Nutrient Impacts on Coral Reefs Captured through Macroalgal Isotopes

Thesis submitted by

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in March 2021

for the degree of Doctor of Philosophy in Biological Science

at Lancaster Environment Centre,

Lancaster University, Lancashire, United Kingdom

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Acknowledgements

The PhD experience has been a truly dynamic, challenging and incredibly rewarding experience for me. I remember getting the email confirming I had got the PhD while actually working in the lab with corals during an internship in Hawai'i and being so excited about the chance to keep working in coral reef science. And now, a few years later, I think it's safe to say that my interests in this field are not only as strong as ever, but have actually grown to cover even more subjects (especially across other disciplines and work areas like social science, environmental monitoring & natural resource management), and that is primarily due to having Nick Graham as my main supervisor. He has been without doubt the best supervisor I've ever had, from all the weekly meetings, in-depth revisions on manuscript drafts, insightful discussions, helping me to sort hundreds of samples during the amazing research cruise around the Seychelles, working on job applications with me, and just being incredibly patient yet constructive whenever I get carried away and try to fit too many ideas down on one page, so thank you, Nick!

This thesis also would not have been possible without my other supervisors, Gareth Williams, Peter Wynn and Phil Barker, so I want to thank them for all their help with concepts, experimental design, and statistics. I especially want to thank Peter, who was added onto my project as my fourth supervisor halfway through first year due to all his help during the planning stages of my first field trip to the Seychelles. Everything I've learnt since about stable isotopes all started from the many meetings and email exchanges I had with Peter, and I would not have been able to pull off nearly as much of the field and lab work in the Seychelles and at LEC as I did without his guidance and expertise. The same could be said for David Hughes, who spent an incredible amount of time helping me to process over a thousand samples, including those that needed to be acidified and re-run!

I also want to thank two other people who were instrumental in bringing this thesis together: Shaun Wilson and Valeriano Parravicini. I really enjoyed working with Shaun in the Seychelles, as he made it such a fun and valuable experience along with Nick and Jan, but he's also given me so much great feedback on manuscript drafts, especially on statistics, and the two papers that have recently been accepted (one pending revisions) show that it clearly paid off. I am also so grateful to Vale for allowing me to work with him and his group at CRIOBE; I really appreciated all the help that he and Alex Merciere gave me in the planning, design, construction and implementation stages of my experiments (including all the trips to the hardware store!). Thank you for also obtaining the SST and benthic data for Mo'orea, as they really made a difference to the paper.

Thank you to everyone else who helped and advised me with both the lab and field work in the Seychelles (especially Nathalie, Stephanie, and Rodney Bonne, my brilliant dive team who helped me collect and organise over a thousand samples!), as well as Seychelles Fishing Authority for logistical support. For the work in Mo'orea, I'd like to thank everyone who provided assistance with lab or field work at CRIOBE, Chris Meyer for bringing my aquarium lights all the way over to Mo'orea from the Smithsonian Institute (the joys of international shipping to remote islands!), and also the researchers from Gump Station, Mo'orea (Russell Schmitt, Sally Holbrook, Tom Adam, Rob Carpenter, Peggy Fong, Cat Fong and Deron Burkepile) who gave me so much insightful advice about running nutrient experiments. I would like to give a special mention to my fellow LEC REEFS PhD student, Samantha Howlett, who I'm so grateful to for running the transplant experiment during the bleaching event in Mo'orea in the midst of all her other experiments – it just goes to show that a casual discussion about experiment ideas over coffee always has the potential to turn into a real collaborative project and even a paper!

Thank you to all the members of LEC REEFS lab, especially Christina, Sal, and the "Coral OG" crew (Jan, Marleen and Anna) who have been through it all with me since the start, along with a bunch of other wonderful people at LEC, Lancaster University and Bangor University. It's been so brilliant working and socialising with you all, and we have so many great memories in so many places, from the various conferences (RCUK, ECRS, WIOMSA), to walks in the Lake District, diving and surfing

in Mauritius, and a brilliant lab retreat in a slightly flooded Cumbria that's made the whole PhD experience even better. I can't wait until we can finally all have a big reunion to celebrate everyone's fantastic achievements in person instead of over Zoom, as it's been far too long! I also want to thank James Robinson, Jeneen Hadj-Hammou, and Casey Benkwitt for their help with statistics advice and troubleshooting.

Finally, I want to thank my Mum, Dad and brother Jamie – you have all been incredibly supportive of my career since the start, even if it means I occasionally have to disappear off to the other side of the world for a while! I'm incredibly lucky to have a mum who's done a PhD, a few post-docs and worked in industry, and a dad who currently works in marine environmental management; your advice and ability to keep my plans and thoughts focused thanks to your own experiences have been invaluable since my undergraduate degree, as well as for life in general. Thank you for putting up with me during the pandemic—something I never imagined factoring in at the start of my PhD—but at least I haven't been the biggest troublemaker in the house! It is slightly ironic, however, that having a mad puppy around again has somehow kept all of us sane while locked in together, so it was definitely worth it. Thank you for everything.

Declaration of contributions from others

My thesis includes collaborations with my supervisors Nick Graham, Peter Wynn, Gareth Williams, and Phillip Barker, and my other co-authors, Shaun Wilson, Valeriano Parravicini, and Samantha Howlett. 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 Nick Graham's Royal Society grant (RG150710), a Royal Society-funded PhD studentship, and a Lancaster University Graduate School travel grant.

Research Support

Field and Laboratory Support and Advice – Seychelles Fishing Authority (SFA), Seychelles

Nathalie Bodin; Stephanie Marie; Rodney Bonne; Rodney Govinden; Jan-Claas Dajka; Jan Robinson, Jude Bijoux, Jacqui; Laurent; Seychelles Marine Parks Authority (SNPA); Global Vision International (GVI); Chris Mason-Parker; SFA R/V *L'Amitie* Crew.

Field and Laboratory Support and Advice – CRIOBE, Mo'orea, French Polynesia

Valeriano Parravicini; Alexander Merciere; Benoit Espiau; Nina Schiettekatte; Beverley Knight; Mathilde Godefroid; Hedrikje Jorissen; Rob Vector; Jennie Pistevos; Maggy Nugues, Christopher Meyer; Gump Field Station researchers, Robert Carpenter, Peggy Fong, Cat Fong, Russell Schmitt, Sally Holbrook, Deron Burkepile, and Tom Adam.

Stable Isotopic and Elemental Analyses, Laboratory Support and Advice – Lancaster Environment Centre, Lancaster, UK

Peter Wynn; David Hughes; Imke Grefe; John Crosse; Catherine Head, Phil Barker.

Permits

The research in Seychelles was undertaken with a research permit from the Seychelles Bureau of Standards (permit number: A0157).

Author contributions by chapter

Supervisors

Professor Nick Graham (NAJG), Dr Gareth Williams (GJW), Dr Peter Wynn (PW), Professor Phillip Barker (PAB)

Other co-authors

Dr Shaun Wilson (SW), Dr Valeriano Paravicini (VP), Samantha Howlett (SJH)

Chapter 1 – The precision and cost-effectiveness of bioindicators for capturing nutrient regimes

EJV AND NAJG conceived the ideas with support from PW, SW, GJW and PAB; , EJV, NAJG and PW designed the methodology; EJV led the sample collection and organised export from the Seychelles; NAJG and SW conducted the benthic surveys; EJV, NAJG and

PW provided the resources; EJV led the stable isotopic analyses; EJV analysed the data; EJV led the writing of the manuscript; All authors contributed critically to the drafts.

Chapter 2 - Species and nutrient history influence the effectiveness of macroalgal bioindicators for passive and active biomonitoring of nutrient runoff.

EJV conceived the ideas with support from NAJG; EJV conducted the sample collection and experiment; EJV led the stable isotopic analyses; VP provided the facilities for laboratory work in CRIOBE; EJV analysed the data. All authors contributed critically to the drafts.

Chapter 3 - The effects of fine-scale spatio-temporal variability of nutrient inputs on two morphologically-similar coral reef macroalgae

EJV conceived the ideas with support from NAJG; EJV conducted the experiment; EJV led the stable isotopic analyses; VP provided the facilities for laboratory work in CRIOBE; EJV analysed the data. EJV and NAJG contributed critically to the draft.

Chapter 4 – Nutrient enrichment in macroalgae following mass coral mortality

EJV and NAJG conceived the ideas with support from GJW and SW; NAJG and SW conducted the benthic surveys in the Seychelles and provided the data; EJV collected the samples in the Seychelles; SJH conducted the experiment in Mo'orea; VP provided the time series and benthic data from Mo'orea; EJV led the stable isotopic analyses. EJV and VP analysed the data. All authors contributed critically to the drafts.

Abstract

Anthropogenic nutrient runoff is a major local stressor on coral reefs but compared to research on global climate change and overfishing, progress has been slower at quantifying its effects, particularly at the ecosystem scale. This is due to the difficulties in cost-effectively capturing the high spatio-temporal variability of bioavailable nutrients in reef systems. In this thesis, I examine common bioindicators and associated methodologies for assessing nutrient regimes as well as the relationships between nutrient and biological responses of the bioindicators. I first compare the precision and cost-effectiveness of five nutrient signatures ($\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N Ratio) in a suite of eight indicators across 21 reefs around the inner Seychelles islands. I show that the congruency between the three most precise types (brown macroalgae, green macroalgae and zoanthids) was low, which was likely due to differences in species-specific ecological strategies (e.g. nutrient uptake and/ or storage capacity). I then test the theory that species within the same functional groups should respond similarly to nutrient enrichment using a) passive biomonitoring (sampling along a nutrient gradient) b) active biomonitoring (*in situ* reciprocal transplant experiment), and c) manipulative laboratory experiments (nutrient supply rates). Overall, these studies suggest that even the responses of morphologically-similar macroalgae with different strategies for nutrient uptake can vary over fine spatio-temporal scales, particularly if they are not nutrient-limited. Finally, I use one of these methodologies in a real-world scenario to investigate the influence of mass coral mortality events on $\delta^{15}N$ signatures of transplanted macroalgae 1) before and after the 2016 bleaching event in the Seychelles, and 2) during the 2019 bleaching event in Mo'orea. Both case studies strongly imply that macroalgae can potentially take up this mass release of dead coral tissue, and possibly locking them into local biogeochemical cycles for up to a year after a bleaching event. I conclude that a traits-based approach, using a

suite of congruent bioindicators with the same functional traits (i.e. rapid nutrient uptake), would be the most cost-effective option for incorporating into broader, more comprehensive monitoring programs.

Publications during the PhD

Peer-reviewed publications

Vaughan EJ, Wynn PM, Wilson SK, Williams GJ, Barker PA, and Graham NAJ (2021)[1]. Precision and cost-effectiveness of bioindicators to estimate nutrient regimes on coral reefs. *Marine Pollution Bulletin*, 170: 112606. https://doi.org/10.1016/j.marpolbul.2021.112606

Vaughan EJ, Wilson SK, Howlett SJ, Parravicini V, Williams GJ, and Graham NAJ (2021)[4]. Nitrogen enrichment in macroalgae following mass coral mortality. *Coral Reefs*, 40, 767-776. https://doi.org/10.1007/s00338-021-02079-w

In preparation for submitting to peer-review

Vaughan EJ, Parravicini V, and Graham NAJ (*in prep*)[2]. Species and nutrient history influence the effectiveness of macroalgal bioindicators for passive and active biomonitoring of nutrient regimes. *Marine Biology*.

Vaughan EJ, Parravicini V, and Graham NAJ (*in prep*)[3]. The effects of fine-scale spatio-temporal variability of nutrient enrichment on two morphologically-similar coral reef macroalgae. *Marine Ecology Progress Series*.

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General Discussion

GENERAL INTRODUCTION

Global and Local Drivers of Coral Reef Degradation

Coral reefs are currently facing large-scale declines due to a multitude of local and global stressors, but the three most prevalent drivers are thought to be climate change, nutrient enrichment from terrestrial runoff, and overfishing (Pandolfi et al., 2003; Bellwood et al., 2004; Hoegh-Guldberg et al., 2007; Howarth, 2008; Jackson, 2008; Hughes et al., 2017; MacNeil et al., 2019). Global climate change causes increases in sea surface temperatures (SST), which can lead to coral bleaching (Hoegh-Guldberg, 1999; Hughes et al., 2018). As the frequency and severity of multiple stressors such as these are not only increasing, but acting synergistically, antagonistically, and additively on coral reefs and reef-associated organisms (Ban et al., 2013; Gil et al., 2016; Fong et al., 2017; Harborne et al., 2017), they are also raising concerns about the future of the ecosystem services they provide (Ainsworth et al., 2019; Williams & Graham, 2019; Woodhead et al., 2019).

The local impacts of overfishing on the abundance of herbivorous organisms, and coastal runoff on the availability of nutrients, are considered to be the primary drivers of macroalgal (Littler & Littler, 1982; McCook, 1999; Burkepile & Hay, 2006) and epilithic turf algal (Hatcher & Larkum, 1983) community dynamics on coral reefs (Duran et al., 2016). The relative and interacting roles of top-down herbivory versus bottom-up nutrient control on algal proliferation have been heavily investigated and debated due to their complexity and differential effects on a range of reef-associated organisms (Smith et al., 2001; Diaz-Pulido et al., 2003; Lapointe et al., 2004b; Littler & Littler, 2006; Littler et al., 2006a; Kopp et al., 2009; Vermeij et al., 2010; Rasher et al., 2012; Jessen et al., 2013; Roth et al., 2015; Clausing

et al., 2016). These controls can influence reef community structure both directly and indirectly, with either positive or negative consequences (Littler et al., 2006a).

Increased nutrient loads from coastal runoff can either have direct limiting effects on coral reefs by inducing physiological stress in corals (i.e. inhibiting growth) (D'Angelo & Wiedenmann, 2014; Silbiger et al., 2018; MacNeil et al., 2019; Zhao et al., 2021), or direct stimulatory effects by enhancing growth of flesh algae and shifting reef community structure (McCook, 1999; Fabricius, 2005; Zhao et al., 2021). In addition, the indirect effects of increased nutrients can influence competitive outcomes for space between algae and hard reef-building (scleractinian) corals (McCook et al., 2001; Jompa & McCook, 2002, 2003; Littler et al., 2006). For instance, a few weedy, opportunistic algal species of low complexity are becoming increasingly dominant on reefs across the globe (Littler & Littler, 1989; Mumby, 2009; Dell et al., 2016; Dajka et al., 2021). If algae become more abundant because of these drivers, it can reduce the growth, reproduction, and survival of corals (Tanner, 1995; Kuffner et al., 2006; Box & Mumby, 2007; Rasher & Hay, 2010, 2013). This may affect the recovery potential of corals, as larval connectivity and settlement are critically important for re-establishing a coral-dominated reef after a disturbance occurs (Nyström et al., 2000; Hoey et al., 2011; Chong-Seng et al., 2014; Dajka et al., 2019). Top-down herbivory control also has a range of direct and indirect impacts on coral reefs: they can directly reduce fleshy algal biomass, which indirectly creates space and allows proliferation of grazer-resistant and calcareous organisms such as reef-building corals and coralline algae (Lewis, 1986; Littler et al., 2006).

It has been suggested that in most cases, herbivory has an overall stronger impact (Stuhldreier et al., 2015; Duran et al., 2016), so much focus has been placed on quantifying the role that

herbivory plays in preventing phase shifts. In some regions, this information has resulted in more sustainable fishing regulations for stocks of key functional groups like algal browsers, or marine protected areas (MPAs) have been created to protect both target and non-target fishes over large areas (Ledlie et al., 2007; McClanahan et al., 2011, 2012; Rasher et al., 2012; Wilson et al., 2012; Graham et al., 2013; Robinson et al., 2019). However, there are limitations to the effectiveness of top-down control. Some algae produce chemical deterrents to reduce the effects of herbivory (Rasher et al., 2011; Rasher & Hay, 2010; 2013), the later successional algal groups (i.e. mature leathery species like *Sargassum* and *Turbinaria*) become unpalatable for most types of herbivores (Burkepile & Hay, 2008; Cheal et al., 2010; Loffler et al., 2015; Bergman et al., 2016; Bittick et al., 2016), herbivory rates become insufficient to control high densities of macroalgae, and herbivorous fishes tend to avoid dense patches for grazing (Williams et al., 2001; Hoey & Bellwood, 2011).

In a few extreme cases, the increase of algal recruitment, inhibition of coral recruitment, and later succession of large fleshy and foliose macroalgae caused by anthropogenic activities can cross what is known as an ecological threshold (Knowlton, 1992; Lapointe, 1997) and lock the reef ecosystem into a feedback loop (Dell et al., 2016; Dajka et al., 2020, 2021). This results in a phase shift in dominance from reef-building, calcifying groups (e.g. scleractinian corals and coralline algae) to non-calcifying algae and invertebrates (Aronson et al., 2004; McManus & Polsenberg, 2004; Bruno et al., 2009; Norström et al., 2009; Nyström et al., 2012; Smith et al., 2016; Johns et al., 2018; Adam et al., 2021). For instance, coral reefs in the Seychelles, western Indian Ocean, were heavily impacted by a mass bleaching event in 1998, and some of these were unable to recover, so phase shifts to macroalgal-dominated states (i.e. dense *Sargassum* beds) have since been observed (Graham et al., 2015; Wilson et al., 2019; Dajka et al., 2021). In other regions, a mass die-off of the grazing sea urchin

Diadema in the Caribbean was thought to be the primary cause of the local proliferation of *Lobophora variegata* (Hughes, 1994; Mumby, 2009), and substantial sewage leakage in Kane'ohe Bay, Hawai'i allowed *Dictyosphaeria cavernosa* to permeate the reefs (Hunter & Evans, 1995; Smith et al., 2001; Stimson et al., 1996, 2001).

Studies on a range of different ecosystems have suggested that even relatively small increases in nutrient loads can exacerbate the effects of climate change and ocean warming on the regulatory mechanisms of some species (Werner et al., 2016), and give other, more stress-resistant benthic organisms a competitive advantage (Martínez et al., 2014). Relying on certain management strategies alone, such as reductions in fishing activities on functionally important herbivorous species and increased number and size of MPAs, may be insufficient to facilitate coral recovery and prevent phase shifts (Graham et al., 2008). These efforts will need to be complemented with localised management and quantification of bottom-up drivers such as nutrient enrichment (Smith et al., 1981; Lapointe, 1997; McCook, 1999; Fabricius, 2005; Mumby et al., 2007; McClanahan et al., 2012; Graham et al., 2013, 2015; Hughes et al., 2017). Therefore, the combined mitigation of local disturbances, through both marine protected areas and local catchment management, and addressing social distal drivers, will be some of the key strategies necessary for managing coral reefs in the Anthropocene, where the impacts of global climate change are predicted to increase in magnitude and frequency (McClanahan et al., 2001; Graham et al., 2015; Hicks et al., 2016; Norström et al., 2016; Hughes et al., 2017; Anderson et al., 2019; McLeod et al., 2019).

Variability of Nutrient Regimes & Biological Responses

There is widespread evidence that reductions in water quality, which includes changes in nutrients, sediment loads, turbidity, light attenuation, and other pollutants such as trace metals, has caused declines in biodiversity and disruption of key ecological processes (Smith, 1981; Hunter & Evans, 1995; Stimson & Larned 2000; Stimson et al., 2001; Loya et al., 2004; Fabricius et al., 2005, 2012; Adam et al., 2021). This phenomenon, which is also sometimes known as eutrophication, not only occurs in tropical shallow-water coral reefs and lagoons, but in other freshwater, estuarine and coastal marine ecosystems around the world (Smith, 2003), such as the temperate and enclosed Black Sea in Europe (Ferreira et al., 2011) and the North Sea, off the east coast of the United Kingdom (Foden et al., 2011; Capuzzo et al., 2018; García-García et al., 2019; Greenwood et al., 2020). Tropical reef ecosystems are typically located in oligotrophic (nutrient-limited) waters, but are highly productive ecosystems (Wyatt et al., 2013), and are therefore very responsive to even moderate changes in nutrient cycles (Howarth et al., 1988; Herbert, 1999; Galloway et al., 2004).

What is often overlooked in many studies is that increases in nutrients can lead to a variety of both positive and negative responses over a range of biological scales (Zehr & Kudela, 2011; Aronson & Precht, 2016), and so is very dependent on the origins and quantity of these nutrients. Healthy coral reef ecosystems can persist over a broad range of natural nutrient environments at the lower end of the concentration scale (D'Angelo & Wiedenmann, 2014), and the increased productivity of phytoplankton and bacterioplankton drive production at higher trophic levels (McCauley et al., 2012) and biogeochemical dynamics (Zehr & Kudela, 2011), respectively. In addition, under certain conditions, slight enrichments can even benefit corals by increasing the physiological performance of both the coral host and their

photosynthesising symbiotic algae (zooxanthellae), as these can increase coral growth rates and induce higher symbiont densities (Bythell, 1990; De'ath & Fabricius, 2010; Burkepile et al., 2013; D'Angelo & Wiedenmann, 2014). The capability of corals to acquire fixed carbon for energy from alternative sources to their symbionts by catching zooplankton suspended in the water column (heterotrophy) can also rise, and this is thought to be an adaptive strategy to resist the effects of bleaching and increase resiliency (Grottoli et al., 2006; Seeman, 2013; Fox et al., 2018; Williams et al., 2018).

Beyond certain thresholds of concentration however, additional inputs of nutrient loads from external pools, from either natural and/ or anthropogenic sources, can easily disturb the balance of natural biogeochemical dynamics (Baker et al., 2013; D'Angelo & Wiedenmann, 2014). Excess loads of nitrogen not only affect the coral-zooxanthellae symbiosis and increase susceptibility to bleaching, due to an imbalance of nitrogen and phosphorous (Wooldridge, 2009b; Wiedenmann et al., 2013; Carnicer et al., 2015; Rosset et al., 2017), and enhance the risk of coral disease (Bruno et al., 2003; Voss & Richardson, 2006; Redding et al., 2013), but they also indirectly influence the reef community structure (Fabricius, 2005; Wild et al., 2011; Naumann et al., 2015; Adam et al., 2021). In addition, the identity of nitrogen source, such as nitrates (NO_3-) versus urea and ammonium (NH_4^+) can also impact coral susceptibility to bleaching and/ or mortality, as it lowers the temperature threshold at which corals can bleach (Burkepile et al., 2019; Donovan et al., 2020). Therefore, if coral reefs are exposed to excess nitrogen, or even to an imbalance of nutrients, it could result in the occurrence of bleaching events even though ambient bleaching thresholds have not been surpassed (Wooldridge, 2009b; Burkepile et al., 2019).

Williams et al. (2015) found that increases in chlorophyll-α, an estimate of phytoplankton biomass, positively correlated with the abundance of calcifiers at unpopulated islands (i.e. no anthropogenically-derived runoff), whereas the reverse was true at populated islands. This implied that human impacts on adjacent reefs can modify natural biophysical relationships (Gove et al., 2015; Jouffray et al., 2019). Phytoplankton blooms also increase the food supply of the larvae of corallivorous Crown-of-Thorns starfish (*Acanthaster* spp.), which results in outbreaks of the adults that decimate large sections of coral-dominated reef (Engelhardt & Lassig, 1997; Fabricius et al., 2010; D'Angelo & Wiedenmann, 2014).

Enhanced nutrient loads not only cause a rise in other primary producing organisms (including macroalgae and phytoplankton), but can consequentially cause an increased abundance of filter-feeding, boring, and bioeroding organisms such as sponges. These types of organisms not only compete with corals and other calcifying organisms for space, but can damage the structural integrity of the reef itself (Smith et al., 1981; Rose & Risk, 1985; Hunter & Evans, 1995; van Woesik et al., 1999; Ward-Paige et al., 2005b; D'Angelo & Wiedenmann, 2014).

The interactions between fleshy organisms and reef calcifiers, such as algae and reef-building corals, are also affected by nutrient enrichment, although it can be difficult to distinguish whether these are competitive or not (McCook, 2001). Direct and indirect physical and chemical interactions reduce the performance of either coral or algae in the presence of the other. Examples include the inhibition of coral fecundity, larval survival and settlement, and juvenile growth (Box & Mumby, 2007; Birrell et al. 2008), formation of lesions (Meesters & Bak, 1993; McCook et al., 2001), algae-mediated and pathogenic microbe-induced coral mortality (Smith et al., 2006; Wild et al., 2010; Barott & Rohwer, 2012; Barott et al., 2012a;

Haas et al., 2016) and the production of allelopathic chemicals by certain macroalgae which damage corals (Rasher et al., 2011; Bonaldo & Hay, 2014). However, coral mortality may also be the result of other disturbances such as storm damage, corallivory, bleaching or nutrient stress. Therefore, it can be difficult to determine whether algae directly or indirectly outcompeted corals, or simply took advantage of the space created following a separate disturbance (McCook et al., 2001).

It is clear that nutrient enrichment causes a range of responses over several biological scales, but progress has been slow at quantifying the complex relationships between physical and ecological controls of coral reef ecosystem dynamics (Williams et al., 2015). This is primarily due to the large spatial and temporal variations in internal nutrient pools and the additional external inputs of nutrients from a vast range of sources as coral reefs are such highly complex, productive, and diverse ecosystems (Briand et al., 2015). Therefore, the relative fluxes and contributions of these different internal and external sources have to be better understood.

Internal (allochthonous) sources of nutrients primarily come in the form of particulate organic matter (POM), and are tightly recycled within reef systems through biological (Rix et al., 2017, 2018) and physical processes (Wyatt et al., 2013; Briand et al., 2015; Lowe & Falter, 2015; Deininger & Frigstad, 2019). Allochthonous POM sources are derived from either living or dead organic materials from different organisms which are suspended in the water column, or can be living organisms (microphytobenthos and meio-infauna) and detritus deposited in the sediments (sedimentary organic matter, SOM) (Umezawa et al., 2008; Wyatt et al., 2013; Briand et al., 2015; Deininger & Frigstad, 2019). POM represents a critical

component of the total nutrient budget (Wild et al., 2004a,b; Deininger & Frigstad, 2019), as it affects coral calcification and resilience to stressors (Grottoli et al., 2006).

Biological processes, such as the excretion and assimilation of nitrogen species from macrofauna and macroflora, respectively, are key contributors to internal nutrient cycling in marine systems (Wild et al., 2011; Burkepile et al., 2013; Fowler et al., 2013; Voss et al., 2013). On a coral-dominated reef, reef fishes can facilitate growth of corals not only by grazing on algae, but also by excreting nutrients. However, these additional "natural" nutrients can also enhance macroalgal growth if corals are significantly impacted by disturbances (Burkepile et al., 2013). Similarly, nitrogen- and phosphorous-rich guano produced by large populations of seabirds on islands adjacent to coral reefs can provide significant nutrient loads to these ecosystems (Young et al., 2010; McCauley et al., 2012; Honig & Mahoney, 2016; Graham et al., 2018). Mangroves can also contribute another natural terrestrial source of nutrients in lagoons and other coastal environments, as up to ~30-50% of their leaves can be exported and broken down into organic matter (Briand et al., 2015).

As structural engineers on coral reefs, scleractinian corals can provide more than just structural complexity or habits to other reef organisms, including the release of POM in the form of mucus. This is an essential source of naturally-derived nutrients that provides sustenance to coral reef ecosystems (Wild et al., 2004a,b; Wyatt et al., 2013; Mumby & Steneck, 2018; Tanaka & Nakajima, 2018). However, after bleaching events, there can be a short spike in mucus release (Coffroth, 1990; Davey et al., 2008; Fitt et al., 2009; Niggl et al., 2009; Wooldridge, 2009a), and if stressful conditions persist, such as increased sea surface temperatures, mass coral mortality can occur and result in the expulsion of coral tissue from

the calcified skeleton (Davey et al., 2008; Leggat et al., 2019). However, there has been very little research into how mass coral mortality events can affect the other organisms on the degraded reef, such as microbial or macroalgal colonisation on the exposed coral skeleton (Diaz-Pulido & McCook 2002; Davey et al. 2008; Haas et al., 2010; Wild et al., 2011) or for how long this natural source of nutrients, driven by anthropogenic events, can persist in local biogeochemical cycles (Rix et al., 2016, 2017, 2018; Mumby & Steneck, 2018; Deininger & Frigstad, 2019; Radice et al., 2020). This is critical to understand, as losing foundation species like scleractinian corals can have substantial impacts on both ecosystem structure and biogeochemical processes (Wild et al., 2011; Wilson et al., 2019).

Microbial processes influence the bioavailability of nutrients for marine organisms, by transforming nitrogen species at different stages of the cycle through processes like nitrogen fixation and denitrification (Howarth et al., 1988; Herbert 1999; Kendall et al., 2007; Voss et al., 2013), which adds a significant amount of internal "new" nitrogen into the system (Cardini et al., 2014; Deininger & Frigstad, 2019). Microbes associated with other benthic organisms, such as the reef-building coral holobiont, cyanobacterial mats, seagrasses, and macroalgae (Smith et al., 2006; Moulton et al., 2016), are also capable of carrying out nitrogen fixation. Human activities like overfishing and nutrient runoff are also the proximal drivers of increasing microbialisation in coral reefs (Barott et al., 2012b; Angly et al., 2016; Haas et al., 2016; Zaneveld et al., 2016; Glasl et al., 2017). These give macroalgae another advantage over calcifying organisms like corals and coralline algae, as they are locked into a positive feedback loop with the former, and dissolved organic matter released from fleshy algae facilities growth of pathogenic bacteria that negatively affect calcifying organisms (Wild et al., 2011; Haas et al., 2016).

The spatial scales of physical drivers of nutrient circulation and transport can vary substantially, from a few millimetres to hundreds of kilometres, so there are many ways in which they can shape biological and chemical processes on coral reefs (Lowe & Falter, 2015). These external oceanographic processes can introduce natural external (autochthonous) sources to reef systems (Wyatt et al., 2013). At the organism to reef-canopy scale, hydrodynamic forcing of water flow can vary due to the complex and diverse structures of reefs (e.g. massive *Porites* vs. branching *Acropora* structures). This not only influences the flow and transfer of nutrients and particles across the boundary layers of the reef, but also how reef heterotrophs adapt to trap and feed on them (Bilger & Atkinson, 1992; Leichter et al., 2013; Lowe & Falter, 2015).

Hydrodynamic processes such as flushing and dilution, accumulation and resuspension of sedimentary organic matter, and internal nutrient storage capacity in organisms' tissues (Fong et al., 1994) drive the retention and removal rates of nutrients within a reef system (Hoeke et al., 2013; Leichter et al., 2013; Lowe & Falter, 2015). A rise in loads from internal and external nutrient pools and increased water residence times in more enclosed seas or lagoons can lead to the formation of blooms of primary producers and decline in water quality, as the concentration and duration of enrichment is higher (Fabricius, 2005; Brodie et al., 2012b; Briand et al., 2015; Deininger & Frigstad, 2019). It is also thought that the further away from an affected coastal area or river mouth a reef is, the more dilute the concentration of nutrients will be (Adjeroud & Salvat 1996; Devlin & Brodie, 2005; Lin & Fong, 2008; Brodie et al., 2010b, 2012b; Devlin et al., 2012). This has been found to be a key driver of spatial variation in reef community structure, particularly along coastlines (Dailer et al., 2010; De'ath & Fabricius, 2010) and along environmental gradients (Adjeroud, 1997; Fabricius et al., 2005; Arévalo et al., 2007; Kürten et al., 2014). For instance, Opunohu Bay in Mo'orea, French

Polynesia is a ~3.5 km bay that is impacted by multiple sources of anthropogenically-derived nutrient runoff, such as shrimp farm and pineapple farm effluents from the river at the base of it, and many studies have shown strong evidence of a gradient in coral reef community from the land-end to the ocean-end (Adjeroud, 1997; Lin & Fong, 2008).

Waves are important oceanographic drivers of rapid exchanges of nutrients on coral reefs, particularly across the Indo-Pacific and Caribbean, as the breaking of surface waves on shallow fore reefs and reef crests dissipates energy and circulates nutrients within the water column (Williams et al., 2013; Adam et al., 2021). However, in more sheltered or deeper areas such as lagoons, wind forcing and tides may play a greater role in driving circulation, as there is a more limited exchange of water with the open ocean. The latter is also a particularly significant driver in areas with large tidal ranges (>3m) (Lowe & Falter, 2015). Natural wind- or current-driven upwelling and internal waves, drives cross-shelf nutrient exchanges between the open ocean and coral reefs bring cooler, nutrient-rich waters to subsurface levels, which may also provide refuge from thermal stress for corals as well as increases in nutrient supply (Viana & Bode, 2013; Williams et al., 2013; Lowe & Falter, 2015; Roth et al., 2015).

The rate of nutrients transported to coastal areas primarily through river discharge is enhanced during extreme weather events, such as periods of heavy rainfall and strong wave forcing caused by storms and tropical cyclones (Russ & McCook, 1999; Anthony et al., 2014; Clausing & Fong, 2016; Fong et al., 2020). However, the latest annual report for inshore water quality monitoring in the Great Barrier Reef (GBR) from the Reef Rescue Marine Monitoring Program (Waterhouse et al., 2021) found variability between catchment areas close to the GBR, due to significant variations in biophysical and socio-economic characteristics (Brodie & Waterhouse, 2012; Schaffelke et al., 2012), as well as in land use

changes over time (Lewis et al., 2021). These factors can subsequently affect the concentrations of different sources from different rivers and/ or flow variability, and even the nutrients that do reach the reefs are often constrained and broken up by island reefs (Devlin & Brodie, 2005; Brodie et al., 2012a&b; Brodie & Waterhouse et al., 2012; Schaffelke et al., 2012; Kroon et al., 2016; Baird et al., 2021; Pearson et al., 2021; Waterhouse et al., 2021). Sedimentary nutrients can also be re-suspended into the water column because of rough weather conditions (Brodie & Waterhouse, 2012; Risk, 2014). There can also be high temporal variability in nutrient regimes on coral reefs due to seasonal changes in some of the local drivers mentioned above, such as the changes in intensity and frequency of heavy rainfall during the monsoonal months of the year (McCook, 1999; Anthony et al., 2014; Edmunds & Gray, 2014; Hernández-Delgado et al., 2014; Clausing et al., 2016; Fong et al., 2020).

Anthropogenically-derived sources of nutrient runoff can also be introduced to the total nutrient budget in reef ecosystems (Howarth et al., 1988; Lapointe et al., 2004a). The key examples include sewage pollution (Pastorok et al., 1985; Costanzo et al., 2001; Jones et al., 2001; Lapointe et al., 2005; Risk et al., 2009), submarine groundwater discharge (Encarnação et al., 2014; Amato et al., 2016), wastewater treatment plant effluent (Dailer et al., 2010; Sawyer et al., 2016; Barnes et al., 2019; Amato et al., 2020), aquaculture and fish farms (Loya, 2004; Lin & Fong, 2008), agricultural fertilisers (Marion et al., 2005; Kroon et al., 2014; Fraser et al., 2017), and atmospheric deposition (Heaton, 1986; Barile & Lapointe, 2005). Terrestrial or river discharge may also contribute to SOM nutrient pools (Brodie et al., 2010b; Briand et al, 2015). More than half of anthropogenic nitrogen is delivered to the marine environment by river input and atmospheric deposition, which now contributes more to nitrogen cycles than natural fixation processes (Vitousek et al., 1997; Fowler et al., 2013;

Voss et al., 2013). Heavily subsidised overuse of fertilisers and pesticides, poor soil management and unregulated animal production systems are the major sources of anthropogenic nitrogen enrichment in the environment, and the manufacturing process of chemical fertilisers itself requires vast quantities of energy from natural gas (Jackson, 2008; Fowler et al., 2013; Kroon et al., 2014; Fraser et al., 2017; Anderson et al., 2019).

Physical measurements of nutrient regimes

A very common approach for assessing general water quality and eutrophication is to measure the physico-chemical components of nutrient loads in the water column over a variety of different scales (Brodie et al., 2010b; Devlin et al., 2020). Measuring these components are key for providing evidence about the spatial extent of any anthropogenic effluent that may have negative impacts on the resilience of vulnerable ecosystems, such as coral reefs and seagrass beds. As ecosystem services are also tightly linked to ecosystem functions (Hicks et al., 2016; Woodhead et al., 2019), public health and/ or the tourism industry could consequently be negatively affected (Hernández-Delgado et al., 2015; Devlin et al., 2020), particularly if the effluent is derived from sewage outfalls (Barnes et al., 2019) or from wastewater treatment plants (Amato et al., 2020). Proper sewage management is lacking in both rich and poor countries across the globe, so long-term monitoring over different spatial scales of coastal pollution is critical to address through both management and policy to find ways to reduce its impacts on coastal ecosystems and the people who depend on them (Brodie & Waterhouse, 2012; Boesch, 2019). This is especially critical for

vulnerable island and coastal nations, including those termed “small island developing states” (SIDS), such as Vanuatu in the Melanesian Pacific Islands (Hafezi et al., 2020a&b; Barnes et al., 2019; Devlin et al., 2021). Devlin et al. (2020) outlined a framework for how these physical measurements can be collected and integrated into environmental monitoring programs, and at what spatial scale they are most appropriate. This ranged from local (e.g. physico-chemical and *in-situ* water quality measurements, habitat mapping), to local-to-national (e.g. hydrodynamic modelling, vulnerable habitat mapping and collaborative data sources) and national-to-regional scale (e.g. remote sensing/ earth observation data, global trends and climate change reporting) and these will be discussed separately in the paragraphs below (see also *Table 0.1*).

On a local scale, a multitude of water quality parameters, such as dissolved inorganic nutrients (DIN), which includes parameters of nitrate + nitrite ($NO_3 + NO_2 = NO_x$), ammonia (NH_4), dissolved inorganic nitrogen (DIN: as the sum of NO_x and NH_4), dissolved inorganic phosphate (DIP), DIN:DIP ratio, and silicate (SiO_4), as well as others such as particulate organic matter (POM), suspended sediment, salinity, bacteria and stable isotopes, can be regularly measured to understand the physical and chemical properties of coastal waters for monitoring programmes (Sigman & Casciotti, 2001; Brodie et al., 2010b; Devlin et al., 2020). These measurements from seawater samples collected from target areas can be particularly useful when assessing the physical extent of any effluent runoff from coastal areas (De’ath & Fabricius, 2010; Fabricius et al., 2012; Zubia et al., 2018; Barnes et al., 2019; Devlin et al., 2020).

“Spot measurements” (i.e. those taken at the time of sampling) of parameters like POM and chlorophyll-a can be collected regularly at coral reef study sites (Wyatt et al., 2013; De’ath

& Fabricius, 2010; Barnes et al., 2019; Devlin et al., 2020), and the isotopic composition of DIN can be analysed from water samples using bacterial denitrifier methods (Sigman et al., 2001). These can reveal vital information about the water quality at the time when other biological variables (e.g. benthic cover) are surveyed, and chlorophyll measurements in particular may provide more information about the bioavailability of nutrients (Blanco et al., 2008; De'ath & Fabricius, 2010). However, these measurements can easily be confounded by additional physical drivers over time and space, such as by enhanced terrestrial or river discharge after heavy rainfall or during wet seasons (Blanco et al., 2008; Devlin & Schaffelke, 2012; Devlin et al., 2012; Clausing & Fong, 2016). Even if water samples are collected periodically over a longer timescale, these discrete measurements alone may not always detect changes in nutrient loads over finer temporal scales than the sampling interval (Fabricius et al., 2012; Clausing & Fong, 2016; den Haan et al., 2016). Therefore, with the exceptions of measurements of bacteria and chlorophyll-a, these parameters alone do not reveal much about the spatial and temporal variability of local nutrient regimes, particularly for the bioavailable nutrients, within the marine system (Costanzo et al., 2001; Fabricius et al., 2012).

The need for autonomous measurements of these parameters in ocean observing systems have encouraged developments in technology that will allow this, including *in situ* autonomous nutrient loggers and sensors (see reviews in Daniel et al., 2020 and Wei et al., 2021). High-resolution *in situ* sensors such as the special compact spectrophotometer MBARI ISUS V3 have been used to characterise physical and chemical properties of large masses of water over large spatial and temporal scales, such as over a number of research ship cruises, which can be validated against laboratory chemical analysis of collected water samples, to help overcome issues with lack of discrete water sampling for chemical analyses (Kaplunenko et

al., 2013). Another study used an UV-based process spectrophotometer (ProPS) during a cruise in the south-eastern North Sea to determine nitrate concentrations by comparing continuous UV optical nitrate measurements against standard wet-chemical analyses (Zielinski et al., 2011). Lan-on-Chip phosphate analysers have also been placed on underwater gliders to understand the marine phosphorus cycle across the northern North Sea (Birchill et al., 2021). Other examples of *in situ* nutrient sensors include wet chemical analysers and electrochemical sensors, and both have different advantages and disadvantages, such as the types of parameters it can measure, measurement frequency, spatial and temporal resolution, robustness, and length of time they can be deployed for in seawater, either on moored/ buoy platforms, profiling floats offshore or on Remotely Operated Vehicles (ROV's) (Daniel et al., 2020). For instance, a Seapoint STM sensor was used to record regular measurements of turbidity and a Seapoint fluorometer sensor for chlorophyll, both of which were components of ESM2, a micro–logger developed by Cefas with standard sensors that can log multiple physico-chemical parameters (Devlin et al., 2020).

Autonomous, *in situ* logging techniques are particularly useful for producing high-quality data outputs for the long-term and large-scale analysis and monitoring of nutrients in the marine environment, as they can not only enhance the spatial and temporal resolution and coverage for the analysis and monitoring of nutrients in the marine environment, but also reduce the time-intensive process of regularly collecting and measuring seawater samples (Nightingale et al., 2015; Daniel et al., 2020). They is even more beneficial when measurements are compared across different technologies and methodologies, as this helps to determine whether or not the results were due to instrument noise or fault. This is particularly important, as there is currently a lack of harmonisation between deployment protocols for different sensors, which makes it difficult to develop a standardised version and build up

global databases (Daniel et al., 2020). In addition, sensors are required to have both high spatial and temporal resolution in order to capture daily or semi-diurnal processes such as episodic and transient events (Mills & Fones, 2012; Daniel et al., 2020).

As with "spot measurements" of water samples, most existing sensors are able to capture physical trends but not corresponding biological processes over the long term, which is critical for understanding temporal variability, and there are currently few instruments able to detect the type of nutrient concentrations typically found in oligotrophic environments such as coral reefs (Daniel et al., 2020). In addition, many of the current sensors need to be calibrated on a regular basis, which is not always practical if they are placed at remote coral reef sites (Fabricius et al., 2012; Mills & Fones, 2012; Daniel et al., 2020). Many of these high-resolution sensors are also still at the prototype stage, and are not yet commercially available or cost-effective enough to produce for researchers on a large-enough scale, so it can reduce the amount of replication across spatial scales. However, significant progress in reducing analysis costs and sampling frequency of sensors has been made over the last decade through the continual development of high-frequency and commercially-available UV optical sensors. For instance, the availability of cheap housing, low-cost controller/ data loggers based on embedded systems as well as low/ no subscription costs for communication systems has been increasing recently (Albaladejo et al., 2012; Daniel et al., 2020; Marcelli et al., 2021; Nehir et al., 2021). Optical sensors such as ISUS, SUNA (Seabird Scientific, United States), NITRATAX plus sc (Hach Lange GmbH, Germany), S::CAN Spectro::lyser (S::CAN Messtechnik GmbH, Austria), ProPS and OPUS (TriOS GmbH, Germany) have been increasingly used in a variety of aquatic environments, including both coastal waters and open ocean (Nehir et al., 2021). Therefore, by integrating the more cost-effective sensors, platforms and communication systems into open science development frameworks, it will

allow either current or future large-scale monitoring programs, even those in developing countries, to incorporate them into their overall monitoring and management strategies (Marcelli et al., 2021).

Measuring the effects of oceanic forcing at the local to national scale using hydrodynamic modelling (Hoeke et al., 2013; Graham et al., 2020; *Table 0.1*) can reveal much about the physical drivers of biological and chemical processes (Williams et al., 2013; Wyatt et al., 2013; Gove et al., 2015; Lowe & Falter, 2015; Burel et al., 2019; Devlin et al., 2020). For instance, seasonal variation in wave circulation was found in Adam et al. (2021) to increase the spatial extent of coastal runoff during the wet season around the island of Mo'orea, French Polynesia, which was due to increases in heavy rainfall and terrestrial discharge (Leichter et al., 2012, 2013). However, many of the different techniques come with their own challenges. For instance, tidal forcing of circulation in enclosed coastal areas is easier to predict, but wave-mediated mixing is more episodic and more prone to extreme weather events, such as tropical cyclones. However, these extreme events are likely to play a key role in shaping reef community structure as it tests the mechanical limits of reef organisms (Lowe & Falter, 2015), particularly as episodic pulse events of rainfall and riverine discharge are predicted to increase in frequency and with climate change (Anthony et al., 2014).

Remote sensing has become a valuable and cost-effective tool for studying geographic and seasonal patterns of physical drivers as well as associated responses of coral reefs (i.e. susceptibility or resilience) over a range of spatial scales by overlaying satellite data onto spatial maps (Mumby et al., 1997, 2004; Herbert, 1999; Brodie et al., 2010a&b; Devlin & Schaffelke, 2011; Demarcq et al., 20012; Devlin et al., 2012; Rowlands et al., 2012; Roelfsema et al., 2013; Knudby et al., 2013, 2014; Purkis, 2018). For instance, plumes of

sediment due to terrestrial discharge can be identified and mapped from imagery captured by satellite data, and complemented with physico-chemical measurements of total suspended sediments (TSS), chlorophyll-a and coloured dissolved and detrital organic matter (CDOM + D) to validate the values determined through the imagery (Siegel et al., 2005; Morel & Bélanger, 2006; Brodie et al., 2010b; Devlin et al., 2012). The type of Earth Observation (EO) data collected from satellite sensors can be categorised into the kind of spatial and temporal variation they can capture (Roelfsema et al., 2013; Lyons et al., 2020). For instance, Sentinel-2 A and different types of Landsat instruments have a medium to high resolution of 5-30m (Mumby et al., 1997; Hedley et al., 2018; Kovacs et al., 2018), while PlanetScope has a very high spatial resolution of 3m (see review for use of EO data for seagrasses: Hossain & Hashim, 2019). Remote sensing can therefore provide a wealth of information over a wide range of spatial scales, which can help to determine areas where reefs are most at risk of degradation, such as those in the northwest and central Indian Ocean, and central west Pacific, and which regions may provide refuges for corals (i.e. high latitude reefs) (Maina et al., 2008; Rowlands et al., 2012; Knudby et al., 2013, 2014).

Remote sensing methods are highly beneficial techniques for investigating biophysical relationships on coral reefs across the world (Hedley et al., 2016). However, these techniques still leave much room for improvement, and could be developed even further. Although technologies and their spatial, spectral and temporal resolution are continually improving (Lyons et al., 2020), higher resolution of remote-sensed data is required, in addition to improvements in remote sensing algorithms and exhaustive field data to ground-truth the satellite data, to capture spatio-temporal variations in environmental forcings over finer scales (i.e. around individual islands and atolls). Conversely, diver-operated, sonar data and airborne imagery (from planes or drones) can capture more information about benthic cover,

geomorphic variation and bathymetry, such as coral bleaching extent on reefs (Andréfouët et al., 2002; Leiper et al., 2014), but these cannot cover the same kind of regional or global scales that satellite imagery can. Therefore, in order to choose the most appropriate, if not cost-effective spatial resolution for specific management goals and/ or monitoring programs, there has been much focus recently on addressing these challenges (Mumby et al., 1997, 2004; Andréfouët et al., 2002; Siegel et al., 2005; Gove et al., 2012; Knudby et al., 2013, 2014; Roelfsema et al., 2013; Hedley et al., 2016, 2018; Hossain & Hashim, 2019; Lyons et al., 2020).

Biological & Biochemical Measurements of Nutrient Regimes

It is still critical to measure physical fluctuations in nutrient loads using the techniques highlighted above, but they should be complemented with other indicators that reflect biological or ecological responses to changes in nutrient regimes over multiple spatio-temporal scales (*Table 0.1*; Jones et al., 2001; Fabricius et al., 2012). Benthic surveys that are conducted in person are commonly used for observing and measuring changes in benthic composition and other biological parameters when repeated over time (Hunter & Evans, 1995; McClanahan, 2011, 2012; 2020a&b; Graham et al., 2015; Rodgers et al., 2015; Horta e Costa et al., 2016; MacNeil et al., 2019; Obura et al., 2019). However, the logistics of monitoring reefs on a regular basis, such as the costs of personnel, equipment, consumables, and transport to the survey sites, can mean finer-scale variability in responses may not be captured (Hoeke et al., 2009). Further, monitoring abundances and distributions of organisms alone does not necessarily capture changes in environmental conditions.

Robust indicators are needed to provide a proxy of the "health" of the marine environment as a result of environmental, social and economic activities (Fichez et al., 2005), and biological indicators (bioindicators) go a step further by capturing a signal of the biological condition of an ecosystem, which can involve investigating individual species, groups of species, or biological processes (Fabricius et al., 2012). These not only provide biologically-relevant responses to physical stressors on an ongoing basis to assess the extent of impacts, but could even offer an early-warning system of pollution or degradation in an ecosystem, which will allow monitoring programs to either halt or mitigate them before critical ecosystem functions and services are lost. This can be achieved through management strategies and/ or by measuring the performance of current policies to determine how effective they are at protecting coastal ecosystems from stressors (Linton & Warner, 2003; Cooper et al., 2009).

Bioindicators have been used in numerous studies as a tool for either communication for management and/ or as a means of measuring environmental conditions and trends, predicting trends and comparing conditions across a range of spatial and temporal scales, as they integrate environmental conditions over time and space (Fong et al., 1998; Jameson et al., 1998; Risk et al., 2001; Linton & Warner, 2003; Dalhoff, 2004; Fichez et al., 2005; Littler & Littler, 2006; Cooper et al., 2009; Ferreira et al., 2011; Birk et al., 2012; Borja et al., 2012; 2016; Fabricius et al., 2012; Goatley et al., 2016; Glasl et al., 2017; Gorman et al., 2017; Bal et al., 2020). Therefore, any observed changes can potentially be linked to changes in the magnitude, frequency or duration of a biological or physical driver (Fabricius et al., 2012). As stress is considered to induce changes in both the structure and functioning of communities, bioindicators can also be categorised into structural (e.g. species diversity and/ or composition) and functional (e.g. photosynthetic activity, growth rate and fecundity) types, although the latter is not measured as frequently in monitoring programs (Linton & Warner,

2003; Cooper et al., 2009). They also can show a gradient that reflects the level of anthropogenically-induced disturbance at which an increase in nutrient loads results in decreased water quality (Fabricius et al., 2005, 2012).

Ideally, bioindicators should be sensitive enough that they can capture low background (or baseline) variability in undisturbed areas (Ferreira et al., 2011). In addition, while physico-chemical measurements may capture large pulse events from heavy rainfall or terrestrial discharge (Brodie et al., 2010b; Devlin et al., 2012; Polónia et al., 2015), they might not detect low intensity, chronic impacts such as low-level pollution. Bioindicators, conversely, can capture this through cumulative biological responses over time (Linton & Warner, 2003; Anthony et al., 2014). They can also help to assess synergistic or additive relationships among impacts, such as climate change and pollution, which is critical for monitoring coral reefs in the Anthropocene (Hughes et al., 2017; Boesch, 2019). In order to realistically measure these responses over large spatio-temporal scales, bioindicators need to be cost-effective, easy to collect and/ or measure, and should be observer independent, but the selection of the most effective bioindicators should be primarily based on the objectives of any monitoring program (Linton & Warner, 2003). Finally, the biological response should contribute to understanding the ecological significance of a particular stressor (Fabricius et al., 2012).

Cooper et al. (2009) consider the validity of several bioindicators across different biological scales and a range of response and recovery times. For instance, for short-term monitoring programs or for environmental impact assessments, they recommend seven bioindicators to quantify the effects of acute changes in water quality at the genetic or colony scale, as these will be the earliest responses to such impacts, These include changes in coral symbiont

physiology (e.g. increased photosynthesis) and coral brightness (e.g. changes in pigmentation). However, the study recognises that responses to stressors at the population or community level can indicate potential community shifts, which may be more important for assessing impacts at the ecosystem-scale. In contrast, for longer-term programs that are investigating the effects of chronic changes in water quality, they suggest 11 bioindicators that capture responses over the scale of months to years, which include changes in coral colony brightness, surface rugosity of massive corals, foraminifera, coral recruitment, macroalgal cover, and taxonomic richness of corals. The criteria defined in this review provide an excellent framework upon which to base decisions on selecting bioindicators depending on the type of response being investigated (*Table 5.1*).

Microalgae have been used as indicators of terrestrial runoff in numerous eutrophication studies, not just around coral reefs (De'ath & Fabricius, 2010), but in temporal and freshwater systems (Livingston, 2007; Litchman et al., 2010; Ferreira et al., 2011; D'Angelo & Wiedenmann, 2014; McQuatters-Gollop et al., 2019; Bedford et al., 2020) as they can cause eutrophication and harmful algal blooms (Angly et al., 2016). These studies have involved estimating phytoplankton biomass by measuring chlorophyll-α concentration, dissolved oxygen (as a measure of productivity in the water column), and phytoplankton composition, and these measurements can also be used to ground-truth chlorophyll data obtained through remote sensing (Demarcq et al., 2012; Lehahn et al., 2018). For instance, Blanco et al. (2008) found that in the inner regions of the Shiraho coral reef of Ishigaki Island (Okinawa, Japan), the phytoplankton communities shifted from diatoms and green microalgae to cyanobacteria (blue-green algae) and cryptophytes due to a combination of detrital decomposition and river discharge brought onto a reef by a typhoon. These primary producers may also provide information about the effects of nutrient regimes on higher

trophic levels that they provide sustenance for (Drinkwater et al., 2010), such as herbivorous, planktivorous fishes, invertebrates like soft corals (Alcyonidae), scleractinian corals, and bivalves (Kürten et al., 2014; Briand et al., 2015).

Many previous studies have also considered macroalgal cover as a rapid and direct bioindicator of nutrient enrichment, as it can demonstrate the spatial extent of terrestrial nutrient discharge (Adjeroud & Salvat, 1996; Cooper et al., 2009; Fabricius et al., 2005, 2010, 2012; De'ath & Fabricius, 2010; Sangil & Guzman, 2016; Gorman et al., 2017; Zubia et al., 2018). The life-history characteristics of fast-growing, or opportunistic macroalgae allow them to take up nutrients rapidly when concentrations are high and assimilate them into their tissues, which quickly reaches their growth rate peaks (Lapointe et al., 1997; Fong et al., 1993, 1994a&b, 1998, 2001, 2003, 2004; Costanzo et al., 2001; Fong & Paul., 2011; McClanahan et al., 2004). Nutrients can often be supplied in episodic pulses, due to variations in rainfall and river discharge, and this kind of nutrient supply (subsidy) is favoured by fast-growing species (Cohen & Fong, 2004; Devlin & Brodie, 2005; Devlin & Schaffelke, 2012; Anthony et al., 2014; Clausing & Fong, 2016; den Haan et al., 2016; Fong et al., 2020).

Fast-growing algae are particularly useful for bioindicator studies as they are more likely to demonstrate stronger responses to nutrient enrichment than larger, slow-growing, and perennial species, which tend to show weaker or mixed responses (Martinez et al., 2014; Duran et al., 2016; Sangil & Guzman, 2016; Zubia et al., 2018). This is because they can internally store nutrients between pulse events so that they can sustain growth rates when conditions are once again nutrient-limited, which gives them the opportunity to dominate the algal community (Fong et al., 2004; McClanahan et al., 2001; Clausing & Fong, 2016; Fong

& Fong, 2017; Adam et al., 2021). Foliose algae, such as and *Ulva* spp. and *Enteromorpha* spp. in temperate estuaries in California, USA, are thought to be effective indicators because they are opportunistic algae with rapid uptake rates (Garcia-Seoane et al., 2018a&b), but are still capable of storing nutrients if the supply is great than the demand, so they can maintain rapid growth rates between pulses (Fong et al., 1994; Cohen & Fong, 2004, 2005; van Alstyne, 2016).

Macroalgal proliferation can still be limited by abiotic controls, such as variations in seasons and rainfall (Payri, 1987; Fong et al., 1996; Stimson et al., 1996; Lirman & Biber, 2000; Lefèvre & Bellwood, 2010; Clausing & Fong, 2016), as well as temperature, light availability, and salinity and carbon dioxide (CO_2) (Littler et al., 1988; Fong et al., 1996; Cohen & Fong, 2004; Chung et al., 2007; Collado-Vides et al., 2007, 2011; Falkenberg et al., 2013; Ober & Thurber, 2017), and biotic factors, like herbivory rates (Burkepile & Hay, 2006; Duran et al., 2016). These factors also affect the spatial distribution of algae, but their succession in certain locations is also dependent on the availability of suitable substrate, wave power (Williams et al., 2013; Gove et al. 2015) and upwelling (Viana & Bode, 2013; Roth et al., 2015). Combined, these biotic and abiotic drivers can result in different algal community patches and changes in benthic composition across reef systems at different times of the year (Duran et al., 2016). It may be that at certain times of the year, some algal groups that are present during all seasons are more responsive to increased nutrients, and these are not always captured during short-term manipulation experiments (Downing et al., 1999; Worm et al., 2000; Littler et al., 2006b; Adam et al., 2021). For instance, many macroalgae in the Great Barrier Reef, eastern Australia, are highly seasonal in their occurrence, growth and reproduction, especially large seaweeds such as *Sargassum*, with peaks in biomass and reproduction during the summer and lowest biomass during the winter due to differences in

prevailing winds and currents (Schaffelke & Klump, 1998; Diaz-Pulido & McCook, 2005; Bijoux, 2013; Dajka et al., 2021). Extensive ephemeral blooms of smaller, fleshy brown macroalgae, such as *Chnoospora* and *Hydroclathrus*, however, have been mostly observed on shallow reef flats during winter and early spring (Cribb, 1973; Schaffelke & Klumpp, 1998; Burgess, 2006; Diaz-Pulido et al., 2007).

In order to quantify time-integrated measurements of specific sources, nitrogen stable isotopes can be analysed from macroalgal tissues and can reveal several months' worth of nutrient loads (Costanzo et al., 2001; Risk et al., 2001; Fichez et al., 2005). Stable isotopes of nitrogen have now been used in nutrient studies for several decades, as it is a highly valuable tool that can identify the origin of nitrogen (Heaton, 1986). There are two naturally occurring atomic forms, or isotopes, of nitrogen, and the most abundant form is the atomically lighter isotope nitrogen-14 (^{14}N). The heavier isotope, nitrogen-15 (^{15}N) is much less common, but the ratios of these isotopes in different materials can be measured and compared to an international standard (atmospheric nitrogen, N_2) to determine the relative amount of ^{15}N, or $\delta^{15}N$, in the material (Heaton 1986; Costanzo et al., 2001). The various sources of nutrient pollution often have distinguishable ^{15}N:^{14}N ratios, so specific signatures can be recognised to identify the source of pollution. For instance, signatures of sewage outfall and animal manure tend to be higher (i.e. ~9-20‰), as these mainly contain ammonium compounds (NH_4^+), which are more enriched in ^{15}N. Conversely, agricultural fertilisers have values closer to that of atmospheric N_2 (i.e. ~0‰) (Heaton, 1986).

The nutrients taken up and assimilated or stored in macroalgal tissues accumulate over time, and so can be measured alongside other ecological parameters, such as growth rates and benthic cover (Lin & Fong, 2008), or even stored in a specimen bank to create a reservoir that

can contribute to long-term monitoring data (Viana et al., 2010). For instance, Dailer et al. (2010) found a range of $\delta^{15}N$ signatures of *Hypnea musciformis* and *Ulva fasciata* tissues during an island-wide survey of Maui, Hawai'i. Across the north-central coast, they found signatures of 9.8‰ and 2.0-3.5‰, which could be linked to leaking cesspools and agricultural fertilisers, respectively, whereas algae located near injection wells of the Wastewater Reclamation Facilities in southern Maui had values of 17.8-50.1‰. This not only strongly implied that reefs around the island were exposed to a range of human activities along different areas of the island coastline, which is valuable for water quality management plans, but that it is also possible to discriminate between the different sources over these spatial scales.

Using only one species of macroalgae as a bioindicator can sometimes have limitations, as there may be spatio-temporal gaps in their distribution (Linton & Warner, 2003), as well as variations in $\delta^{15}N$ between macroalgae found in the same location (Gartner et al., 2002; Gorman et al., 2017; Zubia et al., 2018). Differences in morphologies and physiologies, even amongst macroalgae with the same functional traits, can affect their metabolism of nutrients (Raimonet et al., 2013; Fong & Fong, 2014; Clausing & Fong, 2016), such as variations in preference for N sources (Cohen & Fong, 2004, DeYoe et al., 2007), nutrient uptake rates (Fong et al., 2001), and nutrient storage capacity (Fong et al., 1994a&b, 2003). They therefore are may not always be the most appropriate choice as a bioindicator over all spatial and seasonal scales (Raimonet et al., 2013; Viana & Bode, 2013; Ochoa-Izaguirre & Soto-Jimenez, 2015), which is why some focus has shifted over to other types of benthic reef organisms (e.g. Cooper et al., 2009). Stable isotopes have also been analysed in these different groups to detect and map the source of nutrient loads, particularly across multiple trophic levels (McClelland et al., 1997; Tucker et al., 1999; Smit, 2001; Zanden et al., 2001;

Gartner et al., 2002; Pitt et al., 2009; Boecklen et al., 2011; Kürten et al., 2014; Gorman et al., 2017; Leal et al., 2017; Kristensen et al., 2018; Lachs et al., 2019), but there have been few studies which have simultaneously tested and compared the $\delta^{15}N$ signatures of multiple taxa (Connelly et al., 2013).

Scleractinian (hard, reef-building) corals have also been selected as indicators of longer-term changes in water quality (Heikoop et al., 1998, 2000; Sammarco et al., 1999; Hoegh-Guldberg et al., 2004; Marion et al., 2005; Cooper et al., 2009; Cooper & Fabricius, 2008). However, these bioindicators also require extra laboratory processing time and effort, as the coral tissue and symbiont cells have to be separated first so they can be analysed individually (Hoegh-Guldberg et al., 2004). Sponges are highly efficient at recycling dissolved organic matter (DOM), which not only feed reef consumers, but almost reach the rates of primary production needed for the entire ecosystem (González-Rivero et al., 2011; de Goeij et al., 2013; Rix et al., 2016, 2017, 2018). The increase in phytoplankton load from increased nutrient loads can also stimulate the growth of these filter feeding organisms, which increases competition with corals for space, particularly on degraded reefs (Rose & Risk, 1985; Ward-Paige et al., 2005b; D'Angelo & Wiedenmann, 2014; González-Rivero et al., 2011; Carballo et al., 2013; Kelmo et al., 2013; Powell et al., 2014; Knapp et al., 2016). Different groups of soft corals, such as gorgonians, antipatharians (Sherwood et al., 2005; Ward-Paige et al., 2005a; Baker et al., 2010a&b, 2011; Risk et al., 2009; Risk, 2014) and Alcyonaceae (e.g. *Sarcophyton* spp., Fleury et al., 2000; Costa et al., 2016) have received less attention, but may also be important indicators of water quality, as they are not controlled by herbivory or predation like macroalgae and scleractinian corals (Fabricius et al., 2005).

Sediments, while not biological indicators, could still be useful for looking at nutrient sources and content over larger spatio-temporal scales than organisms like marine algae or filter feeders, whose abundance and distribution can be limited by a range of biological and environmental factors (Umezawa et al., 2008). This could then help to fill in these spatial and seasonal gaps. Sedimentary organic matter (SOM) can also be re-suspended into the water column through biological or physical disturbance, and so has a significant role in nutrient cycling (Stimson et al., 1996; Herbert, 1999; Stimson & Larned, 2000; Umezawa et al., 2008; Brodie et al., 2010b; Clausing et al., 2016).

It may be beneficial to study and apply a suite of bioindicators, as relying on the abundance of a single species and their subsequent responses to stressors can be inconclusive or misleading for monitoring programs, especially if they need to assess a large area for the spatial extent of runoff (Linton & Warner, 2003; Connolly et al., 2013, Kürten et al., 2014; Gorman et al., 2017). A suite of bioindicators may also provide more ecologically-relevant information about the effects of a stressor like pollution on community structure and biodiversity, such as changes in total coral species richness or macroalgal cover (Gartner et al., 2002; Fabricius et al., 2005; Arévalo et al., 2007; Barhartan et al., 2010; De'ath & Fabricius, 2010; Collado-Vides et al., 2011). One particularly comprehensive study, Fabricius et al. (2012), created a bioindicator system for assessing water quality through two different studies in the Great Barrier Reef, Australia, the first of which tested 38 candidate indicators (ranging from coral physiology, benthic composition, coral recruitment and macrobioeroder densities) against a composite index of 13 water quality variables. Following this, 33 of the 38 bioindicators which showed a significant response to the Water Quality Index were also assessed against other water quality gradients, measured using a combination of *in-situ* turbidity and chlorophyll loggers, and found that turbidity was the best predictor of biota.

However, it does emphasise that including a bioindicator system (i.e. with a suite of bioindicators) in monitoring programs may be particularly beneficial in areas where water quality data is not available or is difficult to collect, such as in remote island nations (Devlin et al., 2019).

Using a multiple-isotope approach could also reveal more about the origin of nutrients in a suite of bioindicators, as analysing $\delta^{15}N$ alone may not help to discriminate and interpret isotopic signatures of natural and anthropogenic nutrient sources (Connolly et al., 2013). Sediments, though not biological indicators, store nutrients over time from a variety of sources, so $\delta^{15}N$ and $\delta^{13}C$ signatures can be derived from the surface layers, which contain a combination of decomposed vegetation and other organic matter (Umezawa et al., 2008). In addition, SOM and POM can be detected in the tissues of bioindicators by comparing them to measurements of $\delta^{15}N$ and $\delta^{13}C$ signatures in both surface sediment layers and in the water column. Therefore, this determines the spatial and seasonal distributions of allochthonous and autochthonous organic matter (OM) in coral reefs, as well as exchanges between different ecosystems and trophic levels (Lamb et al., 2012; Kürten et al., 2104; Briand et al., 2015; Lachs et al., 2019), and heterotrophy vs. autotrophy dynamics in scleractinian corals (Grottoli et al., 2006; Seemann, 2013; Fox et al., 2018; Williams et al., 2018) and zooxanthellate soft corals (Fleury et al., 2000; Kürten et al., 2014).

Many previous studies and reviews have attempted a variety of different techniques for modelling the dynamics of macroalgae when exposed to different drivers like sedimentation and nutrient enrichment on coral reefs (Renken & Mumby, 2009), and physical oceanic forcings (Wyatt et al., 2013; Leichter et al., 2012, 2013; Lowe & Falter, 2015; Adam et al., 2021), to laboratory and mesocosm nutrient manipulation methods (see reviews Downing et

al., 1999; Worm et al., 2000; Littler et al., 2006). *in situ* factorial experiments have also been used as a methodology that assesses both the relative and synergistic effects of top-down and bottom-up control (Miller et al., 1999; McCook, 2001; Smith et al., 2001, 2010; Burkepile & Hay, 2006; Jessen et al., 2013; Duran et al., 2016; Fong et al., 2018). There has also been much debate about nitrogen versus phosphorous (N vs. P) limitation on tropical coral reefs, so this has also been widely tested across multiple regions (Wheeler & Bjornsater, 1992; Fong et al., 1993, 2003, 2004; Larned, 1998; Gilbert et al., 2004; McClanahan et al., 2007; Fong & Paul, 2011; D'Angelo & Wiedenmann, 2014; Clausing & Fong, 2016; den Hann et al., 2016; Fong & Fong, 2017; Rosset et al., 2017).

The ENCORE (Effect of Nutrient Enrichment on Coral Reefs) experiment was a large-scale attempt to capture nutrient and community-level dynamics. It was set up in the southern Great Barrier Reef using several automated micro-atolls with controlled additions of dissolved inorganic N and/ or P to both determine eutrophication thresholds and to study the links between nutrients and a range of biological responses (Larkum & Steven, 1994; Koop et al., 2001). However, Bell et al. (2007) argued that the nutrient thresholds at these sites may have already been crossed, so many of the marine plants were already saturated and would not have responded to experimental enrichment as rapidly as expected. This emphasised the need to understand not only the spatio-temporal dynamics of nutrient regimes, but also the metabolic effects on a range of organisms, and their overall ecological significance.

Overall, macroalgal stable isotopes have been widely accepted as bioindicators for anthropogenic nutrient inputs to coral reefs, and can provide highly significant information to help trace these loads back to specific human activities such as wastewater treatment plants or sewage outfalls (Heaton, 1986; Tucker et al., 1999; Costanzo et al., 2001; Marion et al., 2005;

Kendall et al., 2007; Fernandes et al., 2012; Lamb et al., 2012; Kristensen et al., 2018; Lachs et al., 2019). However, the high spatial, temporal and biological variability in macroalgal responses imply that in some instances, other (bio)indicators of water quality may serve the better purpose, especially as the stable isotopic methodologies can still be applied. Other types of marine primary producers and other benthic organisms from different trophic levels, such as sponges, turf algae, and soft corals, also respond very rapidly to increases in nutrient loads, and so need to be considered to help fill in these spatio-temporal gaps. Although this thesis will specifically focus on the application and effectiveness of macroalgal bioindicators, it should still be noted that there are many advantages to using the other types of water quality measurements already mentioned, as some might be more cost-effective or appropriate for a range of different scenarios that have been highlighted above (*see also Table 0.1*). Therefore, using a "toolbox" of indicators, both biological and non-biological, that brings together a diverse variety of tools, measurements, methodologies and scales would be a more holistic management strategy for better quantifying the impacts of nutrient pollution.

A broader approach (e.g. the collective use of bioindicators, ecological surveys, empirical studies, water quality sampling, hydrodynamic modelling, *in situ* sensors, and plume mapping with remote sensing), allows scientists and environmental managers to design and implement long-term monitoring programs to assess such impacts over a range of different biological, temporal and spatial scales (Fabricius et al., 2012; Devlin et al., 2020). In addition, it provides a range of cost-effective tools and methodologies that can be incorporated into and/ or adapted to different management and intervention strategies that are already in place (Duarte & Krause-Jenson, 2018; Anderson et al., 2019). This is key for any scientists or environmental managers working in remote areas or in SIDS where local

communities depend heavily on coral reefs for food, livelihoods, trade and transport but have limited funds, resources, and facilities for research and/ or implementing management strategies and policies (Wilson & Forsythe, 2018; McLeod et al., 2019; Barnes et al., 2021; Hafezi et al., 2021a&b).

Table 0.1. The range of indicators of nutrient enrichment and/or decline in water quality across spatial and temporal scales. The indicators are of biological, biochemical, chemical, biophysical, physical or modelling nature, and are ordered from small- to large-scale application (i.e. molecular to ecosystem-wide), which is shown by the black arrows. The potential costs and logistical issues associated with each bioindicator are also summarised.

Response Scale	Indicator	Responses	Spatial Resolution	Temporal Resolution	Suitable Suite of Indicators?	Costs & Feasibility?	References
	Biological/ Biochemical						
	RNA: DNA Ratio & Gene Expression	Biochemical indicators of condition and metabolic activity used to test nutrient-productivity models – close relationships between nearshore upwelling processes and benthic (intertidal) ecosystems	Molecular to Colony Level	Minutes/ Days	Gene expression; transcriptomes DNA methylation); genomes; metabolic enzyme activity	Lab facilities; resources & equipment; expertise or specific training for personnel, and/or shipping & processing costs to another lab	Dahlhoff (2004) ; Barott et al. (2012)
	Microbial community composition	Disturbance-related shifts from natural microbial communities to opportunistic microbial pathogens & increases in virulence genes. Earlier indicator of reef deterioration than visual changes in benthic cover	Nanometre/Millimetre to Colony Level	Minutes/ Days	Gene expression; RNA: DNA ratio; metabolic profiles	Lab facilities, resources & equipment; expertise or specific training for personnel, and/or shipping & processing costs to another lab	Barott et al. (2012) ; Kelly et al. (2014); Thurber et al. (2014); Haas et al. (2016); Glasl et al. (2017)
	Symbiont photo-physiology	Changes in N:P ratios affect symbiont physiology, e.g. high N and undersupply of P causes malfunctioning of symbiont photosynthesis (& coral bleaching).	Colony	Minutes/ Days	Symbiont density; coral protein & lipid content; chlorophyll content; coral mucus; ultrastructural biomarkers	Lab facilities, resources & equipment; expertise or specific training for personnel, and/or shipping & processing costs to another lab	Hoegh-Guldberg and Smith (1989); Hoegh-Guldberg et al., (2004); Wooldridge (2009a); Rosset et al. (2017)
	Symbiont density	Increased zooxanthellae density in hard corals	Colony	Minutes/ Days	Coral carbohydrate, protein & lipid content; chlorophyll content	Lab facilities, resources & equipment; expertise or specific training for personnel, and/or shipping & processing costs to another lab	Hoegh-Guldberg and Smith (1989)
	Lipid content (fatty acids)	Lipid content in corals under nutrient-enriched conditions was lower than corals under ambient conditions	Colony	Minutes/ Days	Symbiont densities and chlorophyll content, carbohydrate and protein content, symbiont loss rate from corals	Lab facilities, resources & equipment; expertise or specific training for personnel, and/or shipping & processing costs to another lab	Stimson & Kinzie (1991); Achituv et al. (1994)

Micro- and meiobenthic indicators	Increases in cyanobacteria (zeaxanthin: chlorophyll ratio – indicator of the relative contribution of cyanobacteria to phytoplankton biomass) – positive correlations with urea uptake rate; Different ^{15}N/ N sources stimulate different components of algal community; Decreased large symbiont-bearing foraminifera	Community to Reef System-Wide	Months/ Years	$\Delta^{15}N$, Chlorophyll-a, phytoplankton composition	Lab facilities, resources & equipment; expertise or specific training for personnel; expertise in field/ lab ID of organisms; stable isotopic analyses training or expertise, or shipping & processing costs to another lab	Gilbert et al. (2004); Uthicke and Nobes (2008); Blanco et al. (2008); Cooper et al. (2009)
Coral reproduction, larval supply and recruitment	Numbers of larval planulae lower in nutrient-enriched site relative to reference site suggests failure of oocyte maturation, fertilisation and larval development; Reduction in recruitment under decreased water quality	Community to Reef System-Wide	Months/ Years	Larval survival; oocyte and sperm size/ abundance; macroalgal propagule recruitment; Turf & Macroalgal Cover	Access to field sites (boat hire, fuel costs, personnel); expertise or training in coral recruitment studies; field equipment and other resources	Loya et al. (2004); Hoey et al., (2011); Duran et al., (2016); Dajka et al., 2019
Tissue Stable Isotopes	Enriched ^{15}N tissues in macroalgae in areas close to sewage outfall relative to those further from the source	Colony	Months	%N, %C; C:N:P ratios; macroalgal cover	Lab facilities, resources & equipment (e.g. sample drying and crushing); expertise or specific training and/or shipping & processing costs to another lab; access to field sites (boat hire, fuel costs, personnel); permit restrictions (e.g. CITES)	Costanzo et al. (2001); Dailer et al. (2010); Fernandes et al. (2012) ; Barr et al. (2013) Gorman et al., (2017); Adam et al. (2021); Bailes & Gröcke (2020)
	Enriched ^{15}N signatures in coral tissues	Colony	Months	Coral cover; coral species richness		
Skeletal Stable Isotopes (Corals)	Variations in $\Delta^{13}C$ *Porites* 19-year seasonal skeletal records = switch from autotrophy under normal conditions to increased heterotrophy during phytoplankton blooms	Colony/ Reef-wide	Interannual/ Years/ Decades	Remote sensing chlorophyll & SST; benthic cover	Lab facilities, resources & equipment (e.g. sample drying and crushing); expertise or specific training and/or shipping & processing costs to another lab; access to field sites (boat hire, fuel costs, personnel); permit restrictions (e.g. CITES)	Felis et al. (1998); Marion et al. (2005)

Growth Rates	Variable coral linear extension; increased macroalgal growth	Corals: Colony Macroalgae: Individual	Months/ Years Days/ Weeks/ Months	Abundance (benthic cover)	Access to field sites (boat hire, fuel costs, personnel); training or expertise in ecological surveys and physiological measurements	Lough & Barnes (2000)
Benthic Cover	Increases in macroalgal % cover following nutrient enrichment/ across a water quality gradient, relative to hard and soft coral species richness	Community to Reef System-Wide	Temporal intervals of discrete (spot) measurements	Coral species richness; Fish biomass; cryptic species diversity	Access to field sites (boat hire, fuel costs, personnel); training or expertise in ecological surveys (e.g. taxonomy)	Fabricius et al. (2005); De'ath & Fabricius (2010); Sangil & Guzman (2016); Zubia et al. (2018)
Calcifying Organism Cover	Decreases in calcifying organisms (scleractinian corals, calcifying crustose algae etc.)	Community to Reef System-Wide	Months/ Years	Ratio to non-calcifying organisms (and substrata); taxonomic richness	Access to field sites (boat hire, fuel costs, personnel); training or expertise in ecological surveys (e.g. taxonomy)	McClanahan et al. (2011)
Coral Species Richness	Decrease in hard coral and soft coral species richness, respectively, across a water quality gradient	Community to Reef System-Wide	Months/ Years	Benthic cover; fish biomass; cryptic species diversity	Access to field sites (boat hire, fuel costs, personnel); training or expertise in ecological surveys (e.g. taxonomy)	Fabricius et al. (2005); De'ath & Fabricius (2010)
Stable Isotopes in Food Webs	POM and zooplankton ^{15}N enrichment, due to latitudinal environmental gradient and local urban runoff, are primary influencers on $\delta^{13}C$ and $\delta^{15}N$ signatures of reef consumers (e.g. herbivorous, planktivorous and carnivorous fishes, soft corals and bivalves). Also shows variation in heterotrophy vs. autotrophy.	Community & Reef System to Latitudinal Scale	Months/ Years	POM, SIAR model, oceanographic data (SST, salinity), chlorophyll a	Access to field sites (boat hire, fuel costs, personnel); training or expertise in the field; lab facilities, resources & equipment (e.g. sample drying and crushing); expertise or specific training and/or shipping & processing costs to another lab	Grottoli et al. (2006) ; Kürten et al. (2014); Kolasinski et al. (2011); Fox et al., 2018; Kristensen et al. (2018); Lachs et al. (2019)
Chemical						
Organic Matter Composition (DOM, POM)	Parameters: stable isotopes ($\delta^{13}C$, $\delta^{15}N$), C:N Ratios The composition of $\delta^{13}C$-POM (i.e. autochthonous vs. allochthonous sources) and uptake changed consistently in water flowing over reefs	Reef System	Temporal intervals of discrete (spot) measurements	Sedimentary organic matter (SOM) and organism tissue $\delta^{13}C$ & $\delta^{15}N$, %N, %C, C:N:P	Lab facilities, resources & equipment (e.g. sample drying and crushing); expertise or specific training and/or	Kendall et al. (2007); Kolasinski et al., (2011); Fabricius et al. (2012); Wyatt et al. (2013); Pawlik et al.

					shipping & processing costs to another lab;	(2016); Radice et al. (2020)
Dye Fluorescence (Rhodamine WT) – *in situ* tracking of sewage plumes	Used for *in situ* tracking of plumes of sewage outfalls and their dispersion and dilution in real time	System-Wide	Hours/ Days Seasonal comparisons	Radioactive isotope tracers, fluorometer with integrated depth sensor to detect dye etc.	Lab facilities, resources & equipment; expertise or specific training and/or shipping & processing costs to another lab; permits/ risk assessments for using dye in marine environment?	Smith-Evans & Dawes (1996)
Radioactive Isotope Tracers (e.g. Gold, ^{198}Au, and Tritium (^{3}H)) – *in situ* tracking of sewage plumes	Used for *in situ* tracking of plumes of sewage outfalls and their dispersion and dilution in real time	System-Wide	Hours/ Days Seasonal comparisons	Radioactive isotope tracers, fluorometer with integrated depth sensor to detect dye etc.	Lab facilities, resources & equipment (e.g. sample drying and crushing); expertise or specific training and/or shipping & processing costs to another lab; permits/ risk assessments for using radioactive isotopic tracers in marine environment?	Smith-Evans & Dawes (1996)
Biophysical						
Chlorophyll-a (phytoplankton biomass proxy)	Increases in Chlorophyll-a (alongside increases in SST) due to elevated nutrients linked to negative impacts on coral physiology and ecosystem responses across the Indo-Pacific, (i.e. the recovery trajectory of corals negatively linked to increases in chlorophyll-a)	Water samples: Reef-wide; Remote Sensing/ Satellite Data: Regional to Global	Time intervals of discrete (spot) measurements Monthly/ Annual	Satellite data ground-truthed with discrete water sampling (i.e. turbidity: total suspended sediments, coloured DOM (CDOM)) SST, discrete water samples, coral cover	Acquiring satellite/ remote-sensing data at appropriate spectral, temporal and spatial resolutions (costs, personnel, training?); access to field sites for ground-truthing (boat hire, fuel costs, snorkel/ SCUBA gear); training &/ or expertise in water quality sampling and analysis; shipping samples to another lab (costs?)	Brodie et al. (2010b, 2012) ; Devlin et al. (2011, 2012, 2020); Fabricius et al. (2012); Roelfsema et al. (2013); Lyons et al. (2020) Riegl et al. (2015); Gove et al. (2016); Devlin et al. (2011; 2020)

	Dissolved Oxygen	Positive correlations of dissolved oxygen (& other nutrient and trace metal levels) with macroalgal, sponge and zoanthids cover	Sensors: & Water Sampling: Reef system-wide	Temporal intervals of discrete (spot) measurements	Chlorophyll, water clarity (Secchi depth), algae % cover, algal community composition shifts	Access to field sites for ground-truthing (boat hire, fuel costs, snorkel/ SCUBA gear); sampling equipment (e.g. oxygen sensors, water sampling equipment); training &/ or expertise in water quality sampling and analysis; expertise and/or training in ecological surveys	Ferreira et al. (2011); Huang et al. (2011)
	Physical						
	Water Clarity (Secchi disk)	Secchi depths <10m indicate decline in water quality due to sedimentation/ turbidity and/or nutrient enrichment	Reef System-Wide	Temporal intervals of discrete (spot) measurements	Chlorophyll (water samples and remote sensing); benthic cover & species richness	Access to field sites for ground-truthing (boat hire, fuel costs, snorkel/ SCUBA gear); Secchi disk is low-cost equipment for water clarity	De'ath & Fabricius (2010); Ferreira et al. (2011)
	In situ Nutrient Sensors and Loggers (e.g. ISUS, Cycle-PO_4, APNA, "Lab-on-a-Chip"	Records nutrient fluctuations (e.g. NO_3^-, PO_4^-) over time – hourly measures capture tidal fluctuation and allow analysis of river & ocean mixing. Longer-term measurements can detect seasonal fluxes in nutrient concentrations either from rivers/ ocean, or organic matter accumulation & remineralisation	Reef-Wide System (deployed across reefs/ from river mouth to reef)	Continuous *in situ* recordings: Minutes/ Hours/ Months/ Years	CTD Sensors; Remote Sensing SST and Chlorophyll data	Access to field sites for ground-truthing (boat/ large research vessel hire, fuel costs, snorkel/ SCUBA gear); expensive sensors and loggers; possible regular maintenance (e.g. biofouling, battery recharge); laboratory analytical expertise to ground-truth with chemical water quality analyses; few commercially-available prototypes but rapidly improving	Johnson & Coletti (2002); Sakamoto et al. (2009) ; Kaplunenko et al. (2013); Nightingale et al. (2015); Daniel et al. (2020); Devlin et al. (2020); Nehir et al., 2021; Marcelli et al. (2021); Wei et al., 2021

Conductivity-Temperature-Depth Profiles (*in situ* loggers & sensors)	Presence of upwelling processes and vertical profile of water chemistry and quality (e.g. movement of nitrate)	*In-Situ* loggers: Cruise-deployed sensors: 0-1000m	Continuous *in situ* recordings: Minutes/ Hours/ Months/ Years	Nitrate Sensors (e.g. ISUS)	Access to field sites for ground-truthing (boat/ large vessel hire, fuel costs, snorkel/ SCUBA gear); training &/ or expertise in water quality sampling and analysis	Kaplunenko et al. (2013)
Sea Surface Temperature (SST)	Spatio-temporal variations in SST are analogous to those in other water chemistry parameters (e.g. nutrients)	*In Situ* Loggers: Reef System-Wide; Remote Sensing: Regional to Global	Continuous *in situ* recordings: Minutes/ Hours/ Months/ Years	Salinity, CTD, Chlorophyll etc.	Acquiring satellite/ remote-sensing data of the high spectral, temporal and spatial resolutions (costs, personnel, training?); remote-sensing data requires ground-truthing (additional lab/ field costs?)	Gove et al. (2012, 2016)
Modelling						
Mass-Transfer Limiting Nutrient Model	Predictive capacity for mechanisms of water motion that control nutrient uptake by reef communities: Convective transfer of nutrients across turbulent layers formed on surfaces of benthic autotrophs affect nutrient uptake rates	Organism to Reef Canopy Scale	Variable	Variable	Sensors for wave action/ hydrodynamic forces; expertise in statistical modelling (training?)	Bilger & Atkinson (1995); Baird & Atkinson (2003); Falter et al., (2004); Leichter et al. (2013); Wyatt et al. (2013); Lowe & Falter (2015); Graham et al. (2020); Adam et al. (2021)
Wave-Driven Flow Model	Mass and momentum dynamics within and above reef canopies depend on prevailing flow regime, e.g. under strong depth-limited/ wave-driven oscillatory flow, flow within canopy is stronger - driven by pressure gradients	Organism to Reef Canopy Scale	Variable	Variable	Expertise in statistical/ hydrodynamic modelling (training?) If data not already available – sensors for wave action/ hydrodynamic forces etc.	Leichter et al. (2012, 2013); Wyatt et al. (2013); Lowe & Falter (2015)
Eulerian and Lagangian Perspective Models	Determining biogeochemical transformation of reef waters and flow fields: either from circulation/ changing water quality within a fixed frame of reference (Eulerian), or water quality	Community to Reef System Scale	Variable	Variable	Expertise in statistical modelling (training?); If data not already available – sensors for wave action/ hydrodynamic forces;	Falter et al. (2008); Lowe & Falter (2015); Lehahn et al. (2018)

	changes within a defined water parcel flowing through the reef (Lagangian).				water quality sample collections and analyses (lab/ field/ personnel costs)	
Bayesian Belief Network (BBN) Model	Integrating the relative importance of physical and ecological processes on growth dynamics of coral reef macroalgae	Reef System to Regional Scale	Variable	Variable	Expertise in statistical modelling (training?); If data not already available – lab facilities, lab/ field resources, personnel (expertise or training for lab and/or field work)	Renken & Mumby (2007); Hafezi et al. (2020b)
Bayesian Stable Isotope Mixing Model (SIAR)	Net POM uptake by reef community highest over reef crest and higher rates of allochthonous POM supply; variations in $\delta^{13}C$ in higher trophic levels exposed to higher levels of localised urban runoff	Latitudinal/ Regional Scale	Variable	Variable	Expertise in statistical modelling (training?); If data not already available – lab facilities, lab/ field resources, personnel (expertise or training for lab and/or field work, e.g. stable isotope analyses)	Wyatt et al. (2013) Kürten et al. (2014)

Aims and Thesis Outline

In my thesis, I draw on advances in the fields of pollution, bioindicators and coral reef ecology to compare and critique the best approaches for the application of macroalgal stable isotopes to further our understanding of the high spatio-temporal variability of nutrients, both natural and anthropogenic, on coral reefs. I also investigate how the effectiveness of a bioindicator can be influenced by various biological, physical, and logistical factors. I highlight examples where bioindicators would be of great benefit to already established or future monitoring programs. This is particularly important for reefs currently experiencing shifts in biophysical relationships due to cumulative stressors such as pollution and climate change (Williams et al., 2015; Williams & Graham, 2019).

My thesis addresses four overarching research questions relating to what makes a good bioindicator for capturing nutrient regimes on coral reefs, and how can this tool be adapted to account for changes in community structure and ecosystem functions:

1) Is a suite of bioindicators always a more precise and cost-effective management tool for monitoring nutrient regimes than a single-species approach?
2) Are two morphologically-similar brown macroalgae equally as effective as bioindicators for both passive and active biomonitoring methodologies?
3) How do the interacting effects of internal nutrient history and ecological strategies impact how two morphologically-similar macroalgal species respond to different nutrient supply rates?
4) Can macroalgal bioindicators be used to detect changes in local nutrient regimes after mass coral mortality events?

The overarching research questions are addressed in the following research chapters. **Chapter 1** assesses the precision, congruency, and cost-effectiveness of a suite of bioindicators for capturing nutrient regimes across degraded coral reefs in the inner Seychelles that have either undergone a regime shift to a macroalgal-dominated state or recovered to live coral state following a mass coral mortality event. Based on the findings in Chapter 1 that two common macroalgae were precise bioindicators of nutrient regimes but had low congruency between them, **Chapter 2** examines how two species of morphologically-similar macroalgae with different ecological strategies for nutrient uptake and assimilation respond to nutrients *in situ*, comparing both passive biomonitoring (samples collected along a nutrient gradient) and active biomonitoring (reciprocal transplant experiment) methodologies. In addition, **Chapter 3** investigates these same two species in a manipulative laboratory experiment by exposing specimens from different reefs (and therefore different nutrient histories) to pulse versus press variations in nutrient supply. **Chapter 4** applies some of these methodologies to a real-world scenario in two different regions (the Seychelles, western Indian Ocean, and Mo'orea, French Polynesia) to show how nutrients released from corals following mass bleaching and mortality events can be taken up by macroalgae. Overall, all four chapters compare the various benefits and costs of using a suite of bioindicators against a single species for capturing nutrient regimes over different spatial and temporal scales, but also demonstrate how context-dependent it can be. For instance, the costs, abundance and distribution of selected bioindicators, the existence of historical nutrient and ecological data, the spatial and temporal scales required, and ease of access to target sites can all affect the design and implementation of environmental monitoring programs.

Study Regions

Seychelles

The first reg ion of my chosen study sites for assessing large-scale spatio-variability in nutrient regimes was located in the inner Seychelles, specifically on the corals reefs surrounding the granitic islands of Mahé and Praslin (4°30'S, 55°30'E) (*Suppl. Fig. 1.1*). I made this choice based on the long-term coral reef monitoring survey that has been conducted every three years on the same 21 reefs since 1995 (Jennings, 1995), with no monitoring between 1995 and 2005. This monitoring survey has captured two mass coral bleaching events caused by global thermal anomalies (in 1998 and 2016), the first of which caused a loss of > 90 % live coral cover (Goreau et al. 2000; Graham et al., 2006, 2015) and the second which caused ~ 70 % loss in 2016 (Wilson et al. 2019). This long-term data set has also allowed researchers to assess the subsequent effects of these two bleaching events on ecosystem function and services across these reefs (Chong-Seng et al., 2014; Graham et al., 2015; Dajka et al., 2019; Robinson et al., 2019; Wilson et al., 2019; Woodhead et al., 2019). One of the key studies that has come out of this long-term monitoring survey was Graham et al. (2015). This study was the first to establish threshold values for five factors on coral reefs that could predict ecosystem response to a mass bleaching event from temporal data, including the recovery potential of individual reefs. Factors such as high structural complexity, deeper water, relatively high density of juvenile corals and herbivorous fishes, and low nutrient loads (C:N ratios) were all found to all play a large part in dictating ecosystem trajectories. Collectively, these factors have high predictive rate for whether reefs would recover from bleaching or whether they would cross a threshold (tipping point) and undergo a regime shift to a macroalgal-dominated state.

To measure nutrient loads, samples of *Sargassum* sp. tissue were collected from almost all of these sites alongside the ecological surveys in 2014 (two years prior to the 2016 bleaching event) and analysed for nitrogen- and carbon-based stable isotopic and elemental signatures (Graham et al., 2015). Therefore, in 2017, I collected *Sargassum* sp. samples in conjunction with the ecological surveys at the same time and sites so that I could also conduct a direct temporal comparison between nutrient signatures. Of the 21 reefs that I focus on in my thesis, 9 have undergone a benthic regime shift to macroalgal dominance after the 1998 bleaching event, while the other 12 were defined as recovering (Graham et al. 2015). In addition, as three of the sites around Cousin Island, a protected area off the coast of Praslin island, were not accessible in 2017, we surveyed three additional regime-shifted reefs around Praslin to ensure we still had 21 reefs for later analyses. My thesis will contribute to determining large-scale spatio-temporal nutrient regimes around these islands, both prior to and following the 2016 bleaching event, as there is very little existing data on water quality around the Seychelles, particularly on these kinds of spatial scales.

Mo'orea

Mo'orea, a volcanic high island in French Polynesia, central South Pacific (17°30'S, 149°50'W), is a very common study region for coral reef science, with two independent research stations located in the northern region of the island (CRIOBE and the Gump Station). Mo'orea therefore has a wealth of long-term, island-wide monitoring data for both ecosystem processes, community structure and physical drivers of change such as climate change, herbivory, and water quality (Payri, 1987; Stiger & Payri, 1999; Fong & Paul, 2011; Leichter et al., 2012, 2013; Horta e Costa, 2013; Poray & Carpenter, 2014; Burkepile et al., 2019; Vercelloni et al., 2019; Donovan et al., 2020; Hédouin et al., 2020; Adam et al., 2021).

In addition, numerous laboratory and field experiments have been conducted here to test the relationships between these variables, including those between reef-associated organisms and nutrient enrichment (Fong & Fong, 2014, 2017; Fong, 2015; Burkepile et al., 2019; Fong et al., 2020). Therefore, in contrast to the Seychelles, the nutrient history of the marine environment around the coastline of Mo'orea is well known. It also provided an ideal location for studying nutrient gradients, due to the two bays in the northern region of Mo'orea, although I focused on Opunohu Bay for this thesis (*Suppl. Fig. 2.1*). This is because CRIOBE research station, where I conducted my laboratory experiments and sample processing, is situated at the land-end of this bay, and is also adjacent to a 2 ha shrimp farm. Effluent from commercial shrimp feeds drain into the Opunohu River, which then runs into the bottom of the bay and, along with other sources of anthropogenic runoff from a nearby pineapple farm, experimental lumber tree farms, an agricultural school and potentially submarine groundwater discharge, results in very high recordings nutrient loads at the land end. significantly decrease across the bay to the ocean end (Lin & Fong, 2008). Previous studies have shown that nutrient-rich discharge from the river plays an important role in driving spatial patterns of coral reef communities along this bay, although other factors such as reduced light intensity from high turbidity and heavy rainfall during the wet season can also have effects on gradients of community structure and diversity (Adjeroud & Salvat, 1996; Adjeroud, 1997; Poray & Carpenter, 2014).

There is also a substantially different community structure on the forereef along the northernmost coast of Mo'orea (~10m depth) to those along the edges of the bay, and one of the reasons for this is likely due to the significantly lower nutrient levels, as shown in nutrient heat maps in Leichter et al. (2013) and Adam et al. (2021). This particular forereef is well known for rapid coral recovery from previous disturbances, such as rare cyclones and

outbreaks of *Acanthaster* spp., which has been found primarily to be because of the branching coral *Pocillopora* spp., a dominant genus on these reefs (Tsounis & Edmunds, 2016; Donovan et al., 2020; Hédouin et al., 2020; Vercelloni et al., 2020). Overall, I chose Mo'orea to conduct finer-scale spatio-temporal empirical experiments, due to the laboratory facilities at CRIOBE, the presence of a strong nutrient gradient along a ~3.5km bay that was easy to access by powerboat from CRIOBE, which made it possible to bring specimens back to lab and then return them to the field for transplant experiments on the same day. The bleaching event that occurred in 2019, a year after I conducted my experiments at CRIOBE, presented an opportunity to collaborate on a short-term experiment with another PhD student also working at CRIOBE at the time, which then provided supportive evidence for the findings in the Seychelles in **Chapter 4.**

1. PRECISION AND COST-EFFECTIVENESS OF BIOINDICATORS TO ESTIMATE NUTRIENT REGIMES ON CORAL REEFS

1.1 Abstract

Bioindicators are useful for determining nutrient regimes in marine environments, but their ability to evaluate corals reefs in different ecological states is poorly understood. The precision, availability and congruency of eight potential bioindicators (brown macroalgae, green macroalgae, turf algae, cyanobacteria, soft corals, zoanthids, sponges, and sediment) and their stable isotopic and elemental signatures ($\delta^{15}N$, $\delta^{13}C$, %N, %C, and C:N Ratio) were assessed across 21 reefs in the Inner Seychelles. The coefficient of variation (CoV) for $\delta^{15}N$ showed that green and brown macroalgae were highly precise (2.47 ± 0.95, n=11; 4.68 ± 1.33, n=16, respectively), though were less common on coral-mortality reefs relative to macroalgal-dominated ones. Zoanthids were also highly precise for $\delta^{15}N$ (2.98 ± 1.20), but were more readily available regardless of reef state (n=18). Congruency was low among these indicators, suggesting that different physiological mechanisms for nutrient processing have a stronger influence on a bioindicator's effectiveness than reef state.

Published online (18/06/2021):

Vaughan, Wynn, P.M., Wilson, S.K., Williams, G.J., Barker, P.A., and Graham, N.A.J. (2021) Precision and cost-effectiveness of bioindicators to estimate nutrient regimes on coral reefs. *Marine Pollution Bulletin*, 170: 112606.

https://doi.org/10.1016/j.marpolbul.2021.112606

1.2 Introduction

Coral reefs are facing global declines in live coral cover due to climate change (Hughes et al., 2018), and local-scale degradation from overfishing and pollution (Burkepile & Hay, 2006; Littler et al., 2006a; Zaneveld et al., 2016; MacNeil et al., 2019). Increased anthropogenic nutrient loads and reduced herbivory can cause the proliferation of opportunistic species such as fleshy macroalgae, which may lead to a regime shift from a coral-dominated to an algal-dominated reef (McManus & Polsenberg, 2004; Littler et al., 2006a; Hughes et al., 2007; Fulton et al., 2019). Monitoring the state of coral reefs relative to anthropogenic stressors provides insights into causes of decline in reef condition, potentially instigating management actions (Flower et al., 2017). Two particularly widespread local stressors are overfishing and eutrophication (Fabricius et al., 2005; Littler et al., 200a6; Rasher et al., 2012). While there has been significant progress in understanding the effects of overfishing (e.g. Cinner et al., 2018), it has been more difficult to detect and quantify nutrient loads that cause eutrophication in the marine environment, due to high spatio-temporal variability in the water column (Wyatt et al., 2013; Briand et al., 2015; Lowe & Falter, 2015). In addition, understanding the impact of this variability depends on the magnitude, frequency of input, retention ability in the environment, bioavailability and source of nutrients (Fabricius et al., 2005; D'Angelo & Wiedenmann, 2014; Clausing & Fong, 2016). It is therefore critical to identify more cost-effective methods of capturing nutrient enrichment to improve assessments of coral reef health over different spatial scales as part of routine environmental monitoring strategies (Flower et al., 2017; Bal et al., 2020).

Bioindicators are used widely to capture nutrient regimes in tropical marine systems, as they provide an ecologically relevant response to bioavailable nutrients in the surrounding water

column (Fichez et al., 2005; Cooper et al., 2009; Fabricius et al., 2012). As such, bioindicators may be more cost-effective in certain circumstances than periodic measurements of seawater nutrients alone, which can be highly variable and require frequent sampling (Linton & Warner, 2003; Fabricius et al., 2012). To be considered a good bioindicator, the selected organism must: (i) be specific to nutrient impacts, (ii) reflect the intensity and duration of nutrient enrichment, (iii) be consistent at a range of spatial and temporal scales, (iv) be cost-effective, easy to measurement, non-destructive and observer independent, and (v) capture any ecologically relevant changes in environmental conditions (Cooper et al., 2009; *Table 5.1*). Indicators, conversely, are those which can still reflect drivers of change, but not through biological responses (i.e. nutrients stored in reef sediments) (Linton & Warner, 2003; Fichez et al., 2005; Umezawa et al., 2008). Furthermore, measuring stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$) and concentration levels (%N, %C and C:N ratio) in the tissues of a selected bioindicator assesses the source(s) and concentration of nutrient regimes, respectively, and therefore the spatio-temporal variability of nutrient regimes (Costanzo et al., 2001).

Common bioindicators for nutrient enrichment studies are micro- (i.e. phytoplankton, De'ath & Fabricius, 2010) and macroalgae (Zubia et al., 2018). Fleshy macroalgae are particularly useful for such a purpose, because they respond rapidly to high nutrient concentrations by assimilating bioavailable nutrients from their local environment into their tissues over their active growth periods, thereby capturing temporal variation in nutrients (Costanzo et al., 2001; Fong et al., 2004; Dailer et al., 2010; Fernandes et al., 2012). They are also easy to collect and survey in the field, especially in nutrient-rich coastal areas (Zubia et al., 2018). Some of the most common genera used as bioindicators in the literature worldwide are *Ulva* spp., *Cladophora* spp., *Fucus* spp., *Sargassum* spp., and *Padina* spp., and are usually from

the Ochrophyta (brown macroalgae) and Chlorophyta (green macroalgae) phyla, although there are some from Rhodophyta (e.g. *Gracilaria* spp.) (Garcia-Seoane et al., 2018a&b).

One of the main limitations of using a single species of macroalgae are the spatio-temporal gaps in their distribution, which are driven by a number of abiotic factors such as wave exposure, irradiance, temperature, rainfall and seasonality (Linton & Warner, 2003; Williams et al., 2013; Clausing & Fong, 2016; Duran et al., 2016; Fulton et al. 2019), and biotic drivers such as herbivory and competition (Burkepile & Hay, 2006; Rasher et al., 2012; Duran et al., 2016). These limiting factors may also affect the ability of macroalgae to proliferate on some reefs that have experienced significant disturbances (Graham et al., 2015). These distributional gaps can also lead to inconclusive or even misleading findings in any studies or monitoring programs, particularly if they are quantifying the abundance of a particular species across a range of target sites (Linton & Warner, 2003). As such, the utility of alternative bioindicators to capture nutrient regimes is of importance to monitoring programmes.

A range of other marine organisms have been used as bioindicators in water quality or nutrient enrichment studies, such as scleractinian corals (Hoegh-Guldberg et al., 2004), soft corals (Fleury et al., 2000; Risk, 2014), sponges (Ward-Paige et al., 2005), and sediment, though the latter is not a biological indicator (Umezawa et al., 2008). In addition, multiple candidate bioindicators have been used to assess water quality depending upon their response time to a change in their local nutrient environment (Cooper et al., 2009), or on the extent of their abundance and distribution, which also allows the spatial extent of nutrient runoff to be assessed (Fabricius et al., 2012). There are, however, many potential issues with directly comparing measurements among multiple species, for instance because $\delta^{15}N$ signatures differ

between species at different trophic positions due to isotopic fractionation and/ or different nutrient sources (Zanden et al., 2001; Boecklen et al., 2011). Some studies have tested whether patterns in nutrient signatures of different bioindicators are congruent (i.e. they are able to show the same relative trends in isotopic values between indicators) across different spatio-temporal scales (McClelland et al., 1997; Tucker et al., 1999; Gartner et al., 2002; Pitt et al., 2009), though this multi-taxa approach is less common in coral reef studies (Connolly et al., 2013; Kürten et al., 2014; Graham et al., 2018; Lachs et al., 2019). Untested variability in isotopic composition within and between different reef sites, bioindicators, and even studies could therefore reduce the reliability, or else the comparability of large-scale and long-term monitoring assessments.

If multiple bioindicators can demonstrate similarly precise and congruent spatial patterns of $\delta^{15}N$ and $\delta^{13}C$ over a large-scale nutrient gradient, then other taxa, particularly from multiple trophic positions, may become useful proxies in areas where macroalgae are scarce, such as on reefs that are dominated by reef-building corals or turf algae (den Haan et al., 2014; Fulton et al, 2019). However, some bioindicators may take longer to find or process than others, particularly in areas where they are relatively uncommon or rare. Selection of bioindicators should therefore also consider cost-effectiveness of the collection, and subsequent processing of samples (Risk et al., 2001; Drummond & Connell, 2008; Gardner et al., 2008; Bal et al., 2020). This will be especially important for researchers and managers tasked with monitoring water quality over large spatial and temporal scales, such as entire reef systems (De'ath & Fabricius, 2010; Graham et al., 2015).

In this study, the precision and cost-effectiveness of a suite of eight potential bioindicators collected from coral reefs across the Inner Seychelles Islands were each assessed for

measuring nutrient regimes. The specific objectives of the study were to (1) quantify the precision of different bioindicators for measuring stable isotopic and elemental signatures of nitrogen and carbon, (2) determine how much variation exists within bioindicators across different coral reef sites which vary in ecological condition, (3) consider whether there is congruency between selected precise bioindicators based on their nitrogen (N)- and carbon (C)-based measurements, and (4) assess cost-effectiveness of using different bioindicators and the tasks involved.

1.3 Methods

1.3.1 Study Sites and Sample Collections

Bioindicator samples were collected from 21 coral reef sites around the Inner Seychelles Islands between 11th – 22nd April 2017. These sites have been used as part of a 23-year long-term monitoring survey of the coral reefs of the Inner Seychelles Islands (Graham et al., 2015; Wilson et al., 2019), where twelve reefs were defined as "recovering" live coral from a mass bleaching event in 1998, and nine as "regime-shifted" where macroalgae had proliferated. However, another mass bleaching event in 2016 caused mass coral mortality on the recovering reefs (Wilson et al., 2019), and so here we define them as "coral-mortality" reefs. Using nitrogen content of brown algae collected from these sites, Graham et al. (2015) also found that nutrient regimes are one of the key determinants of whether a reef can recover or experience a regime shift after a major disturbance like bleaching.

To assess the availability of potential bioindicators, eight replicate 7-m radius point counts were surveyed along the reef slope at each site, and within each point count area, the percent

cover of benthic groups such as hard coral, soft coral, macroalgae, sand, rubble, and rock was quantified using 10m line-intercept transects (Wilson et al., 2019). Along each transect, the distance of tape occupied by different benthic organisms and substrates was recorded, including live hard coral, soft coral, macroalgae, sponge, cyanobacteria, zoanthids, sand, rubble and rock. For the purpose of this study, the percent cover of dead hard coral and rubble was pooled for a general estimate of turf algae per site. Up to ten replicate samples of eight different bioindicators, each replicate taken from different individuals, were collected haphazardly from within each reef using SCUBA at each site. Bioindicators were selected based on their presence in long-term benthic composition data and their use in previous nutrient enrichment and bioindicator studies (*Suppl. Fig. 1.2*; Risk et al., 2001; Fichez et al., 2005; Cooper et al., 2009; Fabricius et al., 2012). Bioindicators included fronds of mature foliose brown macroalgae with apical tips (*Sargassum* spp., Littler et al., 1991; Schaffelke, 1999; Schaffelke & Klump, 1998; Alquezar et al., 2013), filamentous green macroalgae (*Chlorodesmis* sp., Schaffelke, 1999), cyanobacteria (Charpy et al., 2012; Ford et al., 2018), soft corals (*Sarcophyton* sp., Fleury et al., 2000), turf algal matrix (McCook, 2001; Graham et al., 2018), sponges (Demospongaie: Ward-Paige et al., 2005; Lamb et al., 2012), and zoanthids (*Palythoa* sp., Leal et al., 2017). For turf algae, branches of dead *Acropora* spp. coral densely covered in turf algal assemblages were broken off and scraped with a scalpel to collect enough material to make up ten replicate samples. Marine sediment (< 4 cm depth; Fichez et al., 2005; Umezawa et al., 2008) which are not counted as bioindicators in this study, were also collected from all sites to determine signatures from an important store of nutrients on coral reefs. All samples were frozen at -20°C for up to one month before further processing.

1.3.2 Stable Isotopic and Elemental Analyses

All frozen samples were defrosted, rinsed thoroughly with distilled water and replicate samples were placed in a drying oven for ~48 hr at 60°C at Seychelles Fishing Authority laboratory, Victoria, Mahé, Seychelles. Once dried, samples were each ground into a fine powder using a ball mill and stored in individual airtight containers at SFA. All dried samples were then weighed, alongside the relevant standards, for stable isotopic analyses at Lancaster Environment Centre (LEC), Lancaster University, UK. For bioindicators which contained inorganic carbon material (i.e. calcifying organisms such as soft corals, sponges, and zoanthids), additional acidification was required to remove the inorganic carbonate which can affect carbon-based signatures (Schlacher & Connolly, 2014). ~10g of material was digested in 10% v/v hydrochloric acid (HCl) at room temperature until all constituent carbonate had been removed. Samples were then centrifuged, repeatedly washed until all traces of acidity had been removed, and left to dry prior to analysis for carbon stable isotope composition. The carbon stable isotopic and elemental signatures could not be measured in sediments in this study, because the samples were almost entirely composed of inorganic carbon material, so almost all of the test sediment material dissolved during initial runs of the acidification process. In addition, a subset of all calcified samples were not acidified so they could be used for nitrogen-based stable isotopic signatures, as acidification can alter $\delta^{15}N$ signatures in some organisms (Schlacher & Connolly, 2014).

Stable isotopic and elemental analyses for nitrogen stable isotopes ($\delta^{15}N$), carbon stable isotopes ($\delta^{13}C$), nitrogen content (%N), carbon content (%C), and C:N Ratio (calculated from dividing the values of %C by %N) were undertaken within the Lancaster Environment Centre stable isotope facility, using an Isoprime100 Isotope Ratio Mass Spectrometer (IRMS) linked to an Elementar VARIO MICROcube Elemental Analyser. Combustion of samples within tin

capsules at 950°C yielded N_2 and CO_2 for determination of $\delta^{15}N$ and $\delta^{13}C$ respectively. Analyses were standardised to AIR for $\delta^{15}N$ and VPDB for $\delta^{13}C$ using internal reference materials calibrated to international standards. Within-run replication (1 σ) was <0.3 ‰ for $\delta^{15}N$ and <0.1 ‰ for $\delta^{13}C$ for both standards and samples. The standard for reporting C-based measurements is V-PDB (Vienna-PDB) and for N-based measurements, it is atmospheric nitrogen (AIR) (Duarte et al., 2018).

1.3.3 Cost-Effectiveness Analyses

To evaluate the cost-effectiveness of each of the techniques used to quantify the nutrient signatures in the eight different bioindicators, the time taken for collection, processing and analysis was calculated as follows. Collection time involved the time taken to search for and retrieve samples from up to 21 sites, where the average time recorded for each dive was ~1 h. Processing time included sample drying, crushing, weighing, and/or acidifying. Drying time represented the time taken to completely dry each sample in the drying oven, while crushing time was the time taken to crush each dried sample into a fine power. For weighing, the average time weighing standards for each mass spectrometric analysis was added to the time taken to weigh each individual sample, and stable isotope analysis time represented the time per analysis. The time taken to acidify each sample of the four calcified bioindicators was also included, though these samples had to be run twice to obtain results for both N and C isotopic and elemental signatures, with the first subset of samples unacidified, and the second subset acidified. All recorded and calculated times were then standardised to hours (h). The time taken per unit sample was used as a measure of "cost" instead of monetary value in this study, because the methods used to collect, process and analyse them were the same, except for the carbonate-containing samples which needed to be weighed and analysed twice.

1.3.4 Statistical Analyses

Availability of the bioindicators was assessed in two ways. Firstly, the abundance of the selected groups from the benthic composition data across the 21 sites was averaged and pooled for the two different types of reef state. Secondly, the number of sites that the different bioindicator types were collected from were totalled and categorised according to reef state (i.e. coral-mortality or regime-shifted). The percentage of sites from which each bioindicator was collected, relative to each reef state (i.e. out of 12 for coral-mortality reefs, and out of 9 for regime-shifted reefs), was calculated, as there were different numbers in each category. The mean and standard deviation of the five nutrient signatures ($\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N Ratio) from samples of each bioindicator, collected from up to 21 sites, were then analysed in R (R-Core-Team 2018).

The spatial variation for nutrient signatures of each bioindicator was assessed across all available sites using either linear models (LM) or generalized linear models (GLM). All model fits were inspected for normality using visual plots, and LMs were used on the models with normal distributions, while GLMs were used on those with non-normal distributions. A GLM was used to determine the impact of the bioindicator on the five nutrient signatures (i.e. the response variables), using the following model for each individual signature:

Model 1: Nutrient Signature ~ Bioindicator

Where the nutrient signature was either $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N Ratio, and bioindicator (eight levels) as a fixed factor for each of the five response variables, (C-based signatures in sediment were omitted, as there was no data available). A total of 5 models were therefore run for the overall analysis (alpha = 0.05).

The coefficient of variation (CoV) was used to calculate the overall precision of each bioindicator across all available sites. CoV is the ratio of the sample standard deviation to the same mean, for a given set number of data points, and was used in this study because it is a unitless measure of variation, which is useful when testing the statistical effectiveness (i.e. precision) of the signatures across the different bioindicators. High precision is defined in this study as a small standard deviation compared to the mean, which increases the ability to detect statistical significance, both between the replicate samples of each bioindicator collected at each site, and over all the sites from which each bioindicator was collected. Low precision, conversely, is a large standard deviation compared to the mean (Conquest, 1983). Though there is not one set standard in the literature, it is generally assumed that values of CoV < 5 can be regarded as "precise" (Machin et al., 2010). CoV was calculated from the raw measurements detected in the replicate samples of each bioindicator collected from individual sites. Following this, the CoV of the N- and C-based signatures were compared across all the sites from which each bioindicator was collected with five linear models (Model 2), which were run separately for each nutrient signature:

Model 2: CoV ~ Bioindicator

Where CoV was the CoV value for $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N Ratio, and Bioindicator (eight levels) was the fixed factor. The overall mean and standard deviation for the CoV each bioindicator were also summarised in box-plots.

A principal components analysis (PCA) (PRIMER-E Ltd, V.6.1.5, Plymouth, UK) based on a Bray–Curtis similarity matrix was used to visualise the similarities between averaged values of the five different nutrient measurements and the different bioindicators as a way of assessing the level of congruency of the bioindicators (Clarke & Warwick, 2001). The selection of a subset of bioindicators for this analysis (brown macroalgae, green macroalgae

and zoanthids) was based on their level of precision, and the number of sites used, out of 21, depended upon the availability of each of these three indicators. Therefore nine sites were selected, as they had sufficient replicates of all three bioindicators to compare across sites (n=4), and the nutrient measurements were averaged at site level to compensate for the varying numbers of replicate samples available at each site. However, for C-based signatures, zoanthid samples from one site could not be acidified due to limited material so for these, eight sites were used. A correlation matrix was also constructed to assess the different correlation values between the three selected indicators, where a p-value < 0.05 was considered significant.

To quantify the cost-effectiveness of each bioindicator, another GLM was used (as the data was not normally distributed) to compare the average times taken (per sample per bioindicator) for (a) collecting from the field, (b) drying and crushing of samples, (c) weighing and preparing samples (i.e. acidification) for isotopic analyses, and (d) running isotopic analyses. In this model, "Time" was the response variable, and "Bioindicator" and "Task" were the fixed factors (eight and two levels in each factor, respectively):

Model 3: Time ~ Bioindicator * Task

The interaction between these two fixed factors in Model 3 was also analysed to determine whether the "Bioindicator" (eight levels), "Task" (four-five levels, depending on whether or not the bioindicator was acidified), or the interaction between them affects the time per unit sample. Reef State was also used as a fixed factor (with two levels) during initial statistical analyses, but was not included in this study as it showed no significant effect ($p = 0.16$).

1.4 Results

1.4.1 Sample Collection and Benthic Cover

Across the 21 sites, a total of 150 samples of brown macroalgae (*Sargassum* sp.), 91 green macroalgae (*Chlorodesmis* sp.), 103 cyanobacteria, 59 soft corals, 112 sponges, 134 zoanthids (*Palythoa* sp.), 171 turf algal assemblages, and 204 sediment samples were collected. Availability of bioindicator varied between regime-shifted and coral-mortality reefs, as did the percentage of sites within these two categories where they were present (*Table 1.1*). Average cover of *Sargassum* spp. was significantly higher at the regime-shifted sites where it was an order of magnitude greater than on the coral-mortality sites. As such, there were specimens available at 100% of the regime-shifted sites, whereas they were only found at 58% of regime-shifted reefs. There was a similar percent cover of sediment across sites (along the line-intersect transect) regardless of reef state, and sediment samples were collected from all 21 sites. Percent cover of turf algae on coral-mortality reefs was 32.8 ± 23.8 %, compared to 12.2 ± 8.11 % on regime-shifted reefs, but still had 100% availability in both reef states. Cyanobacteria, soft coral and sponge all had higher percent cover and were also present on a higher percentage of coral-mortality sites than on regime-shifted ones.

Table 1.1. Summary table for percent cover (% cover) of candidate bioindicators (BM = brown macroalgae; CYB = Cyanobacteria; GM = Green Macroalgae; SED = Sediment; SC = Soft Coral; TA = Turf Algae; ZO = Zoanthid) from the line-intercept transect surveys at 21 coral reefs around the Inner Seychelles Islands. Percentage of Sites represents the percentage of sites relative to the total number in each reef state (out of n=12 for "coral-mortality" reefs versus n=9 "regime-shifted" reefs). Mean ± S.D for percent cover.

	Regime-Shifted Sites (n=9)		***Coral-Mortality Sites (n=12)***	
Bioindicator	**Mean ± S.D. (%)**	**Proportion of Sites (%)**	**Mean ± S.D. (%)**	**Proportion of Sites (%)**
Sargassum (BM)	36.9 ± 20.3	100	2.7 ± 8.47	58
Cyanobacteria (CYB)	1.2±2.8	44	2.5 ± 5.0	75
Chlorodesmis (GM)	0.2 ± 0.3	89	0.3 ± 0.4	25
Soft Coral (SC)	0.1 ± 0.8	11	1.2 ± 2.5	67
Sediment (SED)	6.7± 3.4	100	9.52 ± 11.5	100
Sponge (SP)	0.00*	56	1.4 ± 2.1	75
Turf Algae (TA)	12.2 ± 8.1	100	32.8 ± 23.8	100
Palythoa (ZO)	0.2 ± 0.4	67	1.3 ± 1.0	100

1.4.2 Variation of Nutrient Signatures in Bioindicators

The type of bioindicator had variable effects on each of the five nutrient signatures (*Suppl. Table 1.2*). Overall, brown and green macroalgae (BM and GM, respectively) not only had lower average $\delta^{15}N$ signatures than the other indicators, but they also had the smallest variations in signatures across all of their sites (5.58 ± 0.82 and 5.33 ± 0.45‰, respectively. *Fig. 1.1a*). Bioindicators representing higher trophic levels, such as sponges (SP), soft corals (SC), and zoanthids (ZO) (7.51 ± 0.67; 7.61 ± 1.27, and 9.08 ± 0.88‰, respectively) had more enriched average $\delta^{15}N$ signatures, as did the non-biological indicator, sediment (SED) (9.61 ± 1.41 ‰). After acidification, the four bioindicators that contained inorganic carbon (soft corals, sponges, and turf algae (TA)) showed similar signatures of $\delta^{13}C$ on average (-16.3 ±1.29; -17.4 ± 0.38; and -18.5 ± 3.16, ‰, respectively), though it was less negative in zoanthids (-13.7 ± 0.88 ‰). The two types of macroalgae also differed (BM: -16.2 ± 1.58, and GM: -21.3 ± 0.96 ‰) whereas cyanobacteria (CYB) (-21.3 ± 3.36 ‰) was similar to green macroalgae (*Fig. 1.1b*).

Turf algae had a similar average signature for %N (1.53 ± 0.45‰) relative to brown macroalgae (1.10 ± 0.18 %) but green macroalgae had a much higher value (4.32 ± 0.48 %), which was even higher than cyanobacteria (3.31 ± 1.25 %). The N content of brown macroalgae was also most similar to zoanthids (1.06 ± 0.22 %). N content was also much lower in sediment (0.05 ± 0.11 %) (*Fig. 1.1c*). There was much higher C content in green macroalgae than in the other bioindicators (42.2 ± 2.40 %), followed by brown macroalgae (31.0 ± 1.41 %), and cyanobacteria (28.7 ± 5.52 %). Zoanthids had the lowest %C (11.2 ± 2.74) (*Fig. 1.1d*). Brown macroalgae had higher C:N Ratio signatures with a large range due to high %C content and low %N content (28.8 ± 4.99). The other five groups were quite similar to one another, with the exception of sponge (0.85 ± 0.11) (*Fig. 1.1e*). The GLMs

showed that the type of bioindicator had a strong influence on the variability of nutrient signatures, with significance evident across almost all signatures. However, both types of macroalgae were statistically similar for $\delta^{15}N$, as were brown macroalgae, turf algae and zoanthid for %N (*Suppl. Table 1.2*).

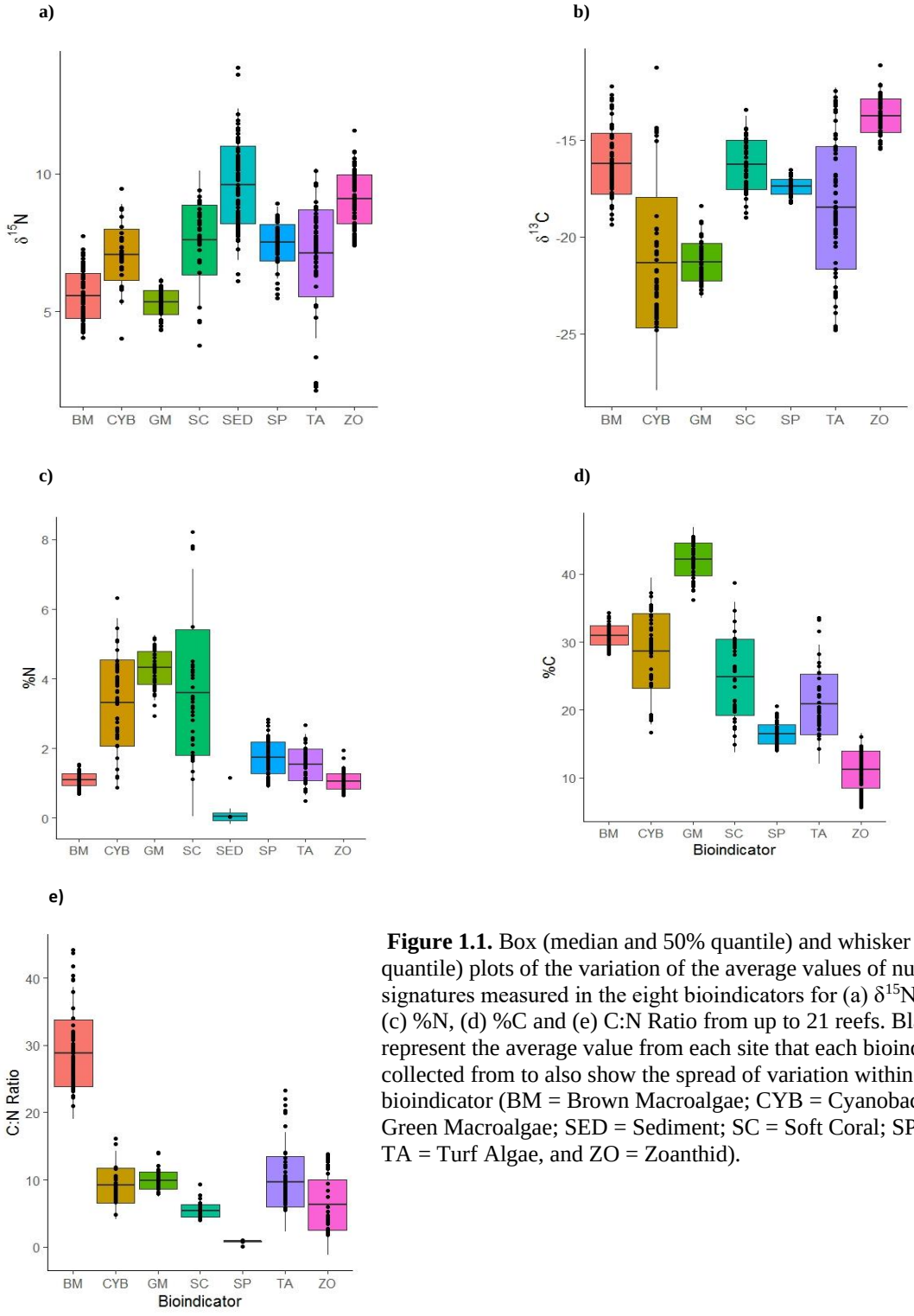

Figure 1.1. Box (median and 50% quantile) and whisker (95% quantile) plots of the variation of the average values of nutrient signatures measured in the eight bioindicators for (a) $\delta^{15}N$, (b) $\delta^{13}C$, (c) %N, (d) %C and (e) C:N Ratio from up to 21 reefs. Black dots represent the average value from each site that each bioindicator was collected from to also show the spread of variation within each bioindicator (BM = Brown Macroalgae; CYB = Cyanobacteria; GM = Green Macroalgae; SED = Sediment; SC = Soft Coral; SP = Sponge; TA = Turf Algae, and ZO = Zoanthid).

1.4.3 Precision of Bioindicators

The precision of the five nutrients within each of the bioindicators was assessed using CoV, as this standardised the nutrient signatures between bioindicators (including the non-biological indicator sediment) controlled for differences in isotopic fractionation in measurements, particularly between trophic levels (summarised in *Fig. 1.2* and *Suppl. Table 1.3*). Green macroalgae had the lowest and most consistent CoV within and across reefs, and therefore the highest precision for all N-based nutrient measurements (δ^{15}N: 2.47 ± 0.95; %N: 7.53 ± 4.29; C:N Ratio: 5.76 ± 5.39), however this pattern was not as distinct for C-only signatures (δ^{13}C: -1.87 ± 1.06 and %C: 3.60 ± 1.67). This was closely followed by brown macroalgae (δ^{15}N: 4.68 ± 1.33 ‰; δ^{13}C: -6.03 ± 3.12; %N: 11.3 ± 4.07; %C: 4.07 ± 1.12, and C:N Ratio: 9.92 ± 3.75). Turf algal assemblages had much more variable average signatures for all five measures, especially those that were N-based (δ^{15}N: 8.30 ± 4.90 ; δ^{13}C: -5.14 ± ; %N: 20.5 ± 20.1; %C: 9.54 ± 10.6, and C:N Ratio: 10.6 ± 10.3) (*Fig. 1.2*).

Zoanthids had lower average CoV values for N-based signatures than higher trophic organisms and were more similar to the two macroalgal types (*$\delta^{15}N$*: 2.98 ± 1.20, and *%N*: 14.3 ± 5.52), as well as for δ^{13}C (-5.14 ± 2.43), though the CoV values for both %C and C:N Ratio were much higher than for any of the other bioindicators (11.8 ± 8.57 and 20.0 ± 24.1, respectively). The other higher trophic level organisms, such as soft corals (δ^{15}N: 6.26 ±4.87; δ^{13}C:-6.20 ± 1.86; %N: 30.4 ± 17.6; %C: 17.4 ± 12.2, and C:N Ratio:11.6 ± 8.68*)* and sponges (δ^{15}N: 6.82 ±5.24; δ^{13}C: -1.44 ± 1.08; %N: 20.0 ± 10.3; C%: 7.24 ± 3.94, and C:N Ratio: 7.58 ± 12.1) showed inconsistent levels of precision across the five signatures. Though sediment had similar precision for δ^{15}N to the other candidates (7.97 ± 3.90), it had the highest range of CoV values for %N (17.4 ± 40.2) (*Fig. 1.2a&c*).

Overall, the CoV analyses showed that both brown and green macroalgae had low average CoV values for N-based signatures, as well as small variations in CoV across the sites. In addition, while the C-based signatures were more variable for zoanthids, the N-based results were more precise compared to the other higher-trophic bioindicators. The statistical models showed variable patterns for each nutrient signature type across the eight bioindicators, however for %C and C:N Ratio, zoanthids were the only bioindicator that significantly differed from brown macroalgae due to its high variation (*Suppl. Table 1.3*).

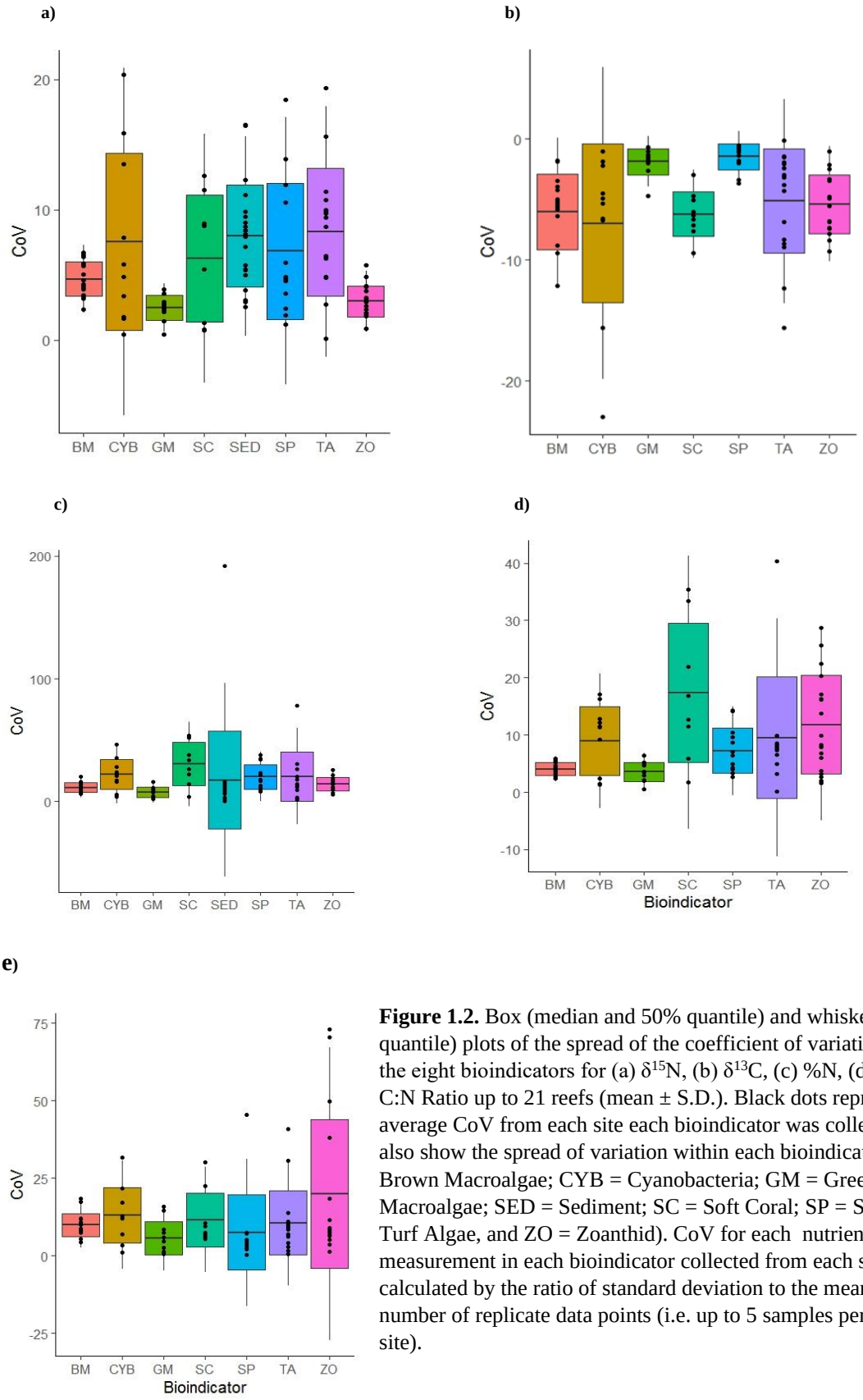

Figure 1.2. Box (median and 50% quantile) and whisker (95% quantile) plots of the spread of the coefficient of variation (CoV) of the eight bioindicators for (a) $\delta^{15}N$, (b) $\delta^{13}C$, (c) %N, (d) %C and (e) C:N Ratio up to 21 reefs (mean ± S.D.). Black dots represent the average CoV from each site each bioindicator was collected from to also show the spread of variation within each bioindicator (BM = Brown Macroalgae; CYB = Cyanobacteria; GM = Green Macroalgae; SED = Sediment; SC = Soft Coral; SP = Sponge; TA = Turf Algae, and ZO = Zoanthid). CoV for each nutrient measurement in each bioindicator collected from each site was calculated by the ratio of standard deviation to the mean of a given number of replicate data points (i.e. up to 5 samples per indicator per site).

1.4.4 Congruency of Bioindicators

A principal components analysis (PCA) was used to assess congruency between the three selected bioindicators. Brown and green macroalgae had low correlation, especially for signatures of N, while zoanthids had no significant relationships with either macroalgae. There were weak positive relationships between N-based signatures of green and brown macroalgae (Table 1.2), but these explain <40% of the variance and are not significant at alpha =0.05 (*Fig. 1.3*). This was also shown by Pearson's correlation analyses between the different combinations of bioindicators, as all data was normally distributed (Table 1.2). The two types that showed the highest similarity for N-based signatures were between brown and green macroalgae for C:N Ratio measurements ($r^2 = 0.61$), closely followed for those of %N ($r^2 = 0.60$) and $\delta^{15}N$ ($r^2 = 0.55$) signatures, though none of these were significantly correlated. However, the highest similarity for C-only signatures was between %C of brown and green macroalgae ($r^2 = 0.81$), but was very low for $\delta^{13}C$ ($r^2 = 0.041$) (Table 1.2).

Table 1.2. Pearson's correlation analyses between the three selected bioindicators (brown macroalgae versus green macroalgae; brown macroalgae versus zoanthids; green macroalgae versus zoanthids) to determine amount of correlation between them (correlation coefficient) The significance level for the p-values is alpha = 0.05.

Bioindicator	**$\delta^{15}N$**	**$\delta^{13}C$**	**%N**	**%C**	**C:N Ratio**
BM vs. GM	0.55	0.041	0.60	0.81	0.61
	(p=0.12)	(p=0.92)	(p=0.09)	(p=0.02)	(p=0.08)
BM vs. ZO	0.10	0.11	0.18	-0.005	0.07
	(p=0.79)	(p=0.80)	(p=0.64)	(p=0.99)	(p=0.68)
GM vs. ZO	0.28	0.64	0.23	-0.23	-0.36
	(p=0.47)	(p=0.09)	(p=0.55)	(p=0.58)	(p=0.34)

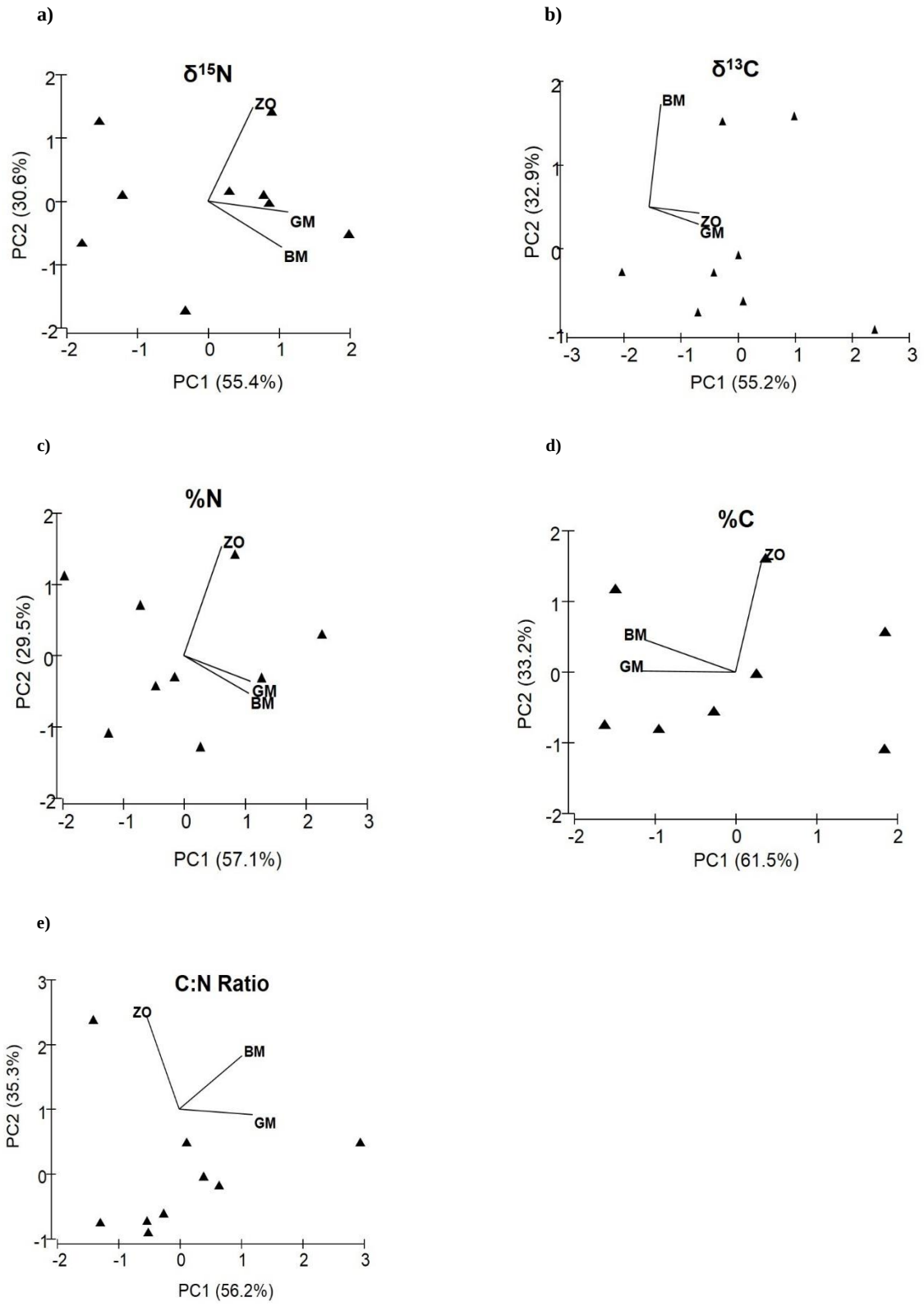

Figure 1.3 Principal Components Analyses (PCA) quantifying congruency between a selection of bioindicators (n =3) (BM = Brown Macroalgae; GM = Green Macroalgae; ZO = Zoanthids) all present at a subset number of sites (n = 9) for measurements of (a) $\delta^{15}N$, (b) $\delta^{13}C$, (c) %N, (d) %C and (e) C:N Ratio.

1.4.5 Cost-Effectiveness of Bioindicators

The time taken for the whole process, from collection to stable isotopic analyses, per unit sample, differed statistically among the eight bioindicators (Table 1.3; summarised in *Suppl. Table 1.4*). The GLMs suggested that both bioindicator and task can have a significant effect on the time taken, per sample, to use each bioindicator for capturing measure nutrient regimes, but reef state does not. Overall the time taken to collect the two macroalgae was similar (BM: 0.038 ± 0.04 h; GM: 0.078 ± 0.078 h), whereas soft corals (0.25 ± 0.31 h), sponges (0.24 ± 0.31 h), turf algae (0.030 ± 0.04 h) and zoanthids (0.18 ± 0.24 h) took significantly longer overall. Sediment, in contrast, took the least time overall to find (Table 1.3). Each task significantly differed as well, with "Drying and Crushing" taking the most time to complete and "Field Collection" taking the least time. The time taken to process the four calcified bioindicators was much greater, because each sample of these indicators required the additional step of "Acidification".

Table 1.3. Summary of the mean time taken (per unit sample, per hour) for each task undertaken to process each bioindicator for the cost-effectiveness. *Acidifying only includes the four bioindicators that were acidified, and thus weighed and analysed in the mass spectrometer twice. Significance Level is $p < 0.05$. Normality inspected using visual plots. Mean ± S.D.

BIOINDICATOR	*FIELD COLLECTION*	*DRYING & CRUSHING*	*ACIDIFICATION*	*WEIGHING*	*STABLE ISOTOPIC ANALYSES*
Brown Macroalgae (BM)	0.038 ± 0.04	24.8 ± 0.5	-	1.5 ± 0.01	0.18 ± 0.03
Cyanobacteria (CYB)	0.35 ± 0.4	23.2 ± 1.4	-	1.5 ± 0.03	0.21 ± 0.1
Green Macroalgae (GM)	0.078 ± 0.08	24.1 ± 0.005	-	1.5 ± 0.01	0.17 ± 0.05
Soft Coral (SC)	0.25 ± 0.3	22.2 ± 1.2	0.17 ± 0.001	3.1 ± 0.06	0.48 ± 0.2
Sediment (SED)	0.015 ± 0.003	22.7 ± 1.2	-	0.14 ± 0.02	0.25 ± 0.1
Sponge (SP)	0.24 ± 0.3	22.6 ± 1.3	0.17 ± 0.0	3.1 ± 0.00	0.37 ± 0.07
Turf Algae (TA)	0.03 ± 0.04	24.6 ± 0.5	0.17 ± 0.002	3.0 ± 0.02	0.34 ± 0.08
Zoanthids	0.18 ± 0.2	23.0 ± 1.5	0.17 ± 0.0	3.0 ± 0.00	0.41 ± 0.03

Although the time taken per sample to collect each bioindicator from the field did not differ between reef states, the availability of samples on each reef did vary (Table 1.1). There was a strong negative correlation between average time taken per sample to collect and the percentage of sites from which each indicator was available on regime-shifted reefs (relative to the total number of sites, i.e. n=9) ($r^2 = 0.94$), whereas there was a very weak negative relationship between average time taken and sample availability on coral-mortality sites ($r^2 = 0.15$; n=12) (*Fig. 1.4*). This suggests that although the time taken varied more among bioindicators on regime-shifted reefs (i.e. it took over an hour, on average, to find one sample of soft coral on a dive), it is a better predictor for finding specific bioindicator(s) on sites dominated by macroalgae. For coral-mortality reefs, in contrast, the times among bioindicators were more similar, but sample availability was more variable. Brown macroalgae had similar collections times between reef states (regime-shifted: 0.01 ± 0.01; coral-mortality: 0.07 ± 0.05 h), but there was 100% availability on regime-shifted sites relative to 58% on coral-mortality sites. Turf algae and sediment, in contrast, not only had 100% availability on both reef states, but they took the least time to collect on regime-shifted sites (TA: 0.04 ± 0.05; SED: 0.01 ± 0.002 h) as well as on coral-mortality ones (TA: 0.02 ± 0.01; SED: 0.01 ± 0.004 h).

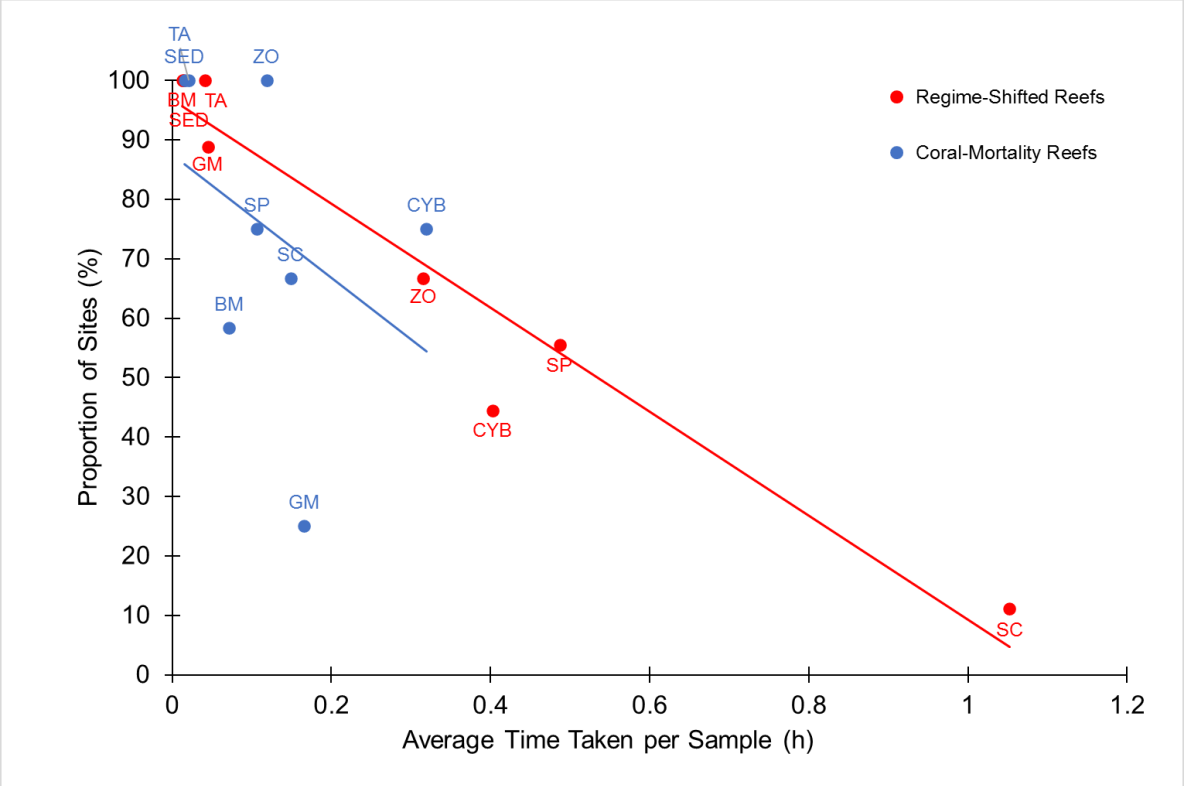

Figure 1.4. The relationships between the average time taken, per unit sample (h) and the availability of samples on both reef states (proportion of sites, %). Each individual point in red represent the total average time, per sample, for the eight bioindicators collected from regime-shifted sites versus the percentage of sites they were available to collect at (n=12), and the individual point in blue represented each indicator from coral-mortality sites. $r^2 = 0.94$ on regime-shifted reefs, and $r^2 = 0.15$ on coral-mortality reefs. BM = Brown Macroalgae; CYB = Cyanobacteria; GM = Green Macroalgae; SED = Sediment; SC = Soft Coral; SP = Sponge; TA = Turf Algae, and ZO = Zoanthid.

1.5 Discussion

The principal aims of this study were to identify precise, cost-effective, and widely available bioindicators for capturing nutrient regimes on coral reefs, particularly over those in different ecological states. Overall, nutrient signatures of brown macroalgae, green macroalgae and zoanthids were considered to meet these criteria, relative to the other candidates. While the macroalgae were more consistent indicators for reefs that have undergone a regime shift, zoanthids were more common on both types of reef state. Turf algae and sediment took the least time to collect and were also the most abundant and available samples across the 21 reefs studied, regardless of reef state, but their utility as bioindicators is limited by their highly variable CoV values. There was low congruency between the three most precise indicators (brown macroalgae, green macroalgae and zoanthids), which suggested that physiological processing of nutrients within each bioindicator has a greater influence on N- and C-based signatures than its local environment. Congruency could be improved by either choosing a suite of indicators from the same functional group, such as macroalgae with comparable nutrient uptake mechanisms, or by tracing the accumulation of nutrient signatures across different trophic levels from the same food chain.

1.5.1 The Precision and Congruency of Nutrient Signatures in Bioindicators

The N- and C-based nutrient signatures of the bioindicators in the current study appear typical of measurements reported in the literature (Atkinson & Smith, 1983; Smit, 2001). For instance, the range of absolute values of $\delta^{15}N$ signatures in all of the bioindicators are quite consistent (5 – 10 ‰), though they are slightly high relative to other marine systems (Sigman & Casciotti, 2001). In addition, the $\delta^{13}C$ signatures reflect that of a carbonate-dominated system, which for instance lies within the range of -10 to -30‰ for most marine macrophytes

(Smit, 2001; Raven et al., 2002). The N-based signatures also follow trophic status whereby those organisms at higher trophic levels are relatively more enriched than those of primary producer status (Boecklen et al., 2011; Lamb et al., 2012). Other studies have found that differences in signatures are not always consistent with distinct sources of nutrient loads (i.e. anthropogenic run-off), which implied that external inputs are not always the cause of variations in nutrient regimes captured in bioindicators (Raimonet et al., 2013).

There were discrepancies found in some of the signatures even between different primary producers in this study, such as between brown (*Sargassum* sp.*)* and green macroalgae (*Chlorodesmis* sp.). For instance, although they had similar $\delta^{15}N$ values across the sites, the other four signatures varied on average between these two bioindicators, particularly for %N, which was much higher in green macroalgae (*Fig. 1.1a&c)*. This could be because nitrogen content in this species is affected by both biological nutrient uptake mechanisms and environmental factors (Fong et al., 2001; Raimonet et al., 2013; Viana & Bode, 2013; Clausing & Fong, 2016) and therefore do not reflect either inorganic concentrations or the $\delta^{15}N$ of their surrounding environment (Viana & Bode, 2013; Ochoa-Izaguirre & Soto-Jimenez, 2015; Gorman et al., 2017). Slower-growing algal species like *Chlorodesmis* have a greater capacity for internal nutrient storage so are not as nutrient-limited, and therefore are less responsive to fluctuations in nutrients as other, more opportunistic species like *Sargassum* (Schaffelke, 1999).

Turf algal assemblages and cyanobacteria are also primary producers that not only take up and utilise bioavailable nutrients but are becoming more prevalent on reefs across a range of reef states, particularly following a disturbance (McManus & Polsenberg, 2004; Charpy et al., 2012; Rasher et al., 2012; Zaneveld et al., 2016), for instance, at the study sites in the

Seychelles (Dajka et al., 2019; Wilson et al., 2019). However, while cyanobacteria have been found to have higher nitrogen fixation rates than turf algal assemblages (den Haan et al., 2016), the extent of the contribution that cyanobacteria make to reef biogeochemical cycles is not known (Ford et al., 2018), and it has been shown that nutrient availability is not associated with cyanobacterial abundance (Thacker & Paul, 2001). This study showed that while cyanobacteria had much more patchy distributions than turf algae, particularly on regime-shifted reefs, both bioindicators had variable precision among the five nutrient signatures, which suggests they are also more influenced by biological factors (i.e. multiple species within the turf assemblage) than their local environment (Raimonet et al., 2013).

Zoanthids are positioned at a higher trophic level than benthic algae and cyanobacteria so their nutrient signatures tend to fractionate and become more enriched (Zanden & Rasmussen, 2001; Fox et al., 2018). There has been little research into zoanthids as potential indicators of nutrient runoff (Leal et al., 2017), but Costa Jr. et al. (2008) found that phosphorus and silica water concentrations had positive effects on both algal and zoanthid growth, and negative effects on coral cover. However, unlike primary producers, zoanthids have to balance auto- and heterotrophic processes for acquiring sources of C and N (Leal et al., 2017), as they not only assimilate sources of nutrition from the food they have consumed (Smit, 2001), but they are also similar to reef-building corals in that they have photosynthetic symbionts in their tissues (Hoegh-Guldberg et al., 2004; Fox et al., 2018). This could explain the large variations in %C and C:N Ratio (*Fig. 1.2d &e*), as they represent the combined signatures from both host and symbiont (Leal et al., 2017). Other higher trophic-level organisms, such as soft corals, can also harbour symbionts (Fleury et al., 2000; Risk, 2014; Baker et al., 2011; Williams et al., 2018). While sponges are not photosynthetic, they do have symbiotic relationships with cyanobacteria, which are supplied with inorganic C by the

sponges, so the $\delta^{13}C$ of sponges also closely reflects that of their diet (Smit, 2001; Lamb et al., 2012).

Although not a biological indicator, sediments can also capture a range of bioavailable nutrients within a reef which can be resuspended within local biogeochemical cycles through biological and/ or physical factors (Fabricius, 2005). Sediments have been used in previous indicator studies, particularly along strong environmental gradients, and have been shown in some locations to reflect the signatures measured in benthic algae (Umezawa et al., 2008). However, in the current study, very little N was detected in the subsamples of sediment analysed even before acidification, so the low precision calculated for it was more likely due to random error than environmental factors, and so was not comparable for either N- or C-based signatures. This is supported by other studies which have found sediments to be an overall poor indicator for nutrients (Fichez et al., 2005).

The congruency among the three bioindicators with the greatest precision (brown macroalgae, green macroalgae and zoanthids) was relatively low, which again could be an example of the effect of the differences in nutrient processing between the different bioindicators. Congruency is important, as a single-species approach may result in an underestimation of spatial patterns in nutrient regimes, and it has been shown across multiple taxa in previous studies (Connolly et al., 2013), but these studies were also conducted along strong nutrient gradients (i.e. with increasing distance from a sewage outfall). This could suggest that the nutrient sources were relatively similar across the study reefs in the Inner Seychelles islands, or else that the biological mechanisms of individual species outweighed the effect of environmental factors on their isotopic and elemental signatures (Raimonet et al., 2013).

1.5.2 Cost-Effectiveness of Bioindicators

Cost-effectiveness is often mentioned as an important criteria in previous bioindicator studies (Fichez et al. 2005; Cooper et al., 2009; Risk et al., 2001). However, analyses are rarely conducted to quantify these in ecological studies (Drummond & Connell, 2008; Bal et al., 2020) even though the "cost" of any particular indicator can be affected by various different factors. For instance, the average time taken to collect an individual sample from a study site depended upon its availability and/ or abundance, which is why there was a significant difference in collection time with reef state. While it only took ~1 to 2 minutes on average to collect samples of turf algae and sediments from each site, regardless of ecological condition, it took significantly less time to collect brown macroalgae from regime-shifted reefs than it did on coral-mortality reefs. Differences in availability on those reefs could be influenced by nutrient loads, abundance of herbivores, depth, structural complexity, and juvenile coral cover (Graham et al., 2015; Dajka et al., 2019). However, reef state did not affect the time taken for the three later processing stages, as samples were treated in the same way after collection, regardless of their original location.

The findings of both the sample collection and the line-intercept survey of benthic cover at the 21 sites illustrated the importance of considering the local abundance of a bioindicator when assessing a specific stressor (Cooper et al., 2009). For instance, turf algae and sediments were ubiquitous at all sites, so could be considered as more "cost-effective" in terms of sampling availability and abundance. However, as turf algae are composed of an assemblage of varying functional groups, and there was very little N detected in the sediment samples, it is difficult to interpret results for nutrient signatures from either (bio)indicator, and therefore to rely on them for capturing nutrient regimes precisely, despite their widespread abundance.

1.5.3 Future Directions in Bioindicator Research

This study investigated novel ways of assessing potential bioindicators for monitoring programs across coral reefs under different ecological states. However precision and effectiveness of bioindicators used in this study could be improved, even if these improvements will increase costs. For instance, to reduce the CoV of diverse turf algae assemblages, cyanobacteria, and symbiotic organisms, future studies could isolate and individually measure the different functional groups within assemblages (Steneck & Dethier, 1994), individual strains of cyanobacteria (Thacker & Paul, 2001), or the host and symbiont fractions in zoanthids and soft corals (Hoegh-Guldberg et al., 2004; Leal et al., 2017) so that the nutrient signatures of each group can be measured and interpreted separately. Conversely, such techniques will increase the time taken to process and analyse samples, and thus will increase their "costs" as a bioindicator. For instance, in the current study, the additional step of acidifying samples of the four calcifying bioindicators increased precision of the $\delta^{13}C$ signatures for their trophic position, but it also took longer to process them as they needed to be weighed and analysed twice (Schlacher & Connolly, 2014; Vaughan, *pers. obsv.*). These factors will need to be carefully considered when choosing a bioindicator, particularly for different reef states.

There have been numerous studies which have used manipulative experiments to determine the responses of potential bioindicators to nutrient enrichment (Schaffelke & Klump, 1998; Fong et al. 2001, 2003; den Haan et al., 2016), and/ or macroalgal bioassays where live specimens are transplanted into areas in which they are not naturally abundant (Costanzo et al., 2001; Lin & Fong, 2008; Fernandes et al., 2012; García-Seoane et al., 2018b). Both techniques can be very useful for understanding the ecological significance of nutrient enrichment, particularly when using macroalgae (Costanzo et al., 2001; García-Seoane et al.,

2018b). However, as either technique requires multiple access to the same sites, this may not always be feasible for all monitoring programs, especially for those working in remote areas or off research vessels, as was the case for the current study. In these instances, it is therefore even more important to establish the precision and cost-effectiveness of a selected bioindicator in advance.

It was also difficult to determine the accuracy of the recorded bioindicator nutrient signatures, as there was little reference data for nutrient levels around the Inner Seychelles islands, even from seawater samples, and especially at the spatial scales investigated in this study. Further research should therefore also investigate the accuracy of cost-effective bioindicators such as macroalgae by additionally measuring stable isotopic signatures in potential point sources (Costanzo et al., 2001), though this could also include seawater samples to potentially help identify nutrient sources. These combined methods could be later applied to coastal areas where agricultural or waste treatment practices take place in order to help track the flow of nutrients from terrestrial to marine systems over large spatio-temporal scales (Dailer et al., 2010; Fernandes et al., 2012). Another approach could entail building up a suite of relatively similar bioindicators by focusing on specific functional group(s), appropriately matched to the scale of the ecological process being investigated (Linton & Warner, 2003; Fong & Fong, 2014; McWilliam et al., 2018). If this option is not possible, for instance when a group of congruent bioindicators (i.e. fleshy macroalgae) is only found on reefs in a certain ecological state, then nutrient signatures could be compared across a suite of bioindicators from different trophic levels within the same food chain. Although nutrient signatures fractionate and become more enriched at higher levels (Smit, 2001; Graham et al., 2018), it might be easier to compare consumers with primary-producing organisms in stable isotopic analyses if they are using the same nutrient sources (Connolly et al., 2013; Kürten et al., 2014).

1.6 Conclusion

In conclusion, the stable isotopic and elemental signatures of fleshy macroalgae were found to be precise and cost-effective bioindicators, as primary producers with widespread distribution and consistent measurements within their tissues. The combination of macroalgal and stable isotopic analyses provide excellent, cost-effective proxies that represent a short-term temporal summary of the average nutrient signatures in the local water column (Costanzo et al., 2001), so if there had been any N enrichment that then fell in the typically higher range of signatures for sewage outfall (~ 9-20 ‰), this would be detectable in the tissues (Heaton, 1986). If the precision of bioindicators can be increased, for instance, through the separation of the host and symbiont in zooxanthellate species such as most corals, it would provide additional opportunities to determine differences in bioavailable nutrient regimes between reefs, although this would also increase time costs. This could be particularly useful in remote coastal areas where environmental management efforts to assess and mitigate the local anthropogenic impacts of coastal run-off and excessive nutrient loads on coral reefs are currently limited, but would be highly beneficial to assessing overall ecosystem health. If remote reefs have been subjected to any large disturbance, such as a mass bleaching event, having a precise and cost-effective bioindicators to detect whether any areas have excessive nutrient loads, could enable better-informed efforts to improve water quality and mediate coral recovery potential.

2. SPECIES AND NUTRIENT HISTORY INFLUENCE THE EFFECTIVENESS OF MACROALGAL BIOINDICATORS FOR PASSIVE AND ACTIVE BIOMONITORING OF NUTRIENT REGIMES

2.1 Abstract

Macroalgae, and the nutrient signatures in their tissues, have been used as bioindicators in both passive (collection of native samples) and active (transplantation) biomonitoring studies, but their effectiveness for both methods have rarely been compared. While some macroalgal species might reflect strong nutrient gradients in their tissues across large spatial scales, species-specific nutrient uptake mechanisms or site-specific nutrient history may limit their suitability for other types of biomonitoring. In this study, coral reef brown macroalgae *Dictyota bartayresiana* and *Padina boryana* were collected from eight sites along a known nutrient gradient across Opunohu Bay, Mo'orea, French Polynesia. Two of these sites, which had distinct nutrient regimes and benthic community structure were then selected for a reciprocal transplant experiment (RTE). $\delta^{15}N$ in both *Dictyota* and *Padina* showed a decline across the bay, but only *Dictyota* was significant ($r^2 = 0.71$, $p = 0.009$, and $r^2 = 0.52$, $p = 0.11$, respectively). However, in the RTE, only *Dictyota* taken from the low-nutrient lagoon showed a 1.4-fold increase in $\delta^{15}N$ when transplanted on the high-nutrient reef. This study found that opportunistic macroalgae were the most responsive to differences in nutrients using both methodologies, and therefore are likely more adaptable as bioindicators than another species within the same broad functional group with different capacities for nutrient uptake. It also shows how finer-scale variation in functional traits can impact a bioindicator's effectiveness, so using a combination of passive and active methods could be used in the initial selection process before bioindicators are applied to large-scale monitoring programs.

In Prep. (28/08/2021):
Vaughan, E.J., Parravicini, V. & Graham, N.A.J. Species and nutrient history influence the effectiveness of macroalgal bioindicators for passive and active biomonitoring of nutrient runoff. *Marine Biology.*

2.2 Introduction

Anthropogenic nutrient runoff is most commonly caused by sewage pollution and agricultural practices along poorly-managed coastlines, and is a local-scale stressor for coastal marine ecosystems across the globe, with both direct and indirect impacts across multiple biological scales (Rasher et al., 2012; D'Angelo & Wiedenmann, 2014; MacNeil et al., 2019). Temporal measurements of a range of water quality parameters from periodic grab water sampling is possible and can provide much information about source and content (Fabricius et al., 2005, 2012; De'ath & Fabricius, 2010; Rouzé et al., 2015; Zubia et al., 2018). However, measuring seawater samples and/ or using *in situ* autonomous loggers can be expensive and time-intensive, particularly in small island developing nations (SIDS) where water quality needs to be assessed over large spatio-temporal scales (Daniel et al., 2020). This has led to calls for more cost-effective methodologies that could provide a more stable measure of nutrient loads on reefs for biomonitoring programs (**Chapter 1;** Fabricius et al., 2012; García-Seoane et al., 2018; Bal et al., 2020). Fast-growing, opportunistic species are widely used as bioindicators for managing and monitoring water quality in marine environments, as they have biological mechanisms that allow them to rapidly take up bioavailable nutrients from the water column and accumulate them in their tissues, which provides a cumulative proxy of local nutrient regimes (Linton & Warner, 2003; Fong et al., 1998; Cooper et al., 2009). One key example of this is fleshy macroalgae (Costanzo et al., 2001).

Macroalgae are an ecologically-relevant functional group on many marine ecosystems across the globe, because they are not only important primary producers for multiple food webs, but some canopy-forming species can also provide habitat for other organisms (Fulton et al, 2019). However, increased nutrient loads can give them a competitive advantage over other, slower-growing species, which can allow them to proliferate in polluted coastal areas (Littler & Littler, 1980; Littler et al., 2006a; Rasher et al., 2012; Zubia et al., 2018). Pairing benthic community structure, including macroalgal cover and/ or species richness, with measurements of stable isotopic and elemental signatures in macroalgae can therefore show short-term responses to nutrient enrichment, even within hours of exposure (Costanzo et al., 2001; Linton & Warner, 2003; Cooper et al., 2009; Fabricius et al., 2012; den Haan et al., 2016; Gorman et al., 2017; Zubia et al., 2018). These signatures provide both source and concentration of nutrients, particularly through nitrogen-based stable isotopic ($\delta^{15}N$) and elemental analyses (nitrogen tissue content, %N), respectively (Costanzo et al., 2001).

In most macroalgal bioindicator studies, only one species is typically used across all monitoring sites for consistency, and in those where multiple species are used, there are rarely quantitative comparisons conducted to determine whether the signatures accurately reflect those of their local environment, or whether they are more influenced by their individual ecological strategies (**Chapter 1;** Connelly et al., 2013; Clausing & Fong, 2016; García-Seoane et al., 2018a&b). In addition, only relying on one species can result in distributional gaps in monitoring surveys if they were absent on some of the target sites where nutrient pollution is either known or suspected, and this can lead to inconclusive and even misleading results about the spatial extent of runoff (Linton & Warner, 2003).

Two of the most common methodologies for using macroalgal bioindicators to assess the impacts of nutrients on coral reefs are passive biomonitoring (García-Seoane et al., 2018a) and active biomonitoring (García-Seoane et al., 2018b). Passive biomonitoring involves collecting wild macroalgae, while active biomonitoring involves transplanting macroalgae on target sites (Rajfur & Klos, 2014). While there are many examples of both passive and active biomonitoring in the literature, there are fewer studies that directly compare the advantages and disadvantages of both in the same area (Lacroix et al., 2015), and none which compare them using a suite of macroalgal species with similar morphologies. However, this may be a faster, more cost-effective way of assessing whether one or more bioindicators are suitable or not for a monitoring program. For instance, passive methods allow researchers and environmental managers to both detect and trace the spatial extent of coastal runoff, such as a nutrient gradient away from river discharge or a sewage outfall (Fabricius et al., 2005), or around an entire island region (Barr et al., 2013). In addition, changes in nutrient signatures can be coupled with ecological data, such as changes in macroalgal cover, which can reveal the impacts of these gradients on community structure and biodiversity (Adjeroud & Salvat, 1996; Adjeroud, 1997; Umezawa et al., 2002; Fabricius et al., 2005, 2012). Another advantage of passive biomonitoring is when sites are in remote locations that are difficult to access more than once, such as for surveys carried out as part of a research expedition (Littler et al., 1992). However, if this method relies on a single-species approach, and this species is not ubiquitous across the whole monitoring area, this can leave significant spatial gaps in recorded water quality data (Linton & Warner, 2003).

Active biomonitoring is an effective way of compensating for gaps in the distributions of native macroalgae, as specimens can be directly transplanted on target sites to ensure sufficient coverage for the monitoring program. This reduces the chance of spatial variability

in captured nutrient signatures, as the specimens will have all been collected from the same original site (García-Seoane et al., 2018a&b). In addition, any stored internal nutrients in the algal tissues can be depleted by placing them in controlled laboratory aquaria for several days before being transplanted onto the target reefs, so that they will be nutrient limited and therefore more responsive to the local environment in which they have been placed (Dailer et al., 2010). For instance, in Lin & Fong (2008), they showed that after ~2 days in outdoor flow-through tanks to deplete internal stores of nutrients, $\delta^{15}N$ in transplanted *Acanthophora* sp. along Opunohu Bay in Mo'orea, French Polynesia captured very similar signatures as those in shrimp farm effluent that was being discharged via the river at the land end of the bay. Examples of large-scale algal transplants include those conducted around the island of O'ahu, Hawai'i (Dailer et al., 2010) and across the central Great Barrier Reef (McCook, 1996). However, there are a few drawbacks to the active method. For instance, it requires multiple visits to the target sites to deploy and collect the algae, which may not be possible if monitoring programs are working off a research vessel in remote areas or cover very large spatial scales (i.e. whole island systems) (**Chapter 1**; Bal et al., 2020). In addition, if bioindicators are transplanted when they have been originally taken from sites with high nutrient levels, as was the case in the ENCORE experiment in the Great Barrier Reef, Australia (Bell et al., 2007), any nutrient signatures captured using the active method might be confounded by the internal nutrients already stored in their tissues (Fong et al., 1994; 2003; Szmant, 2002).

Some studies have also suggested that multiple algal species can be categorised into the same functional-form models, with the assumption that they will show similar patterns in response to environmental drivers (Littler & Littler, 1980). However, different native species may have different ecological strategies that allow them to co-exist within the same environmental

conditions (Tanner et al., 1994; Fong et al., 2001; Gartner et al., 2002; Clausing & Fong, 2016; Fong & Fong, 2014, 2017; Gennaro et al., 2019). This may be due to different nutrient uptake mechanisms, capacities for internal nutrient storage, or being conditioned by their restricted distributions, which can indicate that nutrient signatures reflect macroalgal metabolism rather than environmental variability, and so may not be as effective as initially thought (Umezawa et al., 2002; Raimonet et al., 2013; Fong & Fong, 2014). In addition, the nutrient history of the same species from different reefs may affect how responsive they are to additional nutrient inputs. For instance, those found in areas of high nutrient loads may already have tissues saturated with nutrients, so do not take up any new inputs to the water column as well as those from low-nutrient or oligotrophic reefs might (Fong et al., 1994; 2003). Further investigations into how selected bioindicators take up and utilise bioavailable nutrients is critical to ensure they are accurately reflecting the stressor they were assigned to monitor for multiple types of biomonitoring.

Tropical, shallow-water coral reefs are commonly found along populated coastlines and are currently facing local pressures from poor management of coastal pollution in rich and poor countries alike (Barnes et al., 2019). This makes them a particularly vulnerable ecosystem to the impacts of anthropogenically-derived nutrient pollution and therefore are an excellent study system for investigating their impacts through these two biomonitoring methods (Fabricius, 2005; Dailer et al., 2010; Fernandes et al., 2012; Devlin et al., 2021). The proliferation of macroalgae on reefs can be affected by a number of physical (Fabricius, 2005; Littler et al., 2006; Devlin et al., 2012; Leichter et al., 2013; Williams et al., 2013; Clausing & Fong, 2016; Duran et al., 2016) and biological factors (Littler et al., 2006a; Fong & Paul, 2011; Fong & Fong, 2017), and in Mo'orea, it was recently found that some reefs in

enclosed bays and lagoons have undergone regime shifts to macroalgal-dominated states despite the presence of herbivores (Adam et al., 2021).

This study aims to: 1) assess the effectiveness of native *Dictyota*, a fleshy, fasting growing brown macroalgae, and *Padina*, a slower-growing, lightly calcified brown macroalgae, across a known nutrient gradient in Opunohu Bay, Mo'orea, French Polynesia (passive biomonitoring), 2) assess the effectiveness of *Dictyota* and *Padina* as bioindicators from two sites with distinct benthic communities and nutrient histories with a reciprocal transplant experiment (active biomonitoring), and 3) demonstrate the importance of understanding a bioindicator's local environmental conditions and ecological strategy before selecting it for either type of large-scale biomonitoring methodology.

2.3 Methods

2.3.1 Study Sites

This study was conducted in Opunohu Bay on the north coast of Moorea, French Polynesia, a volcanic high island in the central South Pacific (17°30'S, 149°50'W), in July 2018. Opunohu Bay is ~3.5 km long, and the northern two-thirds of the bay are characterized by fringing and barrier coral reefs along the edges of it. The catchment is characterised by steeply-sloping volcanic mountains that are ~ 900m high, and by a valley where the Opunohu River flows into the bay (Adjeroud & Salvat, 1996; Adjeroud, 1997; Lin & Fong, 2008). At the mouth of the river is a 2 ha intensive shrimp farm that has commercial feed added periodically to the shrimp ponds and effluent drains that empty into the river. The only other sources of coastal nutrient runoff in this area are experimental lumber tree farms, a pineapple

farm, an agricultural school and a small experimental freshwater shrimp farm (Adjeroud & Salvat, 1996; Lin & Fong, 2008). Average rainfall is 325 cm, and tropical cyclones are rare in French Polynesia. Heavy rainfall causes rapid surges in temperature, salinity and light attenuation, primarily due to increased anthropogenic runoff via river discharge, as well as potential submarine groundwater discharge. Freshwater discharge by the river spreads over the surface of the bay and into deeper oceanic waters due to offshore winds along the edges of the bay (Adjeroud, 1997).

Previous studies have demonstrated strong spatial patterns in the benthic community between the river mouth and the ocean along Opunohu Bay (Adjeroud & Salvat, 1996; Adjeroud, 1997; Lin & Fong, 2008; Leichter et al., 2013; Donovan et al., 2020; Adam et al., 2021). There is a spatial gradient in species richness and percent cover for corals and echinoderms along the reef flats at the edges of the bay, with the two taxa almost absent near the river mouth, and increasingly dominant towards the ocean. In contrast, the species richness of macroalgae, molluscs and sponges, and macroalgal coverage, are high in the middle part of the bay, and overall highly variable (Payri, 1987; Adjeroud & Salvat, 1996; Adjeroud, 1997). The gradient in community structure along this bay appears to be closely associated with environmental conditions. For instance, an absence of corals and echinoderms towards the river mouth, where only a few tolerant species occur such as *Porites*, can be explained by low salinity and high turbidity which occur after heavy rains during the wet season (Adjeroud, 1997).

2.3.2 Study Species

Dictyota is a fast-growing opportunistic brown algae in the family Dictyotaceae which has a wide abundance and distribution across almost all areas and zones of reefs (Delgado &

Lapointe, 1994; Beach et al., 2006; Fong & Paul, 2011). In previous studies, *Dictyota* has been shown to respond quickly to enrichment due to a strong capacity for rapid uptake, particularly after pulses of nutrients, which results in rapid growth rates (Littler & Littler, 1980; Fong et al., 2003; Clausing & Fong, 2016). In contrast, while *Padina* is also found in the family Dictyotaceae and is ubiquitous across not only a range of tropical reefs but environmental conditions (Delgado & Lapointe, 1994; Fong & Paul, 2011; Barrow et al., 2015), it is a lightly calcified, foliose brown species that is slower-growing than *Dictyota*, and though it might have limited storage capacities, the former have lower nutrient requirements which allow them to tolerate low nutrient conditions. They are both locally abundant year round in Mo'orea, as well as globally abundant and increasing on reefs worldwide, so both species are suitable candidates as bioindicators for nutrient regimes (Littler & Littler, 1980; Delgado & Lapointe, 1994; Umezawa et al., 2002, 2007; Fong et al., 2003; Fong & Paul, 2011; Fong & Fong, 2014, 2017; Clausing & Fong, 2016).

2.3.3 Passive Biomonitoring – Nutrient Gradient

Samples of *Dictyota bartayresiana* and *Padina boryana* (n=3) (hereafter *Dictyota* and *Padina; Suppl. Fig. 2.1*) were collected at ~1 m depth from eight sites along Opunohu Bay from the river mouth to the ocean-facing reef, and extended to the backreef lagoon "Papetoai", which is close to the north-western head of the bay. *Padina* was only found at six out of the eight sites. This method was used to see if both genera could capture the same nutrient gradient in their tissues (i.e. with changes in $\delta^{15}N$ and %N) over this distance. A detailed sampling point map was created using a global positioning system (QGIS; *Suppl. Fig. 2.1*), and the distance from the Opunohu River mouth at the bottom of the bay to each collection site was calculated.

2.3.4 Active Biomonitoring – Reciprocal Transplant Experiment

After preliminary scoping surveys and stable isotopic analyses of algal tissues, two sites out of the eight were randomly selected for the active monitoring component of this study, as they were found to have both distinctive nutrient regimes and benthic communities which was required for the reciprocal transplant experiment. Site 4 was chosen for the high-nutrient site (hereafter named high-nutrient reef; *Suppl. Fig. 2.1*), as it had low water clarity as well as high macroalgal cover, which was also shown halfway along the fringing reef in Adjeroud (1997). Site 8 (hereafter named low-nutrient reef; *Suppl. Fig. 2.1*) was the Papetoai lagoon, as coral cover and water clarity were both higher there, though there was still a sufficient level of macroalga coverage for this study.

Specimens of *Dictyota* and *Padina* were collected from the two chosen sites (high-nutrient and low-nutrient reef) for the *in situ* reciprocal transplant experiment (RTE) on 23rd July 2018 to assess the effects of nutrient history and morphology on the responses of these two species to a new nutrient environment. 12 specimens of each genera were collected from both sites (n=3 per species, per site; n=24 in total) and were immediately returned to CRIOBE to clean them of epiphytes, epifauna, and sediments, and spun for 1 min in a salad spinner to standardise removal of water. After initial subsamples were taken to be frozen for stable isotopic analyses, they were wet-weighed. Each specimen of *Dictyota* weighed ~5 g, and each specimen of *Padina* weighed ~6 g, as the calcification in the latter required more biomass to approximate equal volumes across species (Fong & Fong, 2017). Each specimen used in the RTE included apical tips, which is where the greatest rate of growth in macroalgae occurs (Clausing & Fong, 2016; Garcia-Seoane et al., 2018a). After being labelled as either “Control” or “Transplant”, individual specimens were attached to small square pieces of plastic grate with cable ties (n = 2 grates per site) and taken back to the same

two sites on the same day on collection. Half of the algae from each site were deployed at the site of origin, and the other half were deployed at the opposite site to control for any effect of transportation or transplantation. Using snorkel, the two mesh grates with the samples were attached to two cinder blocks at ~1 m at each site, before a cage made from chicken wire was secured over the top of each one to minimise the effect of herbivory. Samples were collected after five days and returned to CRIOBE, where they were spun for 1 min and wet-weighed again before final samples were frozen for later stable isotopic analyses.

2.3.5 Benthic Cover

After preliminary scoping surveys and initial stable isotopic analyses, the benthic community structure of the two sites used for the RTE was assessed in more detail. The composition of the benthos was determined using the photoquadrat method. At each site, 2 transects (25 m each) were deployed at a constant depth of ~ 1 m. A total of 5 quadrats placed at 5 m intervals were photographed per transect using a Canon G9 camera (0.5 m^2 total area within each image). Image analysis of the photoquads was completed using Coral Point Count with Excel (CPCe). A total of 100 points were placed in a stratified random design over each image, with the substrate under each point identified to the finest resolution possible (genus for corals, macroalgae, and invertebrates when possible. When no biological cover was noted under a point, the non-biological substrate (e.g. sand) was recorded (Preskitt et al. 2004).

2.3.6 Stable Isotopic Analyses

All frozen samples were defrosted < 2 weeks after collection, rinsed thoroughly with fresh or distilled water to remove any epiphytes or epifauna, and were placed in a drying oven for 48 h at 60°C at CRIOBE. Once dried, samples were returned to Lancaster Environment Centre,

and were ground into a fine powder using a ball mill and stored in individual airtight containers. 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 ($\delta^{15}N$) and elemental analyses (%N) for 2017 were run on an Isoprime100 Isotope Ratio Mass Spectrometer (IRMS) linked to an Elementar VARIO MICROcube Elemental Analyser at Lancaster Environment Centre, Lancaster University. Analyses from both years were standardised using internal reference materials calibrated to international standards. Within-run replication (1 σ) was <0.3 ‰ for $\delta^{15}N$ and <0.1 ‰ for $\delta^{13}C$ for both standards and samples.

2.3.7 Statistical Analyses

A linear regression model was used to determine the relationship between distance from the Opunohu River mouth and the nitrogen-based nutrient signatures ($\delta^{15}N$ and %N) in both *Dictyota* and *Padina* at the eight sites (six for *Padina*) along the established nutrient gradient along Opunohu Bay. In addition, a Spearman's-rank correlation analysis was used to test the congruency between the signatures in *Dictyota* and those in *Padina* along the nutrient gradient, for both $\delta^{15}N$ and %N, as the data was non-normal. The regression slopes between the two species for both nutrient signature were also compared, however as there were only two species, and therefore only two slopes, the differences could not be tested statistically. A two-factor ANOVA was run on $\delta^{15}N$ and %N in both genera to assess the effect of species and river distance on nutrient signatures. For the RTE, a two-factor ANOVA was run four times for $\delta^{15}N$ and %N in *Dictyota*, and for $\delta^{15}N$ and %N in *Padina*, with factors site (two levels: low- and high-nutrient reef) and tissue time-point (two levels: initial tissue samples pre-RTE and final tissue samples post-RTE). Wet weight was not included in this analysis due to loss of material during the RTE. Normality of data was assessed visually using

histograms, and homogeneity of variance for the ANOVAs was assumed with a Levene's test. All statistical analyses were conducted in R (R-Core-Team 2018). Differences between key ecological attributes between high- and low-nutrient reefs (i.e. coral cover, macroalgal cover, and coral: macroalgal ratio). Benthic cover at the different sites was visualised using a principal component analysis (PCA) in PRIMER (PRIMER-E Ltd, V.6.1.5, Plymouth, UK; Clarke & Warwick, 2001).

2.4 Results

2.4.1 Passive Biomonitoring – Nutrient Gradient

For *Dictyota*, the relationship between increases in river distance and decreases in $\delta^{15}N$ signatures was stronger and significant ($r^2 = 0.71$, p=0.009), whereas it was non-significant for *Padina* ($r_S = 0.52$, p=0.11). In summary, as distance from the river increases up Opunohu Bay, $\delta^{15}N$ decreases in both species, but this relationship is weaker in *Padina*. This could be due to higher variation in their signatures, but this could also be because samples were only collected from six of the sites for this species, relative to the eight sites that *Dictyota* samples were taken from. %N values were statistically similar across the Oponuhu Bay nutrient gradient (Dictyota: $r_S = 0.15$, p=0.34, and Padina: $r_S = 0.04$, p=0.72). The slopes of the regression model for $\delta^{15}N$ in the two species across the nutrient gradient (Connolly et al., 2013) were similar (*Dictyota:* -0.35; *Padina:* -0.33), although this was not tested due to only having two species to compare (n=2). In contrast, the slopes for %N in the two species were quite different (*Dictyota:* 0.05; *Padina*: -0.04). The congruency between $\delta^{15}N$ signatures between *Dictyota* and *Padina* was significant but only moderately high ($r_S = 0.53$, p = 0.02), whereas it was much lower and non-significant for comparisons of %N ($r_S = 0.28$, p = 0.28).

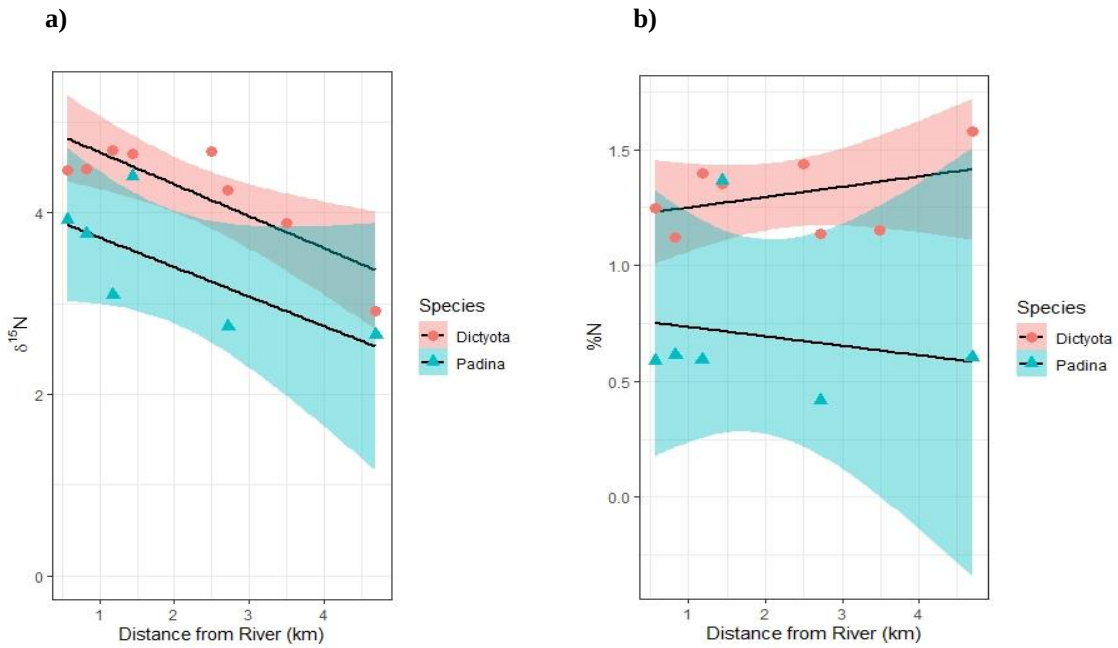

Figure 2.1. The relationship between distance from the mouth of the Opunohu River and (a) $\delta^{15}N$ in *Dictyota* and *Padina*, and (b) %N in *Dictyota* and *Padina* along an established nutrient gradient from the land-end of Opunohu Bay to Papetoai lagoon on the north-western corner of the head of the bay in Mo'orea, French Polynesia. The regression lines and confidence intervals were obtained using linear regression coefficient of determination (r^2); the pink band represents the 95% confidence intervals around the regression line for *Dictyota*, and the blue band represents that for *Padina*.

2.4.2 Active Biomonitoring – Reciprocal Transplant Experiment

In the reciprocal transplant experiment, the greatest significant difference in $\delta^{15}N$ in *Dictyota* was found between sites (low- versus high-nutrient reef, $F_{1, 16} = 119.8$, $p < 0.0001$), followed by tissue time-point (i.e. initial versus final tissue; $F_{1,16} = 17.8$, $p = 0.0006$), and, overall, for treatment (transplant versus control, $F_{1,16} = 12.4$, $p = 0.002$), with a few exceptions. Tukey's post-hoc tests showed that $\delta^{15}N$ in *Dictyota* after transplantation at the high-nutrient site was significantly higher than the initial $\delta^{15}N$ in pre-transplanted algae (3.95 ± 0.2 and 2.92 ± 0.2‰, respectively), as well as in the control algae on the low-nutrient reef, both pre- and post-transplant (2.80 ± 0.2 and 2.88 ± 0.2‰, respectively). Conversely, the final $\delta^{15}N$ in the low-nutrient algae transplanted on the nutrient reef was similar to the final $\delta^{15}N$ in the control

algae on the high-nutrient reef (4.06 ± 0.1, p =0.98). The different interactions between the three factors for $\delta^{15}N$ in *Dictyota* in were all significantly different, including the three-way interaction ($F_{1,16} = 8.30$, $p = 0.01$). Initial tissue samples differed significantly between sites, regardless of their assignment for the RTE (*Fig. 2.2a*). For *Padina*, only site had an effect on $\delta^{15}N$, with the high-nutrient reef specimens having significantly higher signatures, regardless of treatment or tissue time-point ($F_{1,16} = 170.3$, $p < 0.0001$). The post-treatment specimens that were transplanted from the low-nutrient to the high-nutrient reef (2.70 ± 0.4‰) were similar to the pre-transplant algae (2.69 ± 0.3‰), as well as both controls at the low-nutrient reef (Initial: 2.70 ± 0.4 and Final: 2.84 ± 0.5‰).

For *Dictyota*, only the factor "site" had a significant effect on %N ($F_{1,16} = 28.9$, $p < 0.0001$). The post-transplant low-nutrient algae had similar N content (1.66 ± 0.2%) relative to either controls at the same site (Initial: 1.60 ± 0.2 and Final: 1.56 ± 0.1%) or the pre-treatment transplanted algae (1.58 ± 0.2%). The N content of the final high-nutrient algal transplant at the low-nutrient reef was 1.90 ± 0.2%, which was similar to that in the initial transplant algae (1.97 ± 0.1%), as well as the final control for the high-nutrient algae (1.92 ± 0.1%) (*Fig. 2.2c*). For *Padina*, tissue N content was overall lower than in *Dictyota* regardless of site, as initial average %N in both transplant and control algae at the high-nutrient reef were 0.89 ± 0.2 and 0.84 ± 0.2%, respectively (*Fig. 2.2b*). Site was once again the only effect on %N ($F_{1,16} = 25.0$, $p = 0.0001$), with similar concentrations between final transplanted and control algae from the low-nutrient reef (0.54 ± 0.09 and 0.55 ± 0.1%, respectively). Due to the loss of material of *Dictyota* during the RTE, regardless of treatment or site, wet weight could not be calculated. Although *Padina* was less easily fragmented and differences in wet weight could therefore be calculated, no significant effect was found after transplantation, even between sites.

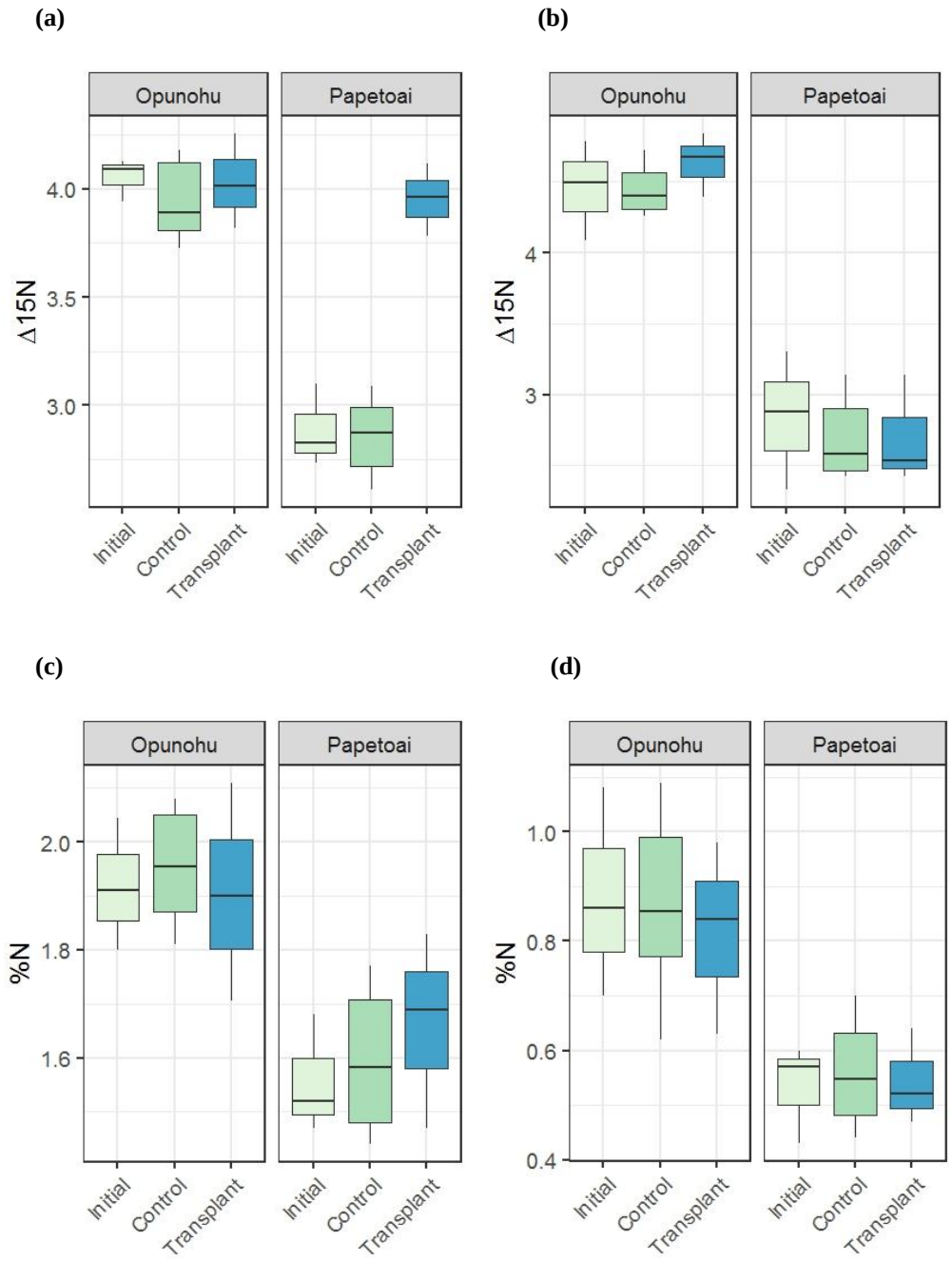

Figure 2.2. Box (median and 50% quantile) and whisker (95% quantile) plots of the stable isotopic and elemental signatures in tissues of *Dictyota* and *Padina* during a reciprocal transplant experiment between a high-nutrient site (Opunohu) and a low-nutrient site (Papetoai) in Opunohu Bay, Mo'orea, French Polynesia. $\delta^{15}N$ signatures are shown in (a) for *Dictyota*, and (b) for *Padina*, and %N is shown in (c) for *Dictyota*, and (d) for *Padina.* Light green boxplots represent a pooled average of initial samples taken immediately after sample collection (from both control and transplant algae), dark green plots represent final samples under control conditions taken after five days transplanted in the corresponding reefs. "Transplant" algae are the subset of specimens which were transplanted on the opposite reef, while "Control" are the subset that were transplanted back on the reef of origin. Note the differences in scale for the Y-axis for the %N plots.

2.4.3 Benthic Cover

The key ecological attributes that have been used in previous studies on the effects of nutrient runoff on coral reefs varied between the two selected sites (*Fig.2.3*). The high-nutrient reef is characterised by macroalgae, turf algae, CCA and Sponge, whereas the low-nutrient reef is characterised by coral cover, a high coral: macroalgal ratio, and sand, and overall, site had a significant effect on benthic cover ($p = 0.02$). On the high-nutrient reef, coral percent cover was 0.82 ± 0.5% (Mean ± S.D.), relative to 4.01 ± 2.2 % on the low-nutrient site ($p = 0.80$). Macroalgal cover, in contrast, was significantly different between the two sites, at 75.2 ± 2.9 % on the high-nutrient reef and 15.4 ± 6.3 % on the low-nutrient reef ($p < 0.0001$), and this was also the case for turf algae, where cover was 45.0 ± 7.9 % and 3.88 ± 1.6 % on high- and low-nutrient reefs, respectively ($p < 0.0001$). For *Dictyota*, percent cover was similar between reefs (8.62 ± 2.3 % and 9.79 ± 4.8 % for the high- and low-nutrient reef, respectively; $p = 0.06$), as was *Padina* percent cover (4.51 ± 2.3 % and 0.21 ± 0.2 %, respectively; $p = 0.81$). Coral:Macroalgae ratio was also similar on both reefs (0.01 ± 0.007% and 0.30 ± 0.2 % ; $p = 0.78$).

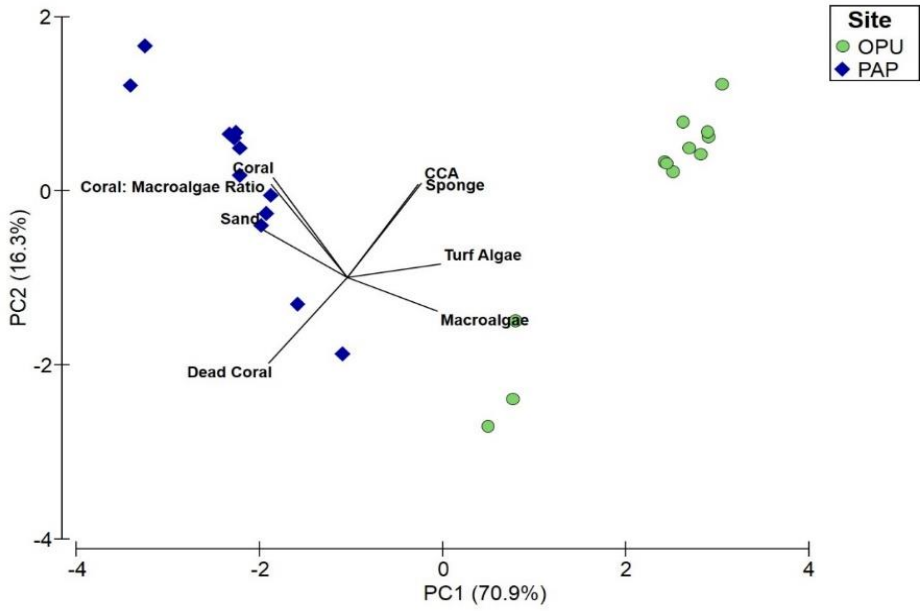

Figure 2.3. Principal Component Analyses for percent cover of the main benthic groups at the two transplant sites OPU (high-nutrient reef) and PAP (low-nutrient reef). CCA = Crustose Coralline Algae.

2.5 Discussion

This study used two brown macroalgal species with similar functional forms to demonstrate how factors such as morphology and internal nutrient history can impact the effectiveness of macroalgal bioindicators for both passive and active biomonitoring methodologies. The fleshy opportunistic macroalgae, *Dictyota*, was not only able to demonstrate a nutrient gradient along Opunohu Bay, but the specimens from the low-nutrient reef were highly responsive to changes in nutrient regimes, as they showed an increase in $\delta^{15}N$ when transplanted on a high-nutrient reef. As this same pattern was not shown when *Dictyota* from the high-nutrient site was transplanted on the low-nutrient site, it suggests that even opportunistic species might not be as responsive to nutrient enrichment if they already have excess nutrients stored in their tissues. Conversely, *Padina*, the slower-growing calcified alga, showed higher variability in nutrient signatures in the native specimens along the nutrient gradient than *Dictyota*, as well as two distributional gaps, and in the RTE, they showed no change in $\delta^{15}N$ or %N after being transplanted on either reef. Although nutrient regimes are not the only driver of benthic community structure on coral reefs, this study shows that Opunohu Bay does have a strong nutrient gradient due to anthropogenic runoff, which is a likely contributor to the different patterns in communities at the two study sites.

These results show that fleshy, opportunistic macroalgae are the most suitable type of macroalgae for assessing responses to nutrient regimes, as they assimilate the nutrients and use it directly for growth. This is also why they tend to proliferate faster on reefs (Littler et al., 2006a). *Dictyota* and *Padina* are two of the most common macroalgal genera on the fringing reef in Moorea and both are categorised as relatively simple functional forms, so functional-form models imply that they should respond similarly to nutrients (Littler & Littler, 1980). In theory, the effects of nutrient enrichment may vary based on species'

competitive abilities but be predictable between ecosystems as a function of productivity (Martínez et al., 2012; Fong & Fong, 2014, 2017). *Dictyota* does, however, have a different ecological strategy to *Padina*, in that it is a fast-growing opportunist, whereas *Padina* is a calcifying species that is likely to be slower-growing (Clausing & Fong, 2016).

This study also showed how macroalgae can record both rapid and non-steady pulses of nutrients, which helps to overcome the issues of capturing spatio-temporal variability of nutrients on coral reefs by relying on spot measurements of seawater samples alone (Costanzo et al., 2001; Fabricius et al., 2012). Tissue signatures of $\delta^{15}N$ were more sensitive to changes in nutrient regimes than %N. Shrimp farm effluent has been found in previous studies to range between 4.2-6‰ (Lin & Fong, 2008), and the $\delta^{15}N$ signatures from algae towards the land-end of Opunohu Bay in the current study fit within this range and gradually decrease towards the head of the bay, especially in *Dictyota* tissues (*Fig. 2.1a*). This supports the findings of Lin & Fong (2008), which suggests the main source of anthropogenic runoff in the bay originates from the shrimp farm rather than from agricultural fertilisers (0‰) or sewage discharges (~10‰). It also demonstrates that the values of $\delta^{15}N$ in both *Dictyota* and *Padina* decreased over the nutrient gradient due to increased mixing of oceanic sources of nutrients (less than 3‰), especially at the low-nutrient reef.

Percent N was a weak indicator for these short-term changes in nutrient regimes in both the passive and active methods for both algal species. Macroalgae may dilute tissue N content during rapid growth and/ or storing excess bioavailable nutrients in tissues instead of using them for growth (Fong et al., 2003), and this was also shown in Lin & Fong (2008). Finally, while the passive method showed that distance from the river mouth had a greater effect on $\delta^{15}N$ than algal type (Umezawa et al., 2002), the differences between *Dictyota* and *Padina*

were more distinct in the reciprocal transplant experiment in the current study, even within sites, which suggested that out of the two species from the low-nutrient reef, the significant changes in $\delta^{15}N$ signatures in *Dictyota* demonstrated a stronger response to a new nutrient regime.

Individual algal species have different capacities for uptake, storage and growth in response to nutrient enrichment, as well as differences in growth and competitive ability to different subsidy regimes (Fong & Fong, 2017), and this can also be affected by their local environmental conditions. These differing capabilities for nutrient uptake and storage dictate the influence of nutrient history and thus drive nutrient responses and therefore may allow species with differing ecological strategies to coexist in a fluctuating environment (Clausing & Fong, 2016). Ideally, in an environmental monitoring program, you can return specimens to a laboratory and allow depletion of internal nutrient stores before deploying them in desirable monitoring locations (Dailer et al., 2010). However, this not always possible, for instance, when research ship cruises are based around remote island systems where sites can only be visited once or else once every few years (**Chapter 1**; Littler et al., 1992). In this case, this study suggests that selecting opportunistic macroalgae from reefs with known low nutrient loads (i.e. either from seawater measurements or previously-used bioindicators) could help alleviate this issue, as they are more likely to be nutrient limited and therefore more responsive to any new influxes (Fong et al., 2003). On nutrient-limited reefs, functionally-similar species respond variably to different enrichment regimes (i.e. to pulse versus press nutrient subsidy regimes, especially with varying rainfall patterns between seasons; Clausing & Fong, 2016; Fong & Fong, 2017) and thus causes differences in macroalgal community structure and diversity. Functional form model predictions may not hold as they don't take into account nutrient subsidy regime when predicting responses to

increasing nutrients (Littler & Littler, 1980; Steneck & Dethier, 1994; Fong & Fong, 2017), so this study suggests that the same could be said for algae from the same functional group with different nutrient histories.

The most common macroalgae used worldwide for active biomonitoring between 1978 and 2017 were reviewed in García-Seoane et al. (2018a), and those most commonly selected for passive biomonitoring were reviewed in García-Seoane et al. (2018b). Therefore, as many of the same genera have been used for the two different biomonitoring techniques across multiple geographic regions, the current study could be expanded to test the effectiveness of the most commonly used genera for both methodologies, specifically those with the same functional trait(s) (i.e. rapid nutrient uptake mechanisms) nested within broader functional groups (Nyström, 2006; McWilliam et al., 2018; Bellwood et al., 2019; Fulton et al., 2019). This could improve understanding on how the ecological strategies and internal nutrient history of a wider range of common bioindicators influence their responses to nutrient enrichment (Sangil & Guzman, 2016; Zubia et al., 2018). For instance, by using this traits-based approach, monitoring programs could build a suite of bioindicators functionally-similar species to obtain more ecologically-relevant information about the impacts of nutrients on a larger spatial scale (Savage et al., 2007; Mouillot et al., 2011; Hevia et al., 2016; McWilliam et al., 2018), particularly as it is less likely to weakened by distributional gaps of one species (**Chapter 1**; Linton & Warner, 2003).

Measuring the relationships of a functionally-specific suite of bioindicators against a diverse range of structural bioindicators (e.g. coral cover, macroalgal cover, macroalgal species composition, opportunistic: perennial macroalgae ratio, and herbivory intensity), in combination with other physical measurements of water quality (e.g. seawater sampling and

in situ sensors), could provide programs with an ecosystem-level response to nutrient impacts, as it has been achieved for other stressors such as overfishing (McClanahan et al., 2011, 2012; Chong-Seng et al., 2012; Hevia et al., 2016; Darling et al., 2017). This could be an important integrative approach in future studies, as it might capture biophysical relationships between changes in nutrient enrichment and ecological processes that are missed by studies that measure the relationships between the same types of structural bioindicators and only one or two water quality measurements in seawater (Fabricius et al., 2005; De'ath & Fabricius, 2010; Fabricius et al., 2012; Devlin et al., 2019, 2020).

While there are a range of widely used, highly responsive macroalgae that are almost ubiquitous on coral reefs, some genera might be more suitable than others for *in situ* experiments if functional responses to nutrient inputs such as growth rate need to be investigated (Lin & Fong, 2008). For instance, in the current study, *Dictyota* specimens were more vulnerable than those of *Padina* to mechanical damage and as a result, fragmented more easily during the transplant experiment, even in the cages, which could have been the result of mild wave action at the sites (Vaughan, *pers. obs.*). However, both have been used successfully in a controlled laboratory or mesocosm setting in previous studies, so that growth rate, or even other physiological measurements such as photosynthetic efficiency, photosynthetic pigment content (e.g. chlorophyll-a) and protein content, can still be measured to gain a more in-depth understanding of responses to nutrients (Downing et al., 1999; Umezawa et al., 2002; Littler et al., 2006b; Teichberg et al., 2010, 2013; Fong & Fong, 2014; Fong, 2015). Therefore, other ecological strategies in addition to nutrient uptake mechanisms could be considered during a bioindicator selection process, especially for active biomonitoring, as some opportunistic species may be as durable as others for measurements such as growth rate. Furthermore, a selected species could be more limited by light

availability than nutrients in a laboratory experiment (Beach et al., 2006), or else may be less vulnerable to additional or synergistic impacts from herbivory when transplanted in the field (Bergman et al., 2016; Clausing et al., 2016; Donovan et al., 2020).

Previous research showed that increased nutrient loads can directly exacerbate the effects of coral bleaching for scleractinian corals (D'Angelo & Wiedenmann, 2014; Burkepile et al., 2019; Donovan et al., 2020), but it is most likely going to have the strongest effect on reefs indirectly through the proliferation of opportunistic fleshy species of macroalgae (Littler et al., 2006a). Furthermore, it has been shown to be one of five strong predictors for whether a degraded reef has the capacity to recover or shift to a macroalgal-dominated state after a mass disturbance like bleaching (Hughes et al., 2007; Graham et al., 2015; MacNeil et al., 2019), so the ability to detect and trace any sources of excess nutrient loads is important for environmental managers hoping to reduce local pressures. However, relationships between parameters of water quality and key ecological attributes of reef status, such as percent cover and species richness of dominant groups like coral and macroalgae, are difficult to quantify, as these are often analysed against averaged levels of nutrients taken directly from the water column, which do not always capture spatio-temporal variability over large spatial scales (De'ath & Fabricius, 2010; Fabricius et al., 2005, 2012). Therefore, modelling tissue $\delta^{15}N$ from opportunistic, nutrient-limited bioindicators against such attributes may demonstrate more biologically-relevant biophysical relationships that may help environmental managers determine the origins and concentrations of any anthropogenic nutrient enrichment that might be detrimental to reef ecosystem health.

2.6 Conclusion

In conclusion, this study shows that while there are advantages and disadvantages to both passive and active biomonitoring, it is important to consider the ecological strategies of the macroalgae chosen as a bioindicator regardless of the methodology applied, as well as the local environment from which specimens are taken. Although species can be depleted of any internal nutrients in their tissues, thus making them nutrient limited, specimens of the same species could still have adapted specifically to their local environment. Therefore, further investigations into their biological mechanisms of nutrient uptake, assimilation and storage should be conducted prior to including them in any large-scale monitoring programs. This is an important first step that is often not considered in biomonitoring studies, which is why testing the selected bioindicator in not only their native environment but in other areas via active transplantation may help determine how accurately they are reflecting their local nutrient regimes, and thus how accurately they can capture any new influxes from anthropogenic sources. Once researchers and environmental managers are confident that the nutrient signatures in macroalgae accurately reflect those of the surrounding water column, they can apply this technique over large spatial scales by transplanting their chosen bioindicator(s), taken from a nutrient-limited site, to assess the spatial extent of any anthropogenic runoff. They can even extend this work even further by using macroalgal $\delta^{15}N$ signatures to determine biophysical relationships with key ecological attributes of reef states. Although nutrients are not the only driver of benthic community structure on reefs, they have still been found to be a key predictor in determining whether a disturbed reef will recover from a disturbance or go through a regime shift to a macroalgal-dominated state. The methods outlined in the current study would therefore tie together several aspects of bioindicator research and provides a more cost-effective and biologically-relevant indication of the ecological effects of nutrients on coral reefs.

3. THE EFFECTS OF FINE-SCALE SPATIO-TEMPORAL VARIABILITY OF NUTRIENT ENRICHMENT ON TWO MORPHOLOGICALLY-SIMILAR CORAL REEF MACROALGAE

3.1 Abstract

The rate of episodic pulse events of coastal runoff is increasing on coral reefs due to rises in the frequency and magnitude of storms and heavy rainfall events. These pulse events can favour opportunistic macroalgae over other, slower-growing species which typically prefer smaller, more frequent press nutrient subsidies. However, it is not known what the responses of morphologically-similar macroalgae with different ecological strategies will be to temporal differences in nutrient supply when they already have differences in internal nutrient history. In this study, the interacting effects of nutrient history and nutrient subsidy type on $\delta^{15}N$, %N, and wet weight of two common coral reef macroalgae, the opportunistic *Dictyota bartayresiana* and the slower-growing *Padina boryana*, were investigated through a multi-factorial laboratory experiment in Mo'orea, French Polynesia. Specimens from one low-nutrient reef (PAP) and one high-nutrient reef (OPU) were tested against the same volume of nutrients, delivered in different quantities over three days. Nutrients were delivered as either one large initial dose of artificial stock solution of $NaNO_3$ and KH_2PO_4 in 'Pulse' treatments (10:1 N:P Ratio), as six separate doses of lower concentration in 'Press' treatments (1.67:0.17 N:P Ratio), or no doses in the ambient seawater in the 'Control' treatments. Overall, only *Dictyota* from PAP showed any significant change in $\delta^{15}N$ in the Pulse treatment, from 2.80 ± 0.2 to 1.85 ± 0.2 ‰ in $\delta^{15}N$ ($p = 0.0002$). However, $\delta^{15}N$ in PAP-*Dictyota* specimens in the Control treatment also decreased from 2.74 ± 0.3 to 1.99 ± 0.3 ‰ ($p = 0.006$), which could have been due to additional isotopic signatures of nutrient sources from the ambient seawater.

Overall, this study highlights the difficulty of capturing both fine-scale spatial and temporal variability of nutrients, particularly in manipulative laboratory experiments, but emphasises the need for further research, including a more comprehensive assessment of nutrient regimes using other types of measurements, on the impacts of increasing nutrient pulses on reef community structure and diversity.

In prep. (28/08/2021):

Vaughan, E.J., Valeriano Parravicini, V., and Graham, N.A.J. The effects of fine-scale spatio-temporal variability of nutrient enrichment on two morphologically-similar coral reef macroalgae. *Marine Ecology Progress Series*

3.2 Introduction

Although tropical coral reefs are typically found in oligotrophic waters, they are also highly dynamic and fluctuating environments, which subsequently helps drive benthic community structure (Gove et al., 2015; Aronson & Precht, 2016; Donovan et al., 2020). Macroalgae, for instance, have been found to co-exist in diverse communities on reefs with high variations in the frequency and magnitude of resource supply, such as nutrients of natural and/ or anthropogenic origin (Anderson et al., 2008; Yang et al., 2008; Clausing & Fong, 2016; Fong & Fong, 2017; Fong et al, 2020). It is the variation in the frequency and concentration of these nutrient supplies which allows species with different ecological strategies to acquire sufficient nutrients for growth and proliferation without directly competing with each other (Tanner et al., 1994; Fong & Paul, 2011; Fong & Fong, 2017). This has even been shown among macroalgae from the same functional group and the same reefs (Littler & Littler, 1980; Steneck & Dethier, 1994; Fong et al., 2001; Fong & Fong, 2014, 2017; Gennaro et al.,

2019). Further, the same species from areas with different local nutrient regimes can have a range of responses to the same supply of nutrients, as their internal nutrient history can also influence nutrient limitation (**Chapter 3;** Fong et al., 2003). This already dynamic, diverse ecosystem can be further impacted by nutrient inputs from external sources, both natural and anthropogenic, which are not supplied in regular concentrations or intervals (Briand et al., 2015).

Episodic upwelling, for example, can provide natural pulsed nutrient supply (Leichter et al., 2003), while anthropogenically-derived nutrient pulses are typically elevated during storms and heavy rainfall events (Clausing & Fong, 2016; Fong et al., 2020). Heavy rainfall events are typically episodic and can drive pulses of anthropogenic nutrients from the coastline or from rivers into the coastal marine environment, and thus over shallow-water coral reefs (Devlin & Brodie, 2005; Brodie et al., 2012b; Devlin et al., 2012; Anthony et al., 2014; Fong et al., 2020). The frequency of nutrient inputs on reefs has been described as a continuum between press subsidies at one extreme and pulse events at the other (Fong & Fong, 2017). Pulse subsidies, for the purpose of this study, are defined as a single, episodic event with high nutrient loads, and press subsidies are defined as a steady supply of nutrient loads of much lower concentrations (Anderson et al., 2008; Yang et al., 2008; Fong & Fong, 2017). These events can occur over a range of timescales, from hours, to days, to weeks (Clausing & Fong, 2016), but there is growing evidence that the frequency and severity of storms and rainfall events are increasing due to climate change (Anthony et al., 2014; Edmunds & Gray, 2014; Hernández-Delgado et al., 2014; Fong et al., 2020). This is concerning for coral reefs already exposed to high nutrient loads, as rainfall-driven nutrient pulse events tend to favour non-native (Lapointe & Bedford, 2011) and opportunistic macroalgae (Fong & Paul, 2011). These species have the capacity to rapidly take up these additional resources and use it for growth,

which can have longer term impacts on community composition and diversity (Littler et al., 2006a). Therefore, it is critical to understand how temporal variation in nutrient availability affects some of the most common macroalgae on coral reefs.

Regular collections of seawater to monitor water quality can provide a lot of information about nutrient concentrations and sources, but relying on this method alone can often miss pulse events due to the coarse resolution of sampling intervals (Costanzo et al., 2001; Fabricius et al., 2012; Clausing & Fong, 2016). To overcome these limitations, autonomous *in situ* logging of nutrients through chemical and optical sensors offer promising potential for monitoring programs, as they have high temporal resolution, and more instruments are becoming commercially available and more affordable with continuing developments in technology over time (Daniel et al., 2020; Devlin et al., 2020; Marcelli et al., 2021; Nehir et al., 2021; Wei et al., 2021). However, for some monitoring programs in small island developing nations (SIDS) with limited funds, most current nutrient sensors might still be too expensive for large-scale monitoring, and also may require regular calibrations with wet chemical analyses from seawater samples, which is both time-intensive and costly (Daniel et al., 2020). Therefore, a more cost-effective alternative in such circumstances is to use reef-associated macroalgae as bioindicators, as they are able to take up nutrients with a quick turnover rate and accumulate them in their tissues, which can quickly be converted into growth (Costanzo et al., 2001; Gorman et al., 2017).

When paired with the measurement of stable isotopic and elemental signatures in their tissues (e.g. $\delta^{15}N$ and %N), macroalgae provide an excellent, cost-effective proxy for both the spatial and temporal variability of nutrients (**Chapter 1 & 3**; Lyngby et al. 1999; Costanzo et al., 2000, 2001; Gartner et al., 2002; Lin & Fong, 2008; Garcia-Seoane et al., 2018a&b; Valiela

et al., 2018; Adam et al., 2021). However, if scientists and environmental managers want to investigate the effectiveness of macroalgal bioindicators for detecting finer-scale variability in frequency and concentration of nutrient subsidies, particularly to episodic pulses (Fong & Fong, 2014, 2017), they require more information than only the cumulative values of $\delta^{15}N$ and %N in macroalgal tissues averaged over a longer period of time. This may therefore require more detailed empirical studies, such as manipulative nutrient enrichment experiments.

Nutrient manipulation experiments are a very common methodology for testing the responses of aquatic organisms, particularly macroalgae and phytoplankton, to nutrients (Downing et al., 1999; Littler et al., 2006b; Fong & Paul, 2011), and can either be conducted in the field (Littler et al., 2006b; Fong et al., 2018), or in the laboratory (Fong et al., 1993, 1994, 2001, 2003). Many nutrient manipulation experiments vary in length, ranging from days to weeks in the field (Yang et al., 2008; García-Seoane et al., 2018a) and minutes, to hours, to days, to weeks in the laboratory (Downing et al., 1999; Yang et al., 2008; Fong & Fong, 2014; Clausing & Fong, 2016; den Haan et al., 2016; Fong & Fong, 2017). As a result, there is huge variation in the responses of macroalgae across different studies, even those conducted in the same areas (Delgado & Lapointe, 1994; Larned, 1998; Schaffelke & Klump, 1998; Schaffelke, 1999; Stimson & Larned, 2000; Thacker et al., 2001; Szmant, 2002; Umezawa et al., 2002; Fong & Paul, 2011; Clausing & Fong, 2016; Fong & Fong, 2017; García-Seoane et al., 2018). The capacity for internal nutrient storage in even morphologically-similar macroalgae can also affect their nutrient limitation, and thus their capacity to show statistically significant responses (**Chapter 2**; Bell et al., 2002; Fong et al., 2003; Raimonet et al., 2013). For instance, macroalgae from eutrophic areas are not nutrient-limited because they can use these reserves for growth even in periods of low external nutrient subsidies, and

therefore do not show responses to any additional input of nutrients (Fong et al., 2001; Viana & Bode, 2013). Clearly, macroalgal responses to nutrient enrichment, both in growth and nutrient signatures, may vary depending on local context, environmental conditions, and ecological strategies (Raimonet et al., 2013; Viana et al., 2013; Fong & Fong, 2014; Ochoa-Izaguirre & Soto-Jimenez, 2015).

This study builds on the work conducted in Clausing & Fong (2016), Fong & Fong (2017) and **Chapter 3** by using *Dictyota bartayresiana*, a fast-growing, fleshy brown macroalgae, and *Padina boryana*, a slower-growing, lightly calcified brown macroalgae from both a low-nutrient reef and a high-nutrient reef in Moorea, French Polynesia. The macroalgal species' responses to Press and Pulse nutrient input treatments, over a 3-day manipulative laboratory experiment, through a) changes in $\delta^{15}N$, b) changes in %N, and c) wet weight. Based on previous literature and the results from **Chapter 3**, *Dictyota* from the low-nutrient reef was expected to be the most responsive species to the pulsed nutrient subsidy and *Padina* to the press nutrient subsidy.

3.3 Methods

3.3.1 Study Species & Sites

Specimens of *Dictyota bartayresiana* (hereafter called *Dictyota*) and *Padina boryana* (hereafter called *Padina*) were collected from two sites with contrasting nutrient regimes for the multifactorial laboratory experiment in the north of Moorea, French Polynesia on 3rd August 2018 (*Suppl. Fig. 2.1*). *Dictyota* and *Padina* are both dominant brown macroalgae in

the family Dictyotaceae, found not only year round across reefs in Mo'orea, but also on other coral reefs across the globe (Littler & Littler, 1980; Payri, 1987; Delgado & Lapointe, 1994; Umezawa et al., 2002, 2008; Beach et al., 2006; Fong & Paul, 2011; Fong & Fong, 2014; Poray & Carpenter, 2014; Barrow et al., 2015; Clausing & Fong, 2016; Fong & Fong, 2017). However, *Padina* is a lightly calcified, foliose brown species that is grows more slowly than the fleshy opportunistic genus *Dictyota*, with limited storage capacities but lower nutrient requirements which allow them to tolerate low nutrient conditions (Delgado & Lapointe, 1994; Umezawa et al., 2002; Fong & Paul, 2011; Fong & Fong, 2014; Clausing & Fong, 2016). *Dictyota*, in contrast, can not only respond quickly to nutrient enrichment, particularly nutrient pulses, but can store nutrients within their tissues more easily and allow them to maintain positive growth even when nutrient concentrations are lower between pulse events (Littler & Littler, 1980; Fong & Paul, 2011; Clausing & Fong, 2016; Fong & Fong, 2014, 2017).

The high-nutrient site was halfway along the right side of Opunohu Bay (hereafter called OPU; 17°29'19.296" S, 149°52'33.92"W), a ~3.5 km long bay which has been found in previous studies to have high nutrient levels due to anthropogenic runoff (Adam et al., 2021), particularly from a 2 ha intensive shrimp farm and a pineapple farm at the bottom of the bay (Adjeroud, 1997; Lin & Fong, 2008). In contrast, the backreef lagoon "Papetoai" on the northwest coast of Mo'orea was selected as the low-nutrient site (hereafter called PAP; 17°29'58.452" S, 149°51'16.343" W), as previous studies have shown this area to have significantly lower nutrient loads than those in Opunohu Bay (**Chapter 2**; *Suppl. Fig. 2.1d&e*; Leichter et al., 2013; Donovan et al., 2020; Adam et al.; 2021).

3.3.2 Specimen Collection & Preparation

A total of twelve specimens of each species were collected from each of the two sites (OPU and PAP) on 3rd August 2018. Samples were immediately returned to CRIOBE Research Station to clean them of all visible epiphytes, epifauna, and sediments, and spun for 1 min at the same rate of spinning in a salad spinner to standardise removal of water, a common method when measuring wet weight (Fong & Fong, 2017). After initial subsamples were taken to be frozen for stable isotopic analyses, they were wet-weighed, then labelled for the different treatments (n = 4 per treatment, per species, per site). Each specimen of *Dictyota* weighed ~5 g, and each specimen of Padina weighed ~6 g, as the calcification in the latter required more biomass to approximate equal volumes across species. Each specimen used in the laboratory experiment included apical tips, which is where the greatest rate of growth in macroalgae occurs (Clausing & Fong, 2016; Garcia-Seoane et al., 2018a&b).

3.3.3 Press-Pulse Laboratory Experiment

In a three-factor fully crossed laboratory experiment, specimens were exposed to different nutrient subsidy treatments (Control, Press, and Pulse) for three days from the day of collection. After initial samples were collected and wet weight was measured, each individual alga was incubated in 1L glass jars that were acid-washed (10% HCl) prior to usage and filled with ambient seawater collected from the forereef off the northern coast of Mo'orea. A total of 48 jars were placed in a randomised array across three connected water tables with a flow-through system to keep the water level and temperature constant in an indoor wet laboratory at CRIOBE. Each jar was constantly aerated with aquarium aeration pumps (Silbiger et al., 2018). The water in the jars was changed every twelve hours (six times in total over the three days), and the position of the jars was also rearranged at each change. Water collected from the forereef was used in the jars instead of the water being pumped into the laboratory from

the bottom of Opunohu Bay, because the former has been found to have high nutrient loads, which would confound the experiment (Lin & Fong, 2008). In addition, previous studies have shown tropical oceanic water to have low nutrient signatures (< 3‰, Costanzo et al., 2001; Lin & Fong, 2008; Adam et al., 2021). The water baths were kept at a constant temperature and under a constant light intensity under lamps that mimicked typical daylight hours, using HOBO Pendant Temperature/ Light loggers (Onset Computer Corp., Borne, MA) (Long et al., 2012; Silbiger et al., 2018). Water temperature (27.2 ± 0.3°C; Mean ± S.D.) and pH (8.0 ± 0.1) were measured daily with a multisensory probe.

The nutrient subsidy regimes were based on a similar study in Fong & Fong (2017), and for the Pulse and Press treatments, the collected seawater was enriched with a ratio of 10:1 $NaNO_3$:KH_2PO_4 artificial stock solutions (hereafter called 10:1 N:P Patio) in a 20 L pre-cleaned carboy and well mixed so that the enriched seawater was distributed evenly among 1L jars. Previous studies have used this ratio as an approximation of nutrient concentrations in areas of Mo'orea with high nutrient loads (Schaffelke, 1998; Fong & Fong, 2014, 2017) and others have used this method of seawater enrichment for similar manipulative experiments (den Haan et al., 2016; Silbiger et al., 2018). Therefore, at the beginning of the Pulse treatment, the seawater in each 1L jar for the Pulse treatment was enriched with a concentration 0.86 mg/L $NaNO_3$ and 0.14 mg/L of KH_2PO_4. For all subsequent five water changes that occurred every 12 h, all seawater was removed from each jar before immediately being replaced by ambient, non-enriched seawater,. This treatment aimed to represent the short burst of high nutrient concentrations from an episodic pulse event, followed by periods of low external nutrient supply, with the assumption that opportunistic, nutrient-limited species like macroalgae will rapidly take up these nutrients into their tissues, thereby removing them from the water column. Opportunistic species are therefore predicted

to have a sufficient enough internal store to maintain positive growth until the next pulse event (Clausing & Fong, 2016; Fong et al., 2018). In contrast, algae for the Press treatment received an N:P ratio of 1.67: 0.17 for each of the six water changes (0.14 mg/L of $NaNO_3$ and 0.02 mg/L KH_2PO_4, per water change), as this was a sixth of the concentration used for the Pulse treatment to expose algae to a more frequent supply of nutrients of lower concentration (Fong & Fong, 2017). The seawater in the jars for the Control Treatment also underwent a full change every twelve hours. At the end of the three-day experiment, each individual specimen was collected and wet weighed for final samples as described above, after which samples were also collected and frozen for final stable isotopic analyses.

3.3.4 Stable Isotopic Analyses

All frozen samples were defrosted, rinsed thoroughly with fresh or distilled water to remove any visible epiphytes or epifauna, and were placed in a drying oven for 48 h at 60°C at CRIOBE. Once dried, samples were returned to Lancaster Environment Centre, and were ground into a fine powder using a ball mill and stored in individual airtight containers. 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 ($\delta^{15}N$) and elemental analyses (%N) for 2017 were run on an Isoprime100 Isotope Ratio Mass Spectrometer (IRMS) linked to an Elementar VARIO MICROcube Elemental Analyser at Lancaster Environment Centre, Lancaster University. Analyses from both years were standardised using internal reference materials calibrated to international standards. Within-run replication (1 σ) was <0.3 ‰ for $\delta^{15}N$ and <0.1 ‰ for $\delta^{13}C$ for both standards and samples.

3.4.5 Statistical Analyses

A three-factor ANOVA was conducted six times to compare change in a) $\delta^{15}N$, b) %N and c) wet weight (biomass), both in *Dictyota* and in *Padina*, to determine whether there were significant differences in the mean of each of the three response parameters due to nutrient subsidy regime, site and tissue type. The three factors were site (2 levels: OPU and PAP), treatment (3 levels: Control, Press, and Pulse), and tissue time-point (2 levels: Initial and Final). Normality of data was assessed visually using histograms, and homogeneity of variance for the ANOVAs was assessed with a Levene's test. All statistical analyses were conducted in R (R-Core-Team 2018).

3.4 Results

3.4.1 Changes in $\Delta^{15}N$

There was a significant effect of site, tissue type and interaction between site and time-point on $\delta^{15}N$ in *Dictyota* (interaction: $F_{2,36} = 32.8$, $p < 0.0001$; *Fig. 3.1a*). The post-hoc Tukey test revealed that there was a significant difference between initial tissue samples of *Dictyota* from PAP and those from OPU ($p < 0.0001$). Although the ANOVA showed only marginal significance for treatment ($F_{2,36} = 3.19$, $p = 0.053$), there was a slight decline in signatures of $\delta^{15}N$ between initial and final Pulse samples from PAP from 2.80 ± 0.2 to 1. ± 0.2 ‰ ($p = 0.0002$; Mean ± S.D.). $\delta^{15}N$ in final PAP-Press samples also declined from 2.79 ± 0.2 to 2.22 ± 0.1 ‰ but was not significant ($p = 0.08$). However, final control samples were similar to both final Pulse and Press samples ($p = 0.99$ and $p=0.97$, respectively), as $\delta^{15}N$ in the final

control samples dropped significantly from 2.74 ± 0.3 to 1.99 ± 0.3 ‰ (p = 0.006). Final OPU control samples were similar to initial OPU-Control (p = 0.99), final OPU-Press (p = 0.99) and final OPU-Pulse (p = 0.99).

For *Padina*, only site had a significant effect on $\delta^{15}N$ ($F_{1,36} = 229.0$, $p < 0.0001$; *Fig. 3.1b*). There was a significant difference between initial samples from PAP and OPU, for instance, $\delta^{15}N$ signatures were 2.19 ± 0.4 and 4.22 ± 0.7 ‰ in initial PAP-Control and initial OPU-Control samples, respectively ($p < 0.0001$). However, $\delta^{15}N$ in PAP-Control samples were not significantly different after the experiment (Final: 2.09 ± 0.4 ‰, p = 0.99), and were similar to final PAP-Press (1.94 ± 0.4 ‰; p = 0.99) and PAP-Pulse (2.26 ± 0.7 ‰; p = 0.99) values. Final values of OPU-Control were also similar to the initial samples (Final: 4.13 ± 0.3 ‰, p = 0.99), as were the final OPU-Press (4.38 ± 0.3 ‰; p =0.97) and OPU-Pulse (4.16 ± 0.2 ‰, p=98).

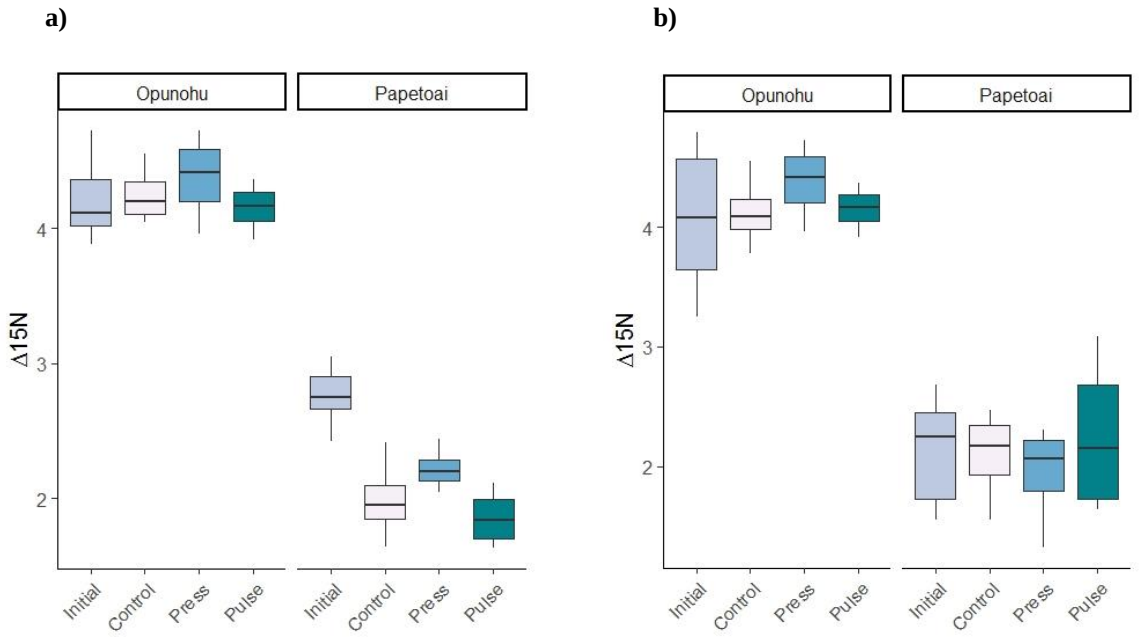

Figure 3.1. Box (median and 50% quantile) and whisker (95% quantile) plots of the changes in the nitrogen stable isotopic signatures ($\delta^{15}N$) in tissues of a) *Dictyota* and b) *Padina* during a manipulative laboratory experiment at CRIOBE, Mo'orea, French Polynesia. Specimens were collected from a high-nutrient site (OPU) and a low-nutrient site (PAP) (n = 12 per species, per site). Purple boxplots represent a pooled average of initial samples taken immediately after sample collection (n=12), light pink boxes represent final tissue samples after submersion in ambient "Control" seawater taken at the end of

the three-day experiment (n=4). Blue boxes represent the "Press" treatment, where ambient seawater was enriched with a 1.67:0.17 N:P Molar ratio (1.67 μM nitrogen (N) and 0.17 μM phosphorus (P) delivered over six water changes every 12 hours; n=4). Teal green boxes represent the "Pulse" treatment, where the same volume of nutrients was delivered at the first water change in a 10:1 N:P Ratio (n=4).

3.4.2 Changes in %N

The %N signature in *Dictyota* tissues differed significantly between site and the interaction between site and treatment (interaction: $F_{2,36} = 3.33$, $p = 0.05$; *Fig. 3.2a*). For example, initial PAP-Control and initial OPU-Control samples had values of 0.94 ± 0.1 and 1.28 ± 0.2 %, respectively ($p = 0.02$), however, final PAP-Control and final OPU-Control samples had similar values of 0.98 ± 0.2 and 1.20 ± 0.1 %, respectively ($p = 0.16$). Values of %N were also similar between final PAP-Control and final PAP-Press ($p=0.99$) and final PAP-Pulse ($p = 0.98$). Similar patterns were found at OPU between final control and Press ($p = 0.98$) and Pulse ($p = 0.99$) samples.

The %N signatures for *Padina* were overall lower than for *Dictyota*, but showed similar results with the ANOVA (*Fig. 3.2b*). However, only site had a significant effect on %N ($F_{1,36} = 46.0$, $p < 0.0001$). Initial and final PAP-Control signatures were similar (0.43 ± 0.04 and 0.46 ± 0.04%; $p = 0.98$), as were those for initial and final OPU-Control samples (0.53 ± 0.06 and 0.54 ± 0.1 %, respectively; $p = 0.99$). %N was also similar between treatments in the final samples for the algae from PAP (Press: 0.44 ± 0.02%, $p = 0.99$; Pulse: 0.45 ± 0.04%, $p =99$) and for OPU (Press: 0.54 ± 0.03%, $p = 0.99$; Pulse: 0.53 ± 0.5%, $p = 0.99$).

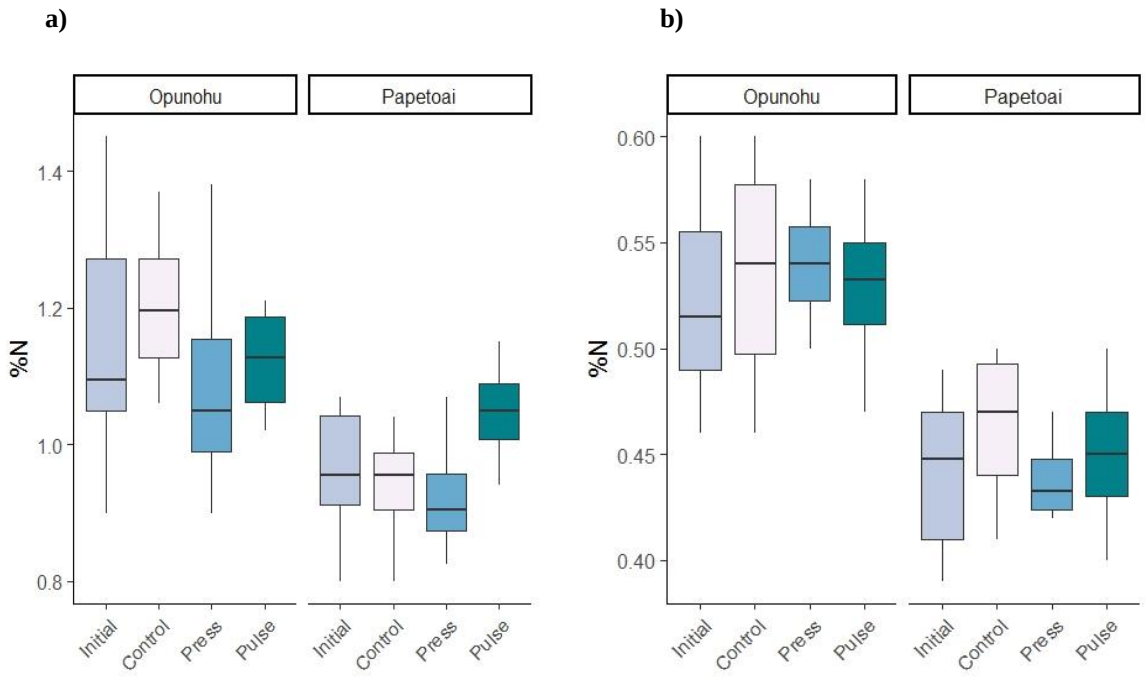

Figure 3.2. Box (median and 50% quantile) and whisker (95% quantile) plots of the changes in nitrogen content (%N) in tissues of a) *Dictyota* and b) *Padina* during a manipulative laboratory experiment at CRIOBE, Mo'orea, French Polynesia. Specimens were collected from a high-nutrient site (OPU) and a low-nutrient site (PAP) (n = 12 per species, per site). Purple boxplots represent a pooled average of initial samples taken immediately after sample collection (n=12), light pink boxes represent final tissue samples after submersion in ambient "Control" seawater taken at the end of the three-day experiment (n=4). Blue boxes represent the "Press" treatment, where ambient seawater was enriched with a 1.67:0.17 N:P Molar ratio (1.67 µM nitrogen (N) and 0.17 µM phosphorus (P) delivered over six water changes every 12 hours; n=4). Teal green boxes represent the "Pulse" treatment, where the same volume of nutrients was delivered at the first water change in a 10:1 N:P Ratio (n=4).

3.4.3 Changes in Wet Weight

Dictyota showed variable changes in wet weight, as there was a significant effect of site, tissue type and the interaction between site and tissue type (interaction: $F_{1,36} = 17.6$, $p = 0.0002$; *Fig.3.3a*). The average wet weight of the final samples from PAP were significantly higher for the Press and Pulse treatments than that of the initial samples. PAP-Press increased from 4.95 ± 0.04 to 5.61 ± 0.3 mg ($p = 0.001$), and PAP-Pulse increased from 4.99 ± 0.07 to 5.83 ± 0.4 mg ($p < 0.0001$). Although there was a slight increase in PAP-Control samples from 5.00 ± 0.01 to 5.31 ± 0.2 mg, it was not significant ($p = 0.47$). There was no significant difference between initial and final wet weight of OPU-Press ($p=0.98$) and OPU-Pulse ($p=0.97$).

Overall, there was no significant effect from any of the factors on the wet weight of *Padina* (*Fig. 3.3b*). The final wet weight of samples from PAP were similar to those of initial samples, regardless of treatment. The wet weights of PAP-Control were 6.29 ± 0.3 and 5.95 ± 0.7 mg for initial and final samples, respectively (p = 0.99). Similarly, the initial and final wet weights of PAP for the Press treatment were 6.08 ± 0.1 and 5.70 ± 0.6 mg, respectively (p = 0.99), and the initial and final wet weights for the Pulse treatment were 5.96 ± 0.1 and 5.83 ± 0.7 mg, respectively (p = 0.96). In addition, the wet weight of *Padina* samples from OPU did not differ between treatments or tissue type. For instance, initial and final OPU-Control wet weight were 5.71 ± 0.2 mg and 5.21 ± 0.3 mg, respectively (p=0.97), initial and final OPU-Press values were 6.05 ± 0.2 and 5.09 ± 0.6, respectively (p=0.99), and initial and final OPU-Pulse values were 5.99 ± 0.2 and 5.77 ± 0.5 mg, respectively (p=99).

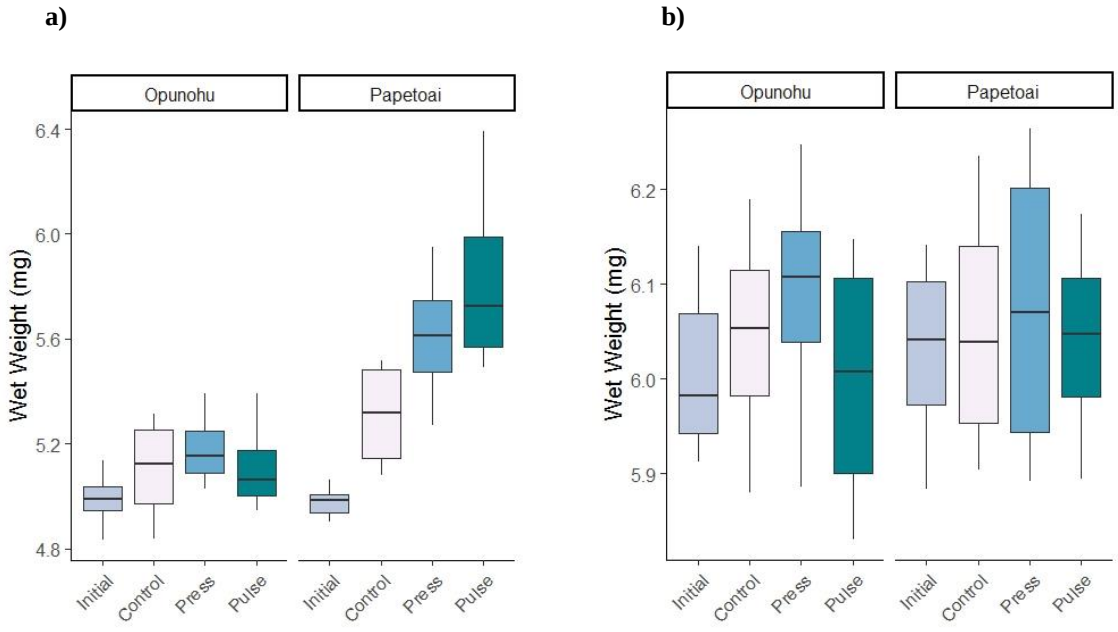

Figure 3.3. Box (median and 50% quantile) and whisker (95% quantile) plots of the changes in wet weight of a) *Dictyota* and b) *Padina* during a manipulative laboratory experiment at CRIOBE, Mo'orea, French Polynesia. Specimens were collected from a high-nutrient site (OPU) and a low-nutrient site (PAP) (n = 12 per species, per site). Purple boxplots represent a pooled average of initial samples taken immediately after sample collection (n=12), light pink boxes represent final tissue samples after submersion in ambient "Control" seawater taken at the end of the three-day experiment (n=4). Blue boxes represent the "Press" treatment, where ambient seawater was enriched with a 1.67:0.17 N:P Molar ratio (1.67 µM nitrogen (N) and 0.17 µM phosphorus (P) delivered over six water changes every 12 hours; n=4). Teal green boxes represent the "Pulse" treatment, where the same volume of nutrients was delivered at the first water change in a 10:1 N:P Ratio (n=4).

3.5 Discussion

This study bridges previous research on both the effects of nutrient history and on nutrient subsidy regimes on coral reef macroalgae by using a manipulative laboratory experiment to demonstrate how these two factors can interact to influence their biological responses to nutrient enrichment, and therefore their effectiveness as bioindicators. It also supports the literature on how functional form models do not account for the differences in ecological strategies that make morphologically similar species react differently to physical drivers of change such as nutrient enrichment. The fleshy opportunistic macroalgae *Dictyota* taken from the low-nutrient reef Papetoai showed a significant response to the nutrient treatments through a decline in $\delta^{15}N$ and increases in %N and wet weight, particularly to the Pulse treatment, although there were also changes in the Control treatment for *Dictyota* from the same site. This implies that although the seawater taken from the surface waters of oligotrophic forereef for the experiment typically has very low nutrient concentrations, the decrease in $\delta^{15}N$ in the Control treatment suggested the algae may have also taken up what little nutrients were available in the surface waters and influenced their isotopic signatures. Therefore, the results of this study are suggestive but not conclusive. In contrast, *Dictyota* from the high-nutrient reef halfway along Opunohu Bay showed no response to nutrient enrichment regardless of treatment, highlighting the importance of understanding the nutrient history of even opportunistic and typically responsive macroalgal bioindicators. As *Padina* is a slower-growing brown macroalgae, previous literature suggested it may be more responsive to Press treatments, with lower concentrations of more frequent nutrient inputs. However, specimens in this study showed no significant change in $\delta^{15}N$, %N or wet weight, regardless of treatment or site, so it could have been affected by other physical factors, such as light intensity.

This study provides further evidence for why it is vital to understand several biological and/ or physical factors that can influence the responses of any chosen bioindicator(s) to nutrient enrichment. One particularly important influence on even opportunistic algae are their ecological strategies (Littler & Littler, 1980; Steneck & Dethier, 1994; Schaffelke, 1999; Martínez et al., 2012; Raimonet et al., 2013; Fong & Fong, 2014, 2017). While **Chapter 1** showed that it might be necessary in some environmental monitoring programs to use a suite of bioindicators if there are distributional gaps of one or more species across an area impacted by anthropogenic runoff, it also highlighted that the two types of macroalgae (*Sargassum* spp. and *Chlorodesmis* spp.) had low congruency between their nutrient signatures across reefs. **Chapter 3** also supported the growing evidence that the definitions of functional form models for morphologically-similar species do not account for these ecological strategies, and therefore for their variation in responses to nutrient enrichment (Fong & Fong, 2014, 2017). It is therefore not surprising that *Dictyota* and *Padina* showed varied responses to treatment, but as the former is a faster-growing opportunist, the nutrient-limited specimens from the Papetoai lagoon also met predictions that it would respond more strongly to the Pulse treatment, shown through changes in $\delta^{15}N$ and wet weight (Delgado & Lapointe, 1994; Fong & Fong, 2014; Clausing and Fong, 2016; den Haan et al., 2016; Fong & Fong, 2017). In contrast, *Padina* showed no significant change in any of the response parameters, not even to the Press treatment. One reason for this could be that specimens from the low-nutrient reef might not have been nutrient-limited enough to respond to the treatments in the current study, indicating that it is not a consistent or reliable species to use as a bioindicator.

There were clear differences in $\delta^{15}N$ between the two reefs in both species, as was also shown in **Chapter.** Meanwhile, %N of *Dictyota* was consistently higher than in that of *Padina*,

regardless of original site or treatment. This is likely due to nutrients typically being stored in macroalgal tissues over the long term when nutrient supply is greater than growth rate, which can dilute the signature of nitrogen content (**Chapter 2**; Lin & Fong, 2008). Opportunistic species which tend to succeed in environments with episodic nutrient pulses can store sufficient nutrients to maintain growth during periods of low concentrations in the water column (Fong & Fong, 2017). Therefore, $\delta^{15}N$ was the most precise nutrient indicator in this study for capturing differences in nutrient regimes over small spatial and temporal scales, as %N was more variable between individual replicates, even if there were some differences between sites, and this was also shown in Lin & Fong (2008). However, %N can still be a useful indicator for detecting differences between morphologically-similar species with different ecological strategies. In addition, changes in wet weight (i.e. growth rate) is an important parameter to include in manipulative nutrient enrichment experiments, as it helps to understand how these different species are actually using the nutrients, whether they are only storing them in their tissues or using them for growth. Other physiological parameters, such as photosynthetic efficiency, respiration rate, chlorophyll content, and protein content, are also useful parameters that can help to assess response at a finer scale (Delgado & Lapointe, 1994; Teichberg et al., 2013), particularly if the nutritional quality of macroalgae after nutrient addition needs to be examined (Ober & Thornber, 2017).

As $\delta^{15}N$ in the final samples of Dictyota from PAP dropped to < 3 ‰, even in the Control samples (Costanzo et al., 2001; Lin & Fong, 2008), it is possible that there were some bioavailable nutrients in the seawater collected from the forereef that were taken up along with the artificial stock solutions (Sigman & Casciotti, 2001; Sigman et al., 2001; Voss et al., 2013; Ochoa-Izaguirre & Soto-Jiménez, 2015). This seems to be the most likely explanation, as the change in wet weight of *Dictyota* in the Control was not significant like it was for the

Pulse and Press treatments. Furthermore, as $\delta^{15}N$ has been shown to be the more sensitive indicator of changes in nutrients, the macroalgae could have accumulated surface-water nutrients into their tissues, but the concentrations were so low, it didn't affect growth as much as the stock solutions did. Conversely, the stable isotopic signatures from nutrients in the forereef seawater, even if low in concentration, could have potentially influenced the average value of the isotopic signatures when combined with the artificial nutrients added in the experiment (Sigman & Casciotti, 2001; Sigman et al., 2001; Hood et al., 2014). For instance, biogeochemical reactions in the nitrogen cycle like ammonia volatization, nitrification and denitrification could have been captured in the nutrient signatures in the final macroalgal tissue (Ochoa-Izaguirre & Soto-Jiménez, 2014).

There is some debate among scientists about using ambient, unfiltered seawater against filtered seawater for laboratory experiments, with the former being less costly in terms of time, resources and/or equipment, and leaving natural microbial communities in the seawater (Magnesen et al., 2013; Voss et al., 2013; *Holbrook, S. & Schmitt, R., pers. obsv., 2018*). However, this must be taken in account when interpreting results of nutrient experiments, especially if there are any nitrogen-fixing bacteria in the seawater (Sigman et al., 2001; Voss et al., 2013). Other key biological factors such as organic matter decomposition (**Chapter 2**; Deininger & Frigstad, 2019; Radice et al., 2020) and physical factors, such as sedimentation and turbidity (Bartley et al., 2014; Fabricius et al., 2013; Risk, 2014; Rouzé et al., 2015; Fong et al., 2020), can also either confound or exacerbate macroalgal responses to nutrient enrichment, so these additional physical stressors should also be tested in future studies. Furthermore, fractionation in nutrient isotopic signatures can also be influenced by geography, light intensity and/ or depth (Marconi et al., 2011; Teichberg et al., 2013; Viana et al., 2013).

If this experiment was repeated, a subset of specimens of the two species from the same two reefs could be placed in filtered seawater as an additional control, to see whether nutrients in oceanic seawater had any effect on nutrient signatures and/ or wet weight among treatments, as demonstrated in Magnesen et al., (2013). Alternatively, the ambient seawater from the forereef could be measured using the bacterial method for the N isotopic analysis of nitrate in seawater (Sigman et al., 2001). In addition, bringing in other measurements to augment those of the macroalgal stable isotope signatures, such as traditional seawater sampling, hydrodynamic modelling, and remote sensing, other potential causes of nutrient enrichment could be linked back to the unexpected changes in $\delta^{15}N$ in the control macroalgae (Amato et al., 2021; Devlin et al., 2020).

High-resolution remote sensing (e.g. primary production, ocean colour) is a highly beneficial technique for monitoring programs at the regional and international scales, as it can help to determine large spatial and temporal extent of plumes from any floods or terrestrial discharge (Devlin & Brodie, 2005; Brodie et al., 2010b; Devlin & Schaffelke, 2012; Devlin et al., 2012). A combination of wave-action modelling, a suite of nutrient-limited, opportunistic macroalgal stable-isotope bioindicators, autonomous *is situ* logging, and traditional seawater sampling (e.g. chlorophyll-a, total suspended solids (TSS), coloured dissolved and detrital organic matter (CDOM + D), and stable isotopic & elemental analyses of nitrates) could also be used to both groundtruth the satellite data and, collectively, to capture the finer-scale temporal variability in nutrient sources and concentrations (Costanzo et al., 2001; Brodie et al., 2010b, 2012; De'ath & Fabricius, 2010; Devlin & Schaffelke, 2011; Devlin et al., 2010; Fabricius et al., 2012; Leichter et al., 2012, 2013; Mills & Fones, 2012; Clausing & Fong, 2016; Fong & Fong, 2017; Daniel et al., 2020; Adam et al., 2021). Therefore, this "toolbox"

approach could subsequently provide more information across a variety of temporal and spatial scales that can help to determine whether even the typically-low waters over the forereef used for ambient seawater in the experiment were affected or not by any coastal runoff at the time of collection (Fabricius et al., 2012; Devlin et al., 2019, 2020). However, the more indicators used in such an assessment, the higher the monetary and time costs, so a decision analysis should also be conducted prior to the experiment to find the most cost-effective solution(s) for any given monitoring program (Barnes et al., 2021).

Chapter 3 compared benthic cover between the high- and low-nutrient reefs and showed that overall macroalgal percent cover, as well as percent cover of *Dictyota* and *Padina*, was significantly higher at OPU, relative to PAP. However, it is difficult to extrapolate the responses of individual algae in a laboratory setting, even if they are predominant species on coral reefs, to community-scale effects of nutrient enrichment in the field (den Haan et al., 2016). Therefore, future research could investigate how differences in the interaction of nutrient history and nutrient supply rates affect macroalgal community composition. For instance, in a mesocosm experiment, Fong & Fong (2017) found that some species, such as *Dictyota*, responded differently to pulsed nutrient subsidies when tested alone compared to when it was part of a macroalgal community, highlighting how macroalgae with varying ecological strategies can co-exist in environments with fluctuating nutrient supplies and concentrations.

It is critical to further understand the greater ecological consequences of nutrient pulses from increased rainfall and storms on coral reefs, particularly if they have been found to shift benthic community structure from coral- to algal-dominated ecosystems (Hughes et al., 1999, 2007; MacNeil et al., 2019; Fong et al., 2020). This can either be achieved by using

microcosms or mesocosms (Fong et al., 1993; Fong & Fong, 2017) in the laboratory, or *in situ* transplant experiments where macroalgal assemblages are either deployed on target reefs with known high nutrient loads, or else on reefs with attached slow-release fertilisers (Littler et al., 2006b; Fong & Fong, 2014; Fong et al., 2018). *in situ* incubation chamber systems are another recent development for field-based experiments that aim to measure organismal responses to biogeochemical fluxes of structurally complex benthic communities. This new technology is designed to be non-invasive, cost-effective, easy to handle and better than mesh cages at keeping out herbivores in (Roth et al., 2018). In addition, for laboratory experiments, stable isotopic tracers can be used to track the flow of nutrients from the water column into macroalgae, although these can be more difficult or expensive to process (Pitt et al., 2008; Naumann et al., 2010; Gilbert et al., 2018; Bailes & Gröcke, 2020).

Although effort was made to mimic environmental conditions in the current study, light could have also been the limiting factor in the laboratory experiment rather than nutrients, particularly for *Padina* specimens that showed no significant response, regardless of treatment or site. Macroalgae from reefs with high nutrient loads and high turbidity, particularly from terrestrial discharge during pulse events, are often limited by other energetic constraining factors such as light, which can restrict growth and even nutrient uptake and storage, regardless of nutrient availability (Szmant, 2002; D'Angelo & Wiedenmann, 2014; Clausing & Fong, 2016; Tuya et al., 2016). Clausing & Fong (2016) discuss how light availability may even have a greater effect on growth in algae in oligotrophic waters than nutrients under certain conditions or even over different spatial scales, such as between rainfall events in the short-term, and between seasons in the long-term. Multiple interacting factors like nutrient and light availability can be manipulated to understand both their individual and their synergistic effects on specific species (Littler et al., 1988, 1992), but

other factors such as sedimentation or herbivory likely also play a vital role in driving algal proliferation, which are harder to manipulate in laboratory or even mesocosm experiments (Rasher et al., 2012; Clausing et al., 2016; Fong et al., 2017, 2020). However, even field transplants can have some influence on algae, such as photoinhibition or photoacclimation, so this should also be considered when interpreting results (Copertino et al., 2006).

Overall, there are a number of drawbacks in only measuring one physical variable on one or more species in laboratory experiments, as there may be other underlying factors that either confound or exacerbate the results. This is one of the primary reasons why results from many laboratory experiments cannot be extrapolated to explain ecosystem- or even community-level responses on reefs, as they are likely not reflective of their native environment (Huston, 1997; Fong & Fong, 2017). This could then have impacts on other types of studies, such as large-scale monitoring surveys which use the same species as bioindicators of nutrient impacts, as they lack important data on the biological responses, including what biological and physical factors other than nutrient availability could potentially reduce their effectiveness. Therefore, in order to bridge large monitoring surveys with empirical studies, *in situ* transplant experiments, as well as additional physical measurements in seawater, may be more informative about responses to local natural variability of both factors (**Chapter 2**; Littler et al., 1991; Clausing & Fong, 2016).

3.6 Conclusion

In conclusion, this study gives suggestive but not conclusive evidence that while macroalgal stable isotopes are a more cost-effective and biologically relevant indicator of average temporal variability of nutrient regimes than regular seawater "spot measurements", it is also important to capture finer-scale episodic pulse events, such as heavy rainfall or river discharge (Brodie et al., 2012a&b; Fabricius et al., 2012; Anthony et al., 2014). Therefore, a broader, more comprehensive "toolbox" approach that brings together multiple indicators and/or measurements at multiple scales, from traditional water sampling, *in situ* loggers, macroalgal bioindicators, to regional-scale remote sensing, could help environmental managers to better understand both the cause and effect of any nutrient pulses on coral reefs (Devlin et al., 2012; 2019, 2020). A good bioindicator, or a suite of bioindicators, need to be capable of responding to differences in the frequency and concentration of nutrient supply even on smaller timescales of hours to days, but their effectiveness can be significantly influenced by both spatial (nutrient history) and temporal (nutrient subsidies) factors. The nutrient-limited *Dictyota* from the low-nutrient reef PAP was more responsive to nutrient enrichment than the morphologically-similar but slower-growing *Padina,* which did not show any significant change in nutrient signatures or growth, regardless of site. However, as there was also a significant decline in the Control specimens of PAP-*Dictyota*, the change in $\delta^{15}N$ could be attributed to more than one nutrient source, so one way to test this could be to use isotopic tracers in future laboratory studies to differentiate between sources (Naumann et al., 2010; Gilbert et al., 2018). However, there also could have been a confounding effect of light limitation in macroalgae in the laboratory, and other factors such as sedimentation and herbivory were not included in the study. More research is therefore needed on the synergistic, antagonistic and additive stressors on coral reefs (Fong et al., 2018), particularly when press-type (chronic) stressors such as pollution and sedimentation interact with

increases in pulse-type (acute) events such as storms and rainfall (Devlin & Brodie, 2005; Anthony et al., 2014; Edmunds & Gray, 2014; Hernández-Delgado et al., 2014; Clausing et al., 2016; Fong et al., 2020). As biophysical relationships between stressors and responses on coral reefs are typically non-linear, any additional anthropogenic-derived impacts will make them increasingly difficult to predict (Huston, 1997; Gove et al., 2015; Jouffray et al., 2019; Williams et al., 2019). Therefore, it is critical to first examine the nutrient history and the ecological strategies of even widely common bioindicators of nutrient regimes before assigning them to larger-scale monitoring programs (Cooper et al., 2009; Flower et al., 2017).

4. NITROGEN ENRICHMENT IN MACROALGAE FOLLOWING MASS CORAL MORTALITY

4.1 Abstract

Scleractinian corals are engineers on coral reefs that provide both structural complexity as habitat and sustenance for other reef-associated organisms via the release of organic and inorganic matter. However, coral reefs are facing multiple pressures from climate change and other stressors, which can result in mass coral bleaching and mortality events. Mass mortality of corals results in enhanced release of organic matter, which can cause significant alterations to reef biochemical and recycling processes. There is little known about how long these nutrients are retained within the system, for instance within the tissues of other benthic organisms. Enrichment of the nitrogen isotopic signatures ($\delta^{15}N$) of macroalgal tissues were detected a) ~1 year after a bleaching event in the Seychelles and b) ~3 months after the peak of a bleaching event in Mo'orea, French Polynesia. In the Seychelles, there was a strong association between absolute loss in both total coral cover and branching coral cover and absolute increase in macroalgal $\delta^{15}N$ between 2014 and 2017 (adjusted $r^2 = 0.79$, $p = 0.004$ and adjusted $r^2 = 0.86$, $p = 0.002$, respectively). In Mo'orea, a short-term transplant experiment found a significant increase in $\delta^{15}N$ in *Sargassum mangarevense* after specimens were deployed on a reef with high coral mortality for ~3 weeks ($p < 0.05$). I suggest that coral-derived nutrients can be retained within reef nutrient cycles, and that this can affect other reef-associated organisms over both short- and long-term periods, especially opportunistic species such as macroalgae. These species could therefore proliferate on reefs that have experienced mass mortality events, because they have been provided with both space and nutrient subsidies by the death and decay of corals.

Published online (12/04/2021):

Vaughan, E.J., Wilson, S.K., Howlett, S.J., Parravicini, V., Williams, G.J., & Graham, N.A.J. (2021) Nitrogen enrichment in macroalgae following mass coral mortality. *Coral Reefs*, 40: 767-776, doi: https://doi.org/10.1007/s00338-021-02079-w

4.2 Introduction

Tropical coral reefs are highly productive ecosystems, but as they are typically surrounded by oligotrophic waters, they require constant recycling and retention of water-borne nutrients and organic matter (Galloway et al., 2004). There are a wide range of physical and biological processes on coral reefs which can retain these essential energetic resources within local biogeochemical cycles for extended periods of time. Thus, these processes can sustain rapid rates of biological activity such as primary productivity, as well as many other key ecosystem functions (Wyatt et al., 2013). For instance, coral-derived particulate organic matter (POM) in the form of mucus can act as an energy carrier and particle trap, so these nutrients may be recycled by benthic and planktonic communities over longer timescales (Ferrier-Pagès et al., 1998; Wild et al., 2004a,b). However, even in a coral-dominated ecosystem, they are not the only natural, or autochthonous, source of bioavailable nutrients (Davey et al., 2008; Wyatt et al., 2013; Tanaka & Nakajima, 2018; Deininger & Frigstad, 2019). Microbes, for instance, are capable of nitrogen fixation (Moulton et al., 2016), and other primary producers, such as phytoplankton and macroalgae, readily take up and store nutrients and dissolved organic matter (DOM) in their tissues (Fong et al., 1994). This DOM is then recycled either through tissue breakdown or through consumption by higher trophic level organisms such as

herbivorous fishes, which in turn recycle significant amounts of nutrients through excretion (Burkepile et al., 2013).

Healthy coral reefs typically persist in suboptimal nutrient concentrations, although nutrient pulses can disrupt the balance of natural biogeochemical dynamics jeopardising reef health. Disturbances such as marine heat waves that cause coral bleaching have a direct negative impact on corals, but can also have indirect consequences for reefs by altering nutrient dynamics (D'Angelo & Wiedenmann, 2014). Branching scleractinian corals are often dominant on a reef, providing structural complexity and micro-habitats for a variety of reef-associated organisms, but they are also particularly vulnerable to heat stress (Hughes et al., 2019). The loss of these vital foundation species therefore has huge implications for the entire ecosystem (Graham et al., 2015; Wilson et al., 2019). Where coral bleaching causes extensive mortality, the metabolic exchange between corals and associated organisms on a reef is reduced, along with the capacity of corals to trap organic matter. This can subsequently trigger the dysfunction of major biogeochemical processes (Glynn, 1993; Wild et al., 2011).

There are few studies assessing how climate-derived disturbances affect mucus release by live corals, and associated processes. Davey et al. (2008) found that in the weeks that follow coral bleaching, a 30-fold higher production of new nitrogen occurred on coral reefs compared to those that did not experience bleaching. Such nitrogen productivity has also been shown in an experimental setting (Niggl et al., 2009). While release rates of mucus-derived POM from corals increase during the early stages of bleaching, providing a burst of nutrients to coral reefs (Coffroth, 1990), these rates can decrease after the initial bleaching response (Fitt et al., 2009; Wooldridge, 2009). If corals recover from bleaching, which can take many weeks to occur (Gates, 1990), there may only be short- to medium-term effects on

biogeochemical processes. However, if corals die, the subsequent mass release of coral tissue into reef environments may also alter biogeochemical processes, and over longer time scales. In addition, colonisation of the exposed coral skeleton by microbial biofilms, turf algae, macroalgae, sponges, cyanobacteria or other invertebrates may not only reduce coral recruitment success, but can also change biogeochemical processes such as nitrogen fixation (Diaz-Pulido & McCook, 2002; Davey et al. 2008; Haas et al., 2010).

In order to identify changes in nutrient regimes due to mass coral mortality, nitrogen stable isotopes ($\delta^{15}N$) and nitrogen content (%N) can be analysed from macroalgal tissues to capture temporally-extensive records of nutrient loads (Costanzo et al., 2001). Stable isotopes of nitrogen have been used in nutrient studies for several decades, helping to identify the origins of nitrogen (Heaton, 1986; Kolasinski et al., 2011). In addition, certain types of marine algae are commonly used in biomonitoring studies due to their widespread distribution and responsiveness to bioavailable pollutants. *Sargassum*, for example, is a genus used worldwide as it has been found to be responsive to nutrient enrichment (McCook, 1996; Schaffelke & Klumpp, 1998; Schaffelke, 2002; García-Seoane et al., 2018). However, marine algae are not the only functional group that can be used to measure isotopic signatures as a proxy of nutrient regimes on reefs. Organisms at higher trophic levels also assimilate nutrients from lower trophic levels, resulting in increasing isotopic enrichment up the food chain (Bierwagen et al., 2018). For instance, corals are at a higher trophic level than primary producers such as macroalgae, and thus have enriched isotopic signatures (Graham et al., 2018). As corals release organic matter into the water column after the death and subsequent decay of tissue following marine heatwave-driven mortality events (Leggat et al., 2019), opportunistic benthic species such as macroalgae may capitalise on this new nutrient source,

assimilate it into tissues for growth and storage, and consequently become more enriched (Pawlik et al., 2016).

In the current study, the temporal effect of coral mass mortality on macroalgal stable isotopic signatures is investigated in two different coral reef systems, over two different time periods. As such, it offers new understanding on whether macroalgae can indicate longer-term effects of coral mortality events on reef nutrient dynamics and biogeochemical cycles. Specifically this study assesses: (1) changes in *Sargassum* sp. nutrient signatures over three years in the inner Seychelles islands, western Indian Ocean, spanning a mass coral bleaching event, and (2) shorter-term changes in *Sargassum mangarevense* nutrient signatures ~3 months after the peak of a severe bleaching event in Mo'orea, French Polynesia, using an *in-situ* three-week transplant experiment.

4.3 Methods

4.3.1 Study Site 1: Seychelles

The inner Seychelles islands experienced two severe coral bleaching events, in 1998 and 2016. In 1998, coral cover dropped by 90%, and though hard coral cover steadily recovered on some study sites (average coral cover of 27% by 2014) (Graham et al., 2015), another global bleaching event in 2016 (Hughes et al., 2018) led to live coral cover declining by 70% on these same sites (Wilson et al., 2019). Around the Inner Seychelles, heat stress reached 4°C-weeks in January 2016, rapidly increased in April and peaked at 11.4°C-weeks in May (Wilson et al., 2019; http://coralreefwatch.noaa.gov/vs/index.php).

Eighteen reefs were surveyed in April 2014, before the mass bleaching event caused extensive coral mortality in 2016 and again in April 2017, a year after the event occurred (Wilson et al., 2019). These reefs form part of a 25-year coral reef monitoring survey around the inner Seychelles, with roughly half the reefs having been defined as "recovering" from a previous mass bleaching event in 1998, and the other half as transitioning to a "regime-shifted" macroalgae-dominated state (Graham et al. 2015). Eight replicate 7-m radius point counts were surveyed along the reef slope on each reef for both survey years. Within each point count area, the percent cover of benthic categories including live hard coral, soft coral, macroalgae, sand, rubble, and rock was quantified using 10m long line-intercept transects (Wilson et al. 2019).

The objectives of this component of the study were to assess the relationship between changes in percent cover of corals between the study years of 2014 and 2017 with differences in $\delta^{15}N$ and %N signatures in tissues of *Sargassum* sp. that were collected from the same sites during the same surveys. Low availability of macroalgae at some reefs meant that macroalgae for stable isotope analyses were not collected from all reefs in both years. A minimum of four replicate *Sargassum* sp. samples were collected from each of the seven "coral mortality" reefs (a subset of the previously termed "recovery reefs", named as such following the impacts of the 2016 bleaching event) and from the six "regime-shifted" reefs in both 2014 and 2017.

4.3.2 Study Site 2: Mo'orea

Mo'orea, an island which is part of the Society Archipelago in French Polynesia, has demonstrated rapid coral recovery from previous disturbances (Vercelloni et al., 2019;

Hédouin et al., 2020). For example, following an outbreak of *Acanthaster* spp. from 2006 to 2009 and a cyclone in 2010, mean coral cover on the outer reefs was reduced to 2% at 10 m depth from a high of 39% in 2005, before recovering to 27% in just four years. The branching coral genus *Pocillopora* spp. was found to be a significant driver in that recovery, as it made up 53% of the re-established coral community (18% cover) (Tsounis & Edmunds, 2016). There were no recorded episodes of abnormally high sea surface temperature (SST) in 1998 in Mo'orea, but it was impacted by the global coral bleaching event in 2016, with heat-sensitive branching corals being the worst affected (Hughes et al., 2019). Donovan et al. (2020) reported that 37% of *Acropora* and 28% of *Pocillopora* colonies exhibited bleaching across all sites, with up to 100% bleaching of *Acropora* on north shore sites. Coral mortality was rare (~1%), as heat stress did not exceed 1.1°C weeks (Hédouin et al., 2020).

Annual surveys of 13 marine areas around Mo'orea were established in 2004 (Service National d'Observation CORAIL). For the purpose of this study, data for the reef slope at the four areas along the north coast of the island, where bleaching was highest and our study site was located, was used (*Suppl. Fig. 4.1*). This includes the site Tiahura which is closest to our study site. The benthic cover of each sample area was quantified at a similar depth to the transplant site (~10 m) using 3 replicate non-permanent 25 m transects (Horta e Costa et al., 2016). The percentage cover of benthic components was sampled every 50 cm using the point intercept transect (PIT) method. Macroalgae was categorised as all the non-coralline algae of large enough size to identify with the naked eye.

Sea surface temperature (SST) was measured hourly using an SBE-56 sensor (Sea Bird Scientific) on the Tiahura forereef at 3m depth from 1998 to 2005. The time series was interrupted for 5 years before being collected continuously again from 2010. In order to

characterise the temperature trend in 2019, relative to that of other years, we calculated weekly means for 2019 and compared this with the average temperature time series and 95% confidence intervals for the entire period. In addition, following Donovan et al. (2020), we calculated cumulative heat stress (in °C weeks) as a 12-wk running sum for all temperatures exceeding 29 °C, a threshold that is considered a good predictor of bleaching in Mo'orea based on previous studies (Pratchett et al., 2013; Donovan et al., 2020; Hédouin et al., 2020). The maximum water temperature during 2019 exceeded 29 °C in March and peaked at ~30°C in April. Patterns of cumulative heat stress peaked at ~6 °C weeks. As the duration of heat stress was much longer in 2019 than in the previous bleaching event (Donovan et al., 2020; Hédouin et al., 2020), the extent of coral mortality was much higher (*Suppl. Fig. 4.2*).

Samples of *Sargassum mangarevense* (n=10) were collected from Papetoai lagoon, a low-nutrient reef in the northwest region of Mo'orea on 6th July 2019 (*Suppl. Fig. 4.1*). These waters were found to typically have low $\delta^{15}N$ and %N values, shown in nutrient heat maps in Leichter et al. (2013), Donovan et al. (2020), and Adam et al. (2021). Specimens were placed in shaded coolers filled with seawater before they were transported back to the CRIOBE research station, Mo'orea. After all visible, larger epiphytes were carefully removed from the fronds using a scalpel, initial tissue samples were taken and frozen at -20°C for later stable isotopic analyses. Algal specimens were then placed in pre-transplant holding tanks for seven days, with water changes every two days. Water changes in the tanks involved surface water collected from the forereef, as it was found to typically be low in $\delta^{15}N$ (< 3.0 ‰, Lin & Fong, 2008, Donovan et al., 2020). This was done to ensure that internal nutrient stores in *S. mangarevense* were depleted before specimens were transplanted on the forereef where there were high levels of coral mortality. Following this seven-day acclimation period, further tissue samples were taken for stable isotopic analyses. For the *in situ* macroalgal bioassay, a

cage was made out of chicken-wire mesh and attached to a cinder block that was already placed on the forereef at ~12 m depth. At the time of the transplant experiment in July 2019, while some corals were still bleached, ~40% had already died (S.J.H., 2020, *pers. obs.*). It was not possible to have a control bioassay, due to restrictions on deploying additional cinder blocks and the lack of non-bleached reefs at that time. The ten macroalgal specimens were deployed on the reef for ~3 weeks from 15th July to 4th August 2019 before they were collected and returned to CRIOBE. Final tissue samples were taken and frozen before stable isotopic analyses were performed.

4.3.3 Stable Isotopic Analyses

All frozen samples from both studies were defrosted, rinsed thoroughly with fresh or distilled water, and placed in a drying oven for 48 h at 60°C. Once dried, samples were each ground into a fine powder using a ball mill and stored in individual airtight containers. 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 ($\delta^{15}N$) and elemental analyses (%N) for both the 2017 samples from the Seychelles study and the 2019 Mo'orea samples were run on an Isoprime100 Isotope Ratio Mass Spectrometer (IRMS) linked to an Elementar VARIO MICROcube Elemental Analyser at Lancaster Environment Centre (LEC), Lancaster University. The samples collected in 2014 from the Seychelles were analysed using a Costech Elemental Analyzer fitted with a zero-blank auto-sampler at James Cook University's Advanced Analytical Centre, Cairns. Analyses from both years were standardised using internal reference materials calibrated to international standards.

4.3.4 Statistical Analyses

For the Seychelles data, four separate two-way analysis of variance (ANOVAs) were used to assess the effect of time period (two levels: 2014 and 2017), reef state (two levels: coral mortality and regime shift) and their interaction on a) total coral cover, b) branching coral cover, c), $\delta^{15}N$, and d) %N across all 13 reefs where *Sargassum* were consistently collected. Based on this analysis and subsequent post-hoc Tukey tests, we found that predominant changes in these response variables were observed on "coral-mortality" reefs, with little response on "regime-shifted" reefs. We therefore include the seven reefs with high levels of coral mortality to investigate the relationship between changes in nutrient signatures against a) absolute and b) branching coral cover loss, using linear regression models. This decision was further supported by coral cover changes on "regime-shifted" reefs, where starting absolute values in 2014 were already very low at 6.69 ± 1.8 % before dropping by ~5% in 2017, and macroalgal cover was very high in both years. Therefore, any influence of coral cover on nutrient signatures in the system would be negligible (*Suppl. Fig. 4.3*; Wilson et al., 2019).

For the Mo'orea data, differences between a) average $\delta^{15}N$ and b) %N signatures in the *Sargassum* specimens in the three treatments (initial, pre-transplanted, and post-transplanted) from the transplant experiment were analysed using a repeated-measures ANOVA. Repeated measures were incorporated into this ANOVA as tissue samples were taken from the same experimental specimens placed under the three different treatments. A time series analysis was conducted to compare the average mean monthly SST in 2019, relative to SST in previous years. Normality of data was assessed visually, and homogeneity of variance for all ANOVAs conducted for both studies was assumed with a Levene's test. All statistical analyses were conducted in R (R-Core-Team 2018), and the time series analyses for Moorea

were performed using ‘zoo’ and ‘xts’ packages to produce *Supplementary Figure 4.2* (Zeileis & Grothendieck. 2005; Ryan & Ulrich, 2020).

4.4 Results

4.4.1 Seychelles

There was a significant effect of year, reef state and interaction on total coral cover across the thirteen reefs (interaction: $F_{1,204} = 37.3$, $p < 0.0001$). The post-hoc Tukey test revealed that there was no significant difference between the pre- and post-bleaching years for the “regime-shifted” reefs ($p = 0.32$; *Suppl. Fig. 4.2*). In contrast, the seven “coral mortality” reefs declined significantly from 27.0 ± 1.5 to 8.01 ± 0.5 % between 2014 and 2017 ($p < 0.0001$; *Fig. 4.1a*). This was mainly due to a loss in branching coral cover on these reefs from 16.0 ± 1.5 to 0.30 ± 0.05 % ($p < 0.0001$; *Fig. 4.1a*). Percent cover of massive corals remained similar between 2014 and 2017 on “coral mortality” reefs, whereas table coral cover declined from 1.27% to 0%. There was also a 0.8 % increase in total macroalgal cover on the seven study reefs between the years.

The $\delta^{15}N$ signature in *Sargassum* tissues differed significantly between 2014 and 2017 across all thirteen reefs (interaction between year and reef state, $F_{1,124} = 11.4$, $p = 0.001$), but only showed a significant difference for the seven “coral mortality” reefs between survey years ($p < 0.0001$, *Fig. 4.1b*; $p = 0.15$ for regime-shifted reefs). Similarly, %N in *Sargassum* tissues was higher in samples collected from “coral morality” reefs in 2017 than in 2014 ($p < 0.0001$, *Fig 4.1c*; significant interaction between year and state $F_{1,124} = 5.0$, $p = 0.03$), although there was no temporal difference in N content in samples collected from “regime-shifted” reefs (p

= 0.20). For the seven "coral mortality" reefs selected for the purpose of this study, there was a significant positive relationship between increase in $\delta^{15}N$ in *Sargassum* tissue and (a) loss of total coral (adjusted r^2= 0.79; p = 0.004; *Fig. 4.2*) and (b) branching coral cover (adjusted r^2 = 0.86; p = 0.002). There was no significant relationship between changes in %N and total coral cover (r^2 = 0.04; p =0.67) or branching coral cover (r^2 = 0.04; p = 0.66).

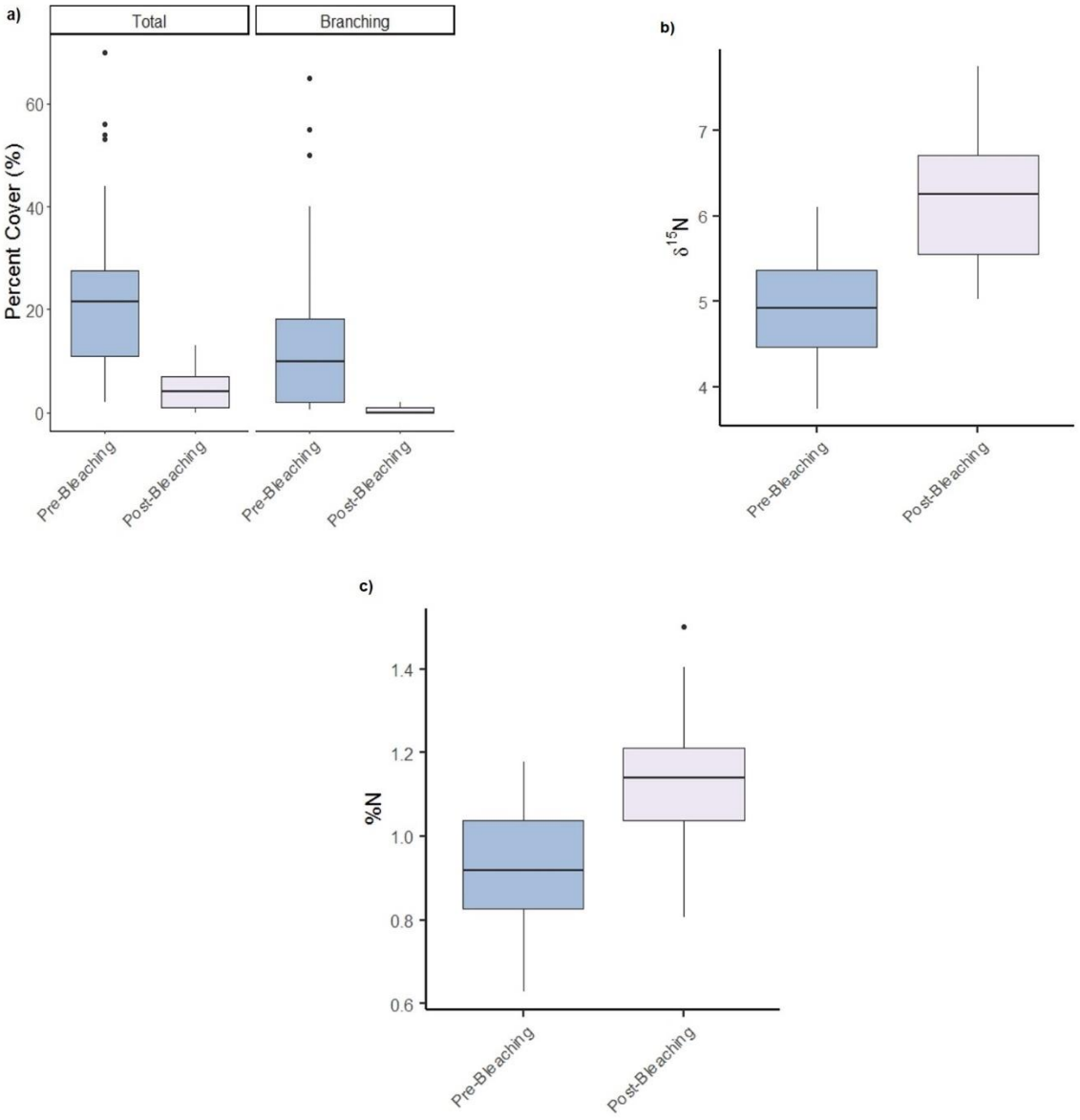

Figure 4.1 Box (median and 50% quantile) and whisker (95% quantile) plots of a) total and branching coral cover in both pre-bleaching and post-bleaching years (2014 and 2017, respectively) on "coral mortality" reefs (n=7), b) the average $\delta^{15}N$ signatures in *Sargassum* sp. tissues in both years, and c) the average percent N (%N) in both years. The pale blue boxes represent the pre-bleaching year and pale pink boxes represent the post-bleaching year.

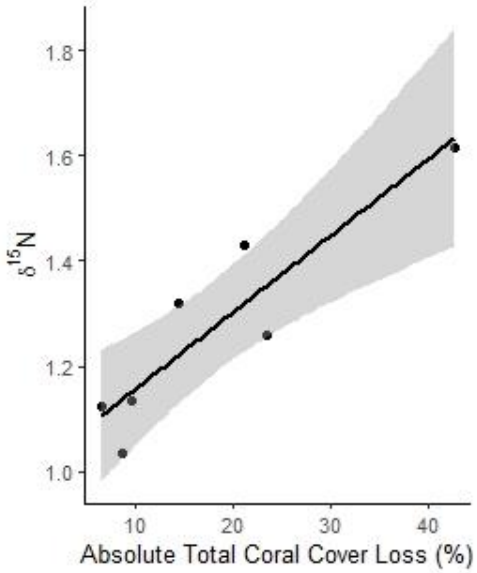

Figure 4.2. Change in absolute total coral cover and the corresponding changes in $\delta^{15}N$ in *Sargassum* tissues across seven coral mortality reefs in the Seychelles between 2014 and 2017. The regression lines and confidence intervals were obtained using linear regression coefficient of determination (r^2); 95% confidence intervals.

4.4.2 Mo'orea

Before the bleaching event peaked in April 2019 (*Suppl. Fig. 4.2*), the benthic cover survey conducted across the outer slopes of the four northern sites of Moorea in March 2019 showed an average of 73.7 ± 2.8% live coral cover, with a significant decline to an average of 36.2 ± 2.9 % in 2020, a year after the event ($p < 0.0001$; Mean ± SE). The closest site to the transplant experiment, Tiahura, had 73.3 ± 5.5% and 36.0 ± 2.0% in live coral cover in 2019 and 2020, respectively. The high coral cover across the four sites in 2019 was primarily due to the abundance of branching coral *Pocillopora* on the forereefs in Mo'orea (Tsounis & Edmunds, 2016). For instance, at Tiahura, there was 60.7 ± 5.7% cover of *Pocillopora* and an average of 55.5 ± 3.3% cover across the four sites in 2019. When the survey was repeated in March 2020, there was a significant decrease in *Pocillopora* to 24.5 ± 1.7% across all four sites ($p < 0.0001$), and a similar pattern was shown at Tiahura ($p < 0.0001$). Other than this

predominant branching coral, no significant differences were found between the years for the other reef-associated organisms, including other coral genera.

In the short-term transplant experiment shortly after the peak of the bleaching event in Mo'orea, treatment had a significant effect on macroalgal $\delta^{15}N$ signatures (repeated-measures ANOVA: $F_{2,27} = 31.71$, $p < 0.0001$; *Fig. 4.3*). Post hoc tests indicated that there were significant differences in $\delta^{15}N$ between all three treatments (initial, pre-transplant, and post-transplant, n=10), which suggested that $\delta^{15}N$ declined in the pre-transplant holding tanks, and then increased substantially on the transplant reef (initial and pre-transplant: $p = 0.003$; initial and post-transplant: $p < 0.0001$; pre-transplant and post-transplant: $p < 0.0001$). However, there was no significant effect of treatment on macroalgal %N (repeated-measures ANOVA: $F_{2,23} = 0.6$, $p = 0.58$; *Suppl. Fig.4.4*). Although it was not possible to include either control sites or reefs with varying levels of bleaching due to permit restrictions, the benthic data shows that the extent of coral mortality across the outer slopes on the northern region of Mo'orea was quite similar.

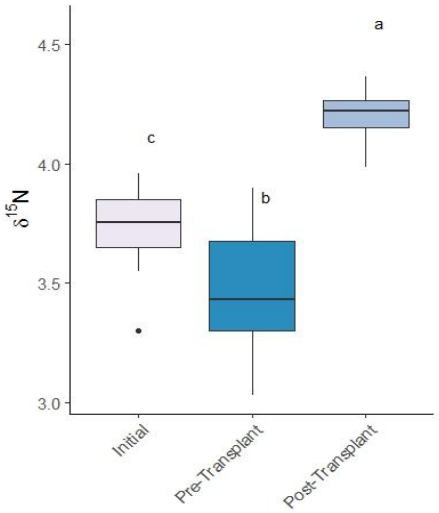

Figure 4.3 Box (median and 50% quantile) and whisker (95% quantile) plots of the median $\delta^{15}N$ in *Sargassum mangarevense* tissue across three treatments from a short-term transplant experiment. Connecting letters indicate significance between treatments. Stable isotopic signatures were measured in subset samples of the same specimens that were collected from a low-nutrient reef (Initial), placed in laboratory aquaria to deplete internal nutrient stores for ~7 days (Pre-Transplant), before they were deployed on the bleached reef for 3 weeks (Post-Transplant) (n=10).

4.5 Discussion

The current study suggests that mass coral mortality events can be detected through nitrogen isotopic signatures in macroalgal tissues, a proxy for nutrient sources, up to a year after a severe bleaching event. Although the exact source of the enrichment could not be traced in this study, a significant increase in $\delta^{15}N$ was shown in both reef systems over different timescales after two separate coral mortality events. For instance, in the Seychelles, there was a strong positive correlation between a decline in total coral cover and an increase in $\delta^{15}N$. This suggested that the N present in algal tissues could be coral-derived. These findings may help improve understanding of how mass disturbances such as coral bleaching impact multiple ecosystem processes on climate-impacted reefs. For instance, the loss of live coral cover, especially branching corals, provides a large amount of new substrate for opportunistic species such as macroalgae and other primary producers to colonise and prevent coral recovery, and may also provide an additional source of nutrients which become locked in the system. Consequently, this could enhance macroalgal proliferation on this colonised space, reinforcing alternative regimes.

The isotopic signature of fleshy macroalgae changed significantly over both short and long timeframes following bleaching events on two different reef systems. The positive relationship between $\delta^{15}N$ and the declines in coral cover suggest that nutrients from dead and decaying corals have contributed to this change of isotopic signatures in macroalgae. While this might be an important natural source of nutrients (Coffroth, 1990; Brown & Bythell, 2005; Bythell & Wild, 2011), any substantial increase could affect or disrupt natural metabolic exchanges between corals and other organisms, not only with their endosymbiotic zooxanthellae, but with sponge, seaweed and microbial communities (de Goeij et al., 2013;

Rix et al., 2016, 2017; Pawlik et al., 2016; Mumby & Steneck, 2018; Leggat et al., 2019). Much of the literature focuses on the mucus released from live corals and how it is recycled within the system (Davey et al., 2008; Naumann et al., 2009; Wild et al., 2004a,b, 2010, 2011), as well as the short term effects of changes in organic matter release after a bleaching event (Niggl et al., 2009; Wooldridge, 2009). Other work such as Radice et al., (2020) supports this by showing that isotopic signatures of particulate organic nitrogen in the water column decreased eight months after a bleaching event. However, there is still little understanding of changes in reef biogeochemical cycles.

Excess nutrients are one of the key factors that can drive a bleached reef towards a regime shift (Graham et al 2015). If the increased release of organic matter through mass coral mortality provides more nutrients to opportunistic species, this may encourage fast-growing macroalgae to proliferate on the exposed coral skeletons. This negative feedback loop can inhibit coral recovery and foster regime shifts to macroalgal-dominated states (Diaz-Pulido & McCook, 2002; Haas et al., 2010; Wild et al., 2011). For instance, the lack of available substrata may reduce the ability for any coral larvae to colonise this space and repopulate reefs, but increases in algal-derived DOM and POM can subsequently increase pathogenic microbial activity through what has been termed the DDAM positive feedback loop (dissolved organic carbon, disease, algae, microorganisms) (Haas et al., 2016). Macroalgae release labile organic matter which benefit pathogenic microbes and together they create unfavourable conditions for corals. For example, they collectively disrupt the function of the coral holobiont, thereby exacerbating death of coral recruits, and maintaining competitive dominance in algae (Wild et al., 2010; Barott & Rohwer, 2012; Pawlik et al., 2016; Mumby & Steneck, 2018).

While mass mortality has the potential to release a substantial source of new nutrients, this type of organic matter is still considered to be internal, or autochthonous (Briand et al., 2015). Excessive nutrient enrichment from external anthropogenic nutrient loads, particularly certain types of nitrogen such as nitrates found in coastal runoff, can further exacerbate changes in biogeochemical cycles on reefs (Burkepile et al., 2019; Donovan et al., 2020). This could accelerate the proliferation of macroalgae and other opportunistic organisms, and further decrease the chance of scleractinian corals re-establishing themselves. In addition, declines in water quality can develop and cause the formation of algal blooms (Fabricius, 2005; Tanaka et al., 2010).

Fleshy macroalgae are important indicators of changes in nutrient cycles because the bioavailable nutrients which are taken up from the water column and assimilated into their tissues can be easily measured over both short and long periods of time (Costanzo et al., 2001). Macroalgae have been used as proxies to study the effects of nutrient enrichment in both laboratory and *in situ* experiments, but these mostly tend to be for investigating anthropogenic sources, such as from coastal run-off (Fong et al., 1994; García-Seoane et al., 2018; Burkepile et al., 2019) and less commonly for natural nutrient inputs, such as seabird guano, deep-water upwelling events or coral-derived organic matter (Schaffelke, 2002; Graham et al. 2018; Williams et al., 2018; Radice et al., 2020).

The kind of nutrient signature used as a bioindicator is also an important factor to consider. Lin & Fong (2008) found $\delta^{15}N$ to be a more sensitive indicator to changes in nutrients in transplanted macroalgae than %N. Nitrogen content is typically diluted during rapid growth of specimens, suggesting that nutrients are only stored in macroalgal tissues over the long term when nutrient supply exceeds growth rate, as they first must assimilate excess nitrogen

into growth. This likely explains why we found no patterns in %N in either the Seychelles regression analysis, or the Mo'orea transplant experiment.

Although the duration of transplant experiments in the literature vary considerably, from hours to ~1 year, García-Seoane et al. (2018) recommended an exposure time of < 1 month, as the uptake kinetics of algal transplants can vary based on the species used or local environmental conditions. The current study suggests that these changes in nutrients may be detected in *Sargassum* tissues up to 12 months after an event, implying that nutrients have been trapped and retained in the system for at least a year. It is also known that *Sargassum* undergoes major seasonal fluctuations in production and biomass that may supplement adjoining ecosystems within the broader seascape (Fulton et al., 2019). This study supports previous literature suggesting macroalgae can easily be deployed in target areas to investigate changes in nutrient loads (Costanzo et al., 2001; Dailer et al., 2010; Fernandes et al., 2012; García-Seoane et al., 2018), but also applies this common technique to capturing energetic resources. Therefore, macroalgal assays have the potential to provide insight into changes in nutrient sources from both natural and anthropogenic events, such as widespread coral bleaching.

There are a number of potential sources of nitrogen that could have influenced these results other than coral-derived nutrients. A strong nutrient gradient from the land-end of Opunohu Bay in Mo'orea to its ocean-end (Lin & Fong, 2008) suggests that the nutrient enrichment from the shrimp farm effluent entering the bottom of the bay was unlikely to affect the isotopic signatures of our specimens. However, storms and heavy rainfall can influence both the spatial extent of run-off and nutrient uptake in reef macroalgae (**Chapter 3**; Clausing & Fong, 2016; Adam et al., 2021). Local upwelling could have provided nutrients and

influenced our results, but Lin & Fong (2008) suggest that the $\delta^{15}N$ of tropical ocean seawater is typically ~3 ‰, which is lower than the signatures found in both the post-transplant and pre-transplant tissue samples. In addition, no *Sargassum* specimens were found at the depth where the bleaching occurred in Mo'orea (~12m), so samples had to be taken from the nearby nutrient-limited lagoon (~1 m). Although this lagoon typically has low nutrient levels (Donovan et al., 2020) and the algal specimens collected from there had low tissue nutrient history, some bleaching was observed in the lagoon at the time of collection, but not in the specific area where the specimens were collected. Even if some coral-derived nutrients were captured by the initial specimens, we accounted for this by depleting tissue nutrient stores in the holding tanks. This resulted in a significant decline in $\delta^{15}N$, followed by a significantly higher signature in the post-treatment algae after they were transplanted at the site where extensive coral bleaching and mortality had occurred. Other factors such as light intensity can also affect algal condition and isotopic signatures (Marconi et al., 2011; García-Seoane et al., 2018), so may have also influenced results in Mo'orea.

Future research could build on this study, and on other studies in the literature (García-Seoane et al., 2018) by applying the above methods to test the degree of influence of coral-derived organic matter on macroalgal nutrient signatures, relative to anthropogenic sources, either in laboratory- or field-based experiments. For instance, macroalgal bioassays could be deployed on bleached reefs with low levels of coastal run-off, such as those in other regions around Mo'orea, and compared to those with significantly higher levels, to test if these effects are synergistic. Clearly assessment of macroalgal isotope signatures across different nutrient loads and levels of coral mortality are required to fully understand nutrient sources before attribution of nitrogen enrichment in macroalgae to nutrients released from dead and decaying corals can be definitively determined.

While this study compared the $\delta^{15}N$ signatures in tissues of *Sargassum* from pre- and post-bleaching years in the Seychelles, no macroalgal samples were collected during the bleaching and the subsequent mortality event in 2016 itself, so it was not possible to compare the stable isotopic results when this mass tissue release was occurring. The short-term experiment in Mo'orea was conducted in part to understand these shorter-term dynamics and to further support these findings. Though the results from the two different reef systems are not directly comparable, this study suggests that macroalgal tissue $\delta^{15}N$ signatures can be affected by mass morality events. However, as the current study only implies that the mass release of dead coral tissue enriched the macroalgal $\delta^{15}N$ signatures, future research could expand on this work by determining the exact source(s) of enrichment (Briand et al., 2015). For instance, enriched stable isotope tracers (^{15}N and ^{13}C) (Naumann et al., 2010; Bailes & Gröcke, 2020) or compound-specific stable isotopes (McMahon et al., 2016) could be used to quantify the flow of organic matter from dead corals to macroalgae in an experimental setting, or seawater from reefs with varying levels of coral mortality could be collected and used to test the responses of macroalgae.

4.6 Conclusion

In conclusion, this study highlights how mass coral mortality events, trigged by marine heat waves, may add additional sources of nutrients into coral reef biogeochemical cycles, which are available to opportunistic macroalgae. These changes in nutrient dynamics could have significant impacts on coral reefs, particularly if those sources are specifically becoming more available because key ecosystem engineers such as scleractinian corals are in decline (Wild et al., 2011). It also suggests that these nutrients can be retained within reefs and can

have both short-term and long-term impacts on their biogeochemical cycles. Although it is not yet known how long these nutrients remain in the system, if other environmental conditions are favourable enough, then corals might still be able to recover (Graham et al., 2015). However, if these same reefs are also facing other local anthropogenic stressors, such as nutrient runoff or overfishing of herbivores, then large coral mortality events may result in competitive advantages to benthic organisms such as macroalgae, leading to a benthic regime shift (Ainsworth et al., 2020). This emphasises the critical need to manage local stressors by detecting and reducing nutrient runoff and other drivers, especially on reefs that do still have high abundance of corals, and/ or have recently bleached.

GENERAL DISCUSSION

Relative to other fields of study that have quantified the impacts of climate change and overfishing on coral reefs (Ledlie et al., 2007; McClanahan et al., 2011, 2012; Wilson et al., 2012; Graham et al., 2013; Hughes et al., 2017, 2018; Robinson et al., 2019), progress has been much slower for understanding the effects of nutrient enrichment. This is due to the current lack of cost-effective sensors, although this is continually improving with research and developments in technology (Daniel et al., 2020), as well as a tendency to only rely on one or two measurements of nutrients, such as periodic collections of seawater "spot measurements" that do not always capture the high spatio-temporal variability of nutrients in the water column (Fabricius et al., 2012). From this, the overall concept of using reef-associated macroalgae, and the stable isotopic and elemental signatures in their tissues, as bioindicators of nutrient regimes (Costanzo et al., 2001; Fong et al., 1998; García-Seoane et al., 2018a&b) formed the initial premise of this thesis. Overall, the findings of my research add novel information to the broad literature on why both macroalgae and stable isotope analyses combined are effective bioindicators over a range of temporal and spatial scales, as are my findings on how their effectiveness can also be influenced by various biological and physical drivers (Raimonet et al., 2013; Fong & Fong, 2014, 2017; Fong et al., 2020). In this thesis, I investigate the effectiveness of several common techniques for understanding nutrient signatures in coral reef ecosystems across a range of biological (single species vs. multi-taxa suite of bioindicators), biochemical (nitrogen- and carbon-based stable isotopic and elemental analyses), spatial (< 10 km to a regional/ multiple-island scale), and temporal (days to years) scales. One of these techniques is then applied to a real-world scenario in which two mass coral bleaching and mortality events occurred in two different biogeographical areas to further our understanding of how nutrient impacts can coincide with other physical stressors, even if indirectly.

As coral reefs are currently facing a multitude of interacting and cumulative impacts from both global and local drivers on their ecosystem functions and closely associated ecosystem services (Ban et al., 2013; Fong et al., 2017; Harborne et al, 2017; Hughes et al., 2017, 2018; Donovan et al., 2018; Jouffray et al., 2019; Williams & Graham, 2019; Woodhead et al., 2019), it is critical that both scientists and environmental managers continually evaluate and improve these methods, using ecosystem-based approaches. This interdisciplinary approach to natural resource management can be achieved through a combination of long-term monitoring of both ecological and physical attributes of ecosystem state (Graham et al., 2015; Flower et al., 2017), ecological modelling to understand the complex links between them (Renken & Mumby, 2009; McClanahan et al., 2011; 2012) and empirical studies to gain a more in-depth understanding of responses to these drivers (Fong et al., 2018). Bioindicators therefore need to be adaptable enough to better quantify these anthropogenically-driven changes on these complex and dynamic ecosystems, particularly for assessing drivers of changes like nutrient enrichment (Littler & Littler, 2006; Flower et al., 2017; McWilliam et al., 2018; Zubia et al., 2018).

Although this discussion is generally structured around the four research questions set out in the **Thesis Aims & Outline**, I will also highlight several links between the key themes addressed across the four chapters to demonstrate how using a wide range of monitoring surveys and empirical studies can provide a more holistic understanding of how to best quantify nutrient regimes on coral reefs. However, the multi-level approach used in this thesis is not exhaustive and there were limitations in each chapter, as well as many knowledge gaps that still need to be addressed in future research. These will be discussed in the **Limitations and wider implications of research.**

The cost-effectiveness of bioindicators for monitoring programs

Before a bioindicator, or the methods used to apply them for capturing nutrient impacts, can be integrated into any current or future monitoring program, several factors regarding costs and benefits need to be considered carefully first. A decision tree can be useful as a non-exhaustive tool to provide hypothetical examples for how scientists and environmental managers could make such critical decisions (*Fig. 5.1*). A decision tree such as the one in Figure 5.1 could be implemented either in the early stages of planning monitoring programs, or in updates to ongoing programs to improve any potential shortcomings, such as any gaps in data or knowledge. These options will be particularly important to consider during initial planning stages if scientists and/ or environmental managers only have limited access to target sites (e.g. during research cruises) or otherwise can visit a site more than once, but do not have access to a laboratory or facilities (e.g. for running laboratory experiments). If it is the latter, an alternative approach could be to use one carefully-selected bioindicator species from one area with historical ecological and water quality data and transplant specimens on all target sites (Costanzo et al., 2001; Fernandes et al., 2012). However, this is also dependent on the existence of long-term monitoring programs in these areas.

In areas where resources, facilities and/ or personnel are often limited, such as in remote areas or in small island developing nations (SIDS) (Singh & Mee, 2008; Barnes et al., 2019; Hafezi et al., 2021a&b), it is also important to consider how else the decision tree (*Fig. 5.1*) could be used to determine not only the most cost-effective but the most feasible options for any current or future monitoring programs. For instance, a program could have the funds and equipment to conduct either passive or active biomonitoring in the field (e.g. boat hire and fuel, survey and sampling equipment), but has no or limited access to a laboratory to process and analyse samples (e.g. drying, crushing, weighing instruments and equipment and/or

running stable isotopic analyses). In addition, a monitoring program could have a lack of personnel with the required training and/ or expertise to undertake complex laboratory experiments or run instruments. In either hypothetical scenario, an alternative option could be to ship samples to another laboratory that does have the appropriate facilities and personnel, either in-country or internationally, and have them analysed there. While this approach would likely generate additional financial (e.g. shipment costs, analysis costs, salaries) and time costs (e.g. obtaining import and export permits; time taken to generate the data from shipment to the analysis stage), it would also open up more possibilities to local scientists and decision-makers that might not otherwise have been able to consider it as a feasible option for their monitoring programs. Decision analyses can then be used to help local scientists and decision-makers, who may be on limited budgets and are therefore struggling to either monitor degraded reefs or to implement any management actions against pollution, to determine the most cost-effective options for their monitoring programs (Barnes et al., 2019).

Another key aspect for local scientists and environmental managers to consider is how to overcome any financial or logistical barriers that prevent them from considering some of the options in the decision tree. One of the more successful, large-scale management strategies is to get multiple local and international stakeholders, (e.g. academics, government agencies, Non-Governmental Organisations (NGOs), local and international industries, lawmakers, and local communities) actively engaged in discussions and collaborations from the outset (Wilson & Forsythe, 2018; Hafezi et al., 2021a&b). This transdisciplinary approach can help in highlighting any areas where there has been a lack of progress in the management of local stressors due to limited resources or capacity (Boesch, 2019), particularly in areas with degraded and poorly managed coral reef ecosystems, or poor wastewater management (Barnes et al., 2019). For instance, these transdisciplinary partnerships can not only help to

improve coral recovery and/ or restoration, but they can inspire local capacity building, subsidise costs when local funds and resources are limited, provide incentives, or create binding agreements when targets for pollution reduction are not being met or certain stakeholders are not fully engaged in monitoring or mitigation strategies (Duarte & Krause-Jensen, 2018; Wilson & Forsythe, 2018; Anderson et al., 2019; Boesch, 2019; McLeod et al., 2019; Hafezi et al., 2020a). Therefore, aligning international processes and local practices from multiple stakeholders gives local monitoring programs greater capacity to enhance both ecological and social resilience (Barnes et al., 2019; Boesch, 2019; McLeod et al., 2019).

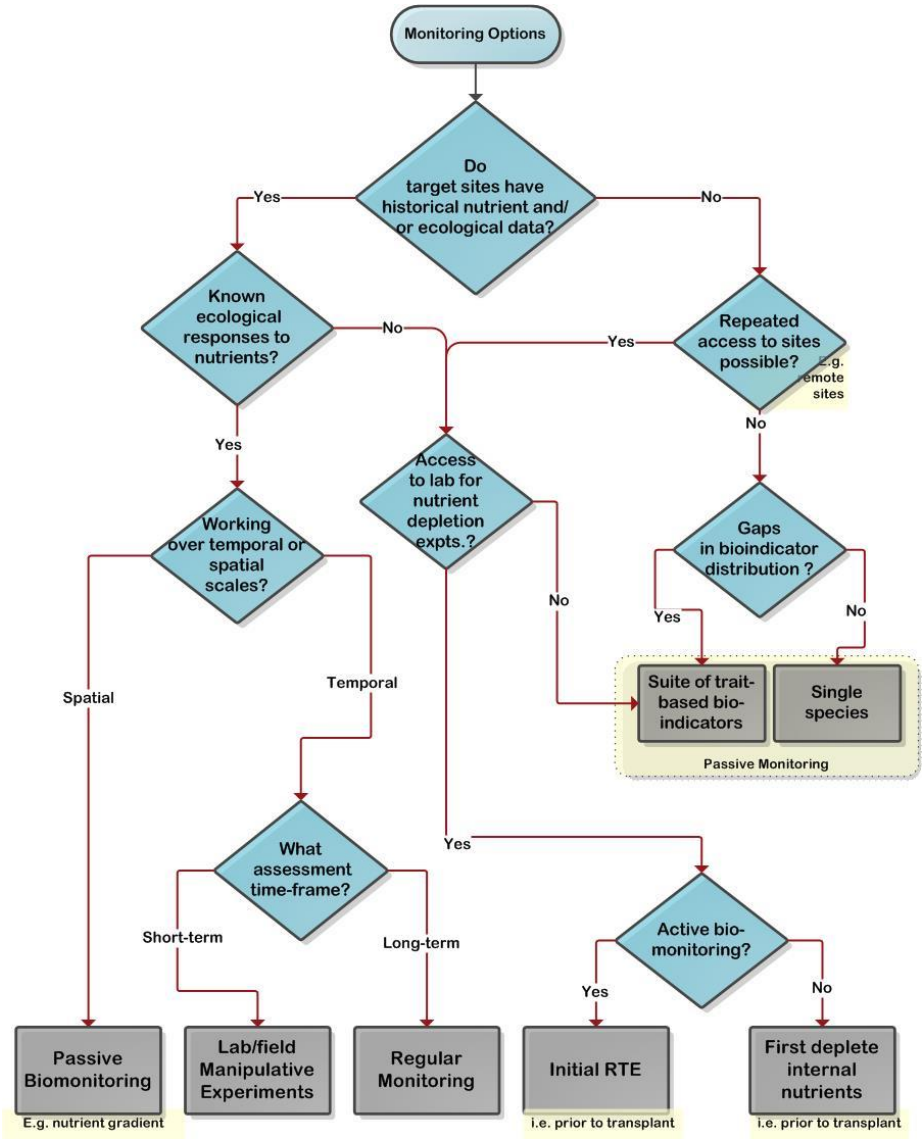

Figure 5.1 Decision framework based on the findings about the uses, costs and benefits of the different bioindicator-related methodologies within and across chapters in this thesis.

What makes a good bioindicator?

Once a decision has been made on the associated methodologies that can make a larger monitoring program more cost-effective, scientists or environmental managers then have to decide which bioindicator(s) will be the most suitable. A number of key criteria have been defined to determine what makes a good bioindicator (Cooper et al. 2009; *Table 5.1*), and when complemented with a number of other key bioindicator-related studies (Linton & Warner, 2003; De'ath & Fabricius, 2010; Ferreira et al., 2011; Fabricius et al., 2012; Gorman et al., 2017; Zubia et al., 2018), provide a useful structural framework for the overall thesis and the aims of the four chapters. These criteria can be applied to both single-species, single-signature approaches as well as to those using a suite of bioindicators, including those with multiple species and multiple nutrient signatures. However, I also expanded on this by evaluating how even good bioindicators can be affected by biological, spatial and/ or temporal variability, as well as where alternative measurements of nutrients may be beneficial, and some of these factors were measured or considered in more than one chapter at different scales. This helped to inform decisions on the type of method(s) to apply to a real-world scenario (i.e. two mass coral mortality events).

Table 5.1 Criteria for selection of bioindicators to assess effects of changes in water quality on corals and coral communities, and linked to the relevant chapters in this thesis. Adapted from Cooper et al. (2009).

CRITERIA	DEFINITION	RELEVANT THESIS CHAPTER(S)
Specificity	Biological response is specific to the stressor of interest and not to other environmental stressors	**1, 2, 3, 4**
Monotonicity	The magnitude of the biological response should reflect the intensity and duration of the stressor of interest	**2, 3**
Variability	Biological responses should be consistent at a range of spatial and temporal scales. Ideally, there should be low background variability although a change in variance can itself be used as an indicator of an impact	**1, 2, 3, 4**
Practicality	Measurements of biological responses should be cost effective, easy to measure, non-destructive and observer independent	**1**
Relevance	Biological response should be ecologically relevant and important in public perception to assist communication	**1, 4**

Single-species, single-signature approach versus a suite of bioindicators

Although there is a growing body of evidence to suggest that the single-species approach for bioindicator systems has many limitations, particularly in terms of abundance and distribution (**Chapter 1**; Linton & Warner, 2003), it may still be more appropriate in certain situations, such as for empirical studies or for monitoring programs with limited time or resources (*Fig. 5.1*). However, this depends heavily on the overall goals and priorities of any study or monitoring program. For instance, it may be more cost-effective to use a single taxonomic species in transplantations across wide spatial areas (active biomonitoring; **Chapters 2 & 4;** Fong et al., 1998; Costanzo et al., 2001; Dailer et al., 2010, 2012; Fernandes et al., 2012; Alquezar et al., 2013; García-Seoane et al., 2018a) or to collect native samples across nutrient gradients or across whole regions (passive biomonitoring; **Chapters 1 & 2**; Barr et al., 2013; García-Seoane et al., 2018,b; Zubia et al., 2018). Costs may influence monetary output for resources, consumables, personnel, transportation, sample analyses etc., but it could also

include sampling effort and timeliness of collecting and processing samples (**Chapter 1**; Barnes et al., 2019; Bal et al., 2020). Conversely, there is also a risk of relying on a single-species and/or single-signature approach to understand nutrient impacts on coral reefs at the ecosystem or community level. They might only be site-specific, or absent at a number of key target sites, and consequently not very generalisable for assessing nutrient regimes on a multitude of sites over larger spatial scales (Linton & Warner, 2003).

A suite of carefully selected bioindicators may be able to inform scientists and environmental managers more about local nutrient regimes than a single species alone, especially if they are hoping to understand the ecosystem-based responses of reefs and reef-associated organisms to nutrient impacts (Zubia et al., 2018). When paired with ecological data, I showed in **Chapter 1** that this approach not only helped to fill in the distributional gaps (i.e. for the two macroalgal species on coral-mortality reefs), but can also reveal more ecologically-relevant information about reef community structure, particularly when comparing reefs in different ecological states (Graham et al., 2015). For instance, although their nutrient signatures had high variability in nutrient signatures, turf algal assemblages were present at all reefs. However, abundance alone is not a strong enough indicator for understanding any links between community structure and nutrient regimes, as was shown in the results for sediment.

A suite of nutrient signatures can provide more information about the origin and amount of the nutrients within the tissues of bioindicators, such as increases in $\delta^{15}N$ and %N, more negative values in $\delta^{13}C$, and low C:N ratios can indicate anthropogenic sources (Atkinson & Smith, 1983; Raven et al., 2002; Vizzini & Mazzola, 2003; Lin & Fong, 2008; Briand et al., 2015; Carnicer et al., 2015). However, **Chapter 1** showed that $\delta^{15}N$ and %N were the most precise measurements of nutrients across the suite of eight bioindicators, particularly in

brown macroalgae, green macroalgae and zoanthids, which supports their use in numerous other studies (Costanzo et al., 2001; Cohen & Fong, 2005; Lin & Fong, 2008; Alquezar et al., 2013). However, even the more precise signatures can still be influenced by trophic level (Kristensen et al., 2018; Shipley & Matich, 2020) and/ or other causes of isotopic fractionation such as light intensity, depth and geographic variability (Marconi et al., 2011; Raimonet et al., 2013; Viana & Bode, 2013; Swart et al., 2014).

Chapters 1, 2 & 3 all showed that biological mechanisms involving nutrient uptake, assimilation and/ or storage in individual species can play a role in how they respond to nutrient enrichment as well as how that is reflected in their tissue nutrient signatures (Fong et al., 2001; Szmant, 2002; Bell et al., 2007; Raimonet et al., 2013; Viana & Bode, 2013; Ochoa-Izaguirre & Soto-Jimenez, 2015). For instance, both the *in situ* and laboratory empirical studies (**Chapters 2 & 3**) showed that the opportunistic brown macroalgae *Dictyota bartayresiana* and the slower growing, lightly-calcified *Padina boryana* had significantly different responses to nutrient enrichment, which was reflected in their nutrient signatures (i.e. higher %N in *Dictyota*), even though they were taken from the same reef and they had been classified as belonging to the same functional group (Littler & Littler , 1980; Delgado & Lapointe, 1994; Umezawa et al., 2002; Fong & Paul, 2011; Clausing & Fong, 2016; Fong & Fong, 2014, 2017).

Impacts on the effectiveness of bioindicators

Biological variability

Another key finding from **Chapter 1** was that, out of the eight initial candidates, the two macroalgal species and zoanthids were the three most precise bioindicators across the coral reefs on which they were present. However, congruency between these three bioindicators was low, even between the brown macroalgae *Sargassum* and the green macroalgae *Chlorodesmis*. This was likely due to a greater internal nutrient storage capacity in the latter (Schaffelke, 1999). Following these results, I reasoned that in order to improve congruency between species in the same suite of bioindicators, macroalgae from similar functional groups should be tested and compared empirically to determine the effect of species-specific responses on nutrient signatures. However, when tested at CRIOBE in Mo'orea, French Polynesia, both **Chapters 2 & 3** showed that responses even between two brown macroalgal species from the same functional form group are heterogeneous and unique. *Dictyota* and *Padina* are classified as morphologically similar genera in the Functional Group Model (FDM) (Littler & Littler, 1980, 1984; Steneck & Dethier, 1994; Fong & Fong, 2014, 2017), but their responses to nutrient enrichment in my studies differed, even when taken from a low-nutrient reef. This supports the surprising findings in Fong & Fong (2014), which led them to suggest that this unexpected variation in responses between two functionally similar macroalgae may have been a result of the breakdown of functional equivalency due to increasing human impacts (i.e. coastal runoff and overfishing of herbivores).

The findings in **Chapters 1, 2 & 3** also support the suggestion that previous definitions FGMs need to be updated to include finer-scale variation in functional traits, such as different ecological strategies for nutrient uptake and/or storage, within the broader functional group

(McWilliam et al., 2018). Recently, there has been a growing body of research into how applying species traits to assess the functional structure of communities (i.e. a traits-based approach) can provide more ecologically-relevant information than the traditional approach of testing relationships between the severity and type of disturbance and the taxonomic structure of communities (Nyström, 2006; Violle et al., 2007; Litchman et al., 2010; Mouillot et al., 2011; Hevia et al., 2016; McWilliam et al., 2018; Silbiger et al., 2018; Bellwood et al., 2019; Brandl et al., 2019). It has also been found to be a more rapid and cost-effective approach for seascape-level estimates of coral reefs, and is more likely to be incorporated into large-scale monitoring programs (Darling et al., 2017). However, Hu et al. (2019) highlighted how macrobenthos functional trait responses to heavy metal pollution are rarely considered in pollution studies, but is something that should be quantified more in future research. For instance, a biological traits analysis (BTA) not only allowed the authors to differentiate the effects of heavy metals between functional traits along an environmental gradient, but to also identify distinct functional trait changes in microbenthic communities which helped explain shifts in species distributions as well as overall ecosystem function. They also found that heavy metal pollution increased certain functional traits (i.e. opportunistic species) and selected against others.

Collectively, these chapters stress the importance of not underestimating the extent to which even common and cost-effective bioindicators of nutrient regimes could be reflecting their own ecological strategies, rather than nutrient sources and concentration in the surrounding water. This is when using *in situ* sampling and/ or autonomous logging, in parallel to the collection and/ or application of one or more bioindicators, could help to confirm whether or not the changes are also occurring in the water column (**Chapter 3**; Fabricius et al., 2012). If there is any correlation between the two methods, then the extra addition of data from

ecological or hydrodynamic modelling (i.e. for the effects of wave action) could also help to determine either the spatial or temporal extent of any changes in nutrient regimes (Graham et al., 2020; Devlin et al., 2020; Adam et al., 2021). Conversely, by not considering these biophysical factors at the individual scale, especially if there is a lack of historical data, could potentially result in over- or underestimations of nutrient sources and concentrations, and could consequently weaken the overall effectiveness and value of selected bioindicators and/or other measurements of water quality (Linton & Warner, 2003; Mixika et al., 2007; Borja et al., 2012, 2016; Bal et al., 2020).

Spatial variability

In this thesis, I looked at a range of spatial scales for understanding nutrient regimes in both the Seychelles and in Moorea:

a) a multi-island scale **(Chapter 1 & 4**);
b) across a ~3.5 km nutrient gradient (**Chapter 2**);
c) between two sites ~ 2.5 km apart with known differences in local nutrient regimes (**Chapter 2, 3 & 4**).

I compared both passive and active biomonitoring methodologies using the same species, using two of the same sites (**Chapter 2**), and I also used different methods of macroalgal transplants, or bioassays, by a) taking local nutrient history and ecological strategies into account in both **Chapters 2 & 3,** and b) depleting internal nutrients in laboratory holding tanks to make them more responsive to nutrient regimes on a target reef in **Chapter 4**. Finally, I also considered the advantages and disadvantages of using *in situ* experiments against manipulative laboratory experiments for the same species from the same sites over the same time period of 3 days (**Chapters 2&3**).

Chapters 1, 2 and 3 all emphasised once again how important the ecological strategies of nutrient uptake (e.g. functional trait) in bioindicators are, particularly when assessing them across different spatial scales (Clausing & Fong, 2016). Local nutrient history of a site is also important, as the same species of macroalgae, including the opportunistic types, can respond differently to nutrient enrichment on different reefs (Fong et al., 1994, 2003; Fong & Paul, 2011). This is because some are already saturated with nutrients from regular exposure to runoff of high concentrations, so they are no longer nutrient limited, and growth is likely more limited by other factors such as reduced light intensity because of increased sedimentation (Beach et al., 2006; Clausing & Fong, 2016; Clausing et al., 2016). However, even on reefs with typically low nutrient concentrations, the slower-growing species *Padina* showed little response to nutrient enrichment in both **Chapters 2 & 3**. Across these chapters, I assessed how nutrient history and ecological strategy impacted their responses to nutrients, both through *in situ* (**Chapter 2**) and in a manipulative laboratory experiment (**Chapter 3**). The experiment lasted the same period of time (3 days) so it made it possible to compare how different methodologies can also have influences on the nutrient signatures of the same species, which is not often tested in the literature.

Another key aspect of a good bioindicator is being able to capture a gradient in the physical driver it is assessing against attributes of community structure (Linton & Warner, 2003; Fabricius et al., 2005; 2012; Arévalo et al., 2007; Lin & Fong, 2008; Devlin & Schaffelke, 2011; Williams et al., 2013; Kürten et al., 2014; Gorman et al., 2017; Zubia et al., 2018; Hu et al., 2019). It was found that correlating distance from the river mouth with $\delta^{15}N$ in both *Dictyota* and *Padina* showed clear spatial patterns of nutrients across Opunohu Bay in Mo'orea, relative to %N. Measuring the spatial extent of nutrient enrichment through collections of bioindicator samples along this gradient, such as from a river mouth or

coastline with known anthropogenic runoff, is also a possibility, both for identifying nutrient sources and for mapping the spatial extent of anthropogenic runoff (Costanzo et al., 2001). This technique has also been used in previous studies for assessing both water quality and ecological attributes along the reefs of Opunohu Bay (Lin & Fong, 2008), as well as around the whole island region of Mo'orea (Adam et al, 2020), but also across reefs in other biogeographic regions and countries such as Japan (Umezawa et al., 2002), the Red Sea (Kürten et al., 2014), and Australia (Costanzo et al., 2005). Therefore, this thesis provides supporting evidence for another widespread and applicable methodology for nutrient bioindicators. It can also be particularly useful if comparing the nutrient signatures between multiple taxa along nutrient gradients (Kürten et al., 2014), even between morphologically-similar species from the same functional group (Petchey & Gaston, 2006; McWilliam et al., 2018; Hu et al., 2019), to also look at the correlations and slopes of regression models between the signatures across the spatial scales being studied (Connolly et al., 2013), as demonstrated in both **Chapters 1 & 2**.

If passive or active biomonitoring methodologies for assessing nutrient impacts (e.g. both bioindicators and other water quality measurements) are coupled with ecological data, such as macroalgal cover, macroalgal species richness (McCook, 2001; Karez et al., 2004; Fabricius et al., 2005; Arévalo et al., 2007; Kürten et al., 2014), proportions of primary producers and consumers (Kristensen et al., 2018), and interactions between algae and corals (Barott et al., 2012b), it may reveal gaps in their distribution across the areas being studied. For instance, in **Chapter 1**, *Sargassum and Chlorodesmis* was absent across a number of the twelve coral-mortality reefs in the Seychelles, but zoanthids were common across both reef states, and turf algal assemblages were present at all 21 reefs. In addition, *Padina* was absent at two sites across a total of eight along the nutrient gradient in Mo'orea (**Chapter 2**), which

thus weakened the statistical power of the correlation between changes in its nutrient signatures and distance from the river mouth.

Active biomonitoring methodologies may be an appropriate option if there are gaps in distribution of bioindicators, especially if scientists and/ or environmental managers have access to a laboratory or facilities to deplete internal nutrients (García-Seoane et al., 2018a). For instance, in **Chapter 4**, *Sargassum* was absent on the section of the forereef being studied, so specimens from the low-nutrient Papetoai lagoon were transplanted there to determine how they responded to local nutrient regimes. Even if they had some nutrients already stored in their tissues from their native environment, allowing them to deplete these in holding tanks for a short period of time means that they will be nutrient limited and therefore much more responsive to any changes in the surrounding water column. This methodology is therefore suitable for use across both small (Opunohu Bay, Mo'orea; Lin & Fong, 2008) and large spatial scales (around the island of O'ahu, Hawai'i; Dailer et al., 2010).

Temporal Variability

If passive biomonitoring methodologies are the preferred option, the tissue samples from one or more selected bioindicators could be collected on a regular basis (e.g. every few months to twice a year). This could be particularly useful to environmental managers if they want to look at long-term temporal variation in community structure (i.e. between wet and dry seasons in the tropics) (Duran et al., 2016; van Alstyne, 2016). Tracking the spatial extent of nutrient run-off over time could be even more informative for assessing the temporal impacts on coral reefs and reef-associated organisms in monitoring programs, as bioindicators capture

a cumulative depiction of nutrient loads over time, and changes in reef community structure and biodiversity can be measured in parallel. However, if scientists or environmental managers wish to assess any changes in trends in nutrient levels or sources, particularly in areas where it is possible to access sites more than once, then transplanting algae (active biomonitoring) could be a very useful alternative. They can not only capture changes in nutrient signatures but can also measure changes in other biological response parameters (i.e. functional traits or indicators), such as growth rate (Linton & Warner, 2003). Once again, this all depends on the initial nutrient history and the ecological strategies of the selected bioindicator(s), as both **Chapter 2 & 3** show that these factors can affect the responsiveness of even fast-growing opportunistic species, so it is important to consider both the bioindicator(s) and the methodology it is being applied to during the early planning stages of any monitoring program.

Macroalgae are not only widely available indicators, but this thesis provides several examples for how they can be adapted for a range of temporal scales:

1) Short-term (3 days, **Chapters 2 & 3**),
2) Moderate-term (3 weeks, **Chapter 4**),
3) Long-term (3 years, **Chapter 4**)

Very little is currently known about the temporal variation in nutrient availability on coral reefs, relative to temperate systems (Fong & Fong, 2017). Therefore, I used the *in situ* reciprocal transplant experiment in **Chapter 2** to compare the same species from the same two sites across a short spatial scale (~3.5km) to see whether fine-scale temporal variability in the bioavailable nutrients from the surrounding water column might also influence their responses. The manipulative laboratory experiment I conducted in **Chapter 3** showed relatively similar results to **Chapter 2**, where *Dictyota* from the nutrient-limited reef

responded to a change in nutrient source/ supply, particularly from the Pulse treatment, as there was a significant decrease in $\delta^{15}N$. There was even a small increase in %N, though it was not significant. This differed slightly from the findings in Fong & Fong (2017), where there was a slight increase in growth in *Padina* after a press treatment, but as there was a significant change in *Dictyota* even in the Control treatment, there could have been other sources of nutrients in the forereef water that complemented the artificial nutrient stock solutions used in the experiment, and thus influenced the average $\delta^{15}N$ in the tissue samples. This also demonstrates how sensitive an indicator $\delta^{15}N$ can be, relative to %N (Lin Fong, 2008; Cooper et al., 2009; *Table 5.1*), if it can also detect background variability, but emphasises the need for further study to see how much of an effect that variability has on bioindicators relative to treatments.

Episodic storm or rainfall events can deliver pulses of high nutrient concentrations to reefs (Brodie et al., 2010b), which can benefit some fast-growing, opportunistic macroalgae over other functional groups, even within macroalgal communities (Lapointe & Bedford, 2011; Fong & Fong, 2017; Fong et al., 2020). However, in Mo'orea, there is a known fluctuating nutrient regime on a continuum from press nutrient subsidies to pulsed nutrient subsidies (Anderson et al., 2008; Nowlin et al., 2008; Yang et al., 2008). As rainfall events are expected to increase in intensity and severity under predicted climate change scenarios, there could be an increase in pulsed events that favour opportunistic macroalgae, with the continual build-up of nutrients in slower-growing species that prefer a steady state of low concentrations (Anthony et al., 2014). This could potentially be advantageous to several algal species with different ecological strategies (functional traits) and responses to nutrient enrichment. This is because it would allow more species to co-exist and even result in a more diverse macroalgal community (Clausing et al., 2016; Fong & Fong, 2017; Fong et al., 2020).

In addition, this brings traditional ecological theories about functional diversity (defined as a diverse range of functional roles) and functional redundancy (defined as multiple species sharing similar arrays of traits) to light. Changes in the diversity of functional roles are increasingly being recognised in the literature as a more useful indicator of ecosystem function and ecosystem services than species richness (Nyström, 2006; Petchey & Gaston, 2006; Hevia et al., 2016; McWilliam et al., 2018). Therefore, it could be possible that while opportunistic species are becoming more dominant on disturbed reefs, there still could be some functional diversity within macroalgal communities in areas where there is temporal variability in nutrient supplies (Petchey & Gaston, 2006). If this were the case, then communities with a diverse range of functions is expected to be more resilient to disturbance, even with the effects of herbivory on reefs, as the cover and/or composition of the algae that reef-associated fish and invertebrates typically consume may change (Cheal et al., 2010, Fulton et al., 2019). In addition, nutrient enrichment can enhance the thickness of the thalli of leathery algae like *Turbinaria ornata* in Moorea (Bergman et al., 2016; Bittick et al., 2016). Increased rainfall can also result in increased turbidity and/ or sedimentation (resuspension) (Anthony et al., 2014), which may supply provide another key source of nutrients to macroalgae, and can reduce light availability, and therefore functional responses like photosynthesis and growth (Risk et al., 2009; Clausing et al., 2016; Fong et al., 2020).

Bioindicators of nutrient regimes for multiple ecosystem states

We are living in a time where coral reefs are in decline because of a number of stressors impacting them, either synergistically, antagonistically, or additively (Fong et al., 2017; Jouffray et al., 2019; Williams & Graham, 2019), it is more critical than ever to not only quantify these stressors on both global and local scales at the ecosystem level, but to also use an ecosystem-based approach for finding solutions to monitor and/ or mitigate them. This is particularly important now, as many reef biological responses to physical drivers are typically non-linear (Gove et al., 2015; Jouffray et al., 2019), but anthropogenic stressors are further modifying and/ or disrupting these natural biophysical relationships (Williams et al., 2015; Williams & Graham, 2019). This can lead to coral reefs shifting from coral-dominated states to alternative stable states, such as macroalgal-dominated reefs (Nyström et al., 2009; Graham et al., 2015). Once the shift has occurred, they become locked in positive feedback loops that are very difficult to break (Dell et al., 2016; Dajka et al., 2020, 2021), as macroalgae can supply themselves and their propagules with resources through tissue breakdown (Diaz-Pulido & McCook, 2005). Therefore, it would be far better to reduce local stressors (i.e. pollution and overfishing) from reefs before a threshold, or a tipping point is reached. **Chapter 4** demonstrates that even large episodic pulses of naturally-derived nutrients, triggered by anthropogenic events, can have impacts on coral reef organisms and local biogeochemical cycles (Mumby & Steneck, 2018; Radice et al., 2020). For instance, even though the results are not conclusive, both case studies that assessed the impacts of the mass mortality events in the Seychelles (2016) and in Mo'orea (2019) strongly suggested that macroalgae rapidly take up large amounts of nutrients from dead coral tissue after it is released into the surrounding water column.

Although it was not tested in **Chapter 4**, the results from both case studies implied that mass coral mortality events not only open up substratum to opportunists such as macroalgae, microbial organisms, cyanobacteria and sponges, but that these species can also then take up these nutrients and recycle them within reef systems (Kolasinski et al., 2011; de Goeij et al., 2013; Pawlik et al., 2016; Rix et al., 2016, 2017, 2018; Mumby & Steneck, 2018), which would likely only exacerbate their proliferation on reefs. This study also suggests that nutrient uptake can occur in macroalgae within a matter of weeks, if not sooner, and can be retained in local reef systems for at least twelve months. This demonstrates one of the advantages of using transplants, or bioassays, of a precise and cost-effective bioindicator for investigating temporal variation in nutrient regimes over periodic collections of seawater nutrients (García-Seoane et al., 2018ab, Bal et al., 2020), as sampling intervals would need to be far less regular, although isotopic enrichment was also detected in seawater POM eight months after a mass bleaching event in Radice et al. (2020). Therefore, using both methods in future studies could provide more conclusive results.

Limitations of thesis and implications for future research

This thesis discusses and empirically tests several methodologies that could be applied to a number of spatial and temporal studies on macroalgal bioindicators of nutrient regimes, but it is by no means exhaustive. For instance, **Chapter 1** assessed the precision and cost-effectiveness of a suite of both ecological and nutrient bioindicators, which is a better way of obtaining an ecosystem-based response to nutrient regimes relative to a single-species, single-signature approach, but the low congruency between the most precise bioindicators (brown macroalgae, green macroalgae and zoanthids) show that species-specific responses to nutrient enrichment still have to be considered. Therefore, a third approach could combine both of the aforementioned methods by selecting a suite of bioindicators within the same functional group to look at the links between drivers of change and ecosystem functions at the ecosystem level. This was tested to some extent in **Chapters 2 & 3,** but future work could take this further by using a more traits-based approach with a greater number of functionally-similar species (i.e. functional redundancy). This would involve building a suite of bioindicators with the same finer-scale functional traits (i.e. rapid, direct nutrient uptake mechanisms) from the same functional group (i.e. opportunistic species) in a hierarchical classification system (Hevia et al., 2017; McWilliam et al., 2018). Theoretically, the species within these groups should then have shared effects and responses to drivers of change such as nutrient enrichment (Litchman et al., 2010).

The traits-based approach is becoming increasingly common in both scientific and environmental monitoring studies (Hevia et al., 2016; Bellwood et al., 2019), so responses cannot be species- or site-specific in order to be applied to reefs needing local monitoring programs across various spatio-temporal scales, or even across vast biogeographic regions

(Linton & Warner, 2003). This would certainly be useful if there are gaps in the distribution of a single species across target sites in passive monitoring studies, as multiple species with congruent responses would compensate for these absences (Linton & Warner, 2003). However, as **Chapter 2 & 3** suggest that fluctuating nutrient regimes can result in the co-existence of multiple species within diverse macroalgal communities, it could be equally interesting to investigate the diversity of responses of macroalgae with a range of nutrient uptake, assimilation and/ or storage mechanisms within the same communities (functional diversity) (Nyström, 2006; Petchey & Gaston, 2006; Christie et al., 2019). This could allow scientists to directly compare functional redundancy against functional diversity to determine which of the two is able to capture a more realistic estimate of the overall high variability of nutrient regimes in these diverse communities (Nyström, 2006; Petchey & Gaston, 2006; Savage et al., 2007; McWilliams et al., 2018). In addition, this could help to improve understanding of how macroalgal community structure may change during a time when multiple stressors increasing in severity impacting these ecosystems, such as increasing rainfall and storms that tend to favour opportunistic algae (Fong & Fong, 2017). Another example of future research using a suite of bioindicators could involve using multiple taxa from several trophic levels within the same food web (Kristensen et al., 2014; Kürten et al., 2014; Shipley & Matich, 2020). Stable isotopes have been used in many trophic ecology studies in the literature (Boecklen et al., 2011; Bierwagen et al., 2018; Kristensen et al., 2018; Bedford et al., 2020), but as food chains have been found to shorten on degraded reefs (Hempson et al., 2017), this approach could provide another ecosystem-based methodology for understanding changes in both nutrient regimes and food webs across reefs in different states of degradation.

Another advantage of using macroalgal stable isotopic and elemental signatures is that they can help to better quantify the biophysical relationships between ecological attributes such as macroalgal cover and water quality along environmental gradients. This is because these signatures are only caused by nutrients that the macroalgae are able to take up, and are therefore more ecologically-relevant (Kristensen et al., 2014; Kürten et al., 2014; Carnicer et al., 2015; Fox et al., 2018; Lachs et al., 2019) than nutrient levels only obtained from seawater "spot measurements" (McCook, 2001; Fabricius et al., 2005, 2012). A lot of studies have measured benthic cover and composition along physical nutrient gradients, however, not many of them tell us at what point (i.e. the nutrient concentration) along that gradient the threshold or the "tipping" point occurs, where reefs start shifting to alternative states (Knowlton, 1992; Lapointe, 1997; Scheffer & Carpenter, 2003; McManus & Polsenberg, 2004; Bell et al., 2007; Mumby et al., 2007; Norström et al., 2009; Wooldridge, 2009b; De'ath & Fabricius, 2010; McClanahan et al., 2011; Graham et al., 2015; Gove et al., 2015). One way to develop this approach could be to use advanced ecological modelling to test the relationships between multiple metrics of ecosystem state, for instance between a range of ecological attributes (e.g. macroalgal cover, coral cover, macroalgal species richness, % herbivory, coral: macroalgae ratio, opportunistic: perennial algal species ratio etc.) and a physical driver (e.g. nutrient loads) (Sangil & Guzman, 2016; Zubia et al., 2018). Models such as Bayesian switch-point analyses (McClanahan et al., 2011), Generalized Additive Models (GAMs; Karr et al., 2015) and Principal Component Analyses (PCAs; Chong-Seng et al., 2012) have been used in the past to determine ecologically-relevant threshold points along gradients of fishable biomass against several key attributes at multi-regional scales, so that thresholds can be generalizable to scales relevant to management (McClanahan et al., 2011; Karr et al., 2015). However, as some studies claim nutrient fluxes are too dynamic to predict a single threshold value, which would therefore not applicable to all reefs under all

conditions (Szmant, 2002; Groffman et al., 2016), this approach could potentially help to capture a range of values in which a tipping point may occur on reefs.

To date, there are no studies that use this sophisticated approach for quantifying the effects of nutrient loads, even with substantial and highly regular collections of seawater nutrient samples from a vast area. Therefore, a more cost-effective and ecologically relevant approach, especially in coastal areas with limited funds and resources, could involve collected macroalgal samples from a wide geographical area, perhaps even across multiple regions and countries, to build up a nutrient gradient within the ecological model. But in order to generate threshold point(s) of nutrient concentrations that can be generalised across large geographical areas, using the traits-based approach for a suite of congruent bioindicators will be more important than ever, as it will likely be impossible to collect the same species at every single site being surveyed (Gartner et al., 2002; Linton & Warner, 2003; Savage et al., 2007; Violle et al., 2007; Fong & Fong, 2014; Darling et al., 2017; Hevia et al., 2017; Bellwood et al., 2018; Zubia et al., 2018; Hu et al., 2019; McQuatters-Gollop et al., 2019; Bedford et al., 2020). Thus, identifying key functional traits in macroalgae (i.e. opportunistic species with rapid nutrient uptake mechanisms) will be key for developing a robust bioindicator system (both structural and functional types of bioindicators; Linton & Warner, 2003) to monitor changes in biodiversity and their effects on ecosystem function and ecosystem services. Furthermore, this ecosystem-based approach can then be incorporated into wider monitoring programs so that target nutrient concentrations can be established and translated into management actions (McClanahan et al., 2011; Chong-Seng et al., 2012; Kroon et al., 2014; Karr et al., 2015; Hevia et al., 2016).

Bioindicators also need to be able to differentiate between responses to natural variability of their local environment and any significant drivers of change (Cooper et al., 2009). For instance, $\delta^{15}N$ of *Dictyota* from the low-nutrient reef Papetoai changed significantly when exposed to a new source of nutrients, whereas in **Chapter 3**, it appeared that it was also reflecting the signatures of background variability in the ambient seawater used in the control, which is possibly why there was a slight decline in the Control specimens. This is another reason why there has been a lag in progress for quantifying nutrient regimes, as unlike temperature, pH or light, nutrient loads are difficult or expensive to monitor in real time (Costanzo et al., 2001). The most common method for this involves measuring changes in nutrient concentrations in experimental seawater across time manually via an autoanalyzer (den Haan et al., 2016), but this can be a timely and costly process, particularly if using individual experimental units like in **Chapter 3**, as it requires substantial numbers of water samples to be processed by personnel with the right expertise. However, it should be noted that there is much that can be still learnt about the origins and concentrations of nutrients from these types of measurements, and so, rather than only using an "either/ or" approach in larger-scale studies or programs, there may be certain occasions, funding and time permitted, where using both methods might be more beneficial overall (Fabricius et al., 2012).

While this thesis has primarily focused on the advantages of using macroalgal stable-isotopic bioindicators over other measurements of water quality, particularly seawater "spot measurements", using multiple methods may equally help to increase the chances of accurately identifying and tracing nutrient loads back to the original source(s). For instance, only using macroalgae in **Chapter 3** meant that the changes in the Control specimens were not detected until the stable isotopic analysis stage. Therefore, complementing the results from the macroalgal tissues with that of seawater measurements taken at the time could have

captured any changes in the local nutrient regime of the forereef waters much sooner. In addition, stable isotopic signatures can also be measured in seawater as well as in POM, which could have provided more evidence for the cause(s) of the significant changes in the N-based signatures, but less so in growth for the control specimens (Sigman et al., 2001; Radice et al., 2020). Furthermore, leaving a wide array of options open for large-scale monitoring programs to potentially use alongside bioindicators, such as hydrodynamic modelling (Adam et al., 2021), high-resolution and cost-effective nitrate sensors (Daniel et al., 2020), or remote sensing (Brodie et al., 2010a&b; Devlin et al, 2012), may increase the chances of successfully detecting and mapping multiple sources of nutrient enrichment and/ or coastal runoff at multiple scales. For instance, it might not always be obvious from the macroalgal signatures alone, such as the suggestive, not conclusive enrichment of *Sargassum* samples from the coral-mortality reefs in the Seychelles in **Chapter 4**. This can then help identify priority areas where management and intervention strategies, such as wastewater management, need to implemented (Barnes et al., 2019; Devlin et al., 2020).

In **Chapter 4**, research permits meant that it was not possible to transplant *Sargassum mangarevense* on more than one reef during the coral bleaching/ mortality event after internal nutrient depletion, including at the original collection site. This was why less emphasis was placed on the results from the Mo'orea case study and more on those from the Seychelles study. Although the former study does support the latter, particularly in a different geographical location, the lack of control made the findings inconclusive as it could not be confirmed that the significant increase in tissue $\delta^{15}N$ was specifically due to the mass release of dead coral tissue or to a combination of multiple sources, such as coastal runoff driven by rainfall or upwelling. However, this chapter does infer some important novel findings for coral reef science, and it is therefore critical to expand on this research by either transplanting

nutrient-limited macroalgal bioindicators on reefs with varying levels of coral bleaching and mortality during such events.

Recommendations for monitoring programs and management

There are many advantages and disadvantages to all the different methods applied in this thesis that could use bioindicators for capturing and quantifying nutrient impacts on coral reefs. However, from my findings in this thesis, I can recommend that the best approach for large-scale environmental monitoring programs with limited access to target sites and/ or laboratories is the passive monitoring methodology. This would involve collecting a suite of bioindicators with the same functional traits and ecological strategies (i.e. for nutrient uptake) across large spatial scales (i.e. around whole island regions or across nutrient gradients) every few months or twice a year if possible, before complementing these sample collections with ecological data from the same sites (Linton & Warner, 2003; Mixika et al., 2007; Borja et al., 2012, 2016; Kroon et al., 2014; Zubia et al., 2018). However, if it is possible to access these sites and/ or laboratories more than once, then active biomonitoring methodologies should be applied to better understand the biological responses of the bioindicators through the transplantation of carefully selected species on target sites, particularly if there are any known gaps in abundance. If the ecological strategies for nutrient uptake or internal history for any potential candidates are not known, then empirical studies could also be conducted prior to any monitoring work. Through this thesis and the previous literature, I would recommend either *in situ* or even mesocosm experiments, rather than laboratory experiments, as it is more difficult and potentially more expensive to monitor and control natural variability in the surrounding water column, and also is more difficult to include other significant co-factors

such as herbivory and sedimentation. However, laboratory experiments can still be valuable for investigating specific responses in individual species and/ or between multiple species without the confounding effects of these other factors that are more difficult to measure *in situ*. In addition, some macroalgae might be less suited to field experiments than others due to their physical fragility or palatability, such as *Dictyota*.

From a management or policy perspective, bioindicators need to be developed with a functional group-based approach in order to apply them to large spatio-temporal monitoring programs rather than be sight specific. This approach is part of the European Union Marine Strategy Framework Directive (MSFD) (Arévalo et al., 2007; Muxika et al., 2007; Bermejo et al., 2012; Borja et al., 2012; 2016; McQuatters-Gollop et al., 2019; Bedford et al., 2020), but has also been adapted to assess the ecological status of coral reefs in areas such as La Réunion island, an overseas French territory (Zubia et al., 2018). Considering the functional traits of one potential bioindicator, or the similar traits of a suite of bioindicators, is especially important if they are being used to understand the effects of drivers of change like nutrient enrichment or climate change at the ecosystem level (Drinkwater et al., 2010; Litchman et al., 2010).

Another vital part of any indicator approach is to reduce and simplify the many complex and often confounding findings from studying such impacts at the ecosystem levels, thus making it easier to communicate any information on state changes to environmental managers and policy-makers (Linton & Warner, 2003; Bedford et al., 2020). In addition, as the inconclusive results in **Chapter 3** showed**,** it is not only critical to set appropriate targets for reducing the impacts of stressors on reefs, but to determine reference conditions using the right methodologies, as previous failures with using bioindicators alone have often been due to the

use of inappropriate methods for setting these conditions (Borja et al., 2012; Brodie & Waterhouse, 2012; Ochoa-Izaguirre & Soto-Jimenez, 2015; Boesch, 2019). However, this is becoming increasingly difficult with baselines shifting in the Anthropocene to novel states and so is an ongoing challenge for environment managers (Jouffray et al., 2019, Williams & Graham, 2019). This thesis highlights the importance of improving functional form models of macroalgae to ensure they also include ecological strategies such as responses to nutrients, as each group should be comprised of taxa with shared effects and responses. Therefore, preliminary work may be needed on a smaller scale first to determine the congruency between species within the selected functional groups, before they can be applied to large-scale monitoring. Empirical, science-based evidence captured through ecosystem-based management is essential to demonstrate the effectiveness of local and international strategies for improving water quality (Flower et al., 2017; Obura et al., 2018).

In order for a monitoring program to be considered "effective", it would require a broader, more comprehensive approach that delivers a "toolbox" of indicators, methods and technologies which can be applied at multiple scales (Devlin et al., 2020). These can range from stable isotopic and elemental signatures in bioindicators, to seawater sampling and *in situ* autonomous logging with nitrate sensors, to hydrodynamic modelling, to large-scale remote sensing, to any combination of these (*Table 0.1*; Fichez et al., 2005; Cooper et al., 2009; Fabricius et al., 2012; Devlin et al., 2020). This holistic approach would thus provide many more viable and flexible options for managers and other decision-makers to apply to their own monitoring programs and management schemes, making it highly adaptable for different coastal regions struggling mitigate multiple sources of pollution, particularly in SIDS (Tsatsaros et al., 2018; Barnes et al., 2019; Anderson et al., 2019; McLeod et al., 2019; Devlin et al., 2019, 2020). However, these decisions should be made at the discretion of local

scientists, organisations and/ or other stakeholders, depending on their individual funding or logistical constraints (i.e. community-based management) (Cummings et al., 2021; Tsatsaros et al., 2021). By creating more of these partnerships, both nationally and internationally, it not only allows these decision-makers to align international processes with local practices, but can help to facilitate local capacity building to ensure long-term success of a monitoring program or management scheme (Behmel et al., 2018; Wilson & Forsythe, 2018; Anderson et al., 2019; Boesch, 2019).

Using adaptive management strategies for reducing nutrient impacts should be combined with efforts to reduce other local stressors, such as overfishing and sedimentation (Risk, 2014; Fong et al., 2017; Boesch, 2019; Devlin et al., 2019; McLeod et al., 2019). Using a holistic approach to tackle cumulative local impacts would therefore help both local communities and coastal ecosystems to cope with global climate change impacts, such as mass coral bleaching and/ or mortality events. By enhancing both social and ecological resilience, it could potentially also prevent any already degraded reefs from undergoing regime shifts to alternative states (Graham et al., 2015; Hughes et al., 2018; Wilson & Forsythe, 2018; McLeod et al., 2019).

Chapter 4 has provided another example to the existing literature on how complex interacting drivers of change are on coral reefs, and how difficult they are to understand individually, let alone synergistically or additively, particularly as natural biophysical relationships are already nonlinear and therefore difficult to predict (Gove et al., 2015; Jouffray et al., 2019). The additional influence of increasing human activities on both global and local scales in the Anthropocene will only exacerbate this problem (Sangil & Guzman, 2016; Williams & Graham, 2019), which is why large-scale and long-term monitoring

programs equipped with a “toolbox” of bioindicators, measurements, and methodologies that can be applied to various different scenarios are more critical than ever (Flower et al., 2017). This will then allow scientists and environmental managers to use an ecosystem-based approach to assess trends over a range of spatial and temporal scales, as well as differentiate between natural variability and anthropogenic influences such as coastal runoff. It is vital to have a better understanding of how ecosystem community structure and biodiversity of organisms, particularly habitat-forming functional types (i.e. scleractinian corals and canopy-forming macroalgae) as well as local biogeochemical cycles, are changing as a result of nutrient impacts (Jackson, 2008; D’Angelo & Wiedenmann, 2014; Hernández-Delgado, 2015; Mumby & Steneck, 2018; Silbiger et al., 2018; Zubia et al., 2018).

GENERAL CONCLUSION

In conclusion, I have found that the use of macroalgae bioindicators, and the methodologies to which they are most commonly applied, can a) help overcome the problem of high spatio-temporal variability of nutrients, making them easier to quantify, b) trace and identify the spatial extent of nutrient runoff, and c) detect the presence of new nutrient inputs on reefs, from either natural or anthropogenic sources, up to a year after introduction. I also show that their effectiveness is context-dependent, and that the type of methodology and/ or bioindicator(s) selected will depend heavily upon the availability of facilities, resources and equipment, funding, training and expertise, access to target sites, prior knowledge of bioindicator functional traits (i.e. nutrient uptake mechanisms), and the presence or absence of historical ecological and water quality data. I have therefore summarised the potential options that both scientists and decision-makers can consider from the decision tree, based on the research conducted in this thesis (*Figure 5.1*). Overall, all four chapters demonstrate that macroalgal bioindicators and the nutrient signatures in their tissues are much more cost-effective, precise and biologically-relevant than routine collections of discrete water samples. I also demonstrate instances when using only one type of measurement or proxy to capture nutrient regimes (i.e. either bioindicators or seawater samples) may present a disadvantage, and thus might require a broader, more comprehensive "toolbox" approach. However, aligning bioindicators to already established long-term monitoring programs would add another, more cost-effective option for scientists and managers in remote areas or small island developing nations that may reveal previously undetected impacts on local nutrient regimes and coral reefs. For instance, they allowed me to detect novel findings on the longer-term impacts of mass coral mortality events on reef-associated organisms and nutrient regimes, which might not have been possible without the data from the long-term monitoring surveys in the Seychelles (Jennings et al., 1995; Graham et al, 2015; Wilson et al., 2019).

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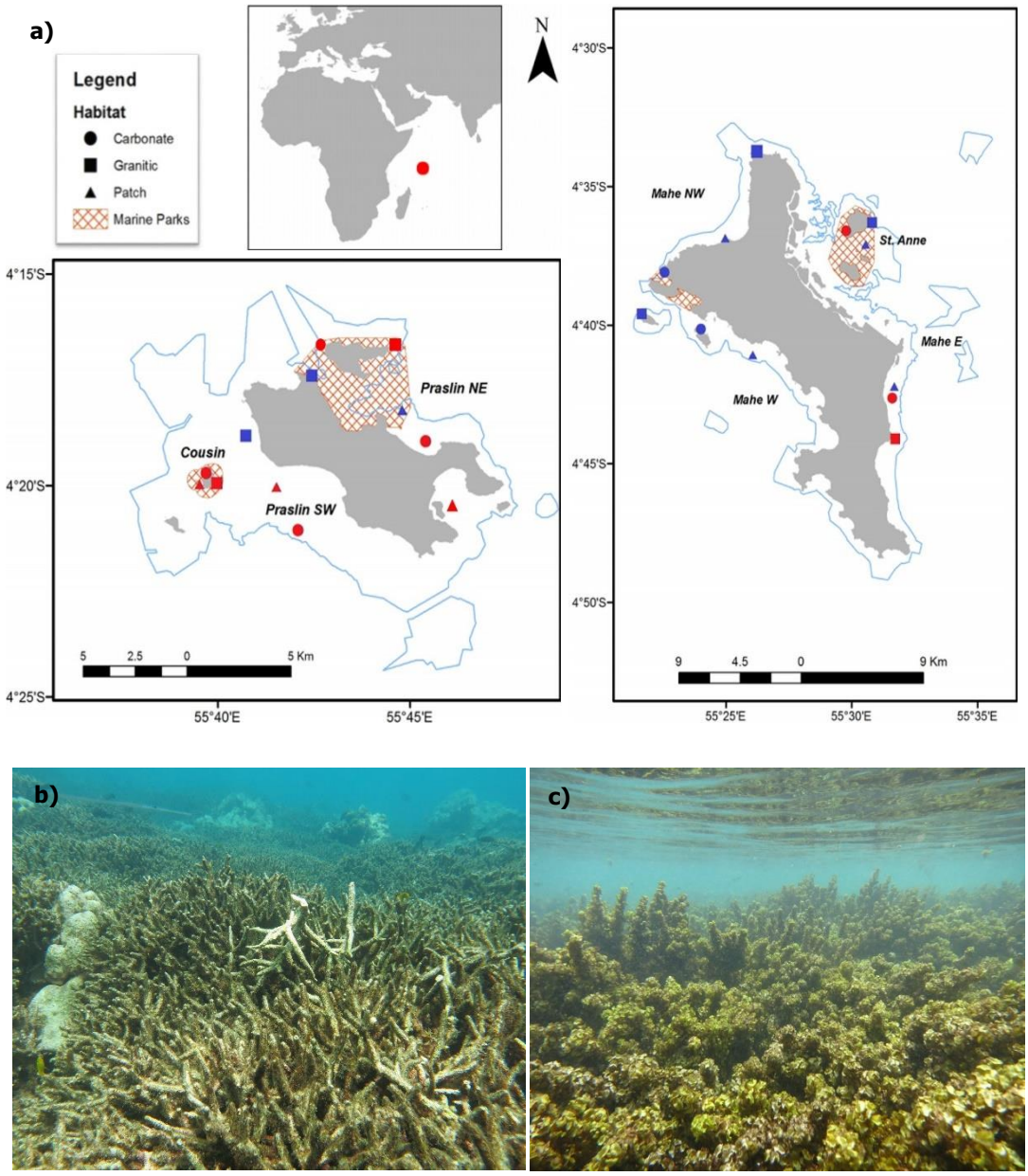
APPENDIX A: SUPPLEMENTARY MATERIAL

Chapter 1



Supplementary Figure 1.1 a) Map of Seychelles showing 21 study sites categorised into previous categorisations regime shifted to macroalgal dominance ("regime-shifted reefs"; red) and previously recovering reefs with high coral mortality ("coral mortality reefs"; blue) from the 1998 bleaching event, adapted with permission from Graham et al. (2015). The three sites in the Marine Park around Cousin Island were not included in the 2017 study, so three additional sites around Praslin island (one of each habitat type) were assessed instead. b) An image of one of the "coral-mortality" reefs (photo credit: Shaun Wilson), and c) an image of one of the "regime-shifted" reefs (photo credit: Jan-Claas Dajka).

Supplementary Table 1.1 Summary of the 21 coral reefs surveyed around the Inner Seychelles islands, including latitude, longitude, habitat type and reef state as categorised in 2017. * denotes the sites added to the 2017 survey in place of the three sites around Cousin Island that were not surveyed that year.

Site	Lat	Long	Habitat Type	Reef State
Mahe West patch reef	-4.684675	55.43472	Patch	CM
Mahe West carbonate	-4.669121	55.40025	Carbonate	CM
Mahe West granitic reef	-4.659828	55.36099	Granitic	CM
Mahe North West carbonate	-4.634994	55.37612	Carbonate	CM
Mahe North West patch reef	-4.614482	55.41627	Patch	CM
Mahe North West granitic	-4.562673	55.43691	Granitic	CM
Ste. Anne granitic reef	-4.605095	55.51353	Granitic	CM
Ste. Anne patch reef	-4.618086	55.5094	Patch	CM
Ste Anne carbonate	-4.609864	55.49636	Carbonate	RS
Mahe East granitic reef	-4.734961	55.52896	Granitic	RS
Mahe East carbonate	-4.710589	55.52704	Carbonate	RS
Mahe East patch reef	-4.703574	55.5282	Patch	CM
Praslin North East patch reef	-4.303653	55.74655	Patch	CM
Praslin North East carbonate	-4.315847	55.75669	Carbonate	RS
Praslin NE granitic reef	-4.290079	55.7075	Granitic	CM
Praslin SW granitic reef	-4.313662	55.67872	Granitic	CM
Praslin SW patch reef	-4.333943	55.69204	Patch	RS
Praslin SW carbonate	-4.350873	55.70152	Carbonate	RS
Curieuse South West carbonate*	-4.28007	55.71199	Carbonate	RS
Curieuse North East granitic reef*	-4.27987	55.74425	Granitic	RS
Baie Ste Anne patch reef*	-4.34278	55.76919	Patch	RS

Supplementary Figure 1.2 Images of the eight candidate bioindicators collected from up to 21 coral reefs around the Inner Seychelles islands. a) Brown Macroalgae (BM), *Sargassum* sp.; b) Cyanobacteria (CYB), c) Green Macroalgae (GM), *Chlorodesmis* sp.; d) Soft Coral (SC), *Sarcophyton* sp.; e) Sediment (SED); f) Sponge (SP), Demospongiae; g) Turf Algae (TA); and h) Zoanthid (ZO), *Palythoa* sp. Photo credit: Images **a), b),** and **c)** by Jan-Class Dajka; image **d)** by Heather Coll (NOAA/NMFS/OPR/ESAICD); and images **f)**, **g)**, and **h)** by EJV.

Supp. Table 1.2. Model (1) for each nutrient measurement and for each bioindicator: Nutrient Signature ~ Bioindicator. Model type was selected for each individual model based on normality of distribution. Sediment (SED) values were not available and so were not included for C-based signatures. Significance is noted as: '***' $p < 0.001$; '**' $p < 0.01$; '*' $p < 0.05$; and ',' $p < 0.1$.

	Model Type (Family)	Intercept	Lower C.I. (5%)	Upper C.I. (95%)	p-value
$\delta^{15}N$					
BM (Intercept)	**GLM (Gamma)**	0.179	0.173	0.185	**<0.0001*** **
CYB	**GLM (Gamma)**	-0.0375	-0.0457	-0.0292	**<0.0001*** **
GM	**GLM (Gamma)**	0.00841	-0.000846	0.0177	0.0766.
SC	**GLM (Gamma)**	-0.0477	-0.0560	-0.0394	**<0.0001*** **
SED	**GLM (Gamma)**	-0.0751	-0.0816	-0.0687	**<0.0001*** **
SP	**GLM (Gamma)**	-0.0459	-0.0534	-0.0385	**<0.0001*** **
TA	**GLM (Gamma)**	-0.0385	-0.0459	-0.0312	**<0.0001*** **
ZO	**GLM (Gamma)**	-0.0690	-0.0757	-0.0624	**<0.0001*** **
$\delta^{13}C$					
BM (Intercept)	**GLM (Gaussian)**	-16.2	-16.7	-15.8	**<0.0001*** **
CYB	**GLM (Gaussian)**	-5.11	-5.82	-4.39	**<0.0001*** **
GM	**GLM (Gaussian)**	-5.08	-5.78	-4.38	**<0.0001*** **
SC	**GLM (Gaussian)**	-0.0533	-0.791	0.643	0.8875
SP	**GLM (Gaussian)**	-1.18	-1.86	-0.508	**<0.0001*** **
TA	**GLM (Gaussian)**	-2.26	-2.89	-1.64	**<0.0001*** **
ZO	**GLM (Gaussian)**	2.48	1.83	3.14	**<0.0001*** **
%N					
BM (Intercept)	**GLM (Gamma)**	0.900	0.709	1.14	**<0.0001*** **
CYB	**GLM (Gamma)**	-0.604	-0.851	-0.383	**<0.0001*** **
GM	**GLM (Gamma)**	-0.675	-0.915	-0.465	**<0.0001*** **
SC	**GLM (Gamma)**	-0.629	-0.877	-0.407	**<0.0001*** **
SED	**GLM (Gamma)**	20.2	16.1	24.9	**<0.0001*** **
SP	**GLM (Gamma)**	-0.331	-0.603	-0.071	**0.0146***
TA	**GLM (Gamma)**	-0.255	-0.545	0.033	0.0816.

ZO	**GLM (Gamma)**	0.0333	-0.275	0.343	0.8316
%C					
BM (Intercept)	**GLM (Gamma)**	0.0323	0.0311	0.0335	**<0.0001*** **
CYB	**GLM (Gamma)**	0.00258	0.000558	0.00464	**0.0134***
GM	**GLM (Gamma)**	-0.00857	-0.0102	-0.00695	**<0.0001*** **
SC	**GLM (Gamma)**	0.00798	0.00555	0.0104	**<0.0001*** **
SP	**GLM (Gamma)**	0.0285	0.0257	0.0314	**<0.0001*** **
TA	**GLM (Gamma)**	0.0157	0.0131	0.0183	**<0.0001*** **
ZO	**GLM (Gamma)**	0.0568	0.0533	0.0603	**<0.0001*** **
C:N RATIO					
BM (Intercept)	**GLM (Gamma)**	0.0347	0.0322	0.0372	**<0.0001*** **
CYB	**GLM (Gamma)**	0.0743	0.0638	0.0855	**<0.0001*** **
GM	**GLM (Gamma)**	0.0663	0.0573	0.0757	**<0.0001*** **
SC	**GLM (Gamma)**	0.149	0.132	0.167	**<0.0001*** **
SP	**GLM (Gamma)**	1.14	1.04	1.24	**<0.0001*** **
TA	**GLM (Gamma)**	0.0685	0.0608	0.0766	**<0.0001*** **
ZO	**GLM (Gamma)**	0.125	0.112	0.138	**<0.0001*** **

Supp. Table 1.3. Model (2) for the CoV of each nutrient measurement in each bioindicator across all sites: CoV ~ Bioindicator. Model type selected for each individual model based on normality of distribution. Sediment (SED) values were not available and so were not included for C-based signatures. Significance is noted as: '***' $p < 0.001$; '**' $p < 0.01$; '*' $p < 0.05$; and ',' $p < 0.1$.

	Model Type (Family)	Intercept	Lower C.I. (5%)	Upper C.I. (95%)	p-value
$\delta^{15}N$					
BM (Intercept)	**Linear Model**	4.68	2.73	6.64	**<0.0001*** **
CYB	**Linear Model**	2.86	-0.296	6.01	0.0753.
GM	**Linear Model**	-2.21	-5.28	0.855	0.156
SC	**Linear Model**	1.58	-1.81	4.97	0.358
SED	**Linear Model**	3.29	0.694	5.89	**0.0135***
SP	**Linear Model**	2.14	-0.785	5.06	0.150
TA	**Linear Model**	3.61	0.848	6.38	**0.011***
ZO	**Linear Model**	-1.70	-4.39	0.986	0.212
$\delta^{13}C$					
BM (Intercept)	**Linear Model**	-6.03	-7.75	-4.32	**<0.0001*** **
CYB	**Linear Model**	-0.930	-3.62	1.76	0.494
GM	**Linear Model**	4.16	1.47	6.85	**0.00283****
SC	**Linear Model**	-0.171	-3.03	2.69	0.906
SP	**Linear Model**	4.59	1.97	7.21	**0.000794*** **
TA	**Linear Model**	0.90	-1.50	3.29	0.459
ZO	**Linear Model**	0.65	-1.78	3.07	0.598
%N					
BM (Intercept)	**Linear Model**	11.3	1.13	21.4	**0.0297***
CYB	**Linear Model**	10.9	-5.21	25.6	0.185
GM	**Linear Model**	-3.73	-19.6	12.1	0.642
SC	**Linear Model**	19.1	1.57	36.8	**0.0331***
SED	**Linear Model**	6.13	-7.33	19.6	0.369
SP	**Linear Model**	8.71	-6.43	23.9	0.256
TA	**Linear Model**	9.19	-6.29	24.7	0.242
ZO	**Linear Model**	3.04	-10.90	17.0	0.666
%C					
BM (Intercept)	**Linear Model**	4.07	-0.395	8.54	0.0734.
CYB	**Linear Model**	4.84	-2.16	11.8	0.0172

GM	**Linear Model**	-0.470	-7.47	6.53	0.894
SC	**Linear Model**	-0.909	-8.35	6.53	0.809
SP	**Linear Model**	0.452	-6.37	7.27	0.895
TA	**Linear Model**	-1.83	-8.15	4.48	0.565
ZO	**Linear Model**	27.4	21.0	33.7	**<0.0001*** **
C:N RATIO					
BM (Intercept)	**Linear Model**	9.92	3.54	16.3	**0.00271** **
CYB	**Linear Model**	3.26	-7.04	13.6	0.531
GM	**Linear Model**	-4.16	-14.2	5.84	0.411
SC	**Linear Model**	1.72	-8.92	12.4	0.749
SP	**Linear Model**	-2.33	-12.1	7.41	0.635
TA	**Linear Model**	0.671	-8.22	9.56	0.881
ZO	**Linear Model**	10.1	1.06	19.1	**0.0290***

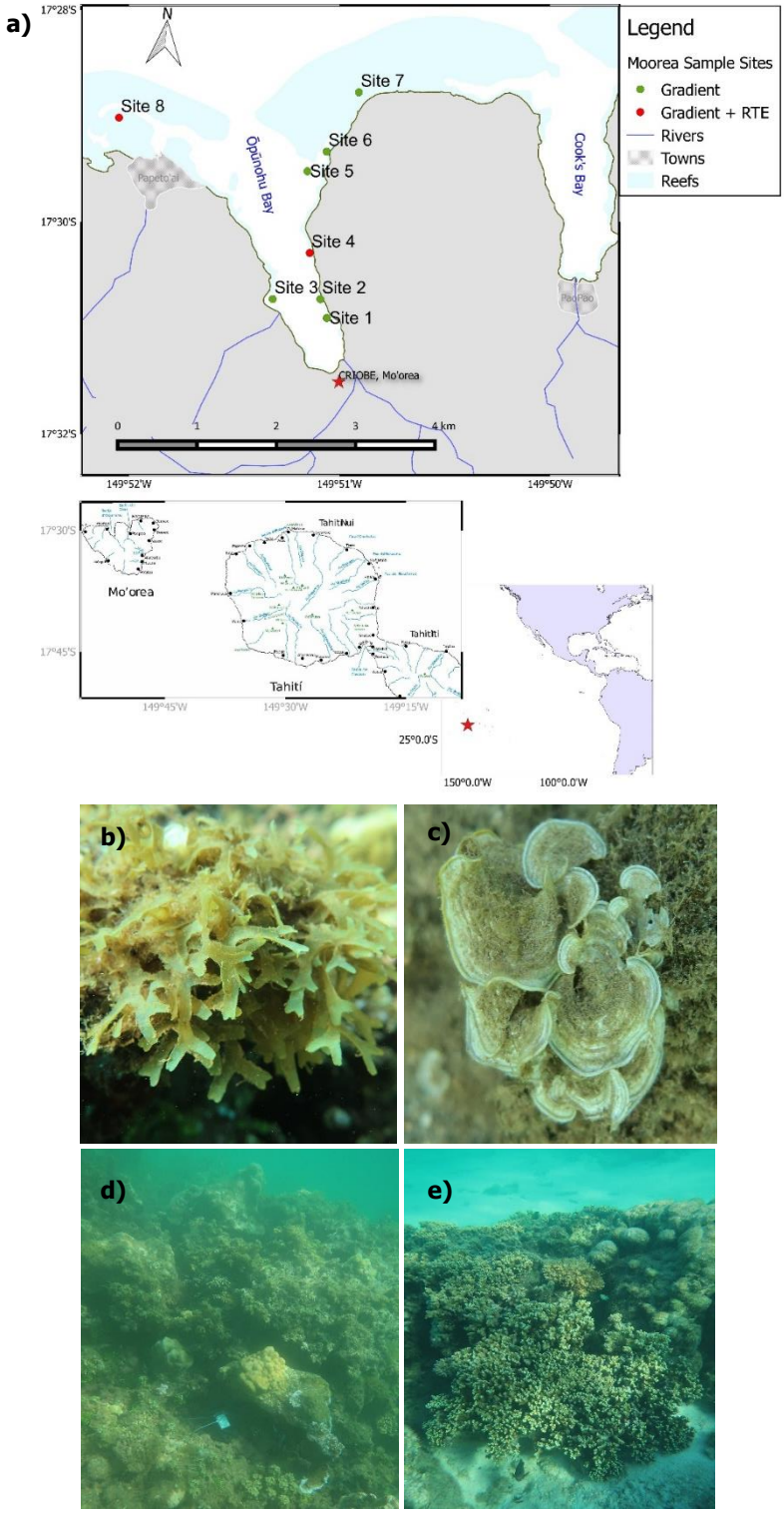
Supp. Table 1.4. Generalised Linear Model (3) for the cost-effectiveness analyses to determine the effect of Bioindicator, Task, Reef State and the interaction between them on the time per unit sample (per hour): Time ~ Bioindicator * Task * Reef State. Normality inspected using visual plots. Significance is noted as: '***' $p < 0.001$; '**' $p < 0.01$; '*' $p < 0.05$; and ',' $p < 0.1$.

	Intercept	Lower C.I. (2.5%)	Upper C.I. (97.5%)	p-value
BM (Intercept)	-1.33	-1.89	-0.767	**<0.0001*** **
CYB	0.0109	-0.498	0.520	0.966
GM	0.00803	-0.689	0.704	0.982
SC	1.50	0.831	2.16	**<0.0001*** **
SED	-1.37	-1.85	-0.891	**<0.0001*** **
SP	1.49	0.838	2.15	**<0.0001*** **
TA	1.45	0.864	2.13	**<0.0001*** **
ZO	1.49	1.01	1.97	**<0.0001*** **
DRY-CRUSH	26.0	25.3	26.7	**<0.0001*** **
FIELD	1.40	0.719	2.08	**<0.0001*** **
SIA	1.51	0.830	2.19	**<0.0001*** **
WEIGH	2.84	2.43	3.25	**<0.0001*** **
REEF STATE-REGIME SHIFT	0.00376	-0.873	0.881	0.993
CYB-DRY	-1.91	-2.63	-1.19	**<0.0001*** **
GM-DRY	-0.562	-1.55	0.424	0.264
SC-DRY	-3.94	-4.79	-3.094	**<0.0001*** **
SED-DRY	-0.681	-1.36	-0.00183	**0.0500***
SP-DRY	-3.83	-4.66	-3.00	**<0.0001*** **
TA-DRY	-1.48	-2.27	-0.684	**0.000293*** **
ZO-DRY	-3.10	-3.78	-2.42	**<0.0001*** **
CYB-FIELD	0.237	-0.483	0.957	0.519
GM-FIELD	0.0864	-0.899	1.072	0.864
SC-FIELD	-1.42	-2.27	-0.573	**0.00109** **
SED-FIELD	1.32	0.636	2.00	**0.000166*** **
SP-FIELD	-1.46	-2.29	-0.629	**<0.000620*** **
TA-FIELD	-1.55	-2.34	-0.753	**<0.000152*** **

ZO-FIELD	-1.45	-2.13	-0.767	**<0.0001*** **
CYB-SIA	0.0336	-0.686	0.753	0.927
GM-SIA	0.0158	-0.970	1.00	0.975
SC-SIA	-1.26	-2.12	-0.410	**0.00380** **
SED-SIA	1.40	0.720	2.08	**<0.0001*** **
SP-SIA	-1.29	-2.12	-0.459	**0.00246** **
TA-SIA	-1.43	-2.22	-0.635	**0.000461*** **
ZO-SIA	-1.25	-1.92	-0.566	**0.000361*** **
CYB-WEIGH	NA	NA	NA	NA
GM-WEIGH	NA	NA	NA	NA
SC-WEIGH	0.0198	-0.632	0.672	0.953
SED-WEIGH	NA	NA	NA	NA
SP-WEIGH	0.0476	-0.585	0.675	0.889
TA-WEIGH	0.00659	-0.576	0.590	0.982
ZO-WEIGH	NA	NA	NA	NA
CYB-REGIME	-0.00667	-0.799	0.785	0.987
GM-REGIME	-0.0157	-0.868	0.837	0.971
SC-REGIME	-0.00710	-1.39	1.38	0.992
SED-REGIME	0.0110	-0.665	0.687	0.975
SP-REGIME	-0.00376	-1.046	1.038	0.994
TA-REGIME	-0.00710	-0.991	0.976	0.989
ZO-REGIME	-0.00376	-0.721	0.713	0.992
DRY-REGIME	0.203	-0.811	1.22	0.695
FIELD-REGIME	-0.0627	-1.08	0.951	0.904
SIA-REGIME	-0.00429	-1.02	1.01	0.993
WEIGH-REGIME	<0.0001	-0.714	0.714	1.00
CYB-DRY-REGIME	1.13	0.0128	2.25	**0.0480***
GM-DRY-REGIME	-0.194	-1.40	1.01	0.752
SC-DRY-REGIME	-0.313	-2.14	1.50	0.737
SED-DRY-REGIME	0.0877	-0.869	1.04	0.857
SP-DRY-REGIME	0.518	-0.771	1.81	0.431
TA-DRY-REGIME	-0.343	-1.54	0.850	0.573
ZO-DRY-REGIME	-0.176	-1.90	0.838	0.734

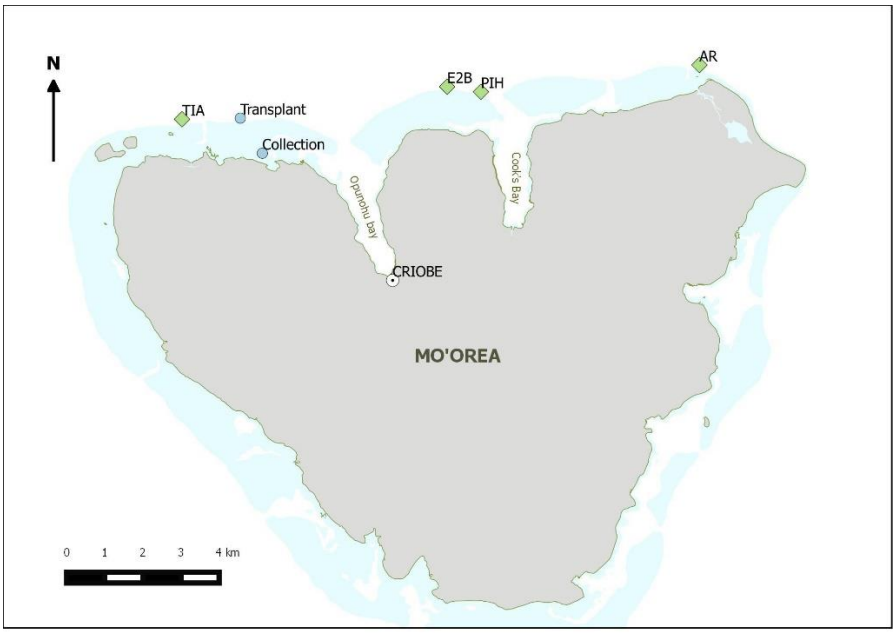
CYB-FIELD-REGIME	0.149	-0.972	1.27	0.795
GM-FIELD-REGIME	-0.0465	-1.25	1.16	0.940
SC-FIELD-REGIME	0.969	-0.854	2.79	0.298
SED-FIELD-REGIME	0.0462	-0.910	1.00	0.925
SP-FIELD-REGIME	0.443	-0.846	1.73	0.501
TA-FIELD-REGIME	0.0873	-1.11	1.28	0.886
ZO-FIELD-REGIME	0.259	-0.755	1.27	0.617
CYB-SIA-REGIME	-0.0562	-1.18	1.06	0.922
GM-SIA-REGIME	-0.0279	-1.23	1.18	0.964
SC-SIA-REGIME	0.520	-1.30	2.34	0.577
SED-SIA-REGIME	0.0742	-0.882	1.03	0.879
SP-SIA-REGIME	-0.0365	-1.33	1.25	0.956
TA-SIA-REGIME	0.233	-0.970	1.42	0.714
ZO-SIA-REGIME	0.0749	-1.09	0.939	0.885
CYB-WEIGH-REGIME	NA	NA	NA	NA
GM-WEIGH-REGIME	NA	NA	NA	NA
SC-WEIGH-REGIME	0.164	-1.51	1.84	0.848
SED-WEIGH-REGIME	NA	NA	NA	NA
SP-WEIGH-REGIME	- <0.0001	-1.07	1.07	1.00
TA-WEIGH-REGIME	0.0133	-0.939	0.965	0.978
ZO-WEIGH-REGIME	NA	NA	NA	NA

Chapters 2 & 3

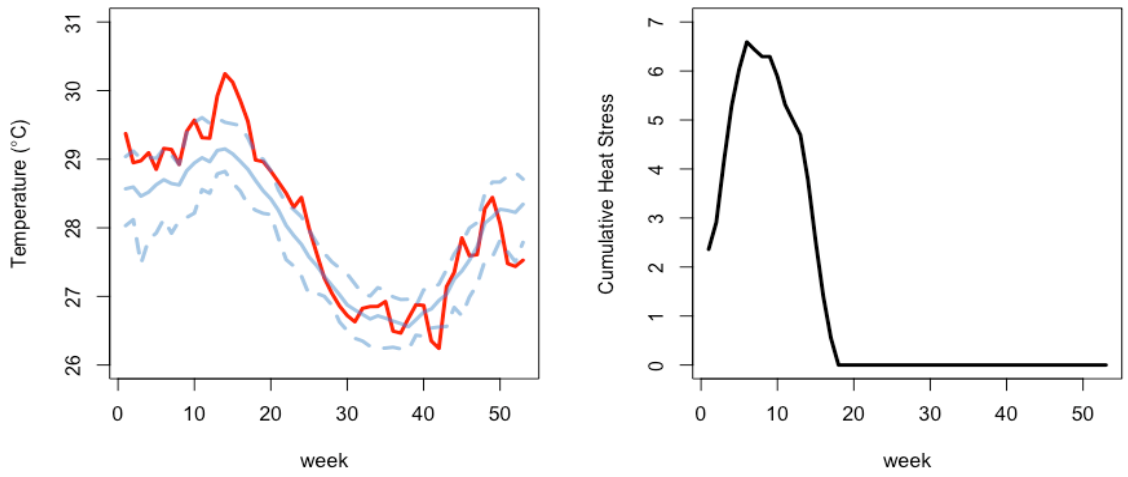


Supplementary Figure 2.1 a) Map of Mo'orea, French Polynesia showing the eight study sites (green points) where samples of b) *Dictyota bartayresiana* and c) *Padina boryana* were collected along the established nutrient gradient in Oponuhu Bay using the passive biomonitoring method (Lin & Fong, 2008). The red points for d) Site 4 and e) Site 8 denote the two contrasting sites where both passive and active biomonitoring methods were applied (García-Seoane et al., 2018a,b), both sample collection for the nutrient gradient and the reciprocal transplant experiment (Site 4 = high-nutrient reef; Site 8 = low-nutrient reef). Image credit: EJV. Map created in QGIS

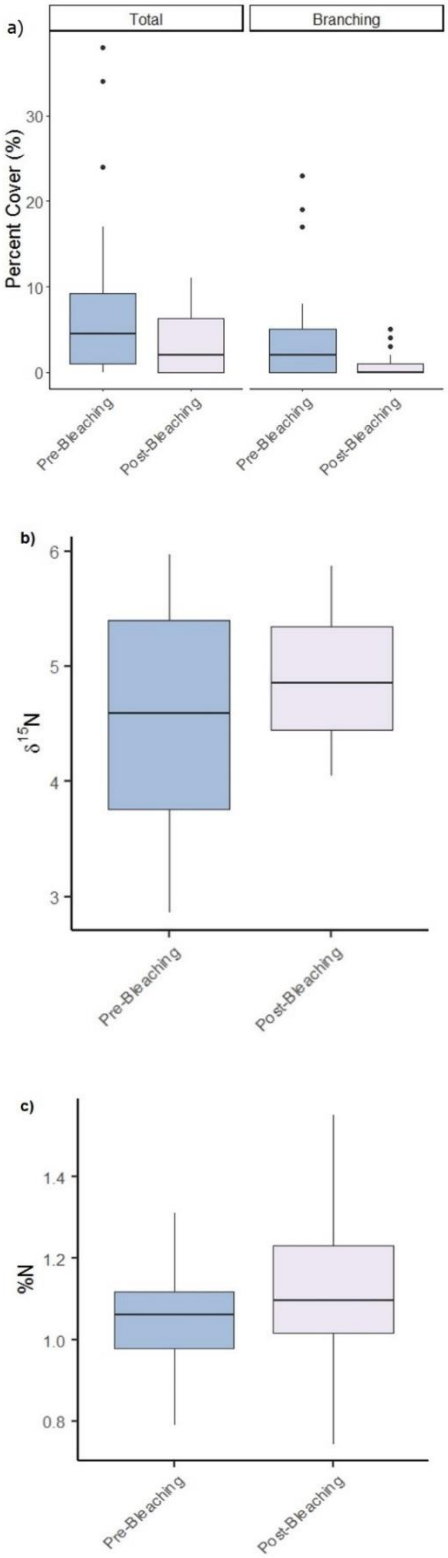
Chapter 4



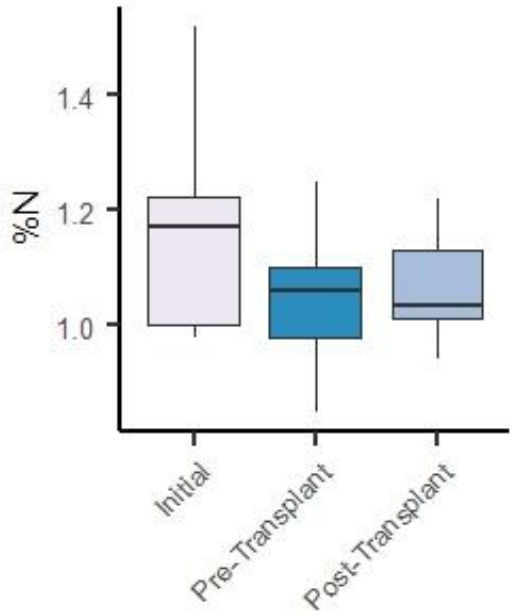
Supplementary Figure 4.1. Mo'orea, French Polynesia. The sites for collection and transplantation of *Sargassum mangarevense* specimens (n=10) are labelled with the blue circles, and the four sites with benthic cover from the Service National d'Observation CORAIL are labelled in abbreviated capital letters (TIA = Tiahura; E2B = Entre Deux Baies; PIH = Pihaena; AR = Aroa) with green triangles. CRIOBE research station, where the specimens were depleted of internal nutrients for 7 days, is indicated with a white circle and black dot.



Supplementary Figure 4.2. Average Temperature patterns from *in situ* temperature loggers at Tiahura reef on the north shore of Moorea in 2019. (A) In 2019 (blue line) temperatures exceed the maximum monthly mean of 29 °C during the Austral summer, and it was much warmer than the average long-term seasonal pattern (blue line with 95% confidence intervals as dashed blue lines). (B) Cumulative heat stress, measured as a 12-week running sum for all temperatures exceeding the maximum monthly mean, peaked in April 2019.



Supplementary Figure 4.3. Box plots of the median a) total and branching coral cover in both pre-bleaching and post-bleaching years (2014 and 2017, respectively) on regime-shifted reefs (n=6), b) the average $\delta^{15}N$ signatures in *Sargassum* sp. tissues in both years, and c) the average percent N (%N) in both years. The pale blue boxes represent the pre-bleaching year and pale pink boxes represent the post-bleaching year, which both show the third quartile (Q3) and first quartile (Q1) range of the data and data outliers.



Supplementary Figure 4.4. Box and whisker plots of the median %N in *Sargassum mangarevense* tissue across three treatments from a short-term transplant experiment, showing the third quartile (Q3) and first quartile (Q1) range of the data, the whiskers (95% quartile) and data outliers. Connecting letters indicate significance between treatments. Nutrient signatures were measured in subset samples of the same specimens that were collected from a low-nutrient reef (initial), placed in laboratory aquaria to deplete internal nutrient stores for ~7 days (pre-transplant), before they were deployed on the bleached reef for 3 weeks (post-transplant) (n=10).

Marine Pollution Bulletin 170 (2021) 112606

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Precision and cost-effectiveness of bioindicators to estimate nutrient regimes on coral reefs

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ARTICLE INFO

Keywords:
Pollution
Stable isotopes
Macroalgae
Environmental monitoring
Regime shifts

ABSTRACT

Bioindicators are useful for determining nutrient regimes in marine environments, but their ability to evaluate corals reefs in different ecological states is poorly understood. The precision, availability and congruency of eight potential bioindicators (brown macroalgae, green macroalgae, turf algae, cyanobacteria, soft corals, zoanthids, sponges, and sediment) and their stable isotopic and elemental signatures ($\delta^{15}N$, $\delta^{13}C$, %N, %C, and C:N Ratio) were assessed across 21 reefs in the Inner Seychelles. The coefficient of variation (CoV) for $\delta^{15}N$ showed that green and brown macroalgae were highly precise (2.47 ± 0.95, n = 11; 4.68 ± 1.33, n = 16, respectively), though were less common on coral-mortality reefs relative to macroalgal-dominated ones. Zoanthids were also highly precise for $\delta^{15}N$ (2.98 ± 1.20), but were more readily available regardless of reef state (n = 18). Congruency was low among these indicators, suggesting that different physiological mechanisms for nutrient processing have a stronger influence on a bioindicator's effectiveness than reef state.

1. Introduction

Coral reefs are facing global declines in live coral cover due to climate change (Hughes et al., 2018), and local-scale degradation from overfishing and pollution (Burkepile and Hay, 2006; Littler et al., 2006; Zaneveld et al., 2016; MacNeil et al., 2019). Increased anthropogenic nutrient loads and reduced herbivory can cause the proliferation of opportunistic species such as fleshy macroalgae, which may lead to a regime shift from a coral-dominated to an algal-dominated reef (Littler et al., 2006; Hughes et al., 2007; Fulton et al., 2019). Monitoring the state of coral reefs relative to anthropogenic stressors provides insights into causes of decline in reef condition, potentially instigating management actions. Two particularly widespread local stressors are fishing and eutrophication (Fabricius et al., 2005; Burkepile and Hay, 2006; Littler et al., 2006; Zaneveld et al., 2016). While there has been significant progress in understanding the effects of fishing (e.g. Cinner et al., 2018), it has been more difficult to detect and quantify nutrient loads that cause eutrophication in the marine environment, due to high spatio-temporal variability in the water column (Fabricius et al., 2005; Wyatt et al., 2013; D'Angelo and Wiedenmann, 2014; Briand et al., 2015; Lowe and Falter, 2015; Clausing and Fong, 2016; MacNeil et al., 2019). It is therefore critical to identify more cost-effective methods of capturing nutrient enrichment to improve assessments of coral reef health over different spatial scales as part of routine environmental monitoring strategies (Fabricius et al., 2012; Bal et al., 2020).

Bioindicators are used widely to capture nutrient regimes in tropical marine systems, as they provide an ecologically relevant response to bioavailable nutrients in the surrounding water column (Fichez et al., 2005; Cooper et al., 2009; Fabricius et al., 2012). As such, bioindicators are cost-effective alternatives to direct measures of seawater nutrients, which can be highly variable and require frequent sampling that do not always capture fine-scale temporal variation or wider ecological impacts (Fabricius et al., 2012). Suitable bioindicators are defined in Cooper et al. (2009) as those with biological responses that are a) specific towards a driver of change or stressor, b) reflective of the magnitude of any changes, c) consistent across different scales, d) cost-effective, and e) ecologically relevant. Non-biological indicators, conversely, are those which can still reflect drivers of change, but not through biological

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https://doi.org/10.1016/j.marpolbul.2021.112606
Received 30 April 2020; Received in revised form 1 June 2021; Accepted 4 June 2021
Available online 18 June 2021

responses (i.e. nutrients stored in reef sediments) (Linton and Warner, 2003; Fichez et al., 2005).

Previous studies have measured the presence: absence ratio of selected bioindicators to investigate water quality (Fichez et al., 2005; Cooper et al., 2009), however, using this type of methodology alone does not take into account other biophysical factors that may influence their abundance (Linton and Warner, 2003). Therefore, measuring stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$) and concentration levels (%N, %C and C:N ratio) in the tissues of a selected bioindicator allows scientists and environmental managers to assess both the source(s) and concentration of nutrient regimes, respectively, better determine the spatio-temporal variability of nutrient regimes and detect and map the spatial ecological impacts (Costanzo et al., 2001). Fleshy macroalgae are widely used for such a purpose, because they respond rapidly to high nutrient concentrations by assimilating bioavailable nutrients from their local environment into their tissues over their active growth periods, thereby capturing temporal variation in nutrients (Costanzo et al., 2001). They are also easy to collect and survey in the field, especially in nutrient-rich coastal areas (Fichez et al., 2005; García-Seoane et al., 2018a, 2018b; Zubia et al., 2018).

One of the main limitations of using only a single species of macroalgae, even with stable isotopic analyses, are the spatio-temporal gaps in their distribution, which are driven by a number of abiotic factors such as wave exposure, irradiance, temperature, rainfall and seasonality (Linton and Warner, 2003; Williams et al., 2013; Clausing and Fong, 2016; Duran et al., 2016; Fulton et al., 2019), and biotic factors such as herbivory and competition (Burkepile and Hay, 2006; Duran et al., 2016). These limiting factors may also affect the ability of macroalgae to proliferate on some reefs that have experienced significant disturbances (Littler et al., 1991; Graham et al., 2015). These distributional gaps can also lead to inconclusive or even misleading findings in any studies or monitoring programs, particularly if they are quantifying the abundance of a particular species across a range of target sites (Linton and Warner, 2003). As such, the utility of alternative bioindicators to capture nutrient regimes is of importance to monitoring programs.

A range of other marine organisms have been used as bioindicators in water quality or nutrient enrichment studies, such as scleractinian corals (Hoegh-Guldberg et al., 2004), soft corals (Fleury et al., 2000; Risk, 2014), and sponges (Ward-Paige et al., 2005). In addition, multiple candidate bioindicators have been used to assess water quality depending upon their response time to a change in their local nutrient environment (Cooper et al., 2009), or on the extent of their abundance and distribution, which also allows the spatial extent of nutrient runoff to be assessed (Fabricius et al., 2012). Some bioindicators may take longer to find or process than others, particularly in areas where they are relatively uncommon or rare. Selection of bioindicators should therefore also consider the cost-effectiveness of the collection and subsequent processing of samples (Risk et al., 2001; Drummond and Connell, 2008; Bal et al., 2020). This will be especially important for researchers and managers tasked with monitoring water quality over large spatial and temporal scales, such as entire reef systems (Déath and Fabricius, 2010; Graham et al., 2015).

Few studies have tested whether patterns in nutrient signatures of different bioindicators are congruent (i.e. they are able to show the same relative trends in isotopic values between bioindicators) across different spatio-temporal scales or gradients (Tucker et al., 1999; Gartner et al., 2002; Pitt et al., 2009), and this multi-taxa approach is even less common in coral reef studies, (Connolly et al., 2013; Kürten et al., 2014; Graham et al., 2018). Untested variability in isotopic composition within and between different reefs, bioindicators, and even studies could therefore reduce the reproducibility, or else the comparability of large-scale and long-term monitoring assessments (Pitt et al., 2009; Connolly et al., 2013).

If multiple bioindicators can demonstrate similarly precise and congruent spatial patterns of nutrients over a large-scale gradient, then other taxa, particularly those from multiple trophic positions, may become useful proxies in areas where macroalgae are scarce, such as on reefs that are dominated by reef-building corals or turf algae (den Haan et al., 2014; Fulton et al., 2019). However, some of these bioindicators may not be directly comparable with others due to the way they take up and process nutrients internally or how other biophysical drivers could potentially influence their signatures (Raimonet et al., 2013; Viana and Bode, 2013; Clausing and Fong, 2016). In addition, species at different trophic levels have different $\delta^{15}N$ signatures due to isotopic fractionation (Boecklen et al., 2011). This may therefore impact the overall effectiveness of a suite of bioindicators, so additional measures are needed to directly compare their compatibility before they can be used for monitoring programs.

In this study, we investigated the precision and cost-effectiveness of a suite of eight potential bioindicators collected from coral reefs across the Inner Seychelles Islands for measuring nutrient regimes. The specific objectives of the study were to (1) quantify the precision of different bioindicators for measuring stable isotopic and elemental signatures of nitrogen and carbon, (2) determine how much variation exists within bioindicators across different coral reef sites which vary in ecological condition, (3) consider whether there is congruency between selected precise bioindicators based on their nitrogen (N)- and carbon (C)-based measurements, and (4) assess cost-effectiveness of using different bioindicators and the tasks involved.

2. Methods

2.1. Study sites and sample collections

The inner Seychelles islands (43°S, 55°30′E) are comprised of high granitic islands with well-developed carbonate fringing reefs (Littler et al., 1991; Dajka et al., 2019). Bioindicator samples were collected from 21 coral reef sites around the populated islands of Mahé and Praslin, between 11th and 22nd April 2017. These sites have been used as part of a 23-year long-term coral reef monitoring survey, of the reefs of the Inner Seychelles Islands (Suppl. Table 1; Graham et al., 2015; Wilson et al., 2019). The 21 reefs in this study were formed on habitats of either granite, contiguous carbonate or patches that are surrounded by sand or rubble. Twelve of these reefs were defined as “recovering” live coral from a mass bleaching event in 1998, and nine as “regime-shifted” where macroalgae had proliferated (Wilson et al., 2019). However, another mass bleaching event in 2016 caused mass coral mortality on the recovering reefs (Wilson et al., 2019), and so here we define them as “coral-mortality” reefs. Using nitrogen content of brown macroalgae collected from these sites, Graham et al. (2015) also found that nutrient regimes are one of the key determinants of whether a reef can recover or experience a regime shift after a major disturbance like bleaching.

To assess the availability of potential bioindicators, eight replicate 7-m radius point counts were surveyed along the reef slope at each site, and within each point count area, the percent cover of benthic groups such as hard coral, soft coral, macroalgae, sand, rubble, and rock was quantified using eight replicate 10 m line-intercept transects (Wilson et al., 2019). Along each transect, the distance of tape occupied by different benthic organisms and substrates was recorded, including live hard coral, soft coral, macroalgae, sponge, cyanobacteria, zoanthids, sand, rubble and rock. For the purpose of this study, the percent cover of dead hard coral and rubble was pooled for an estimate of turf algae per site. Up to ten replicate samples of eight different bioindicators (i.e. each replicate was a separate individual or sample) were collected haphazardly using SCUBA from within the same area used for the benthic surveys on each reef. However, there were not always ten available replicate samples at all sites, and some reefs had none of some types at all. Bioindicators were selected based on their presence in long-term benthic composition data and their use in previous nutrient enrichment and bioindicator studies (Risk et al., 2001; Fichez et al., 2005; Cooper et al., 2009; Fabricius et al., 2012). Bioindicators included whole

fronds of mature foliose brown macroalgae with the apical tips (*Sargassum* sp., Littler et al., 1991; Schaffelke, 1999; Schaffelke and Klumpp, 1998), filamentous green macroalgae (*Chlorodesmis* sp., Schaffelke, 1999), cyanobacteria (Ford et al., 2018), soft corals (*Sarcophyton* sp., Fleury et al., 2000), turf algal matrix (Graham et al., 2018), sponges (Demospongaie: Ward-Paige et al., 2005; Lamb et al., 2012), and zoanthids (*Palythoa* sp., Leal et al., 2017). For turf algae, branches of dead *Acropora* spp. coral densely covered in turf algal assemblages were broken off and scraped with a scalpel to collect enough material to make up ten replicate samples. Marine sediment (<4 cm depth; Fichez et al., 2005; Umezawa et al., 2008) which was considered as a non-biological indicator in this study, was also collected to determine nutrient signatures as an important store of nutrients on coral reefs. All samples were frozen at −20 °C for up to one month.

2.2. Stable isotopic and elemental analyses

Sample processing and preparation for isotopic analyses were conducted between the Seychelles Fishing Authority laboratory (SFA), Victoria, Mahé, Seychelles and Lancaster Environment Centre (LEC), Lancaster University, UK. All frozen samples were defrosted, rinsed thoroughly with distilled water and replicate samples were placed in a drying oven for ~48 h at 60 °C. Once dried, samples were each ground into a fine powder using a ball mill and stored in individual airtight containers at SFA. For bioindicators which contained inorganic carbon material (i.e. calcifying organisms such as soft corals, sponges, and zoanthids), additional acidification was required to remove the inorganic carbonate which can affect carbon-based signatures (Schlacher and Connolly, 2014). ~10 g of material was digested in 10% v/v hydrochloric acid (HCl) at room temperature until all constituent carbonate had been removed. Samples were then centrifuged, repeatedly washed until all traces of acidity had been removed, and left to dry prior to analysis for carbon stable isotope composition at LEC. The carbon stable isotopic and elemental signatures could not be measured in sediments in this study, because the samples were almost entirely composed of inorganic carbon material, so almost all of the test sediment material dissolved during initial runs of the acidification process. In addition, a subset of all calcified samples were not acidified so that they could be used for nitrogen-based stable isotopic signatures, as acidification can alter $\delta^{15}N$ signatures in some organisms (Schlacher and Connolly, 2014).

Stable isotopic and elemental analyses for nitrogen stable isotopes ($\delta^{15}N$), carbon stable isotopes ($\delta^{13}C$), nitrogen content (%N), carbon content (%C), and C:N Ratio (calculated from dividing the values of %C over %N) were undertaken within the LEC stable isotope facility, using an Isoprime100 Isotope Ratio Mass Spectrometer (IRMS) linked to an Elementar VARIO MICROcube Elemental Analyser. Combustion of samples within tin capsules at 950 °C yielded N_2 and CO_2 for determination of $\delta^{15}N$ and $\delta^{13}C$ respectively. Analyses were standardised to AIR (for $\delta^{15}N$) and VPDB (for $\delta^{13}C$) using internal reference materials calibrated to international standards. Within-run replication (1σ) was <0.3‰ for $\delta^{15}N$ and <0.1‰ for $\delta^{13}C$ for both standards and samples.

2.3. Cost-effectiveness analyses

To evaluate the cost-effectiveness of each of the techniques used to quantify the nutrient signatures in the eight different bioindicators, the time taken for collection, processing and analysis was calculated as follows. Collection time involved the time taken to search for and retrieve samples from up to 21 sites, where the average time recorded for each dive was ~1 h. Processing time included sample drying, crushing, weighing, and/or acidifying. Drying time represented the time taken to completely dry each sample in the drying oven, while crushing time was the time taken to crush each dried sample into a fine power. For weighing, the average time weighing standards for each mass spectrometric analysis was added to the time taken to weigh each individual sample, and stable isotope analysis time represented the time per analysis. The time taken to acidify each sample of the four calcified bioindicators was also included, though these samples had to be run twice to obtain results for both N and C signatures, with the first subset of samples unacidified, and the second subset acidified. All recorded and calculated times were then standardised to hours (h). The time taken per unit sample was used as a measure of "cost" instead of monetary value in this study, because the methods used to collect, process and analyse them were the same, except for the carbonate-containing samples which needed to be weighed and analysed twice.

2.4. Statistical analyses

Availability of the bioindicators was assessed in two ways. Firstly, the abundance of the selected groups from the benthic composition data across the 21 sites was averaged and pooled for the two different types of reef state. Secondly, the number of sites that the different bioindicator types were collected from were totalled and categorised according to reef state (i.e. coral-mortality or regime-shifted). The percentage of sites from which each bioindicator was collected, relative to each reef state (i.e. out of 12 for coral-mortality reefs, and out of 9 for regime-shifted reefs), was calculated, as there were different numbers in each category. The mean and standard deviation of the five nutrient signatures ($\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N Ratio) from samples of each bioindicator, collected from up to 21 sites, were then analysed in R (R Core Team, 2018).

The spatial variation for nutrient signatures of each bioindicator was assessed across all available sites using generalized linear models (GLM). All model fits were inspected for normality using visual plots, and GLMs were used on those with non-normal distributions. A GLM was used to determine the impact of the bioindicator, reef state and individual site on the five nutrient signatures (i.e. the response variables), using the following model for each individual signature:

$$\text{Model 1}: \text{Nutrient Signature} \sim \text{Bioindicator} + \text{Reef State} + \text{Site}$$

where the nutrient signature was either $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N Ratio, and bioindicator (eight levels), reef state (two levels) and site (up to 21 levels) as fixed factors for each of the five response variables, (C-based signatures in sediment were omitted, as there was no data available). A total of 37 models were therefore run for the overall analysis (alpha = 0.05).

The coefficient of variation (CoV) was used to calculate the overall precision of each bioindicator across all available sites. CoV is the ratio of the sample standard deviation to the same mean, for a given set number of data points, and was used in this study because it is a unitless measure of variation, which is useful when testing the statistical effectiveness (i.e. precision) of the signatures across the different bioindicators. High precision is defined in this study as a small standard deviation compared to the mean, which increases the ability to detect statistical significance, both between the replicate samples of each bioindicator collected at each site, and over all the sites from which each bioindicator was collected. Low precision, conversely, is a large standard deviation compared to the mean (Conquest, 1983). Though there is not one set standard in the literature, it is generally assumed that values of CoV < 10 can be regarded as "precise". CoV was calculated from the raw measurements detected in the replicate samples of each bioindicator collected from individual sites. Following this, the CoV of the N- and C-based signatures were compared across all the sites from which each bioindicator was collected with five linear models (Model 2), which were run separately for each nutrient signature:

$$\text{Model 2}: \text{CoV} \sim \text{Bioindicator} + \text{Reef State} + \text{Site}$$

where CoV was the CoV value for $\delta^{15}N$, $\delta^{13}C$, %N, %C and C:N Ratio, and Bioindicator (eight levels), reef state (two levels) and site (up to 21 levels) were the fixed factors. The overall mean and standard deviation for the CoV each bioindicator were also summarised in box-plots.

A principal components analysis (PCA) (PRIMER-E Ltd., V.6.1.5, Plymouth, UK) based on a Bray–Curtis similarity matrix was used to visualise the similarities between averaged values of the five different nutrient measurements and the different bioindicators as a way of assessing the level of congruency of the bioindicators (Clarke and Warwick, 2001). The selection of a subset of bioindicators for this analysis (brown macroalgae, green macroalgae and zoanthids) was based on their level of precision, and the number of sites used, out of 21, depended upon the availability of each of these three indicators. Therefore nine sites were selected, as they had sufficient replicates of all three bioindicators to compare across sites (n = 4), and the nutrient measurements were averaged at site level to compensate for the varying numbers of replicate samples available at each site. However, for C-based signatures, zoanthid samples from one site could not be acidified due to limited material so for these, eight sites were used. A correlation matrix was also constructed to assess the different correlation values between the three selected indicators, where a p-value < 0.05 was considered significant.

To statistically assess the cost-effectiveness of each bioindicator, another GLM was used (as the data was not normally distributed) to compare the average times taken (per sample per bioindicator) for (a) collecting from the field, (b) drying and crushing of samples, (c) weighing and preparing samples (i.e. acidification) for isotopic analyses, and (d) running isotopic analyses. In this model, "Time" was the response variable, and "Bioindicator" and "Task" were the fixed factors (eight and two levels in each factor, respectively):

Model 3 : Time ~ Bioindicator*Task

The interaction between these two fixed factors in Model 3 was also analysed to determine whether the "Bioindicator" (eight levels), "Task" (4–5 levels, depending on whether or not the bioindicator was acidified), or the interaction between them affects the time per unit sample. Reef State was also used as a fixed factor (with two levels) during initial statistical analyses, but was not included in this study as it showed no significant effect.

3. Results

3.1. Sample collection and benthic cover

Across the 21 sites, a total of 150 samples of brown macroalgae (*Sargassum* sp.), 91 green macroalgae (*Chlorodesmis* sp.), 103 cyanobacteria, 59 soft corals, 112 sponges, 134 zoanthids (*Palythoa* sp.), 171 turf algal assemblages, and 204 sediment samples were collected. Availability of bioindicator varied between regime-shifted and coral-mortality reefs, as did the percentage of sites within these two categories where they were present (Table 1). Average cover of *Sargassum* sp. was significantly higher at the regime-shifted sites where it was an order of magnitude greater than on the coral-mortality sites. As such, there were specimens available at 100% of the regime-shifted sites, whereas they were only found at 58% of regime-shifted reefs. There was a similar percent cover of sediment across sites (along the line-intersect transect) regardless of reef state, and sediment samples were collected from all 21 sites. Percent cover of turf algae on coral-mortality reefs was 32.8 ± 23.8%, compared to 12.2 ± 8.11% on regime-shifted reefs, but still had 100% availability in both reef states. Cyanobacteria, soft coral and sponge all had higher percent cover and were also present on a higher percentage of coral-mortality sites than on regime-shifted ones.

3.2. Spatial variation of nutrient signatures in bioindicators

The type of bioindicator had variable effects on each of the five nutrient signatures. Overall, brown and green macroalgae (BM and GM, respectively) not only had lower average $\delta^{15}N$ signatures than the other indicators, but they also had the smallest variations in signatures across all of their sites (5.58 ± 0.82 and 5.33 ± 0.45‰, respectively. Fig. 1a).

Table 1
Summary table for percent cover (% cover) of candidate bioindicators (BM = brown macroalgae; CYB = cyanobacteria; GM = green macroalgae; SED = sediment; SC = soft coral; TA = turf algae; ZO = zoanthid) from the line-intercept transect surveys at 21 coral reefs around the Inner Seychelles Islands. Percentage of sites represents the percentage of sites relative to the total number in each reef state (out of n = 12 for "coral-mortality" reefs versus n = 9 "regime-shifted" reefs). Mean ± S.D. for percent cover.

Bioindicator	Regime-shifted reefs (n = 9)		Coral-mortality reefs (n = 12)	
	Mean ± S. D. (%)	Percentage of sites (%)	Mean ± S. D. (%)	Percentage of sites (%)
Sargassum (BM)	36.9 ± 20.3	100	2.7 ± 8.47	58
Cyanobacteria (CYB)	1.2 ± 2.8	44	2.5 ± 5.0	75
Chlorodesmis (GM)	0.2 ± 0.3	89	0.3 ± 0.4	25
Soft coral (SC)	0.1 ± 0.8	11	1.2 ± 2.5	67
Sediment (SED)	6.7 ± 3.4	100	9.52 ± 11.5	100
Sponge (SP)	0.00	56	1.4 ± 2.1	75
Turf algae (TA)	12.2 ± 8.1	100	32.8 ± 23.8	100
Palythoa (ZO)	0.2 ± 0.4	67	1.3 ± 1.0	100

Bioindicators representing higher trophic levels, such as sponges (SP), soft corals (SC), and zoanthids (ZO) (7.51 ± 0.67; 7.61 ± 1.27, and 9.08 ± 0.88‰, respectively) had more enriched average $\delta^{15}N$ signatures, as did sediment (SED) (9.61 ± 1.41‰). After acidification, the four bioindicators that contained inorganic carbon (soft corals, sponges, and turf algae (TA)) showed similar signatures of $\delta^{13}C$ on average (−16.3 ± 1.29; −17.4 ± 0.38; and −18.5 ± 3.16‰, respectively), though it was less negative in zoanthids (−13.7 ± 0.88‰). The two types of macroalgae also differed (BM: −16.2 ± 1.58, and GM: −21.3 ± 0.96‰) whereas cyanobacteria (CYB) (−21.3 ± 3.36‰) was similar to green macroalgae (Fig. 1b).

Turf algae had a similar average signature for %N (1.53 ± 0.45%) relative to brown macroalgae (1.10 ± 0.18%) but green macroalgae had a much higher value (4.32 ± 0.48%), which was even higher than cyanobacteria (3.31 ± 1.25%). The N content of brown macroalgae was also most similar to zoanthids (1.06 ± 0.22%). N content was also much lower in sediment (0.05 ± 0.11%) (Fig. 1c). There was much higher C content in green macroalgae than in the other bioindicators (42.2 ± 2.40%), followed by brown macroalgae (31.0 ± 1.41%), and cyanobacteria (28.7 ± 5.52%). Zoanthids had the lowest %C (11.2 ± 2.74) (Fig. 1d). Brown macroalgae had higher C:N Ratio signatures with a large range due to high %C content and low %N content (28.8 ± 4.99). The other five groups were quite similar to one another, with the exception of sponge (0.85 ± 0.11) (Fig. 1e).

The GLMs showed that the type of bioindicator had a strong influence on the variability of nutrient signatures, with significance evident across almost all signatures. However, both types of macroalgae were statistically similar for $\delta^{15}N$, as were brown macroalgae, turf algae and zoanthid for %N (Suppl. Table 2). However, the effect of reef state varied among both bioindicators and nutrient signatures. For instance, differences in $\delta^{15}N$ signatures in BM ($p = 0.0002$), CYB ($p = 0.002$), GM ($p < 0.0001$), SED ($p = 0.01$), TA ($p = 0.02$) and ZO ($p < 0.0001$) were significant, whereas the difference in %N for GM between reef states was not ($p = 0.93$). Reef state was also significantly different for $\delta^{13}C$ in cyanobacteria ($p = 0.002$), green macroalgae ($p < 0.0001$), sediment ($p = 0.01$), turf algae ($p = 0.02$) and zoanthids ($p < 0.0001$). For %N, reef state also significantly differed in BM ($p < 0.0001$), CYB ($p < 0.0001$) and ZO ($p = 0.04$). For %C, reef state differed significantly for CYB ($p < 0.0001$) and ZO ($p = 0.01$), and for C:N Ratio, only BM ($p = 0.04$) and TA ($p = 0.0002$) differed significantly.

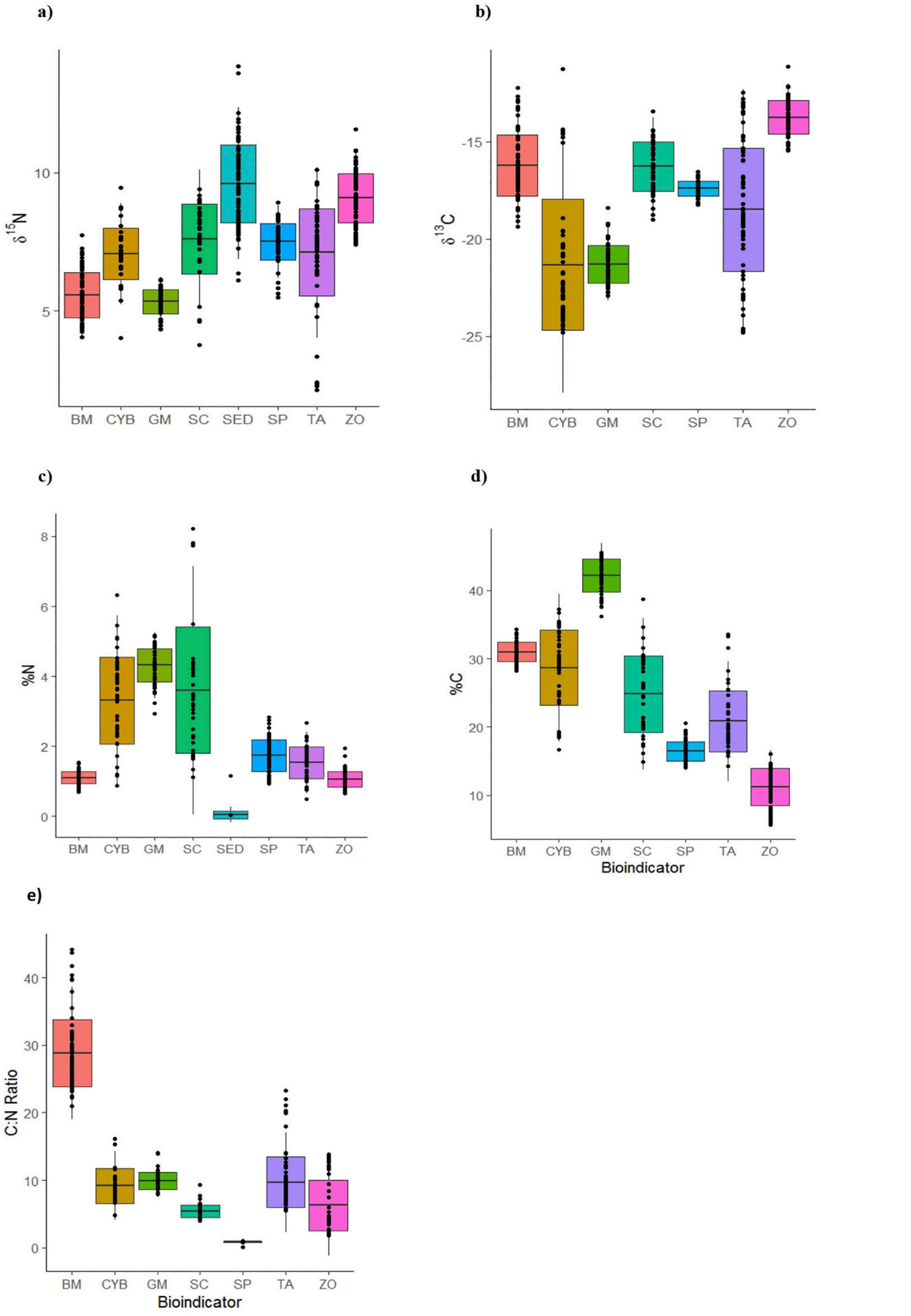

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Fig. 1. Box (median and 50% quantile) and whisker (95% quantile) plots of the variation of the average values of nutrient signatures measured in the eight bioindicators for (a) $\delta^{15}N$, (b) $\delta^{13}C$, (c) %N, (d) %C and (e) C:N ratio from up to 21 reefs. Each black dot represents the average value from an individual site that each bioindicator was collected from to also show the spread of variation within each bioindicator (BM = brown macroalgae; CYB = cyanobacteria; GM = green macroalgae; SED = sediment; SC = soft coral; SP = sponge; TA = turf algae, and ZO = zoanthid). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Precision of bioindicators

The precision of the bioindicators was assessed using CoV, as this standardised the nutrient signatures between bioindicators (including the non-biological indicator sediment) and controlled for differences in isotopic fractionation in measurements, particularly between trophic levels. Green macroalgae had the lowest and most consistent CoV within and across reefs, and therefore the highest precision for all N-based nutrient measurements ($\delta^{15}N$: 2.47 ± 0.95; %N: 7.53 ± 4.29; C:N ratio: 5.76 ± 5.39), however this pattern was not as distinct for C-only signatures ($\delta^{13}C$: −1.87 ± 1.06 and %C: 3.60 ± 1.67) (Fig. 2). This was closely followed by brown macroalgae ($\delta^{15}N$: 4.68 ± 1.33; $\delta^{13}C$: −6.03 ± 3.12; %N: 11.3 ± 4.07; %C: 4.07 ± 1.12, and C:N ratio: 9.92 ± 3.75). Turf algal assemblages had much more variable average signatures for all five measures, especially those that were N-based ($\delta^{15}N$: 8.30 ± 4.90; $\delta^{13}C$: −5.14 ± 4.30; %N: 20.5 ± 20.1; %C: 9.54 ± 10.6, and C:N ratio: 10.6 ± 10.3).

Zoanthids had lower average CoV values for N-based signatures than higher trophic organisms and were more similar to the two macroalgal types ($\delta^{15}N$: 2.98 ± 1.20, and %N: 14.3 ± 5.52), as well as for $\delta^{13}C$ (−5.14 ± 2.43), though the CoV values for both %C and C:N ratio were much higher than for any of the other bioindicators (11.8 ± 8.57 and 20.0 ± 24.1, respectively). The other higher trophic level organisms, such as soft corals ($\delta^{15}N$: 6.26 ± 4.87; $\delta^{13}C$: −6.20 ± 1.86; %N: 30.4 ± 17.6; %C: 17.4 ± 12.2, and C:N ratio: 11.6 ± 8.68) and sponges ($\delta^{15}N$: 6.82 ± 5.24; $\delta^{13}C$: −1.44 ± 1.08; %N: 20.0 ± 10.3; C%: 7.24 ± 3.94, and C:N ratio: 7.58 ± 12.1) showed inconsistent levels of precision across the five signatures. Though sediment had similar precision for $\delta^{15}N$ to the other candidates (7.97 ± 3.90), it had the highest range of CoV values for %N (17.4 ± 40.2) (Fig. 2a).

Overall, the CoV analyses showed that both brown and green macroalgae had low average CoV values for N-based signatures, as well as small variations in CoV across the sites. In addition, while the C-based signatures were more variable for zoanthids, the N-based results were more precise compared to the other higher-trophic bioindicators. There was also no overall significant effect of reef state or site-level variation on CoV for any of the five nutrient signatures, suggesting that precision did not vary over different spatial scales or between the coral-mortality and regime-shifted reefs. The statistical models showed variable patterns for each nutrient signature type across the eight bioindicators, however for %C and C:N Ratio, zoanthids were the only bioindicator that significantly differed from brown macroalgae due to its high variation (Suppl. Table 3).

3.4. Congruency of bioindicators

A principal components analysis (PCA) was used to assess congruency between the three selected bioindicators. Brown and green macroalgae had low correlation, especially for signatures of N, while zoanthids had no significant relationships with either macroalgae. There were weak positive relationships between N-based signatures of green and brown macroalgae (Table 2), but these explain <40% of the variance and are not significant at alpha = 0.05 (Fig. 3). This was also shown by Pearson's correlation analyses between the different combinations of bioindicators (Table 2). The two types that showed the highest similarity for N-based signatures were between brown and green macroalgae for C:N ratio measurements ($r^2 = 0.61$), closely followed for those of %N ($r^2 = 0.60$) and $\delta^{15}N$ ($r^2 = 0.55$) signatures, though none of these were significantly correlated. However, the highest similarity for C-only signatures was between %C of brown and green macroalgae ($r^2 = 0.81$), but was very low for $\delta^{13}C$ ($r^2 = 0.041$) (Table 2).

3.5. Cost-effectiveness of bioindicators

The time taken for the whole process, from collection to stable isotopic analyses, per unit sample, differed among the eight bioindicators (Table 3; Suppl. Table 4). The GLMs suggested that both bioindicator and task can have a significant effect on the time taken, per sample, to use each bioindicator for capturing measure nutrient regimes, but reef state does not. Overall, it took a similar amount of time to collect the two macroalgae and cyanobacteria, whereas soft corals, sponges, turf algae and zoanthids took significantly longer to find. Sediment, in contrast, took the least time overall to find and collect (Table 3). Each task differed significantly as well, with "Drying and Crushing" taking the most time to complete and "Field Collection" took the least time, but significance varied between the bioindicators. The time taken to process the four calcified bioindicators was much greater, because each sample of these indicators required the additional step of "Acidification".

Although the time taken per sample to collect each bioindicator from the field did not differ between reef states, the availability of samples on the different reef did (Table 1). There was a strong negative correlation between average time taken per sample to collect and the percentage of sites from which each indicator was available on regime-shifted reefs (relative to the total number of sites, i.e. n = 9) ($r^2 = 0.94$), whereas there was a very weak negative relationship between average time taken and sample availability on coral-mortality sites ($r^2 = 0.15$; n = 12) (Fig. 4). This suggests that although the time taken varied more among bioindicators on regime-shifted reefs (i.e. it took over an hour, on average, to find one sample of soft coral), it is a better predictor for finding specific bioindicator(s) on sites dominated by macroalgae. For coral-mortality reefs, in contrast, the times among bioindicators were more similar, but sample availability was more variable. Brown macroalgae had similar collection times between reef states (regime-shifted: 0.01 ± 0.01; coral-mortality: 0.07 ± 0.05 h), but there was 100% availability on regime-shifted sites relative to 58% on coral-mortality sites. Turf algae and sediment, in contrast, not only had 100% availability on both reef states, but they took the least amount of time to collect .

4. Discussion

The principal aims of this study were to identify precise, cost-effective, and congruent bioindicators for capturing nutrient regimes on coral reefs, particularly over those in different ecological conditions. Overall, nutrient signatures of brown macroalgae, green macroalgae and zoanthids were considered to meet these criteria, relative to the other candidates. While the macroalgae were more consistent indicators for reefs that have undergone a regime shift, zoanthids were more common for both types of reef state. Turf algae and sediment took the least amount of time to collect and were also the most abundant and available samples across the 21 reefs studied, regardless of reef state, but their utility as indicators was limited by their highly variable CoV values. There was low congruency between the three most precise indicators (brown macroalgae, green macroalgae and zoanthids), which suggested that biological processing of nutrients within each bioindicator has a greater influence on N- and C-based signatures than their local environment does. Congruency between multiple taxa could be improved by either choosing a suite of indicators from the same functional group,

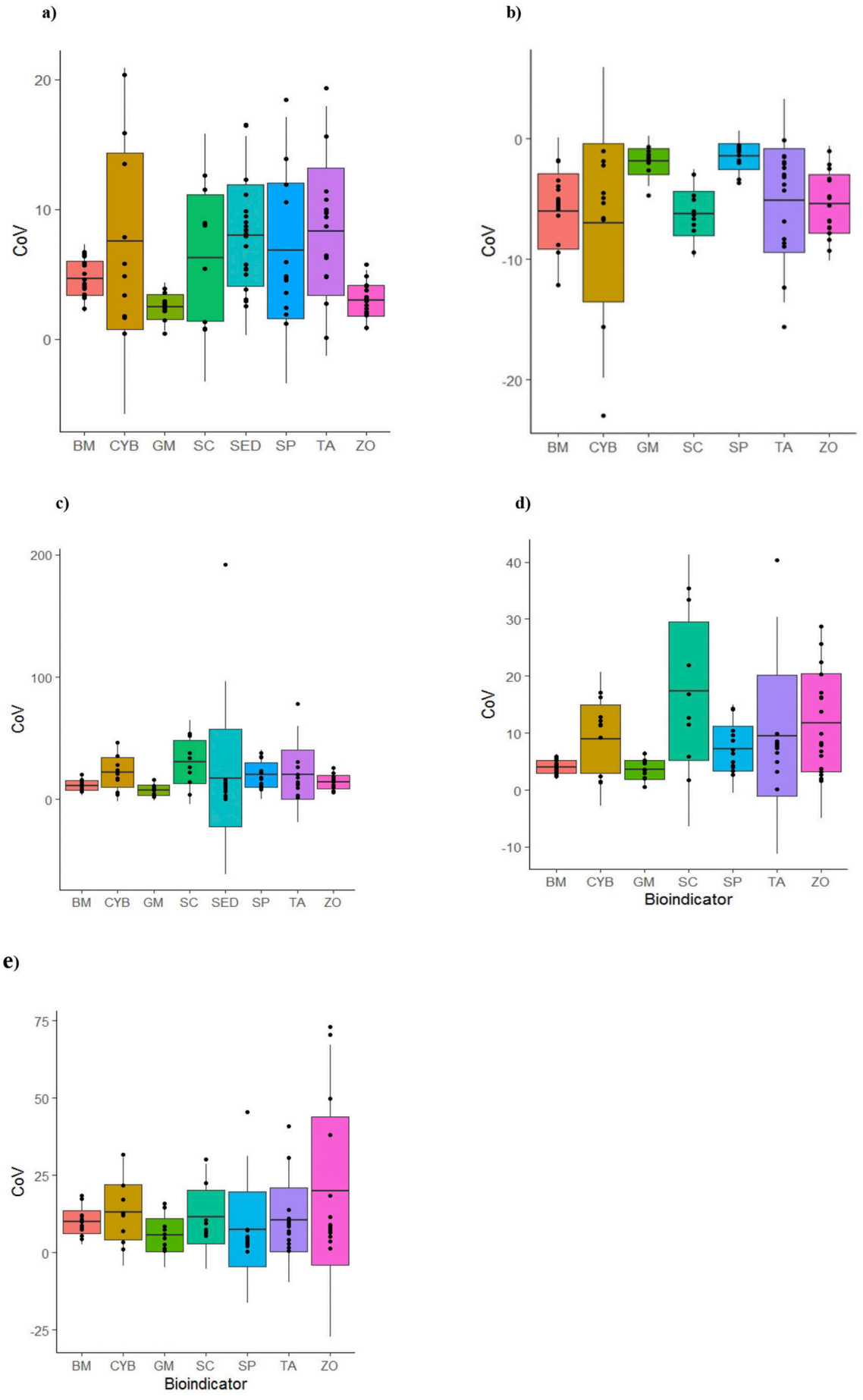

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Fig. 2. Box (median and 50% quantile) and whisker (95% quantile) plots of the spread of the coefficient of variation (CoV) of the eight bioindicators for (a) $\delta^{15}N$, (b) $\delta^{13}C$, (c) %N, (d) %C and (e) C:N ratio up to 21 reefs (mean ± S.D.). Each black dot represents the average CoV from the individual sites from which each bioindicator was collected to also show the spread of variation within- and among sites (BM = brown macroalgae; CYB = cyanobacteria; GM = green macroalgae; SED = sediment; SC = soft coral; SP = sponge; TA = turf algae, and ZO = zoanthid). CoV for each nutrient measurement in each bioindicator collected from each site was calculated by the ratio of standard deviation to the mean of a given number of replicate data points (i.e. up to 5 samples per indicator per site). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Pearson's correlation analyses between the three selected bioindicators (brown macroalgae (BM) versus green macroalgae (GM); brown macroalgae versus zoanthids (ZO); green macroalgae versus zoanthids) to determine amount of correlation between them (correlation coefficient). The significance level for the *p*-values is alpha = 0.05.

Bioindicator	$\delta^{15}N$	$\delta^{13}C$	%N	%C	C:N ratio
BM vs. GM	0.55 ($p = 0.12$)	0.041 ($p = 0.92$)	0.60 ($p = 0.09$)	0.81 ($p = 0.02$)	0.61 ($p = 0.08$)
BM vs. ZO	0.10 ($p = 0.79$)	0.11 ($p = 0.80$)	0.18 ($p = 0.64$)	−0.005 ($p = 0.99$)	0.07 ($p = 0.68$)
GM vs. ZO	0.28 ($p = 0.47$)	0.64 ($p = 0.09$)	0.23 ($p = 0.55$)	−0.23 ($p = 0.58$)	−0.36 ($p = 0.34$)

such as macroalgae with comparable nutrient uptake mechanisms, or by tracing the accumulation of nutrient signatures across different trophic levels from the same food chain.

4.1. Spatial variation, precision and congruency of nutrient signatures in bioindicators

The N- and C-based nutrient signatures of the bioindicators in the current study appear typical of measurements reported in the literature (Atkinson and Smith, 1983; Smit, 2001). For instance, the range of absolute values of $\delta^{15}N$ signatures in all of the bioindicators are quite consistent (5–10‰), though they are slightly high relative to other marine systems (Sigman and Casciotti, 2001). In addition, the $\delta^{13}C$ signatures reflect that of a carbonate-dominated system, which for instance lies within the range of −10 to −30‰ for most marine macrophytes (Smit, 2001; Raven et al., 2002). The N-based signatures also follow trophic status whereby those organisms at higher trophic levels are relatively more enriched than those of primary producer status (Boecklen et al., 2011; Lamb et al., 2012).

Spatial variation of the different nutrient signatures, both within and among reefs, varied widely across the inner Seychelles. The N-based signatures also showed a significant difference between coral-mortality and regime-shifted reefs for a number of the bioindicators, including $\delta^{15}N$ in the two macroalgae and zoanthids, whereas signatures tended to be more similar across sites for the C-based signatures. Being able to capture variability in nutrient regimes, especially across different spatial scales or even different reef states, is another important aspect of a good bioindicator (Cooper et al., 2009), so this study provides supporting evidence that $\delta^{15}N$ and %N are particularly effective proxies of nutrient regimes (Lin and Fong, 2008). For instance, Littler et al. (1991) found that nutrient concentrations in a number of algal species were generally higher on reefs around the high granitic, populated islands like Mahe and Praslin, relative to the low, remote carbonate atolls in the wider Seychelles Archipelago. In a related study in Vaughan et al. (2021), the use of macroalgal $\delta^{15}N$ helped to determine that the dead coral tissue released into the water column after the 2016 coral bleaching event in the Seychelles may have been subsequently taken up and retained by macroalgae like *Sargassum* on the coral-mortality reefs. However, the high variability shown across nutrient signatures in the current study, particularly in $\delta^{15}N$, may not be solely due to differences in local sources of nutrients. Other studies, for example, have found that differences in signatures are not always consistent with distinct sources of nutrient loads (i.e. in areas with known anthropogenic run-off), which implied that external inputs are not always the cause of variations in nutrient regimes captured in bioindicators (Raimonet et al., 2013; Viana and Bode, 2013).

There were discrepancies found in some of the signatures even between different primary producers in this study, such as between brown (*Sargassum* sp.) and green macroalgae (*Chlorodesmis* sp.). For instance, although they had similar $\delta^{15}N$ values across the sites, the other four signatures varied on average between these two bioindicators, particularly for %N, which was much higher in green macroalgae, although it was similar between reef states (Fig. 2a & c). This could be because nitrogen content in *Chlorodesmis* is affected by both biological nutrient uptake mechanisms and environmental factors (Fong et al., 2001; Raimonet et al., 2013; Viana and Bode, 2013; Clausing and Fong, 2016), and therefore does not reflect either inorganic concentrations or the $\delta^{15}N$ of their surrounding environment (Viana and Bode, 2013). Slower-growing algal species like *Chlorodesmis* have a greater capacity for internal nutrient storage so are not as nutrient-limited, and therefore are less responsive to fluctuations in nutrients as other, more opportunistic species like *Sargassum* (Schaffelke, 1999; García-Seoane et al., 2018a, 2018b).

Zoanthids are positioned at a higher trophic level than benthic algae so their nutrient signatures tend to fractionate and become more enriched (Fig. 1a; Zanden and Rasmussen, 2001; Fox et al., 2018). There has been little research into zoanthids as potential indicators of nutrient runoff (Leal et al., 2017), but Costa Jr. et al. (2008) found that phosphorus and silica water concentrations had positive effects on both algal and zoanthid growth, and negative effects on coral cover. However, unlike primary producers, zoanthids have to balance auto- and heterotrophic processes for acquiring sources of C and N (Smit, 2001; Leal et al., 2017) because, similarly to scleractinian corals, they have photosynthetic symbionts in their tissues (Hoegh-Guldberg et al., 2004; Fox et al., 2018). This could explain the large variations in %C and C:N ratio, both within- and among-reefs in this study (Fig. 2d & e; Suppl. Table 2), as they represent the combined signatures from both host and symbiont (Leal et al., 2017).

Even though the three most precise bioindicators (brown macroalgae, green macroalgae and zoanthids) all showed significant differences in $\delta^{15}N$ between the two reef states for the spatial variation analyses, their CoV values did not. This suggests that these bioindicators are not only consistently precise among reefs and reef states, but are also able to detect differences in nutrient regimes across the same areas, which is why $\delta^{15}N$ is such a versatile tool for monitoring water quality (Costanzo et al., 2001; Lin and Fong, 2008). However, when compared directly, the congruency among these three bioindicators was relatively low. This could be due to the differences in nutrient processing between the different bioindicators. Congruency is important, as a single-species approach may result in an underestimation of spatial patterns in nutrient regimes (Linton and Warner, 2003), and it has been shown across multiple taxa in previous studies (Connolly et al., 2013), but these studies were also conducted along strong nutrient gradients (i.e. with increasing distance from a sewage outfall) (Fernandes et al., 2012). This suggests that in the current study, the biological mechanisms of individual species may have outweighed the effect of environmental factors on their isotopic and elemental signatures.

The other (bio)indicators included in this study were found to have variable and inconsistent nutrient signatures across sites and the two reef states, which was why they were not included in the congruency analyses. Like macroalgae, turf algal assemblages and cyanobacteria are

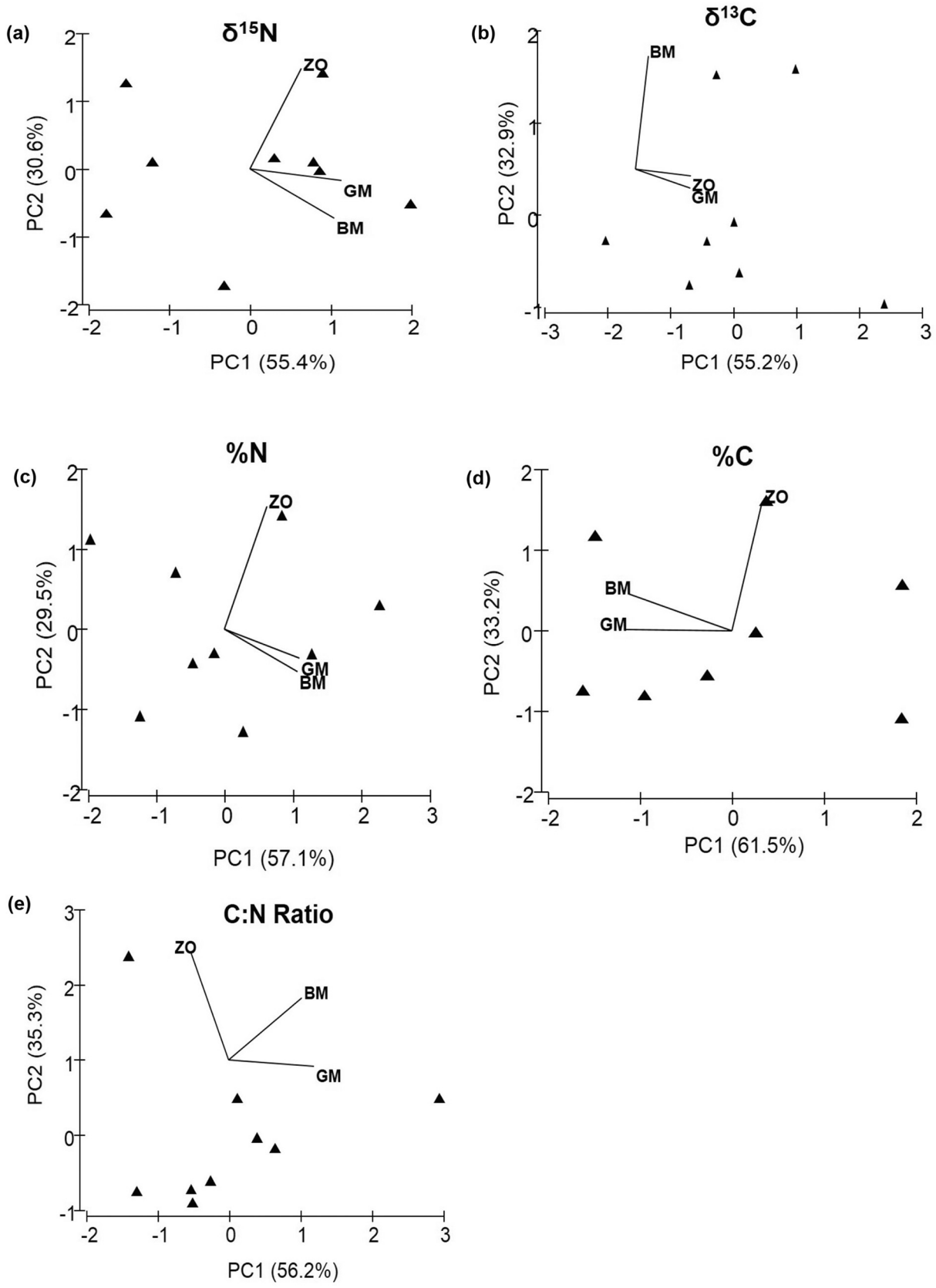

Fig. 3. Principal components analyses (PCA) quantifying congruency between a selection of bioindicators (n = 3) (BM = brown macroalgae; GM = green macroalgae; ZO = zoanthids) all present at a subset number of sites (n = 9) for measurements of (a) $\delta^{15}N$, (b) $\delta^{13}C$, (c) %N, (d) %C and (e) C:N ratio. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Summary of the mean time taken (per unit sample, per hour) for each task undertaken to process each bioindicator for the cost-effectiveness. Acidification only includes the four bioindicators that were acidified, and thus weighed and analysed in the mass spectrometer. Significance level is $p < 0.05$. Normality inspected using visual plots. Mean ± S.D.

Bioindicator	Field collection	Drying & crushing	Acidification	Weighing	Stable isotopic analyses
Brown macroalgae (BM)	0.038 ± 0.04 ($p < 0.0001$)	24.8 ± 0.5 ($p < 0.0001$)	-	1.5 ± 0.01 (N/A)	0.18 ± 0.03 ($p < 0.0001$)
Cyanobacteria (CYB)	0.35 ± 0.4 ($p = 0.52$)	23.2 ± 1.4 ($p < 0.0001$)	–	1.5 ± 0.03 (N/A)	0.21 ± 0.1 ($p = 0.93$)
Green macroalgae (GM)	0.078 ± 0.08 ($p = 0.86$)	24.1 ± 0.005 ($p = 0.26$)	–	1.5 ± 0.01 (N/A)	0.17 ± 0.05 ($p = 0.98$)
Soft coral (SC)	0.25 ± 0.3 ($p = 0.001$)	22.2 ± 1.2 ($p < 0.0001$)	0.17 ± 0.001 ($p < 0.0001$)	3.1 ± 0.06 ($p = 0.95$)	0.48 ± 0.2 ($p = 0.004$)
Sediment (SED)	0.015 ± 0.003 ($p = 0.0002$)	22.7 ± 1.2 ($p = 0.05$)	–	0.14 ± 0.02 (N/A)	0.25 ± 0.1 ($p < 0.0001$)
Sponge (SP)	0.24 ± 0.3 ($p = 0.0006$)	22.6 ± 1.3 ($p < 0.0001$)	0.17 ± 0.0 ($p < 0.0001$)	3.1 ± 0.00 ($p = 0.89$)	0.37 ± 0.07 ($p = 0.002$)
Turf algae (TA)	0.03 ± 0.04 ($p = 0.0002$)	24.6 ± 0.5 ($p = 0.0003$)	0.17 ± 0.002 ($p < 0.0001$)	3.0 ± 0.02 (p = 0.98)	0.34 ± 0.08 ($p = 0.0005$)
Zoanthids	0.18 ± 0.2 ($p < 0.0001$)	23.0 ± 1.5 ($p < 0.0001$)	0.17 ± 0.0 ($p < 0.0001$)	3.0 ± 0.00 (N/A)	0.41 ± 0.03 ($p = 0.0004$)

primary producers that not only take up and utilise bioavailable nutrients but are becoming more prevalent on reefs across a range of reef states, particularly following a disturbance (den Haan et al., 2014; Zaneveld et al., 2016; Ford et al., 2018). However, this study showed that both bioindicators had variable precision among the five nutrient signatures with no clear spatial patterns between reefs, which implied they were also more influenced by biological factors (i.e. multiple species within the turf assemblage) than their local environment (Steneck and Dethier, 1994; Raimonet et al., 2013). Similarly to zoanthids, soft corals can also harbour symbionts (Fleury et al., 2000; Risk, 2014; Williams et al., 2018), and while sponges are not photosynthetic, they do have symbiotic relationships with cyanobacteria, which is reflected in their $\delta^{13}C$ signatures (Smit, 2001; Lamb et al., 2012). Sediments can also capture a range of nutrients within a reef, which can be resuspended within local biogeochemical cycles through various biophysical factors and thus provide an additional source (Fabricius, 2005; Umezawa et al., 2008). However, some studies have found sediments to be an overall poor indicator (Fichez et al., 2005). In the current study, for instance, very little N was detected in the subsamples of sediment analysed even before acidification, so the low precision calculated for it was more likely due to random error than environmental factors, and so was not comparable for either N- or C-based signatures.

4.2. Cost-effectiveness of bioindicators

Cost-effectiveness is often mentioned as an important criteria in previous bioindicator studies (Fichez et al., 2005; Cooper et al., 2009; Risk et al., 2001). However, analyses are rarely conducted to quantify these in ecological studies (Drummond and Connell, 2008; Bal et al., 2020) even though the "cost" of any particular indicator can be affected by various different factors. For instance, the average time taken to collect an individual sample from a study site depended upon its availability and/or abundance, which is why there was a significant difference in collection time with reef state. While it only took ~1 to 2 min on average to collect samples of turf algae and sediments from each site, regardless of ecological condition, it took significantly less time to collect brown macroalgae from regime-shifted reefs than it did from coral-mortality reefs. Differences in availability on those reefs could be influenced by nutrient loads, abundance of herbivores, depth, structural complexity, and juvenile coral cover (Graham et al., 2015; Dajka et al., 2019). The findings of both the sample collection and the line-intercept survey of benthic cover at the 21 sites illustrated the importance of considering the local abundance of a bioindicator when assessing nutrient regimes (Cooper et al., 2009; Fabricius et al., 2012). For instance, turf algae and sediments were ubiquitous at all sites, so could

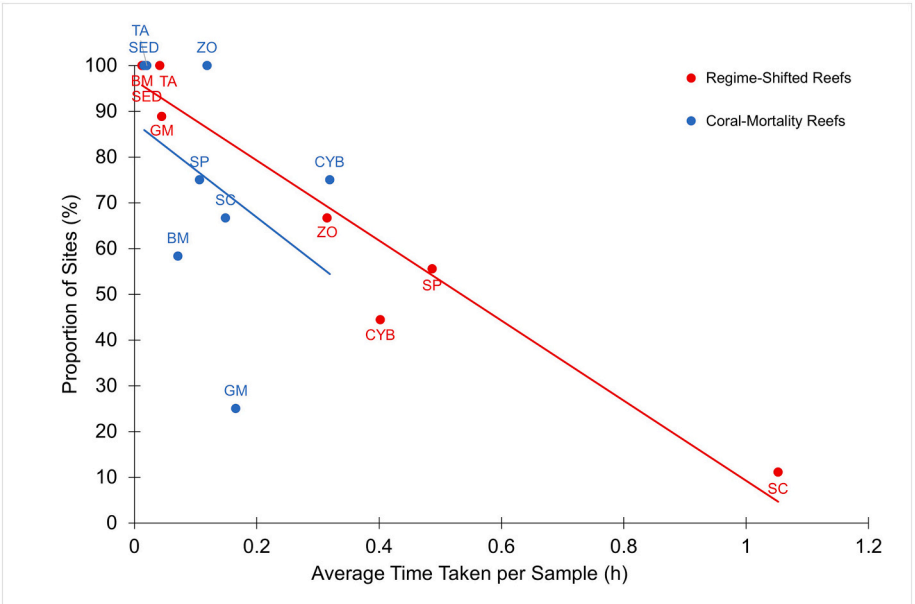

Fig. 4. The relationships between the average time taken, per unit sample (h) and the availability of samples on both reef states. Each individual point in red represent the total average time, per sample, for the eight bioindicators collected from regime-shifted sites versus the percentage of sites they were available to collect at (n = 12), and the individual point in blue represented each indicator from coral-mortality sites. $r^2 = 0.94$ on regime-shifted reefs, and $r^2 = 0.15$ on coral-mortality reefs. BM = brown macroalgae; CYB = cyanobacteria; GM = green macroalgae; SED = sediment; SC = soft coral; SP = sponge; TA = turf algae, and ZO = zoanthid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

be considered as more "cost-effective" in terms of sampling availability and abundance. However, as turf algae are composed of an assemblage of varying functional groups, and there was very little N detected in sediment, it is difficult to interpret results for nutrient signatures from either bioindicator, and therefore to rely on them for capturing nutrient regimes precisely, despite their widespread abundance.

4.3. Future directions in bioindicator research

This study investigated novel ways of assessing potential bioindicators for monitoring programs across coral reefs under different ecological states. However precision and effectiveness of bioindicators used in this study could be improved, even if these improvements will increase costs. For instance, to reduce the CoV of turf algal assemblages, cyanobacteria, and symbiotic organisms, future studies could isolate and individually measure the different functional groups within assemblages (Steneck and Dethier, 1994), individual strains of cyanobacteria (Thacker and Paul, 2001), or the host and symbiont fractions in zoanthids and soft corals (Hoegh-Guldberg et al., 2004; Leal et al., 2017) so that the nutrient signatures of each group can be measured and interpreted separately. Conversely, such techniques will increase the time taken to process and analyse samples, and thus will increase their "costs" as a bioindicator.

It was also difficult to determine the accuracy of the bioindicator nutrient signatures, as there is little reference data for nutrient levels around the inner Seychelles Islands, even from seawater samples, and especially at the spatio-temporal scales required for this study. Further research should therefore also investigate the accuracy of cost-effective bioindicators such as macroalgae for capturing either natural or anthropogenic sources by additionally measuring stable isotopic signatures of potential point sources (Costanzo et al., 2001; Dailer et al., 2010; Fernandes et al., 2012; den Haan et al., 2014). Another approach could entail building up a suite of relatively similar bioindicators by focusing on specific functional group(s), appropriately matched to the scale of the ecological process being investigated (Fong and Fong, 2014). If this option is not possible, for instance, when a group of congruent bioindicators (i.e. fleshy macroalgae) is only found on reefs in a certain ecological state, then nutrient signatures could be compared across a suite of bioindicators to see the accumulation of this energy source across different trophic levels within the same food chain (Smit, 2001; Pitt et al., 2009; Connolly et al., 2013; Kürten et al., 2014; Graham et al., 2018).

5. Conclusion

In conclusion, the stable isotopic and elemental signatures of fleshy macroalgae were found to be precise and cost-effective bioindicators across coral reefs in the inner Seychelles, as primary producers with widespread distribution and consistent measurements within their tissues. If the precision of bioindicators can be increased, it would provide additional opportunities to determine differences in bioavailable nutrient regimes between reefs. This could be particularly useful in remote coastal areas where environmental monitoring efforts to assess the local anthropogenic impacts of coastal run-off and excessive nutrient loads on coral reefs are currently limited, but would be highly beneficial to assessing overall ecosystem health. If remote reefs have been subjected to any large disturbance, such as a mass bleaching event, having precise and cost-effective bioindicators to detect whether any areas have excessive nutrient loads, could enable better-informed efforts to improve water quality and mediate coral recovery potential.

Funding

The project was funded via grants from the Royal Society (UF140691 and RG150710).

CRediT authorship contribution statement

Eleanor J. Vaughan: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Peter M. Wynn:** Methodology, Resources, Supervision. **Shaun K. Wilson:** Funding acquisition, Investigation, Writing – review & editing. **Gareth J. Williams:** Supervision, Writing – review & editing. **Philip A. Barker:** Supervision, Writing – review & editing. **Nicholas A.J. Graham:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

We thank Seychelles Fishing Authority, Seychelles Marine Parks Authority and Jan Dajka for field and laboratory assistance in the Seychelles, and David Hughes and Jon Crosse from Lancaster Environment Centre for extensive help with preparations for fieldwork and with the subsequent stable isotopic and elemental analyses. James Robinson and Jeneen Hadj-Hammou gave advice on statistical analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112606.

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Coral Reefs (2021) 40:767–776
https://doi.org/10.1007/s00338-021-02079-w

REPORT

Nitrogen enrichment in macroalgae following mass coral mortality

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Received: 25 July 2020 / Accepted: 11 March 2021 / Published online: 12 April 2021

Abstract Scleractinian corals are engineers on coral reefs that provide both structural complexity as habitat and sustenance for other reef-associated organisms via the release of organic and inorganic matter. However, coral reefs are facing multiple pressures from climate change and other stressors, which can result in mass coral bleaching and mortality events. Mass mortality of corals results in enhanced release of organic matter, which can cause significant alterations to reef biochemical and recycling processes. There is little known about how long these nutrients are retained within the system, for instance, within the tissues of other benthic organisms. We investigated changes in nitrogen isotopic signatures ($\delta^{15}N$) of macroalgal tissues (a) $\sim$ 1 year after a bleaching event in the Seychelles and (b) $\sim$ 3 months after the peak of a bleaching event in Mo'orea, French Polynesia. In the Seychelles, there was a strong association between absolute loss in both total coral cover and branching coral cover and absolute increase in macroalgal $\delta^{15}N$ between 2014 and 2017 (adjusted $r^2 = 0.79$, $p = 0.004$ and adjusted $r^2 = 0.86$, $p = 0.002$, respectively). In Mo'orea, a short-term transplant experiment found a significant increase in $\delta^{15}N$ in *Sargassum mangarevense* after specimens were deployed on a reef with high coral mortality for $\sim$ 3 weeks ($p < 0.05$). We suggest that coral-derived nutrients can be retained within reef nutrient cycles, and that this can affect other reef-associated organisms over both short- and long-term periods, especially opportunistic species such as macroalgae. These species could therefore proliferate on reefs that have experienced mass mortality events, because they have been provided with both space and nutrient subsidies by the death and decay of corals.

Keywords Climate change · Macroalgal bioindicators · Coral bleaching · Stable isotopes · Biogeochemical cycles · Coral reef ecology

Topic Editor Andrew Hoey

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00338-021-02079-w.

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Introduction

Tropical coral reefs are highly productive ecosystems, but as they are typically surrounded by oligotrophic waters, they require constant recycling and retention of waterborne nutrients and organic matter (Galloway et al. 2004). There are a wide range of physical and biological processes on coral reefs which can retain these essential energetic resources within local biogeochemical cycles for extended periods of time. Thus, these processes can sustain rapid rates of biological activity such as primary productivity, as well as many other key ecosystem functions (Wyatt et al.

2013). For instance, coral-derived particulate organic matter (POM) in the form of mucus can act as an energy carrier and particle trap, so these nutrients may be recycled by benthic and planktonic communities over longer timescales (Ferrier-Pagès et al. 1998; Wild et al. 2004a, b). However, even in a coral-dominated ecosystem, they are not the only natural, or autochthonous, source of bioavailable nutrients (Davey et al. 2008; Wyatt et al. 2013; Tanaka and Nakajima 2018). Microbes, for instance, are capable of nitrogen fixation (Moulton et al. 2016), and other primary producers, such as phytoplankton and macroalgae, readily take up and store nutrients and dissolved organic matter (DOM) in their tissues (Fong et al. 1994). This DOM is then recycled either through tissue breakdown or through consumption by higher trophic level organisms such as herbivorous fishes, which in turn recycle significant amounts of nutrients through excretion (Burkepile et al. 2013).

Healthy coral reefs typically persist in low nutrient waters, although nutrient pulses can disrupt the balance of natural biogeochemical dynamics jeopardising reef health. Disturbances such as marine heat waves that cause coral bleaching have a direct negative impact on corals, but can also have indirect consequences for reefs by altering nutrient dynamics (D'Angelo and Wiedenmann 2014). Branching scleractinian corals are often dominant on a reef, providing structural complexity and micro-habitats for a variety of reef-associated organisms, but they are also particularly vulnerable to heat stress (Hughes et al. 2019). The loss of these vital foundation species therefore has huge implications for the entire ecosystem (Graham et al. 2015; Wilson et al. 2019). Where coral bleaching causes extensive mortality, the metabolic exchange between corals and associated organisms on a reef is reduced, along with the capacity of corals to trap organic matter. This can subsequently trigger the dysfunction of major biogeochemical processes (Glynn 1993; Wild et al. 2011).

There are few studies assessing how climate-derived disturbances affect mucus release by live corals, and associated processes. Davey et al. (2008) found that in the weeks that follow coral bleaching, a 30-fold higher production of new nitrogen occurred on coral reefs compared to those that did not experience bleaching. Such nitrogen productivity has also been shown in an experimental setting (Niggl et al. 2009). While release rates of mucus-derived POM from corals increase during the early stages of bleaching, providing a burst of nutrients to coral reefs (Coffroth 1990), these rates can decrease after the initial bleaching response (Fitt et al. 2009; Wooldridge 2009). If corals recover from bleaching, which can take many weeks to occur (Gates 1990), there may only be short- to medium-term effects on biogeochemical processes. However, if corals die, the subsequent mass release of coral tissue into reef environments may also alter biogeochemical processes, and over longer time scales. In addition, colonisation of the exposed coral skeleton by microbial biofilms, turf algae, macroalgae, sponges, cyanobacteria or other invertebrates may not only reduce coral recruitment success, but can also change biogeochemical processes such as nitrogen fixation (Diaz-Pulido and McCook 2002; Davey et al. 2008; Haas et al. 2010).

In order to identify changes in nutrient regimes due to mass coral mortality, nitrogen stable isotopes ($\delta^{15}N$) and nitrogen content (%N) can be analysed from macroalgal tissues to capture temporally-extensive records of nutrient loads (Costanzo et al. 2001). Stable isotopes of nitrogen have been used in nutrient studies for several decades, helping to identify the origins of nitrogen (Heaton 1986; Kolasinski et al. 2011). In addition, certain types of marine algae are commonly used in biomonitoring studies due to their widespread distribution and responsiveness to bioavailable pollutants. *Sargassum*, for example, is a genus used worldwide as it has been found to be responsive to nutrient enrichment (Schaffelke and Klumpp 1998; Schaffelke 2002; García-Seoane et al. 2018). However, marine algae are not the only functional group that can be used to measure isotopic signatures as a proxy of nutrient regimes on reefs. Organisms at higher trophic levels also assimilate nutrients from lower trophic levels, resulting in increasing isotopic enrichment up the food chain (Bierwagen et al. 2018). For instance, corals are at a higher trophic level than primary producers such as macroalgae, and thus have enriched isotopic signatures (Graham et al. 2018). As corals release organic matter into the water column after the death and subsequent decay of tissue following marine heatwave-driven mortality events (Leggat et al. 2019), opportunistic benthic species such as macroalgae may capitalise on this new nutrient source, assimilate it into tissues for growth and storage, and consequently become more enriched (Pawlik et al. 2016).

In the current study, the temporal effect of coral mass mortality on macroalgal stable isotopic signatures is investigated in two different coral reef systems, over two different time periods. As such, it offers new understanding on whether macroalgae can indicate longer-term effects of coral mortality events on reef nutrient dynamics and biogeochemical cycles. Specifically this study assesses: (1) changes in *Sargassum* sp. nutrient signatures over three years in the inner Seychelles Islands, western Indian Ocean, spanning a mass coral bleaching event, and (2) shorter-term changes in *Sargassum mangarevense* nutrient signatures $\sim$ 3 months after the peak of a severe bleaching event in Mo'orea, French Polynesia, using an *in-situ* three-week transplant experiment.

Methods

Study Site 1: Seychelles

The inner Seychelles islands experienced two severe coral bleaching events, in 1998 and 2016. In 1998, coral cover dropped by 90%, and though hard coral cover steadily recovered on some study sites (average coral cover of 27% by 2014) (Graham et al. 2015), another global bleaching event in 2016 (Hughes et al. 2018) led to live coral cover declining by 70% on these same sites (Wilson et al. 2019). Around the Inner Seychelles, heat stress reached 4 °C weeks in January 2016, rapidly increased in April and peaked at 11.4 °C weeks in May (Wilson et al. 2019; http://coralreefwatch.noaa.gov/vs/index.php).

Eighteen reefs were surveyed in April 2014, before the mass bleaching event caused extensive coral mortality in 2016 and again in April 2017, a year after the event occurred (Wilson et al. 2019). These reefs form part of a 25-year coral reef monitoring survey around the inner Seychelles, with roughly half the reefs having been defined as "recovering" from a previous mass bleaching event in 1998, and the other half as transitioning to a "regime-shifted" macroalgae-dominated state (Graham et al. 2015). Eight replicate 7-m radius point counts were surveyed along the reef slope on each reef for both survey years. Within each point count area, the percent cover of benthic categories including live hard coral, soft coral, macroalgae, sand, rubble and rock was quantified using 10-m-long line-intercept transects (Wilson et al. 2019).

The objectives of this component of the study were to assess the relationship between changes in percent cover of corals between the study years of 2014 and 2017 with differences in $\delta^{15}N$ and %N signatures in tissues of *Sargassum* sp. that were collected from the same sites during the same surveys. Low availability of macroalgae at some reefs meant that macroalgae for stable isotope analyses were not collected from all reefs in both years. A minimum of four replicate *Sargassum* sp. samples were collected from each of the seven "coral mortality" reefs (a subset of the previously termed "recovery reefs", named as such following the impacts of the 2016 bleaching event) and from the six "regime-shifted" reefs in both 2014 and 2017.

Study Site 2: Mo'orea

Mo'orea, an island which is part of the Society Archipelago in French Polynesia, has demonstrated rapid coral recovery from previous disturbances (Vercelloni et al. 2019; Hédouin et al. 2020). For example, following an outbreak of *Acanthaster* spp. from 2006 to 2009 and a cyclone in 2010, mean coral cover on the outer reefs was reduced to 2% at 10 m depth from a high of 39% in 2005, before recovering to 27% in just four years. The branching coral genus *Pocillopora* spp. was found to be a significant driver in that recovery, as it made up 53% of the re-established coral community (18% cover) (Tsounis and Edmunds 2016). There were no recorded episodes of abnormally high sea surface temperature (SST) in 1998 in Mo'orea, but it was impacted by the global coral bleaching event in 2016, with heat-sensitive branching corals being the worst affected (Hughes et al. 2019). Donovan et al. (2020) reported that 37% of *Acropora* and 28% of *Pocillopora* colonies exhibited bleaching across all sites, with up to 100% bleaching of *Acropora* on north shore sites. Coral mortality was rare ($\sim$ 1%), as heat stress did not exceed 1.1 °C weeks (Hédouin et al. 2020).

Annual surveys of 13 marine areas around Mo'orea were established in 2004 (Service National d'Observation CORAIL). For the purpose of this study, data for the reef slope at the four areas along the north coast of the island, where bleaching was highest and our study site was located, were used (Suppl Fig. 1). This includes the site Tiahura which is closest to our study site. The benthic cover of each sample area was quantified at a similar depth to the transplant site ($\sim$ 10 m) using 3 replicate non-permanent 25 m transects (Horta e Costa et al. 2016). The percentage cover of benthic components was sampled every 50 cm using the point intercept transect (PIT) method. Macroalgae were categorised as all the non-coralline algae of large enough size to identify with the naked eye.

Sea surface temperature (SST) was measured hourly using an SBE-56 sensor (Sea Bird Scientific) on the Tiahura forereef at 3 m depth from 1998 to 2005. The time series was interrupted for 5 years before being collected continuously again from 2010. In order to characterise the temperature trend in 2019, relative to that of other years, we calculated weekly means for 2019 and compared this with the average temperature time series and 95% confidence intervals for the entire period. In addition, following Donovan et al. (2020), we calculated cumulative heat stress (in °C weeks) as a 12-wk running sum for all temperatures exceeding 29 °C, a threshold that is considered a good predictor of bleaching in Mo'orea based on previous studies (Pratchett et al. 2013; Donovan et al. 2020; Hédouin et al. 2020). The maximum water temperature during 2019 exceeded 29 °C in March and peaked at $\sim$ 30 °C in April. Patterns of cumulative heat stress peaked at $\sim$6 °C weeks. As the duration of heat stress was much longer in 2019 than in the previous bleaching event (Donovan et al. 2020; Hédouin et al. 2020), the extent of coral mortality was much higher (Suppl Fig. 2).

Samples of *Sargassum mangarevense* ($n = 10$) were collected from Papetoai lagoon, a low-nutrient reef in the northwest region of Mo'orea on 6th July 2019

(Suppl Fig. 1). These waters were found to typically have low $\delta^{15}N$ and %N values, shown in nutrient heat maps in Leichter et al. (2013) and Donovan et al. (2020). Specimens were placed in shaded coolers filled with seawater before they were transported back to the CRIOBE research station, Mo'orea. After all visible, larger epiphytes were carefully removed from the fronds using a scalpel; initial tissue samples were taken and frozen at −20 °C for later stable isotopic analyses. Algal specimens were then placed in pre-transplant holding tanks for seven days, with water changes every 2 days. Water changes in the tanks involved surface water collected from the forereef, as it was found to typically be low in $\delta^{15}N$ (< 3.0 ‰, Lin and Fong 2008; Donovan et al. 2020). This was done to ensure that internal nutrient stores in *S. mangarevense* were depleted before specimens were transplanted on the forereef where there were high levels of coral mortality. Following this 7-day acclimation period, further tissue samples were taken for stable isotopic analyses. For the in situ macroalgal bioassay, a cage was made out of chicken-wire mesh and attached to a cinder block that was already placed on the forereef at ∼ 12 m depth. At the time of the transplant experiment in July 2019, while some corals were still bleached, ∼ 40% had already died (S.J.H., 2020, pers. obs.). It was not possible to have a control bioassay, due to restrictions on deploying additional cinder blocks and the lack of non-bleached reefs at that time. The ten macroalgal specimens were deployed on the reef for ∼ 3 weeks from 15th July to 4th August 2019 before they were collected and returned to CRIOBE. Final tissue samples were taken and frozen before stable isotopic analyses were performed.

Stable isotopic analyses

All frozen samples from both studies were defrosted, rinsed thoroughly with fresh or distilled water, and placed in a drying oven for 48 h at 60 °C. Once dried, samples were each ground into a fine powder using a ball mill and stored in individual airtight containers. 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 ($\delta^{15}N$) and elemental analyses (%N) for both the 2017 samples from the Seychelles study and the 2019 Mo'orea 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. The samples collected in 2014 from the Seychelles were analysed using a Costech Elemental Analyser fitted with a zero-blank auto-sampler at James Cook University's Advanced Analytical Centre, Cairns. Analyses from both years were standardised using internal reference materials calibrated to international standards.

Statistical analyses

For the Seychelles data, four separate two-way analysis of variance (ANOVAs) were used to assess the effect of time period (two levels: 2014 and 2017), reef state (two levels: coral mortality and regime shift) and their interaction on (a) total coral cover, (b) branching coral cover, (c), $\delta^{15}N$ and d) %N across all 13 reefs where *Sargassum* were consistently collected. Based on this analysis and subsequent post hoc Tukey tests, we found that predominant changes in these response variables were observed on "coral mortality" reefs, with little response on "regime-shifted" reefs. We therefore include the seven reefs with high levels of coral mortality to investigate the relationship between changes in nutrient signatures against a) absolute and b) branching coral cover loss, using linear regression models. This decision was further supported by coral cover changes on "regime-shifted" reefs, where starting absolute values in 2014 were already very low at 6.69 ± 1.8% before dropping by ∼ 5% in 2017, and macroalgal cover was very high in both years. Therefore, any influence of coral cover on nutrient signatures in the system would be negligible (Suppl Fig. 3; Wilson et al. 2019).

For the Mo'orea data, differences between (a) average $\delta^{15}N$ and (b) %N signatures in the *Sargassum* specimens in the three treatments (initial, pre-transplanted and post-transplanted) from the transplant experiment were analysed using a repeated measures ANOVA. Repeated measures were incorporated into this ANOVA as tissue samples were taken from the same experimental specimens placed under the three different treatments. A time series analysis was conducted to compare the average mean monthly SST in 2019, relative to SST in previous years. Normality of data was assessed visually, and homogeneity of variance for all ANOVAs conducted for both studies was assumed with a Levene's test. All statistical analyses were conducted in R (R-Core-Team 2018), and the time series analyses for Mo'orea were performed using "zoo" and "xts" packages to produce Supplementary Fig. 2 (Zeileis and Grothendieck. 2005; Ryan and Ulrich 2020).

Results

Seychelles

There was a significant effect of year, reef state and interaction on total coral cover across the thirteen reefs (interaction: $F_{1,204} = 37.3$, $p < 0.0001$). The post hoc Tukey test revealed that there was no significant difference

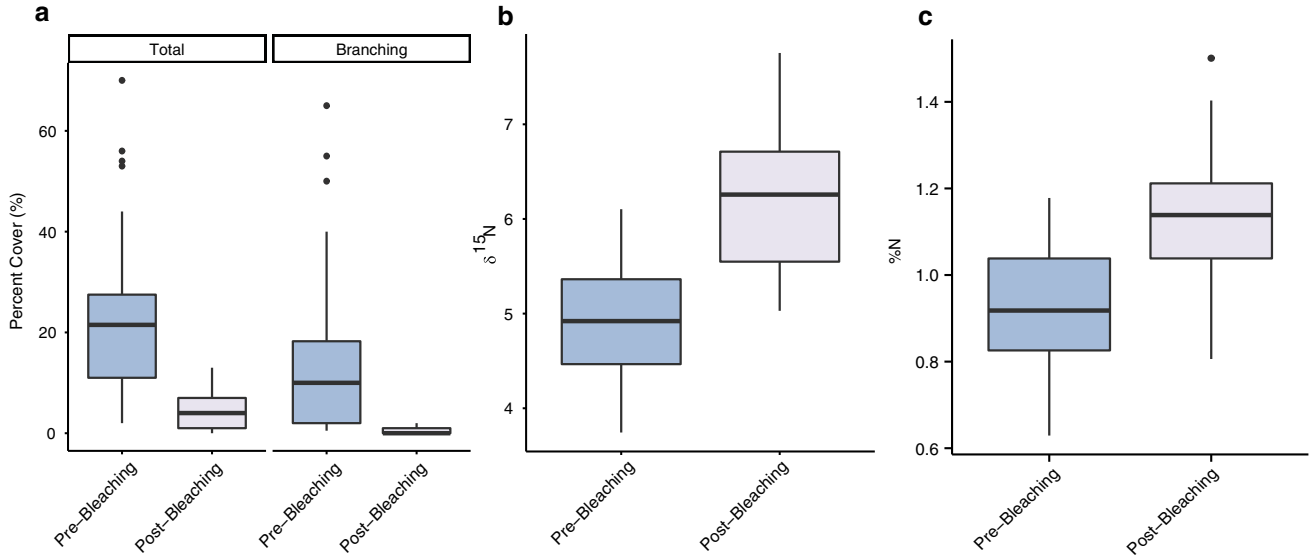

Fig. 1 Box and whisker plots of the median **a** total and branching coral cover in both pre-bleaching and post-bleaching years (2014 and 2017, respectively) on "coral mortality" reefs ($n = 7$), **b** the average $\delta^{15}N$ signatures in *Sargassum* sp. tissues in both years, and **c** the average percent N (%N) in both years. The pale blue boxes represent the pre-bleaching year and pale pink boxes represent the post-bleaching year, both showing the third quartile (Q3) and first quartile (Q1) range of the data, the whiskers (95% quartile) and data outliers

between the pre- and post-bleaching years for the "regime-shifted" reefs ($p = 0.32$; Suppl. Fig. 2). In contrast, the seven "coral mortality" reefs declined significantly from 27.0 ± 1.5 to 8.01 ± 0.5% between 2014 and 2017 ($p < 0.0001$; Fig. 1a). This was mainly due to a loss in branching coral cover on these reefs from 16.0 ± 1.5 to 0.30 ± 0.05% ($p < 0.0001$; Fig. 1a). Percent cover of massive corals remained similar between 2014 and 2017 on "coral mortality" reefs, whereas table coral cover declined from 1.27% to 0%. There was also a 0.8% increase in total macroalgal cover on the seven study reefs between the years.

The $\delta^{15}N$ signature in *Sargassum* tissues differed significantly between 2014 and 2017 across all thirteen reefs (interaction between year and reef state, $F_{1,124} = 11.4$, $p = 0.001$), but only showed a significant difference for the seven "coral mortality" reefs between survey years ($p < 0.0001$, Fig. 1*b*; $p = 0.15$ for regime-shifted reefs). Similarly, %N in *Sargassum* tissues was higher in samples collected from "coral morality" reefs in 2017 than in 2014 ($p < 0.0001$, Fig. 1c; significant interaction between year and state $F_{1,124} = 5.0$, $p = 0.03$), although there was no temporal difference in N content in samples collected from "regime-shifted" reefs ($p = 0.20$). For the seven "coral mortality" reefs selected for the purpose of this study, there was a significant positive relationship between increase in $\delta^{15}N$ in *Sargassum* tissue and (a) loss of total coral (adjusted $r^2 = 0.79$; $p = 0.004$; Fig. 2) and (b) branching coral cover (adjusted $r^2 = 0.86$; $p = 0.002$). There was no significant relationship between changes in %N and total coral cover ($r^2 = 0.04$; $p = 0.67$) or branching coral cover ($r^2 = 0.04$; $p = 0.66$).

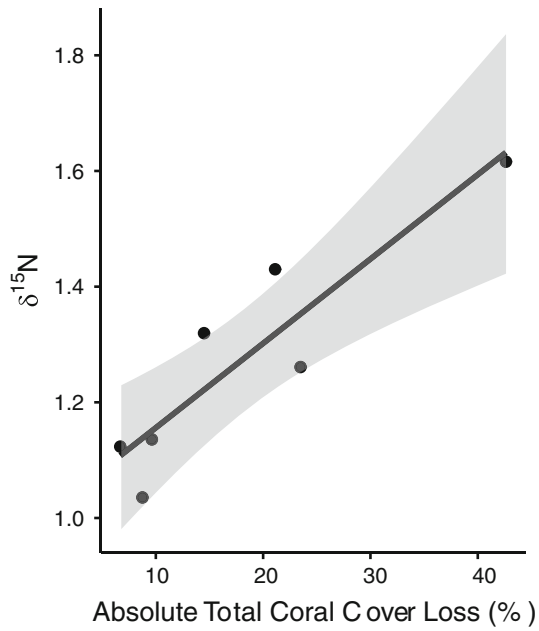

Fig. 2 Change in absolute total coral cover and the corresponding changes in $\delta^{15}N$ in *Sargassum* tissues across seven coral mortality reefs in the Seychelles between 2014 and 2017. The regression lines and confidence intervals were obtained using linear regression coefficient of determination (r^2); 95% confidence intervals

Mo'orea

Before the bleaching event peaked in April 2019 (Suppl Fig. 2), the benthic cover survey conducted across the outer slopes of the four northern sites of Mo'orea in March 2019 showed an average of 73.7 ± 2.8% live coral cover,

with a significant decline to an average of 36.2 ± 2.9% in 2020, a year after the event ($p < 0.0001$; Mean ± SE). The closest site to the transplant experiment, Tiahura, had 73.3 ± 5.5% and 36.0 ± 2.0% in live coral cover in 2019 and 2020, respectively. The high coral cover across the four sites in 2019 was primarily due to the abundance of branching coral *Pocillopora* on the forereefs in Mo'orea (Tsounis and Edmunds 2016). For instance, at Tiahura, there was 60.7 ± 5.7% cover of *Pocillopora* and an average of 55.5 ± 3.3% cover across the four sites in 2019. When the survey was repeated in March 2020, there was a significant decrease in *Pocillopora* to 24.5 ± 1.7% across all four sites ($p < 0.0001$), and a similar pattern was shown at Tiahura ($p < 0.0001$). Other than this predominant branching coral, no significant differences were found between the years for the other reef-associated organisms, including other coral genera.

In the short-term transplant experiment shortly after the peak of the bleaching event in Mo'orea, treatment had a significant effect on macroalgal $\delta^{15}N$ signatures (repeated-measures ANOVA: $F_{2,27} = 31.71$, $p < 0.0001$; Fig. 3). Post hoc tests indicated that there were significant differences in $\delta^{15}N$ between all three treatments (initial, pre-transplant and post-transplant, $n = 10$), which suggested that $\delta^{15}N$ declined in the pre-transplant holding tanks, and then increased substantially on the transplant reef (initial and pre-transplant: $p = 0.003$; initial and post-transplant: $p < 0.0001$; pre-transplant and post-transplant: $p < 0.0001$). However, there was no significant effect of treatment on macroalgal %N (repeated-measures ANOVA: $F_{2,23} = 0.6$, $p = 0.58$; Suppl Fig. 4). Although it was not possible to include either control sites or reefs with varying levels of bleaching due to permit restrictions, the benthic data show that the extent of coral mortality across the outer slopes on the northern region of Mo'orea was quite similar.

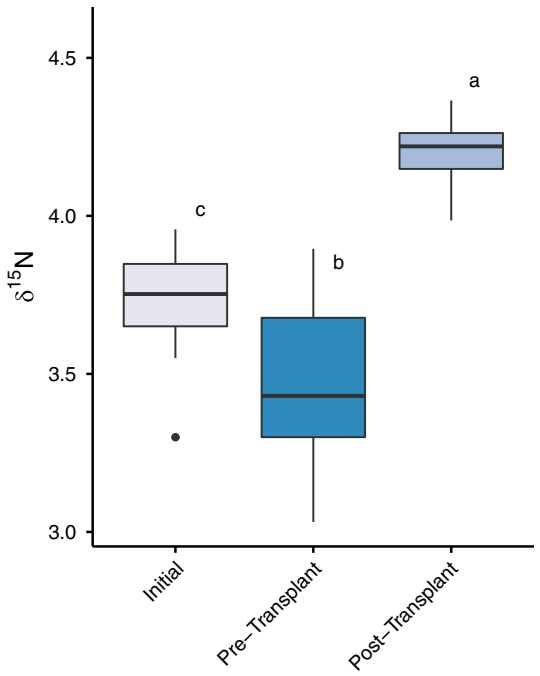

Fig. 3 Box and whisker plots of the median $\delta^{15}N$ in *Sargassum mangarevense* tissue across three treatments from a short-term transplant experiment, showing the third quartile (Q3) and first quartile (Q1) range of the data, the whiskers (95% quartile) and data outliers. Connecting letters indicate significant differences between treatments. Stable isotopic signatures were measured in subset samples of the same specimens that were collected from a low-nutrient reef (initial), placed in laboratory aquaria to deplete internal nutrient stores for ~ 7 days (pre-transplant), before they were deployed on the bleached reef for 3 weeks (post-transplant) ($n = 10$)

Discussion

The current study suggests that mass coral mortality events can be detected through nitrogen isotopic signatures in macroalgal tissues, a proxy for nutrient sources, up to a year after a severe bleaching event. Although the exact source of the enrichment could not be traced in this study, a significant increase in $\delta^{15}N$ was shown in both reef systems over different timescales after two separate coral mortality events. For instance, in the Seychelles, there was a strong positive correlation between a decline in total coral cover and an increase in $\delta^{15}N$. This suggested that the N present in algal tissues could be coral-derived. These findings may help improve understanding of how mass disturbances such as coral bleaching impact multiple ecosystem processes on climate-impacted reefs. For instance, the loss of live coral cover, especially branching corals, provides a large amount of new substrate for opportunistic species such as macroalgae and other primary producers to colonise and prevent coral recovery, and may also provide an additional source of nutrients which become locked in the system. Consequently, this could enhance macroalgal proliferation on this colonised space, reinforcing alternative regimes.

The isotopic signature of fleshy macroalgae changed significantly over both short and long timeframes following bleaching events on two different reef systems. The positive relationship between $\delta^{15}N$ and the declines in coral cover suggest that nutrients from dead and decaying corals have contributed to this change of isotopic signatures in macroalgae. While this might be an important natural source of nutrients (Coffroth 1990; Brown and Bythell 2005; Bythell and Wild 2011), any substantial increase could affect or disrupt natural metabolic exchanges between corals and other organisms, not only with their endosymbiotic zooxanthellae, but with sponge, seaweed and microbial communities (de Goeij et al. 2013; Rix et al. 2016, 2017; Pawlik et al. 2016; Mumby and Steneck, 2018; Leggat et al. 2019). Much of the literature focuses on the

mucus released from live corals and how it is recycled within the system (Davey et al. 2008; Naumann et al. 2009; Wild et al. 2004a, b, 2010, 2011), as well as the short term effects of changes in organic matter release after a bleaching event (Niggl et al. 2009; Wooldridge, 2009). Other work such as Radice et al. (2020) supports this by showing that isotopic signatures of particulate organic nitrogen in the water column decreased 8 months after a bleaching event. However, there is still little understanding of changes in reef biogeochemical cycles.

Excess nutrients are one of the key factors that can drive a bleached reef towards a regime shift (Graham et al. 2015). If the increased release of organic matter through mass coral mortality provides more nutrients to opportunistic species, this may encourage fast-growing macroalgae to proliferate on the exposed coral skeletons. This negative feedback loop can inhibit coral recovery and foster regime shifts to macroalgal-dominated states (Diaz-Pulido and McCook 2002; Haas et al. 2010; Wild et al. 2011). For instance, the lack of available substrata may reduce the ability for any coral larvae to colonise this space and repopulate reefs, whilst increases in algal-derived DOM and POM can subsequently increase pathogenic microbial activity through what has been termed the DDAM positive feedback loop (dissolved organic carbon, disease, algae, microorganisms) (Haas et al. 2016). Macroalgae release labile organic matter which benefit pathogenic microbes and together they create unfavourable conditions for corals. For example, they collectively disrupt the function of the coral holobiont, thereby exacerbating death of coral recruits, and maintaining competitive dominance in algae (Wild et al. 2010; Barott and Rohwer 2012; Pawlik et al. 2016; Mumby and Steneck 2018).

While mass mortality has the potential to release a substantial source of new nutrients, this type of organic matter is still considered to be internal, or autochthonous (Briand et al. 2015). Excessive nutrient enrichment from external anthropogenic nutrient loads, particularly certain types of nitrogen such as nitrates found in coastal runoff, can further exacerbate changes in biogeochemical cycles on reefs (Burkepile et al. 2020; Donovan et al. 2020). This could accelerate the proliferation of macroalgae and other opportunistic organisms, and further decrease the chance of scleractinian corals re-establishing themselves. In addition, declines in water quality can develop and cause the formation of algal blooms (Fabricius 2005; Tanaka et al. 2010).

Fleshy macroalgae are important indicators of changes in nutrient cycles because the bioavailable nutrients which are taken up from the water column and assimilated into their tissues can be easily measured over both short and long periods of time (Costanzo et al. 2001). Macroalgae have been used as proxies to study the effects of nutrient enrichment in both laboratory and in situ experiments, but these mostly tend to be for investigating anthropogenic sources, such as from coastal run-off (Fong et al. 1994; García-Seoane et al. 2018; Burkepile et al. 2020) and less commonly for natural nutrient inputs, such as seabird guano, deep-water upwelling events or coral-derived organic matter (Schaffelke, 2002; Graham et al. 2018; Williams et al. 2018).

The kind of nutrient signature used as a bioindicator is also an important factor to consider. Lin and Fong (2008) found $\delta^{15}N$ to be a more sensitive indicator to changes in nutrients in transplanted macroalgae than %N. Nitrogen content is typically diluted during rapid growth of specimens, suggesting that nutrients are only stored in macroalgal tissues over the long term when nutrient supply exceeds growth rate, as they first must assimilate excess nitrogen into growth. This likely explains why we found no patterns in %N in either the Seychelles regression analysis, or the Mo'orea transplant experiment.

Although the duration of transplant experiments in the literature varies considerably, from hours to $\sim$ 1 year, García-Seoane et al. (2018) recommended an exposure time of $<$ 1 month, as the uptake kinetics of algal transplants can vary based on the species used or local environmental conditions. The current study suggests that these changes in nutrients may be detected in *Sargassum* tissues up to 12 months after an event, implying that nutrients have been trapped and retained in the system for at least a year. It is also known that *Sargassum* undergoes major seasonal fluctuations in production and biomass that may supplement adjoining ecosystems within the broader seascape (Fulton et al. 2019). This study supports previous literature, suggesting that macroalgae can easily be deployed in target areas to investigate changes in nutrient loads (Costanzo et al. 2001; García-Seoane et al. 2018), but also applies this common technique to capturing energetic resources. Therefore, macroalgal assays have the potential to provide insight into changes in nutrient sources from both natural and anthropogenic events, such as widespread coral bleaching.

There are a number of potential sources of nitrogen that could have influenced our results other than coral-derived nutrients. A strong nutrient gradient from the land-end of Opunohu Bay in Mo'orea to its ocean-end (Lin and Fong 2008) suggests that the nutrient enrichment from the shrimp farm effluent entering the bottom of the bay was unlikely to affect the isotopic signatures of our specimens. However, storms and heavy rainfall can influence both the spatial extent of run-off and nutrient uptake in reef macroalgae (Clausing and Fong 2016; Adam et al. 2021). Local upwelling could have provided nutrients and influenced our results, but Lin and Fong (2008) suggest that the $\delta^{15}N$ of tropical ocean seawater is typically $\sim$ 3 ‰, which

is lower than the signatures found in both the post-transplant and pre-transplant tissue samples. In addition, no *Sargassum* specimens were found at the depth where the bleaching occurred in Mo'orea (~ 12 m), so specimens had to be taken from the nearby nutrient-limited lagoon (~ 1 m). Although this lagoon typically has low nutrient levels (Donovan et al. 2020) and the algal specimens collected from there had low tissue nutrient history, some bleaching was observed in the lagoon at the time of collection, but not in the specific area where the specimens were collected. Even if some coral-derived nutrients were captured by the initial specimens, we accounted for this by depleting tissue nutrient stores in the holding tanks. This resulted in a significant decline in $\delta^{15}N$, followed by a significantly higher signature in the post-treatment algae after they were transplanted at the site where extensive coral bleaching and mortality had occurred. Other factors such as light intensity can also affect algal condition and isotopic signatures (Marconi et al. 2011; García-Seoane et al. 2018), so may have also influenced results in Mo'orea.

Future research could build on this study, and on other studies in the literature (García-Seoane et al. 2018) by applying the above methods to test the degree of influence of coral-derived organic matter on macroalgal nutrient signatures, relative to anthropogenic sources, either in laboratory- or field-based experiments. For instance, macroalgal bioassays could be deployed on bleached reefs with low levels of coastal run-off, such as those in other regions around Mo'orea, and compared to those with significantly higher levels, to test if these effects are synergistic. Clearly, assessment of macroalgal isotope signatures across different nutrient loads and levels of coral mortality is required to fully understand nutrient sources before attribution of nitrogen enrichment in macroalgae to nutrients released from dead and decaying corals can be definitively determined.

While this study compared the $\delta^{15}N$ signatures in tissues of *Sargassum* from pre- and post-bleaching years in the Seychelles, no macroalgal samples were collected during the bleaching and the subsequent mortality event in 2016 itself, so it was not possible to compare the stable isotopic results when this mass tissue release was occurring. The short-term experiment in Mo'orea was conducted in part to understand these shorter-term dynamics and to further support these findings. Though the results from the two different reef systems are not directly comparable, this study suggests that macroalgal tissue $\delta^{15}N$ signatures can be affected by mass morality events. However, as the current study only implies that the mass release of dead coral tissue enriched the macroalgal $\delta^{15}N$ signatures, future research could expand on this work by determining the exact source(s) of enrichment (Briand et al. 2015). For instance, enriched stable isotope tracers (^{15}N and ^{13}C) (Naumann et al. 2010) or compound-specific stable isotopes (McMahon et al. 2016) could be used to quantify the flow of organic matter from dead corals to macroalgae in an experimental setting, or seawater from reefs with varying levels of coral mortality could be collected and used to test the responses of macroalgae.

In conclusion, this study highlights how mass coral mortality events, trigged by marine heat waves, may add additional sources of nutrients into coral reef biogeochemical cycles, which are available to opportunistic macroalgae. These changes in nutrient dynamics could have significant impacts on coral reefs, particularly if those sources are specifically becoming more available because key ecosystem engineers such as scleractinian corals are in decline (Wild et al. 2011). It also suggests that these nutrients can be retained within reefs and can have both short-term and long-term impacts on their biogeochemical cycles. Although it is not yet known how long these nutrients remain in the system, if other environmental conditions are favourable enough, then corals might still be able to recover (Graham et al. 2015). However, if these same reefs are also facing other local anthropogenic stressors, such as nutrient runoff or overfishing of herbivores, then large coral mortality events may result in competitive advantages to benthic organisms such as macroalgae, leading to a benthic regime shift (Ainsworth et al. 2019). This emphasises the critical need to manage local stressors by detecting and reducing nutrient runoff and other drivers, especially on reefs that do still have high abundance of corals, and/ or have recently bleached.

Acknowledgements We thank Seychelles Fishing Authority, Seychelles Marine Parks Authority and Jan Dajka for field and laboratory assistance in the Seychelles, Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) and Service National d'Observation CORAIL data and assistance in Mo'orea, and David Hughes and Jon Crosse from Lancaster Environment Centre for extensive help with preparations for fieldwork and with the subsequent stable isotopic and elemental analyses. The project was funded via a grant from the Royal Society (RG150710).

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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