ACCLIMATING ACROSS HEALTHY AND DEGRADED REEFS

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Presented to
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By

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ACCLIMATING ACROSS HEALTHY AND DEGRADED REEFS

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SUMMARY

As a result of human activities, many environments are becoming fragmented into areas with different community compositions and selective regimes. The coral reefs of Fiji for example, are divided into 'fished areas' (fragments subjected to fishing and trampling) and 'protected areas' (fragments with little human pressure) that occur in close proximity and now have differing community compositions and selective regimes. Theory predicts that the species able to survive in such conditions should have highly plastic genotypes allowing them to acclimatise to diverse habitats without the time lag required for local adaptation. Here we use two species -*Epinephelus merra* (a small grouper) and *Sargassum polycystum* C. Agardh (a brown macroalga)- which are found in both fished and protected reefs, to investigate this plastic response and understand how these species cope in healthy versus degraded environments.

We found that the fish *E. merra* exhibits plasticity in diet and feeds lower in the food chain in fished reefs than similarly sized conspecifics in protected reefs. The seaweed *S. polycystum* exhibits plasticity in defensive traits and is able to induce increased defenses in response to being partially consumed. In addition, we found that dense stands of *S. polycystum* increased the survival and growth of both recruit-sized and mature-sized *S. polycystum* ramets, suggesting that *Sargassum* beds protect conspecifics from grazing by herbivorous fishes and construct conditions that facilitate their growth. Implications for management are discussed.

CHAPTER ONE

INTRODUCTION

Human are having pervasive impacts on ecosystems (Palumbi 2001; Steffen et al. 2011), the ramifications of which may include degradation of habitat, severe alterations in community composition, and fragmentation of systems into smaller, isolated areas often differing considerably in species composition (Jackson *et al.* 2001; Morrison *et al.* 2007; Halpern *et al.* 2008; Vitousek *et al.* 2014). Coral reef ecosystems for example have undergone dramatic declines, with reports of 80% loss of coral cover in the Caribbean (Gardner et al. 2003) and 50% in the indo-Pacific (Bruno & Selig 2007) with concomitant declines in species abundance and diversity (Dulvy et al. 2004; Bellwood et al. 2004).

One of the measures commonly taken to mitigate such impacts is the establishment of Marine Protected Areas (MPAs) which, when properly enforced, can reverse such declines and lead to a recovery of coral cover, species diversity, and abundance (Bellwood et al. 2004; Selig & Bruno 2010; Simpson 2010; Rasher et al. 2013). However, the median size of these MPAs is small (~4 km^2; Halpern 2003), meaning that reefs can become fragmented into areas of low human pressure embedded within a region subjected to high exploitation, and often degradation. Consequently, areas of

differing community composition and selective pressures can occur in close proximity. As an example, properly enforced MPAs have higher diversity and abundance of corals and fishes (Roberts 2003; Lester et al. 2009; Rasher et al. 2013), while the degraded reefs where fishing is permitted (non-MPAs), may contain a small subset of these species (Rasher et al. 2013).

What enables some species to occupy both degraded and healthy habitats when others tend to be found only in healthy areas? Theoretical predictions suggest that the species persisting in degraded habitats will be 'ecological generalists' with highly plastic genotypes (Sultan 2001), allowing them to acclimatise to diverse habitats without the time lag required for local adaptation. Here we use two species found in both protected and degraded reefs in Fiji to investigate this plastic response and understand how these species cope in healthy versus degraded environments. *Epinephelus merra* is a small grouper found in both healthy and degraded reefs, while *Sargassum polycystum* C. Agardh is a brown macroalga that can dominate degraded reefs and is uncommon, but present on healthy reefs.

We found that the fish *E. merra* exhibits plasticity in diet and feeds lower in the food chain in degraded reefs than similarly sized conspecifics in protected reefs. The seaweed *S. polycystum* exhibits plasticity in defensive traits and is able to induce increased defenses in response to being partially consumed. Consequently, those individuals growing in the protected areas are less palatable than those growing on the unprotected reefs.

In addition, we found that dense stands of *S. polycystum* increased the survival and growth of both recruit-sized and mature-sized *S. polycystum* ramets, suggesting that *Sargassum* beds protect conspecifics from grazing by herbivorous fishes (Hoey & Bellwood 2011) and construct conditions that facilitate the growth of *S. polycystum*. Thus through a reinforcing feedback *S. polycystum* is able to perpetuate its own success. This could be a key factor explaining *S. polycystum* dominance and persistence on degraded reefs.

The study on diet plasticity of *E. merra* has the potential to be used as an indicator of food web integrity and hence also MPA efficacy. Furthermore, that *S. polycystum* maintains a self-reinforcing feedback is a potential mechanism enhancing the phase-shift to macroalgal dominance on coral reefs. Thus these findings have direct application in management and conservation.

As humans continue to alter the environment, it is species such as these with a within-generation capacity to adjust, which are most likely to persist. In the case of *S. polycystum* in Fiji, this species is persisting and dominating large areas of degraded reefs which is hindering reef recovery (Nuges et al. 2004; Kuffner et al. 2006; Foster et al. 2008; Rasher & Hay 2010; Rasher et al. 2011; Thurber et al. 2012; Dixson et al. 2014; Webster et al. 2015). Hence these areas may need to be actively managed if macroalgae are to be controlled and their impact mitigated.

This dissertation therefore adds to the growing body of literature signally that MPAs alone are not enough (Bellwood et al. 2004; Coelho & Manfrino 2007; Adam et al. 2015). Management should take measures to reduce the presence of macroalgae on

degraded reefs through selective fishing bans and restoration of urchin populations, as well as increase the attractiveness of those areas to recruiting larvae by transplanting more resilient coral species (Adam et al. 2015).

If the degraded areas are to be managed effectively, we need to understand more about the species that survive there. This dissertation helps decipher the processes maintaining degraded reefs in their current state and contributes to the restoration and sustainable management of these valuable resources.

CHAPTER TWO

EFFECT OF MARINE PROTECTED AREAS (MPAS) ON CONSUMER DIET: MPA FISH FEED HIGHER IN THE FOOD CHAIN

Introduction

Overfishing has pervasive impacts on marine ecosystems, ranging from species extinctions to fundamental alterations of ecosystem processes (Jackson et al. 2001; Worm et al. 2006). A common strategy for protecting marine communities from overfishing is the establishment of no-take marine protected areas (MPAs). Their effectiveness has been debated (Roberts & Polunin 1993, Bruno & Selig 2007), but where they are well enforced, MPAs can facilitate recovery of enclosed communities (Lester et al. 2009), enhancing abundance and diversity of fishes, as well as the ecosystem's mean trophic level (Libralato et al. 2010; Rasher et al. 2013; Bonaldo et al. submitted). Hence, MPAs can alter the composition of species assemblages, but their impacts on the feeding behavior and trophic biology of individual species are relatively unexplored.

By protecting larger consumers, MPAs may facilitate trophic cascades that alter lower trophic level species' behavior and access to resources. In the Caribbean, Stallings (2008) found the presence of large grouper caused smaller grouper to spend less time

foraging and to have lower growth rates than when the larger grouper were absent. Recruitment of lower trophic level species was also higher when the larger grouper were present. Through such interactions, MPAs might alter fishes' feeding biology and potentially their trophic position within the food web. This possible effect of MPAs on fish behavior and resource use has rarely been investigated.

Here we use stable isotope analysis to ask if the trophic biology of a mid-level consumer (the small grouper *Epinephelus merra*) differed depending on whether the individual was living in the no-take MPA or in the adjacent fished area, only a few hundred meters away. Stable isotope analysis has been widely used to elucidate connections in food webs and species' trophic positions. This technique has the advantage that it need not be destructive (one can use fin clips as opposed to gut content analysis) and it integrates the sources of nitrogen assimilated (Cocheret de la Moriniere et al. 2003) over periods of weeks to years (Hobson 1999, MacNeil et al. 2006); rather than producing a 'snapshot' view as provided by stomach content analysis (Harmelin-Vivien & Bouchon 1976). It is also able to give an accurate representation of energy flow (Post 2002) and the relative importance of differing food sources or feeding strategies, such as omnivory (Post 2002). As a result, analyses of carbon and nitrogen isotopic ratios have been able to answer some questions at greater resolution, or beyond the scope of other methods.

Here we chose the grouper *Epinephelus merra* as our focus species because it is one of the only site attached predatory fish that is common in both MPAs and non-MPAs along the coast of Fiji (Clements et al. 2012), making it a possible integrator of the food chain up to its level in both MPAs and fished areas.

Methods

Study Site & Species

Along the Coral Coast of Fiji's main island, local villages have established and enforced no-take MPAs. Thus multiple, small MPAs occur scattered within the unprotected back-reef (non-MPA) which is subject to artisanal fishing using hand lines, nets and spears. The MPAs and non-MPAs we investigated are 2-1.5m deep at low tide, occupy an 11km stretch of continuous coastline (Fig. 2-1) and thus are impacted by the same oceanic waters and similar terrestrial influences. Our study focused on three pairs of protected (MPA) and fished reefs associated with the villages of Votua, Vatu-o-lailai and Namada. The MPAs cover areas of ~0.8km^2, ~0.5km^2 and ~0.5km^2 respectively and are located between ~300m and ~1km from the adjacent fished site (Fig. 2-1). The MPAs were established in 2003-2003 (Simpson 2010) and now differ greatly from their associated unprotected reefs. Live coral cover in the MPAs is 38-56% on hard substrates, but only 4-16% in the non-MPAs, while macroalgal cover is 2-3% in the MPAs but 49-91% in the non-MPAs (Rasher et al. 2013). Fish diversity, density, biomass and recruitment are also suppressed in the non-MPAs relative to the MPAs of these villages (Rasher et al. 2013, Bonaldo and Hay 2014, Dixson et al. 2014, Bonaldo et al. submitted).

We chose the brown macroalga *Turbinaria conoides* to give an indication of ambient nitrogen conditions at each location because it is longer-lived and thus integrates fluctuating conditions. It is also one of the few macroalgal species found in both the MPAs and non-MPAs at most locations. We selected the small grouper *Epinephelus merra* as the consumer of focus because it is a mid-level, generalist carnivore, common in both the protected and fished habitats of the reef-flat (Clements et al.

2012). It is reported to have a limited home range (47.7±11 m^2; To 2009) and therefore should feed predominantly - if not exclusively – in the area of collection, thus providing a localized dietary signal. Its diet consists of small fishes, crabs and a small percentage of shrimps and cephalopods, the proportions of which vary with ontogeny and feeding period (Harmelin-Vivien & Bouchon 1976). Harmelin-Vivien & Bouchon (1976) found that diets contained a higher proportion of crabs after nocturnal feeding periods and a higher proportion of small fishes during diurnal feeding periods. They also reported that smaller individuals consumed more crustaceans, while the larger ate more fish.

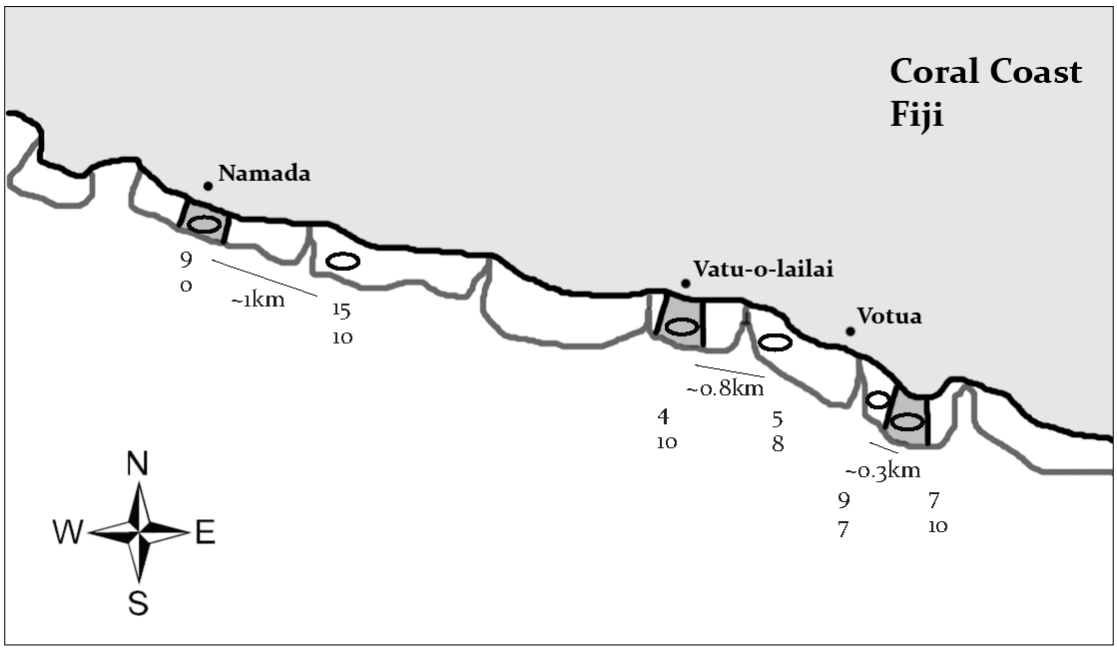


Figure 2-1: Map of sampling locations on Fiji's Coral Coast. MPAs are in grey, the fished area is in white and distances between the protected and fished collection sites are shown for each village. The pairs of numbers are the sample size for each site: the number of algal samples is below the number of fish samples.

Sample Collection

As the MPAs were no-take reserves, we non-lethally sampled individuals by clipping fins, which has been shown to be a viable alternative to muscle sampling and correlates strongly with results from muscle tissue (Suzuki et al. 2005; Sanderson et al. 2009). Fin tissue has the additional benefit that it contains collagen which integrates dietary signal over the individual's lifetime and thus provides a representation of *E. merra's* overall trophic history, rather than sampling only the preceding few days or weeks (as some tissues such as liver or whole blood would; Hobson 1999). The outer 0.5cm of the pectoral fin margin was cut so that the total size of each sample was $<1cm^2$ meaning that samples were composed of webbing tissue, fin rays and skin covering the fin. Samples were shaken vigorously in seawater to remove any particulates (none noted) prior to being stored at -20°C until processing in the lab. Between four and 15 fish were caught ~100m from shore using baited hand lines at a depth of ~1m at each site in May-June 2012.

The top 2cm of *T. conoides* were collected in May-June 2011 from seven to ten randomly collected replicates from each site except Namada's MPA where *T. conoides* was not found, potentially due to heavy grazing (Rasher et al. 2013). Like the fin clips, samples were shaken vigorously in seawater to remove epibionts or other surface-attached particulates (none noted). All samples were collected ~100m from shore at ~1m depth in each site and frozen at -20°C until processing. Collecting only the uppermost sections meant all samples were of recent growth, so minimizing temporal differences and avoiding the potentially confounding effects of fouling organisms that are found on older growth. Phaeophytes such as *T. conoides* have previously been used in calculations of trophic position and Carassou et al. (2008)

found close agreement between estimates of *E. merra's* trophic position based on brown macroalgae versus particulate organic matter.

Constraints of field time resulted in collections of algae occurring one year prior to the collections of fish. Since collagen integrates the isotopic signature over the individual's life (Stenhouse & Baxter 1976, Hobson 1999), fish samples will include the time period represented by the algae. Moreover, both seaweed and fish were sampled at the same time of year, so limiting seasonal differences.

Sample Preparation and Lab Analyses

No lipid treatment was performed on the fin clips as mean C:N ratios from all sites were all around 4 so correction from lipid-normalization would be minimal (McConnaughey and McRoy 1979; Sanderson et al. 2009). Fin clips were not acid treated because fin rays are composed primarily of collagen fibers (Nagai and Suzuki 2000).

To minimize the impact of epibionts in the analysis of *T. conoides*, only the newest growth - the uppermost 1cm of the algal ramet - was prepared for isotopic analysis. Both the fin clips and algal samples were dried to a constant weight at 70°C and ground with a pestle and mortar into a fine powder before analysis.

Samples were analyzed in triplicate by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Carlo Erba NC2500 elemental analyzer interfaced to a Micromass Optima mass spectrometer. Each analytical run included a series of elemental (methionine) and isotope (peptone) standards to correct for blanks and

instrumental drift. We conservatively estimate an analytical precision of ±0.2‰ for our isotopic measurements. Isotope abundances are expressed as $\delta^{15}N$ and $\delta^{13}C$ values relative to atmospheric N_2 and Vienna Pee Dee Belemnite (VPDB) respectively.

Statistical Analysis

Statistical analyses were performed using SPSS version 16.0. Contrasts of isotopic signature between the MPA and non-MPA areas were assessed with an ANOVA blocked by 'village' where 'protection status' was the main effect. No post-hoc tests were necessary because there were only two levels of 'Protection Status'. Within village MPA/non-MPA differences were analyzed with Independent Samples T-tests. Assumptions of normality and homogeneity of variance were examined using the Shapiro-Wilk test and the Levene's Test respectively, with $\alpha = 0.05$. No data sets violated these assumptions (algal $\delta^{13}C$ p= 0.179; algal $\delta^{15}N$ p= 0.574; fish $\delta^{13}C$ p= 0.943; fish $\delta^{15}N$ p= 0.672; fish total length p= 0.685).

Linear regression analysis evaluated whether there were correlations between $\delta^{15}N$ and total length, as well as between $\delta^{13}C$ and total length for the fish *E. merra*.

Fish trophic position (TP) was calculated using the following equation (Post 2002, Carassou et al. 2008):

$$\mathrm{TP}_{fish} = \lambda + \frac{\Delta\delta^{15}N}{3.4}$$

Where:

λ = TP of algae

$\Delta\ \delta^{15}N = \delta^{15}N_{fish} - \delta^{15}N_{algae}$

$\delta^{15}N_{algae}$ in this equation refers to the mean algal $\delta^{15}N$ ratio for each site. This was subtracted from each individual fish $\delta^{15}N$ value to give an estimate of trophic position.

Results

From the non-MPAs, 25 pieces of *T. conoides* and 29 *E. merra* fin clips were collected and analyzed. Twenty algal samples and 20 fin clips were collected and analyzed from the MPAs. Mean variation between our duplicate samples was only 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Jardine & Cunjak (2005) proposed a limit of 0.5‰, so this indicates that our samples were well homogenized and the potential difference in isotopic signature between fin webbing and fin ray would not confound our analyses.

Algal $\delta^{13}C$ was significantly higher in the MPAs versus the fished sites (Blocked ANOVA, $p<0.001$), while we detected no significant differences in the $\delta^{15}N$ signatures between the MPAs and non-MPAs ($p = 0.067$), although the trend was towards higher values in the fished areas. Individual values of $\delta^{13}C$ ranged from -8.3‰ to -6.1‰ (mean -7.1‰ ±0.14) in the MPAs and from -9.8‰ to -7.3‰ (mean -8.7‰ ±0.16) in the non-MPAs, while algal $\delta^{15}N$ ranged from -4.2‰ to 5.7‰ (mean -0.6‰ ±0.62) in the MPAs and from -4.5‰ to 5.3‰ (mean -0.2‰ ±0.49) in the non-MPAs (Fig. 2-2).

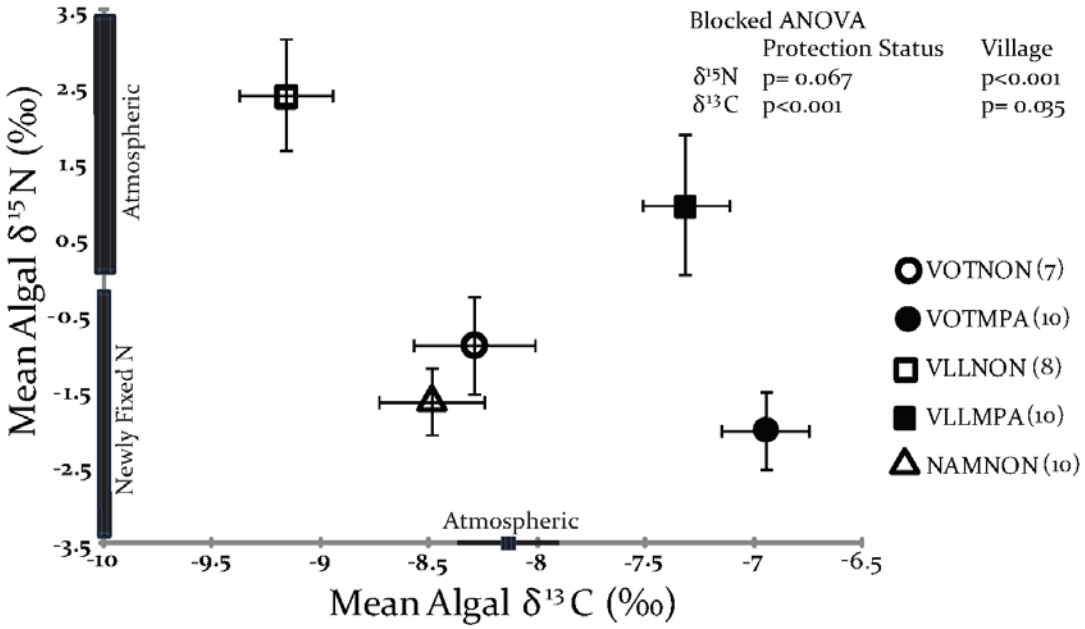


Figure 2-2: Isotopic cross plot showing the relationship between algal $\delta^{15}N$ and $\delta^{13}C$ (mean± 1SE) by village and protection status. Villages are abbreviated as: Votua (VOT), Vatu-o-lailai (VLL) and Namada (NAM) with protected area (MPA) and fished area (NON) in each village. *T. conoides* was absent from Namada's protected site (NAMMPA). N for each location is indicated in parentheses in the legend. Analysis by Blocked ANOVA found no significant difference in $\delta^{15}N$ (p=0.067), while algae from the MPA were significantly enriched in ^{13}C (p<0.001).

For the fish *E. merra*, individuals from the MPAs were significantly enriched in both ^{13}C (Blocked ANOVA, p<0.001) and ^{15}N (p<0.001) compared to individuals from the adjacent non-MPAs. The mean fish $\delta^{13}C$ values from the MPAs were greater than those from the non-MPAs by 1.0‰, 0.3‰ and 1.0‰ in Votua, Vatu-o-lailai and Namada respectively, which gave a mean difference of 0.8‰. The mean fish $\delta^{15}N$ values from the MPAs were greater than those from the non-MPAs by 0.4‰, 1.1‰ and 0.5‰ in Votua, Vatu-o-lailai and Namada respectively, which gave a mean difference of 0.7‰. $\delta^{13}C$ values in individual fin clips ranged from -9.9‰ to -7.0‰ (mean -8.1‰ ±0.15) in the MPAs and from -11.4‰ to -7.9‰ (mean -8.9‰ ±0.13) in the non-MPAs. Individual values for $\delta^{15}N$ ranged from 6.9‰ to 8.4‰ (mean 7.6‰

±0.08) in the MPAs and from 6.1‰ to 7.8‰ (mean of 7.0‰ ±0.07) in the non-MPAs (Fig. 2-3).

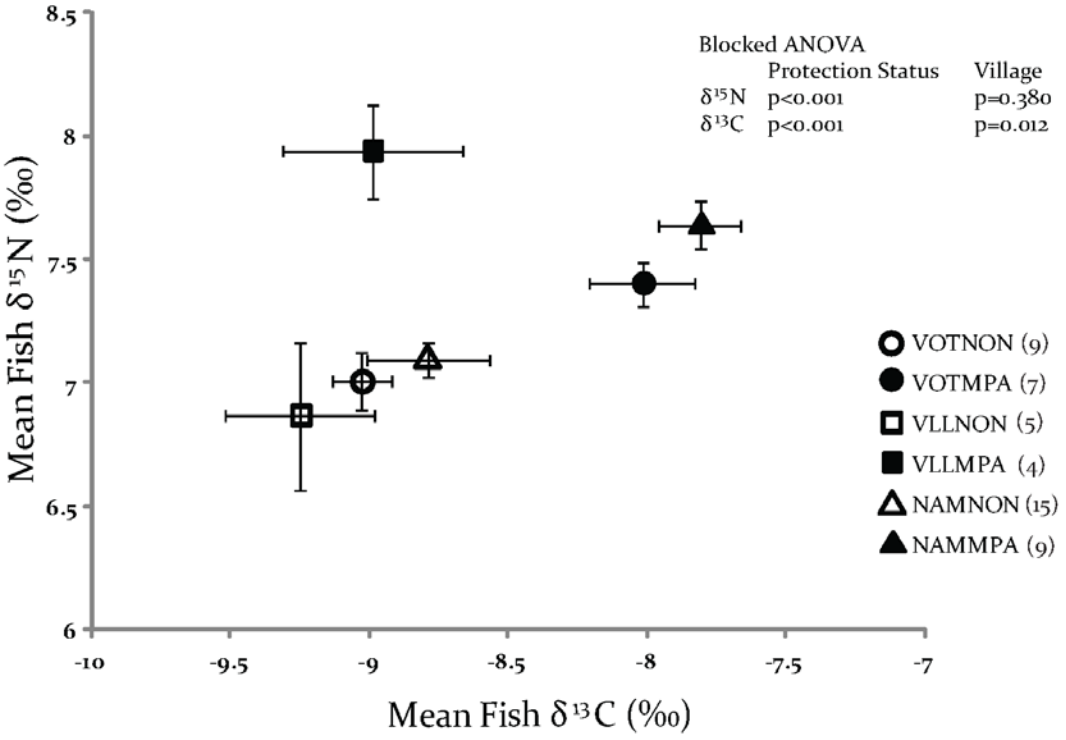


Figure 2-3: Isotopic cross plot showing the relationship between fish $\delta^{15}N$ and $\delta^{13}C$ (mean± 1SE) by village and protection status. Symbols, analyses and site abbreviations as in Figure 2; analysis by Blocked ANOVA found fish from the MPA were significantly enriched in ^{13}C ($p<0.001$) and ^{15}N ($p<0.001$).

Although the range of fish total length was similar in the non-MPAs and MPAs (12.6cm to 19.7cm and 10.6cm to 20.1cm, respectively) and comparisons by Independent Samples T-tests were not significant for any of the villages, when data were pooled across all villages, the mean total length of fish from the non-MPAs was a significant 7.5% greater (mean= 16.0cm ±0.39 for the non-MPAs and 14.8cm ±0.48 for MPAs; p=0.036, blocked ANOVA, Fig. 2-4). Nevertheless, this difference in length would not have confounded isotopic values because there were no correlations

between fish total length and either $\delta^{13}C$ or $\delta^{15}N$ (r^2=0.001, p=0.823 and r^2=0.005, p=0.622, respectively), in addition the Beta coefficients were low (-0.033 and -0.072, respectively; Figs. 2-5 and 2-6).

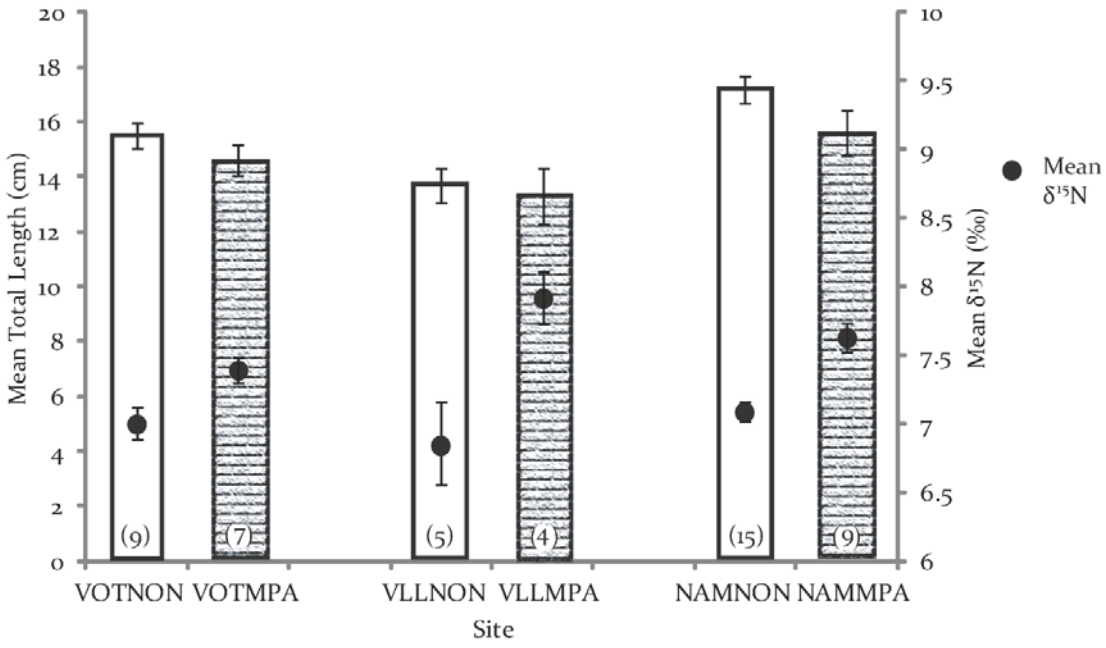


Figure 2-4: Mean (± 1SE) fish total length and mean $\delta^{15}N$ in each site; N is shown in parentheses at the base of each bar. The dashed columns are the protected sites in each village; site abbreviations as in Figure 2. Analysis by blocked ANOVA found individuals from the MPA were significantly smaller (p=0.036) and significantly enriched in ^{15}N (p<0.001).

Analyses of algal $\delta^{13}C$ for MPA versus non-MPA samples by Independent Samples T-tests detected significant differences in Votua (p= 0.001) and Vatu-o-lailai (p< 0.001), but not $\delta^{15}N$ (Votua p= 0.180, Vatu-o-lailai p= 0.260). The comparison could not be made in Namada due to the absence of *T. conoides* in its MPA. For the fish, Independent Samples T-tests were possible for all three villages and found the difference between MPA and non-MPA $\delta^{13}C$ significant for Votua (p<0.001) and

Namada (p= 0.004), but not Vatu-o-lailai (p= 0.550). For $\delta^{15}N$, MPA non-MPA differences were significant for all three villages (Votua p= 0.022, Vatu-o-lailai p= 0.026, Namada p<0.001).

Calculated trophic position of *E. merra* was about half a trophic level higher in the MPAs of each village than in the corresponding fished areas. Trophic position was calculated to be 3.3, 2.3 and 3.6 in the non-MPAs and 3.8, 3.1 and 4 in the MPAs of Votua, Vatu-o-lailai and Namada respectively. This gives a mean trophic position of 3.1 for fish from the non-MPAs and 3.6 for those from the MPAs.

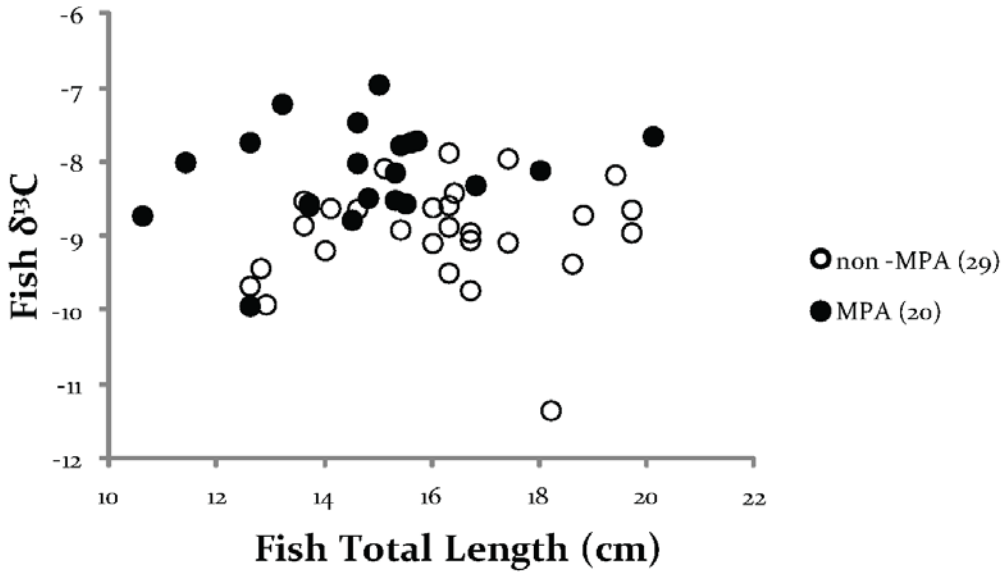


Figure 2-5: Plot of fish $\delta^{13}C$ against total length for all sites
For the entire dataset, regression of fish total length with $\delta^{13}C$ as the dependent variable, yielded a Beta coefficient of -0.033 (r^2= 0.001; p =0.823). When divided by protection status, the Beta coefficient of $\delta^{13}C$ against fish total length was 0.262 (r^2= 0.069; p =0.264) for fish from the MPA. For fish from the non-MPA, the Beta Coefficient was 0.066 (r^2= 0.004; p =0.733)

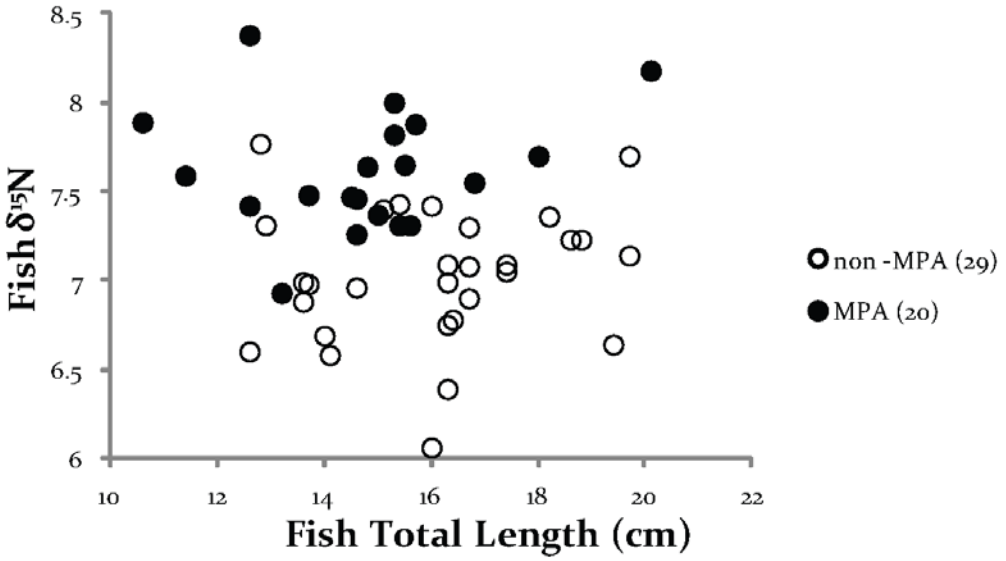


Figure 2-6: Plot of fish $\delta^{15}N$ against total length for all sites
When all data were pooled, regression of fish total length with $\delta^{15}N$ yielded a Beta coefficient of -0.072 (r^2= 0.005; p =0.622). When the data were split by protection status, the Beta coefficients with $\delta^{15}N$ against total length were 0.164 (r^2= 0.027; p = 0.489) for fish from the MPA and 0.140 (r^2= 0.020; p =0.470) for fish from the non-MPA

Discussion

We documented isotopic signatures for the grouper *E. merra* that indicate individuals from three different MPAs are feeding higher in the food chain than individuals collected from spatially-paired non-MPAs located 300-1000m away. Thus, in addition to altering fish density, biomass, and species composition (Clements et al. 2012; Rasher et al. 2013; Bonaldo et al. submitted), MPAs can also alter a species' trophic biology relative to conspecifics living in nearby fished reefs. Both the $\delta^{13}C$ and the $\delta^{15}N$ values of the grouper were significantly higher in individuals from the MPAs (Blocked ANOVA, $p<0.001$ for both $\delta^{13}C$ and $\delta^{15}N$; Fig. 2-3). This difference was not caused by a difference in ambient nitrogen conditions, since the algal samples

indicated there were no significant differences between the MPA and non-MPA within each village.

If the algal samples are used as representative of autotrophs at the base of the food web, calculations show the fish from the MPAs fed about half a trophic level higher than those from the corresponding non-MPAs: these estimates of trophic position ranged from 2.3 to 3.6 in the non-MPAs and 3.1 to 4 in the MPAs. Following the values used by Bozec et al. (2004), this reflects a shift in the diet of *E. merra* from primarily invertebrates in the non-MPAs to primarily fishes in the MPAs. This seems reasonable given that the density of small recruiting fishes (prey for *E. merra*) is 5-8 times higher in the MPAs than non-MPAs (Dixson et al. 2014). The enriched isotopic signal in fish from the MPAs may result from greater prey choice and availability in those areas. This is despite the likelihood of higher competition and predation in the MPAs, as densities of *Epinephelus spp.* specifically (Clements et al. 2012; Bonaldo et al. submitted) and piscivores generally (Bonaldo et al. submitted) were significantly higher in the MPAs.

A study of 7 individuals in New Caledonia (also using brown macroalgae as indicative of the basal trophic level) estimated *E. merra's* trophic position to be 2.5 to 2.9 (Carassou et al. 2008). No mention was made regarding protection status or fishing pressure in their sites, but our data suggest they may have been sourced from reefs where their diet was more reliant on invertebrates than fishes.

There are alternative, but improbable, explanations for the higher $\delta^{15}N$, and consequent estimate of trophic position, in *E. merra* from the MPAs. Firstly, this

difference in isotopic signature could result from differing physical and chemical environments. This seems unlikely because these reef flat sites are all of similar depth, distance from shore and are interspersed within an 11km stretch of continuous coastline that is subject to the same oceanic waters and similar terrestrial inputs. Furthermore, analysis of algal $\delta^{15}N$ by blocked ANOVA failed to find any significant differences among these sites and intra-village comparisons were also not significant (Independent Samples T-tests p= 0.180 for Votua and p= 0.260 for Vatu-o-lailai, Fig. 2-1). In addition, the MPAs were far smaller in area than the corresponding non-MPAs, so a positive relationship between trophic position and volume, as reported by Post et al. (2000), would not have produced our results.

Secondly, *E. merra* has been reported to shift from a crustacean-rich to a fish-rich diet as it grows (Harmelin-Vivien & Bouchon 1976). Thus, a higher $\delta^{15}N$ may be expected from larger individuals. However in our study, the fish from the non-MPAs were slightly larger (blocked ANOVA p=0.036) and were significantly less enriched in ^{15}N. Indeed the site with the largest fish; Namada's non-MPA; also had the lowest $\delta^{15}N$ of all 6 sites; thus differences in fish size within the range we investigated, cannot have generated our findings and indeed may have reduced the magnitude of isotopic difference we documented between MPA and non-MPA sites. Nevertheless, it was surprising to find the fish from the MPAs were smaller in length and this may be related to lower growth rate in those areas with higher trophic level consumers and greater risk of predation, as reported by Stallings (2008).

Harmelin-Vivien & Bouchon (1976) found the diet of *E. merra* between 6 & 9cm in length contained only 35% fish, while individuals between 10 & 24cm consumed fish as 68% of the diet. Every fish in our study was from the latter size class, so we would

expect a comparable diet, and thus isotopic signature, in all individuals if they had equal access to prey. This makes the significant difference in isotopic ratio all the more interesting, as it suggests that diet is not determined by consumer length, within the size range we sampled. Moreover, regressions of fish total length against isotopic signatures found low Beta coefficients that were not significantly different from zero. In addition, the r^2 values of 0.001 and 0.005 suggest no relationship, as do plots of fish total length versus isotopic signature (Figs. 2-5 & 2-6).

Finally, although we did not examine gender of the fish, it is unlikely this would have confounded our results because *E. merra* is a protogynous hermaphrodite. Pothin et al. (2004) found 75% of individuals smaller than 23.5cm to be female and since our largest specimen was 20.1cm long, it is probable that most, if not all, specimens in our study were female.

Thus, it seems that human activities like fishing can simplify habitats and communities and in this case, limit trophic options and lower the isotopic signature of the mid-level consumer *E. merra*. The establishment and enforcement of MPAs can cause dramatic changes in species diversity, abundance, biomass (Lester et al. 2009, Russ et al. 2008, Rasher et al. 2013), growth rate (Stallings 2008), recruitment (Dixson et al. 2014), longevity and age at sexual maturity (McClanahan & Omukoto 2011). Here we see that feeding biology and trophic level are also impacted. The greater prey choice and availability for *E. merra* in the MPAs may indicate more complete food webs there. This is in agreement with other reports of a return of trophic links in response to protecting large predators (Shears et al. 2002). Briand & Cohen (1987) found ecosystem dimensionality to be a significant factor in food chain

length, with longer food chains found in ecosystems of greater topographic complexity. The coral dominated MPAs are more topographically complex than the fished areas, so MPAs in these villages may enhance community integrity and food chain length as well as preventing direct removal of species through over-harvesting. More research is necessary to support this hypothesis, but it is an exciting possibility. Stable isotope analysis of a relatively sessile, mid-level generalist carnivore (such as small grouper or possibly lizardfish) may thus be able to provide a simple, integrative means of assessing food web integrity and efficacy of MPAs. Extension of this approach to additional species and locations will provide a critical test of this hypothesis.

In summary, stable isotope analysis indicates that a common mid-level predator on Pacific coral reefs fed higher in the food chain when living in well-enforced MPAs than when living in fished areas only a few hundred meters away. This did not appear to be related to environmental differences or to ontogenetic shifts in diet, as the fish in the MPAs were slightly, but significantly, smaller in size. Fish collected from the three MPAs we investigated were feeding about half a trophic level higher than conspecifics in the adjacent non-MPAs. Establishment of MPAs may thus not only enhance fish biomass and species richness, but may also impact the trophic function of some fishes. Here we investigated only one mid-level consumer, but if the values we documented for *E. merra* are a reflection of the local food web, then it is possible that this is not just a species trait, but one generated by altered food-web structure. Stable isotope analysis could thus provide a rough measure of community integrity in such scenarios. Investigations of more species and locations will be needed to rigorously evaluate this possibility.

CHAPTER THREE

SPATIAL VARIANCE IN PALATABILITY ASSOCIATED WITH HERBIVORY

Introduction

A review from 20 years ago noted, 'There are too few studies of chemical induction in marine prey to adequately evaluate the relative importance of large, mobile versus small, sedentary consumers in inducing chemical defences' (Hay 1996). Today this is still the case. Publications in the intervening years have documented induced defences in response to grazing by small, sedentary mesograzers (e.g. amphipods, isopods, and snails), but investigations of induction due to mobile, macroherbivores have not been published.

Since Lewis et al. (1987) showed *Padina* induced morphological escapes in response to fish grazing, there seem to have been only a few published studies documenting a correlation between morphology or metabolite concentration and fish grazing intensity (Coen & Tanner 1989; Paul & van Alstyne 1988), but no experimental tests. Likewise, reviews (Harvell 1990; Hay 1996) and meta-analyses (Koricheva et al. 2004; Toth & Pavia 2007) have not covered induction by fishes, apparently because too few studies exist. It has become the 'accepted wisdom' that mesograzers induce

defences and large, mobile grazers do not (Hay 1996; Pavia & Toth 2000; Toth et al. 2007).

In aquatic ecology the thinking supportive of this stems from Hay (1996) who hypothesised that mesograzers would induce defences because they feed on small spatial scales, may remain on one plant for extended periods of time and rarely cause prey mortality. Thus, hosts would have time to induce defences before they were completely consumed and the herbivores inducing the defence would be affected by it. In contrast, large, mobile grazers may completely consume a palatable individual before the individual could induce defence. Thus herbivorous fishes that were large and mobile relative to their algal prey were thought to select for constitutive, rather than induced, defences. There are also parallels in terrestrial systems where induced chemical defences in response to invertebrate grazers are common and well studied, whereas those induced in response to vertebrate herbivores are either rare or unstudied (Karban & Baldwin 1997). In contrast, the defences induced in response to terrestrial vertebrate grazers, appear to be structural (such as thorns or silica) rather than chemical (Hanley et al. 2007).

However despite the general patterns discussed above, several observations suggest that it in some situations it may be beneficial for macroalgae to induce defences in response to large, mobile herbivores. Firstly, fish grazing is not spatially or temporally uniform (Hay et al. 1983). While relatively palatable algae in areas constantly accessible to grazers may be rapidly consumed to extinction, those in areas of spatial or temporal escapes may experience periodic and non-fatal bouts of feeding, allowing time to induce defences before another feeding bout commences. Variability

in grazing by herbivorous fishes has been reported due to many factors, including wave action (Verges et al. 2011), turbulence (Hay 1981), as well as diurnal (Hay et al. 1983; Michael et al. 2013) and seasonal changes (Carpenter 1986). This variation provides temporal or spatial escapes and in such situations authors have noted it may be advantageous to induce rather than exhibit constitutive defences as this would avoid the cost of continuous production and maintenance of defence (Tollrian & Harvell 1999).

Secondly, most seaweeds are clonal, with basal sections that exist within structural refuges in the reef and so escape consumption by larger herbivores. These protected portions might be able to regenerate induced tissue following bouts of even intense grazing (Harvell 1990).

Thirdly, most reef fish are resident to an area of reef and feed within a defined home range (Sale 1980; Welsh & Bellwood 2014). So they are likely to return to a previously grazed alga, and be affected by the macroalgal defences they induce (Harvell 1990).

Published results to date suggest induction by mesograzers may be more common than by macrograzers (Hay 1996; Pavia & Toth 2000; Toth et al. 2007). However there are too few published tests of macrograzer induction to rigorously assess whether this is a genuine pattern or an artefact of bias in publication, interest, or greater ease of conducting experiments with small, less mobile invertebrates. In this study we investigated 1) whether herbivorous fishes preferentially fed on *S. polycystum* fronds growing in areas with few herbivorous fishes versus areas with

many, 2) whether the difference in palatability we observed resulted from an induced response to fish grazing, and 3) whether differential tissue toughness or nutritional value played a role in feeding preference as has been demonstrated in some other studies (Littler & Littler 1980; Coley 1983; Burkepile and Hay 2009; Chan et al. 2012).

Methods

Study Sites

Our study sites were three pairs of marine protected areas (MPAs) and their adjacent fished areas (non-MPAs) in the villages of Votua (VOT), Vatu-o-lailai (VLL) and Namada (NAM) on the Coral Coast of Fiji's main island, Viti Levu (Fig. 3-1). These sites occur along an 11km stretch of continuous reef flat habitat, between 1 and 3 metres depth at high tide. The MPAs of these 3 villages were established in 2003-2003 and cover areas of ~0.8km^2, ~0.5km^2 and ~0.5km^2 respectively (Simpson 2010). Surveys conducted near the time of MPA establishment indicated that MPA and non-MPA areas were similarly low in coral cover and high in macroalgal cover (Simpson 2010), suggesting similar physical and biological regimes and selective pressures. However, in the 10+ years since MPA establishment, community composition of the MPAs and associated non-MPAs came to differ dramatically. Biomass and diversity of herbivorous fishes are 7 to 17 times and 2 to 3.3 times greater in the MPAs than the adjacent non-MPAs, respectively (Rasher et al. 2013). Macroalgal cover and diversity are respectively 27 to 61 times and 2.6 to 3.6 times greater in the non-MPAs, while coral cover is ~3 to11 times greater in the MPAs than the non-MPAs (Rasher et al. 2013). Thus while physical parameters are similar between adjacent sites, there is now

considerable difference in the selective pressure imposed by the different densities and diversities of herbivorous fishes. Paired MPA and non-MPA sites at each village were separated by ~0.3 to 1km and replicate villages were separated from each other by 3.3 to 7.6km (Fig. 3-1). Data presented here were collected between May 2012 and January 2015.

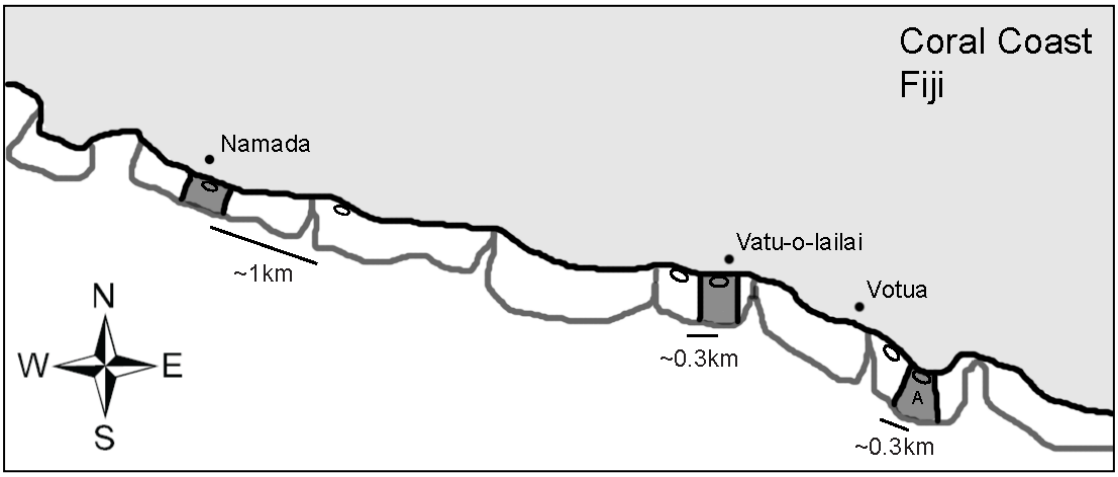


Figure 3-1: Site Map showing the collection areas inside the MPAs (grey) and non-MPAs modified from Dell et al. (2015). The study site is located between 18°11'23"S, 177° 37'15"E (Namada's MPA) and 18°12'58"S, 177°43'01"E (Votua's MPA). Distances between the protected and fished areas are shown for each village and 'A' indicates the location where all feeding assays were conducted

Study Species

To assess potential differences in palatability and possible induction of defence that might result from differing grazing pressure, we selected the brown macroalga *Sargassum polycystum* C. Agardh which is palatable to fishes (Rasher et al. 2013) and occurs in both the MPAs and non-MPAs. *S. polycystum* is a one of the most common macroalgae in the coastal, tropical and sub-tropical Pacific (Chiang et al. 1992). On Fijian reefs, *S. polycystum* begins to regenerate from perennial holdfasts in December and can grow to 1.5m long and dominate the reef (Rasher et al. 2013) by the end of its

growing season (June-July in Fiji). At this point the species may reproduce sexually and senesce (as per De Wreede 1976).

Although 29 species of larger herbivorous fishes occur in the MPAs, two unicorn fishes (*Naso unicornis* and *N. Lituratus*) are the primary consumers of *S. polycystum*, and brown macroalgae in general (Rasher et al. 2013).

Fish Feeding Assays

To assess the palatability of MPA versus non-MPA *S. polycystum* to herbivorous fishes, fronds from both the MPA and non-MPA of each village were collected, assembled into pairs of ropes and these rope pairs put in Votua's MPA to be grazed. *S. polycystum* occurs throughout the non-MPAs but in the MPAs is largely restricted to near-shore, shallow areas. It was therefore first collected from the MPA in each village from as far off shore as possible, and then from a comparable location in the adjacent non-MPA of that village. The upper 15cm of each frond was collected and rinsed vigorously in seawater to remove epiphytes and particulates. Fifteen to 20g of this was spun in a salad spinner for 15 revolutions to remove excess water, weighed to the nearest 0.1g, and affixed as two pieces ~10cm apart on one end of a rope 40cm long. The same was then performed with algae from the other site so that one rope held algae from the MPA and the other from the non-MPA. The weight of algae on each rope was matched to within 1gram and assembled to be visually similar. Twenty rope pairs were prepared, attached to cement weights, and distributed in the MPA to be grazed. Ropes of a pair were separated from each other by ~50cm and pairs were positioned ≥2m apart. Pairs were removed when at least 50% of the macroalgae on either member of a rope pair had been eaten. Pairs were omitted from subsequent

analysis if <10% or >90% of both ropes had been eaten. This rarely occurred and eliminated replicates in which preference would have been obscured by inadequate or excessive fish grazing. Grazing assays lasted 1 to 4 hours and the rope pairs were monitored continuously during this time to ensure loss of mass was due to grazing and not physical dislodgement from the rope (none noted). Following the assay, the algae were then spun again and weighed to measure grazing as grams lost. This assay was repeated with *S. polycystum* collected from each village multiple times between May 2012 and May 2013, and for one village in January 2015.

Induction Experiment

This experiment was conducted to determine whether differential palatability of *S. polycystum* might be due to induction of defence following initial grazing by herbivorous fishes. In January 2015, we collected eight ramets 15cm in length from each of 25 *S. polycystum* holdfasts in Votua's non-MPA, moved them to the MPA where we exposed half to grazing and protected the rest. Four of the eight ramets from each holdfast were threaded through a rope and affixed to the sides of cages (40cm x 15cm x 15cm with mesh size of 1cm) so that the upper 5 cm protruded through the mesh while the remainder of the thalli were protected inside the cage. The remaining four ramets were threaded through ropes and positioned in separate cages so that they were entirely protected from grazing. All cages (n=25 pairs) were then distributed in the MPA and left for two days so the treatment algae could be grazed while the control remained protected but in the same environment. After the two day grazing period, the entire 5cm section protruding from the cage had been consumed. The ropes were then transferred to separate cages (three for the treatment ropes and three for the controls, each ~1.5m x 1.5m x 1m in size) where the algae could grow

protected from grazing for two weeks. After this period, new growth from three of the four ramets from each rope (~7g spun wet mass in each replicate) was used in a feeding assay as described above.

The new growth from the remaining ramet from each rope was measured with a penetrometer (as in Littler & Littler [1980] and detailed below) to determine if changes in physical toughness could explain differences in palatability. As some thalli detached from the rope and washed against the side of the cages where they may have been grazed, we excluded them from the subsequent feeding assay leaving n=16 rather than 25.

Penetrometer Measurements of Blade Toughness

To investigate the potential impact of blade toughness on fish grazing preference, *S. polycystum* was collected from the MPA and non-MPA of each village in January 2015 for measurement with a Penetrometer as per methods detailed in Littler and Littler (1980). Such measures of 'leaf toughness' have been considered important determinants of grazing in both marine and terrestrial systems (Littler & Littler 1980; Coley 1983).

Briefly, the alga's blade is clamped between two plexiglass sheets that have a predrilled hole where a metal pin supporting a small plastic cup can be positioned so that the pin rests directly on the algal blade. Small lead beads are then slowly added to the cup until the pin pierces the algal blade. The lead beads, needle, and cup are then weighed to determine the mass needed to pierce the blade, thus giving a measurement of algal 'toughness'. Eighteen to 20 *S. polycystum* ramets were collected from the

MPA and non-MPA of Votua and Vatu-o-lailai and between six and eight were collected from Namada's MPA and non-MPA depending on *S. polycystum* availability. For consistency, the needle was positioned in the centre of the widest part of a blade growing 1cm from the apical meristem.

To assess whether fish grazing induced a change in blade toughness in *S. polycystum*, one of the four ramets from each rope in the induction experiment was reserved for penetrometer measurements (while the remaining three were used in a feeding assay). A total of 23 control and 23 induced ramets from this experiment were measured with the Penetrometer.

Nitrogen Content and C:N Ratio

As nitrogen can be limiting to herbivores (Mattson 1980), we measured nitrogen content and C:N ratios of *S. polycystum* fronds to determine whether MPA and non-MPA thalli differed in nutritional value. The top 5cm from five *S. polycystum* ramets were collected from each of the six sites in May 2012. These were rinsed vigorously in seawater to remove particulates and epiphytes and frozen at -20°C for transport to the lab. There the samples were dried at 70°C to a constant weight, ground into a fine powder with a pestle and mortar and analysed by mass spectrometer for carbon and nitrogen content. Further processing methods are detailed in (Dell et al. 2015).

Statistical Analyses

Assumptions of normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene's Test, respectively. Statistical analyses were completed using SPSS version 16.0. Means are reported ± 1SE; α was set to 0.05.

Data from the fish feeding assays satisfied the assumption of normality, or were successfully square-root transformed to do so, and were analysed with independent sample t-tests or t-test for unequal variance when data failed the assumption of homogeneity of variance. All penetrometer data satisfied assumptions of normality (or were successfully log10 transformed to do so) and homogeneity of variance and were analysed by independent samples t-test. Percent nitrogen and C:N data were analysed with Mann-Whitney U 2-tailed tests. Difference scores from the induction experiment satisfied the assumption of normality, so data were analysed by a paired t-test.

Results

When *S. polycystum* was collected from MPAs and non-MPAs and placed in the MPA to be grazed, fish consumed significantly more of the non-MPA relative to MPA algae in 11 of the 13 paired assays conducted at five time points over three years ($p \leq 0.036$; Fig. 3-2). This difference in consumption was on average ~18% (between ~8 to ~30% over the 11 trials). The preference for non-MPA algae was consistent in the villages of Vatu-o-lailai and Namada, whereas in Votua it occurred in two of the four trials, which were those run later in the year (in May; Fig. 3-2).

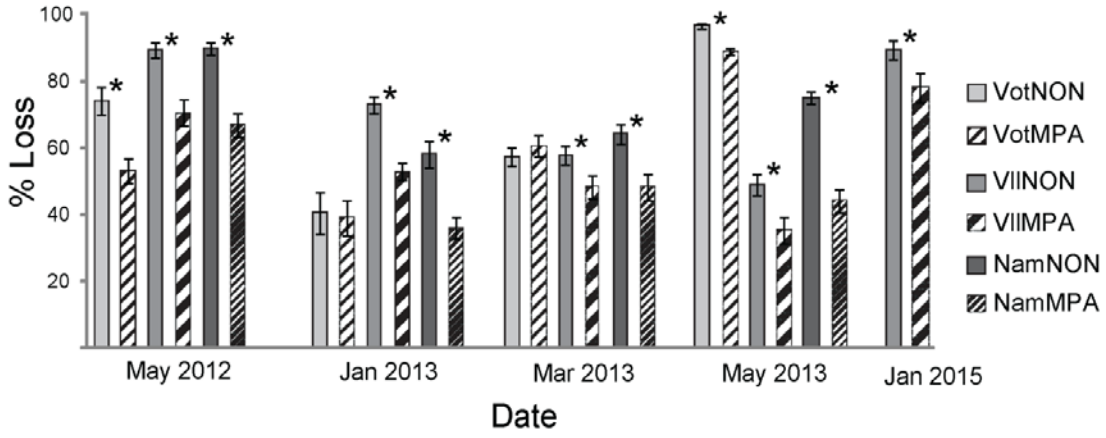


Figure 3-2: Results of Feeding Assays Comparing Herbivorous Fish Preference for *Sargassum polycystum* from the non-MPAs versus the MPAs over multiple years. In 11 out of the 13 trials, fishes significantly preferred *S. polycystum* from the non-MPAs, there were no significant differences in the other two trials. Solid bars indicate non-MPA sites, striped bars indicate MPA sites; n= ~20 for each assay; asterisks indicate significance at P= <0.05 from independent samples t-tests; error bars are ±1SE

Toughness of *S. polycystum* blades did not differ significantly between MPA and non-MPA sites ($p \geq 0.214$; Fig. 3-3) and thus did not correlate with feeding differences. Nutritional patterns did differ between MPA and non-MPA sites, but in a direction opposite to that of feeding preference: the more nutritious, nitrogen-rich *S. polycystum* occurred in the MPAs, and was consumed significantly less than the lower quality individuals from the non-MPAs. Despite being the less preferred foods, algae from the MPAs were significantly higher in percent nitrogen (dry mass) than those from the associated non-MPAs for the villages of Vatu-o-lailai and Namada ($p=0.016$, $p=0.009$, respectively; Mann-Whitney U tests); this contrast was not significant for Votua but trended in the same direction ($p=0.076$; Fig. 3-4). Similarly, contrasts for Vatu-o-lailai and Namada showed the algae from the MPAs had significantly lower C:N ratios than algae from the non-MPAs ($p=0.028$, $p=0.009$ respectively); again this difference was not significant for Votua ($p=0.602$; Fig. 3-4).

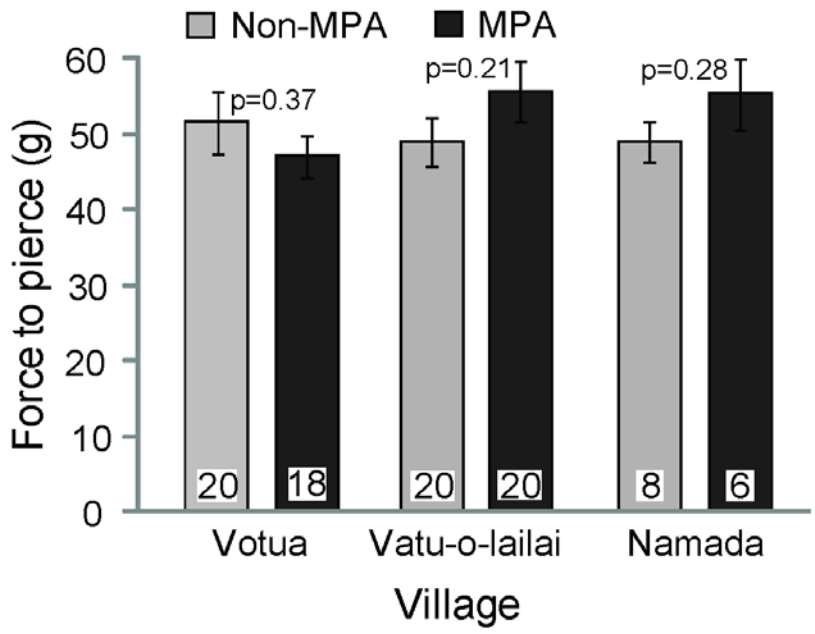


Figure 3-3: Blade Toughness as Measured with a Penetrometer. Light grey bars indicate blades from non-MPA and dark bars indicate blades from MPA sites; n is at the base of each column; P-values are from independent sample t-tests; error bars are ±1SE

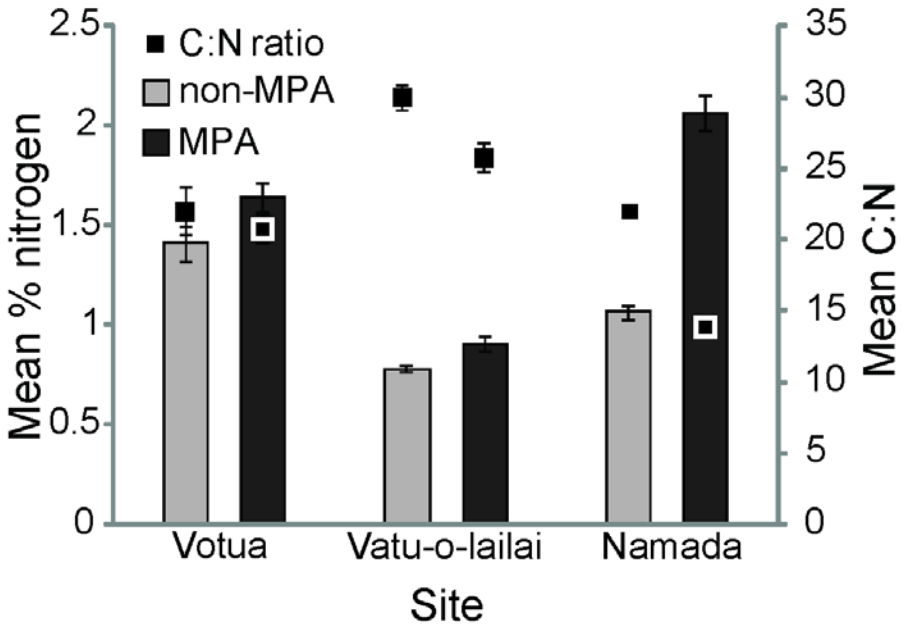


Figure 3-4: Mean Nitrogen Content and C:N Ratio for *S. polycystum*. Percent nitrogen (dry mass) was significantly higher in *S. polycystum* from the MPA in two of the three villages and trended this way in the other (Votua P=0.076; Vatu-o-lailai P=0.016; Namada P=0.009; Mann Whitney U tests); significance in C:N ratio on the secondary x-axis followed the same pattern. Light grey bars indicate non-MPA sites, dark bars

indicate MPA sites and filled squares represent C:N ratio for each site. N= 5; error bars are ±1SE

When algal thalli growing in the non-MPA were moved to the herbivore-rich MPA and either protected from or exposed to limited grazing, and then used in a feeding assay, thalli previously protected from attack were consumed a significant 78% more than those that had experienced previous grazing ($p<0.001$, paired t-test; Fig. 3-5). As with the previous feeding assays (MPA versus non-MPA contrasts), this difference in palatability was not correlated with increased toughness as penetrometer measurements were not significantly different between the two groups (induced= 41.4g ±2.17; control= 44.7g ±2.56; n=23; p=0.337; independent sample t-test).

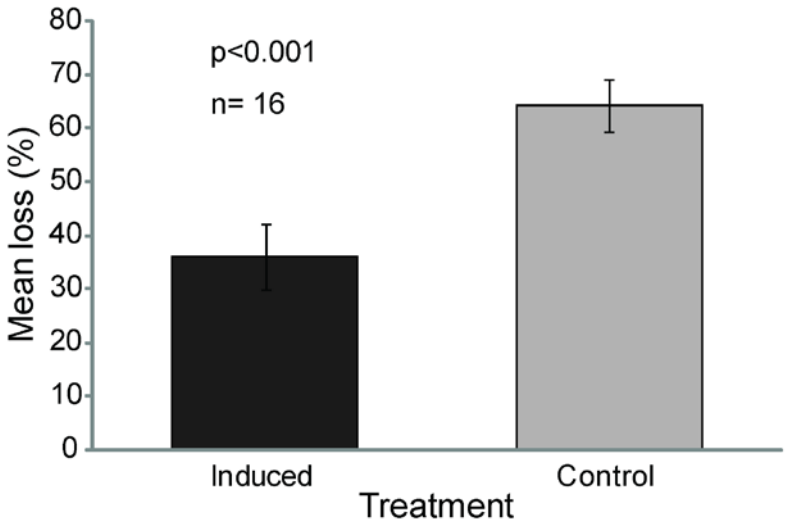


Figure 3-5: Fish Feeding Preference on *S. polycystum* from the Two Week Induction Experiment showing fish preference for the control (light grey bar) over the previously grazed algae (dark grey bar); n= 16; $P<0.001$ from paired samples t-test; error bars ±1SE

Discussion

Over five sampling periods and across three replicate pairs of MPAs and non-MPAs, we found herbivorous fishes significantly preferred *S. polycystum* from the non-MPAs in 11 out of 13 trials (Fig. 3-2). This occurred despite these *S. polycystum* fronds growing only a few hundred metres apart on the same reef flat (Fig. 3-1). Preferential feeding did not correlate with differences in blade toughness (Fig. 3) or nutritional value as measured by nitrogen content (Fig. 4). Nitrogen is often a limiting resource for herbivores (Mattson 1980) and in field assays, herbivorous fishes have commonly selectively consumed food choices with added nitrogen (Burkepile and Hay 2009; Chan et al. 2012). In contrast, fishes in our assays were selectively feeding on non-MPA algae which had lower nitrogen content (significantly so for Vatu-o-lailai and Namada; Fig. 4).

The lower nitrogen content in *S. polycystum* from the non-MPAs could be caused by greater competition among algae for nutrients. Macroalgal cover in the non-MPAs is 27 to 61 times greater than in the MPAs (Rasher et al. 2013), so increased competition for nutrients could occur and limit nutrient content in non-MPA algae. Furthermore, fish biomass is 7 to 17 times higher in the MPAs than the non-MPAs (Rasher et al. 2013), so there is greater potential for nitrogenous enrichment from fish excretion in the MPAs (Meyer et al. 1983; Burkepile et al. 2013).

While feeding choices did not correlate with changes in blade toughness or nitrogen content, we found they could be explained by induction of defence in *S. polycystum* after partial grazing (Fig. 5). When *S. polycystum* ramets were taken from the non-MPA (where there are fewer herbivorous fishes; Rasher et al. 2013) and half were

allowed to be partially grazed while the other half were protected from grazing, the new growth from the protected ramets was preferred to that from the previously grazed algae despite originating from the same holdfast. Penetrometer measurements of these two groups did not differ significantly and there was no noticeable difference in morphology. This suggests that the induced defences were chemical, but further investigation is required to confirm this hypothesis.

That the magnitude of fish preference (effect size) was greater in the induction experiment (Fig. 5) than the un-manipulated MPA/non-MPA feeding contrasts (Fig. 3-2), is perhaps attributable to the higher percentage of induced fronds in assays with the former. While 100% of the algae in the induced treatment had been previously grazed by fishes, this was perhaps not the case with the fronds in the MPA treatment, since they only survive in shallow MPA areas where fish grazing appears to be lower. Herbivorous fishes on these reefs feed predominantly at low tide (*pers. obs.*), and at these times, the areas of the MPA where *S. polycystum* occurs are so shallow that access by fishes is restricted. Additionally, in the induction experiment one third of each frond had been grazed, whereas grazing on the individuals growing in the MPA may have been less severe, perhaps leading to a lower level of induction. Either of these scenarios could explain the stronger fish preference in the induction experiment relative to the MPA/non-MPA contrasts.

There are numerous previous demonstrations of induced seaweed defences in response to grazing by small, less mobile herbivores (mesograzers such as amphipods, isopods and gastropods), but few demonstrations in response to grazing by larger, more mobile herbivores. Induction of morphological defence in response to grazing

by herbivorous fishes has been reported (Steneck & Adey 1976; Lewis et al. 1987), but there appears to have been no experimental tests since then, although some studies have suspected it may be occurring (Coen & Tanner 1989; Paul & van Alstyne 1988). This highlights a gap in current knowledge and a potential misconception regarding macroalgae not inducing defences in response to grazing by larger herbivores. We found that large, mobile grazers could induce reduced palatability in a macroalga, but more evaluations are needed across more species to determine whether such induction is common or rare. Those species capable of rapid growth and with extensive portions protected from consumption within the substratum (such as *S. polycystum*) may be more likely to utilise induced defences because the basal portions may be able to produce induced upright portions in response to attack, even if the attack is severe.

Other studies have documented intraspecific variance in algal palatability to fishes, so induction of defence in response to fish grazing may be more common than currently realised. For example, Keeley et al. (2015) documented that fish consumed *Sargassum* transplanted from the intertidal six times more than conspecifics grown <15m away in the subtidal. This could result from higher grazing in the deeper site causing induction of defence in algae growing there, while less grazing in the intertidal (no fish were observed in that region during the study) left the algae there un-induced.

Although the role of mesograzers in inducing macroalgal defences is well established (Toth & Pavia 2007), differential grazing by invertebrates would not have been responsible for induction in our study. The algae used in the induction experiment were collected from the same holdfast and randomly assigned to treatment or control

groups so initial invertebrate densities would not have differed. Similarly, during the experiment, cages were checked during both day and night periods for the presence of herbivorous invertebrates and none were seen. Scars attributable to invertebrate grazing also were not observed, suggesting invertebrate herbivores were uncommon in this experiment. Their absence is most likely attributable to the unsuitable habitat provided by the low abundance of *S. polycystum* in the cages, since invertebrate density often scales with macroalgal biomass (Carpenter 1986) and cover (Roff et al. 2013). Additionally, the mesh size used in the cages would not have prevented small, invertivorous fishes (such as wrasses) from entering the cages and controlling invertebrate numbers.

Induction of defence by macroalgae in response to grazing is one example of how community structure drives phenotypic responses. In the case of mesoherbivores, this has consequent effects on community dynamics because the algae are a habitat as well as a food source and algae with greater defences are often used preferentially as hosts because they are safer sites (as they less likely to be consumed by fishes; Hay et al. 1990; Hay 1996). Hence it would be interesting to assess the interplay between induction by macro- and meso- herbivores and the consequences this has on population or community level dynamics.

As a final observation, the only previous studies to document induced macroalgal defences in response to vertebrate grazing both detected induction of morphological traits (Steneck & Adey 1976; Lewis et al. 1987). Likewise in terrestrial systems, morphological or structural defences seem to be induced in response to vertebrate grazing (thorns or silica; Hanley et al. 2007), while induction of chemical defences

(which appears common in response to invertebrate grazing) is rare to absent (Bryant et al. 1991). It is unclear whether this is a real pattern in marine and terrestrial systems, or a result of bias in investigation as investigators may focus on invertebrate grazers due to their ease of use in laboratory settings and their importance in agricultural systems.

Here we present one example of induction of macroalgal defence by vertebrate grazing. Investigations into induction of algal defences have been performed since the 1980s, yet this appears to be one of the few published studies to address induction due to fish grazing. Whether this stems from a bias in interest or the difficulty of publishing negative results is unknown. But regardless of the reason, we are still unable to adequately assess the relative roles of meso- versus macro- grazers in inducing seaweed defences (Hay 1996). More publication of both positive and negative results would assist in this.

CHAPTER FOUR

POSITIVE FEEDBACKS ENHANCE MACROALGAL RESILIENCE ON DEGRADED CORAL REEFS

Introduction

Degeneration of coral reefs is commonly correlated with an increase in macroalgal cover (Mumby & Steneck 2008; Hughes et al. 2010). This is an undesirable shift in the community composition because macroalgal dominated reefs lose topographic complexity, support fewer species, and provide fewer ecosystem services than coral dominated reefs (Alvarez-Filip et al. 2009). Macroalgal dominance is also a key factor preventing the recovery of corals (Wilson et al. 2012; Dixson et al. 2014) and it is thought this may be due to the establishment of feedback mechanisms that reinforce the dominance of the new macroalgal dominated state (Mumby & Steneck 2008; Nystrom et al. 2012). Because of this, there is a need for greater understanding of the mechanisms that hinder reef recovery, most particularly of feedbacks potentially enforcing the degraded state (Hughes et al. 2010; Nystrom et al. 2012).

To date, known feedback mechanisms function by negatively affecting corals and fishes. Macroalgae reduce growth of coral recruits (Webster et al. 2015) and adults (Thurber et al. 2012), negatively impact fecundity (Foster et al. 2011), and increase

coral mortality via allelopathy (Rasher & Hay 2010; Rasher et al. 2011) and by vectoring coral disease (Nugues et al. 2004). Macroalgae also deter recruitment of both corals and fishes (Kuffner et al. 2006; Dixson et al. 2014). In addition, rapid macroalgal growth can overtake the grazing ability of herbivorous fishes (Williams et al. 2001; Mumby et al. 2007) leading to the formation of dense macroalgal stands that can deter browsing fishes (Hoey & Bellwood 2011). Furthermore, mature algae are often less palatable than young recruits (Van Alstyne et al. 1999), so if not grazed at this stage algae can develop into sizes or morphologies that suppress grazing (Hay 1981; Hoey 2010). This may further promote the persistence of mature macroalgal beds.

However all of these mechanisms focus on the impact macroalgae have on other species; the effect macroalgae have on their own species is relatively un-investigated. Our aims were therefore: 1) to investigate the processes controlling macroalgal populations, 2) to understand the impacts of these processes on recruit-sized versus mature-sized algal ramets, 3) to evaluate how these processes varied between areas of different community compositions (fished areas dominated by macroalgae versus protected reefs dominated by corals), and 4) to determine whether these processes might generate feedback mechanisms that facilitate macroalgal persistence. Specifically, we tested for the effects of herbivory and the presence of conspecifics on the survival and growth of mature-sized and recruit-sized macroalgae, and used comparisons between coral dominated Marine Protected Areas (MPAs) and adjacent fished reefs (non-MPAs dominated by macroalgae) in Fiji to assess whether the influence of these factors varied between these habitats. Additionally, because population divergence has been documented to occur within a few generations when

selection is strong (Conover & Munch 2002; Stockwell et al. 2003), we accounted for the possibility of rapid evolution in this system by examining growth and survival by algal origin (MPA vs. non-MPA) and also by analysing microsatellite loci to determine if populations were differentiated by site.

Methods

Study Site & Species

This study was conducted between January and May in 2013 and 2015 on the coral coast of Fiji's main island, Viti Levu, in the villages of Votua and Vatu-o-lailai (18°12'32S, 177°42'00W and 18°12'13S, 177°41'29W respectively; Fig. 4-1). These villages are ~3km apart and each has jurisdiction over their stretch of reef flat; a habitat ranging between ~1.5 and 3m deep at high tide and between ~0 and 1.5m deep at low tide. In 2002, these villages established small areas (0.8km^2 in Votua and 0.5 km^2 in Vatu-o-lailai; Fig. 4-1) as no-take MPAs (Simpson 2010). Though MPA and non-MPA areas were initially similar in coral and macroalgal cover (34-42% macroalgal cover; 4-12% coral cover; Simpson 2010), MPAs now differ significantly from the adjacent non-MPAs in benthic cover and fish diversity and abundance. MPAs now have ~56% live coral cover on hard substrate, ~2% macroalgal cover, ~8 fold higher biomass of fishes, and higher recruitment of both fishes and corals than the non-MPAs (Rasher et al. 2013, Dixson et al. 2014). Meanwhile the non-MPAs have lower fish biomass, 5-16% live coral cover on hard substrates and 51-92% macroalgal cover, the majority of which is comprised by Phaeophytes (primarily *Sargassum polycystum* C. Agardh; Rasher et al. 2013). In the MPAs, macroalgal cover is restricted to the shallowest, most shoreward areas (where access by

herbivorous fishes appears limited), whereas macroalgal cover in the non-MPAs extends throughout the habitat. Thus, over distances of only a few hundred metres, there are dramatic differences in community composition that may impact the efficacy of factors controlling macroalgal populations, without the confounding factors of space or time.

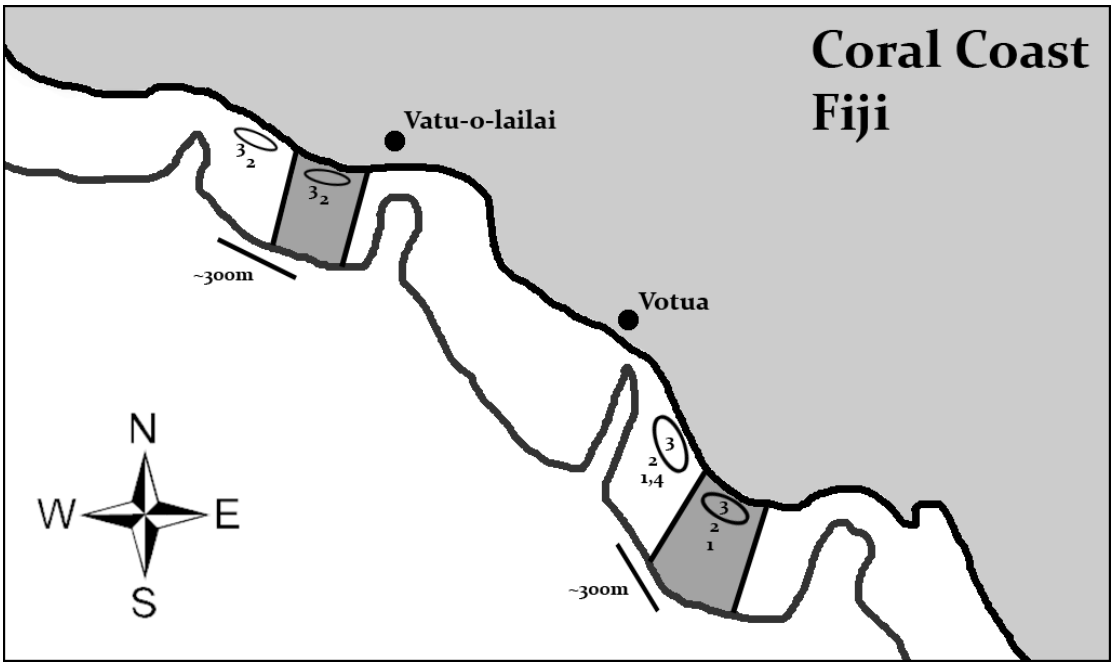


Figure 4-1: Locations of villages, MPAs, sample collections, and experiments. Ovals represent the area in which samples were collected for microsatellite analysis and where experiments involving caging of mature *S. polycystum* fronds and transplanting of recruit-sized ramets were conducted. Experiments caging recruit-sized ramets and transplanting adults were run slightly seaward. Modified from Dell et al. (2015)

We used *Sargassum polycystum* as a study organism because it is often the most conspicuous macroalgal species on degraded Pacific reefs and can grow to dominate large areas (Hughes et al. 2007 in Australia; Mattio et al. 2009 throughout the South Pacific; Rasher et al. 2013 in Fiji; N'Yeurt & Iese 2014 in Tuvalu). On reefs lacking adequate herbivory, *S. polycystum* can reach 8.55 kg wet weight per square metre

(Hughes et al. 2007) and its odour can suppress both fish and coral recruitment (Dixson et al. 2014), potentially suppressing reef recovery. In Fiji, perennial holdfasts start regenerating in December and by the end of its growing season in June, *S. polycystum* commonly dominates large expanses of the unprotected reef flats (Mattio et al. 2009; Rasher et al. 2013). Around this time it may reproduce sexually via spores that disperse only one to three metres (Kendrick & Walker 1995), suggesting the potential for reduced connectivity between even nearby sites. After June, *S. polycystum* senesces leaving the perennial rhizomes sheltered within the reef structure. Populations in our study area will have undergone about 10 generations since MPA establishment, which has been shown to be adequate time for population differentiation among some species if selection is strong (Kinnison & Hendry 2001; Stockwell et al. 2003).

Effect of Habitat and Origin on the Survival and Growth of Mature *S. polycystum* fronds

The dearth of *S. polycystum* in the MPAs and its high abundance in the non-MPAs could be due to differing physical conditions in those locations. To investigate the role of physical conditions and to test whether *S. polycystum* in these areas was acclimatising to the different local conditions, a reciprocal transplant experiment was performed between the MPA and non-MPA to measure survival and growth of mature *S. polycystum* as a function of origin (from the MPA or non-MPA) and habitat (placed in the MPA or non-MPA) when the fronds were protected from herbivory in cages.

The uppermost 15 centimetres of an *S. polycystum* frond was collected from 40 separate holdfasts in the MPA and 40 in the non-MPA of the villages of Votua and

Vatu-o-lailai. To minimise the likelihood of collecting multiple fronds from a single clone, the holdfasts were separated by at least two metres. The lowest five centimetres of each frond were defoliated, the fronds were then blotted dry with paper towels and weighed to the nearest 0.1g. The top of the defoliated section was marked by piercing the thallus with a needle and tying a thread at this 5cm point to set a standard from which to measure growth in length. One strand of *S. polycystum* from the MPA and one from the non-MPA were affixed 20cm apart in the centre of a 50cm piece of 3-strand rope. The lowest 5cm of each algal stipe was threaded through the rope to anchor the strand in place. Four ropes were affixed in each of five cages (dimensions 1m x 1m x 0.8m constructed of 1cm mesh) by the two 10cm end sections of each rope so that the rope's centre, holding the algae, was raised a few centimetres above the substrate. Five cages were anchored at a depth of ~1.2m at low tide in both the MPA and non-MPA so that cages at each location were separated by a minimum of two metres. After one month, the length (from the threaded point) and mass of each frond were measured to assess growth.

Change in length was measured in centimetres after two and four weeks. As mass measurements required removing the fronds from the water, to minimise stress to the organism change in mass was measured in grams only after four weeks. Because significant effects were the same in each of these data sets, only results from height change at week four are reported. A mean change in length was calculated separately for the MPA and non-MPA adults in each cage, yielding an n=5 for each location. As difference scores were normally distributed, a paired t-test was run separately for each location testing the effect of origin on growth over the four weeks.

To investigate the effect of habitat (MPA or non-MPA) on growth, a Mann-Whitney U test compared MPA originated fronds transplanted into both habitats; the same was done for non-MPA originated fronds. All analyses were conducted in SPSS version 16.0 with α=0.025 to account for the multiple contrasts.

Effects of Habitat, Origin and Herbivory on Survival of Recruit-sized S. *polycystum*

In parallel to the above experiment, we addressed the effects of origin, habitat, and herbivory on survival of recruit-sized *S. polycystum*, by performing a reciprocal transplant between Votua's MPA and non-MPA.

Small *S. polycystum* ramets ~1cm long (range between 0.5cm and 1.5cm) were collected from both the MPA and non-MPA using a nail and hammer so that a small piece of bedrock remained attached to each alga's holdfast, allowing four ramets from either the MPA or the non-MPA to be affixed to ~25cm^2 tiles by attaching the rock pieces using aquarium glue (Ecotech Marine, USA). The ramets were selected so that the four on each tile were of equal origin and size and were arranged in a square pattern 1cm distance from each other. The tiles were placed in coolers, containing a few centimetres of seawater and left for 12 hours in the shade to allow the glue to set before moving the tiles to the reef. The tiles were paired so the MPA and non-MPA ramets were of equal size and one tile of each was affixed in a cage so they were 30cm from each other.

These cages were either complete, so the ramets would be protected from fish grazing, or open-sided, so the ramets would be exposed to fish grazing. The open cages lacked the 2 walls parallel to the current direction so that fish access was

permitted, while cage effects would be as similar as possible between treatments in terms of effects on flow and shading. The base of each cage was 0.75m x 0.75m, the height was 0.75m and the mesh size was $1cm^2$ thus excluding all but the smallest fishes and invertebrates. Ten replicates of each treatment were distributed in Votua's MPA and 10 in Votua's non-MPA so that the complete and open cages were paired and the cages in each pair were about one metre apart, while the distance between pairs was ≥ two metres. These cages were distributed ~25 to 50m from shore at a depth of ~1 to 1.5m at low tide.

The experiment was established mid- January 2013, ran for 4 months (112 days), and was checked for ramet mortality every 3 days for the first month and then every week. If an alga was missing but the stone remained, this was noted as mortality. If the stone was also missing this could have been due to failure of the glue, dislodgement by turbulence, or some unknown agent, so we recorded these as 'lost' and excluded them from analysis. Only ten ramets (3.1%) were lost which reduced the total number of ramets in the experiment from 320 to 310.

Despite running for four months, we could detect no growth in this experiment so we report only survivorship. Duration of survival was calculated as the average number of days survived by the four MPA ramets and by the four non-MPA ramets in each cage, giving n=10 for each treatment in each habitat. Difference scores were normally distributed ($p \geq 0.200$; Shapiro-Wilk) so the effect of origin was analysed by paired t-test run separately for each treatment in each location.

Comparisons of the two treatments (caged or grazed) were performed by independent samples t-tests as all datasets satisfied the assumptions of normality and homogeneity of variance or were successfully log2 transformed to do so. This analysis was run separately for each origin (MPA and non-MPA) in each habitat. As data were analysed twice, we applied the Bonferroni correction with α=0.025 and ran analyses using SPSS version 16.0.

Effect of Conspecifics on Survival and Growth of Mature Fronds

To assess whether conspecific density might facilitate the survival and growth of mature fronds, we transplanted mature fronds into the centre of *Sargassum* beds and into nearby exposed habitats where they were isolated from others. Growth and duration of survival were measured over a two week period. Due to logistical constraints this experiment was only conducted in Votua's non-MPA.

Eight 10cm fronds of *S. polycystum* were removed from the centre of one holdfast, assuring genetic uniformity. Four were threaded through a three-strand rope (secured 5cm apart and 10cm from each end of the rope), and returned to the centre of the *Sargassum* bed (crowded condition) ~75m from shore at a depth of ~1m at low tide. The other four were threaded through a separate rope and tied in an area devoid of *Sargassum* two to four metres away (isolated condition). The ends of the rope were tied to the substrate to hold the rope in place. Twenty such rope pairs were set up with a total of 80 *S. polycystum* pieces in each of the crowded and isolated treatments. After two weeks the ropes were collected, the number of remaining fronds was counted and their length was measured. The initial length was subtracted from the final so that fronds that had been grazed in excess of growth were recorded as

negative change. An average change in length was calculated per rope from the four fronds in each rope giving an n=20. This measurement included those that had been grazed and hence included negative change. These data were analysed with a Wilcoxon signed-rank test.

Grazing was either complete (such that none of the frond remained except the section of thallus held between strands of the rope) or absent (such that the entire frond remained including the apical meristem); there were no fronds with portions missing that might indicate partial grazing. Thus, fronds that were grazed or ungrazed could be easily identified. Consequently, we also calculated an estimate of growth from only the ungrazed fronds that had retained their apical meristems and survived the experiment. This permitted a comparison of growth between the crowded and isolated conditions when herbivory appeared to be absent. Once again, the initial length was subtracted from the final length of each ungrazed frond and an average change in length per rope was calculated. As all fronds were completely grazed on four ropes, those pairs were excluded leaving n=16 in this dataset. Difference scores satisfied the assumption of normality ($p=0.161$; Shapiro-Wilk) so data were analysed by a paired t-test. Both analyses were run in SPSS version 16.0 with $\alpha=0.05$.

Effect of Conspecifics, Origin and Habitat on Survival and Growth of Recruit-sized Ramets

We investigated the effect of conspecifics on the survival and growth of recruit-sized ramets in conjunction with the effect of origin when ramets were not protected from herbivory. Because *Sargassum* beds in the MPAs only exist near shore and we did not want to confound distance from shore with treatment, we conducted this experiment

at a depth of ~0.5m (at low tide) between ~10m to 20m from shore in both Votua and Vatu-o-lailai (Fig. 4-1).

As in the previous experiment that also used recruit-sized ramets, small algal recruits (0.5 to 1.5cm tall) were detached from the substrate so that a small piece of bedrock remained attached to the alga's holdfast and these rock pieces were affixed to tiles using Ecotech coral glue. Two MPA and two non-MPA ramets were attached onto each tile in a square pattern 1cm distance from each other. As before, the ramets were chosen so that the four on each tile were of equal size and the tiles were arranged so there was similar size representation of ramets in each treatment. In each location, tiles were placed within established *Sargassum* beds (crowded condition) or placed in open areas (isolated condition) ~2 metres away.

A total of 30 tiles were affixed in the MPA and 30 in the non-MPA within each village, 15 in crowded and 15 in isolated areas. This design ensured there were two origins (MPA or non-MPA) and two density conditions (crowded or isolated) in each of the MPA and non-MPA habitats of both Votua and Vatu-o-lailai.

The tiles were out-planted at the end of February 2013, monitored every 3 days for the first month and then weekly for two subsequent months for mortality and loss. As in the previous tile experiment, if the stone to which the ramet was attached was missing, those individuals were recorded as lost and excluded from subsequent analyses. Of the initial 240 ramets deployed in each village, 16 and 15 individuals were lost (6.7% and 6.2%) from Votua and Vatu-o-lailai respectively.

At the end of three months, change in height and change in mass were recorded for each ramet. The initial measurement from each ramet was subtracted from its final, meaning the ramets that died were recorded as negative change. An average final height and average final mass were calculated from the two sub-samples (the two MPA and two non-MPA ramets) on each tile giving an n=15 for each density (isolated/crowded) in each location. These data were analysed by Permutations Analysis of Variance blocked by tile, with origin and density as main effects plus the interaction between the two. This analysis was run separately for each of the four locations using the package lmperm (Wheeler 2010) on R version 2.15.3 with α=0.05. As significant effects were the same for height as for mass data, only results from the height data are shown.

Microsatellite Analysis

To investigate whether differences in *S. polycystum* growth and survival correlated with genetic variation among populations, we assessed allelic variation among the four populations (the MPA and non-MPA areas of Votua and Vatu-o-lailai) at five neutral microsatellite loci. Full details of sample collection, sequencing and laboratory methodology for microsatellite analysis are in the supplementary material (S1 Appendix).

The statistic Fst reflects how much of the total population genetic variance is contained in a subpopulation and is thus used as a measure of the genetic divergence between subpopulations (Wright 1951). Fst ranges between 0 and 1, where 0 indicates there is no genetic differentiation among populations and 1 indicates complete

absence of gene flow among populations. Fst was calculated from the five microsatellites in the four populations using GenePop V4.3 (Rousset 2008).

Results

Effect of Habitat and Origin on the Survival and Growth of Mature *S. polycystum* fronds

When protected from grazing, survival of mature fronds was 100% regardless of location. *S. polycystum* originating in the MPA grew significantly more (~40%) than those from the non-MPA in three of the four locations, suggesting a considerable effect of algal origin on growth of mature fronds ($p \leq 0.013$; paired t-tests; Fig. 4-2). Conversely in Votua's non-MPA, the MPA and non-MPA fronds grew at indistinguishable rates ($p=0.214$; Fig. 4-2).

An effect of habitat on growth was observed in Votua, where both MPA and non-MPA-originated algae grew a significant 1.8 and 3 times more, respectively, in the MPA than in the non-MPA ($p \leq 0.016$; Mann-Whitney U tests) suggesting a strong effect of habitat on growth in this village. In contrast, in Vatu-o-lailai there was no effect of habitat on growth ($p>0.347$; Fig. 4-2).

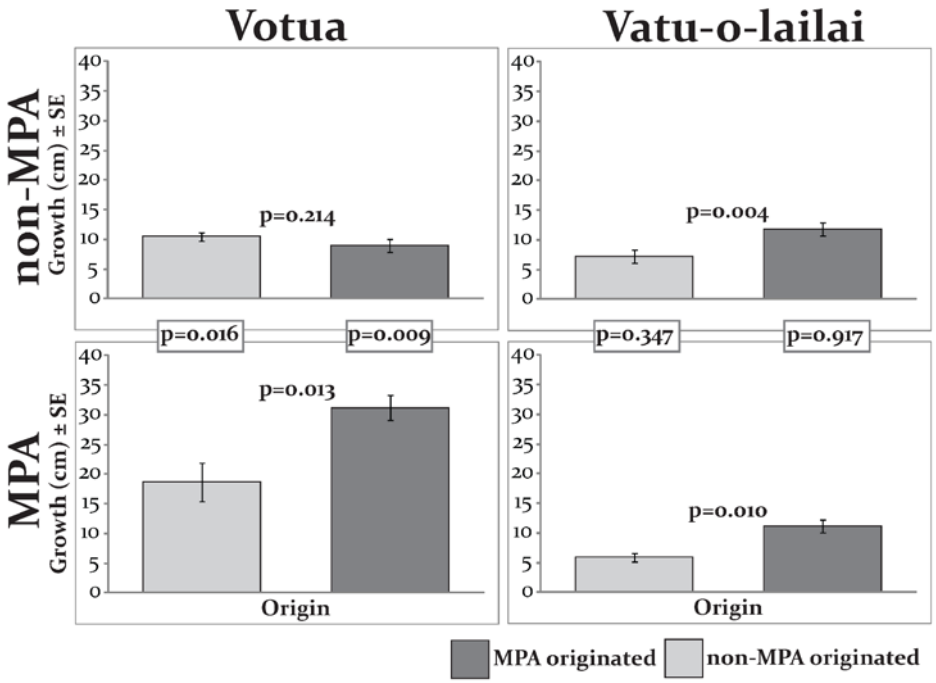


Figure 4-2: Growth of mature, caged *S. polycystum* fronds from MPAs and non-MPAs when reciprocally transplanted p-values above the bars are from paired t-tests assessing the effects of origin. The boxed p-values are from Mann-Whitney U tests assessing the effect of habitat (placement within the MPA or non-MPA) on growth. N=5 at each location; α=0.025 to correct for multiple contrasts

Effects of Origin, Habitat and Herbivory on Survival of Recruit-sized S. *polycystum*

In contrast to the significant effect of origin on growth of mature *S. polycystum* fronds (Fig. 4-2), origin did not affect duration of survival of recruit-sized ramets in any location ($p \geq 0.401$; paired t-tests; Fig. 4-3).

Of the 320 initial recruit-sized ramets deployed in the reciprocal transplant experiment, 10 (3.1%) were lost (they and their basal substrate were missing). Of the remaining 310, 271 (87.4%) died or appeared to be consumed and 39 (12.6%) remained alive at the end of the 4 months. Of the 39 survivors, 25 (64.1%) were in the MPA closed-cages while none survived in the MPA open-sided cages. In the non-

MPA, 7 ramets (17.9%) survived in the complete cages and 7 survived in the open cages.

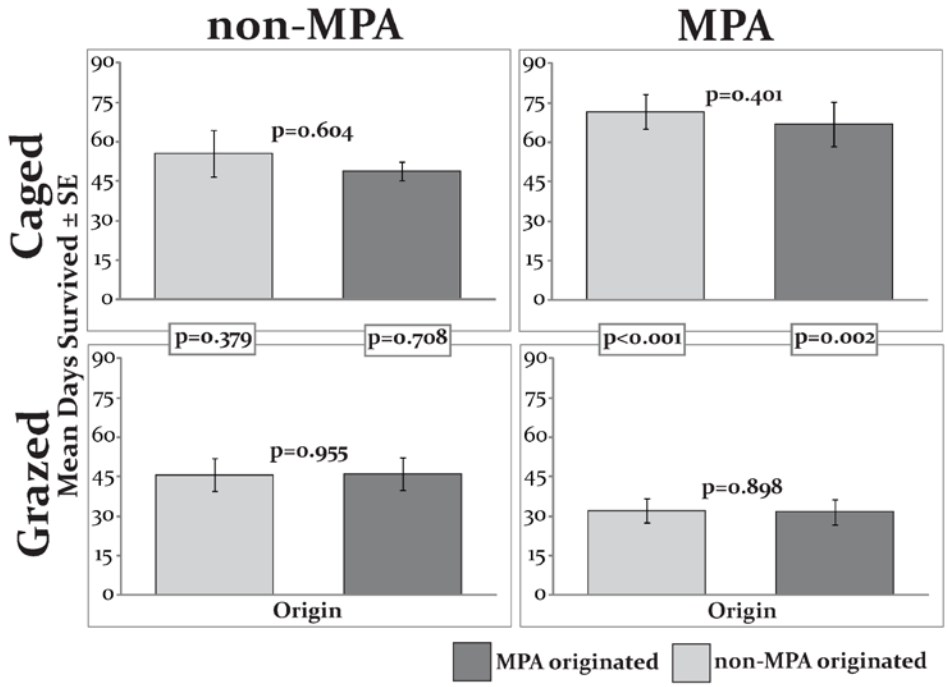


Figure 4-3: Survival of recruit-sized ramets reciprocally transplanted between MPA and non-MPA when caged or exposed The experiment ran for 112 days; p-values above bars are from paired t-tests comparing the two origins within each treatment and location. Boxed p-values are from unpaired t-tests comparing ramets of the same origin in the caged and grazed treatments. N=10 for each treatment and α=0.025 to correct for multiple contrasts

When duration of survival was assessed, protection from herbivory strongly increased the duration of ramet survival in the MPA: juvenile-sized ramets protected from fish grazing survived more than twice as long as those that were unprotected in the open sided cages ($p \leq 0.002$; independent samples t-tests; Fig. 4-3). Conversely, caging had no effect on duration of survival in the non-MPA ($p \geq 0.379$; Fig. 4-3).

Effect of Conspecifics on Survival and Growth of Mature Fronds

There was a clear difference in survival and growth of adult fronds placed into crowded versus isolated areas. We found that adult fronds experienced increased survival and growth when placed within *Sargassum* beds compared to nearby isolated areas. In the crowded condition, 78 out of 80 (97.5%) fronds remained at the end of the two week experiment; only two had been grazed (2.5%) and these were completely consumed. In the isolated treatment only 47 out of 80 (58.8%) remained while 33 (41.2%) were grazed. None had dislodged from the ropes as the basal stipes were still entwined within the rope strands for all fronds, including those removed by grazing. When all fronds were analysed, those in the crowded condition increased an average of ~2cm (~20%), while those in the isolated condition declined by ~3.5cm (~35%; $p<0.001$; $n=20$; Wicoxon signed-rank test; Fig. 4-4a). When only the survivors were analysed, those in the crowded conditions still increased significantly more than those in the isolated conditions (crowded ~2.1cm; isolated ~1.4cm; $p=0.002$; $n=16$; paired t-test) indicating that even when herbivory is minimal, conditions in the *Sargassum* beds are more suitable for *S. polycystum* growth than conditions in isolated areas.

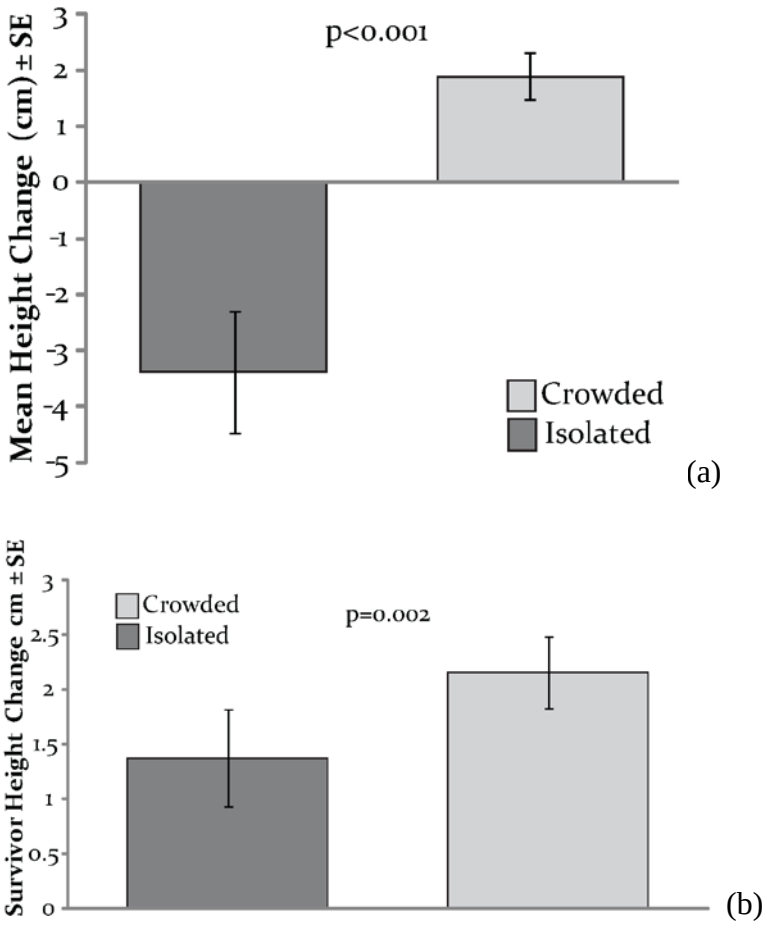


Figure 4-4: Growth of all (a) and surviving (b) mature *S. polycystum* fronds transplanted into or outside *Sargassum* beds For (a) n=20 and p-value is from a Wilcoxon signed-rank test. For (b), n=16 and p-value is from a paired t-test

Effect of Conspecifics on Survival and Growth of Recruit-sized Ramets

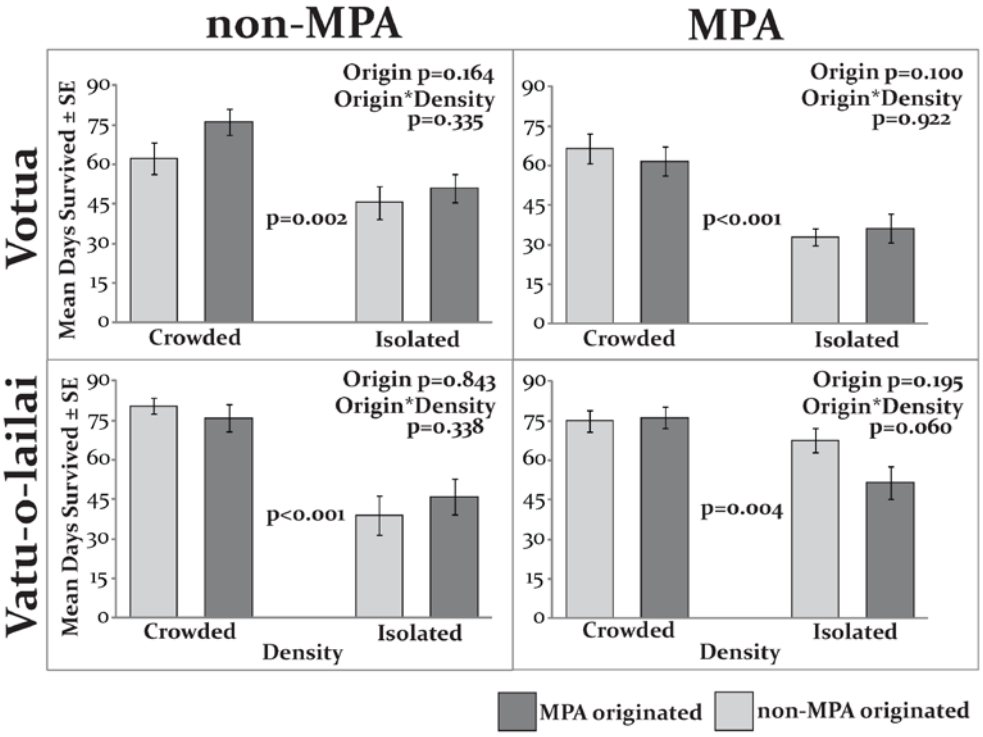
When we transplanted recruit-sized ramets in a similar experiment, once again survival and growth were higher when ramets were crowded by conspecifics. By the end of the three month experiment in Votua, 16 individuals were lost from the initial 240, leaving 224. Of these 224, 86 survived the duration of the experiment, of which 67 (78%) were in the established *Sargassum* beds (crowded condition). In Vatu-o-lailai, 15 individuals were lost leaving a total of 225. Of these 225, 116 survived of which 79 (68%) were within the algal beds.

Not only was proportion of survivors higher in the crowded condition, but when survival was measured as number of days survived, ramets survived longer when crowded by conspecifics (average between 62 and 80 days) than when they were isolated (between 33 and 68 days; $p \leq 0.004$; Fig. 4-5a).

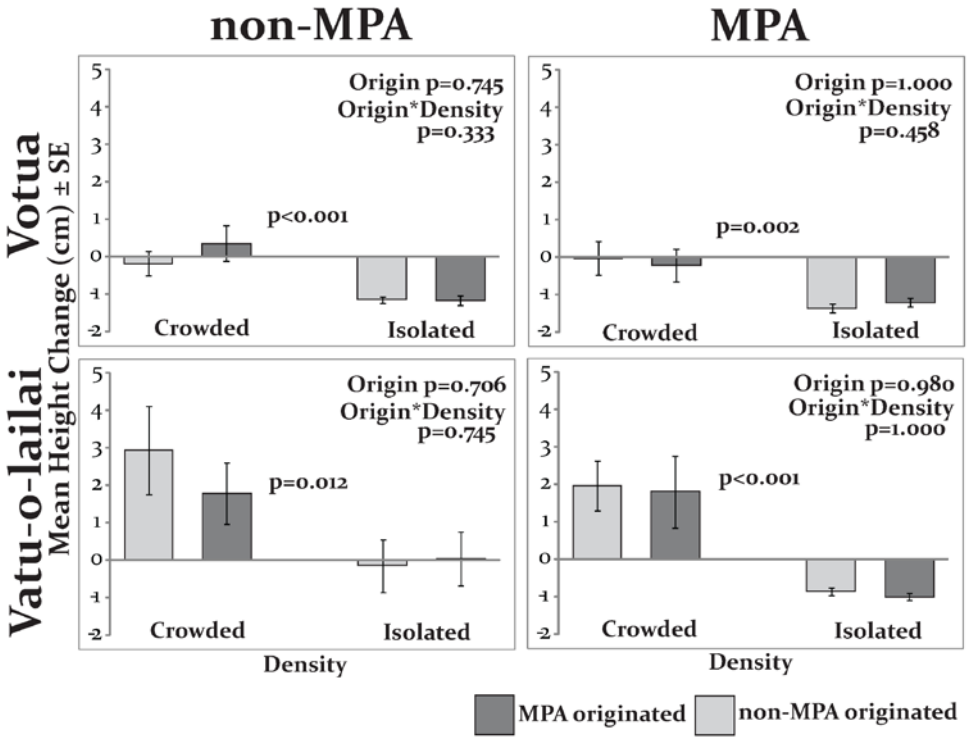
Net change in height was also significantly greater in the crowded (average height change between -0.2 and 3 cm) than in the isolated condition (-1.4 and ~0 cm; $p \leq 0.012$; Fig. 4-5b).

Similar to the previous experiment using recruit-sized ramets (Fig. 4-3), ramet origin was not a significant factor here: neither duration of survival nor growth varied by origin in any location (Origin $p \geq 0.100$ Fig. 4-5a; Origin $p \geq 0.706$ Fig. 4-5b; permutation ANOVA). The interaction was also not significant in any location (Origin*Location $p \geq 0.060$).

Thus regardless of where the ramets originated and whether they are placed in MPA or non-MPA habitats, both survival and growth were higher when surrounded by conspecifics.



(a)



(b)

Figure 4-5: Survival (a) and growth (b) of recruit-sized ramets growing in crowded or isolated densities N=15; initial height was subtracted from final height for all ramets, meaning those that died were included as negative values; statistical analyses were by Permutation ANOVA

Microsatellite Analysis

The estimate of Fst was <0.05 for each individual locus (S1 Appendix) and 0.0048 over the five loci combined, indicating negligible differentiation across these four populations.

Discussion

The MPAs and non-MPAs we investigated differ dramatically in coral cover, macroalgal cover and fish biomass (Rasher et al. 2013), despite being situated adjacently in a ~4km stretch of continuous coastline. The dominance of *S. polycystum* in the non-MPAs suppresses the recovery of those reefs to a coral dominated state, but the processes controlling *S. polycystum* are relatively un-investigated.

Of the factors we assessed, herbivory was the dominant process negatively affecting *S. polycystum* survival and growth, while the presence of conspecifics was the dominant positive influence. That these factors apply to both mature and recruit-sized ramets and in habitats of vastly different community composition (intact and degraded reefs), suggests they are major factors influencing populations of *S. polycystum* in this region. The origin of the alga and the habitat in which it grew were of secondary importance in influencing *S. polycystum* survival and growth.

In the MPAs, *S. polycystum* is only found in shallow, spatial refuges close to shore, putatively where access by fish is restricted. In contrast, *S. polycystum* is abundant and broadly distributed in the non-MPAs, where biomass and diversity of herbivorous fishes is low (~0% cover in mid-reef to outer areas of the MPAs versus 21 to 44% in

the non-MPAs; Rasher et al. 2013). We find that this is not due to favourable physical conditions in the non-MPA as growth of mature fronds was generally higher in the MPA when they were protected from grazing (Fig. 4-2). Instead, this discrepancy in *S. polycystum* abundance results from 1) herbivore escape in the non-MPAs (Fig. 4-3), and 2) positive feedbacks that *Sargassum* beds generate facilitating conspecific survival and growth (Figs. 4-4 and 4-5).

The common appreciation for the negative effects of intraspecific competition often obscures the fact that elevated plant density can produce positive feedbacks that more than compensate for competitive costs, especially when plants are in stressful physical or biological circumstances (He et al. 2013). Just as animals achieve positive benefits by aggregating into herds, schools, or flocks (Parrish et al. 1999; Sumpter 2006), experiments are increasingly demonstrating similar positive effects of "herding" or dense aggregations in organisms as diverse as seaweeds (Hay 1981), marsh plants (Silliman et al. 2015), mangroves (Kumara et al. 2010), oysters (Schulte et al. 2009), and microbes (Darch et al. 2012). Once *Sargassum* beds are established, they clearly suppress herbivory on juveniles and adults growing within the bed versus those a few metres outside the bed (Figs. 4-4a and 4-5a). However, in addition to reducing herbivory, *Sargassum* beds also appear to generate a positive physiological effect on congeners; growth of mature fronds placed outside the *Sargassum* beds was only 2/3 of the growth of those fronds placed inside the bed (when we evaluated only those fronds that appeared ungrazed; Fig. 4-4b). Numerous studies on aggregated conspecifics find advantages due to protection from consumers or enhanced awareness of resource patches or dangers, but these advantages often have to counterbalance a physiological cost due to increased intraspecific competition. Here

we detected a group advantage of reduced attack by consumers, but we also detected a physiological advantage, rather than cost, to intraspecific crowding. Reasons for this are unknown, but previous studies on plant aggregations have sometimes found that aggregated plants are better than individuals at lessening physical stresses such as desiccation, anaerobic soils, or erosion (He et al. 2013; Silliman et al. 2015), resulting in a physiological benefit of aggregation.

In the habitats where we worked, light is high and turbulence and flow are often considerable, meaning that light resources may be plentiful and nutrient replacement high, which would minimise intraspecific competition. Hence, our results could be different in lower light (deeper) conditions or habitats with less flow to break down diffusion gradients. However, it is also possible that *Sargassum* crowding produces direct positive effects for members of the group. Possible hypotheses include: 1) shading within the bed reduces light shock or photorespiration in these shallow waters, 2) baffling of wave force reduces damage due to sand scour or other physical processes, 3) retention of DOC or other leached metabolites within the bed enhance beneficial microbes or suppress damaging microbes and increase the net growth of *Sargassum* individuals in the group, or 4) other unknown benefits generated by positive feedbacks from *Sargassum* density. Although additional work is needed to clarify the mechanism of protective benefit from conspecifics, our data indicate that established *Sargassum* beds act as a positive feedback, or stabilising mechanism (Fong & Paul 2011; Nystrom et al. 2012), that promotes algal growth and persistence and potentially also expansion. These positive effects on *Sargassum* likely enhance the resilience of macroalgal dominated reefs and suppress recovery of the coral dominated state (Mumby & Steneck 2008; Nystrom et al. 2012).

Welsh & Bellwood (2015) reported mobile grazers were attracted to algal aggregations of low density while Hoey and Bellwood (2011) reported they were deterred by dense macroalgal stands. Therefore it may be that the effects of macroalgal aggregation vary in a density dependent manner: at lower densities they may be attractive to mobile herbivores, whereas at high densities, such as established algal beds, they may deter grazers. Thus these positive feedback mechanisms may function in a similarly density-dependent fashion and *Sargassum* may have to reach a critical mass before such mechanisms operate (Nystrom et al. 2012). That macroalgal stands cause avoidance behaviour of herbivorous fishes in Fiji as well as Australia (Orpheus Island; Hoey & Bellwood 2011) and the Caribbean (Hay 1981) illustrates that this is not a site-specific behaviour, but may be a general process on reefs (Nystrom et al. 2012).

Although we saw no influence of algal origin on survival or growth of recruit-sized ramets, we did detect a significant effect of algal origin on growth of mature fronds. Mature fronds from the MPA grew significantly more than those from the non-MPA in three of our four locations (Fig. 4-2). Isotopic analyses of macroalgae from these locations also suggested that those in the MPAs were growing more rapidly (Dell et al. 2015). It is possible that competition for nutrients is limiting *S. polycystum* growth in the non-MPAs since previous work found that sparsely distributed *S. polycystum* growing near shore in the non-MPAs had significantly lower nitrogen content (Dell & Hay submitted). The nitrogen content of *S. polycystum* growing within the *Sargassum* beds remains to be determined, so we are unable to accurately compare nutrient levels within and outside the beds.

It is surprising that mature fronds exhibited an effect of origin while recruits did not. We speculate this could either be because mature fronds have accumulated nutrient stores and can rely on them post-transplant, or alternatively, because recruit-sized ramets are able to acclimatise by responding rapidly to new conditions, while mature fronds cannot. Another potential explanation is that this difference results from genetic differences or maternal effects that were not detected in the five microsatellite loci we analysed (S1 Appendix) or our experiments on earlier life stages due to the low number of survivors. However all Fst values from our microsatellite analysis were below 0.05 (S1 Appendix), which suggests minimal genetic differentiation (Weir 1996). At such small spatial scales, we would only expect to see genetic differences if selective pressures were strong and distinct, and we found herbivory and the presence of conspecifics to be major forces in both the MPAs and non-MPAs. Additionally, previous research has found little genetic differentiation in this species across large spatial scales (Chan et al. 2013; Kantachumpoo et al. 2013). Thus we think it most likely that *S. polycystum* in this region is one population that is responding phenotypically to different drivers in different locations (although perhaps only when recruit-sized). This is in agreement with other studies that have reported plasticity in growth rate without concomitant variation in genetics (Trtikova et al. 2010).

When faced with environmental variation, populations of sessile organisms can either respond plastically or evolve (Smith et al. 2014). In a landscape such as Fiji's Coral Coast, where such different habitats occur within hundreds of metres, theoretical models predict the former (Sultan & Spencer 2002) and indeed this appears to be how

S. polycystum has responded. With rapid change expected due to increasing habitat fragmentation and climate change, species able to respond plastically -such as *S. polycystum*- may be the best able to cope (Somero 2005; Canale & Henry 2010). Such capacity for phenotypic plasticity may be especially advantageous given the high mortality rate of recruits observed in these experiments. The low growth and percent survival of recruit-sized ramets deployed in our experiments parallels the high mortality found in other studies (Kendrick & Walker 1995; Leung et al. 2014), and suggests that the energy reserves stored in rhizomes may be critical for the rapid early growth and persistence of this species.

Although we have not addressed how *Sargassum* beds become established, we do see that they are a stabilising mechanism (Fong & Paul 2011) that promotes continued *S. polycystum* dominance via positive feedbacks and may be preventing the recovery of coral reef communities (Kuffner et al. 2006; Dixson et al. 2014). It is plausible that removal of herbivores from the non-MPAs through extensive fishing has created spatial refuges from herbivory which allowed the initial establishment of *S. polycystum*. Once established, this species creates a positive feedback that enhances the fitness of both recruit-sized and mature conspecifics, making macroalgal dominated areas resilient, and less likely to revert back to coral domination. *S. polycystum's* ability to store reserves in rhizomes that are protected within the reef structure may further enhance its resilience in the non-MPAs (Grime & Hunt 1975).

Research is increasingly focused on how to reverse phase-shifts from coral to macroalgal dominance (Adam et al. 2015). Protection of herbivorous fish populations has been the major tool endorsed to accomplish this (Mumby & Steneck 2008), but

this strategy has not always had the anticipated effect (Guarderas et al. 2011). Our results suggest that measures reducing the size or density of *Sargassum* beds could work synergistically with fish protection to aid reef recovery since *S. polycystum* benefits greatly from a self-generating positive feedback. Although *S. polycystum* dies back in June-July, the rhizomes remain perennially within the reef structure and produce new fronds in December. Therefore it may be more appropriate to manage *S. polycystum* more like a fungus, where the visible tissue is only a small part of the organism and extensive tissue grows protected out of sight. Thus measures that deplete the rhizome reserves and prevent them from producing regenerative fronds (which will replenish the reserves) may prove more effective. This suggests that if manual removal (Adam et al. 2015) is employed, it may have to be repeated frequently.

This case study illuminates an interesting interplay between the different population controls operating in this system. Of the factors we addressed, herbivore escape appears to be the primary factor promoting *S. polycystum* survival and growth. The difference in *S. polycystum* abundance between the MPAs and non-MPAs is dramatic, but this does not arise from the non-MPAs being a more favourable environment since growth is lower in the non-MPAs. Instead, the combination of reduced herbivory and increased algal density in the non-MPAs act in a positive feedback manner (Nystrom et al. 2012) to promote *S. polycystum's* persistence and enhance the resilience of the degraded reef state. We detected no negative effects of intra-specific competition as growth and survival were greater in areas of high *Sargassum* density even when herbivory appeared to be minimal. This study highlights the positive influence *Sargassum* beds have on their own species and thus how they generate

positive feedback mechanisms that stabilise macroalgal dominance on reefs and suppresses recovery to coral and fish dominated systems.

APPENDIX

Sample Collection for Microsatellite Analysis

In May 2013, the uppermost three leaves of 50 *S. polycystum* ramets were collected from the MPAs and non-MPAs in Votua and Vatu-o-lailai (Fig. 4-1), maintaining a minimum distance of 3 metres between each sample to reduce the possibility of sampling clones. Samples were collected a minimum of ~40m from the boundaries of the MPA and non-MPA borders to avoid edge effects. To minimize potential differences in physical conditions, collecting sites in the MPA and non-MPA areas were chosen to have comparable depth and distance from shore. Samples were shaken to remove particulates and preserved in molecular grade ethanol, which was replaced after the first 48 hours.

DNA Extraction, Amplification & Analysis

DNA was isolated from 3-3mm^2 of between 29-43 *S. polycystum* samples per site using DNeasy Blood & Tissue Kit (Qiagen, The Netherlands) and purified with DNA, RNA & Protein Purification kit (Macherey-Nagel, Germany). All polymerase chain reactions (PCR) were run on an Eppendorf AG Thermal Cycler with a total volume of 10µL which contained: 6.8 µL DI water; 1 µL 10x PCR buffer; 1µL diluted DNA; 0.2µL of each dNTP, forward primer, reverse primer and GO Taq polymerase buffer (5u/µL Promega) and 0.4 µL $MgCl_2$ (25mM, Thermo Scientific; except primer 38 which contained 0.8µL $MgCl_2$). Primer 9 was amplified with the following profile: initial denaturation at 95°C for 2 minutes; 45 cycles of 95°C for 30 seconds, 48°C for

1 minute and 72°C for 1 minute; followed by a final extension at 72°C for 6 minutes. All other primers were amplified with the following profile: denaturation at 95°C for 2 minutes, followed by 40 cycles of 95°C for 30 seconds, 50°C for 1 minute and 72°C for 1 minute. PCR products were analysed by Nevada Genomics (Applied Biosystems Prism 3730 DNA Analyser; University of Nevada, Reno) using GeneScan 500 LIZ size standard (Applied Biosystems, USA) and read using Peak Scanner Software 2 (Applied Biosystems, USA).

Table S1: Microsatellite Primer Sequences: Sequences and Fst values for the five microsatellite loci. Fst was not estimable for locus 42 as there was no allelic variation at that locus.

Primer	Forward Sequence	Reverse Sequence	Fst
1	AGG CAA GCA ACA AAC GAG TT	CAG GAT TGC AAC CAT ACC CG	0.049
9	AGGACGGGAAAAGGGAATAG	AGTTTCGGAAAGCGTTCTCA	-0.01
24	ATG GGC AGT GGG TAG ACA AT	GAT TGG TTT GAC AGA GCC GG	0.002
38	CCA ACA ACC ACT GAT GTC CC	ACC CGG CTC TGT CAA ACT AA	0.001
42	CAA CTC GCC CTG TCA AAC TA	TAG TCG TCA CCC TTT CCG G	-

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