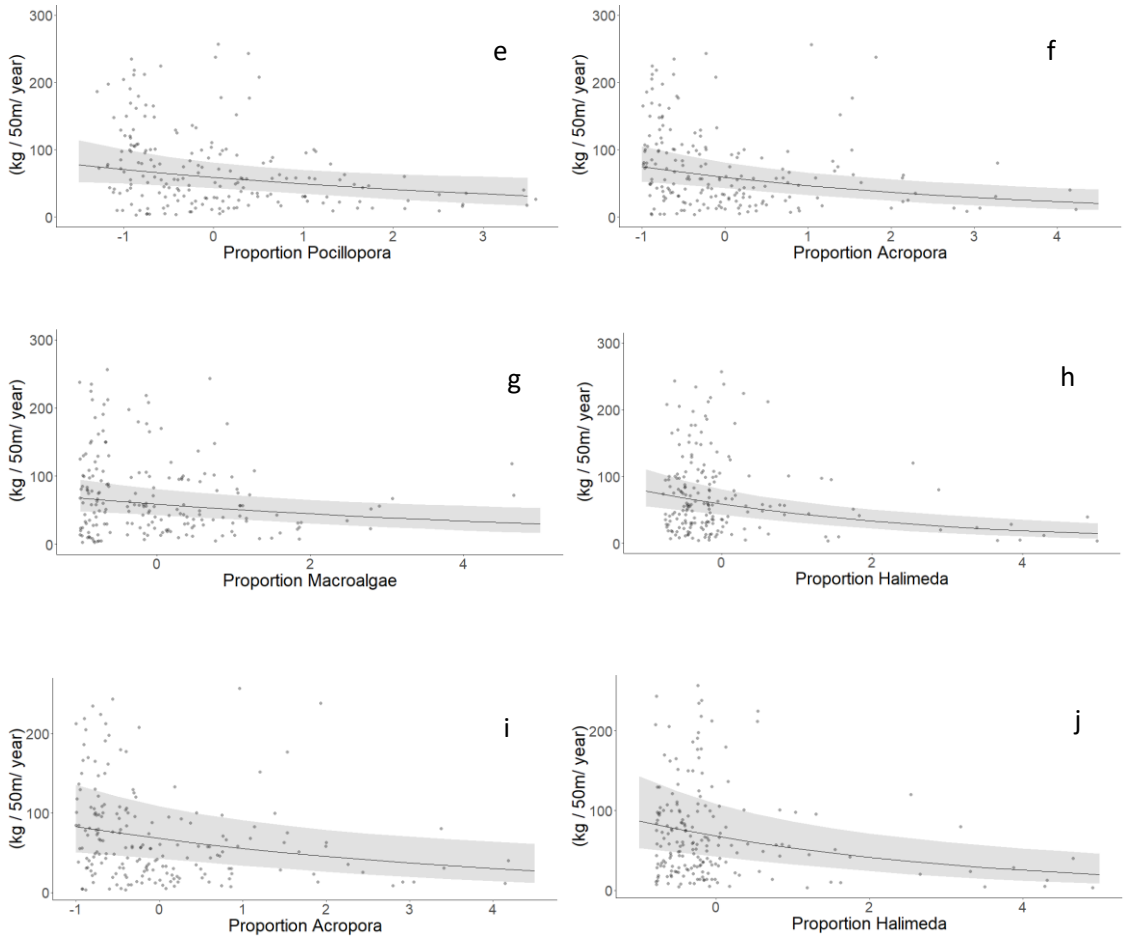


Figure 1.5. Partial plots of predicted functional rates against main benthic drivers on functions. Proportions of benthic cover are the percentage cover for the transect. a) – d) scraping rates, e) – h) bioerosion rates including *C. microrhinos* with y axis limited to focus on main trends and i) and j) are bioerosion rates excluding *C. microrhinos*.

1.4 Discussion

The benthic communities of the outer reefs of Mo'orea went through continuous changes throughout the fifteen-year data set. The primary trends were loss of live coral cover in response to the COTs outbreak and then Cyclone Oli. Live coral was mostly converted to pavement, with some increases in rubble also. Coral dominance had returned by 2016, however these communities were distinguished from the previous coral dominated community by less *Acropora*, *Porites* and other corals, to a greater proportion of *Pocillopora* and *Monitpora*. All metrics of the parrotfish community (biomass, scraping and bioerosion rates) increased in response to the start of the disturbance period in the time series. This was driven by a big

increase in the density of fish on the reef following the first year of COTs; primarily within the small size category; which translated to the highest year for scraping rates. Bioerosion rates showed a lag effect that mirrored those of fish size, with erosion rates peaking four years later. This shows that the two metrics of parrotfish functioning responded differently to disturbances, with scraping being the first ecosystem process to respond and is driven by rapid recruitment to the population. This increase in density of small, rapidly biting fishes is likely the main mechanism behind this. Bioerosion rates peak later as those fishes grow and enhance the larger size spectrum of the community. Communities comprised of proportionally smaller fishes showed higher scraping rates at all levels of biomass, however for bioerosion the highest rates were found when the community was comprised of large bodied individuals, and especially with those of *C. microrhinos*. The transitions between live coral cover and available substrate were supported as a primary driver of parrotfish functioning with both the GLMM models at the coarse scale. Fine scale GLMM models did not find pavement as a significant driver for either function, and instead found that an increase in dominant coral types were more influential, as well as the presence of *Halimeda*.

Our study supports previous research linking changes to parrotfish abundance and functioning with amount of available grazing space, especially with loss of live coral cover following large scale disturbances (Russ et al. 2015, Emslie & Pratchett 2018, Questel & Russ 2018). However, this was only clearly shown when benthic data were aggregated at the coarse scale or when benthic trends were summarised into principle components. Comparing functions with data kept at the fine scale found limited relationships with an increase in substrates associated with algal turfs, except for the association between scraping and rubble. Associations between rubble areas and high scraping rates was also found in Yarlett et al. (2020) which mapped the spatial distribution of scraping versus bioerosion at the island scale. Instead, at the fine scale, trends of parrotfish functions were more related to increases in live coral cover and dominant coral types. *Montipora* and *Acropora* had negative effects on both scraping and bioerosion, with an increase in *Pocillopora* coverage also being associated with decreases in bioerosion rates. As

Pocillopora and Montipora are becoming more prominent in recent periods of coral dominance, this negative association; if driven by the corals themselves, rather than simply an increase to live coral cover; has implications for future functioning. Hoey & Bellwood (2008) found that *B. muricatum* avoided feeding from *Montipora* substrates, which they argue could be due to its flat/concave surfaces making bites challenging. Brandl & Bellwood (2016) show how microtopographic variations can influence grazing patterns of certain fish, meaning that if these corals become grazing space they may exclude certain species, especially as skeletons of branching corals such as A*cropora* or *Pocillopora* regularly beak down into rubble beds following mortality. Shifts in coral types and lifeforms affect reef topography and microhabitat variation, which has been shown to influence niche size for different parrotfish (Brandl & Bellwood 2014). However, these trends with coral coverage may simply reflect the trend that as coral cover goes up, grazing space goes down, and these three coral types are the most dominant throughout the time series. The presence of macroalgae has been shown to negatively affect herbivorous activity (Robinson et al. 2019), however in this study only *Halimeda* (Caulerpaceae) had a negative effect on both scraping and bioerosion. This is a calcified green alga with hard segmented thalli (Adey & Loveland 2011) and was mostly associated with transitional communities immediately post disturbance.

Lack of structural complexity has been shown to be an influencing factor on parrotfish and herbivore distributions (Graham et al. 2006, Nash et al. 2016), however there were limited trends to suggest that this was the case in this study. While structural complexity was not directly measured within this study, patterns or complexity recovery with exposure categories was not found to be influential, and mean size of fish continued to increase following the impact of the cyclone which flattened the reef structure. However, Graham et al. (2007) found lag effects between loss of live coral cover and changes to size structure within fish communities.

Previous work comparing trends in biomass of parrotfish without discussing size spectra has been shown to overlook changes in functional rates (Bonaldo & Bellwood 2008, Shantz et al.

2020.) This study supports the idea that assuming functioning by biomass alone can miss variations driven by the abundance and size structure of the community (Robinson et al. 2019). Density of individuals is the primary metric driving scraping rates in this study, with one large *S. psittacus* (40 cm) scraping 103 cm^2 per min, whereas three small *S. psittacus* (12 cm) will scrape 2528 cm^2 per min, which is twenty-four times that of a single large fish. The influence in counts holds true for *C. spilurus*, where one large individual will scrape 290 cm^2, whereas three small *C. spilurus* scrape 1634 cm^2, which is five times that of a large individual. There are conflicting general trends that bite rate decreases with fish size, but area scraped increases with size. Overall, scraping rates per individual increase with fish size, however the rate at which this increases and whether this is maintained at larger sizes varies between species. For example, for *S. oviceps* the relationship between size and area scraped is an exponential curve, indicating that area scraped increases rapidly for an individual at a larger size, where the increase to bite area has a greater influence than loss of bite rate. However, for *S. psittacus* the trend is more like that of a flattened parabola, with area scraped dropping off in larger sizes due to a reduction in bite rates as fish sizes reach a maximum which is not effectively countered for by an increase to bite area, and with limited relative increase between the minimum and the maximum values for scraping. This means that increases in counts of *S. psittacus* will have a much greater impact on scraping rates than an increase to size, whereas an increase in counts will have less of an effect on scraping performed by *S. oviceps*. This is demonstrated by the fact that a single large (31-40 cm) *S. oviceps* will scrape 2496 cm^2 per min, however three small (11-20 cm) *S. oviceps* will only scrape 1878 cm^2 per min, which is 1.3 times less than one large individual.

For bioerosion, our study supports previous research in that this ecosystem process is closely linked to body size, so that larger individuals will contribute more than a similar weight of fish but comprised of a smaller size category (Ong & Holland 2010). For comparison for interspecific variation, one large (40cm) *C. spilurus* will erode 20kg per year, whereas three small (12cm) *C. spilurus* will erode only 2.5kg, with a single large individual eroding eight

times more than that of multiple small individuals. In terms of regularity across the fifteen years in this study, bioerosion is currently primarily performed by *C. spilurus*, which is a similar pattern observed in other regions (Yarlett et al. 2020). However, the presence of *C. microrhinos*, especially individuals larger than 30 cm, rapidly dwarfs any bioerosion rates contributed by *C. spilurus*. *C. microrhinos* is a dominant bioeroder, with one large individual (40cm) eroding 248 kg per year, which is 12 times higher than that of *C. spilurus* of the same size. The high variation in values driving functions indicates the need for greater species resolution across feeding metrics (Lange et al. 2020). While good data was available on bite rates per species and size within this study, a larger data set on scar sizes would add further clarity to trends. For the species that I have, the data suggests that scar area increases similarly with fish size across species, however many studies show high variation in scar metrics (Lokrantz et al. 2008, Yarlett et al. 2018, Lange et al. 2020). The majority of variation is likely due to scar depth (drivers of variation in bioerosion rates) and bite rates (variation in scraping and bioerosion), as Lange et al. (2020) also found similar trends in scar area, yet high variation in bite rate and volume. Bioerosion rates show high levels of variation with size across species (Yarlett et al. 2018, Lange et al. 2020), but this is likely more linked to jaw myology and osteology which allows them to bite deeper, rather than have a wider bite which covers more area.

I found no loss of parrotfish functioning across the span of a fifteen-year data set, and whether a site was protected or not had no effect on functional rates. However, these recent rates of bioerosion are unlikely to be comparable those of historical rates (Bellwood et al. 2012). The presence of *C. microrhinos* decreases in counts and size throughout time, indicating that this species was once in higher numbers, and therefore bioerosion rates would likely have been significantly higher than current levels. The presence of *Cetoscarus spp.* also has a huge impact on erosion rates, with one 40cm individual eroding 176 kg per year; 8.6 times that of *C. spilurus*. However, with just a single observation within the data set, it is not possible to make any assumptions as to whether this species would be more abundant historically. The idea that

modern ecosystems, communities and associated rates of functions are diminished as a result of anthropogenic impacts has been shown in a number of studies (Bellwood et al. 2012, Cramer et al. 2016). The loss of regional historical functioning is most understood with declines in the largest parrotfish species *Bobometopon muricatum*, where the size of these adults mean that they have the ability to remove large chunks of reef substrate per bite, making them prominent bioeroders (Hoey & Bellwood 2008). These fish have historically suffered heavy fishing pressure, resulting in local extinctions (Myser 1999, Donaldson & Dulvy 2004) and resulting in the loss of the majority of bioerosion rates in certain areas (Bellwood et al. 2003). This study suggests that current bioerosion performed by *C. spilurus* is able to withstand current levels of fishing pressure, however *C. microrhinos* is not. These relationships between species, size and abundance supports the idea that bioerosion is typically dominated by key species, whereas scraping is more varied and shows greater functional complimentarity (Yarlett et al. 2020). The response of parrotfish communities to disturbances is varied in the literature (Emslie & Pratchett 2018), with Taylor et al. (2019) arguing that in general parrotfish numbers reach a peak occurrence two years following a disturbance. While counts of fish did peak again three years following Cyclone Oli, this increase was far less than the initial growth in response to the first year of COTs, potentially due to widescale loss of structural complexity following the cyclone (Carlot et al. 2020).

This study highlights the importance of two stages of parrotfish response to disturbances. The initial rapid recruitment of individuals to the adult population, and then the persistence and growth of individuals that then contributes to latter spikes in bioerosion rates. However, characterising habitats and benthic change in purely an aspect of adult foraging space only tells one half of the story to parrotfish response to disturbances. While the increase in parrotfish abundance is a key mechanism to counteract the sudden increase in available grazing space following large scale disturbances, ample foraging areas alone are not enough to promote recruitment to the population. Juvenile parrotfish utilise different reef habitats to adults, and frequently rely on macroalgal and seagrass beds shortly after metamorphosis (Paddack &

Sponaugle 2008, Sievers et al. 2020). In Mo'orea, most reef fish species initially recruit to these areas within the lagoon (Lecchini 2005), so community comparisons with benthic trends in these reef areas, such as temporal trends in macroalgal beds, would help to understand changes to habitat influencing settlement. Paddack & Sponaugle (2008) found that recruitment of *Sparisoma viride* increased in conjunction with an increase in *Dictyota spp.*. However, most juvenile parrotfish species are challenging to visually ID (Bellwood & Choat 1989), with most individuals < 10 cm simply being recorded in this study broadly as parrotfish juvenile or "*Scarus spp*.", therefore hindering effective species level monitoring of parrotfish recruitment. Applying modern molecular approaches is one way of navigating such a challenge (Sievers et al. 2020). Greater detail in UVC surveys for life phase of parrotfish also would help improve estimations of functions. With this differentiation of habitat use at different life stages, benthic changes on the outer reef will only influence parrotfish population dynamics that apply to recruited juveniles and adults. Even though these areas saw the biggest changes in terms of shifts between live coral cover, parrotfish population dynamics are likely driven largely by availability of quality habitat for recruitment. Understanding temporal trends and how large-scale disturbances affects the availability of recruiting habitat for settling juveniles is the next major step.

More needs to be understood in relation to quality of food and productivity patterns. Biomass levels of parrotfish had returned to pre-disturbance levels by 2014, however live coral cover was still lower and available substrate still higher than pre-disturbance levels, meaning that biomass does not always directly correspond with available grazing space and that this is not an accurate measure of parrotfish grazing resources. This suggests a need for greater knowledge in characterising grazing spaces and turf communities. Many studies view new grazing space synonymously with grazing space that existed prior to the COTs outbreaks and the cyclone. However, this view overlooks temporal variations in algal species and EAM composition both spatially, and temporally through the process of colonisation. Studies indicate that cyanobacteria are prevalent in the early stages of colonisation following COTs predation

(Larkum 1988), and as parrotfish are now widely considered to be microphages (Clements et al. 2017) this suggests that they stand to benefit more during this time. Turf algal communities themselves can vary in their attractiveness for parrotfish feeding with variable levels of sedimentation and their potential to yield organic matter (Gordon et al. 2015), all of which will likely vary as algal community composition develops through the colonisation process. These changes have been identified as an important area for future research (Clements et al. 2017).

This study is the first to apply feeding metrics to historical UVC data to break down community changes into patterns of ecosystem functioning through time, and compare rates of functioning with patterns in benthic change. This approach has given insights into the varying response of parrotfish functioning to biomass patterns which is dependent upon size structure and abundance. This two-step process; initial rapid recruitment of individuals followed by growth of these fish with a resulting latter spike in bioerosion; may be key in understanding why live coral recovery was so rapid on the outer reefs of Mo'orea (Edmunds 2016). Hypothetically, while counts of fish may have been shown to increase in other study areas following disturbances, intense fishing pressure in some areas may prevent these additionally recruited individuals from eventually reaching sizes to effectively contribute to bioerosion. While scraping may be maintained, there would be a loss in the modifications to the reef that come from bioerosion such as alterations to reef complexity and wave action (Bozec et al. 2015, Glynn & Manzello 2015, Perry et al. 2018). Graham et al. (2007) found that while fish size increased following bleaching, counts of fish in smaller categories were greatly reduced, meaning a rapid drop in recruitment was imminent.

While this approach has provided insights that fit with wider knowledge on parrotfish functioning, as with all modelling approaches these come with assumptions. One criticism of such approaches is that UVC assumes that fish present equates to fish feeding in that area. Studies that have used un-baited Remote Underwater Video to measure realised grazing pressure rather than estimated grazing pressure from UVC found patchiness of herbivore feeding and functioning (Streit et al. 2018, Yarlett et al. 2020). Bite rates are likely to be highly

variable by different regions and habitats, since they will be influenced by quantity and quality of food, therefore there is a need for data to be regional, and made relevant to the habitat. For example, Russ (2003) found that grazing rates were more closely linked to productivity rather than standing mass. Productivity has been shown to be seasonal on coral reefs (Diaz-Pulido & Garzón-Ferreira 2002), and influenced by local factors such as terrestrial run-off (McCook 1999). Furthermore, simply the proportion and patchiness of available grazing substrates can affect parrotfish distribution and feeding behaviour (Nash et al. 2012, Tootell & Steele 2016). A study by Tootell & Steele (2016) suggests that *C. spilurus* is food limited on Mo'orea, which may mean that bite rates are more concentrated on specific areas here than in other areas where bite rate data was collected. Therefore, it is important to build data sets where bite rate metrics are specific to each region and time. The use of video to capture grazing rates in different reef areas would help to address questions such as observed grazing rates versus estimates from UVC, grazing rates in comparison to available grazing substrates. It would negate issues associated with divers being able to effectively estimate fish size underwater and would allow for footage to be re-watched indefinitely, thereby improving species ID across life phases. Furthermore, this study combines multiple data sets across a number of different studies around the world, which all add their own degrees of uncertainty. However, this study provides a current best guess using existing data available, and the results can be verified in the future with further analyses as data sets develop.

The increase to parrotfish biomass and functions following disturbance is clearly an important positive feedback response necessary to prevent ecosystem transitions away from coral dominated states. This study helps to understand how parrotfish community dynamics following a large-scale disturbance translates to provisioning of functions within the most recent periods of disturbance and recovery for the island of Mo'orea. The main benthic drivers to parrotfish functioning were related to shifts between live coral cover and available grazing space, however some negative associations between dominant coral types (*Monitpora, Pocillopora*) suggest that future shifts towards these genera may have a growing impact on

parrotfish communities. It could be argued that biomass, scraping and bioerosion rates are fairly resilient and that parrotfish functions are resilient to fishing pressure, as there were similar between periods of coral dominance. However, while this may be the case for scraping rates it is more likely that bioerosion rates have been simply been reduced to be functionally non-existent. This is alone is concerning, however the significant reduction to mean fish size over time suggests that there is potential for both functions to be reduced in the future. Such studies must be continued as the rate of disturbances increases, and data sets characterising feeding metrics of different species need to be developed and made relevant to local regions to improve estimates.

2. Using stereo video cameras to identify drivers of herbivore functional rates

2.1 Abstract

Grazing by fishes on coral reefs provides important ecosystem functions that enhance resilience by preventing algae from dominating and clearing substrate to allow for coral recruitment. Large scale disturbances that result in the loss of huge amounts of coral cover, such as bleaching events and cyclones, are set to increase due to climate change. Therefore, understanding drivers to ecosystem functions that facilitate resilience, and identifying ways to enhance them are important tools. Due to variations in feeding styles and the way that material is removed, herbivory in general can be broken down into sub-functions, and here I considered cropping and scraping. Understanding the functional capacity of these communities, how they are structured and identifying factors that enhance ecosystem functioning are important tools for managing for resilience. I deployed stereo camera frames within the lagoon reefs around Mo'orea, French Polynesia, to estimate bite rates and size measurements for surgeonfish and parrotfish species. Using fish size estimates obtained thanks to the stereo-camera system, I then converted this data into area grazed (cm^2 per min per m^2) for parrotfish species, and grams of carbon removed daily (g C per m^2 per day) for surgeonfishes. I compared micro habitat variation (within quadrat video frame) with landscape scale features such as nutrient enrichment (average total nitrogen) and estimated fishing pressure to see which features were associated with higher herbivory rates. Other than the total number of species present, cropping and scraping rates were associated with different drivers. Where high cropping rates were associated with higher levels of nutrients and a greater proportion of pavement to live coral, high scraping rates were located on fringing rather than barrier sites within the lagoon, and were associated with a greater proportion of crustose coralline algae and less sand and rubble. This study supports the concept that greater biodiversity enhances ecosystem functioning, and that managing to promote biodiversity will support reef resilience.

2.2 Introduction

On tropical coral reefs, grazing fishes provide important ecosystem functions by consuming algae and clearing reef substrate which enhances coral recruitment (Cheal et al. 2010). Understanding what drives these ecosystem processes is of vital importance as reefs are set to face an increase in the frequency and severity of climate related disturbances (Hughes et al. 2017, Graham et al. 2011). Due to differences within feeding styles, the amount and type of matter removed through feeding varies, therefore herbivory in general can be broken down into a set of unique functions. Within these, cropping species such as surgeonfishes only remove standing plant biomass or detritus, whereas parrotfish species scrape away all epilithic material as they feed (Tebbett et al. 2017, Wainright & Price 2018). This means that both croppers and scrapers vary in the amount of algal material processed, with croppers being more selective, and with parrotfish contributing more towards sediment dynamics (Tebbett & Bellwood 2019).

To translate community data into estimates of these different types of function, a widely used approach is to apply feeding metrics to individual fish recorded during regular community surveys (Hoey & Bellwood 2008, Hamilton et al. 2014, Duran et al. 2019, Yarlett et al. 2020). For parrotfish that leave visible grazing scars, measurements of scar area can be multiplied by bite rate to get estimates of area grazed by an individual fish in the unit time (Hoey & Bellwood 2008, Lange et al. 2020, Yarlett et al. 2020). Since surgeonfish do not leave such scars, it is more logical to quantify algal material processed, such as estimating grams of carbon per day (Marshell & Mumby 2015). Scar area and the amount of algae processed by individuals has been shown to increase exponentially with size (Lokrantz et al. 2008, Marshell & Mumby 2015, Robinson et al. 2019, Lange et al. 2020). Therefore, accurate size estimates of individuals within a community is important for effectively quantifying functional rates. The use of stereo cameras is one way to account for all of these measures without biases associated to the observer. The use of two cameras facing towards a focal point allows for the calculation of

depth of field, and accurate measurements to within millimetres, which is not possible using single camera approaches (Bozeman & Grossman 2019). Thus, the use of remote cameras for assessing fish communities, including grazing potential has increased in popularity in coral reef research (Houk & Van Woesick 2006, Dumas et al. 2009, Safuan et al. 2015)

The herbivorous reef fish community that primarily target the epilithic algal matrix (EAM) of turf algal communities is diverse, and the specific materials that they target, and how they gain nutrition from the EAM varies. For example, within the surgeonfish family Acanthuridae, members are divided between either being considered croppers, browsers or detritivores. These definitions are dependent on feeding behaviour and the biomechanics of their feeding apparatus which define how material is removed and how it is digested (Wilson et al. 2003, Marshell & Mumby 2012, Clements et al. 2017, Tebbett et al. 2017). Recent research however, indicates that parrotfish primarily gain nutrition from microphages such as cyanobacteria (Clements et al. 2017). Regardless of how they feed, this diverse community is primarily supported by these fine, filamentous and multi-species algal communities. EAM communities are highly productive, and are characterised by low standing mass with a rapid turnover, which is believed to be one of the main drivers that facilitates high fish biomass on reefs (Carpenter 1985, Klump & McKinnon 1989, Russ 2003, Bellwood et al. 2018). Reefs are typically found in oligotrophic waters; therefore, any input of external nutrients such as terrestrial run-off can boost productivity to the point where fish communities can no longer keep these algal communities to short, productive turfs, and instead upright fleshy macroalgal communities can dominate (Hughes et al. 2007, Fulton et al. 2019). A variety of biotic and abiotic features can influence reef fish community composition. Communities of herbivorous reef fish can be driven by available grazing space (Robinson et al. 2019), structural complexity (Pratchett et al. 2008, Tebbet et al. 2019) and wave exposure (Bejarano et al. 2017, Karkarey et al. 2020). Since top down pressures such as fishing and predation typically alter the abundance and size structure of communities (Mumby et al. 2007a, Robinson et al. 2017, Bellwood et al 2012), this can alter the functional potential of these communities. For example, biomass comprised of small bodied

individuals may increase scraping rates (Robinson et al. 2019), but the same does not hold true for functions such as bioerosion that relies on key large bodied species (Bellwood et al 2012). Designating areas as Marine Protected Areas (MPAs) is a tool that managers use to safeguard key areas from fishing affects, which theoretically should enhance the abundance of large bodied, highly fecund individuals (McClanahan et al. 2007, Wilson et al. 2010, Beldade et al 2012, Tsoukali et al. 2016). These areas then should enhance surrounding reefs through spill-over effects (Goni et al. 2007, Stobart et al. 2009). However, this does not always hold true, especially if MPA size is not designed with target species in mind (Garcia-Rubies et al. 2013, Howlett et al. 2016).

Top down and bottom up pressures have been shown to influence functional groups differently on a macro-ecological scale (Robinson et al. 2019). However, high variation in human population density and associated pressures, and variation in local environmental features means that the strength of these relationships will vary geographically. For managers to make decisions, identifying drivers of rate of functions provided by communities must be assessed at the local scale. To quantify rates of functioning performed by herbivorous reef fish communities, we deployed fixed stereo camera frames to capture species composition, size and bite rates of individuals present on reefs around Mo'orea, French Polynesia. We measured small scale habitat features such as percentage coverage of benthic types, and combined this with seascape scale data on nutrient loads and fishing pressure, to determine the key drivers to functional rates performed by herbivorous reef fish at the island scale. Specifically, I ask the following questions: 1) to what extent do small scale and seascape scale habitat features influence functional rates provided by herbivorous reef fish? 2) Does this vary by functional group?

2.3 Methods

2.3.1 Study Site

In May-July 2019, 3 paired reef sites; 3 sites within MPAs and 3 adjacent non-MPA locations; were studied across Mo'orea island, French Polynesia (17.5388° S, 149.8295° W). Reef sites were selected from the **Plan de Gestion de l'Espace Maritime (**PGEM) list of surveyed sites (Service de l'urbinisme, 2019). The MPAs; Aroa, Pihaena and Taotaha, were chosen as they represent zones that were "fully protected" (Horta et al. 2016) compared to other less managed areas around the island. These were then paired with non-MPA sites: Vaiare, Hilton and Haapiti, that were also chosen from the PGEM list to be as close geographically as practically feasible. This would allow us to test whether designation as an MPA and restriction from fishing enhanced grazing rates. Within each site, two zones within the lagoon were surveyed: barrier and fringing reef. Three camera frames with two Go Pros facing forwards were deployed at each site (n per site =3). At each camera frame location, a PVC quad of $1m^2$ was temporarily placed in the focal point in front of the frame, with the quad location being selected to contain at least 50% substrate other than sand. The frames were positioned so that the focal point of the two cameras rested on the quad. A photograph of the quad was taken from above to quantify microhabitat variation before the quad was removed prior to filming grazing behaviour. Rugosity for the quad location was quantified by assigning a value of 1 – 5, with a bare flat surface with minor bumps having a score of 1, and complex substratum with branching corals having a score of 5 (Gratwicke & Speight 2005). Visually estimating reef rugosity has been a commonly used method as it rapid, low cost and minimises impact to reef substrate (Dustan et al. 2013). Cameras were left filming for one hour, and were deployed between 11:00 – 14:00 to account for midday peak feeding (Ferreira et. al, 1998, Bonaldo et al. 2017, Yarlett et al. 2018). The study was undertaken between May- July in 2019.

2.3.2 Video analysis

Videos were analysed using Vidsync (Neuswanger et al. 2016). Clips were calibrated for wide angle distortion and the 3D coordinate system (*x, y*, z) was set up prior to the start of the analysis. A 20-minute acclimation period was used from when the quad was removed from view in order to decrease disturbance effects on fish feeding behaviour and to allow fish to

return (Lefcheck et al. 2019). Following the acclimation period, the videos were analysed for 30-minutes, whereby any fish that took a bite from within the $1m^2$ area of where the quad had been placed was sized, and the number of bites that individual took was recorded. Surgeonfish (*Acanthuridae*) and parrotfish (*Scaridae*) were recorded to species level, and total length was recorded for each individual. This was done by selecting a point in the video whereby the individual fish was as side on to the camera as possible, and both standard length and total length were recorded with size being rounded up to the nearest cm. As GoPro footage is stored in clips, each 30-minute video was analysed in separate 10-minute clips, giving three clips per camera frame, and three frames per site (total clips n= 108). This gave a sample size of 9 clips per site and reef zone. While this does give a low sample size that can result in a higher risk of type II errors (Harmon & Losos 2005), this is a common issue with ecological studies and requires replicate studies to add support to findings (Lemoine et al. 2016).

2.3.3 Data analysis

I used allometric relationships to convert surgeonfish bite rates into grams of carbon (g C) removed through EAM consumption (Marshell & Mumby, 2015). These values were summarised as daily grams of carbon processed per m^2 (g C per m^2 per day). Area scraped per bite was calculated as a function of body size, where area scraped increased exponentially with size (Robinson et al. 2019), giving a value of cm^2 per min per m^2. The substrate type and percentage coverage of each quad photo were estimated to the nearest 5% from each quad photograph for the corresponding camera frame. “Live Coral”, “Dead Coral”, “Bleached Coral”, “Pavement”, “Crustose Coralline Algae” (CCA), “Rubble”, “Sand” and “Macro Algae” were the main benthic features identified. Consolidated reef matrix such as pavement, with no other obvious benthic coverage, is the primary reef surface for turf algae. Hence, in this study, pavement is synonymous with primary grazing space. Nutrient values were taken from a study exploring the influence of nutrients associated with terrestrial runoff on the extent of bleaching in 2019 (Donovan et al. 2020). Exploratory analysis showed that both δ^{15} N and percentage nitrogen of algal tissues were highly correlated. The models were initially run independently

with both values having similar effects. I chose to present the results using percentage nitrogen as I felt like this value is easier for readers to understand than isotopic ratios. Indication of potential localised fishing pressure was taken from Thiault et al. (2017), who used a combination of participatory survey approaches with local fishermen, and socioeconomic approaches of fishing capacity for an area to map likely fishing effort. This data is simply an estimate of fishing intensity without knowledge on species targeted. It is a different way of exploring the impact of fishing pressure than MPA as it is more related to fishermen's personal choices, which tended to driven by factors such as the proximity of fishing areas to both deep water channels and ease of access. It also explores the statement by locals that MPAs are not well enforced in this area (Howlett 2019, pers. comms.), as a fisherman may choose not to fish somewhere due to factors other than MPA designation on paper. I conducted a kernel interpolation using a fifth order polynomial kernel function within the Geostatistical analysis toolbox of ArcPro to extrapolate point data for fishing and nutrient values across the lagoon reefs. Fishing estimates were log transformed prior to analysis. To get an idea of feeding diversity, the total number of unique species feeding within each camera frame was summarised. To compare functional rates, quad features and species diversity between reef zones and sites, I summarised features to each site, zone and camera frame. Cropping and scraping values were both positively skewed, therefore I ran a Kruskal-Wallis to see if there were differences, followed up by a pair wise Wilcoxon test using Benjamin & Hochberg (1995) method to adjust p values to identify where differences were found.

Prior to modelling I checked for collinearity between variables. Nutrient values of average total nitrogen and average δN15 were highly correlated (Pearson correlation, $r= 0.80$, $t= 6.75$, $df= 34$, $p< 0.01$), as were both measurements of nutrients with bleaching (average total N; $r= 0.7$, $t= 5.74$, $df= 34$, $p< 0.01$, δN15; $r= 0.6$, $t=3.88$, $df= 34$, $p< 0.01$), therefore I only included total percentage nitrogen in our analysis. I found that live coral cover and pavement were also highly negatively correlated ($r= -0.8$, $t= -0.89$, $df= 34$, $p< 0.01$), as were CCA and fishing estimates ($r= -0.6$, $t= -4.54$, $df= 34$, $p< 0.01$). To account for this, and not lose too much data by

simply dropping variables, I ran a principle component analysis (PCA) for the habitat quad data. Benthic data was scaled and centred prior to running the PCA, with PC1 and PC2 explaining 31.3 and 20.1% of variation, respectively.

2.3.4 Statistical analysis

Due to the presence of multiple clips with zero values for both cropping and scraping functions, data for each function was analysed using a zero inflated hurdle modelling approach (Dajka et al. 2019). Presence only data was analysed as a continuous gamma distribution model, while presence-absence values of functions were analysed in a binomial model. I used generalised mixed effects models to model functions as gradients in benthic habitat summarised by extracted values for PC1 and PC2, and against values for average total nitrogen and estimated fishing pressure. I included MPA protection (categorical variable; (protected, unprotected), our rugosity scoring, and whether or not the zone was barrier or fringing. To place modelled effect sizes on a common scale, I scaled and centred all continuous covariates to a mean of zero. For the presence only cropping model, I removed 21 zero values out of 108 observations, giving a zero inflation of 19.44%. For our scraping presence only model, I removed 65 zero values out of 108 observations, giving a zero inflation of 60.18%. Prior to all models being run, I ran a forward and backwards stepwise selection to identify key variables, and those that were retained were then included in our mixed effects models. For the cropping presence only gamma model, average total nitrogen, total species present and PC1 were retained. For the cropping presence-absence binomial model, protection; unprotected, average total nitrogen, total species present and fishing pressure were retained. For the scraping presence absence binomial model, total species present PC2 were retained. For the scraping presence only gamma model, stepwise selection did not retain any variables, with the final model calling scraping ~ 1. I ran a full model with all variables, and used likelihood ratios to identify significant variables. For the random effects, I had camera frame nested within site. I used likelihood ratios to identify which variables in our models had a significant effect by comparing all full models (all variables selected for by stepwise selection) with null models. Prior to

running models, any potential outliers were identified using box and dot plots. Model fit was checked by viewing diagnostic plots. For all models, variance inflation values were checked using the car package (Fox & Weisberg 2019), with values for all models excluding the scraping presence only model less than 2. For the presence only scraping model, VIF values were less than 4. All analyses were done using R version 4.1.1 (R Core Team 2021). Data cleaning and organising was done using the tidyverse package (Wickham et al. 2019). Graphs and plots were produced using ggplot2 (Wickham 2016), and GLMM models were run using lme4 (Bates et al. 2015). The package ggeffects was used to create prediction plots from the mixed effects models (Lüdecke 2018).

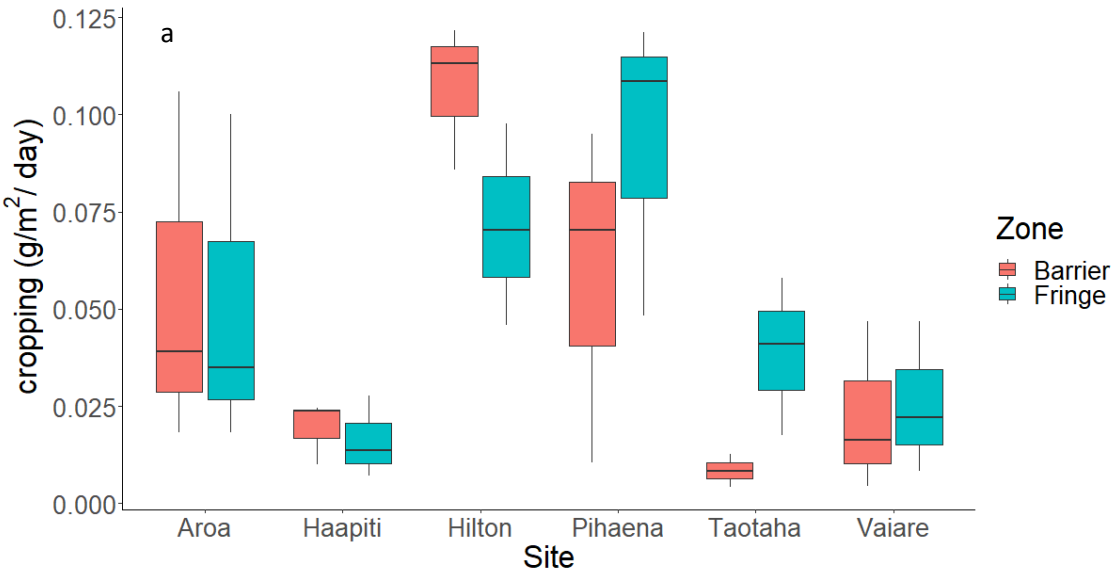
2.4 Results

Acanthuridae were present in all videos analysed, excluding one camera frame deployed at Taotaha barrier. Parrotfish were present within 24 of the 36 cameras deployed, meaning that roughly a third of videos lacked parrotfish feeding activity. Summarising values to each clip for each site and zone showed that there was no significant difference in cropping rates (χ^2= 1.12, df= 1, p= 0.29) between reef zones, however scraping rates were significantly higher (χ^2= 7.33, df= 1, p< 0.01) in fringing reef zones (adjusted p-value< 0.05, Figure 2.1). To explore the effect of sample size on the possibility of type II error I ran a power analysis using the pwr package in R (Champely 2020) using an effect size calculated from both the cropping and scraping data between reef zones, which estimated a sample size of 67 for scraping rates and 289 for cropping rates, suggesting a high likelihood of a type II error risk, which must be taken into consideration when interpreting these results.

There was a significant difference in cropping rates between sites (χ^2= 43.47, df= 5, p< 0.01), with Pihaena having higher rates than Haapiti (adjusted p-value< 0.05), Taotaha (adjusted p-value= 0.01) and Vaiare (adjusted p-value= 0.03). Hilton also had higher cropping rates than Haapiti (adjusted p-value< 0.05), Taotaha (adjusted p-value< 0.05) and Vaiare (adjusted p-value< 0.05). Aroa also had higher cropping rates than Haapiti (adjusted p-value= 0.02). There

was a significant difference of scraping rates between sites (χ^2= 16.31, df= 5, p< 0.01) with Hilton having significantly higher rates than both Haapiti (adjusted p-value = 0.02) and Vaiare (adjusted p-value= 0.03, Figure 2.1b). Species diversity was highest at Hilton fringe camera 3 with a total of 7 species, with a mean value of 3.1 species across all sites.

The site with maximum coral cover (70%) was recorded in Taotaha fringing reef, with a mean coral coverage of 24.03% across all sites. The highest coverage of pavement (90%) was detected at Aroa barrier reef, with a mean of 50.97% pavement coverage across all sites. The highest rugosity score was 4, and was attributed to 11 filming locations out of 36. The mean rugosity score across sites was 3.06. Differences along the PC1 axis were primarily driven by the relationship between live coral and pavement, with low values of PC1 corresponding to high coverage of pavement, and high values corresponding with greater coverage of live coral (Figure 2.2). The PC2 axis was primarily driven by rubble and sand versus sites with high coverage of CCA and live coral. High values of PC2 correspond to sites with greater coverage of sand and rubble, whereas low PC2 values correspond with sites with high CCA and live coral cover (Figure 2.2).



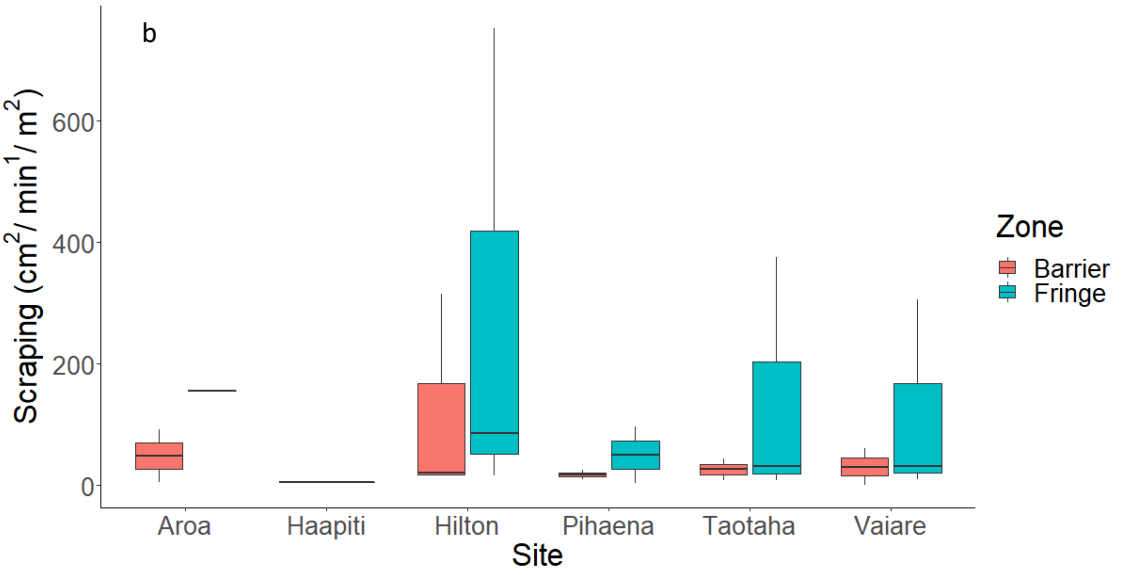


Figure 2.1. Summary plots of how cropping (a) and scraping (b) rates varied by site and zone. The MPA sites consisted of Aroa, Pihaena and Taotaha

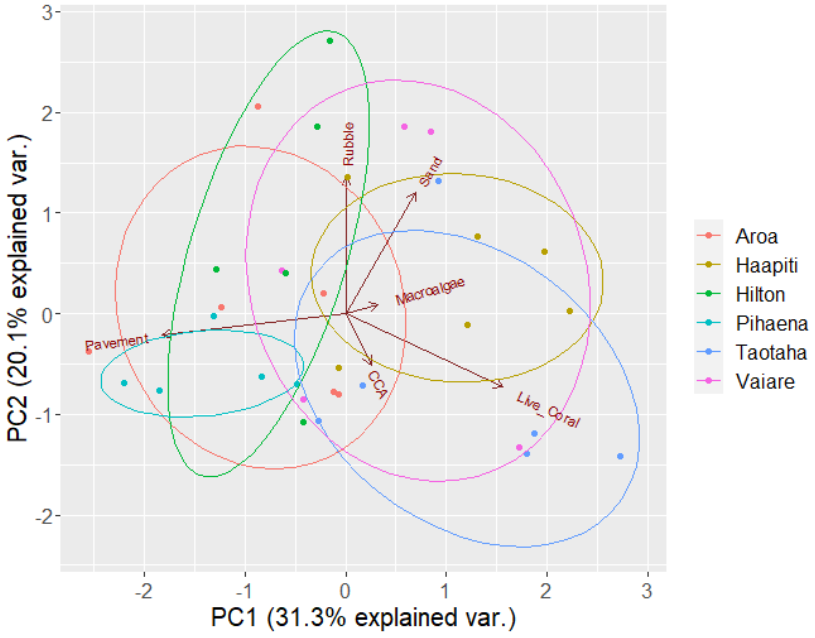


Figure 2.2. Principle Component Analysis of coverage of benthic types within the quads, grouped by site. Benthic variables were scaled and centred prior to analysis.

For the presence only cropping model, total number of species (X^2 = 14.95, p< 0.01) and average total nitrogen (X^2 = 5.94, p= 0.02) were significantly associated with cropping rates, raising them by 0.27 g C per m^2 per day ± 0.07, and 0.25 g C per m^2 per day ± 0.08 respectively (Figure 2.3, Figure 2.5). PC1 also had a significant effect, with higher cropping rates being

associated with lower values for PC1 (X^2 = 7.68, p< 0.01). PC1 negatively affected cropping rates by 0.23 g C per m^2 per day ± 0.07, with lower values of PC1 relating to areas with a greater proportion of pavement and less live coral coverage. When data was aggregated as presence-absence and run as a binomial model, only number of species (X^2 = 12.39, p< 0.01) and fishing (X^2 = 4.52, p= 0.03) had an effect on whether cropping was present. Protection (X^2 = 1.16, p= 0.28) and average total nitrogen (X^2 = 1.47, p= 0.23) had no effect (Figure 2.3, Figure 2.5).

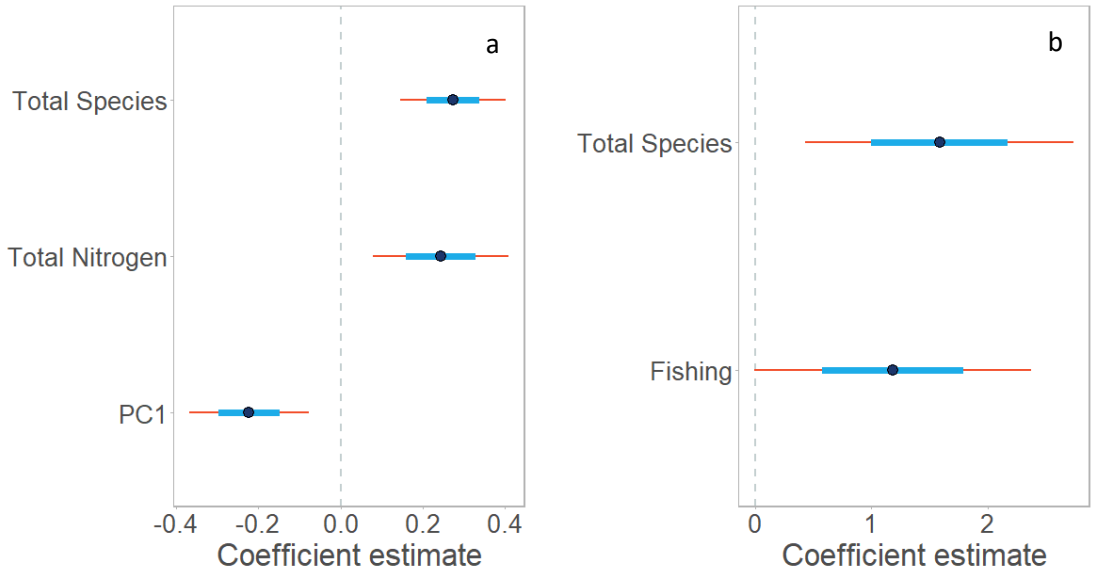


Figure 2.3. Effect size estimates of predictor coefficients of cropping in the gamma model (a) and the binomial model (b) with standard error (thick line) and 95% confidence intervals (thin line). For the binomial model, positive coefficient estimates predict cropping presence (1) and negative coefficient estimates predict cropping absence (0).

For modelling scraping rates using presence only data under a gamma distribution, only zone (X^2 = 10.77, p< 0.01) and PC2 (X^2 = 6.73, p= 0.01) had a significant effect (Figure 4, Figure 6). Scraping rates were higher on fringing reefs than barrier reefs by 1.02 cm^2 per min per m^2 ± 0.30, and PC2 lowered scraping rates by 0.40 cm^2 per min per m^2 ± 0.15. High values of PC2 were associated with a greater coverage of rubble and sand, while low values of PC2 were associated with a greater proportion of CCA coverage. Number of species (X^2 = 0.12, p= 0.73), protection (X^2 = 1.19, p= 0.28), average total nitrogen (X^2 = 2.44, p= 0.12), fishing pressure (X^2 = 1.51, p= 0.22) and PC1 (X^2 = 0.88, p= 0.35) had no effect. When scraping rates were

analysed as presence absence in the binomial model, only total number of species had a significant effect ($p < 0.01$), raising scraping rates by 1.59 cm^2 per min per m^2 ± 0.35, with PC2 having no effect (($X^2 = 3.30$, $p = 0.07$).

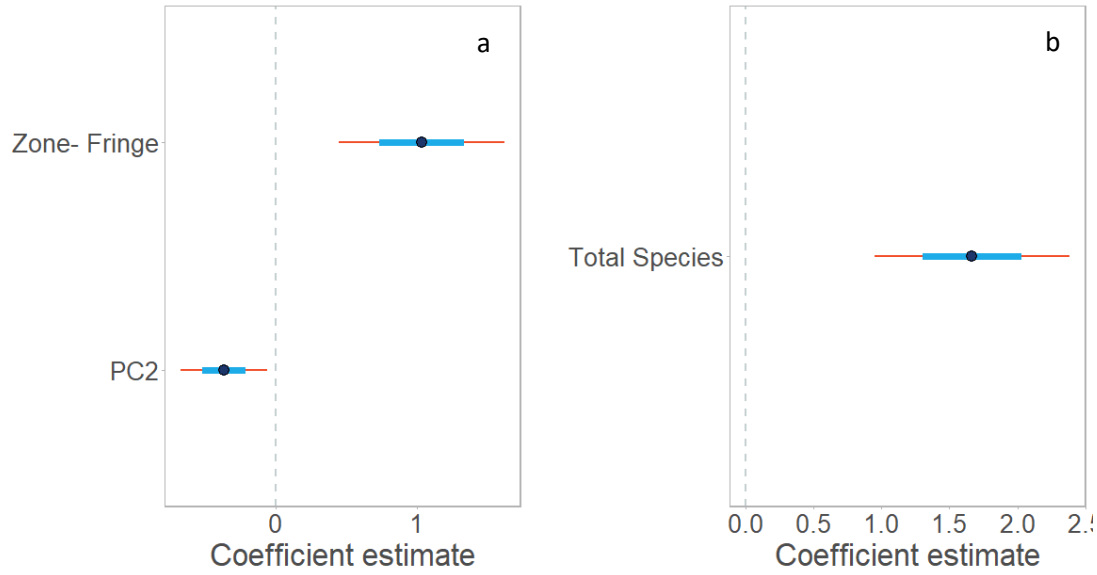
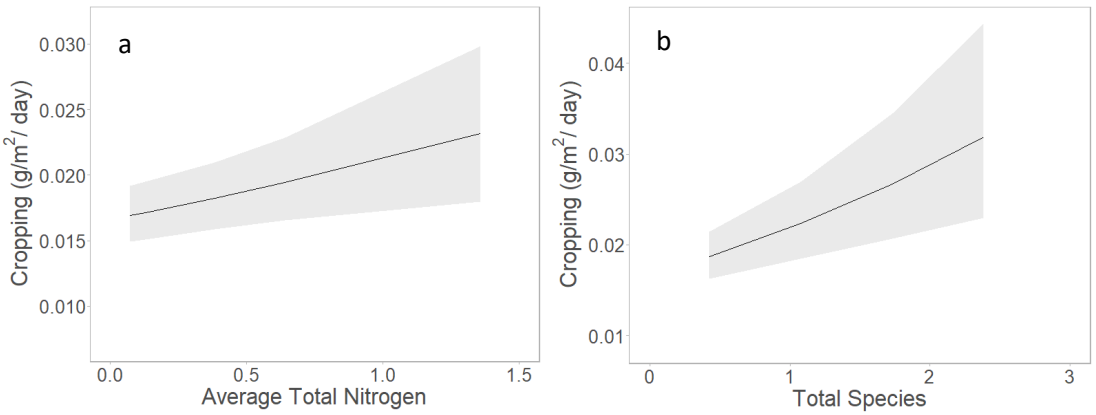


Figure 2.4. Effect size estimates of predictor coefficients of scraping in the gamma model (a) and the binomial model (b) with standard error (thick line) and 95% confidence intervals (thin line). For the binomial model, positive coefficient estimates predict scraping presence (1) and negative coefficient estimates predict scraping absence (0).



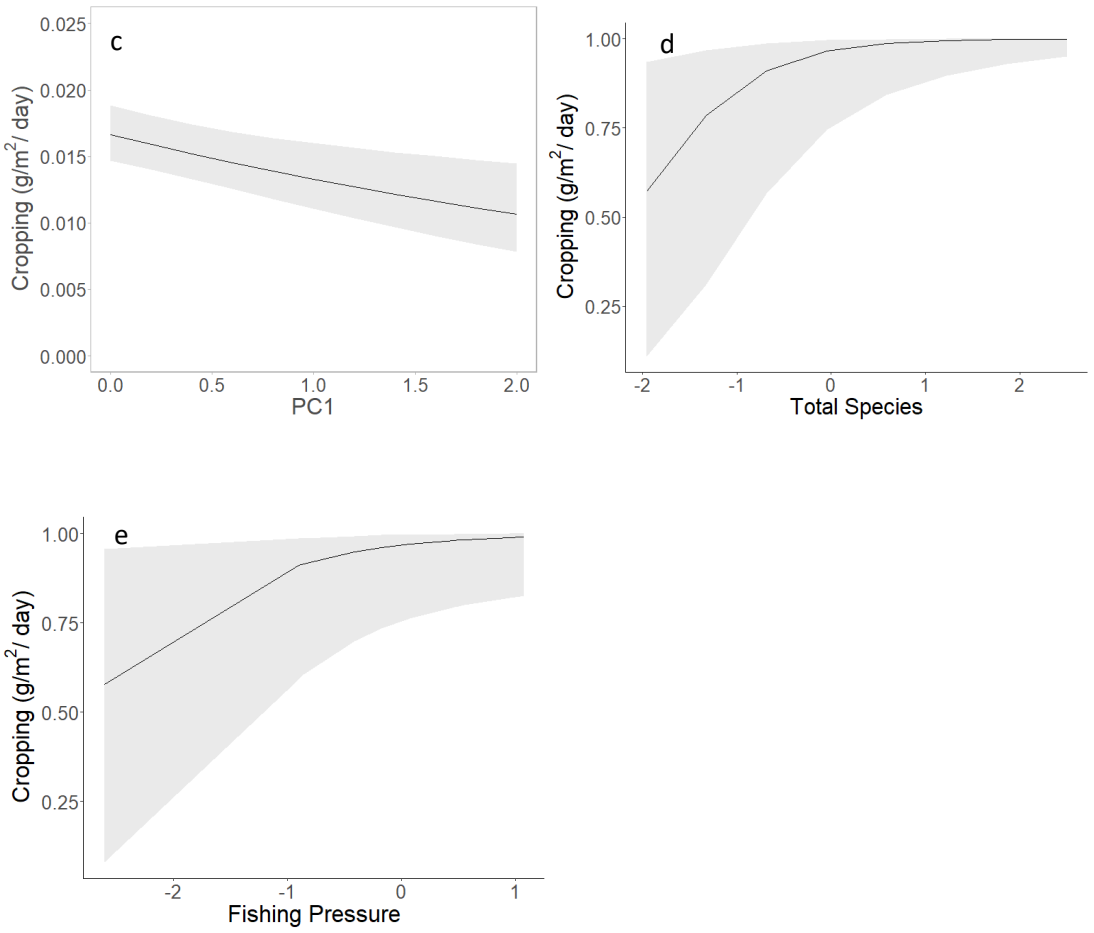
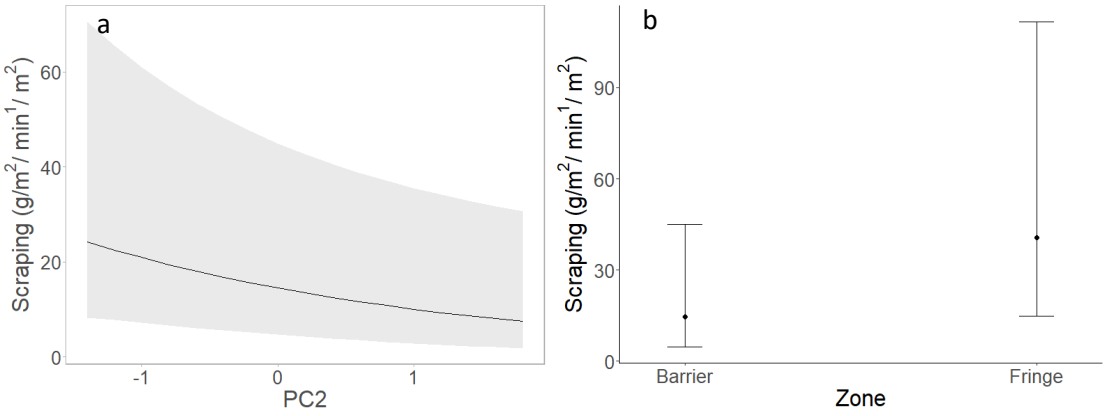


Figure 2.5. Predictions of cropping values for the a, b & c) presence only gamma model, and d & e) presence absence binomial model, showing the fitted trend line and 95% confidence intervals.



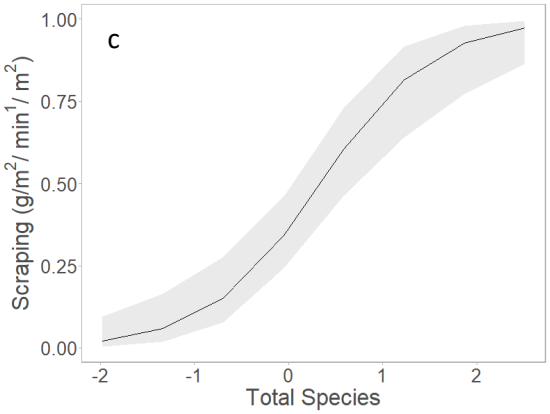


Figure 2.6. Predictions of scraping values for the a) presence only gamma model, and b & c) presence absence binomial model, showing the fitted trend line and 95% confidence intervals.

1.5 Discussion

These results support other studies in that cropping and scraping functions vary in their drivers (Robinson et al. 2020). Where cropping rates were associated with nutrient input, a high proportion of pavement to live coral and were positively associated with estimated fishing pressure, scraping rates were higher in fringing reef zones with a greater coverage of CCA. However, greater species diversity was universal in enhancing both ecosystem functions provided by herbivorous reef fish.

Increasing biodiversity has been shown to augment ecosystem functioning in a variety of ecosystems (Tilman et al. 2014, Clements & Hay 2021). A greater number of species can result in greater trait diversity (McWilliam et al 2020, Micheli et al. 2014, Pombo-Ayora et al. 2020, Clements & Hay 2021), which can enhance functional complementarity (Brandl & Bellwood 2014). Variations in traits can maximise resource use and minimize competition (Adam et al. 2015, Kelly et al. 2016). Due to these variations in feeding styles and prey preferences, diverse herbivore communities can be more effective at maintaining low standing, highly-productive algal biomass (Burkepile & Hay 2010).

Consolidated reef structure covered primarily by fine, filamentous turfs (in this study referred to as pavement) is the dominant grazing space on reefs for herbivorous fishes (Tebbet & Bellwood 2019), therefore it is unsurprising that at least one functional rate was associated with high pavement coverage. The interaction between herbivore reef fish and the substratum is a two-way process, whereby availability of resources will define whether or not an individual may be present there, but also the grazing pressure exerted by individuals shapes the environment (Burkepile & Hay 2010). High grazing pressure by herbivorous reef fish keeps algal turf communities short and highly productive, however changes to abiotic conditions can alter this, such as an increase to sediment loads or ambient nutrients (Hughes et al. 2007, Fulton et al. 2019, Tebbet & Bellwood 2019). Nitrogen is thought to be most limiting to algal growth on coral reefs (Hatcher & Larkum 1983), therefore inputs from terrestrial sources can enhance algal productivity and push reefs towards dominance by fleshy macroalgae (Fulton et al. 2019, Dajka et al 2021). Average total nitrogen was also related to higher cropping rates; therefore, it is likely that this input of nutrients boosts productivity in these areas, thereby supporting higher densities of individuals.

CCA are grazing tolerant, due to calcareous structures and their ability to rejuvenate from grazing damage (Steneck 1985). In areas with high nutrients and high grazing rates, CCA tends to dominate on reefs (Littler & Littler 1984, Johnson & Carpenter 2018). However, in this study, there was no evidence that nutrients were correlated with CCA cover. Grazing by herbivorous reef fish enhances CCA cover through removal of fleshy macroalgal competitors (Belliveau & Paul 2002, O'Leary & McClanahan 2010). Some studies have shown that parrotfish tend to selectively graze from CCA more than surgeonfishes (Francini-Filho et al. 2010), which may be why PC2 had a significant effect on scraping rates, whereas cropping rates were related more to pavement versus live coral cover.

While studies have shown that structural complexity and reef rugosity can be important features for herbivore reef fish communities (Graham et al. 2006, Nash et al. 2016), interestingly rugosity was not found to be influential in this study. This may possibly be due to the coarse

level of measurement used. There are a variety of ways that rugosity and structural complexity can be quantified. One method estimates the ratio of a straight line transect to that of a flexible chain draped over the reef (Risk 1972), however this method has the potential to damage the reef and increases the amount of weighted gear that a diver must carry, which is not possible when also diving with heavy camera frames. It can also be argued that it is an imprecise descriptor of structural complexity across a wide range of scales (Dustan et al. 2013). This concept of scale may also be applicable to our $1m^2$ quads. New technological advances also allow for measuring structural complexity by mapping the dimensional structure of reef areas through methods such as photogrammetry (Carlot et al. 2020). Mapping a greater proportion of the reef would be more reflective of the reef scape within which roving fish interact.

Studies have shown that the survey methods used can influence the ability to detect different fish species. Physical and behavioural features can influence which species can be favoured, and studies have shown that video surveys typically result in lower values for density and diversity than other approaches such as UVC (Tessier et al. 2005, Pelletier et al. 2011, Holmes et al. 2013). Parrotfish were found in a lower abundance than surgeonfishes in the videos in this study, therefore they may be especially sensitive to this survey approach. Comparisons with UVC data would indicate whether parrotfish are less dominant in the community in comparison to surgeonfishes, or if there is reduced detection for these species using this approach.

We found no evidence that top down pressures such as fishing had a negative effect on functions. Indeed, whether a site was a protected area or not had no influence on functional rates. Furthermore, not only was high estimated fishing pressure not negatively associated with functions, but it actually had a positive effect on whether cropping was present or not. The most likely explanation behind why MPAs had no effect on functional rates is due to the small size of the MPAs on Mo'orea, and that fishing adjacent to these areas negates any potential effect due to the transient nature of many species (Gell & Roberts 2003, Howlett et al. 2016). The estimate of fishing pressure by Thiault et al. (2017) is a broad estimate based on fisher surveys, social structure and habitat features, as measuring actual landings by fishermen is almost

impossible due to the small-scale artisanal fishery with no centralised fish market. Fishermen in this region tend to favour spear fishing, and target larger, more palatable species such as parrotfish over surgeonfish (Pers. comms.). This may mean that any potential lacking data or coarse level of these estimates may prevent associations being identified with the functional rates from our videos. This may also mean that parrotfish are especially wary of diver presence in this area, and may partially explain the lower detection rates of parrotfish within the videos. It may even be possible that the fishing of parrotfish reduces competition of these species with surgeonfish, perhaps explaining why cropping was more likely to be present at sites with a high estimated fishing pressure. However, since our values of high total species diversity were associated with higher rates for both functions, this is unlikely.

Our approach of using stereo camera footage to quantify functional rates of reef fish communities at the island scale shows that functions vary in their drivers, however species diversity uniformly has a positive effect. This supports the idea that managing to optimise biodiversity protects ecosystem functioning (Griffin et al 2009, Topor et al. 2019). Our study suggests that, currently, management approaches such as MPAs around Mo'orea are not enhancing the functions of reef fish communities, however the strength of this conclusion is hindered by the fact that this a small study with limited sampling, and the potential for confounding factors. Since many of the relationships between grazing functions and their associated drivers identified here have been found in the wider literature, it reduces the likelihood that these are type II errors, however further studies are needed to confirm the results of this study. Furthermore, accurate measures of top-down pressures such as fishing are currently lacking, and more information on target species and sizes is needed to understand the influence that this has on the fish community. Functional rates relate directly to key habitat features such as available grazing space, and this is also coupled with nutrient input and potential increase to primary productivity.

3 Micro-habitat variation and bleaching response influences algal successional processes following widescale coral bleaching

3.2 Abstract

Large-scale coral bleaching events rapidly and drastically alter benthic composition, with the subsequent development of algal communities providing new resources for reef species. I used an experimental approach to explore the development of algal communities on corals that were thermally stressed in aquaria and then deployed on a reef. I sampled at 14 days, 50 days and 90 days following coral mortality. Both *Pocillopora* and *Porites* genera were used, to see if bleaching response and/or colony morphology influenced trajectories of algal communities.

I found that coral type and bleaching response influenced organic and inorganic loads, which were independent to canopy heights. Rapid mortality of *Pocillopora* fragments resulted in an instantaneous loss of organic material, whereas partial mortality across the colony surface of *Porites* resulted in a high retention of organic material, and limited changes through the time series to organic and inorganic loads. Community analysis found differences to colonising communities by coral type and treatment using both microscopy and the 16S metabarcoding marker. For 23S, a large proportion of reads were low quality and coarsely assigned, with high overlap across colonised substrates. However, comparisons with live coral communities still showed some evidence of community progression through time using this marker, with transitions to the rhodophyte community and ochrophytes being more prominent in later stages.

This study confirms that bleaching response of coral types and their morphology influences colonising algal community composition. However, the mass release of organic matter by *Pocillopora* into the local area during the bleaching event (shown through stable isotope analysis), alongside protection from grazing likely had a strong influence on colonisation trajectories. This study is one of the first to directly measure algal community transitions following a bleaching event alongside associated features that are known to influence grazing rates.

3.2 Introduction

Coral reefs are important marine habitats as they are home to a quarter of all marine species, and provide nutrition, income and cultural identity for local communities (White et al. 2000, Koehn et al. 2022, Woodhead et al. 2021). However, in the face of growing anthropogenic pressures coral reefs are shifting away from coral dominated states, which has implications for biodiversity and ecosystem functioning (Hughes et al. 2017, Williams et al. 2019, Perry et al. 2019, Dajka et al. 2021). One of the primary processes for reef resilience and return towards a coral dominated state is grazing by reef fish. Through the action of feeding they not only consume algae but also clear the substrate to facilitate coral recruitment (Bellwood & Choat 1990, Bonaldo et al. 2014). It has been widely shown that once areas shift towards algal dominated states, there are a number of feedback processes that occur, which makes it difficult to reverse towards a coral dominated ecosystem (Tebbet & Belwood 2019, Dajka et al. 2021). This means that such situations can become locked in, providing different resources for species and thereby altering the community structure (Bellwood et al. 2006, Graham et al. 2013).

Many studies have shown increases to herbivore communities following widescale bleaching and coral loss (Han et al. 2016, Graham et al. 2020), and these increases are typically linked to increases to grazing space and food availability (Taylor et al. 2020). This rapid recruitment to the population is believed to be a key mechanism that prevents algal proliferation in such situations. There is little data on whether this increase is related to breeding rates or survival of juveniles, and has mostly been attributed to greater resources for adult fish through benthic conversion. However, there is a lot of literature now that shows that there are a variety of features associated with turf algal communities that can either encourage or hinder grazing rates (Tebbet & Bellwood 2019). Features such as canopy heights (Goatley & Bellwood 2013), species composition (Pfeffer 1963), sedimentation and standing organic mass (Bellwood & Fulton 2008, Goatley & Belwood 2012) have been shown to influence feeding behaviour of various reef fish, and that these features vary depending on a wide variety of biotic and abiotic

features. This has important implications for resilience, as the attractiveness for herbivore feeding will influence recovery trajectories.

The establishment of algal communities following widespread coral mortality is a vital time for reefs, yet surprisingly, we still have very little knowledge of the colonisation process and how communities establish post-bleaching. Most studies on colonisation patterns of algae on reefs involve the use of artificial substrates, since this makes standardising and quantifying easier (Fricke et al. 2011, Ceccarelli et al. 2011). However, microstructure variation, such as surface rugosity and porosity of the material, can influence the settlement and survivorship of epibiota (Davis 2009, Mallela et al. 2017). Furthermore, applying such patterns to post coral mortality assumes that there is no influence on colonisation processes from either organic material shed by dying corals, or the structure of the microbiome prior to and during coral death. Living corals vary in their corallite size and structure, micro-rugosity of their colony surface and thickness of tissue layer (Hoegh-Guldberg 1999, Babcock et al. 2003). Furthermore, corals respond to bleaching in different ways, namely the temperatures at which they bleach and the degree to which they release mucus and other organic material (Richman et al 1975, Hoegh-Guldberg & Savat 1995, Mumby et al. 2001, Gardner et al 2018). Their microbiome varies by coral type, and changes in response to bleaching stress (Gardner et al. 2018, Morrow et al. 2018, van Oppen & Blackall 2019). This all suggests that at the point of coral mortality when algal communities are establishing, coral type could influence how algal communities establish themselves.

To date, most *in situ* studies simply characterise communities as broad functional groups, like macroalgae and turf (Diaz-Pulido & McCook 2002, Burkepile & Hay 2010, Doropoulos et al. 2013). This is likely due to the fact that identifying algal species, especially within turf communities, is challenging, requires specialist knowledge and is time consuming. The application of metabarcoding techniques can be one way to overcome these challenges, since this utilises DNA present within the sample and identification can be made even with limited material (Robba et al. 2006).

During bleaching events, the release of organic matter by corals has been shown to greatly increase (Niggl et al. 2008), with stable isotope analyses showing that these nutrients are retained within biogeochemical cycles (Vaughan et al. 2021). Studies using algal transplants suggest that this uptake could be rapid, and monitoring of macroalgal communities shows that these signals can persist for a year following the disturbance event (Vaughan et al. 2021). However, little is known regarding how these nutrients spatially disperse; i.e. are they tied to local substrates, such as the coral that released them, or are they widely taken up by algal communities within the adjacent area? Furthermore, there are currently no studies to date exploring whether coral derived nutrients increase primary productivity following widescale coral bleaching, and whether these nutrients affect colonisation patterns such as algal community composition, as has been shown experimentally with artificial nutrients (McClanahan et al. 2003, McClanahan et al. 2007, Duran et al. 2016).

To explore to what degree coral structure, bleaching response and release of organic material influenced the colonisation process of algal communities, I experimentally induced coral mortality through heat stress in two genera of corals; *Pocillopora* and *Porites*, and sampled developing communities at 14 days, 50 and 90 days after deployment on the reef. I compared bleached corals with control substrates (corals with their tissue removed leaving only the bare skeleton), to see whether coral derived nutrients were retained locally to the individual substrate from which they were released to see if these influenced colonisation patterns. I used metabarcoding approaches to determine community composition for algae and bacteria, and measured features of turf communities that influence feeding behaviour of grazing fishes, such as canopy heights and loading of organic and inorganic (sediment) material. Finally, I used stable isotope analysis to see how the isotopic signature of developing algal communities compared to that of healthy live coral tissue, to see if these nutrients were being taken up by developing algal communities, and whether this varied by coral type and treatment.

Specifically, I asked the following questions; post bleaching:

- Is algal community development influenced by coral genus?

- Is bacterial community development influenced by coral genus?
- Do colonisation processes vary between bleached corals and clean bare substrates of those coral types?
- Do sedimentation and organic loads vary through time by coral types?
- Are nutrients from coral tissue being recycled into primary productivity?

3.3 Methods

3.3.1 Study Site

The study was carried out on Mo'orea (French Polynesia), which is located in the Pacific Ocean between 17.4714° and 17.6058° south and 149.7522° and 149.9269° west. Historically, widescale coral loss in this region has been attributed to outbreaks of *Acanthaster plancii* or cyclones (Berumen & Pratchett 2006; Lamy et al. 2016, Adjeroud et al. 2018), with mortality from bleaching being comparably minimal (Hédouin et al. 2020). However, during the spring and summer of 2019 when the experiment was conducted, a mass bleaching event occurred within French Polynesia, with the peak of the bleaching occurring between March and April (Péres-Rosales 2021). The northern reefs of Mo'orea where the experiment was set up saw some of the highest bleaching levels around the island, with up to 50% coral mortality in some areas (Donovan et al. 2020, Vaughan et al. 2021). This meant that my experiment was not run as patches of isolated bleaching on a healthy reef, but rather was part of an area that was undergoing extensive bleaching and coral mortality. Thus, my experiment became more of a focus on whether or not large-scale bleaching had a generic effect on turf algal communities developing on a variety of substrate types (*Pocillopora* versus *Porites*; bleached versus controls; explained below), or whether we could still see variations in turf algal communities and associated features by substrate type.

I selected a site for deployment of corals that was situated on the fore reef, and adjacent to a reef lagoon that has been shown to have low inputs of terrestrial nutrient sources (Leichter et al. 2013, Donovan et al. 2020), to ensure that the majority of changes in nutrients detected would

be solely from the bleaching event. *Pocillopora* as a dominant coral genus in recent years following recovery from last major disturbance (Tsounis and Edmunds 2016).

3.3.2 Substrate collection, tank set up and deployment on the reef

Healthy corals were collected from the north-west outer reef at approximately 10 m using a hammer and chisel to loosen them at the base. *Porites* colonies were collected that were approximately the size of a fist, and individual large branches were taken from *Pocillopora* colonies. Determining corals to species level is an involved process that usually takes examination of corallite structure under the microscope (Babcock et al. 2003). Based on colony morphology and location, *Porites* corals were likely either *Porites lutea* or *Porites lobata*, with the size and structure of *Pocillopora* colonies likely to be *Pocillopora grandis*. However, since this was not determined we simply refer corals to genus level within this study. Since we were using healthy live corals from the reef, we kept the sample size small (n=6) to minimise impact. Corals that were taken from the reef were placed into a cooler filled with local sea water on the boat for transportation back to the research base. Corals were wrapped in clean towels soaked in sea water, then carefully drilled into the base to place screws to hold them to plastic frames. Once fixed on frames, they were placed into 70 L closed circuit tanks in aquariums that were kept under artificial lighting under a L12:D12 cycle. Corals were acclimated for 48 hours at 28°C, and to check that no mortality occurred due to handling.

To induce mortality through intense heat stress, tanks containing *Pocillopora* fragments were kept at 30/31 °C for 7 days. Initial *Porites* corals were incubated in the same tanks at 30/31 °C for 7 days and then the temperature was increased to 33 °C for an additional heat pulse. However, after deployment on the reef the majority of *Porites* colonies recovered their symbodinium within the two-week period prior to the first sampling date. The corals that recovered were returned to the laboratory and this time were incubated at 33 °C for 7 days. In nature, coral mortality through heat stress normally occurs through loss of symbodinium and

reduced energy intake for corals (refs). However, Hughes et al. (2018) found that in 2016 on the northern Great Barrier Reef, corals exposed to excessively high temperatures died rapidly within the space of two weeks, indicating that temperature was the cause of mortality, not starvation or prolonged stress. While these conditions are not common, such intense heating events are possible in nature and could become more frequent as the Anthropocene advances (Hughes et al. 2017). While this second incubation led to increased mortality for *Porites*, this still only largely resulted in partial bleaching across the colony surface (which applied to approximately 80% of substrates), with establishment of turf algae patchy in nature, interspersed between bleached or recovered coral tissue.

Following incubation, corals were transplanted back out onto the north-west outer reef and placed into cages that were attached to blocks that had been placed in rubble beds. Herein these substrates are referred to as bleached corals within this study. Studies have shown that the density and identity of grazing fishes influences trajectories of algal succession (Burkepile & Hay 2010). Furthermore, after a trial experiment yielding limited material at 14 days, I decided to cage substrates. The concept was that this would then put the focus on the competitive interaction between algae without the confounding effect of herbivore grazing pressure, and provide more standing mass to be split between analyses. Three blocks were placed per rubble bed, with three rubble beds being set up resulting in a total of 9 blocks spread across three locations/rubble beds. Cages with a 1 cm mesh size were attached to the blocks, with the plastic frames with the corals attached to the base inside. Cages were cleaned weekly during the experiment by carefully removing the plastic frames whilst wearing gloves, and scrubbing them using brushes. The plastic frames holding the substrates were moved aside to avoid being covered in material released from cleaning, and the frames themselves were carefully brushed also.

To test whether organic material released from coral mortality influenced the following colonisation process, corals were also collected for comparison to be viewed as controls. These were carefully cleaned of all coral tissue to represent ‘bare’ substrates with no decomposing

organic material. To do this, corals were collected from the same sites at the same depth in the same manner. At the research base, coral tissue was removed using a high-pressure water jet. After a thorough washing, corals were left to air dry before drilling and were then placed out on the reef in the cages on the plastic frames. At each rubble bed, one of the three blocks were dedicated to controls only, to minimise transfer of material from bleached corals influencing colonisation. Herein, these substrates are referred to as control substrates within this study.

Following deployment, all coral substrates were left for either 14 days, 50 days or 90 days in the cages on the reef. Due to the re-incubation of the majority of *Porites*, this meant that sampling became staggered; i.e. the colonies which did not recover their symbodinium were left out for the full 90 days, whereas the colonies that were re-incubated were placed out for 14 and 50 days in order to run the experiment within the time frame. Furthermore, fewer tanks were available, so incubations took place in batches, with the 50-day *Porites* incubated and deployed first and then later on the 14-day *Porites*. The first sampling that took place was of the 14-day *Pocillopora* fragments. This sampling was used as a trial run to streamline and finalise the sampling process, and it was decided not to include the data from this period. Therefore, additional fragments were collected, incubated and redeployed for the 14-day sampling for *Pocillopora*. This meant that the final order of sampling for bleached corals ran as follows; *Pocillopora* 50 days, then 90 and finally 14; for *Porites* it ran 50 day, then 14 and finally 90. Control substrate deployment and collection ran in this order for both *Pocillopora* and *Porites*; 50 days, then 90 days and finally 14 days. Due to time restrictions in running the stereo videos in July, the control substrates that were deployed on the reef for 14 days were only deployed at the end of July, nearly two months after the other substrates were deployed and at a time when the bleaching event had ended. Due to this, I excluded these substrates from some of the stable isotope analyses, as it was felt that conditions on the reef had changed. This was shown through stable isotope data exploration, where these samples appeared as outliers. The full experiment took place over a four-month period, with the first sampling taking place in May 2019 and the final sampling for the 14-day controls in August 2019.

3.3.3 Substrate processing and collection of surface material

Substrates were placed into individual Ziplock bags at point of collection from the cages on the reef, with fragments stored in water from the site. These were closed and placed into a cooler box on the boat filled with local sea water for transportation back to the lab. Canopy height was measured using callipers at the laboratory before removing the fragments from their bags. A measurement was taken from areas visually appearing longest, shortest and then three randomly across the surface (canopy measurements per fragment n=5). This would capture the maximum, minimum and general variation across the substrate surface. Material was removed from the entire surface of the fragment by using a high-pressured water jet of filtered sea water (with a particle retention of 0.2 µ) using a Waterpik nano water flosser. This was collected in the Ziplock bag to include any sediment that was lost during transport. This was then filtered using a vacuum pump and a glass microfibre filter (0.7 µ). For all substrates excluding the 14-day substrates, two filters were required to capture all the material, which was combined together across analyses for each sample. Gloves were worn during all stages, and tools were cleaned between each sample by first being placed into a bleach bath for at least 5 minutes and then rinsing in a Milli-Q bath. Once all surface material was collected onto the filter, the sample was split between the four analyses methods listed below.

3.3.4 Organic and inorganic loads

A wet weight of the filter was taken by wetting the filter and then removing excess water with the pump. This was subtracted from the final wet weight to get weight of sample. Samples collected at 14 days and 50 days were split into three, with one portion of the material taken for assessment of organic/ inorganic mass, one portion going for Stable Isotope Analysis and the rest of the material going for metabarcoding. Samples at 90 days were split into four, due to additional mass available at this time, with an additional portion going for microscopy analysis.

Wet weights were taken for each subsample to establish the percentage of the overall sample that went for ashing. Samples were dried at 60°C until at a constant weight and weighed to the nearest 0.001 g. The organic load (or ash free dry weight—AFDW) and inorganic load of each sample was determined by reweighing the sample after ashing at 550 °C for 1 h (Fang et al. 2013, Marshell & Mumby 2015). Due the nature of the pressure washing and filtering, the sample was viewed as homogenised with no major variations between each of the subsamples. After washing, photogrammetry was used to determine the surface area of each substrate, and this was used to calculate g per cm^2 for both organic and inorganic weights. Photogrammetry is the process by which a large quantity of images of a structure from various angles are combined within a software creating a 3D image (Ferrari et al. 2017). By using a base with known measurement lines, this allows us to calculate size, volume and surface area. Photogrammetry was done using Agisoft Metashape 1.7. These values were then divided by percentage of the overall sample taken for ashing to standardise as total g per cm^2 across each sample.

3.3.5 Stable isotope analysis of colonised substrates

Samples were freeze dried in Falcon tubes for transport. Once at the lab, they were rinsed thoroughly by filling the tube with Milli Q water to remove any excess salt residue. The sample was agitated and then left to settle for a few hours. Once all the material had settled, the water was decanted off taking care not to disturb the material in the bottom. This was repeated and then the Falcon tubes were left open to dry at 60 °C. Due to the high content of water within the samples after washing, this took between 5 – 7 days until the samples were fully dried. Once dried, samples were each ground into a fine powder using a pestle and mortar and liquid nitrogen to break down the tough algal tissue. All dried samples were weighed, alongside the relevant standards, for stable isotopic analyses. Samples were then run on an IsoPrime Dual Analyser to determine signatures of stable isotopes and elemental content. The stable isotopic (δ15N) and elemental analyses (%N) samples were run on an Isoprime100 Isotope Ratio Mass

Spectrometer (IRMS) linked to an Elementar VARIO MICROcube Elemental Analyser at Lancaster Environment Centre, Lancaster University.

3.3.6 Live coral samples

To compare microbial and algal communities of healthy corals, 5 colonies of Porites and 5 fragments of *Pocillopora* (separate colonies) were collected at the beginning of the experiment (May). Colonies/fragments were collected from the same area of reef at the same depth, and were placed into Ziplock bags directly on the reef. These were sealed in water from the site, placed into a cooler full of site water and transported back to the lab. At arrival at the lab, the Ziplock bags were drained of water and placed immediately into -80 °C freezer for later processing. To break the fragments/colonies down into pieces for metabarcoding, the frozen substrates were placed in-between two large steel plates within a plastic tub. The top plate was struck with a hammer to shatter the fragment. This was repeated until there were pieces small enough for collection into cryovials. Pieces were taken randomly from across the full surface of the colony/fragment, with Porites samples targeting the surface layer and leaving older internal layers behind. Both the tub and the plates were first washed thoroughly, and then soaked in a bleach bath for a minimum of 5 minutes and then placed into a Milli-Q bath. All materials were cleaned in this way between each sample with gloves changed also.

3.3.7 Stable isotope analysis of live coral tissue

To collect coral tissue for comparison of healthy corals, 5 *Porites* colonies (similar size to before) and 5 *Pocillopora* fragments from separate colonies were collected from the reef and transported back to the laboratory in the same manner as above. To minimise the amount of mucus produced by the corals in the cleaning stage, the corals were placed directly into the -80°C freezer prior to processing for one week. Corals were brought out of the freezer and left to

defrost in their bags for 30 minutes, and then tissue was removed by using a high-pressure water jet of Milli-Q water. The washing was collected in the sample bag, and transferred to 50 ml Falcon tubes at the end. For most corals there was approximately 200 ml of washing (4 Falcon tubes). These were then vortexed using a pipette before centrifuging at 4 °C at 1500 RCF (relative centrifuge force) for 10 minutes to separate the coral tissue. The supernatant containing the tissue was poured off into a new 50 ml Falcon tube being careful to retain the algal pellet in the bottom of the initial tube, if required for future analysis. The supernatant was then centrifuged again at 7197 RCF for 5 minutes. Following the second centrifuge 15 ml of supernatant was retained for freeze drying, the rest was discarded.

3.3.8 Metabarcoding

When the surface material had finishing filtering, the portion that was to go for metabarcoding was placed into a cryovial and snap frozen using liquid nitrogen to fix microbial communities as quickly as possible. Samples were then stored in -80° until extraction. Samples were homogenised using pestle and mortar, and DNA was extracted using a PowerPlant RNA isolation kit (Qiagen, Hilden, Germany). Sample homogenisation and DNA extraction was conducted at the CRIOBE research base in Mo'orea.

A portion of the chloroplast was PCR amplified from each genomic DNA sample using the p23SrV_f1 and Diam23Sr1 23S primers (forward primer: GGACAGAAAGACCCTATGAA; reverse primer: TGAGTGACGGCCTTTCCACT). Both forward and reverse primers also contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. Each 40 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 12.5ul Master Mix, 0.5 µM of each primer, 1.0 µl of gDNA, and 10.5 µl DNase/RNase-free H2O. DNA was PCR amplified using the following conditions: initial denaturation at 94 °C for 3 minutes, followed by 40 cycles of

30 seconds at 94 °C, 45 seconds at 55 °C, and 1 minute at 72 °C, and a final elongation at 72 °C for 10 minutes.

A portion of mitochondrial 16S rDNA gene was PCR amplified from each genomic DNA sample. Both forward and reverse primers (forward primer: GTGYCAGCMGCCGCGGTAA; reverse primer: GGACTACNVGGGTWTCTAAT) contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. Each 25 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 12.5ul Master Mix, 0.5 µl of each primer, 1.0 µl of gDNA, and 10.5 µl DNase/RNase-free H2O. DNA was PCR amplified using the following conditions; initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 45 seconds at 95 °C, 1 minute at 50 °C, and 90 seconds at 72 °C, and a final elongation at 72 °C for 10 minutes.

To determine amplicon size and PCR efficiency, each reaction was visually inspected using a 2% agarose gel with 5µl of each sample as input. Amplicons were then cleaned using the UltraClean 96 PCR Cleanup Kit (384) (cat#12596-4) (QIAGEN, Germantown, MD) according to the manufacturer's protocol. A second round of PCR was performed to give each sample a unique 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 µM of each primer and 2 µl of template DNA (cleaned amplicon from the first PCR reaction) and consisted of an initial denaturation of 95 °C for 3 minutes followed by 8 cycles of 95 °C for 30 sec, 55 °C for 30 seconds and 72 °C for 30 seconds. 5µl of indexing PCR product of each sample were visualized on a 2% agarose gel to ensure the success of the barcoding PCR. Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA). 25µl of PCR amplicon is purified and normalize using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol. Samples are then pooled together by adding 5µl of each normalized sample to the pool. Sample library pools were sent for sequencing on an Illumina MiSeq (San Diego, CA) in the Texas A&M Sequencing Center using the v2 500-cycle

kit (cat# MS-102-2003). Necessary quality control measures were performed at the sequencing center prior to sequencing.

16S amplicons were processed via a joint QIIME (Caporaso et al. 2010) and UPARSE (Edgar 2013) pipeline similar to that of Andrei et al. (2015), with modification. Sequences were demultiplexed by taking advantage of Golay barcodes (Caporaso et al 2012) via QIIME v1.9.1 (Caporaso et al. 2010). The following options were used to output raw unfiltered fastq files for both forward and reverse reads: split_libraries_fastq.py -q 0 --max_bad_run_length 250 --min_per_read_length_fraction 0.0001 --sequence_max_n 250 --store_demultiplexed_fastq…. Paired-ends where then merged by the –fastq_mergepairs option of usearch v8 (Apprill et al. 2015). Primer sequences were then trimmed using cutadapt v1.8.1 (Martin 2011) to remove the primers 515fB (5'- GTGYCAGCMGCCGCGGTAA -3') and 806rB (5'- GGACTACNVGGGTWTCTAAT -3') (Apprill et al. 2015, Parada et al. 2015, Walters et al. 2016). Sequences were discarded if either primer was not detected or the final merged sequence length was less than 100 base-pairs. From here, general quality filtering and OTU table construction was completed as per the UPARSE pipeline (Edgar 2013) by clustering reads at 97% sequence similarity using de novo chimera detection defaults. The following alterations to the pipeline were implemented: the –minh option of -uchime_ref was set to 1.5 for reference-based chimera removal; to reduce the false positive detection of chimeras. The OTU table was generated by mapping quality filtered reads back to the OTU seeds by setting the following –usearch_global parameters: -maxaccepts 64 -maxrejects 1024. These parameters help to avoid over-inflation of specific OTU counts and ensure that individual reads are correctly mapped to their respective OTUs. Consensus taxonomy was assigned via the RDP classifier (Wang et al. 2007) on a custom-made SILVA v128 database (Pruesse et al. 2007).

On completion of the first run there was limited amplification for samples, despite presence of DNA (minimum= 9.8 ADN MetF ng/µl, max= 124 ADN MetF ng/µl, mean= 62.2 ADN MetF ng/µl). The full protocol was run again, however with less DNA during the PCR process. For 16S, each 25 µL PCR reaction was mixed using 12.5ul Master Mix, 0.5 µl of each primer,

however this time with only 0.5 µl of gDNA, and 11.0 µl DNase/RNase-free H2O. For 23S, each 40 µL PCR reaction was mixed including 12.5ul Master Mix, 0.5 µM of each primer, and with 0.5 µl of gDNA, and 11.0 µl DNase/RNase-free H2O. All other steps remained the same.

3.3.9 Summarising ASVs

Prior to analysis, full data on amplicon sequence variants (ASV's) was only included if the sequence match was 97% or higher. Callahan et al. (2017) argue that analysis of sequencing data in the form of amplicon reads (single DNA sequences recovered) over clustering reads into Operational Taxonomic Units (OTUs) is more exact and informative, rather than applying arbitrary dissimilarity thresholds. For those samples with a sequence match of >80% but ≤97%, these were assigned the broadest taxonomic classification at the phylum level. Values for ASV's were converted into Relative Read Abundance (RRA) by dividing by total number of ASV values for that sample (Deagle et al. 2018). Following this, data was grouped and RRA was summarised at each taxonomic level. To visualize the metabarcoding data, we used non-metric dimensional scaling (NMDS) plots. Since these are rank ordered, they are viewed as robust comparisons for relative read abundance data (Casey et al. 2019). I tested for differences between communities using a PERMANOVA, and then taxa driving changes were identified using a SIMPER analysis (Benkwitt et al. 2018).

While running the sample with a lower quantity of DNA improved the level of amplification for the majority of *Porites*, there was still limited amplification for *Pocillopora* samples. Of the 5 samples run, only 1 of each sampling type ran successfully. Due to this, only those samples from bleached corals at 50 days were included for *Pocillopora* in the 23S analysis as 3 of the 5 samples ran successfully. For those samples that failed to amplify, DNA was present in the sample, as this was tested prior using a qubit drop. There were some issues with amplification for *Porites*, however these only applied to the 14-day controls and the 90-day controls, where

only 1 out of 5 samples successfully amplified. Because of these issues, 23S NMDS analysis was only run on bleached *Porites* samples, and bleached *Pocillopora* samples at 50 days.

For 16S, amplification was improved across samples after diluting DNA, however this improvement was still reduced for *Pocillopora* samples in comparison to *Porites*. Samples for *Pocillopora* at 50 days was sufficient for both substrate types (2 or more samples out of 5 for both bleached and control). All samples for *Porites* successfully amplified (2 or more out of 5 for both bleached and control), excluding 90-day controls. One of the two samples for *Porites* bleached substrates that amplified for 90 days only yielded 2 ESVs, with both of those having low resolution. Due to this, samples for *Porites* 90 days was excluded from the analysis. This resulted in a final NMDS analysis including samples for *Porites* for healthy live corals and controls for 14-day and 50-day samples. *Pocillopora* samples (both bleached and control) at 50 days were also included.

3.3.10 Morphological assessment of algae

The portion of subsample from samples collected from 90-day substrates to go for microscopy analysis was preserved in a solution of buffered formaldehyde in seawater (3%). Slide preparation and identification of macrophyte and cyanobacteria species based on morphology was done according to Zubia et al. (2016, 2018). The data was recorded as presence absence, since relative abundance values can be challenging to quantify using such approaches (Zubia et al. 2016, Zubia et al. 2018). Communities were again visualised using NMDS plots as these are robust to presence absence data using Bray-Curtis distances (Bennett et al. 2008). The main differences driving communities was identified using SIMPER.

3.3.11 Statistical analyses

To compare differences in means for canopy heights, organic material, inorganic material and ratios of organic to inorganic through time for each treatment throughout the time series we used Kruskal Wallis test, followed up by a pair wise Wilcoxon test using Benjamin & Hochberg (1995) method to adjust p values to identify where differences were found. Running multiple statistical tests does increase the risk of a Type I error, however approaches such as Bonferroni or the Hochberg correction account for this to some degree with the adjusted p-value considering the number of statistical tests used (Andrade 2019). There are debates in the literature around the utility of altering the alpha level from 0.05 to account for Type I errors (Bender & Lange 2001), therefore I chose to keep it at 0.05. Differences between canopy heights of bleached and control corals within each sampling period was done using a t-test, with data either being log or square root transformed to account for skewness. Due to the small sample size, I kept to simple analyses over complex models. There were 30 observations per coral type for canopy heights (5 measurements for 6 samples), whereas organic and inorganic loads only had one measurement per substrate (n= 6). Normality for transformed data was tested using Shapiro-Wilk test. 14-day controls failed normality after both log and square root transformations, so the non-parametric Mann Whitney test was used instead. Canopy heights for control 90-day samples were square root transformed for normality, the rest were log transformed. For comparisons between organic load, inorganic load and ratios between treatments and coral types within sampling periods, the non-parametric Mann Whitney test was used as low sample size meant that transformations to deal with skewness were not applicable.

To compare between $\delta^{15}N$ and total % nitrogen of coral types, Mann Whitney test was used due to low sample size (n=5). Visualising trends by length of deployment (14 days- 50 days -90 days) showed limited trends by all coral types and treatments for both $\delta^{15}N$ and total % nitrogen. Due to the extensive bleaching at the beginning of the experiment, I hypothesised that sampling proximity to the bleaching could be the major driver of trends. I then compared both $\delta^{15}N$ of samples and total % nitrogen with the chronological order of number of days that a sample was collected to see if collection time since the peak of bleaching had an effect. Day 1

was for the first samples collected, which were bleached *Poclliopora* samples that had been deployed for 50 days. I run a full linear model with all variables (number days, deployment duration, coral type and treatment), and then compared with null models using ANOVA to see which variables had significant effects. This was done for both $\delta^{15}N$ and total % nitrogen. I also included data points for two sampling periods of *Pocillopora* colonies that bleached and died naturally during the bleaching event (14 days n=4, 55 days n=3). I also dropped the two sets of 14-day controls as these were deployed on the reef two months later than the other substrates, and the bleaching event had ended by this point, likely explaining why $\delta^{15}N$ was lower in these substrates.

All analyses were done using R version 3.6.1 (R Core Team 2019). Data cleaning and organising was done using the tidyverse package (Wickham et al. 2019), graphs and plots were done using ggplot2 (Wickham 2016). Kruskal-Wallis tests and pairwise Wilcoxon rank sum tests were run using rstatix package (Kassambara 2021). GLMM models were run using lme4 (Bates et al. 2015). Species accumulation curves, NMDS plots, SIMPER and PERMANOVA were done using the vegan package (Oksanen et al. 2020).

3.4 Results

3.4.1 Canopies and loadings

Canopy heights were significantly higher on bleached corals than for controls at 14 days for *Pocillopora* (t= 2.80, df= 50.96, p< 0.01) and Porites (t= 3.62, df= 43.32, p< 0.01, Figure 3.1). There was no significant difference by treatment (bleached versus control) on canopy heights for *Pocillopora* at 50 days (t= 1.92, df= 42.13, p= 0.06) or 90 days (t < -.01, df= 52.77, p= 0.99), with the same holding true for Porites at 50 days (t= -1.46, df= 51.03, p= 0.15) and 90 days also (5= 1.38, df= 56.62, p= 0.17). No significant difference was found between mean canopy height throughout the time series for bleached corals, both for *Pocillopora* (H(2)= 4.05,

p= 0.13) and for *Porites* (H(2)= 0.41, p= 0.81). For the control substrates, the 14-day communities were significantly lower for *Pocillopora* (H(2)= 15.61, p< 0.01) however there was no difference between 50 and 90-day communities (p< 0.01). For *Porites* controls, canopy heights were significantly different at all time periods (H(2)= 19.87, p< 0.01), with the 14-day being significantly lower than both 50-day (p< 0.01) and 90-day (p= 0.01), and 50-day being significantly higher than 90-day (p= 0.04). There was no significant difference in canopy heights between bleached *Pocillopora* or bleached *Porites* at 14-days (t= -1.36, df= 45.5, *p*= 0.18), however mean canopy heights were significantly higher on bleached *Porites* than bleached *Pocillopora* at 50-days (t= -3.21, df= 64, *p*< 0.01) and at 90-days (t= -2.07, df= 60.1 *p*= 0.04). Canopy heights were not significantly different for 14-day controls between coral types (W= 289, *p*= 0.15), or for 90-day controls (t= -0.21, df= 56.8 *p*= 0.84), however they were significantly higher on *Porites* controls at 50 days than for *Pocillopora* (t= -2.0, df= 47.1 *p*= 0.05).

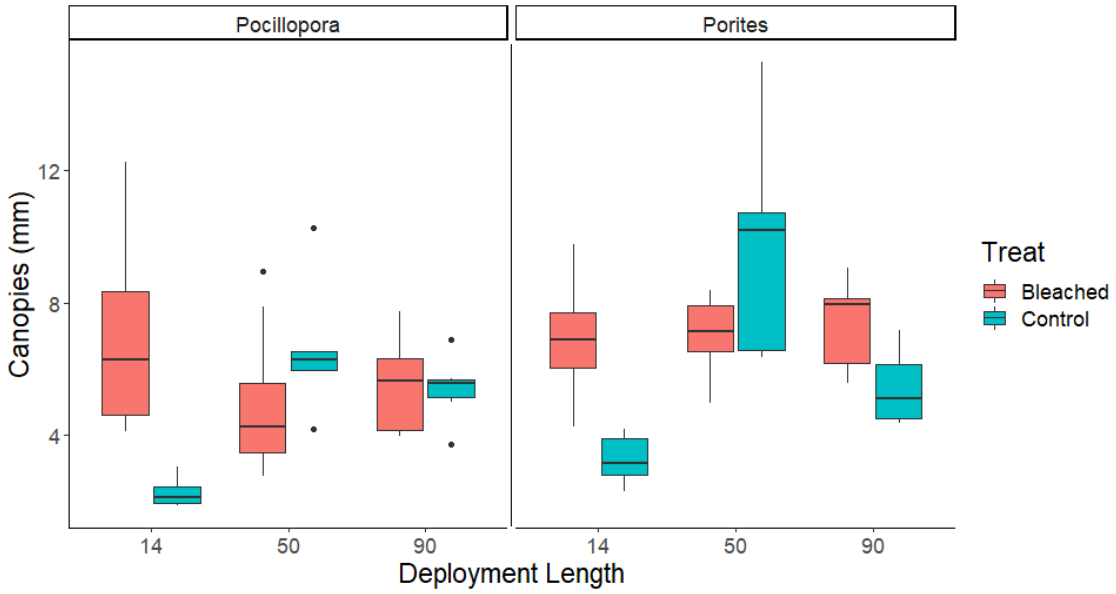


Figure 3.1 Canopy heights for each coral type and treatment over the 3 sampling periods (n= 5 for each sample).

For organic loads, boxplots revealed that there were two outliers for organic loads for bleached *Pocillopora* (values > 0.0025 g / cm^2), with one observation for 50-day and one for 90-day that were removed from the analysis. Treatment (bleached versus control) had no effect on organic

loads for *Pocillopora* at all time periods; for 14 days (W= 18, p= 0.06), 50 days (W= 10, p= 0.41) and 90 days (W= 31, p= 0.68, Figure 3.2a). For *Porites*, organic loads were significantly lower on control substrates than bleached corals at 14 days (W=15, p= 0.04), however there was no significant difference at 50 days (W= 8, p= 0.46) or at 90 days (W= 15, p= 1). Mean organic load for bleached *Pocillopora* increased through time, however, due to high variation at 14-day this was only significant (H(2)= 6.95, p= 0.03) between 50-day and 90-day samples (p= 0.05). There was no significant difference between mean organic loads for bleached *Porites* (H(2)= 0.50, p= 0.80). For control substrates, 14-day samples were the lowest, for both *Pocillopora* (H(2)= 6.80, p= 0.03) and *Porites* (H(2)= 8.10, p =0.02). Mean organic load for control 14-day *Pocillopora* was lower than both 50-day and 90-day (H(2)= 8.42, p= 0.54 for both), with mean organic load of 14-day control *Porites* being lower than 50-day and 90-day (H(2)= 8.10, p= 0.26 for both). There was no significant difference between organic loads for bleached corals between *Pocillopora* and *Porites* at 14 days (W= 5, *p*= 0.55), 50 days (W= 17, *p*= 0.62) or at 90 days (W= 32, *p*= 0.43). There was aslo no significant difference between organic loads for control *Pocillopora* or *Porites* at 14 days (W= 7, *p*= 1), at 50 days (W= 6, *p*= 0.40) or at 90 days (W= 20, *p*= 0.81).

Inorganic loads were not influenced by treatment (bleached versus control) for either coral type (Figure 3.2b). This applied to both *Pocillopora* at 14 days (W= 12, p= 0.71), 50 days (W= 15, p= 1) and 90 days (W= 32, p= 0.60), and for *Porites* at 14 days (W= 10, p= 0.55), 50 days (W= 9, =p 0.59) and 90 days (W= 14, p= 0.93). Even though mean inorganic loads appeared to decrease through time for bleached *Pocillopora*, due to high variation there was no significant difference between each sampling period (H(2)= 2.03, p= 0.36). This trend was the same for *Pocillopora* controls (H(2)= 01.22, p= 0.55). There was no significant difference in inorganic loads between time periods for bleached *Porites* also (H(2)= 1.98, p= 0.37). Inorganic loads were significantly lower (H(2)= 6.89, p= 0.03) at 14 days on control *Porites* substrates than on 50 days (*p*= 0.05), where mean loads were at their highest. Mean loads decreased gradually by

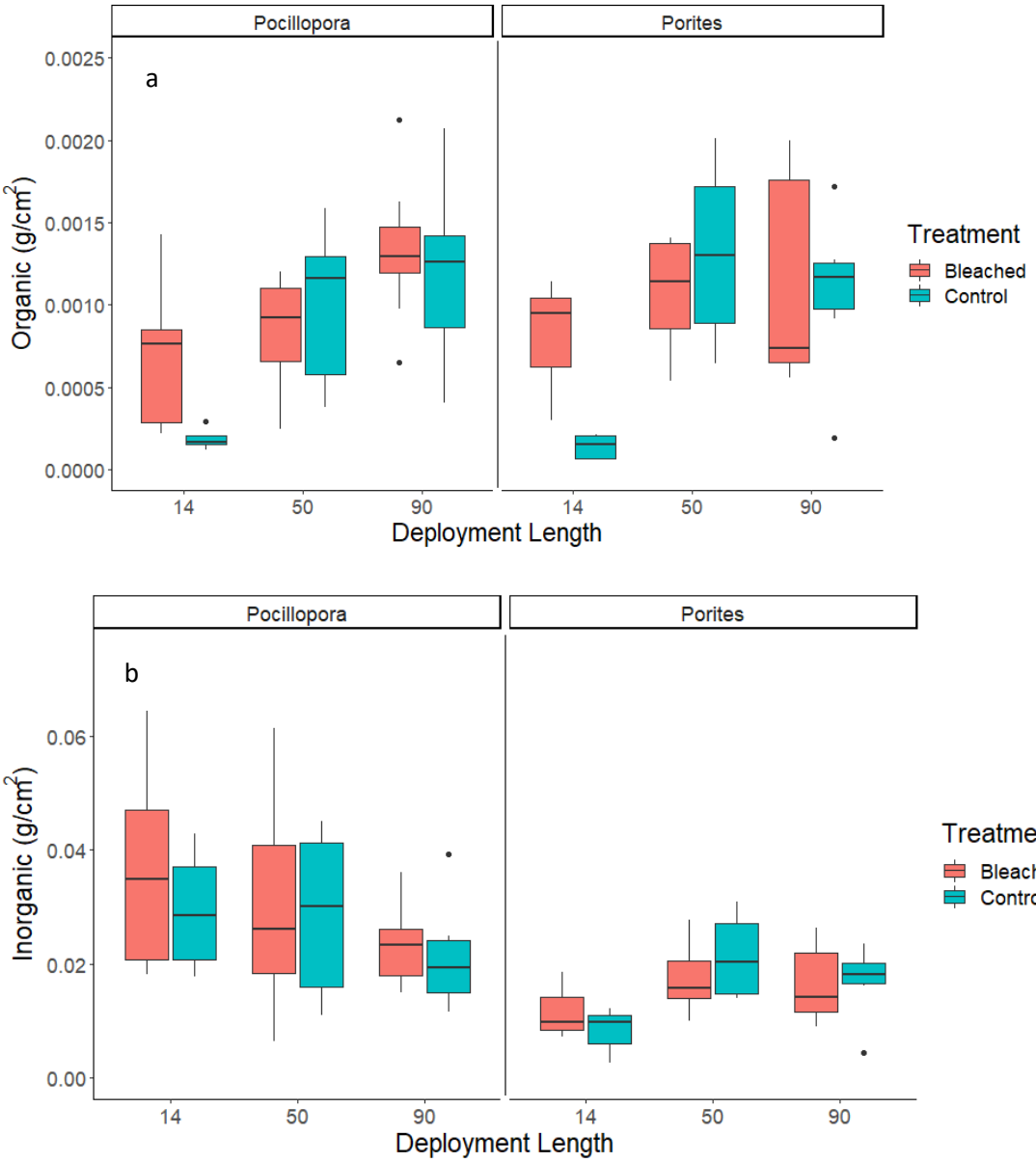
90 days, with no significant difference between 14 days and 90 days (p= 0.08) or 50 and 90 days (p= 0.76) for control *Porites* substrates.

Even though mean inorganic loads were higher on control *Pocillopora* (0.0245 g/ cm^2) than for control *Porites* (0.0082 g/cm^2) at 14 days, there was no significant difference due to high variation in *Pocillopora* samples (W= 15, p= 0.27). Mean inorganic loads were slightly higher on *Pocillopora* controls (0.029 g/ cm^2) than for *Porites* (0.022 g/ cm^2) at 50 days, however due to high variation in *Pocillopora* samples this was not significant (W= 13, p= 0.54). The same was observed for control substrates at 90 days, with mean organic load for *Pocillopora* at 0.022 g/ cm^2 and 0.018 g/ cm^2 for *Porites*, with no significant difference between the two substrates (W= 21, p= 0.69). For bleached corals, there was no significant difference between inorganic loads at either 14 days (W= 14, p= 0.07), 50 days (W= 27, p= 0.17) or at 90 days (W= 34, p= 0.14).

Due to gradual decreases to inorganic through time, and gradual increase to organic, mean ratio of organic matter to inorganic matter increased through time for bleached *Pocillopora* (H(2)= 13.67, p <0.01, Figure 3.2c). Mean ratios were significantly lower at 14 days than at both 50 days (p= 0.05) and 90-day (p< 0.01), with day 50 also being significantly lower than 90-day (p< 0.01). A similar trend was observed for control *Pocillopora* also (H(2)= 8.69, p= 0.01), where 14-day was significantly lower than both 50 days (p= 0.05) and 90 days (p= 0.05) mean ratios. However, due to high variation at 90-day, this was not significantly different from 50-day (p= 0.08). For control *Porites*, ratio of organic to inorganic load was significantly lower (H(2)= 9.71, =< 0.01) at 14 days than both day 50 (p= 0.02) and day 90 (p= 0.01), however there was no difference between day 50 and day 90 (p= 0.48). For bleached Porites, there was no significant difference in mean ratio of organic to inorganic load for any time period (H(2)= 0.07, p= 0.96). Treatment (bleached versus control) had a significant effect on the ratio of organic to inorganic loads for *Pocillopora*, with ratios of organic to inorganic loads being higher on bleached corals than controls at 14 days (W= 20, p= 0.02), but not at 50 days (W= 15, p= 1) or 90 days (W=31, p= 0.68). The same held true with *Porites*, where the ratio or organic

to inorganic loads was higher on bleached corals than controls at 14 days (W= 15, p= 0.04), but not at 50 (W= 15, p= 0.59) or 90 days (W= 17, p= 0.78).

For bleached substrates, there was a significantly higher ratio of organic material to inorganic material on *Porites* (mean = 0.067 g/ cm^2/ g inorganic) than on *Pocillopora* (mean= 0.018 g/ cm^2/ g inorganic, W= 0, *p*= 0.04) at 14 days. This was also true at 50 days (W= 4, *p*= 0.03), with mean ratios 0.032 g/ cm^2 for *Pocillopora*, and 0.064 g/ cm^2/ g inorganic for *Porites*. However, by 90 days there was no significant difference in ratios of organic to inorganic loads between bleached *Porites* (0.067 g/ cm^2/ g inorganic) and *Pocillopora* (0.064 g/ cm^2/ g inorganic, W= 19, *p*= 0.69). For control substrates, ratio of organic to inorganic loads were significantly higher on *Porites* substrates than for *Pocillopora* at 14 days (W= 0, *p*= 0.02) and at 50 days (W= 0, *p*= 0.02). However, by 90 days there was no significant difference in ratios of organic to inorganic loads (W= 14, *p*= 0.58).


Pocillopora
Porites
a
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
Organic (g/cm2)
14
50
90
Deployment Length
Treatment
Bleached
Control
b
0.06
0.04
0.02
0.00
Inorganic (g/cm2)

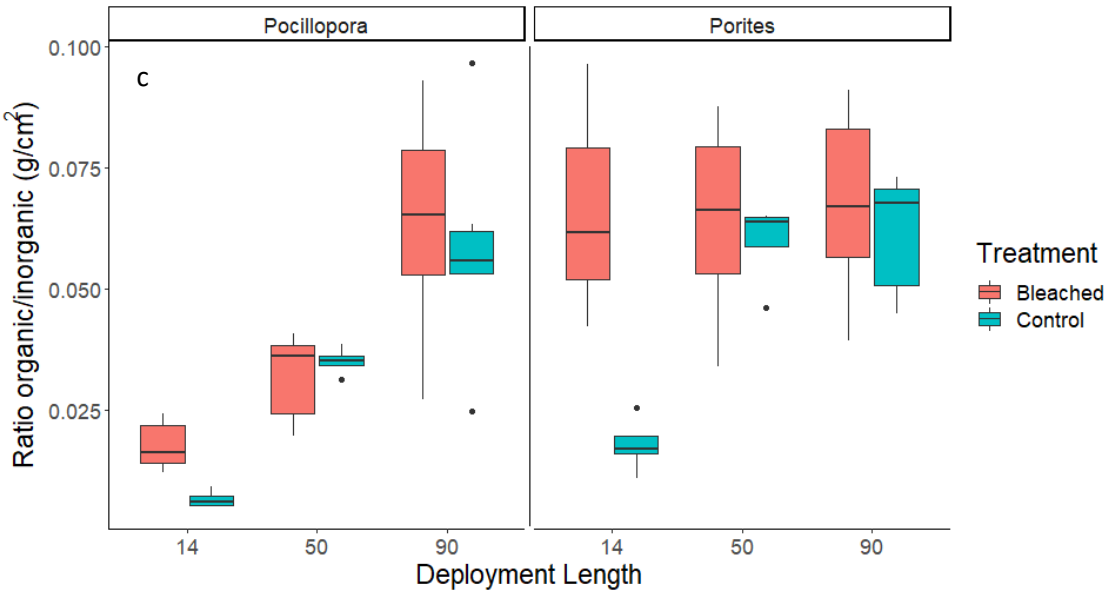


Figure 3.1. Comparisons between coral type and treatment for each sampling time period (number of days of deployment on the reef) showing a) organic loadings, b) inorganic loadings and c) ratio of organic to inorganic.

3.4.2 Stable isotope analysis

Comparisons of $\delta^{15}N$ for healthy living coral tissue varied by coral type (Figure 3.3). *Pocillopora* had a significantly higher amount of $\delta^{15}N$ than *Porites* (W= 25, p= 0.01), with total nitrogen % being significantly higher for *Pocillopora* also (W= 25, *p*= 0.01). Visualising trends of $\delta^{15}N$ chronologically according to day sampled across all samples found a significant positive linear relationship with collection date (t= 7.03, df= 76, *p*< 0.01, Figure 3.4), which corresponds to proximity to the peak of the bleaching event. Likelihood ratio tests between null models against the full model found that both duration of deployment (χ^2= 0.04, df= 1, *p*= 0.84) and coral type and treatment (χ^2= 0.00, df= 1, *p*= 1) did not have a significant effect, with chronological sampling day being the only significant variable (χ^2= 35.10, *p*< 0.01), associated with increased $\delta^{15}N$ by 0.03 ± 0.004 se. Total % nitrogen decreased through time when compared with chronological sampling day (t= -6.67, df= 54, *p*< 0.01). Again, likelihood ratio tests showed that only sampling day was significant (χ^2= 25.03, *p*< 0.01), with deployment

duration (χ^2= 1.87, p= 0.17) and coral type and treatment (χ^2= 0.02, p= 0.89) having no effect (Figure 3.4). Sampling day was associated with decreased total nitrogen % by 0.04 ± 0.007 se.

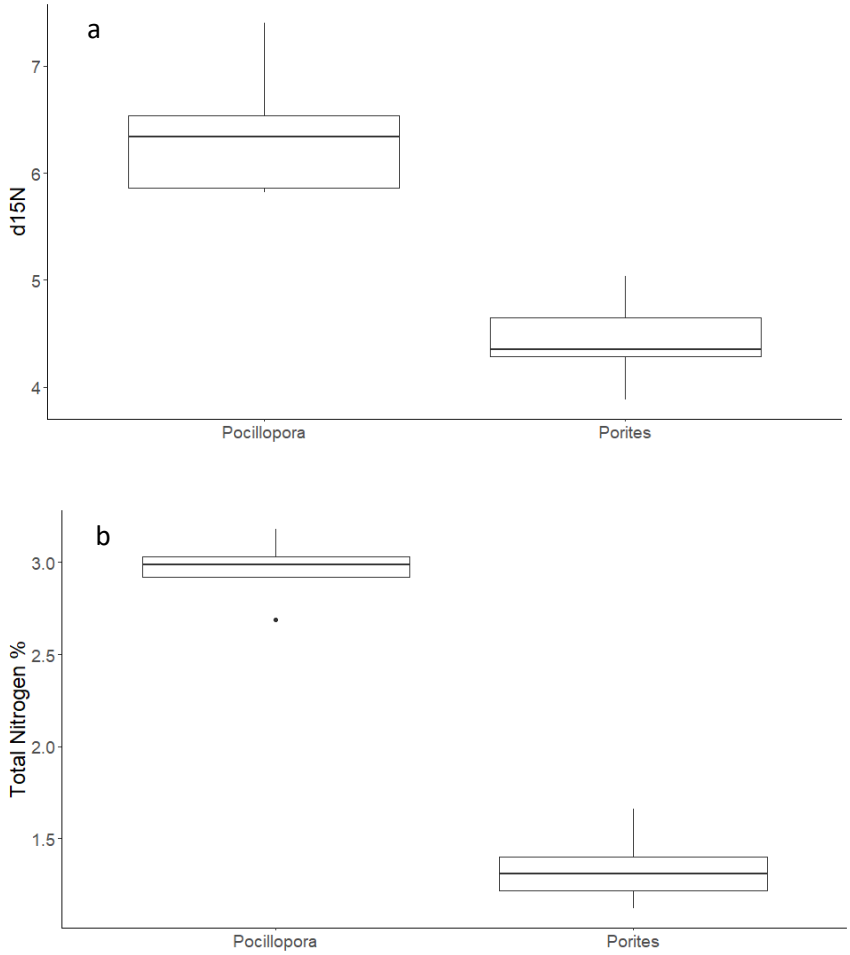
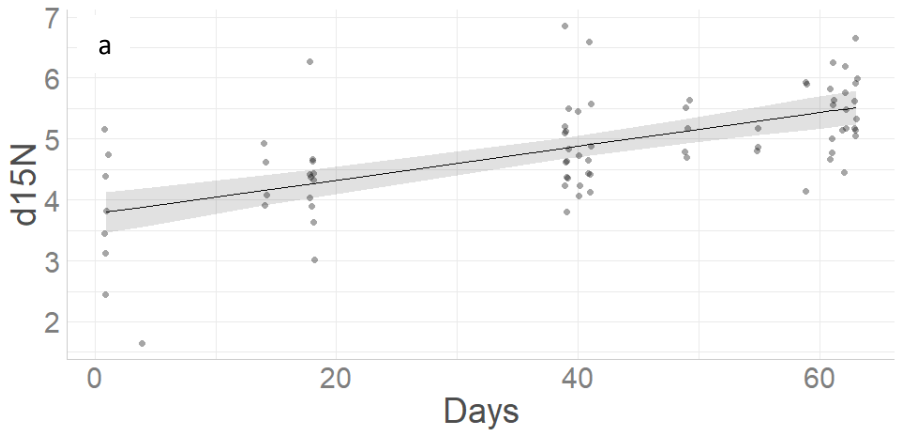


Figure 3.2. Comparisons between *Pocillopora* and *Porites* healthy live coral tissue for a) δ^{15}N and b) total nitrogen %.



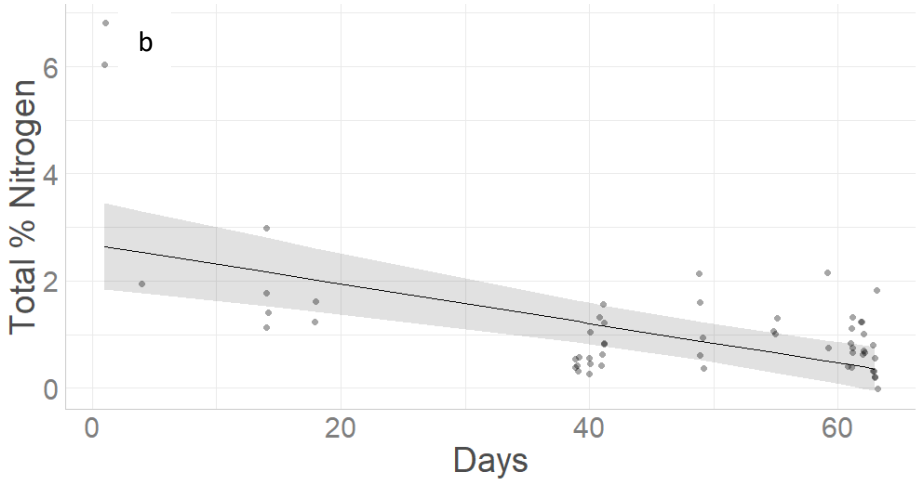


Figure 3.3. Partial plots from the linear models of the number of days of chronological sampling and a) $\delta^{15}N$ and, and b) total nitrogen %. All samples from all substrate types and treatments were used, excluding 14-day controls. Peak of bleaching occurred March/ April, with the first sample collected (day 1) on 04/06/19.

3.4.3 Metabarcoding

For 23S I found a total of 848 unique ESVs across all samples, with 395 ESVs with a sequence match of ≥97%, and 453 ESVs with a sequence match of <97% but ≥80%. For 16S, I found a total of 1829 unique ESVs using 16S across all samples. Of these, 1283 had a sequence similarity match of ≥97%, with 505 ESVs having a sequence match of between <97% and ≥80%, with 27 ESVs having a sequence match of <80% and being dropped from the analysis. This gave 70% of the data for 16S at high resolution, but only 47% of the data for 23S.

3.4.3.1 23S results

There was a significant difference between communities analysed with 23S by PERMANOVA when all samples including healthy live corals were included (F= 2.46, p= 0.001, Figure 5a). However, when these were dropped, and only colonised communities were compared, there was no significant difference (F= 1.34, p= 0.14, Figure 5b). The results from the SIMPER analysis from the NMDS with all samples is discussed further, primarily comparing how

colonised substrates compare with live coral communities as this still described some community progression, especially with bleached *Porites* substrates through time.

A large portion of reads using 23S were unassigned Rhodophytes, which were the highest on bleached *Porites* substrates at 14 days, due to a sequence similarity match of <97% (Figure 6). Unassigned reads were also prominent within Streptophyta and Chlorophyta. The main differences in samples identified by the SIMPER analysis were the presence of Apicomplexa only in live corals and not in any colonised substrates. *Cylindrotheca closterium* of the order Bacillariales was also more prevalent in live corals, as was an unassigned Chlorophyte. Bleached *Porites* at 14-days a higher proportion of unassigned rhodophytes and ESVs assigned to the cyanobacteria *Moorea producens* within the order oscillatoriales. Most of the ESVs driving changes for bleached *Porites* at 50 days were within the rhodophyte community. These were mostly *Ceratodictyon* within the rhodymeniales, *Heterosiphonia crispella* within ceramiales and *Peyssonnelia inamoena* within gigartinales. Bleached *Porites* substrates at 90-days showed a shift in the rhodophyte community. *Heterosiphonia crispella* still drove changes between bleached substrates and live corals, however this time *Wrangelia elegantissima* and *Laurencia* sp. were also observed. One ESV with a sequence match of <97% within the phylum ochrophyta was also higher on bleached Porites at 90 days.

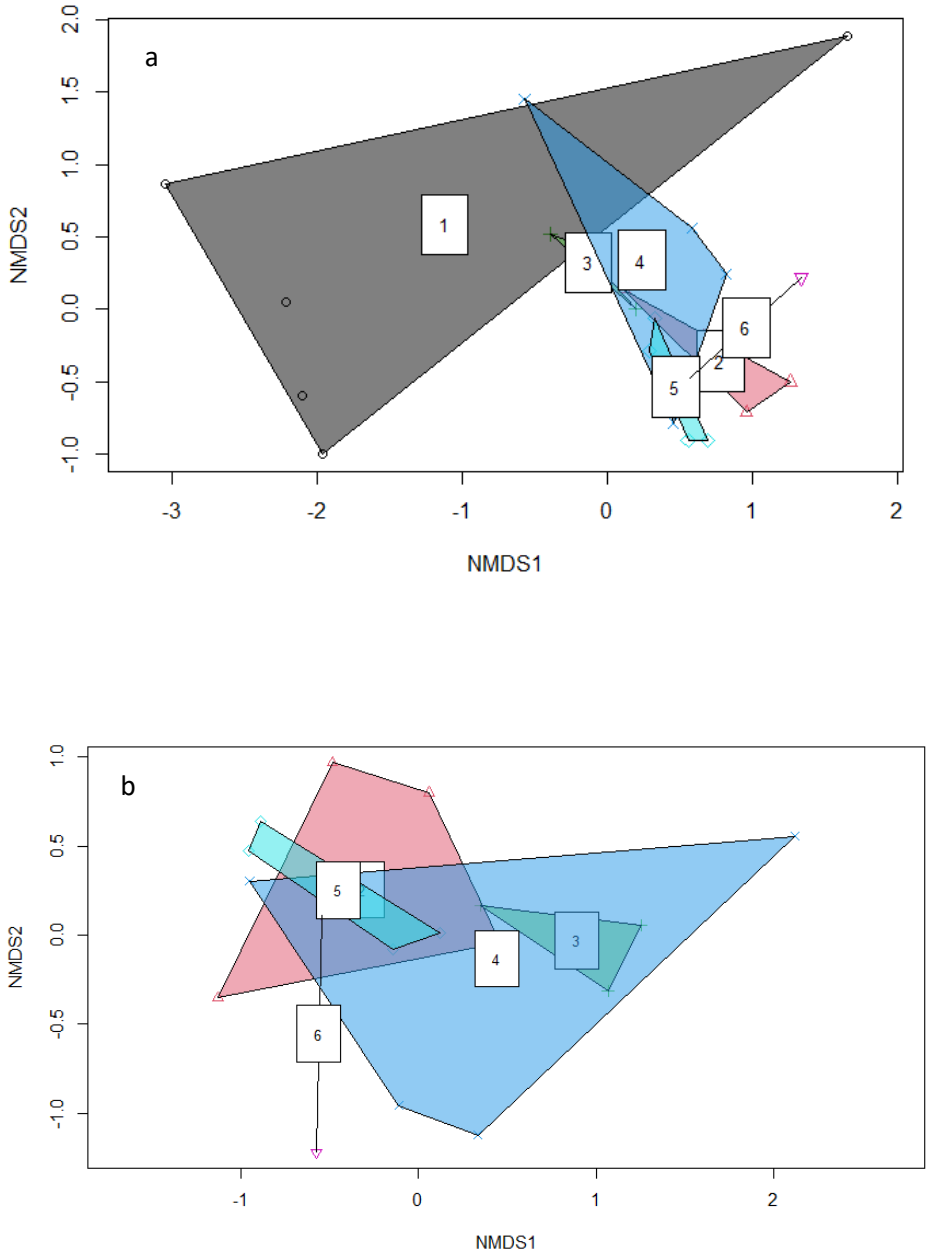


Figure 3.4. NMDS plot for 23S metabarcoding data, for all samples including healthy live Porites samples (1, Figure 5a), and then the reduced plot excluding healthy live corals and including bleached Porites at 14 days (2), 50 days (4) and 90 days (6). Control Porites substrates were also included for 50 days (5), as well as bleached Pocillopora samples at 50 days (3). Stress was 0.1.

Phylum	Order	PR	PR14	PR50	PC50	PR50CT	PR90
Apicomplexa	NA						
Cercozoa	NA						
Streptophyta	Gleicheniales						
	Poales						
Cyanobacteria	NA						
	Nostocales						
	Oscillatoriales						
	Pleurocapsales						
	Synechococcales						
Haptista	NA						
NA	NA						
Bacillariophyta	Bacillariales						
	Cymbellales						
	Licmophorales						
	Melosirales						
	NA						
	Naviculales						
Chlorophyta	Bryopsidales						
	Chloropicales						
	NA						
	Ulvales						
Ochrophyta	Dictyotales						
	Ectocarpales						
	Florenciellales						
	NA						
	Pelagomonadales						
	Sphacelariales						
Rhodophyta	Bonnemaisoniales						
	Ceramiales						
	Erythropeltidales						
	Gelidiales						
	Gigartinales						
	NA						
	Rhodymeniales						

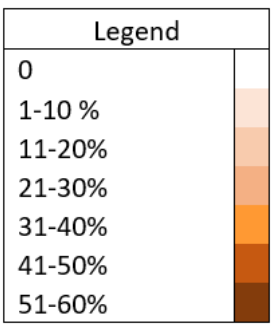


Figure 3.5. Heatmap of relative read abundance using the 23S marker, summarised at the level of order and phylum for each sampling period, substrate type and treatment. Numbers denote the number of days that the substrates were deployed, PR are for *Porites* corals, PC is for *Pocillopora*, and CT for control substrates. NA denotes those that were unassigned. RRA was summarised across samples at each level and then divided by the number of samples.

Unsurprisingly, the biggest differences between live *Porites* coral communities and colonised substrates arose from samples for bleached *Pocillopora* at 50 days, and control *Porites* at 50 days. Mean number of ESVs driving changes from the SIMPER analysis that were higher for bleached *Porites* through time (14, 50 and 90 days) was 9.33. The number of ESVs driving changes between colonised substrates and live corals, which were higher in colonised substrates for bleached *Pocillopora* at 50 days was 24 ESVs, and 75 ESVs for control *Porites* at 50 days.

For *Pocillopora* 50-day samples, the ESVs driving changes with live coral communities were comprised of diverse phyla (10 ESVs for rhodophytes, 8 ESVs for bacillariophytes, 5 ESVs for cyanobacteria and 1 ESV for ochrophytes). A broad selection of ESVs within rhodophytes drove changes across ceramiaceae, rhodomelaceae, rhodymeniales and gigartinales. The diatom community also showed diverse changes for bacillariaceae within the order naviculales. Cyanobacteria were either for *Moorea producens*, or unidentified past phylum level.

Porites controls showed the majority of changes between live corals within the diatom community, with 65 out of the 75 ESVs driving changes being assigned as bacillariophyta. Of these, 24 were unassigned, with the majority of assigned ESVs being within naviculales and bacillariales. Of the remaining ESV diversity, 4 were unassigned cyanobacteria, 3 were unassigned ochrophytes and 1 ESV for scytosiphonaceae within ectocarpales.

3.4.3.2 16S results

There was a significant difference between communities analysed with 16S by PERMANOVA (F= 5.07, p= 0.001). Similar to 23S, the biggest differences between communities arose from healthy live *Porites* and then colonised substrates, however with 16S this was even more pronounced (Figure 7a). Due to this, I ran the PERMANOVA and SIMPER analysis again, this time excluding healthy live *Porites* samples in order to explore differences between colonised substrates. Comparing communities excluding healthy live corals still showed a significant difference (F= 1.99, p= 0.008). To compare how bleached *Porites* substrates compared to

communities associated with healthy live *Porites* corals, we used SIMPER outputs from the full model (Figure 6a). When comparing between colonised substrates, we used the model output excluding communities associated with healthy live corals (Figure 7b).

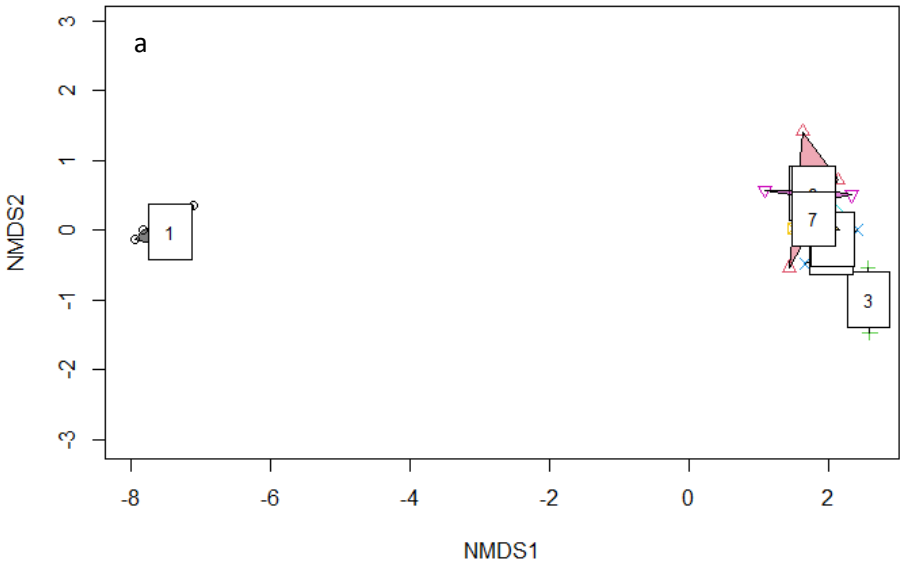
There were a high proportion of reads unassigned even at phylum level, and these were especially prominent for live corals (Figure 8). Other than this, most of the variation found between ESVs were primarily within proteobacteria and cyanobacteria. ESVs driving changes that were found proportionally higher in live corals was lower than for those that were found proportionally higher in colonised substrates (number of ESVs driving changes that were higher in live corals = 4, higher in bleached *Porites* at 14 days= 22, bleached *Porites* at 50 days = 61). Healthy live *Porites* substrates had a higher proportion of unassigned cyanobacteria ESVs, and *Endozoicomonas* sp. within gammaproteobacteria. Bleached 14-day *Porites* consisted of unassigned cyanobacteria ESVs, along with bacteroidia, alphaproteobacteria and acidimicrobiia. Control *Porites* at 14 days had a high number of ESVs within cyanobacteria (44), the majority of which were unassigned, as well as a high number of ESVs driving changes within proteobacteria (36). These were diverse and spread between both alphaproteobacteria and gammaproteobacteria.

Bleached *Porites* at 50 days had a higher proportion of reads from the archea *Nitrosopumilus* sp, as well as a high number of unassigned cyanobacteria. Those that were assigned were from the family nodosilineaceae. For the 14 ESVs driving changes within proteobacteria, the majority were for gammaproteobacteria, with 2 ESVs for alphaproteobacteria. Control *Porites* at 50 days showed a higher proportion of reads from the family puniceicoccaceae within verrucomicrobiae, as well as a few ESVs associated with unassigned cyanobacteria.

Comparing bleached *Pocillopora* and control *Pocillopora* at 50 days, the number of ESVs driving changes were lower on controls (5) than on bleached substrates (14). The ESVs that were higher on controls were split between alphaproteobacteria and gammaproteobacteria, whereas for bleached substrates the majority of ESVs (12) were unassigned cyanobacteria, with the remaining ESVs within gammaproteobacteria. Comparing bleached *Pocillopora* at 50 days

with bleached *Porites*, *Porites* were once again differentiated by the archea *Nitrosopumilus* sp., as well as a high number of ESVs for gammaproteobacteria.

Comparing controls at 50 days, this time *Porites* showed a lower number of ESVs driving changes (9) than for *Pocillopora* (28). *Porites* were dominated by unassigned cyanobacteria, whereas *Pocillopora* showed a diverse community within proteobacteria across both alphaproteobacteria and gammaproteobacteria.



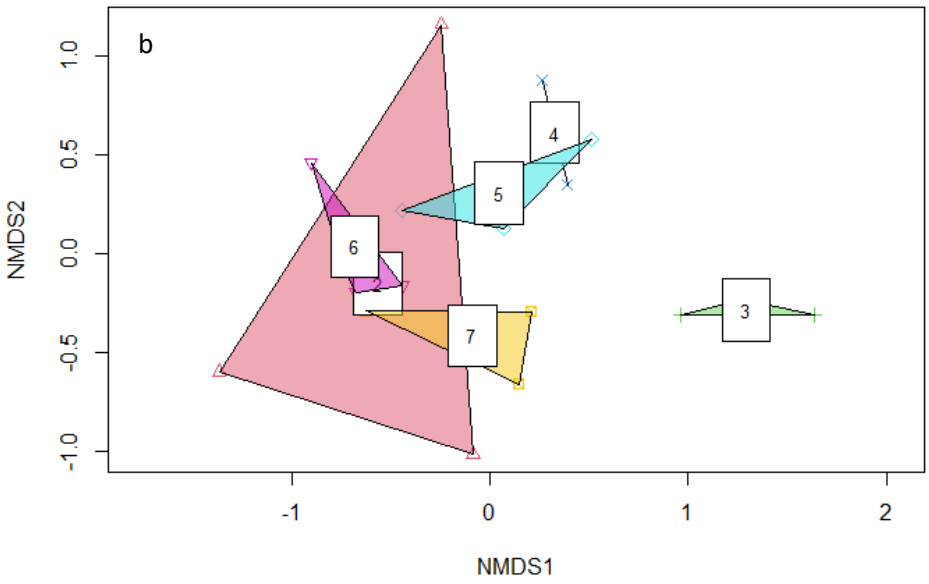


Figure 3.6. NMDS plots for 16S metabarcoding data, for the full model including healthy live *Porites* samples (1; Figure76a), and then the reduced model excluding healthy live *Porites* (Figure 7b), including bleached *Porites* at 14 days (2) and 50 days (7). Control *Porites* substrates were also included for14 days (3) and 50 days (6), as well as bleached *Pocillopora* samples at 50 days (4) and control *Pocillopora* samples at 50 days (5). Stress was 0.08 for the full model and 0.2 for the model excluding live *Porites* corals.

Phylum	Class	PR	PR14	PR14CT	PC50	PC50CT	PR50	PR50CT
Acidobacteriota	Acidobacteriae							
	Blastocatellia							
	NA							
	Subgroup 26							
	Thermoanaerobaculia							
Actinobacteriota	Acidimicrobiia							
	NA							
Bacteroidota	Bacteroidia							
	NA							
	Rhodothermia							
Bdellovibrionota	Bdellovibrionia							
	NA							
	Oligoflexia							
Campylobacterota	Campylobacteria							
Chloroflexi	Anaerolineae							
	NA							
Chlorophyta	Ulvophyceae							

Crenarchaeota	Nitrososphaeria	
Cyanobacteria	Cyanobacteriia	
	NA	
	Vampirivibrionia	
Dadabacteria	Dadabacteriia	
Dependentiae	NA	
Desulfobacterota	Desulfobacteria	
	Desulfobulbia	
	Desulfovibrionia	
Firmicutes	Bacilli	
	Clostridia	
	NA	
Fusobacteriota	Fusobacteriia	
Gemmatimonadota	PAUC43f	
Myxococcota	NA	
	Polyangia	
NA	NA	
NB1-j	NA	
Ochrophyta	Phaeophyceae	
Patescibacteria	NA	
Planctomycetota	NA	
	OM190	
	Phycisphaerae	
	Pla3 lineage	
	Planctomycetes	
Proteobacteria	Alphaproteobacteria	
	Gammaproteobacteria	
	NA	
Verrucomicrobiota	NA	
	Verrucomicrobiae	
WPS-2	NA	

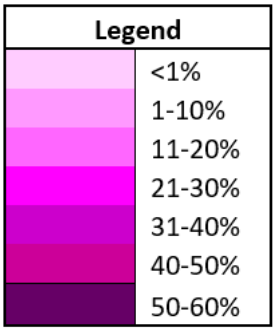


Figure 3.7. Heatmap of relative read abundance using the 16S marker, summarised at the level of phylum for each sampling period, substrate type and treatment. Numbers denote the number of days that the substrates were deployed, PR are for *Porites* corals, PC is for *Pocillopora*, and CT for control substrates. NA denotes those that were unassigned. RRA was summarised across samples at each level and then divided by the number of samples.

3.4.4 Morphological assessment of algae

Communities on colonised substrates at 90 days were found to be significantly different using PERMANOVA (F= 4.94, p= 0.001). Communities on control Porites were more distinct from the others, and these differences arose from the rhodophyte *Dasya iyengarii* within ceramiales oonly being found on *Porites* controls, whereas the cyanobacteria *Hydrocoleum* within oscillatoriales which was widely found across most substrates was less commonly found on *Porites* controls. Brown algae within ochrophytes were common across substrates, and mostly consisted of *lobophora* and *sphacelaria rigidula*.

I ran additional PERMANOVAs to see if coral type (F= 3.94, p= 0.002) and treatment type (F= 2.56, p= 0.012) had an effect, and these were both found to be significant. For the effect of coral type, the var. *laxa* of the rhodophyte *Heterosiphonia crispella* and the chlorophyte *Boodlea composita* within the order cladophorales were more commonly found on *Pocillopora* substrates, with neither of these being identified on *Porites* controls. For treatment type, an unidentified species of cladophorales and the ochrophyte *Hincksia* within ectocarpales were more commonly found on *Porites* substrates. In terms of treatment effects, the rhodophyte *Antithamnion* sp. within the order ceramiales was more common on bleached substrates, and was not found on control substrates. The cyanobacteria *Phormidium* within the order oscillatoriales was more common on control substrates than on bleached corals.

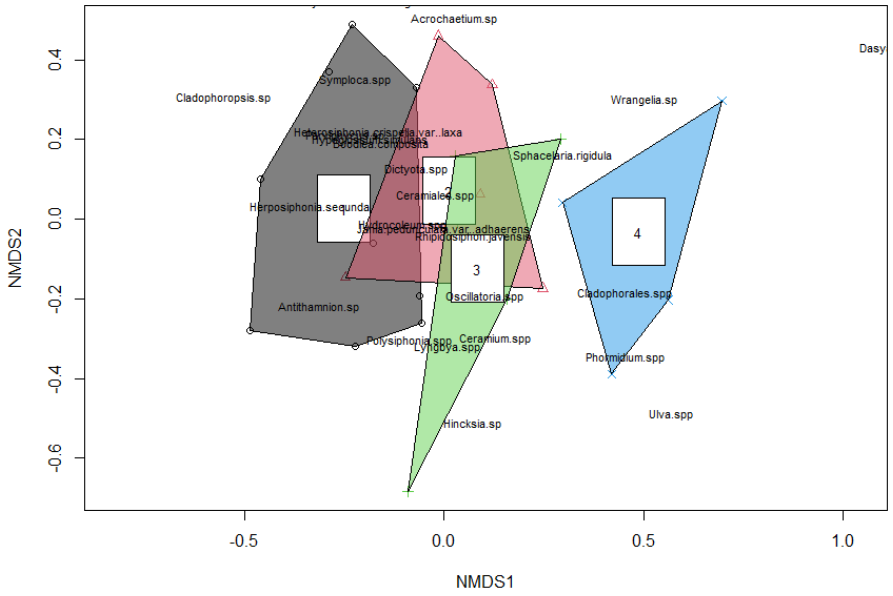


Figure 3.8. NMDS plots for the presence / absence community data from the microscopy analysis of samples at 90 days showing community composition for *Pocillopora* substrates that were bleached (1) or controls (2), and for *Porites* substrates that were either bleached (3) or controls (4). Stress was 0.23.

3.5 Discussion

Coral type and bleaching response affected patterns of organic and inorganic loads in developing algal communities, as well as community composition. Bleached *Pocillopora* substrates behaved similarly to controls in terms of canopy heights and organic and inorganic loads, whereas bleached *Porites* showed little to no variation in such features, indicating that partial mortality of the colony surface had a stabilising effect on these features. *Porites* controls showed that canopy heights and ratio of organic to inorganic load increased in the early stages (from 14 days to 50 days), however appeared to even out around this point. McClanahan (1997) found that algal community composition can stabilise over different time periods under varying grazing conditions, with some stabilising as early as at 50 days. Therefore, it is likely that *Porites* control substrates under a low grazing intensity stabilise at this time.

Longer turfs with greater canopy heights have been shown to trap more sediment (Bonaldo & Bellwood 2011, Gordon et al. 2016b, Purcell 2000, Purcell & Bellwood 2001). However, sediment trapping potential tends to decrease as algal communities shift away from fine filamentous turfs and begin to move towards macro-algae, and is no longer related to canopy height (Steneck 1997, Tebbett & Bellwood 2019). There was no evidence of late stage 90-day *Pocillopora* communities having a greater presence of brown algal ochrophytes over communities establishing on *Porites*, which did not show an obvious reduction to sedimentation loads through time. Microscopy showed that ochrophytes such as *Lobophora and S. rigidula* were common across all late stage communities, with the only difference arising from *Hincksia* being more commonly found on *Porites* substrates. *Pocillopora* communities at 90 days were characterised by *H. siphonia* and *B. composita* which show fine, densely packed branches which give them a fuzzy appearance. It may be that the presence of these species acts to slow water movement, as microhabitat variation and canopy heights have been shown to affect water flow speeds (Carpenter & Williams 1993), which in turn affects sedimentation rates (Gowan et al. 2014).

The majority of corals that bleached on the north coast of Mo'orea during the event were predominantly *Pocillopora* sp. (Edmunds et al. 2018), and I show through time the signature of $\delta^{15}N$ increasing to match that of healthy live *Pocillopora* corals. Nutrient uptake of material released was shown to occur gradually following the peak of bleaching regardless of substrate and treatment type. This supports previous studies that nutrient uptake can be rapid (Vaughan et al. 2021a). However, Vaughan et al. (2021a) show that these elevated signatures can be observed for up to one-year post bleaching. In this study, substrates deployed after the peak showed a lower uptake in these nutrients, suggesting that this occurs as a short pulse event and that all organic matter released has either already been taken up or dispersed. This elevated signature is therefore maintained through biogeochemical cycles, such as stored in algal tissues (Fong et al. 1994, Vaughan et al. 2021b) or reintroduced through excretion (Burkepile et al. 2013).

Measuring organic matter release and how this varies by benthic community composition and environmental features has been the focus of a number of studies (Haas et al. 2013, Courtial et al. 2018, Radice 2021), as has the introduction of new nutrients through cyanobacteria fixation in establishing algal communities (Davey et al. 2008). Niggl et al. (2009) show that organic matter release by corals increases in response to bleaching. However, to date there have been no studies that directly quantify this at the point of mortality. Comparisons of organic and inorganic trends in this study on *Pocillopora* fragments suggest that this is rapid and complete, since trends were the same on substrates which had manually had their tissue removed. Total nitrogen went down as $\delta^{15}N$ went up after the bleaching event. This supports other studies that show that coral release organic matter in response to bleaching that is depleted in nitrogen, however this was only found under elevated ultraviolet radiation levels (Courtial et al. 2018).

I found limited clustering of colonised communities and high overlap using 23S, which is likely in part due to the fact that the majority of samples were from *Porites* that showed a high level of partial mortality across colony surfaces, which may have masked transitional changes. Partial mortality of the colony surface typically results in the expansion of turfs and sediments (Nugues & Roberts 2003). Furthermore, the extent of bleaching and subsequent mortality across the colony surface of *Porites* has been found to influence algal community composition (Diaz-Pulido & McCook 2002). However, the fact that there was also high overlap between controls and bleached *Pocillopora* corals suggests that this may be indicative of generic successional patterns for algae that are not overly influenced by coral type or treatment. In this way, substrates likely go through typical successional patterns which are driven more by evolutionary strategies (k versus r selected species/ rapid growth, able to exploit new space versus slow growing competitors).

Previous studies show that cyanobacteria and diatoms tend to be early colonisers with ochrophytes dominating later communities that shift towards macro-algae (Hixon & Brostoff 1996, Diaz-Pulido & McCook 2002). While brown algae within ochrophytes were observed more in later stage communities, cyanobacteria were fairly prevalent across time periods and

substrate types within this study. Diaz-Pulido & McCook (2002) found that *Polysiphonia* sp. become more prevalent in later stage communities. This rhodophyte was common across all 90-day substrates using morphological approaches in this study, indicating that it was associated with late stage communities regardless of coral type or treatment. This shows that, while microhabitat variation may alter certain community traits, some species are driven more by time and successional stages, rather than small biotic variations.

Caging the samples likely had strong effects on successional patterns, as studies have shown that grazing pressure is highly influential of algal community composition (Sammarco 1983, Hixon & Brostoff 1998, Ceccarelli et al. 2011) which may explain why there was a high level of overlap of communities using 23S. This may also explain why features such as canopy heights and organic and inorganic loads changed little between 50 days and 90 days for many substrates. Most experimental studies show that grazing reduction tends to encourage communities to shift towards phaeophytes (Sotka & Hay 2009, Doropoulos et al. 2013, Duran et al. 2016). This is supported by this experiment, as ochrophytes were found to be higher on *Porites* communities at 90 days using 23S, with microscopy showing ochrophytes being common across all substrates at 90 days.

Apicomplexa are obligate intracellular parasites (Lévêque & Besterio 2016) that are commonly associated with corals and vertebrates in reef environments (Janouškovec et al. 2012, Keeling et al. 2021), hence why these were only found associated with living coral communities. Gammaproteobacteria were more common in earlier stage communities in this study, with an increase in alphaproteobacteria in later stage communities. This fits with research that shows that live corals and algae vary in the composition and amount of dissolved organic carbon that they exude, which in turn alters the community composition and metabolic rate of microbial communities within the surrounding water column (Haas et al. 2011, Haas et al. 2013, Nelson et al. 2013). Bacterioplankton communities associated with macroalgae have been shown to have a higher proportion of gammaproteobacteria from families such as Vibrionaceae (Nelson et al. 2013). These are known coral pathogens (Kvennefors et al. 2012, Blackall et al. 2015),

hence why these were likely found in earlier stage communities when corals were at their most stressed. Alphaproteobacteria have been associated with the decomposition and breakdown of lignin (Tao et al. 2020), which may be indicative of less coral tissue and more plant matter being recycled during this time.

The high proportion of unassigned taxa or low resolution reads for 23S shows that more work to develop the barcode database is needed in order to improve classification of species with retrieved sequences that tends to be higher for more standard markers like 16S and 18S rDNA (Yilmaz & Glöckner 2015, Marcelino & Verbruggen 2016). Comparisons of morphological approaches with molecular ones have been validated for invertebrates (Shackleton et al. 2020), however such studies are limited with turf algal communities. Such studies are necessary as the database is expanded, and to potentially identify gaps.

In conclusion, I found that coral bleaching response and micro-habitat variation influenced colonisation communities on establishing algal communities. This is one of the first studies to relate these community changes with features that are known to influence grazing behaviours of reef fishes, thereby linking benthic change following bleaching events with drivers to grazing rates and ecosystem functions. The small sample size within this study does raise the risk of type II errors with low statistical power. However, sample size was intentionally kept low in order to not have an impact on the reef since live corals were used. This was a trade off between low sample size and using natural substrates that would be a true representation of processes that occur in response to bleaching. Further studies are required to support the findings of this study, and similar approaches to this study should be applied to naturally bleached substrates under normal grazing pressure to corroborate these results and to explore to what extent experimental design may have influenced colonisation trajectories. The results of this study show that mass bleaching events rapidly release a huge amount of organic matter that is then taken up into colonising communities. Future shifts to dominance of coral types and their bleaching responses under thermal anomalies (such as an increased likelihood of *Porites*

bleaching and mortality) may influence establishing algal communities and their ability to provide attractive feeding sites for grazing fish.

4 Early stage successional turf communities following coral mortality and implications for juvenile reef fish grazing and fitness

4.1 Abstract

Mass coral mortality from bleaching events rapidly and drastically changes the foraging landscape for herbivorous reef fish. Availability and quality of resources for these fish affects grazing potential of fish communities and therefore affects resilience. However, there is still little detailed knowledge on how algal communities establish after bleaching events, nor how features that have been shown to influence grazing behaviour develop during this time. I document colonisation changes to turf algal communities developing on recently bleached corals using metabarcoding techniques, and measure canopy heights, organic and inorganic loading of the turf communities. I then link these changes to grazing preference and use respirometry as an indicator of fitness on fish fed specifically newly establishing turf communities and turf communities already established on rubble substrates prior to the bleaching event.

Using 23S and 16S markers, I found significant changes to community composition and associated features between turf communities developing on corals at approximately 2 months after peak bleaching compared to those that were present in 4 months after bleaching, as well as with established turf communities on rubble substrates. Fish actively avoided feeding from colonising communities 2 months after bleaching and selected rubble substrates, however at 4 months after bleaching there was no selection between establishing communities and rubble

substrates. Increases to inorganic loads appeared to be the main deterrent to grazing, which was not related with canopy heights and was likely driven by changes to algal community composition. Fish that fed exclusively on establishing turf communities at 4 months post bleaching had a higher standard metabolic rate than those that fed on rubble substrates, showing greater energy reserves for activity and growth.

My study shows that turf communities change rapidly during the colonisation process, varying their impact on feeding behaviours for young *Acanthurus triostegus*. As establishing turf communities develop, changes to community composition and increases to organic to inorganic ratios indicates that these turf communities become important grazing substrates for fish, helping to boost secondary productivity and act as a mechanism for resilience.

4.2 Introduction

Mass coral bleaching events that are linked to climate change and high sea surface temperatures can rapidly and drastically alter the foraging landscape for herbivorous reef fish (Hédouin et al. 2020). High rates of coral mortality result in not only a huge increase in available substrate for grazing (Hoegh-Guldberg 1999, Hughes et al. 2007, Harris et al. 2014, Holbrook et al. 2018) but they also alter the algal community composition temporally (Diaz-Polido & McCook 2002) and can affect reef sediment dynamics through loss of complexity (Tebbett et al. 2020). This transitional period while newly available substrate is colonised following wide-spread coral mortality may also affect nutritional pathways through a sudden input of nitrogen through fixation by cyanobacteria in the early colonisation stages (Webb et al. 1975, Davey et al. 2008, Niggl et al. 2009). The community of fine, turfing algae that grows on bare substrates is known as the epilithic algal matrix (EAM), capturing the high diversity of algal species and associated organic and inorganic material that is found within these communities (Wilson et al. 2003). The EAM is the primary food source for grazer and detritivore species, providing nutrition through either the algal tissue itself, or the associated detritus and microbial communities (Choat et al. 2002, Wilson et al. 2003, Clements et al. 2017).

Bare substrate is a rare commodity on reefs, therefore following bleaching events, competition on newly available space will be high. During the colonisation processes algal communities will change through time, with newly developing turf communities likely being different to established areas of climax turf communities (Diaz-Polido & McCook 2002, Mumby et al 2005). The colonisation process itself will vary, and may be influenced by the microbial communities present while the coral was alive or through the stress of bleaching (Ritchie & Smith 2004, Bourne et al. 2008). Hydrodynamic and localised sedimentation flows (Purcell 2000), levels of herbivory (Burkepile & Hay 2010, Clausing et al. 2014), and the degree to which particulate organic matter which is released through mortality is retained locally or dispersed (Coffroth 1990, Ritchie & Smith 2004) can also play a role. Sedimentation within algal turfs has been shown to reduce herbivory rates (Bellwood & Fulton 2008, Goatley & Bellwood 2012) by restricting access to food (Adam et al. 2018) or through the dilution of organic material (Gordon et al. 2016a, Tebbett et al. 2017). However, this varies between functional groups and feeding styles (Gordon et al. 2016a). Longer turfs with greater canopy heights have been shown to trap larger amounts of sediment (Bonaldo & Bellwood 2011, Gordon et al. 2016b, Purcell 2000, Purcell & Bellwood 2001), meaning that the potential for turf communities to trap sediment will likely change throughout the colonisation process as communities grow. Such studies show that successional patterns on reef substrata are likely more complex than previously imagined, therefore viewing recently deceased corals simply as 'available bare substrate' in terms of the nutritional landscape for herbivores, overlooks important fine scale details that will subsequently scale up to affect ecosystem processes (Clements et al. 2017).

When high coral cover reefs experience extensive coral mortality, grazer / detritivore species go from a scenario of being food limited, due to the relatively low initial availability of grazing substrate, to having food excess. This is thought to drive a number of herbivorous fish species to increase in both numbers and growth rates following coral mortality (Pratchett et al. 2008, Adam et al. 2011, Taylor et al. 2020). Despite these documented patterns, we still have limited

understanding of how benthic changes following coral mortality influence herbivore communities. If recently colonised turf communities vary in the amount and quality of food available to fish, the types, quality and abundance of resources could influence survival and fitness, particularly of juvenile fish. The influence of food abundance and quality on the health and body condition of fish can be captured through either lipid storage in the liver (Berumen et al. 2005) or through comparisons of metabolic rate (Auer et al. 2015, Zeng et al. 2018). The ability of an individual to convert food into a useable form of energy (ATP) is a fundamental process for survival and growth (Seebacher et al. 2010). While metabolic rate has been shown to display high plasticity (Auer et al. 2015, Norrin & Metcalfe 2018, Pettersen et al. 2018), studies have shown that many animals go into 'energy saving modes' with a reduced resting metabolic rate when food quality, abundance and predictability is low (Maldonado 2001, Cavieres & Sabat 2008, Langer et al. 2018). Greater food availability has been shown to increase standard metabolic rate (SMR), and in turn has been linked with increased growth rates in juvenile fish (Auer et al. 2015, Zeng et al. 2018).

Given the importance of grazing by fishes in mediating coral-algal competition, and how they interact with developing communities of the EAM, there are still surprisingly few studies characterising the short-term development of the colonisation process of these substrates in detail, and how these changes can shape herbivory rates. Many studies on the variation in turf communities are snapshot studies and focus on spatial variation at different scales (Bonaldo & Bellwood 2011, Tebbett et al. 2019), or are mechanistic studies that experimentally look at specific drivers such as nutrient (Smith et al 2001, Vermeij et al. 2010) or sediment enrichment (Clausing et al. 2014), and how this affects feeding behaviour by fishes (Tebbett et al. 2017). Few studies consider how these factors vary naturally and temporally, especially considering the rapid colonisation process following coral mortality. While some studies have documented patterns of algal community change through time following coral bleaching (Diaz-polido & McCook 2002), there still lacks fine scale taxonomic detail over broad functional characteristics, and short-term observational studies of natural processes on the initial and

dynamic early stages of colonisation, even though this is a crucial time in defining the outcome of these communities. Given the diverse and complex nature of turf algal communities, defining fine scale taxonomic and short-term changes to species composition can be challenging. One approach to deal with this is to use metabarcoding techniques, which overcomes the problematic nature of identifying items under microscopic examination by utilising the presence of DNA (Hays et al 2018). Metabarcoding is a burgeoning field in ecological studies that takes specific genetic markers that can be recovered from a broad variety of taxa and uses high-throughput sequencing to characterise community composition (Tabarlet et al. 2012, Thomas et al. 2016). Since identification can be made with limited material, especially when key distinguishing features are not present, it allows for greater detection of cryptic diversity (Robba et al. 2006).

Large scale bleaching events are forecast to increase in frequency and severity as the Anthropocene progresses (Hughes et al. 2018), with algal turfs poised to become increasingly more dominant (Tebbett & Bellwood 2019). Building our understanding of how the colonisation process affects turf communities temporally on newly available coral substrates, and the influence that these community changes have on the feeding rates and fitness of recruiting reef fishes will give greater insights into the processes involved for ecosystem resilience. Here, I ask the following questions: How do newly establishing turf algal communities on recently deceased corals following a bleaching event change within the period of two months, characterised by both community composition and organic and inorganic loading?; Do fish show any feeding preference for old established turfs or newly establishing turf communities on recently deceased corals, and does this vary through time?; To what extent is feeding influenced by variations in organic and inorganic loads, and/or species composition?; and does this influence metabolic rate/fitness of recruiting fish?

4.3 Methods

4.3.1 Study Site

The study took place on the island of Mo'orea (17°30′S, 149°50′W), located 17 km to the northwest of Tahiti in the Society Archipelago, which is part of the Windward Island group in French Polynesia. The island is encircled by a barrier reef which is approximately 500 – 700 m wide for the majority of the island, excluding the northern shore where it is closer. Tides are semidiurnal with an amplitude of less than 0.3 m (Chazottes et al. 1995; Leichter et al. 2013). The reefs of Mo'orea have faced a number of disturbances over recent decades, with the primary loss of live coral during this time arising from either cyclones or crown of thorns (COTs) outbreaks (Lamy et al. 2016). While Mo'orea has seen periods of elevated sea surface temperatures (SST), in terms of coral mortality this area was largely unaffected from some of the major global bleaching events in recent times, such as in 1998 and 2016 (Spencer et al. 2000, Berkelmans et al. 2004, Monroe et al. 2018, Koester et al. 2020). However, in 2019 outside of an El Niño year, this area saw a long period of elevated SST with widespread bleaching, and more than 50% coral mortality in some areas (Vaughan et al. 2021).

4.3.2 Substrate sampling

Coral colonies of the genus *Pocillopora* were tagged on the outer northern reef slope at the initial peak of bleaching (16/04/19) and fragments were later taken from each colony that had died (n= 5). This area was selected not only due to the high intensity of bleaching, but also because previous studies have shown that this area of the reef is least affected by terrestrial run-off (Leichter et al. 2013, Donovan et al. 2020.), meaning that algal communities should be less affected by external sources of nutrients. Three rubble beds at this site were used for collecting fragments of existing rubble. Both rubble and recently deceased corals were taken from the same upper part of the outer reef slope (10m depth) between 8 – 9 am. Resampling fragments from the same site prevents variation in sedimentation within turfs that is associated with location and depth changes (Purcell 2000, Gordon et al 2016b, Tebbett & Bellwood 2019).

Recently dead coral (DC) substrates were sampled at approximately 2 months after the peak of bleaching in June and 4 months after bleaching in August (n= 5), however rubble substrates were only sampled in August which equated to 4 months after the peak of bleaching (n= 3). However, with rubble substrates being viewed as established communities dominated primarily by climax species, it could be fair to assume that significant changes did not occur to the community within the two-month time period.

4.3.3 Substrate processing

Random fragments were selected from the substrate collection to process for organic and inorganic load. Fragments were placed into individual Ziplock bags at point of collection underwater with fragments stored in water from the site. Algal canopy height was measured using callipers at the laboratory before removing the fragments from their bags. A measurement was taken from areas visually appearing longest, shortest and then three random points across the surface (canopy measurements per fragment n=5). This aimed to capture the maximum, minimum and general variation across the substrate surface. Material was removed from the entire surface of the fragment by using a high-pressured water jet of filtered sea water (with a particle retention of 0.2 µ). This material was collected in the Ziplock bag to include any sediment that was lost during transport. This was then filtered using a vacuum pump and a glass microfibre filter (0.7 µ). A wet weight of the filter was taken by wetting the filter and then removing excess water with the pump. This was subtracted from the final wet weight to get weight of sample. The sample was split into half, with half the material taken for assessment of organic/ inorganic mass, and the rest of the material going for metabarcoding. Wet weights were taken for each subsample to establish the percentage of the overall sample that went for each analysis. After washing, photogrammetry was used to determine the surface area of each fragment, and this was used to calculate g per cm^2 for both organic and inorganic weights. Photogrammetry is the process by which a large quantity of images of a structure from various angles are combined within a software creating a 3D image (Ferrari et al. 2017). By using a

base with known measurement lines, this allows us to calculate size, volume and surface area. Photogrammetry was done using Agisoft Metashape 1.7. These values were then divided by percentage of the overall sample taken for ashing to standardise as total g per cm^2 across each sample. Samples were dried at 60°C until at a constant weight and weighed to the nearest 0.001 g. The organic load (or ash free dry weight—AFDW) and inorganic load of each sample was determined by reweighing the sample after ashing at 550 °C for 1 h (Fang et al. 2013, Marshell & Mumby 2015). Due the nature of the pressure washing and filtering, the sample was viewed as homogenised with no major variations between the two halves of the sample.

4.3.4 Metabarcoding

When the sample had finishing filtering, approximately half was collected and placed into cryovials and snap frozen using liquid nitrogen to fix microbial communities as quickly as possible. Samples were then stored in -80° until extraction. Samples were homogenised using pestle and mortar, and DNA was extracted using a PowerPlant RNA isolation kit (Qiagen, Hilden, Germany). Sample homogenisation and DNA extraction was conducted at the CRIOBE research base in Mo'orea.

Initial PCR 16S

A portion of mitochondrial 16S rDNA gene was PCR amplified from each genomic DNA sample. Both forward and reverse primers contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. Each 25 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 12.5ul Master Mix, 0.5 µl of each primer, 1.0 µl of gDNA, and 10.5 µl DNase/RNase-free H2O. DNA was PCR amplified using the following conditions; initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 45 seconds at 95 °C, 1 minute at 50 °C, and 90 seconds at 72 °C, and a final elongation at 72 °C for 10 minutes.

Initial PCR 23S

A portion of the chloroplast was PCR amplified from each genomic DNA sample using the p23SrV_f1 and Diam23Sr1 23S primers. Both forward and reverse primers also contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. Each 40 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 12.5ul Master Mix, 0.5 µM of each primer, 1.0 µl of gDNA, and 10.5 µl DNase/RNase-free H2O. DNA was PCR amplified using the following conditions: initial denaturation at 94 °C for 3 minutes, followed by 40 cycles of 30 seconds at 94 °C, 45 seconds at 55 °C, and 1 minute at 72 °C, and a final elongation at 72 °C for 10 minutes.

To determine amplicon size and PCR efficiency, each reaction was visually inspected using a 2% agarose gel with 5µl of each sample as input. Amplicons were then cleaned using the UltraClean 96 PCR Cleanup Kit (384) (cat#12596-4) (QIAGEN, Germantown, MD) according to the manufacturer's protocol. A second round of PCR was performed to give each sample a unique 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 µM of each primer and 2 µl of template DNA (cleaned amplicon from the first PCR reaction) and consisted of an initial denaturation of 95 °C for 3 minutes followed by 8 cycles of 95 °C for 30 sec, 55 °C for 30 seconds and 72 °C for 30 seconds. 5µl of indexing PCR product of each sample were visualized on a 2% agarose gel to ensure the success of the barcoding PCR. Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA). 25µl of PCR amplicon is purified and normalize using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol. Samples are then pooled together by adding 5µl of each normalized sample to the pool. Sample library pools were sent for sequencing on an Illumina MiSeq (San Diego, CA) in the Texas A&M Sequencing Center using the v2 500-cycle kit (cat# MS-102-2003). Necessary quality control measures were performed at the sequencing center prior to sequencing.

16S amplicons were processed via a joint QIIME (Caporaso et al. 2010) and UPARSE (Edgar 2013) pipeline similar to that of Andrei et al. (2015), with modification. Sequences were demultiplexed by taking advantage of Golay barcodes (Caporaso et al 2012) via QIIME v1.9.1 (Caporaso et al. 2010). The following options were used to output raw unfiltered fastq files for both forward and reverse reads: split_libraries_fastq.py -q 0 --max_bad_run_length 250 --min_per_read_length_fraction 0.0001 --sequence_max_n 250 --store_demultiplexed_fastq…. Paired-ends where then merged by the –fastq_mergepairs option of usearch v8 (Apprill et al. 2015). Primer sequences were then trimmed using cutadapt v1.8.1 (Martin 2011) to remove the primers 515fB (5'- GTGYCAGCMGCCGCGGTAA -3') and 806rB (5'- GGACTACNVGGGTWTCTAAT -3') (Apprill et al. 2015, Parada et al. 2015, Walters et al. 2016). Sequences were discarded if either primer was not detected or the final merged sequence length was less than 100 base-pairs.

From here, general quality filtering and OTU table construction was completed as per the UPARSE pipeline (Edgar 2013) by clustering reads at 97% sequence similarity using de novo chimera detection defaults. The following alterations to the pipeline were implemented: the –minh option of -uchime_ref was set to 1.5 for reference-based chimera removal; to reduce the false positive detection of chimeras. The OTU table was generated by mapping quality filtered reads back to the OTU seeds by setting the following –usearch_global parameters: -maxaccepts 64 -maxrejects 1024. These parameters help to avoid over-inflation of specific OTU counts and ensure that individual reads are correctly mapped to their respective OTUs. Consensus taxonomy was assigned via the RDP classifier (Wang et al. 2007) on a custom-made SILVA v128 database (Pruesse et al. 2007).

On completion of the first run there was limited amplification for samples at 2 months and for rubble substrates, despite presence of DNA (minimum 33 ADN MetF ng/µl). The full protocol was run again, however with less DNA during the PCR process. For 16S, each 25 µL PCR reaction was mixed using 12.5ul Master Mix, 0.5 µl of each primer, however this time with only 0.5 µl of gDNA, and 11.0 µl DNase/RNase-free H2O. For 23S, each 40 µL PCR reaction

was mixed including 12.5ul Master Mix, 0.5 µM of each primer, and with 0.5 µl of gDNA, and 11.0 µl DNase/RNase-free H2O. All other steps remained the same.

Summarising ASVs

Prior to analysis, full data on amplicon sequence variants (ASV's) was only included if the sequence match was 97% or higher. For those samples with a sequence match of >80% but ≤97%, these were assigned the broadest taxonomic classification at the phylum level. Values for ASV's were converted into Relative Read Abundance (RRA) by dividing by total number of ASV values for that sample (Deagle et al. 2018). Following this, data was grouped and summarised at each taxonomic level.

4.3.5 Fish collection and husbandry

Acanthurus triostegus is a common species found within the reefs of French Polynesia (Besson et al. 2017). In its adult form it feeds on and derives its nutrition from algae, which has been defined both through microscopic examination of stomach contents and stable isotope analysis (Frédérich et al. 2012) and is shown by its spatulate teeth for cropping fine algae (Holzer et al. 2017, Tebbett et al. 2017). This species recruits from the larval stage around the new moon, and have previously been collected and studied for developmental properties in this region (Frédérich et al. 2012, Besson et al. 2017, Holzer et al. 2017). These fish expend a great deal of energy going through metamorphosis from larval pelagic stage where they are planktivorous, to their juvenile stage where they become algal grazers (Holzer et al. 2017). The process involves changing not only their outer morphology, but also internal structures to adapt to this diet change, such as growing new sets of teeth and elongating their digestive tract (Holzer et al. 2017).

Newly recruiting *A. triostegus* were collected using hand nets from rock pools at the tide line around the new moon on the night of 05/06/19 for the June experiment (2 months after peak

bleaching) and 29/07/19 for the August experiment (4 months after peak bleaching). Fish were kept in 35L closed circuit tanks with a continuous flow through. Mean temperature of the tanks was 28.1 ± 0.9°C and they were kept under artificial lighting under a L12:D12 cycle. Tank temperatures rose slightly at midday due to increase in daily temperature, but this variation was similar between tanks. Fish were also collected on the 08/07/19 for respirometry and a trial of the fitness experiment.

4.3.6 Choice experiment

Individual fish were placed into a tank with one piece of each substrate type of visually similar sizes, with a GoPro either clipped to the edge of the tank or standing on a tripod. All cameras faced down onto the substrates from above and fitted both substrates into the frame. Cameras were left recording for 30 mins, with each fish being provided with their own new substrates. Each time a fish moved forwards and attached its jaws to the substrate this was recorded as a bite. A feeding foray was defined as a bout of successional bites where the fish only took time to reattach its jaws to the substrate, without looking around and with minimal movement. The time was recorded at the first bite of a foray, or for individual bites. The total number of bites was summarised per fish and was divided by the number of minutes of recording following the first bite once the fish was deemed acclimatised to give a bite rate per minute. Fish were defined as acclimatised once a fish was utilising the entire space of the tank so that it was aware of and comfortable with both substrates. Bites were not recorded, and clips were not used if fish were hiding and only briefly feeding on whichever substrate was closest, or patrolling along one side of the tank. If a fish repeatedly slipped back into anxious behaviour then the fish was excluded from the analysis. For analysis, clips were only included when the fish had settled and didn't display anxious hiding or patrolling behaviour. Out of the 6 fish collected in June (2 months post peak bleaching), 2 were excluded from the analysis due to excessive hiding/patrolling behaviour (final n= 4), whereas out of the 11 fish assessed for feeding behaviour in August (4 months post peak), 4 were excluded (final n= 7). Once a fish had been

recorded it was placed into a new tank of mixed substrates to avoid re-filming the same fish. This experiment was run for both June and August new moon recruitments, and was the first assessment after collection prior to respirometry.

4.3.7 Fitness experiment

For the trial run in June at 2 months post peak bleaching, fish were acclimated in tanks for 48 hours following collection with a mixture of substrates collected from the field, and then starved for 24 hours. Fish were then measured and weighed, and assessed for metabolic rate using intermittent-flow respirometry (Killen et al. 2021). Fish were weighed by placing briefly within a small dish on scales that had been tared to 0. For measuring body length, they were briefly placed into clear plastic bags with a small amount of water to lay them flat. Standard length and full length were then recorded using callipers. These fish were divided up for a trial for the fitness experiment (n per tank =9), which ran for two weeks. In the second week, fish displayed obvious aggression and territorial behaviour, and subsequently some fish began to lose visible body condition and die. Due to this, the experiment was stopped and no final respirometry was run on these fish.

For the second run in August at 4 months post peak bleaching, the experiment was run for a shorter duration (one week) with fewer fish per tank (n=7). Respirometry was also only assessed at the end of the experiment to limit stress prior to the start if the experiment, and I used the baseline metabolic rate post collection from the fish collected in July. This meant that fish did not go through a period of starvation prior to the start of the experiment. Fish were divided up for the experiment immediately after collection from the field, after the choice experiment was run. Food was provided for *ad libitum* as close as possible by providing many substrates within the tanks, and refreshing these daily from the reef. The number and approximate size of substrates was visually estimated to be comparable to avoid one tank having more grazing substrate than the other. Mean total length of fish in the recently dead

coral tank was 33 mm ± 1, and 33.5 mm ± 0.56 for the rubble tank. Studies have shown that *A. triostegus* completes ontogenetic changes at around 35 – 40 mm, indicating that these fish were still in the final phases of metamorphosis (Frédérich et al. 2012). Therefore, all fish in this study can be considered as post-larval. Mean weight of fish was slightly higher in the rubble tank at 5.6×10^{-4} g ± 2.47×10^{-5} than for the dead coral tank with 5.1×10^{-4} g ± 3.50×10^{-5}. However, there was no significant difference in weight of fish between tanks (Mann Whitney W=12, p= 0.379), or between mean total length of fish between treatments (W=12, p=0.371). To explore the effect of small sample size on the possibility of type II error I ran a power analysis using the pwr package in R (Champely 2020) using an effect size calculated from the fish weight data which estimated a sample size of 33. A sample size of 6 gave a power of 0.22, so a fair likelihood of a type II error risk, which must be taken into consideration when interpreting these results. After being kept in their treatments with a particular food source for a number of days, fish were placed into an empty clean tank and starved for 24 hours prior to respirometry. Fish within the rubble tank were kept for five days with their substrates, whereas due to competition for equipment use, the fish within the dead coral tank were kept for six.

4.3.8 Respirometry

Intermittent-closed respirometry experiments to measure oxygen uptake rate (MO_2) were run to quantify standard metabolic rate (SMR) (Steffensen 1989, Clark et al. 2013). Fish were starved for 24hr in aquaria prior to being placed into 0.38 L chambers. The intermittent respirometry cycles consisted of a measurement (closed) period followed by an open period during which the respirometry chambers were flushed with fully aerated water from the ambient tank. Measurement and flush periods lasted 2 and 3 minutes respectively.

4.3.9 Statistical analyses

Comparisons between means used Mann Whitney non-parametric tests as data was right skewed and only consisted of small sample sizes meaning that transformations for parametric comparisons were not feasible. This applied to comparisons of organic and inorganic loadings (n= 5 for dead coral, n = 3 for rubble), to the comparison of mean bites per minute per substrate type in the choice experiment (2 months n= 7, 4 months n= 4) and to comparisons of SMR in the fitness experiment (for both dead coral and rubble tanks, n= 6). Community analysis of metabarcoding data used non-metric dimensional scaling (NMDS) plots. Since these are rank ordered they are widely viewed as robust comparisons for relative read abundance (RRA) for ASVs (Casey et al. 2019). Difference between communities was tested using PERMANOVA, and taxa driving changes were identified using SIMPER analysis (Benkwitt et al. 2018). Species accumulation curves were summarised at the level of ASV and were run for both 16S and 23S with all samples that successfully amplified included together. All analyses were done using R version 3.6.1 (R Core Team 2019). Data cleaning and organising was done using the tidyverse package (Wickham et al. 2019), and boxplots were done using ggplot2 (Wickham 2016). Species accumulation curves, NMDS plots, SIMPER and PERMANOVA were done using the vegan package (Oksanen et al. 2020).

4.4 Results

4.4.1 Substrates

Mean canopy height was lowest for rubble substrates at 0.88 mm ± 0.21 (Figure 4.1a). Both 2-month and 4-month dead coral mean canopy heights were significantly higher than those for rubble (W= 305, p= 0.001, W= 312.5, p< 0.001 respectively). There was no significant difference in mean canopy heights for dead coral substrates between communities at2 months and 4 months (W= 373, p= 0.24). Mean organic load for rubble was 5.45×10^{-4} g ± 2.88×10^{-4}, however due to high variability this was not significantly different from either 2-month dead coral substrates (W = 10, p = 0.55) or for 4-month dead coral (W = 11, p = 0.37, Figure 4.1b).

Mean organic load at 2 months was 5.65×10^{-4} g ± 1.29×10^{-4} for dead coral, which increased to 7.62×10^{-4} g ± 1.54×10^{-4} at 4 months, however there was no significant difference between these two time periods (W= 18, p= 0.29).

Inorganic loads were highest for the dead coral samples at 2 months, with mean load at 2.49×10^{-2} g ± 4.23×10^{-3} (Figure 4.1c). This dropped in the following period to a mean load at 1.20×10^{-2} g ± 1.59×10^{-3}. Mean inorganic load for rubble was 2.06×10^{-2} g ± 5.30×10^{-3}. Inorganic loadings were significantly higher for dead coral communities sampled at 2 months than at 4 months (W= 23, p= 0.03), but there was no significant difference between rubble and dead coral values at 4 months (W= 13, p= 0.13) or between rubble substrates and dead coral at 2 months (W= 5, p= 0.55).

Mean ratio of organic to inorganic material for rubble was 2.33×10^{-2} g ± 6.65×10^{-3}, which was similar to that of dead coral communities at 2 months of 2.59×10^{-2} g ± 7.56×10^{-3} (Figure 4.1d). However, due to an increase in organic load and a decrease in inorganic, this ratio increased to 6.87×10^{-2} g ± 1.93×10^{-2} for dead coral by 4 months. There was no significant difference in organic to inorganic ratios between rubble and dead coral communities at 2 months (W= 9, p= 0.75), however dead coral communities at 4 months were significantly higher than rubble (W= 0, p= 0.03). Due to greater variability for ratio values for dead corals at 2 months, there was no significant difference between ratios for dead coral at 2 or 4 months (W= 3, p= 0.06).

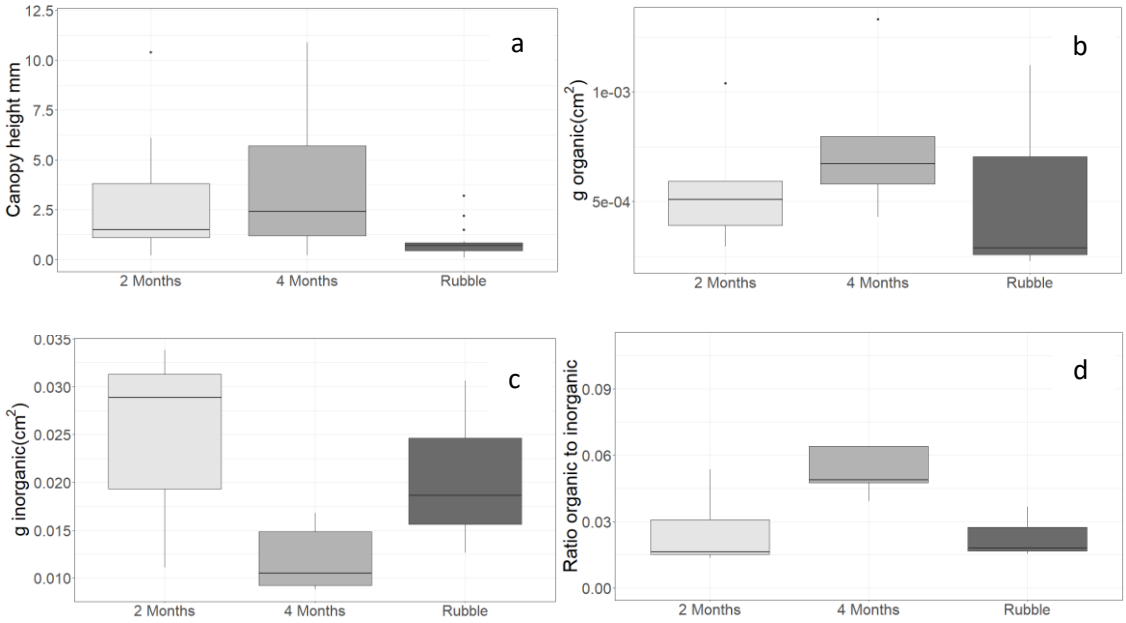


Figure 4.1. Values of organic and inorganic loadings for each substrate type. Weights were divided by the surface area of the fragment and then by the percentage of overall sample used and finally multiplied by 100 to standardise values. a) Canopy height measurements from substrates, b) grams of organic (mass lost through ashing), c) grams of inorganic material (mass left from ashing), d) organic weights were divided by inorganic weights to get a ratio of organic to inorganic. Horizontal lines show the median values, with whiskers showing highest and lowest values that are within 1.5 * Inter Quantile Range (IQR).

4.4.2 Metabarcoding

23S

Out of a total of 13 samples (5 samples for 2 months post peak bleaching, 5 for 4 months and 3 for rubble), 11 samples successfully amplified; 4 samples from the 2-month sampling, 5 samples from 4 months and 2 for rubble. Across these 11 samples, we found a total of 403 unique ASVs for 23S. Of those, 146 had a sequence similarity of ≥97%, with 257 with a sequence similarity of ≥80%, resulting in 63.77% of the data having broad classifications. One sample from samples collected at 2 months only consisted of samples with a sequence similarity of ≥80%. Mean ASVs across all samples was 61. Mean number of ASVs was higher in samples collected at 4 months post peak bleaching (77.6) than at 2 months (47.25), however

a t-test found no significant difference (t = 1.7034, df = 4.735, p = 0.15). The mean number of ASVs for the two rubble samples was 48.

There was a significant difference between communities analysed with 16S by PERMANOVA (*F*= 2.73, *p*= 0.001, Figure 4.12). Community differences identified by the SIMPER analysis showed that ASVs assigned to *Lobophora variegata* were higher at 4 months than at 2 months, whereas month 2 communities had higher proportions of reads from unassigned rhodophytes and the genus *Gayliella* within ceramiaceae (Figure 4.3). The number of ASVs driving changes between rubble substrates and dead corals (both sampling periods) were higher than for comparisons between colonising communities (16, 21 and 9 ASVs respectively). In comparison to rubble substrates, colonising communities at 2 months had higher reads for *Polysiphonia* sp. and *Herposiphonia* sp., as well as for the diatom *Cylindrotheca closterium* within bacillariaceae.

Comparing rubble samples with dead corals sampled at 4 months, most of the ASVs identified were higher in rubble samples than 4-month samples (15 out of 21). Of these, the majority were predominantly diatoms in the order bacillariales. The rhodophyte community had a higher proportion of reads from *Polysiphonia* sp. and *Herposiphonia* sp. on rubble substrates DC samples at 4 months. Within cyanobacteria, *Xenococcus* sp. were higher in colonised communities, however rubble samples had a higher proportion of reads for unassigned cyanobacteria.

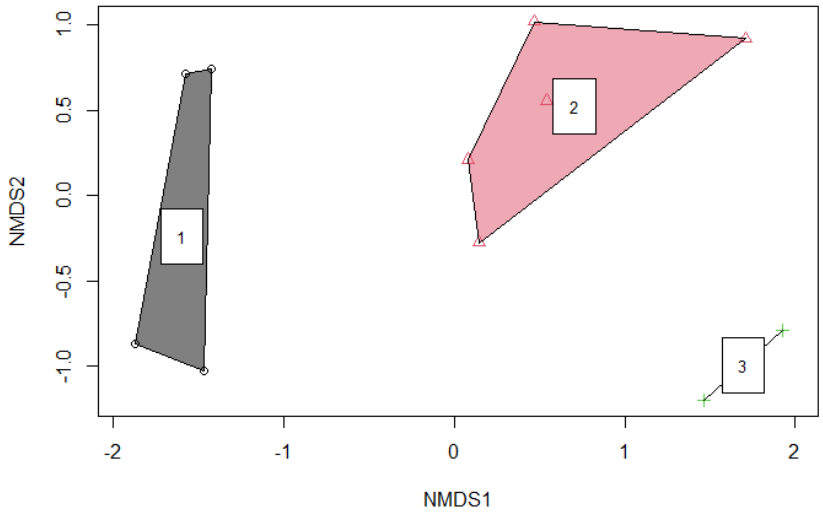


Figure 4.2. NMDS plots of 23S data comparing algal communities between dead coral samples collected at 2 months (1) and then at 4 months (2) and then rubble samples (3). Stress level for the NMDS was 0.08.

From the SIMPER analysis, turf communities on DC substrates at 4 months had the least amount of variation with ASVs within cyanobacteria, and reads within this were dominated by unassigned (Figure 4.5). DC samples at 2 months and rubble samples showed more variation in ASVs in this phylum within the orders of cyanobacteriales, phormidesmiales and eurycoccales, however these were found in higher proportions in rubble samples than on DC.

The 16S marker also found high variation within proteobacteria. Again, DC samples at 2 months and rubble samples had higher proportion of reads from alphaproteobacterial than DC samples at 4 months, namely from the orders rhodobacterales, rhizobiales and caulobacterales, and again these were higher in rubble than on DC. Rubble samples also had a higher proportion of ASVs from gammaproteobacteria, mostly with enterobacterales and steroidobacterales, with one ASV from pseudomonadales. Rubble substrates had a higher proportion of the Archea nitrososphaeria within crenarchaeota than colonised substrates, whereas DC samples at 2 months had a higher proportion of Microtrichales within Actinobacteriota than DC samples at 4 months.

Phylum	Order	2 Months	4 Months	Rubble
Bacillariophyta	Bacillariales			
	Licmophorales			
	NA			
	Naviculales			
	Surirellales			
Cercozoa	NA			
Chlorophyta	Bryopsidales			
	NA			
	Ulvales			
Cyanobacteria	NA			
	Nostocales			
	Oscillatoriales			
	Pleurocapsales			
NA	NA			
Ochrophyta	Dictyotales			
	NA			
Rhodophyta	Bonnemaisoniales			
	Ceramiales			
	Erythropeltidales			
	Gelidiales			
	Gigartinales			
	NA			
	Rhodymeniales			

Legend	
	0
	1-5%
	6-10%
	11-15%
	16-20%
	21-25%
	26-30%

Figure 4.3. Heatmap of relative read abundance using the 23S marker, summarised at the level of phylum and order for each substrate type. NA denotes those that were unassigned. RRA was summarised across samples at each level and then divided by the total number of samples.

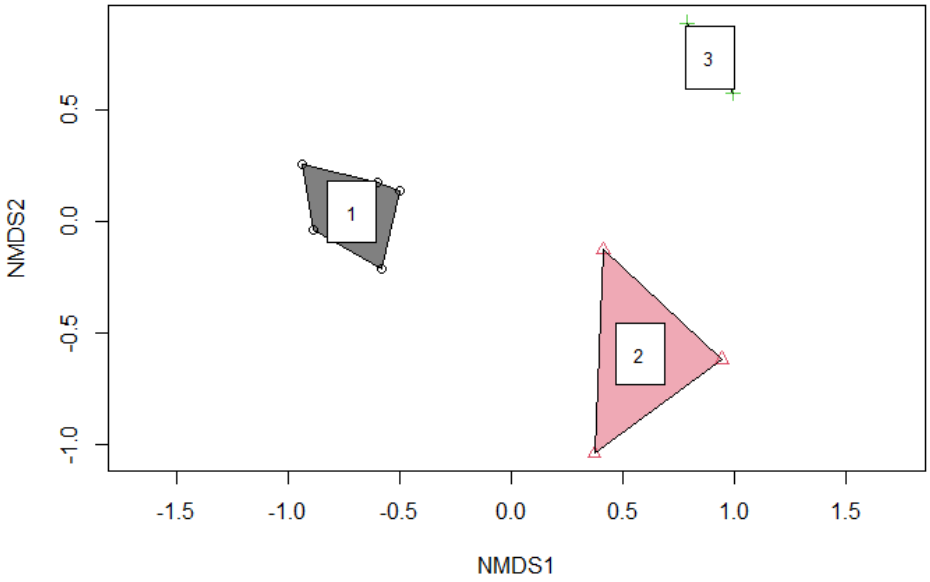


Figure 4.4. NMDS plots of 16S data comparing turf communities between dead coral samples collected at 2 months (1) and then at 4 months (2) and rubble samples also (3). Stress level for the NMDS was 0.06.

Phylum	Order	4 Months	2 Months	Rubble
Acidobacteriota	PAUC26f			
	Vicinamibacterales			
	Microtrichales			
Bacteroidota	Chitinophagales			
	Cytophagales			
	Flavobacteriales			
	NA			
	Rhodothermales			
Bdellovibrionota	NA			
Chloroflexi	Ardenticatenales			
	NA			
	SBR1031			
Crenarchaeota	Nitrosopumilales			
Cyanobacteria	Chloroplast			
	Cyanobacteriales			
	Eurycoccales			
	Limnotrichales			
	NA			
	Phormidesmiales			
	Synechococcales			

Entotheonellaeota	Entotheonellales	
Firmicutes	Lachnospirales	
	Mycoplasmatales	
Myxococcota	Haliangiales	
	NA	
	Polyangiales	
NA	NA	
Ochrophyta	Dictyotales	
Patescibacteria	NA	
Planctomycetota	NA	
	Phycisphaerales	
	Pirellulales	
Proteobacteria	Alphaproteobacteria Incertae Sedis	
	Arenicellales	
	Caulobacterales	
	Enterobacterales	
	Gammaproteobacteria Incertae Sedis	
	Kiloniellales	
	Kordiimonadales	
	NA	
	Parvibaculales	
	Pseudomonadales	
	Puniceispirillales	
	Rhizobiales	
	Rhodobacterales	
	Rickettsiales	
	Sphingomonadales	
	Steroidobacterales	
	Tenderiales	
	Thalassobaculales	
	Tistrellales	
	UBA10353 marine group	
	uncultured	
Verrucomicrobiota	Chlamydiales	
	Methylacidiphilales	
	NA	
	Opitutales	
	Verrucomicrobiales	
Xenacoelomorpha	NA	

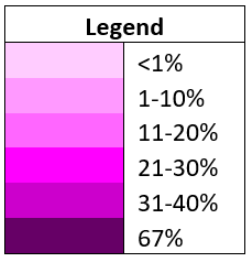


Figure 4.5. Heatmap of relative read abundance using the 16S marker, summarised at the level of phylum for each substrate type. NA denotes those that were unassigned. RRA was summarised across samples at each level and then divided by the total number of samples.

4.4.3 Choice experiment

For the feeding preference experiment conducted in June (2 months after peak bleaching), fish showed a preference for rubble substrates, with a higher mean bites per minute for rubble at 87.6 bites per minute ± 13.5 se, than that for dead coral at 35.8 bites per minute ± 7.91 ($W= 0$, $p= 0.02$). However, by August (4 months after peak bleaching), this trend switched and mean bite rate became lower for rubble substrates (8.58 bites per minute ± 3.40 se), compared to the mean bite rate for dead coral (24.10 ± 10.8 se, Figure 4.6a). However, due to high variation there was no significant difference for bite rates between the two substrates at this time period ($W= 36$, $p= 0.16$). Comparing general bite rates (for all substrates combined), mean bite rates were significantly lower in August ($W= 14$, $p< 0.01$), with a mean bite rate of 58.3 bites per minute ± 11.4 se for June, dropping to 16.3 ± 5.87 se in August.

To account for standing mass and estimate mass ingested, bites per minute was multiplied by the mean value for organic matter for that substrate type per sampling period (Figure 4.6b). Mean mass ingested for rubble in June was significantly higher at 2.73×10^{-4} g/ per min ± 5.85×10^{-5} se, than for dead coral at 1.12×10^{-4} g/ per min ± 2.74×10^{-5} se ($W= 11$, $p= 0.03$). Mean mass ingested for dead coral in August was 1.44×10^{-4} g/ per min ± 6.85×10^{-5} se with rubble being 3.65×10^{-5} g/ per min ± 1.22×10^{-5} se, with no significant difference between substrate types ($W= 54$, $p= 0.25$).

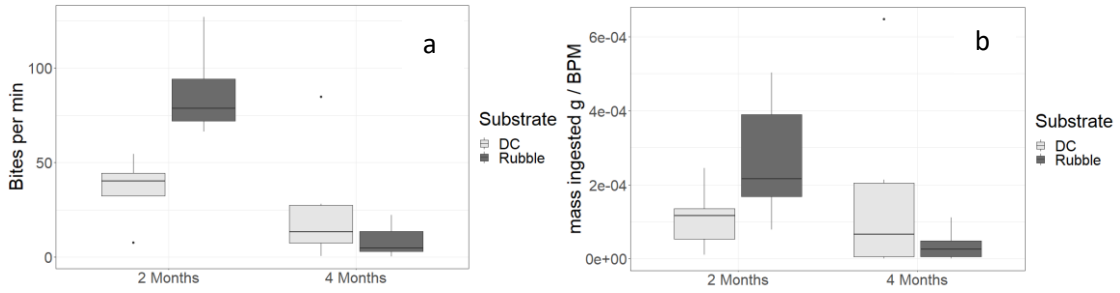


Figure 4.6. Bites per minute (BPM) per substrate type (a), and mass adjusted bites per minute (b). DC relates to samples collected from dead corals.

4.4.4 Respirometry

Standard Metabolic Rate (SMR) was mass adjusted by dividing SMR by fish weight (SMR_m) to account for how this varies with fish size. Mean initial SMR_m of fish collected in June was (630 ± 22.3 MO_2, Figure 7). This dropped to 532 ± 30.4 se for dead coral and to 475 ± 19.8 se for rubble. While both treatments lost significant levels of fitness in comparison to the initial SMR_m rates in June (dead coral, W= 66, p= 0.02, rubble W= 77, p< 0.01), fish within the rubble tank lost significantly more fitness than those within the dead coral tank, even though fish within the dead coral tank were kept for an additional day (W= 31, p= 0.04).

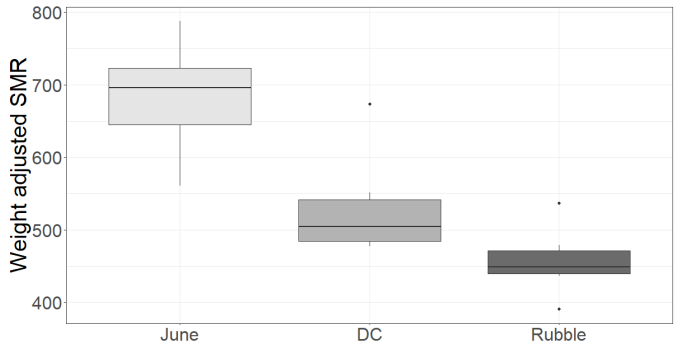


Figure 4.7. Weight adjusted SMR (SMR_m) values for fish at the point of recruitment (June) and then at the end of the experiment for fish fed only either dead coral (DC) communities at 4 months or rubble substrates.

4.5 Discussion

The results of this study highlight the importance of early successional changes to algal and microbial communities following wide-scale coral mortality, and how features associated with these communities can influence grazing behaviour of reef fish. Interestingly in this study, canopy heights were not shown to influence the sediment trapping potential of turf algal communities. There was no difference in mean canopy heights between turf algal communities sampled at 2 months and 4 months, and the amount of inorganic material present actually decreased during this time. Greater canopy heights have been linked with higher rates of sedimentation in turf algal communities in a number of studies (Bonaldo & Bellwood 2011, Gordon et al. 2016b, Purcell 2000; Purcell & Bellwood 2001). This connection between canopy height and sedimentation is perceived to be so strong that some researchers have begun to partition communities as either short productive algal turfs (SPATs) and long sediment laden algal turfs (LSATs) by whether or not canopy height exceeds 5mm (Tebbett & Bellwood 2019). Within this study, mean canopy height did not exceed 5 mm, therefore all communities could be considered as SPATs. However, I show that even categorising communities within this general description can be problematic, since there are differences between the potential for communities to trap sediment, and that feeding preferences by fish vary that are unrelated to canopy heights. It is likely that changes to species composition of algal communities, discussed below, are the major drivers here.

My study supports previous research that increases in the amount of sediment within algal turfs can be a deterrent for grazing fishes (Bellwood & Fulton 2008, Goatley et al. 2012, Gordon et al. 2016a, Tebbett et al. 2017). Levels of inorganic matter were highest on dead coral substrates at 2 months, and fish actively avoided feeding from these and chose rubble instead. Work by Tebbett et al. (2017) found that increases to sediment had varying affects within the surgeonfish family, reducing feeding rates of *Ctenochaetus striatus* and not *Acanthurus nigrofuscus*. The authors attribute this to the fact that *A. nigrofuscus* is a cropper, and takes short nipping bites from the tips of algal fronds, whereas *C. striatus* is a detritivore with comb-like teeth that allows them to ingest fine particulate matter (Purcell & Bellwood 1993), meaning that *A.*

nigrofuscus has both physical and behavioural adaptations that prevent it from ingesting a lot of inorganic material in comparison. However, *A. triostegus* is also a cropper with the same dentition and feeding style as *A. nirgofuscus* (Holzer et al. 2017), meaning that increases in inorganic load may still deter grazing even for fish within this feeding mode.

Mean organic load from dead coral communities increased slightly from 2 months post peak bleaching to 4 months, however it was a significant drop in inorganic load that increased the ratio of organic to inorganic by 4 months. Fish avoided feeding from dead coral substrates that had a low ratio of organic to inorganic, but fed more regularly from those with a higher ratio. Conceptually, this supports work by Gordon et al. (2016a) and Tebbett et al. (2017) that sediments can 'dilute' turfs and make them less attractive for feeding substrates for fishes, which may be why I observed such low bite rates for dead coral at 2 months. However, even with a significant increase in the ratio of organic to inorganic load, bite rates were the same between the dead coral at 4 months and rubble substrates, so that while fish were no longer actively avoiding dead coral substrates, they were no more attractive for feeding over rubble. If ratio of organic to inorganic was influential to fish feeding behaviours, then we would expect them to show a preference for dead coral substrates over rubble, which was not observed. One of the reasons that fish may still select rubble substrates is that canopy heights were significantly lower on rubble substrates compared to dead corals at both time periods, indicating that these substrates are kept in a short, cropped state by grazers. Intense herbivory has been shown to keep primary producers in fast-growing forms that increases production and yield to grazers (Carpenter 1986, Burkepile 2013), which is believed to be facilitated by reducing self-shading and selecting for fast growing species (Bruno et al. 2006). In terrestrial and seagrass systems, this faster growth rate stimulated by grazing has also been shown to increase the quality of food items too, by increasing nitrogen content within tissues (Green & Detling 2000, Aragones 2006). Regular cropping of seagrass leaves has also been shown to alter carbohydrate and starch content within tissues (Aragones 2006). It may be that intense regular cropping of established turf communities creates more productive food resources,

which, while less abundant, gives more nutrition. Future studies could also look at C:N ratios within turf algal communities which is a method for comparing protein to energy ratios, and track how these change through time to give an indication of quality of food sources (Wilson et al. 2003). Studies on browsing reef fish have shown that enrichment of nitrogen and phosphorous within algal tissues increased the bite rate of a variety of fish, with different species reacting differently to treatments (Shantz et al. 2017). This was also found to affect younger fish more, likely because growth rates of fish are higher when they are younger, and more micronutrients are required (Hood et al. 2005, Sugiura et al. 2005).

It is unlikely that rubble substrates were more nutritious for juvenile *A. triostegus*, due to the results of the fitness experiment. While fish in both treatments showed a reduction in weight adjusted standard metabolic rate in comparison to the SMR_m of fish directly after collection from the reef, this was less for those within the dead coral tank, indicating that these fish had higher energy reserves that could be allocated towards bodily processes, foraging and growth (Norrin & Metcalfe 2018). Increases to food availability and quality has not only been linked to increases in metabolic rate in juvenile fish, but to increases in growth rates also (Zeng et al. 2018). While growth rates were not measured in this short-term study, increases to growth rates following large scale disturbances has been shown to occur in parrotfish following widescale bleaching events (Taylor et al. 2020). However, even with a constant supply of substrates, all fish still showed a reduction in resting metabolic rate, indicating that daily substrate refreshment was either not frequent enough, or that there were some criteria not being met within the tanks. Other studies of post-larval *A. triostegus* have kept fish in similar densities (Besson et al. 2017, Gache et al. 2021), however such studies did not keep these individuals for such a long time period as within this experiment. Being kept in close proximity for a long duration may have meant that individuals lost energy through guarding and challenging behaviour (Besson pers. comms., pers. obs.). If highly cropped and productive rubble substrates are more nutritious, then the lack of standing organic matter, or limited ratio of organic to inorganic was significant enough that substrates were rapidly grazed down in

comparison to those of dead substrates, meaning that more substrate area is required to support a given number of fish. This means that following widescale coral bleaching, at certain time periods the algal communities can support a greater density of individuals than existing turf communities prior to bleaching. Further work is required to see if/how this trend continues past three/four months post bleaching.

Metabarcoding of the two dead coral communities using the 23S marker showed that significant changes had occurred to the composition of the algal communities within the space of two months, and that these two communities differed to rubble communities. While mean number of ESVs was highest on dead coral (DC) communities at 4 months, analysis using SIMPER showed that rubble communities had more distinct ESVs. Compared to both dead coral samples, rubble samples had a higher proportion of reads from ESVs belonging to bacillariophytes, predominantly Bacillariophyceae. Microalgae such as diatoms and cyanobacteria are some of the most productive primary producers on coral reefs (Larkum 1983, Hatcher 1990), and since studies have shown that high grazing rates can relate more to productivity than standing mass (Russ 2003), this greater diversity of bacillariophytes may be an explanation behind why rubble was consistently a popular grazing substrate for fish in this study. Rubble substrates also had significantly more ESVs relating to *Herposiphonia* and *Polysiphonia*, also explaining why these substrates remained popular feeding substrates for both experiments. *A triostegus* has an elongated digestive tract (Holzer et al.) and utilises gut symbionts to help breakdown plant matter (Clements & Bullivant 1991). Early studies on choice experiments with *A. triostegus* in Hawai'i found that they preferred to feed on the red algae *Polysiphonia* (Randall 1961), which is supported by studies that have shown that *A. triostegus* can more readily digest the proteins of *Polysiphonia*, over those of brown algae such as sargussum (Pfeffer 1963). The bacillariophyte *Cylindrotheca closterium* was identified as higher on rubble substrates than both stages of recently colonised dead coral substrates using 23S. This is interesting, as studies have shown that this species can be used as an indicator for nutrient enriched or stressed conditions (Penessi & Danovaro 2017). One possible explanation

for this is that the stressed and dying corals from the bleaching event released en masse a large amount of nutrients into the surrounding environment, which enriched existing surrounding algal communities. Mass coral bleaching mortality events have been widely shown to introduce novel nutrients, through either organic matter shed by dying corals (Niggl et al. 2008, Vaughan et al. 2021), or through nitrogen fixation by colonising cyanobacteria (Davey et al. 2008). This release of nutrients might theoretically suggest that bacterial activity would be higher on recently deceased coral substrates, however I saw no evidence of this in this study. The majority of this material would likely be coral tissue and excreted mucus, which would be animal in origin, however the majority of distinct ESVs at 2 months were related to proteobacteria, specifically alphaproteobacteria which have been associated with the decomposition and breakdown of lignin (Tao et al. 2020). This further supports the fact that all organic material from live corals is released during mortality, with little material being retained to the substrate itself.

Microbial communities are highly influenced by environmental conditions, and have been shown to be regionally distinct along latitudinal gradients (Pearman et al. 2020). My results support other studies that have found that Proteobacteria, especially Alphaproteobacteria and Gammaproteobacteria, are dominant in coral reef sediments and as symbionts with corals and sponges (Uhticke et al. 2007, Rusch et al. 2009, Olsen & Kellogg 2010). Kelly et al. (2014) found that reefs with a higher coral coverage had a greater proportion of the Alphaproteobacteria *Rhodobacterales,* yet those with a greater proportion of macroalgae show greater proportions of Gammaproteobacteria. My study supports other research that shows that benthic community composition has been shown to affect microbial communities present on reefs (Kelly et al. 2014), and that this is highly variable (Rohwer et al. 2002).

Studies have shown that cyanobacteria tend to be prominent in the early stages of substrate colonisation on reefs (Diaz-polido & McCook 2002, Davey et al. 2008). While 16S showed a high diversity of ESVs associated with cyanobacteria on dead coral substrates at 2 months samples in comparison to 4 months, when comparing 2-month DC samples with rubble, rubble

had a higher diversity of cyanobacteria ESVs driving changes between the two communities. This was further supported by 23S, which showed that there was greater variation in cyanobacteria ESVs driving changes in rubble communities than recently deceased corals being colonised. Specifically, this related to Xenococcus sp. These are a nitrogen fixing species (Brocke et al. 2018), hence potentially a reason why rubble substrates had a higher presence of cylindrotheca.

The shifts to algal community composition may explain why the amount of inorganic material decreased from month 2 to month 4, even though canopy heights remained similar. The dominant algae driving changes at 4 months was *Lobophora variegata,* within the ochrophyte phylum and the class phaeophyceae, which are the fleshy brown algae which are typically associated with climax communities that dominate at later stages on reefs that shift towards algal dominance (Chong-Seng et al. 2014, Roff et al. 2015, Viera et al. 2019). Phaeophyceae includes common genera such as *turbinaria*, *sargassum*, *lobophora* and *dictyota*, however of these only *lobophora* was present in our samples for DC communities at 4 months. *Lobophora* typically has thick, lobed thalli with smooth surfaces (Viera et al. 2019), and was visually dominant in the sample. In comparison, at 2 months the red algae of the genus *Gayliella* within ceramiaceae was the primary algae driving changes, as were the two red algae from rhodomelaceae for rubble substrates. The community with primarily foliose, lobed and leaf-like structures dominating trapped the least sediment, whereas those communities with fine, filamentous branching structures trapped more (Baweja et al. 2016, Muguerza et al. 2017). However, this is only considering those ESVs that were successfully determined to lower taxonomic level, and further work that would classify those unassigned taxa would be needed to support this theory.

23S has widely been used as a complimentary marker in multi-marker approaches (Marcellino & Verbruggen 2016). While a multi-marker approach does offer a more comprehensive overview of community composition across a range of taxa, many smaller research projects are limited financially, placing restrictions on sample sizes and the number of markers compared.

Single marker approaches can still yield a high level of information on fine-scale community composition, in comparison to traditional approaches, such as microscopy (Abad et al. 2016, Rimet et al. 2018). 23S is becoming increasingly common as the primary marker for single marker studies determining algal communities as the barcode database is expanded (Brandl et al. 2020, Nalley et al. 2021a, Nalley et al 2021b, Stamoulis et al. 2017). In this study, in comparison to 16S, 23S had a high proportion of ESVs that were assigned broad taxonomic classifications. This is likely due to the fact that the 16S has a more expansive barcode database. The high variation of unassigned ESVs indicates that turf algal communities are more diverse than we think, and further emphasises the need for development of the 23S barcode database.

Caveats

I acknowledge that small sample size is a limitation within this study, and that further work with higher replication should be done with greater statistical power to support these results. However, as a pilot study trialling this methodology, I identified a key gap in knowledge in that the colonisation process on dead corals changes rapidly through time, and that changes to algal community composition can affect its attractiveness for feeding for fishes in a variety of ways. Application of this work to see how these trends compare with other species of fish would shed more light on how changes to algal communities and their associated microbial communities drive foraging rates and nutrition for roving herbivorous fishes. For example, patterns in feeding response to different algal communities would likely vary if such an experiment was conducted using Scaridae versus Acanthuridae, since these species tend to target microphages rather than the algal itself (Clements et al. 2017).

While bite rates have been a common method for measuring feeding preferences of fish (Pratchett 2013, Feitosa & Ferreira 2015), it does not actually capture how much material an individual actually ingests. Fish may bite rapidly to ingest a similar proportion of material that may be yielded from another substrate. Fish may have needed to take less bites from DC substrates at 4 months because each bite would yield a higher ratio of organic material, which

may have also driven down bite rates in general for DC substrates at 4 months. Measurements of gut throughput could be a more accurate way of estimating actual food intake of individuals and mass ingested, rather than simple bite rates (Polunin 1988, Marnane & Bellwood 1997).

This study sheds new light on how rapidly turf algal communities can change in the initial stages of colonisation following coral mortality. It also shows how these community changes can affect fish feeding behaviour, either directly through changes to species composition, or indirectly by affecting sediment trapping potential and quality of organic material. Over time these changes can potentially enhance the fitness of recruiting fish, through either an increase in the amount and/or quality of food available. I show that algal community composition is likely a key driver in sediment dynamics for algal turfs and their attractiveness for feeding fishes. This helps link changes to benthic properties and the foraging landscape, and the potential and implications for newly recruited fish to acquire resources. This study paves the way for further testing of these methods, a wider application into how colonisation processes develop further, and how they vary regionally or among other species of coral and fish.

General Discussion

Grazing by fishes has been shown to be a vital function on coral reefs that inhibits algal proliferation and promotes coral dominance (Ledlie et al. 2007, Cheal et al. 2010, Graham et al. 2015, Bennett et al. 2015). Disturbance events linked to climate are becoming more frequent and acute (Hughes et al. 2017, Williams et al. 2019b), meaning that the ability of the community to provide such functions will become increasingly import as the Anthropocene progresses. Predicting to what degree such ecosystem functions will be maintained in the future is made challenging by the interaction of top-down (fishing and predation) and bottom-up (habitat features) drivers that can vary spatially (Nash et al 2016, Robinson et al. 2020, Oakley-Cogan et al. 2020) and seasonally (Clifton 1995, Diaz-Polido 2002, Lefèvre & Bellwood 2010), as well as long-term gradual shifts to communities under climate change (Adjeroud et al. 2008, Li et al. 2011, Edmunds 2018) and anthropogenic pressures (Graham et al. 2015, Robinson et al. 2017, Adam et al. 2021). This shows the need for studies on site specific drivers, and the application of long-term monitoring data to look at trends to functional rates through time. Furthermore, while large-scale impacts associated with climate have been shown to have temporary beneficial effects to grazing populations through the provision of additional grazing spaces (Bellwood et al. 2018, Robinson et al. 2020, **Chapter 1**), there have still been no studies to date linking community changes that occur through colonisation processes with algal community features that are known drivers to grazing (Tebbet & Bellwood 2019). My aim in this thesis was to find ways to bridge these gaps and identify mechanisms by which habitat features drive functional rates provided by grazing fishes and their secondary productivity.

Main Findings

Drivers to functions provided by grazing reef fish

The body of literature applying bite rate metrics to survey data in order to calculate functions related to grazing fishes is fairly well developed, especially when looking at how individuals

can contribute disproportionally dependent on species, size and function (Lokrantz et al. 2008, Yarlett et al. 2018, Lange et al. 2020), how functions differ by reef zone (Hoey & Bellwood 2008, Yarlett et al. 2020), how they vary spatially in response to human pressures (Bellwood et al. 2012, Robinson et al. 2017) and what habitat features are associated with higher functional rates (Yarlett et al. 2018, Robinson et al. 2019, **Chapter 2**). Such studies are important as they enable community data to be broken down into trends which are not apparent by simply looking at biomass trends alone (Hoey & Bellwood 2008, Robinson et al. 2020, Lange et al 2020, **Chapter 1**).

For example, while studies such as Han et al. (2016) and Graham et al. (2020) have shown that populations of grazing fishes increase following disturbances, it was still unknown how this related to the various functions associated with herbivory. **Chapter 1** is the first study to my knowledge to show that two different functions provided by parrotfish respond contrarily following a large-scale disturbance. Since scraping rates are driven primarily by high densities of rapidly biting smaller individuals, this function responds immediately to initial recruitment of small bodied individuals. In comparison, bioerosion rates rely on individuals attaining larger body sizes, therefore this function gradually increases through time showing a much later peak. While literature on individual bite metrics may suggest that such trends could be predicted, this is the first study to actually show such an effect. This is pertinent as peaks to bioerosion rates following disturbances rely on recruited individuals being afforded the opportunity to grow, which may not be the case in heavily fished systems.

The temporal approach with estimated functions also allowed me to test whether there was any evidence of weakening to functional rates through time. While Bellwood et al. (2012) compare current bioerosion rates with historical estimates, these can only be taken as estimates due to lack of scientific data. Long-term monitoring sets of a standardised format provide comparable data to allow us to make accurate assessments. The results of this showed two key things: firstly, parrotfish are likely resource limited, as their biomass and functions were strongly associated with available substrate versus live coral cover, and once this had returned to similar

levels as the previous coral dominated period, then so too did parrotfish biomass and functional rates. This supports studies that have found similar trends for herbivorous reef fish (Mumby et al. 2007b, Williams & Polunin 2001). Secondly, while there was no significant difference between biomass and functions between periods of coral dominance suggests long-term resilience, there is also evidence that this may not be the case, due to a reduction to mean fish size in the second period of coral dominance., Furthermore, the initial response to bleaching in 2019 coincided with a reduced rate of recruitment in comparison to 2006. This suggests that a period of 15 years in insufficient to truly identify long-term trends in this region, and that ongoing monitoring is vital in the face of multiple disturbances, especially as these potentially increase in frequency and severity (Hughes et al. 2017). The reduction to mean size of parrotfish may have driven the reduced recruitment in 2019 through allometric relationships with fecundity (Barneche et al. 2018), and it would be interesting to explore this in future studies.

Chapters 1 and 2 also deepen the discussion on top-down effects (in this study fishing) on functions performed by grazing fish, both by supporting existing studies, and raising novel concepts to be explored. Fishing has been shown to have a strong influence on functions related to parrotfish (Bellwood 2012, Hoey and Bellwood 2008, Robinson et al. 2019), and this was supported with evidence that bioerosion rates are likely reduced in comparison to historical rates through loss of large bodied key species and a reduction to mean fish size through time. Bellwood et al. (2012) suggest that bioerosion is functionally non-existent today on Mo'orea in comparison to estimated rates in the 1960's. My study supports this, due to the fact that the majority of bioerosion is currently being conducted by a small bodied *Chlorurus* species, *C. spilurus*. The occasional observation of large bodied *Chlorurus* such as *C. microrhinos* in the data set give an indication of how high rates could be with greater populations of these species. Currently it appears that the high bite rate of smaller individuals is likely compensating for the loss of large bodied individuals, as there was no significant difference between bioerosion

functions between the two periods of coral dominance. However, since this function is closely tied to body size, this may not hold true if mean size continues to decrease into the future.

Contrarily, fishing was found to have a positive effect on cropping rates by surgeonfish. The mechanisms behind this are uncertain, and may be related to fishing selectivity. On Mo'orea, spearfishing is a common method for targeting reef fish, which favours large bodied parrotfishes over small bodied parrotfishes and surgeonfishes. This may suggest competition and limited functional redundancy. This may be possible if parrotfishes are resource limited, which may perhaps apply to surgeonfish also. However, this is unlikely due to a number of factors. Firstly, one of the main outcomes from **Chapter 2** was that a greater number of species was associated with higher functional rates, both for scraping and cropping. This indicates that, rather than competition between species for existing grazing space being a factor, the limiting factor is the proportion of substrate available for grazing. This is supported by Brandl & Bellwood (2014) that found high levels of niche overlap for parrotfishes and surgeonfishes. Furthermore, even though both parrotfish and surgeonfish may preferentially target the same algal turfs, their physical and physiological traits mean that the material that they ingest and draw nutrition from varies (Choat et al. 2002, Wilson et al. 2003, Crossman et al. 2005, Clements et al. 2009, Kelly et al. 2016, Clements et al. 2017). Not only does this mean that competition between species is unlikely, but also explains why grazing fish communities and their associated functions respond differently to habitat features (**Chapter 2**, Robinson et al. 2021).

Colonisation patterns following wide-scale bleaching

The literature on the effects of sediment on grazing behaviour is also fairly well developed. Increases to sediment can negatively affect both surgeonfish and parrotfish (Bellwood & Fulton 2008), however even within this there are variations by feeding styles (Tebbett et al. (2017). Features of sediments, such as grain size, can further influence grazing behaviour (Gordon et al.

2016a). Turf algal communities can be highly variable spatially (Scott & Russ 1987, Harris et al. 2015) and temporally (Diaz-Polido & McCook 2002), and be influenced by small-scale habitat variations such as orientation (Duran et al. 2018). To date, no studies had attempted to quantify such features through transitional stages following large-scale disturbances, even though herbivory rates during such times are vital. The aim of the last two chapters was to monitor how features such as sedimentation and canopy heights developed through time and to directly link these to changes to community composition.

In **Chapters 3 and 4** I combine a variety of methods to determine community composition on post-bleaching coral substrates. Metabarcoding techniques were helpful in understanding the potential extent of high variation occurring between some samples which may not have been captured by morphological assessment alone, especially comparing microbial communities using 16S. However, it also showed the potential limitations of taking a single marker approach for algal community definition (using 23S for algal communities) and the necessity to include morphological assessments if a multi-marker approach (Marcelino & Verbruggen 2016, da Silva et al. 2019) is not financially feasible. In hindsight, such an approach should have been applied to the naturally bleached substrates colonising under ambient grazing pressure and with higher replication. However, due to the fact that the bleaching event was unexpected, and the work for **Chapter 3** had already begun at this time, data collection priority was given to **Chapter 3**, which would not have been the case if I could run these again. In hindsight Chapter 3 was too ambitious, as I tried to control too many variables which made inferences challenging.

Regardless of the challenges, the results in **Chapters 3 and 4** highlighted some key findings that require further exploration. Firstly, even though communities in my studies could be classed as short productive algal turfs due to mean canopy heights (Goatley et al. 2016, Tebbett & Bellwood 2019), and according to Purcell (2000) canopy heights should be closely related to sedimentation rates, both **Chapters 3 and 4** show that this is not always true, and that the divergence in this relationship suggested by Steneck (1997) can occur much earlier. One factor

that I wanted to explore but did not get the chance to look at was alterations in primary productivity in response to nutrient release during the bleaching event, which may have pushed production rates above those to which herbivore communities could cope, thereby shifting towards macro-algae species becoming more prominent. This would be a key step for future studies linking primary production with secondary production.

Secondly, **Chapter 4** gives insights into how benthic changes following bleaching can influence secondary productivity. This is the first study to show that transitional communities of colonising algae can go from active avoidance of grazing fish in early stages to being beneficial feeding sites that can support higher densities of fish than established, highly cropped substrates. The main drivers behind this identified in this study are increases to organic to inorganic ratios, and shifts towards more palatable/ digestible species. However, since there is a growing body of evidence that nutrients released from dying corals are taken up by primary productivity (Vaughan et al. 2021, **Chapter 3**), and that nutritional content of algal tissues can influence grazing intensity and fish nutrition (Shantz et al. 2017), this is the next major step for research within this field. Understanding how habitat changes drive secondary productivity is imperative for fisheries associated with these species.

Studies such as Robinson et al. (2019) show that productivity of nominally herbivorous fish can remain high long after a disturbance subsides, regardless of whether or not habitat conversion from coral to macro-algae occurs (Morais et al. 2020a). High productivity may be in response to fishing pressure and loss of predators (Morais et al. 2020b). Such trends are independent of those related to biomass (Hooper et al. 2005, Brandl et al. 2019), since they also are heavily influenced by the size structure of the community. Understanding to what degree recycling of nutrients within biogeochemical cycles within this time, and how it could influence productivity and nutrition are key future questions.

My results from **Chapter 3 and 4** show just how important community changes are in their ability to influence grazing rates in various ways. By beginning to define these drivers and explore relationships, I also identify areas that need further definition. Understanding how the

nutritional landscape for grazing reef fishes changes through time, especially in response to large-scale disturbances, is still in its infancy and offers many avenues for further research.

Future research

Through the process of identifying research questions and analysing my results, this gave rise to additional themes that need developing in the future. For example, there are a number of features that influence rate of functions calculated using bite metrics. There are strong allometric relationships between size and scar area and depth for parrotfish (Lokrantz et al. 2008, Hoey & Bellwood 2008, Ong & Holland 2010), as there are for bite rate and size (Lange et al. 2020). Scar area tends to increase with size in a similar way across species (Robinson et al. 2020, Lange et al. 2020), which is likely limited to gape size which appears to offer little variation. Scar depth, bite rate and substrate preference appear to be more variable (Hoey & Bellwood 2008, Yarlett et al. 2018, Lange et al. 2020). Due to the growing evidence of parrotfish being resource limited (Tootel & Steele 2016, **Chapter 1**), bite rate may also be influenced locally by available grazing substrate. Currently data on bite rate metrics is not complete for all species and cross a wide range of regions, making the application of existing data extremely generalised. Also, such an approach does not account for how bite rate may vary seasonally, or if primary productivity and nutritional content of algal communities varies following bleaching events. This indicates the need for more data on species and across regions, and to try to gauge bite rate as a function of habitat quality.

The mass release of nutrients following bleaching events has been proven to be rapidly taken up by primary productivity (Vaughan et al. 2021, **Chapter 3**), however how such a flux of nutrients influences secondary productivity is still largely undetermined. The concept of using control substrates that were located away from bleached substrates in **Chapter 3** was designed to explore this, however the mass release from the bleaching event that occurred nullified such an approach. One avenue that I wanted to pursue was attempting to quantify how the nutritional

quality of prey items changed through time in response to coral bleaching and community change. With a greater sample size, I could have measured the content of algal tissues of establishing communities to see how carbon to nitrogen ratios change as this can be a measurement of protein to carbohydrate ratios (Wilson et al. 2003). However, a small sample size, and limited material yielded from substrates at 2 months meant that only analyses for nitrogen were possible.

Another interesting trend that I observed was the inverse relationship through time of the take up of both the percentage of nitrogen and $\delta^{15}N$. Where a shift towards the heavier element was observed, the total percentage of nitrogen decreased. Courtial et al. (2018) found that organic matter released by *Pocillopora* corals was depleted in nitrogen in response to bleaching, but only under elevated ultraviolet radiation levels. They argue that the corals ability to take up nitrogen becomes impaired, however, such a relationship is so far unproven and requires further investigation. This is interesting considering that an influx of terrestrial based nutrients increases both $\delta^{15}N$ and the total percentage of nitrogen (Donovan et al. 2020).

Understanding feeding preferences and the nutritional effects of algal community changes on parrotfish communities would also be a key topic for future studies, as the populations of these species have been shown to respond the most to large scale disturbances, and display increased growth rates also (Han et al. 2016, Taylor et al. 2020). This shows that some element of benthic change favours these species. I wanted to use parrotfish for the choice and respirometry experiment, but it became apparent that collecting them and housing them successfully in aquaria would be challenging. Hence, I opted for a species that was easy to collect and had previously been kept in such aquaria before, especially as I was running multiple studies concurrently.

Parrotfish are widely believed to be microphages, targeting protein rich microorganisms rather than algal material (Clements et al. 2017). Studies have shown that cyanobacteria can dominate in early communities (Diaz-Polido & McCook 2002, Arthur et al. 2005, Houk et al. 2010). In my thesis, **Chapters 3 and 4 both** showed that cyanobacteria can remain diverse up to 90 days

post mortality. In **Chapter 4**, 23S found that cyanobacteria were generally more prominent on rubble substrates, however 16S found that cyanobacteria were more prominent on colonised substrates. It may be that these dense, colonising algal communities provide a better environment for microorganisms than short, highly cropped established turf communities, however this is purely speculative and requires further investigation. In **Chapter 3** I found that microbial communities were diverse across colonising substrates, and tended to shift away from gammaproteobacteria (associated with coral pathogens) towards alphaproteobacteria (associated with the breakdown of lignin) as plant matter increased. Future research should aim to link changes to microbial community composition with the role that they play in recycling nutrients released from coral mortality, and how this could influence parrotfish communities.

Metabarcoding allowed for some interesting insights, however, there are some issues associated with these techniques that need to be developed in the future. Many studies identify that some markers can be biased towards certain taxa (Kirkham et al. 2011, Marcelino & Verbruggen 2016, da Silva et al 2019, Martins et al. 2020, van der Loos & Nijland 2021), and there can also be amplification bias which can make abundance inferences challenging (Nicholls et al. 2018). Incomplete DNA databases inhibit taxa resolution, but this will improve through time as databases grow. The high overlap observed for *Porites* substrates was more of an issue related to their physiological response to bleaching, whereas in **Chapter 3** we had some issues which were more likely associated with procedural steps. These could have been related to preservation issues, contamination or errors during extraction. There is also the possibility to use different extraction kits in the future. For example, we used the Qiagen DNeasy Powerplant pro kits as these were believed to be appropriate for breaking down tough algal tissues, however van der Loos & Nijland (2020) strongly suggest using the PowerSoil kits for any samples containing traces of sediment as these can contain inhibitors. However, this is unlikely to be the issue, as the samples with the least amount of sediment in **Chapter 3** (*Pocillopora* at 90-days) were the ones which were associated more with limited amplification. In truth, there are many steps along the way that could have caused the issues that we saw, and there is

currently no one reason as to why this issue occurred to such a high degree with *Pocillopora* samples. In the future, increasing sample size, using a combination of markers and including morphological assessments will increase the ability to make inferences with such data. Furthermore, as DNA databases are developed, the application of single markers (such as 23S) for algae will improve.

Concluding remarks

This thesis applies a variety of existing approaches to address novel and pertinent questions surrounding drivers to functions provided by grazing fishes on coral reefs, and linking benthic changes with grazing rates and secondary productivity. The overall value of this thesis is in adding to our knowledge on how ecological processes will be shaped in the Anthropocene, and by bridging some of the gaps in successional theory on reefs.

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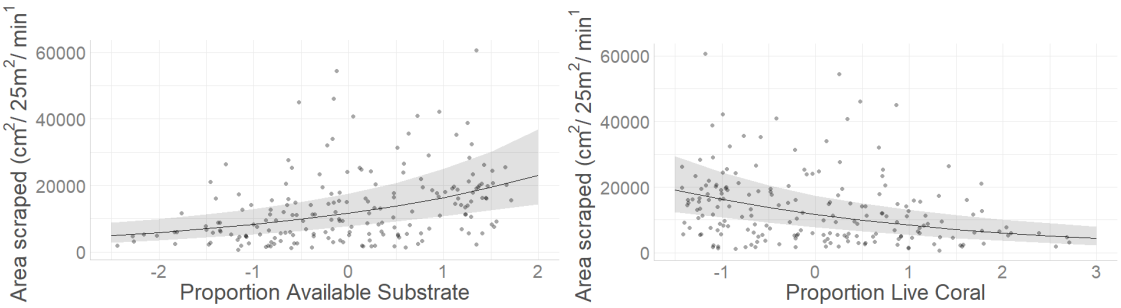
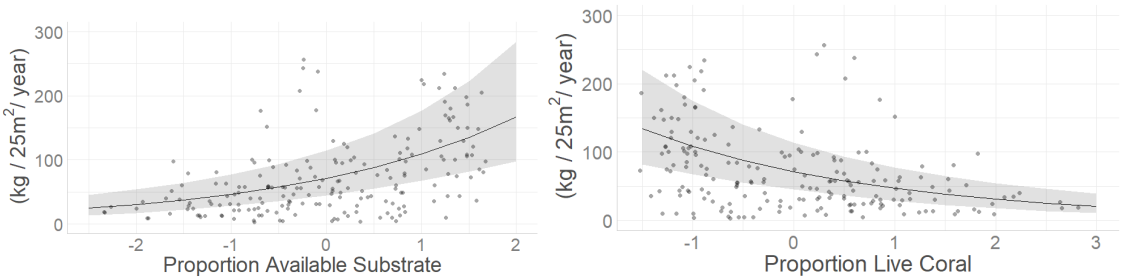
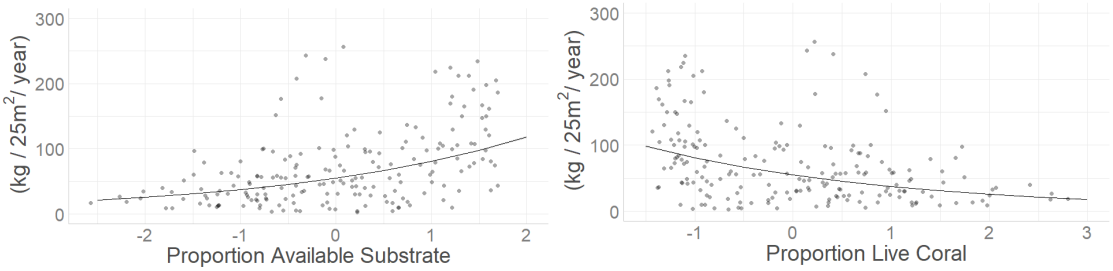
Appendix A – Supplementary material



Supplementary Figure 5.1. Partial plots of predicted scraping rates against either available substrate (pavement and rubble combined) or live coral cover (all coral types combined) for the coarse scale model.



Supplementary Figure 5.2. Partial plots of predicted bioerosion values including observations of *C. microrhinos* against values for either available substrate (pavement and rubble combined) or live coral cover (all coral types combined) for the coarse scale model.



Supplementary Figure 5.3. Partial plots of predicted bioerosion values excluding observations of C. microrhinos against values for either available substrate (pavement and rubble combined) or live coral cover (all coral types combined) for the coarse scale model.

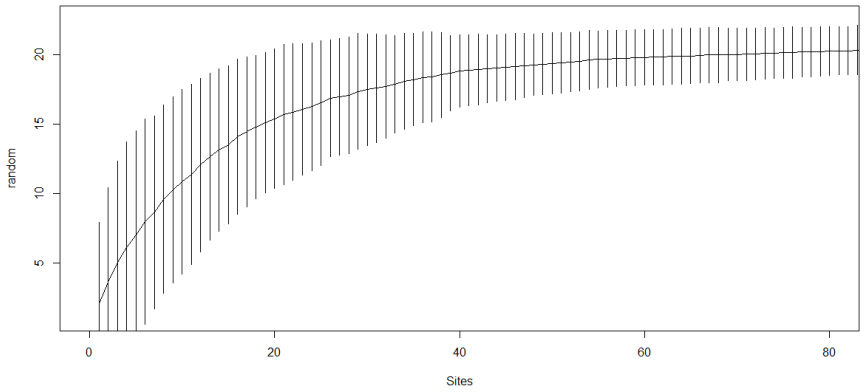


Figure 5.3. Species accumulation plot for all samples run at the level of ASV using the 23S marker in Chapter 3.

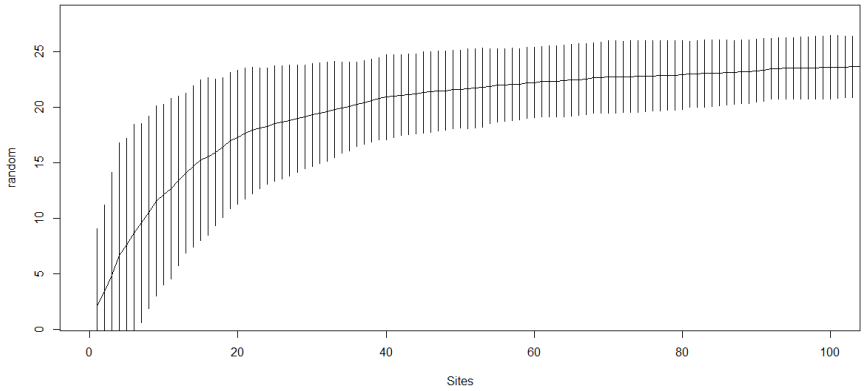


Figure 5.4. Species accumulation plot for all samples run at the level of ASV using the 16S marker in Chapter 3.

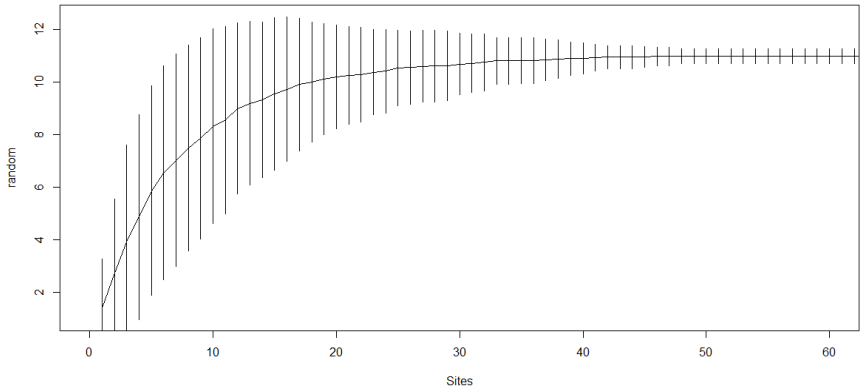


Figure 5.5. Species accumulation plot for all samples run at the level of ASV using the 23S marker in Chapter 4.

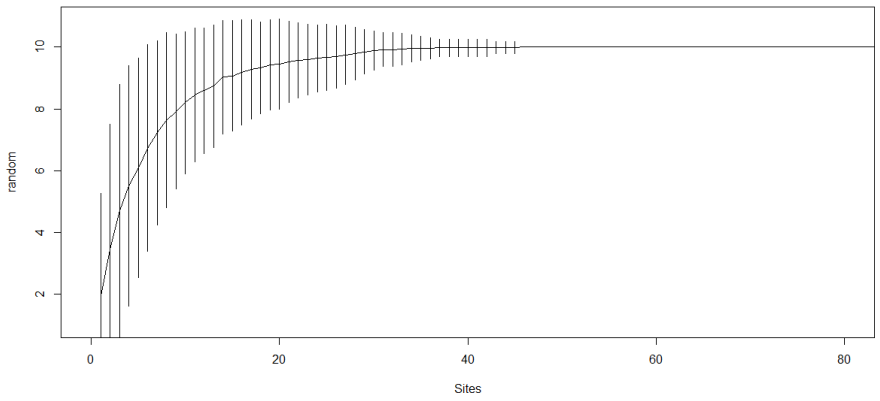


Figure 5.6. Species accumulation plot for all samples run at the level of ASV using the 23S marker in Chapter 4.