Ontogenetic variation in blade toughness may contribute to the dominance and spread of *Turbinaria ornata* across the South Pacific

Austin M. Betancourt*[1], Ileana F. Fenwick*[2], Hunter B. Howard*[3δ], Alexys E. Long*[4], Peggy Fong[5], Paul H. Barber[5], Caitlin R Fong[6§]

*these authors contributed equally and are co-first authors. First author order was determined alphabetically.

[§] corresponding author fong@nceas.ucsb.edu

[1] San José State University

[2] Hampton University

[3] University of Maryland Eastern Shore

[4] Norfolk State University

[5] University of California, Los Angeles

[6] National Center of Ecological Analysis and Synthesis

[δ] current affiliation, University of Puerto Rico Mayagüez

Running Head: Ontogenetic variation in blade toughness

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Abstract

Coral reefs are shifting from coral to algal-dominated ecosystems worldwide. Recently, *Turbinaria ornata*, a marine alga native to coral reefs of the South Pacific, has spread in both range and habitat usage. Given dense stands of *T. ornata* can function as an alternative stable state on coral reefs, it is imperative to understand the factors that underlie its success. We tested the hypothesis that *T. ornata* demonstrates ontogenetic variation in allocation to anti-herbivore defense, specifically that blade toughness varied nonlinearly with thallus size. We quantified the relationship between *T. ornata* blade toughness and thallus size for individual thalli within algal stands (N=345) on 7 fringing reefs along the north shore of Moorea, French Polynesia. We found that blade toughness was greatest at intermediate sizes that typically form canopies, with overall reduced toughness in both smaller individuals that refuge within the understory and older reproductive individuals that ultimately detach and form floating rafts. We posit this variation in blade toughness reduces herbivory on the thalli that are most exposed to herbivores and may facilitate reproduction in dispersing stages, both of which may aid the proliferation of *T. ornata*.

Introduction

Coral reefs worldwide are shifting from coral to macroalgal dominance, with limited recovery (Bellwood et al 2004), necessitating research on the mechanisms that underpin success of the emergent macroalgal-dominated communities. While algae are essential to provisioning herbivorous fish communities (Borowitzka 1981, Vroom 2011), a phase shift to macroalgal dominance has detrimental community and ecosystems-level impacts and threatens the capacity of reefs to persist (Hughes et al. 2003). *Turbinaria ornata* is a brown macroalga that readily establishes after disturbances to reefs of the South Pacific (Stiger & Payri 1999) and functions as a secondary foundational species (Bittick et al. 2016). Like other species of macroalgae, dominance by *T. ornata* may be detrimental to the settlement and subsequent survival of coral recruits (Bulleri et al 2018, Schmitt et al. 2022). As *T. ornata* can be an alternative stable state on reefs in the South Pacific (Schmitt et al. 2019), it is critical to understand what traits facilitate the persistence of this species on tropical reefs.

Turbinaria ornata has anti-herbivory defenses, grows in dense stands, and spends a portion of its life cycle rafting, traits that may facilitate its success on tropical reefs by enhancing both resistance to top down control and dispersal (Stiger et al. 2004, Stewart 2006 a,b, Bittick et al. 2016, Davis 2018, Bittick et al. 2019, Sirison & Burnett 2020). In particular, physical toughness of blades (Bittick et al. 2016) and chemical anti-herbivore defenses (Stiger et al. 2004) of *T. ornata* likely facilitate persistence of this species on tropical reefs where herbivory pressure is typically strong (Bellwood et al. 2004). Dense stands of *T. ornata* form canopies that provide shelter for understory algae, including smaller conspecifics that are more vulnerable to herbivores (Davis 2018). In the largest individuals, the stipe weakens, the thallus becomes more buoyant, and individuals detach and float (Stewart 2006a). Winds and currents form and transport extensive rafts of mature, reproductive thalli as they release gametes (Sirison & Burnett 2019, Stewart 2006 a,b), likely aiding dispersal. This shift from strong physical defenses deterring herbivory to weak structural support that allows rafting and dispersal suggests *T. ornata* may allocate differential effort toward blade toughness across its lifespan.

Here we explore the relationship between blade toughness (a relative metric used by Bittick et al. 2016 and Bergman et al. 2016 defined as the weight (g) required to pierce a blade with a needle) and size of *T. ornata* in Moorea, French Polynesia. We hypothesize a nonlinear relationship between size and toughness such that the blades on the smallest thalli are weakest as they experience reduced herbivory in the understory of dense adult stands (Davis 2018). As thalli grow larger and emerge from the understory, blades become tougher and more resistant to herbivory, a known inducible response (Bergman et al. 2016). Finally, the blades of the largest individuals that are about to enter the rafting stage will also be weaker, representing a shift in strategy away from defense.

Methods

To quantify the relationship between blade toughness and thallus size, we collected *Turbinaria ornata* and measured height and toughness of individual blades. Thalli were haphazardly collected from seven fringing reef sites along the north shore of Moorea, French Polynesia, with a maximum depth of 5m. Overall, we aimed to collect at least 10 thalli on the smaller end of the size range and 10 on the larger end—the goal was to sample a range of thalli sizes, not to reflect the size structure of the populations. Twenty to 55 individuals were collected from each site, depending on availability (N=345). Thalli were returned the lab, placed into flow-through seawater tables, and processed within the same day; all work was done in June and July, 2018.

Following collection, we measured the height of each thallus (base of the holdfast to the tip of the thallus) to the nearest millimeter. We then used a penetrometer to measure blade toughness following Bergman et al. (2016). To avoid potential confounding effects of blade age on toughness, for all thalli we tested individual blades from the second whirl from the tip of each thallus. To limit potential variation in toughness across individual blades, we standardized placement of the needle of the penetrometer (Supplemental Figure 1) in the middle of the blade surface. To measure toughness, we then added weight incrementally (< 1 gram in steps) to the penetrometer until the needle visibly pierced the blade surface; weight needed to penetrate each blade was recorded (*sensu* Bittick et al. 2016, Bergman et al. 2016). A single observer conducted all thallus toughness measurements to avoid inter-observer variability.

To test our specific hypotheses that blade toughness varied with thallus size, we used a model comparison approach, comparing a linear, quadratic, negative exponential, and spline fits between size and toughness. To explore whether this relationship was affected by site differences, we tested whether blade toughness varied across sites with a Kruskal-Wallis test because data did not meet assumptions of parametric statistics even with transformation.

Results and Discussion

We found no significant differences in height across sites (Kruskal-Wallis, $p = 0.87$), even though there was considerable variability in the size of thalli collected in each site. This result confirms that we met our aim to collect thalli of all sizes at each site. It also confirmed that the differences in blade toughness were not driven by differences that may have been associated with site.

Table 1. Results of model selection approach.

Model	AIC	R2	p
Linear	2961.958	0.1925	<0.0001
Quadratic	2915.043	0.2992	<0.0001
Negative exponential	2946.223	0.3110	<0.0001
Spline regression	**2908.402**	**0.3165**	**<0.0001**

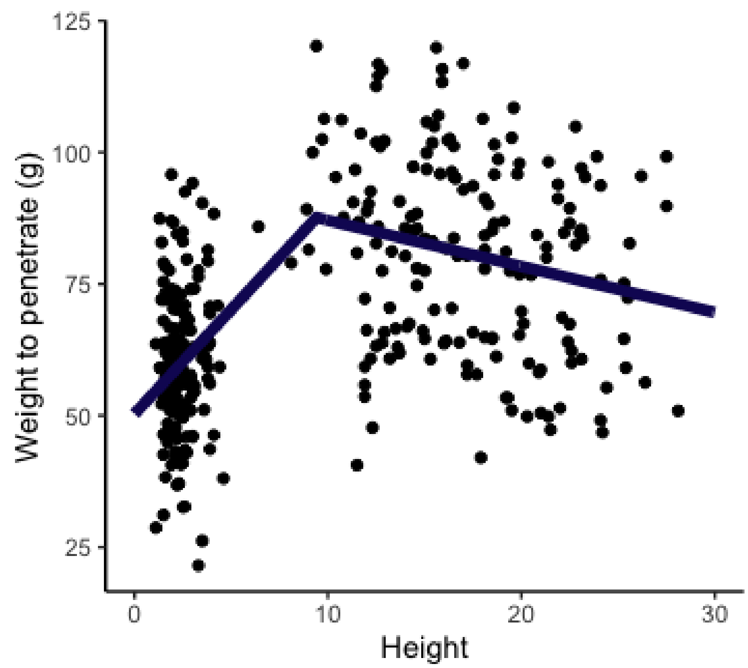

Figure 1. Relationship between thallus height (cm) and weight required to penetrate the blade (g). Blue line represents the spline fit.

We found a spline regression was the best fitting model of the relationship between size of *T. ornata* and blade toughness, with a knot at ~9.6 cm tall (Figure 1). Overall, toughness increased with size for thalli <9.6 cm but decreased with height when thalli were >9.6 cm tall, and this fit explained ~32% of the variation in toughness (Table 1). Overall, small thalli were weakest; for example, our fitted curve predicts 4 cm thalli require approximately 66 g of weight to penetrate. In contrast, our fitted curve predicts thalli around 16 cm tall were approximately 33% tougher, requiring approximately 82 g of weight to penetrate. Finally, the largest thalli were weaker than the intermediate height thalli, such that our fitted curve predicts a 28 cm tall thallus required approximately 71 g of weight to penetrate. Given that it is well-established that toughness of *T. ornata* blades is extremely variable, responding to nutrient supply (Bittick et al. 2016) and history of herbivory (Bergman et al. 2016), explaining 30% of the variance in these populations across sites is notable.

Variation in toughness across size may contribute to the successful expansion in geographic range and habitat usage of *T. ornata* across the South Pacific. While there was substantial variation in the data, overall we found that blades on the smallest individuals were the least tough on average, aligning with evidence that the smallest individuals are most vulnerable to herbivory (Davis 2018). However, small thalli of *T. ornata* are protected from herbivory when in the understory of dense stands of adults (Davis 2018); this same associational refuge was found for a diversity of understory algae (Bittick et al. 2010). Previous research demonstrates blade toughness is inducible in response to herbivory and nutrient context. This trait may allow *T. ornata* thalli to escape herbivory (Bittick et al. 2016, Bergman et al. 2016) once they outgrow the understory refuge. Bittick et al. (2019) found that average thalli size in dense stands was between 15-20cm, within the height range we found to be toughest. Thus, we posit *T. ornata* allocate energy primarily to growth when small and switch to invest more in toughness after ~10 cm, when thalli begin to emerge from the canopy and are exposed to increased herbivory pressure.

However, as thalli grow even larger, results show that blades weaken. We posit there is a possible shift in investment away from herbivory defense and to reproduction as thalli become very large, which would increase the per capita output of germlings of rafting *T. ornata* once thalli have detached. Thus, the weaker blades of the largest thalli demonstrate an ontogenetic shift in strategy away from investment in defense and possibly to reproduction prior to the rafting component of the lifecycle (Stewart 2006a). Further, we suggest that weakened blades are likely concomitant with a weakened stipe. This weakening of the stipe may facilitate detachment and thus allow for transitions to the floating component of the lifecycle. During rafting, herbivory pressure is likely reduced making blade toughness less critical.

Overall, *T. ornata* exhibits substantial variation in thallus toughness in response to environmental drivers, which has been implicated as an important contributor to their recent expansion and success (Bittick et al. 2016, Bergman et al. 2016, Sirison and Burnett 2019). Here, we empirically test one aspect of this process, demonstrating that the physical defenses of *T. ornata* increase ontogenetically as young thalli outgrow the herbivory refuge provided by adult stands and that defenses are then decreased as thalli grow larger still, likely as energy is shifted to reproduction during the rafting portion of the lifecycle. This capacity to shift allocation to rapidly ramp up physical defense then downscale later in life may help explain the dispersal, proliferation and persistence of *T. ornata* on tropical reefs.

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CRediT

AMB Conceptualization; Data curation; Investigation; Validation; Visualization; Roles/Writing – original draft. **IF** Conceptualization; Data curation; Investigation; Validation; Visualization; Roles/Writing – original draft. **HBH** Conceptualization; Data curation; Investigation; Validation; Visualization; Writing – original draft. **AEL** Conceptualization; Data curation; Investigation; Validation; Visualization; Writing – original draft. **PF** Conceptualization; Funding acquisition; Investigation; Methodology; Supervision; Validation Writing – Review & Editing. **PHB** Conceptualization; Funding acquisition; Supervision; Validation Writing – Review & Editing**.** **CRF** Conceptualization; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing – Review & Editing.

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