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Nutrients induce and herbivores maintain thallus toughness, a structural anti-herbivory defense in *Turbinaria ornata*

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ABSTRACT: The loss of coral cover, frequently driven by anthropogenic disturbances, can result in a phase shift to dominance by fleshy macroalgae, many of which contain anti-herbivore defenses. Using field surveys, a mesocosm experiment, and field experiments, we evaluated whether 2 human impacts—nutrient enrichment and reduction in herbivory—affected production and maintenance of thallus toughness, a physical defense of the brown macroalga *Turbinaria ornata* that has recently expanded across the South Pacific. In contrast to our expectations, there was a weak negative relationship between herbivorous fish abundance and thallus toughness. This relationship was driven by greater toughness in algae collected at the more eutrophic sites, which also had lower herbivore abundances. A mesocosm experiment confirmed a positive relationship between nutrients and thallus toughness with no measurable cost to growth. Mechanical damage simulating herbivory maintained thallus toughness in cages, with a significant trade-off in growth. In addition, reduction of herbivory through caging in a transplant experiment resulted in a reduction in thallus toughness; however there was no measurable benefit to growth, possibly due to a concurrent change in environmental context from the transplant. While reduction in herbivory via overfishing allows this alga to trade energy normally spent on defense for increase growth, nutrient enrichment provides *T. ornata* with additional resources to increase defenses. As anthropogenic impacts become increasingly prominent in coral reef systems, it is critical that we understand the processes that may facilitate the expansion and dominance of coral reef algae, especially those with inducible anti-herbivore defenses.

KEY WORDS: Herbivory · Macroalgae · Nutrient addition · Physical defense · Energy allocation · Phenotypic plasticity

INTRODUCTION

Globally, natural and anthropogenic disturbances have shifted benthic cover of many tropical reefs from coral to macroalgal dominance (e.g. Bellwood et al. 2004), stimulating research on processes that maintain this alternative state. On pristine coral reefs herbivory is intense; thus, the only algae that can proliferate often have defenses that deter herbivores

and release algae from top-down control (for a review see Fong & Paul 2011). Anti-herbivory defenses, which are common adaptations to herbivory pressure in both terrestrial and aquatic producer communities, can be either structural or chemical (see Coley et al. 1985 for terrestrial, Hay & Fenical 1988 for marine). Algae from all 4 taxonomic divisions contain secondary metabolites that may deter herbivore feeding (Hay & Fenical 1988). However, a single mechanism

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of defense may not be effective in tropical reefs subjected to abundant and diverse herbivore communities; as a result, many marine macroalgae employ multiple means of defense (Hay & Fenical 1988, Fong & Paul 2011). While chemical defenses in marine macrophytes have been studied extensively (Hay & Fenical 1988, Paul et al. 2007), physical defenses have received less attention (but see Steneck 1986, Padilla 1989 for the role of calcification, Campbell et al. 2014 on links between acidification, calcification, and herbivory). Therefore, more research focused on the mechanisms underlying physical defenses in tropical systems is needed.

The presence of anti-herbivore defenses can either be constitutive or induced, and these strategies may have different costs and benefits (for a review see Strauss et al. 2002). While constitutive defenses provide permanent deterrents to herbivores (e.g. Wittstock & Gershenson 2002), induced defenses are plastic and activated based on context (Dicke & Hilker 2003). Theory predicts that induced defenses may have fewer overall costs if the risk of herbivory is infrequent or sublethal (Wittstock & Gershenson 2002). For example, *Padina jamaicensis*, a common brown Caribbean macroalga, adopts a prostrate encrusting morphology in reef zones subjected to high herbivory pressure and a foliose morphology in areas with less herbivory (Lewis et al. 1987). In terrestrial systems, induction may require the presence of, or actual attack by specific herbivores, or may be a general response to physical damage that simulates herbivory (Dicke & Hilker 2003). Similarly, in aquatic systems, physical damage simulating grazing on the marine macroalga *Fucus vesiculosus* caused a clear, rapid, induced resistance to grazing (Hemmi et al. 2004) while chemical cues from specific consumers were required to induce herbivore defenses in a freshwater unicellular alga (Pohnert et al. 2007). Regardless of the type of defense or mechanism of induction, herbivore defenses are likely costly because they use resources that would otherwise be available for growth or reproduction (for reviews see Bazzaz et al. 1987, Strauss et al. 2002, Wittstock & Gershenson 2002).

The underlying principle of energy allocation is that an individual organism must partition limited resources among key metabolic processes (for review see Strauss et al. 2002) and that use of resources within an individual is exclusive; thus, resources that are invested in one metabolic subsystem are no longer available for use in another subsystem (Perrin & Sibily 1993). Production of herbivore defenses may incur a wide variety of costs, including reduction in

growth. Growth–defense trade-offs have been widely demonstrated across many terrestrial ecosystems including grasslands and tropical forests (Fine et al. 2006, Lind et al. 2013). One meta-analysis found 82% of studies on crop plants demonstrated significant costs associated with herbivore resistance (Strauss et al. 2002). It is important to understand if marine macroalgae are also able to shift energy allocation between growth and defense, a strategy that may be highly advantageous for algae in human-impacted environments.

While a meta-analysis for terrestrial plants suggested the costs of resistance to herbivores may vary with resource availability (Strauss et al. 2002), the relationship between nutrients and anti-herbivory defenses for macroalgae is not clear. For example, nutrient addition did not affect growth or herbivore resistance of *F. vesiculosus*, though concentrations of chemicals thought to act as deterrents decreased (Hemmi et al. 2004). Similarly, nutrient enrichment of the tropical alga *Turbinaria conoides* reduced anti-herbivore compounds and did not affect growth, although differences in resistance to herbivory were not tested (Mayakun et al. 2013). Further, while one study documented decreased palatability of *Turbinaria ornata* following nutrient addition (Bittick et al. 2016), nutrients increased palatability of a congener, *T. turbinata* in another study (Chan et al. 2012). A positive relationship between nutrients and herbivory rate was found for other tropical macroalgae, such as *Acanthophora spicifera* (e.g. Boyer et al. 2004). Finally, a field study in Tahiti demonstrated the concentration of anti-herbivore compounds in *T. ornata* was higher at sites with more grazers and higher nutrients (Stiger et al. 2004). Most of these studies focused on the causal link between nutrients and chemical defenses. We know of only one study that considered physical anti-herbivore defenses (Bittick et al. 2016); these authors found that nutrients bolstered physical defenses in the form of thallus toughness that deterred herbivory of *T. ornata*. However, Bittick et al. (2016) did not evaluate algal responses to a gradient of nutrient supplies or algal defense/growth trade-offs. Thus, more research is needed to understand the relationship between resources and defenses in marine macroalgae.

Our overall objective was to evaluate the effects of herbivory and nutrients on physical defenses in the marine alga *T. ornata* on fringing reefs of Mo'orea, French Polynesia. *T. ornata* has recently increased in distribution and abundance throughout the South Pacific (Stiger et al. 2004, Martinez et al. 2007), and is considered an invasive species due to its rapid ex-

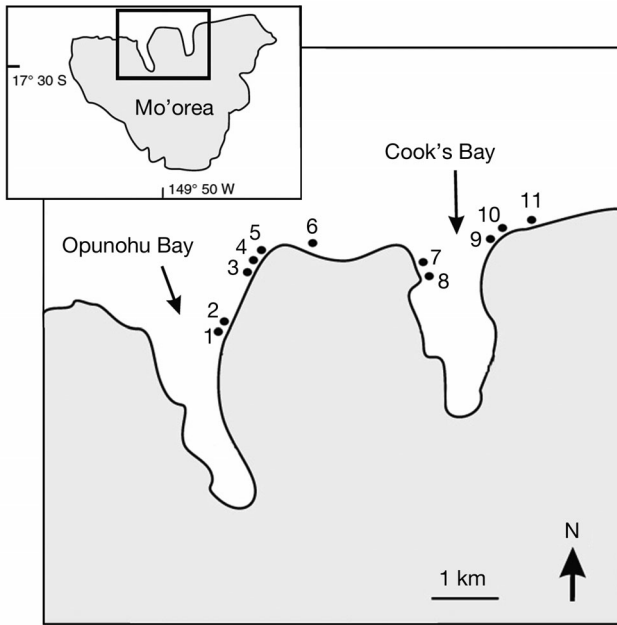


Fig. 1. Study sites on fringing reefs along the north shore of Mo'orea, French Polynesia

pansion (Stiger & Payri 1999, Stewart 2008). Recent evidence has demonstrated that *T. ornata* may out-compete coral, especially under high flow conditions (Brown & Carpenter 2015). While this alga is characterized as palatable on the Great Barrier Reef (Mantyka & Bellwood 2007, Hoey & Bellwood 2010), only a few herbivorous fish species have been observed to consume it, including 2 unicorn fish, *Naso lituratus* and *N. unicornis*, and 1 rabbitfish, *Siganus doliatus* (Robertson & Gaines 1986, Meyer et al. 1994, Mantyka & Bellwood 2007). However, numerous juvenile herbivorous fishes (scarids and acanthurids) on fringing reefs of Mo'orea were observed to graze epiphytes on *T. ornata* blades, leaving grazing scars (S. J. Bittick et al. unpubl. data). In this study, we evaluated the effects of herbivory and nutrients on *T. ornata*'s physical defenses (thallus toughness) to address 3 questions: (1) Does natural herbivory or mechanical damage simulating herbivory induce physical defenses? (2) Does nutrient enrichment induce physical defenses? (3) Is there a trade-off between defense and growth of the alga?

MATERIALS AND METHODS

All research was conducted between January and May 2014 on fringing reefs of Mo'orea (17° 32' S, 149° 50' W), a small volcanic island in the Windward group of the Society Islands in French Polynesia. Mo'orea has been subjected to a series of disturban-

ces, including an outbreak of *Acanthaster planci*, a major coral predator, from 2003 to 2010 that opened benthic substrate to colonization by algae (Kayal et al. 2012).

To evaluate whether herbivory and/or nutrients affected thallus toughness of *Turbinaria ornata* (Payri et al. 2004) and to investigate a possible plastic trade-off between physical defense and growth rates, we conducted (1) a field survey relating herbivorous fish abundance to thallus toughness, (2) a mesocosm experiment evaluating the relationship between nutrients and thallus toughness, and (3) 2 field experiments, one excluding herbivores and the other simulating herbivory. We selected 11 sites along the north shore and within the 2 large northern bays for the field surveys (Fig. 1). Sites 1 and 2 were located within Opunohu Bay, which is inhabited by fewer people, is less influenced by agriculture, and has lower mean annual flow from riverine sources than Cook's Bay (Letourneur et al. 2013 and references therein), where Sites 7 and 8 were located. Additional nutrient sources to Cook's Bay include waste from a fruit juice factory near Sites 7 and 8—although to our knowledge, the effects of this source have not been quantified. Although Opunohu Bay receives effluent from a shrimp farm, the effects on nutrient supplies were detected only a few hundred meters into the bay (Lin & Fong 2008) and likely did not affect our sites. The remaining sites were either at the mouth of the bays or along the more exposed north shore.

We conducted surveys of herbivorous fish and collected algal thalli from all 11 sites to evaluate the relationship between herbivorous fish abundance and *T. ornata* thallus toughness in April and May 2014. Sites were shallow, with *T. ornata*-dominated patch reefs just behind the fringing reef crest at depths of 1 to 2 m. Herbivorous fish were counted by swimmers on snorkel in 12 replicates of a 2 × 50 m transect per site (100 m² at each site) between 09:00 and 11:00 h. Surveys included only parrotfish (Scaridae) and surgeonfish (Acanthuridae), as rabbitfish (Siganidae) and sea chubs (Kyphosidae) were never observed in the transects. Herbivores that directly consume *T. ornata* (unicorn fish and rabbitfish; Robertson & Gaines 1986, Meyer et al. 1994, Mantyka & Bellwood 2007) were never encountered at our sites, though monitoring data show unicorn fish occur in deeper zones of fringing and fore reefs, and at similar depths of back reefs (datasets provided by the Mo'orea Coral Reef Ecosystem LTER, Brooks 2016). In contrast, smaller herbivorous fish (juvenile acanthurids and scarids) that have been observed to tar-

get epiphytes and leave grazing scars (S. J. Bittick et al. unpubl. data) were abundant. Dominant species of acanthurids were *Zebrasoma scopas* (Brandl et al. 2014 found that *Z. scopas* abundance has a strong negative correlation with young *T. ornata* abundance) and *Ctenochaetus striatus*. All scarid species were grouped, as the majority were small juveniles that were difficult to distinguish from each other. Damselfish (mostly Pomacentridae) were present, but were not counted in our surveys.

A total of 20 *T. ornata* thalli, ranging from 5 to 10 cm in height, were collected from 5 algal patches at each site. These patches, located at the same depth (1 to 2 m) and of similar density (10 to 20 thalli patch⁻¹), were haphazardly selected at ~10 m intervals within the same 100 m² area used for the fish surveys. Four thalli were collected from each patch. Care was taken to avoid damselfish territories. Height and wet weight were recorded for each thallus and a penetrometer was used to measure toughness by the mass (in g) required to penetrate a young blade selected haphazardly from the first ring of blades around the apical meristem. The penetrometer consisted of a cup to which weight was added until the attached pin pierced the algal blade (adapted from Duffy & Hay 1991). Thus, our measure of thallus toughness estimated the effort needed to bite into a thallus blade rather than being a measure of tensile strength (force to break a stipe or detach a blade). We measured 3 of the 4 thalli from each of the 5 patches and averaged the weight required to penetrate a thallus blade over a site ($n = 15 \text{ site}^{-1}$). Total herbivorous fish abundance (Scaridae + Acanthuridae) was averaged for each site ($n = 12 \text{ site}^{-1}$) and regressed (using Microsoft Excel) against the average weight needed to penetrate *T. ornata* blades.

We conducted a mesocosm experiment (April to May 2014) at the UC Berkeley Gump South Pacific Research Station in Mo'orea to determine if nutrient addition influenced growth and/or thalli toughness of *T. ornata*. While these experiments did not simulate natural field conditions (nutrient supply, flow, competition) and thus are not predictors of algal growth on the reef, results can be compared among treatments. A total of 50 thalli (selected using the size and plot criteria described above) were collected haphazardly at Sites 7 and 8. Each thallus was wet weighed and subjected to nutrient addition treatments in experimental mesocosms ($n = 10$). Nutrient treatments consisted of water collected from Site 7 immediately prior to the experiment and enriched to make final concentrations of 0+0, 10+1, 20+2, 30+3, and 40+4 μM nitrate (NaNO_3) + μM phosphate (NaH_2PO_4),

respectively; thus, the concentration of nutrients supplied to the mesocosms was varied, but the N:P molar ratio remained 10:1. These are the same concentrations used in Fong & Fong (2014) for similar experiments on Mo'orean algae. Concentrations of N as high as 24 μM have been measured entering Cook's Bay in Mo'orea (datasets provided by the Mo'orea Coral Reef Ecosystem LTER; Alldredge & Carlson 2013). Thus, our highest nutrient treatments exceeded the highest field values measured. Each experimental unit consisted of an individual *T. ornata* thallus placed in a clear plastic 1 l container with 800 ml of the appropriate treatment water. Individual mesocosms were haphazardly arranged in an outdoor, flow-through water bath with water supplied from the reef for temperature control. Nylon window screening covered the water table to reduce light by ~30% (Fong et al. 2006). Experimental units were open to wind-driven circulation but not continuously mixed, and treatment water remained unchanged for the duration of the experiment. After 5 d, *T. ornata* samples were collected and reweighed, and their toughness measured as described above. Five days was demonstrated to be long enough to detect treatment effects on growth and thallus toughness in field experiments (Bittick et al. 2016). Growth was calculated as percent change from initial biomass. The effect of nutrient treatments on weight required to penetrate blades and growth were analyzed with 1-factor ANOVA and Tukey's HSD post hoc analysis when the ANOVA was significant.

To evaluate whether herbivory affected growth and physical defenses of *T. ornata*, in January and February 2014 we caged plots containing algal thalli and simulated herbivory via mechanical damage of blades. Herbivory was simulated to evaluate whether algal defense responses are triggered by damage to their blades caused by epiphyte grazers, or if actual herbivory on the algal thalli itself is required to elicit a response (as in Rohde et al. 2004). Experimental units consisted of 10 caged plots (20 × 20 × 20 cm, with a 5 cm skirt and 1 cm mesh openings) with natural densities of *T. ornata* of ~25 thalli plot⁻¹. To assess initial toughness, 5 thalli were collected from randomly chosen plots and their toughness was measured (as above). Prior to initiation of experimental treatments, heights of 2 randomly selected and marked thalli per plot were recorded. One thallus was subjected to simulated herbivory while the other was not damaged. Herbivory was simulated every other day using wire cutters to damage the blades of each algal thallus assigned to the damaged treatment. Blade edges were cut 12 times, and blade sur-

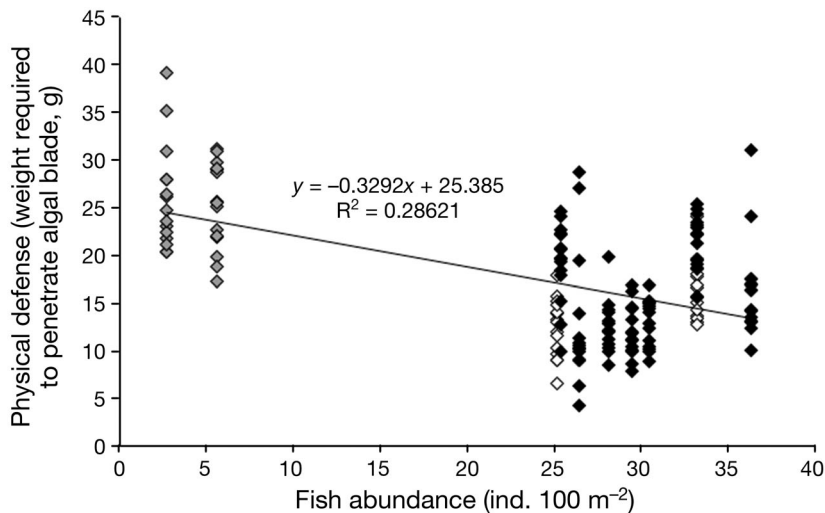


Fig. 2. Field survey results relating total abundance of herbivorous fish (site means, $n = 12$ site⁻¹) to thallus toughness measured as weight required to penetrate *Turbinaria ornata* blades ($n = 15$ site⁻¹). Simple linear regression, $R^2 = 0.28621$, $p < 0.0001$. Grey symbols: sites from Cook's Bay; white symbols: Opunohu Bay; black symbols: remaining sites

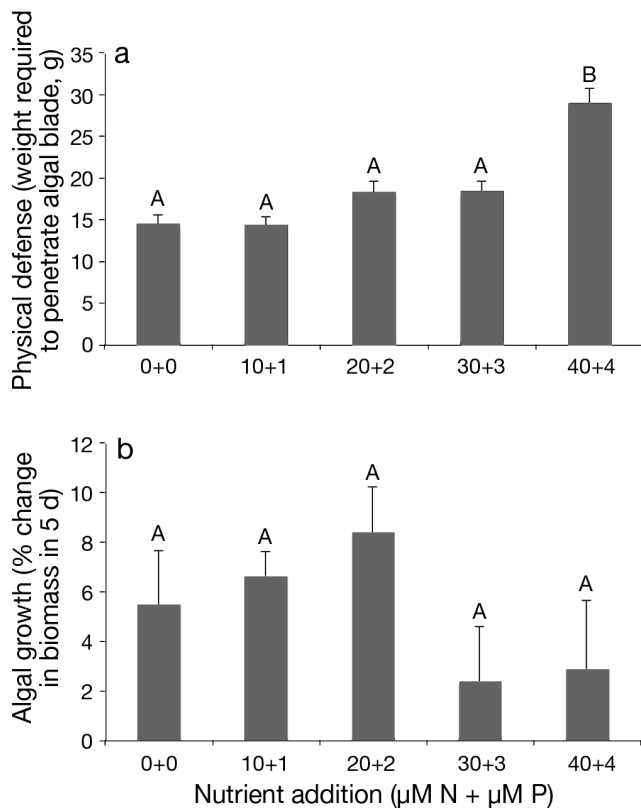


Fig. 3. Results of mesocosm experiment results testing whether nutrients affected (a) toughness and (b) growth of *Turbinaria ornata*. Nutrient treatments were as described in the 'Materials and methods'. Bars are means \pm SE ($n = 10$). Different letters above bars denote statistical differences among means

faces scratched 3 times to match grazing scars found on *T. ornata*. After 10 d, we measured the height of both damaged and undamaged thalli in each plot and quantified structural defenses using the penetrometer (as above). Differences in mean growth (% change in height) and physical toughness (weight to penetrate blade) between the treatments were detected with paired *t*-tests.

We conducted a transplant experiment in April and May 2014 to determine whether natural rates of herbivory at Site 4, which had higher abundances of herbivores, influenced growth and structural defenses of *T. ornata* from Site 7, which had lower herbivore abundances. A total of 25 thalli (5 to 10 cm in height) were randomly selected from Site 7, and heights and physical toughness were measured for 5 randomly

selected thalli (as described above). The remaining 20 thalli were transported to the higher herbivory site (Site 4), where half of the thalli were caged while the other half was subjected to natural herbivory. After 10 d, changes in height and physical toughness were measured and differences between treatments analyzed with a Wilcoxon signed-rank test, as assumptions for parametric analyses were not met.

RESULTS

There was a significant but weak negative relationship between average herbivorous fish abundance and the weight necessary to penetrate *Turbinaria ornata* blades ($R^2 = 0.2821$, $p < 0.0001$), suggesting that the alga's toughness decreased in the presence of increased herbivory (Fig. 2). However, this relationship was strongly driven by the 2 eutrophic Cook's Bay sites (Sites 7 \times 8, symbols in grey) that also had the lowest numbers of herbivores.

Nutrient addition increased the toughness of *T. ornata* in the mesocosms (Fig. 3a). *T. ornata* blades subjected to 40 μM N + 4 μM P additions were significantly tougher than blades grown in lower nutrient concentrations (ANOVA, $p < 0.0001$, Tukey's HSD, $p < 0.05$ for 40:4 μM N:P compared to all other treatments). Although there was a trend towards decreased growth with increased physical defenses, suggesting an energetic trade-off, these differences were not significant (1-factor ANOVA, $p = 0.1874$; Fig. 3b), likely related to high variability in algal growth.

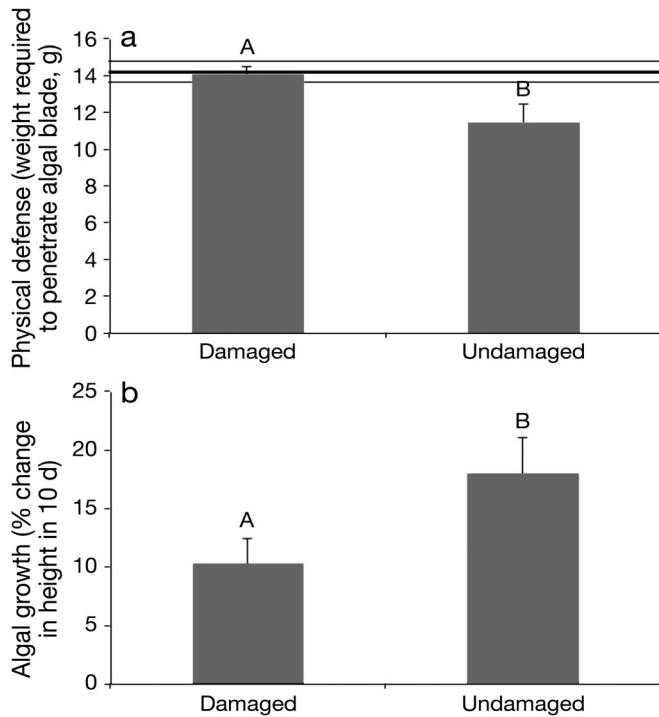


Fig. 4. Results of field experiment testing whether simulated herbivory affected (a) toughness and (b) growth in *Turbinaria ornata*. All thalli were protected from natural herbivory by cages. Herbivory was simulated by damaging assigned blades every other day. Bars are means \pm SE ($n = 10$); bars that share letters (A/B) are not different from each other. Horizontal lines in (a) show initial toughness values (black line) \pm SE (grey lines)

Simulated herbivory in the form of physical damage to blades of *T. ornata* maintained initial levels of structural anti-herbivore defenses at a cost to growth (Fig. 4). Significantly more weight was needed to penetrate *T. ornata* blades subjected to experimentally simulated herbivory (caged and damaged) compared to undamaged and caged blades (paired *t*-test, $p = 0.034$). Blades that were undamaged decreased their thallus toughness by $\sim 20\%$ from initials and from damaged treatments. In contrast, thallus growth was almost double in undamaged algae with reduced toughness, compared to algae with simulated herbivory (damaged) and greater toughness ($p = 0.005$; Fig. 4b), demonstrating a trade-off between blade toughness and growth.

Natural levels of herbivory at Site 4 also maintained blade toughness of transplanted algae (Fig. 5), while exclusion of herbivorous fish via caging significantly reduced the weight necessary to penetrate blades (Wilcoxon test, $p = 0.045$; Fig. 5a). In contrast to the simulated herbivory experiment, there was no significant difference in algal growth between treatments exposed to and protected from natural her-

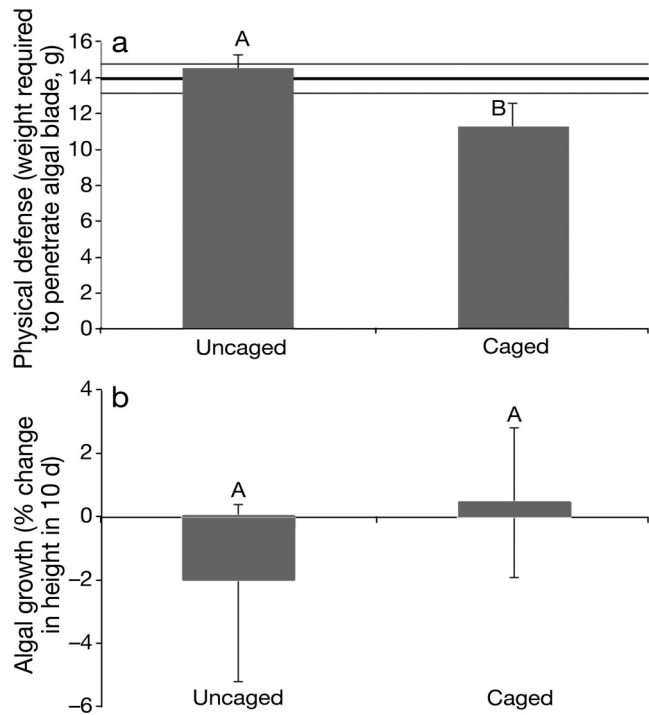


Fig. 5. Results of field experiment testing whether different levels of natural herbivory affected (a) toughness and (b) growth in *Turbinaria ornata*. Thalli were either protected from herbivory (caged) or open to herbivores (uncaged). Bars are means \pm SE ($n = 10$); bars that share letters (A/B) are not different from each other. Horizontal lines in (a) show initial toughness values (black line) \pm SE (grey lines)

bivory (Wilcoxon test, $p = 0.523$; Fig. 5b). Thus, a trade-off between defense and growth was not demonstrated in this experiment.

DISCUSSION

Our results demonstrated that reduction in herbivory allowed *Turbinaria ornata* to reduce its physical anti-herbivore defenses, which we measured as toughness of the thallus surface. Likewise, a meta-analysis found strong relationships between tissue damage—by both simulated and natural herbivory—and induced protection from further herbivory for many other marine brown algae, though whether resistance was chemical or physical was not investigated (Toth & Pavia 2007). It is likely that higher nutrient supplies, such as those found in Cook's Bay, resulted in *T. ornata* thalli that were initially tough, as toughness did not increase when thalli were transplanted to a site where they were exposed to higher herbivory. Rather, toughness decreased in all algal thalli protected from herbivory,

regardless of whether they were transplanted or remained at the site of origin. In contrast, the temperate brown alga *Ascophyllum nodosum* increased tensile strength (force to break) rather than toughness (force to pierce) in response to relatively low levels of experimentally simulated herbivore damage (Lowell et al. 1991), and this increased strength reduced subsequent loss to wave action. However, herbivore damage resulted in a reduction in chemical defenses, making the alga more edible and suggesting a trade-off between the costs of different types of herbivore defenses (Lowell et al. 1991). Likewise, simulated and natural herbivory caused a rapid resistance to herbivory that was unrelated to concentrations of chemicals usually associated with anti-herbivore defenses in a temperate species of *Fucus* (Hemmi et al. 2004), suggesting that some other chemical defense or a physical defense was activated. Taken together, these studies and our own findings suggest that several brown algal species are able to adapt physical anti-herbivore defenses to changes in herbivory pressure and thereby facilitate their proliferation on reefs subjected to overfishing of herbivores.

We also provided some evidence for a trade-off between physical defense and growth. Several studies have shown that terrestrial plants exhibit energetic trade-offs between growth and defenses, when defenses were induced by herbivory (e.g. Strauss et al. 2002, Dicke & Hilker 2003). However, evidence for trade-offs between physical defense and growth is rare for brown algae, possibly because existing studies have focused on chemical defenses. While chemical defenses may have trade-offs with growth, the chemicals often have both primary (cell walls) and secondary (anti-herbivore) functional roles, so may not represent a simple 'trade-off' (Arnold & Targett 2003). In contrast, our results suggest that under some conditions, there may be a direct and simple trade-off between physical defenses and growth for this brown alga. However, in our transplant experiment we found no evidence of a trade-off; rather, growth was negligible regardless of herbivory treatment. Energy allocation theory offers one possible explanation for this difference as it predicts optimal allocation strategies may change with age, stage, or environmental context (Perrin & Sibly 1993). Furthermore, other studies have found the phenotype of *T. ornata* can be extremely variable and plastic, depending on physical conditions such as wave action, light, and possibly nutrients (Stiger & Payri 1999, Stewart 2006, 2008). Thus, it is likely that in our transplant experiment the algae were adapting not only to differences in herbivory but to a suite of new environmental conditions.

Thus, the energy saved on defenses may have been allocated to processes other than growth in this experiment. Overall, the ability of *T. ornata* to reduce investment in defenses and increase growth, at least under some environmental conditions, may enhance this alga's success in human-impacted environments.

Our findings from the mesocosm experiment imply that anthropogenically enhanced nutrient supplies may also play a role in the recent expansion of *T. ornata* by inducing physical anti-herbivore defenses in the form of blade toughness. In contrast, a different experimental study showed that nutrient addition increased the palatability of a congener in the Caribbean (Chan et al. 2012). While many studies relate higher nutrient levels from anthropogenic sources (such as urban development and industrial runoff) to increased algal growth (e.g. Lapointe et al. 2005, Killberg-Thoreson et al. 2013) and invasion success (Stimson et al. 2001, Inderjit et al. 2006), our study found evidence that nutrients may also induce physical defenses with a weak cost to growth. While it is unknown if nutrient supplies have been increasing over the South Pacific, there is some evidence nutrient supplies have increased on Mo'orea's north shore due to increased runoff from watersheds (Letourneur et al. 2013 and references therein) and a shrimp farm in Opunohu Bay (Lin & Fong 2008). This suggests that the ability of *T. ornata* to increase physical defenses against consumption in response to high nutrient concentrations may contribute to its success in human-impacted environments.

In summary, our study suggests that the responses of *T. ornata* to increased nutrients and decreased herbivory may have facilitated its expansion across the South Pacific. While reduction of herbivory via overfishing allows this alga to trade energy normally spent on defense to increase growth, nutrient enrichment provides *T. ornata* with additional resources to increase defenses. As anthropogenic impacts become even more prominent in coral reef systems, it is critical that we understand the processes that may facilitate the expansion and dominance of coral reef algae, especially those that contain inducible anti-herbivore defenses.

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