

NOTE

*TURBINARIA ORNATA* (PHAEOPHYCEAE) VARIES SIZE AND STRENGTH TO MAINTAIN ENVIRONMENTAL SAFETY FACTOR ACROSS FLOW REGIMES<sup>1</sup>

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Water motion in coastal areas can produce hydrodynamic forces that damage or dislodge benthic macroalgae if the tissues of macroalgae are not sufficiently strong. Some macroalgae vary their morphology and strength in response to ambient water motion, but little is known of how morphology and strength of macroalgae change relative to one another across flow regimes. Here, we use *Turbinaria ornata*, an ecologically important macroalga, to study how both the morphology and strength of macroalgae vary with ambient water motion. Typically, *T. ornata* exhibits weakening of its stipe when sexually mature, leading to breakage from the substratum and dispersal, which is beneficial for reproduction. Across three flow regimes, adult *T. ornata* increased its size but decreased its strength as water motion decreased. However, the strength of *T. ornata* relative to the maximum hydrodynamic forces it is expected to encounter (the environmental safety factor) did not differ between flow regimes. Our results showed that *T. ornata* can conform to its local flow habitat by varying both size and strength, similar to other macroalgae. Varying multiple traits between flow regimes suggested that *T. ornata* is capable of surviving a wide range of flow conditions, which may permit more control over the timing of its weakening, breakage from the substratum, and dispersal, even with future increases in flow velocities (e.g., large waves from storms) that are expected to occur frequently with climate change.

**Key index words:** biomechanics; environmental safety factor; hydrodynamic force; macroalgae; morphology

**Abbreviations:**  $A_{blade}$ , projected area of blade;  $A_{projected}$ , projected area; BA, back reef A site; BB, back reef B site;  $C_{ds}$ , drag coefficient; ESF, environmental safety factor;  $F_{break}$ , breaking force;  $F_{max\ drag}$ , maximum drag force; FR, fringing reef site;  $N_{blades}$ , number of blades on thallus exposed to flow;  $\rho$ ,

water density;  $u_{max}$ , maximum water velocity;  $V$ , velocity exponent

Many macroalgae have mechanical designs that allow them to reduce and/or withstand the hydrodynamic forces in their habitat without being damaged or dislodged from the substratum (Martone et al. 2012; Burnett and Koehl 2019). Mechanical designs can encompass morphological features (e.g., size, shape, strength), while hydrodynamic forces are dependent on the size and shape of the macroalgae and the water's velocity (Gaylord et al. 2008). Whether macroalgae are damaged or dislodged depends on the magnitude of the hydrodynamic forces they experience relative to the strength of their tissues or attachment to the substratum (Johnson and Koehl 1994).

Variation in mechanical designs of macroalgae and the local water velocity can be linked to the life histories of macroalgae. We can quantify how well-suited a macroalga is for its flow habitat at a specific point in its lifetime by calculating its environmental safety factor (ESF): the force required to break the macroalga at a particular life stage divided by the maximum hydrodynamic forces the macroalga is likely to encounter at that same life stage (Johnson and Koehl 1994). For instance, the macroalga *Nereocystis luetkeana* maintains the same ESF in different flow regimes by varying its size and strength (Johnson and Koehl 1994). Macroalgae with low ESFs (e.g., near 1) are likely to suffer tissue breakage, but this can be advantageous if broken; fragmented tissues are reproductive and carried by ocean currents to establish the macroalgae in new habitats (Ceccherelli and Cinelli 1999). Overall, mechanical designs and ESFs of macroalgae can vary in many ways (e.g., size, strength), and these traits can be strongly tied to life-history traits such as reproduction and survival.

In contrast to macroalgae with mechanical designs that minimize hydrodynamic forces and the risk of damage or dislodgement, the macroalga *Turbinaria ornata* synchronizes its reproduction with

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decreases in its mechanical strength and ESF, enabling adult thalli (including reproductive tissues) to break from the substratum and disperse on ocean currents to new habitats (Stewart 2006b). This “programmed” weakening and breakage from the substratum in *T. ornata* usually occurs in the tissues of the stipe rather than separation of the holdfast from the substratum (Stewart 2006b). *Turbinaria ornata* is an annual brown alga that is common on reefs across subtropical and tropical regions of the Indian and Pacific Ocean (Payri and N’Yeurt 1997). An individual *T. ornata* is made up of a discoid holdfast and an upright stipe extending from the holdfast with rows of turbinate blades forming a thallus that is approximately cylindrical (Fig. 1). Blades on adult thalli can contain airbladders that provide buoyancy. In addition to reducing its mechanical strength as it ages, *T. ornata* also varies the way in which it remains upright in the water column based on local water motion; individuals in wave-exposed areas grow flexible, buoyant thalli, whereas individuals in wave-protected areas grow stiff, less-buoyant thalli (Stewart 2004, 2006a). We expand on previous studies of *T. ornata* by examining how morphology, mechanical strength, and ESF of *T. ornata* from one ontogenetic stage change between three flow habitats. This study uses reproductively mature adult *T. ornata*, classified as thalli containing reproductive receptacles with less than 50% epiphytic coverage, following guidelines from Stewart (2006b), from different flow habitats to test the following hypotheses: (1) flow conditions are different between flow habitats, (2) mechanical strength and morphology of *T. ornata* are different between flow habitats, and (3) the ESF of *T. ornata* is different between flow habitats.

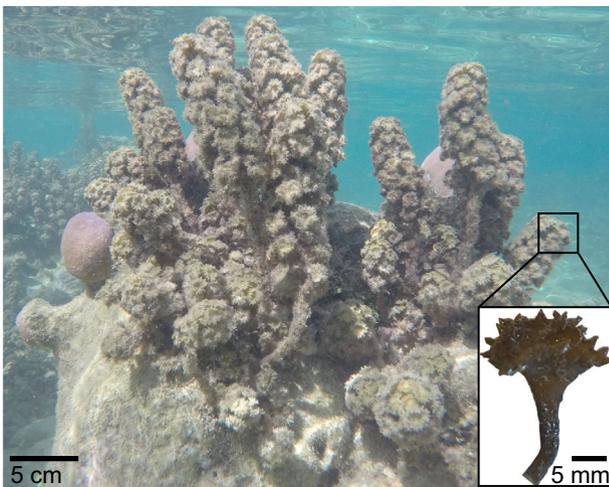


FIG. 1. Morphology of *Turbinaria ornata*. Thalli grow on coral heads in shallow waters and have numerous turbinate blades (inset) that can contain buoyant air bladders.

This study was conducted in Paopao Bay on Mo’orea, French Polynesia, where *Turbinaria ornata* is abundant and ecologically important (Payri and N’Yeurt 1997). Paopao Bay encompasses a lagoon-pass system with a reef crest dividing the bay and ocean. The study was confined to two parts of the reef system: the fringing reef (connected to the shoreline) and the back reef of the reef crest. The fringing reef and the back reef were separated by a deep lagoon (Monismith et al. 2013). In this reef system, the maximum water velocities (i.e., the flow exerting the largest hydrodynamic forces on *T. ornata*) can reach approximately  $4.0 \text{ m} \cdot \text{s}^{-1}$  (Monismith et al. 2013). Each reef was interspersed with coral heads that were lined with *T. ornata*.

We studied *Turbinaria ornata* in three flow habitats that spanned the fringing and back reefs of Paopao Bay in depths  $\leq 3 \text{ m}$ . The sites were the Fringing Reef (“FR”;  $17^{\circ}29'02.3'' \text{ S}$ ,  $149^{\circ}49'02.5'' \text{ W}$ ) and Back Reefs A and B ( $17^{\circ}28'53.3'' \text{ S}$ ,  $149^{\circ}49'06.0'' \text{ W}$ ). Back Reef A (BA) extended from the southwest, shoreward corner of the back reef and continued along the reef’s edge for 30 m northwest, away from shore, and Back Reef B (BB) continued along the reef’s edge an additional 30 m northwest. To examine whether the sites had similar flow conditions, we compared the dissolution rates of plaster of Paris modules (“clod cards”) deployed at each site. Clod cards were constructed using blocks of plaster of Paris (DAP Products Inc., Baltimore, MD, USA; mass ranged from 21 to 23  $\text{g} \cdot \text{card}^{-1}$ ; Thompson and Glenn 1994). Each clod card was attached with cyanoacrylate glue to a plastic tray. Trays were anchored at each deployment site with 2 kg masses. At each site, six clod cards were deployed for 24 h on coral heads (depths  $\leq 1 \text{ m}$ ) adjacent to patches of *T. ornata*. Before deployment, dry weights of clod cards were measured to the nearest 0.001 g (JC1003PL, Joan Lab Equipment, Zhejiang, China). Dry weights were measured after collection and drying for 24 h at  $23^{\circ}\text{C}$ . Rates of change in clod card mass were used to calculate dissolution rates, and thereby quantify the relative differences in flow regimes between the study sites (Thompson and Glenn 1994).

Between October 6 and 20, 2018, we collected *Turbinaria ornata* from each site to test whether morphology, mechanical strength, and ESF of the macroalga differed between sites. At each site, we sampled whole thalli along a 30 m transect with a  $0.25 \text{ m}^2$  quadrat placed at 10 m intervals (i.e., 3 quadrats  $\cdot \text{transect}^{-1}$ ). All *T. ornata* were collected from each quadrat by manually dislodging the thalli, including holdfasts, from the substratum. Samples were held in seawater (salinity = 33.8, temperature =  $25^{\circ}\text{C}$ ). From the collections made in each quadrat, we used a random number generator to select 10 adult thalli in approximately the same ontogenetic stage to measure morphology and mechanical

strength (Stewart 2006b). All measurements were taken within 10 h of collection.

We compared the morphological feature of projected area ( $A_{\text{projected}}$ ) of *Turbinaria ornata* between sites because hydrodynamic forces acting on macroalgae are proportional to their  $A_{\text{projected}}$  (Vogel 1994). We considered the contribution that each turbinate blade on the side of the thallus exposed to flow made to  $A_{\text{projected}}$  with:

$$A_{\text{projected}} = A_{\text{blade}} N_{\text{blade}} \quad (1)$$

where  $A_{\text{blade}}$  is the mean projected area of a blade and  $N_{\text{blade}}$  is the number of blades on the side of the thallus exposed to moving water (i.e., half the total number of blades on the entire thallus). We approximated each blade's shape as a cone with a maximum radius of  $0.8 \times 10^{-3}$  m, a typical blade size reported by Stewart (2006a), and assumed the blades were fully reconfigured in moving water (e.g., Martone et al. 2012). Thus,  $A_{\text{blade}}$  was calculated as a circle with a radius of  $0.8 \times 10^{-3}$  m (i.e.,  $A_{\text{blade}} = \pi [0.8 \times 10^{-3} \text{ m}]^2$ ).

The mechanical strength (i.e., force to break,  $F_{\text{break}}$ ) influences whether *Turbinaria ornata* can withstand the hydrodynamic forces in its habitat, so we compared  $F_{\text{break}}$  between sites. Because *T. ornata* typically breaks from the substratum by breaking at the stipe (Stewart 2006b), we measured the stipe's  $F_{\text{break}}$  using a custom-built rig that applied loads along the stipe's long axis until the tissue failed (Simonson et al. 2015). Each sample was suspended by the base of its stipe using a metal clamp, and a plastic container was clamped to the free-hanging end of the seaweed. Masses (0.05 kg) were added at 15 s intervals to the container until the stipe broke. The total mass (container + masses) that broke the stipe was recorded and multiplied by  $9.81 \text{ m} \cdot \text{s}^{-2}$  to find  $F_{\text{break}}$  (Stewart 2006b). We discarded measurements when the stipe broke at the clamps.

Environmental safety factor (ESF) describes how well-suited an organism is to hydrodynamic forces in its habitat, so we compared ESF of *Turbinaria ornata* between sites. We calculated ESF as:

$$\text{ESF} = \frac{F_{\text{break}}}{F_{\text{max,drag}}} \quad (2)$$

where  $F_{\text{max,drag}}$  is the maximum drag force that the macroalga will experience at a life stage. Drag is the hydrodynamic force that acts parallel to the direction of water motion and is the major hydrodynamic force acting on benthic organisms (Gaylord et al. 2008). Drag was calculated by:

$$F_{\text{drag}} = 0.5 C_d \rho A_{\text{projected}} u_{\text{max}}^V \quad (3)$$

where  $C_d$  is the drag coefficient describing the macroalga's shape. Because  $C_d$  describes shape,  $C_d$

can be similar between macroalgae with similar shapes. Macroalgae with shapes similar to *T. ornata* have a  $C_d$  near 0.15 at flow velocities of  $3\text{--}4 \text{ m} \cdot \text{s}^{-1}$  (see below; Carrington 1990).  $\rho$  is the density of the seawater ( $1025 \text{ kg} \cdot \text{m}^{-3}$  at  $20^\circ\text{C}$ ; Vogel 1994), and  $u_{\text{max}}$  is the maximum water velocity at that life stage ( $4 \text{ m} \cdot \text{s}^{-1}$  in wavy conditions in Paopao Bay; Monismith et al. 2013). We used this maximum water velocity for Paopao Bay and the ratios of median clod card dissolution rates between sites (Fig. 2A; based off Back Reef B, which had the most rapid dissolution) to estimate  $u_{\text{max}}$  at each site: Fringing Reef ( $3.0 \text{ m} \cdot \text{s}^{-1}$ ), Back Reef A ( $3.6 \text{ m} \cdot \text{s}^{-1}$ ), and Back Reef B ( $4.0 \text{ m} \cdot \text{s}^{-1}$ ). For rigid objects in flow, the velocity exponent  $V$  equals 2, but for flexible objects that can reconfigure into increasingly streamlined shapes as the water velocity increases, the velocity exponent is  $< 2$ . We set  $V$  to 1.5 based on measurements of slender, flexible macroalgae that can reconfigure in flow similar to how *T. ornata* reconfigures in flow (Stewart 2004, Gaylord et al. 2008).

All statistical tests were done in R Statistical Software (R Core Team 2019). We compared clod card dissolution rates and the  $A_{\text{projected}}$ ,  $F_{\text{break}}$ , and ESF of *Turbinaria ornata* between sites using Kruskal–Wallis tests with *post hoc* Dunn's tests and Bonferroni  $P$  value adjustments for multiple comparisons ("FSA" package; Ogle et al. 2018).

Clod cards' dissolution rates differed between sites, indicating different flow regimes between the sites. Clod cards at Back Reef B showed the most rapid dissolution (median =  $15.0 \text{ g} \cdot \text{d}^{-1}$ ) and were significantly faster than dissolution at Fringing Reef (median =  $11.3 \text{ g} \cdot \text{d}^{-1}$ ; Kruskal–Wallis test,  $X^2_2 = 11.556$ ,  $P = 0.003$ ; Dunn's test,  $Z = 3.353$ ,  $P = 0.002$ ), while clod cards at Back Reef A showed intermediate rates of dissolution (median =  $13.5 \text{ g} \cdot \text{d}^{-1}$ ; Fig. 2A). We also considered maximum dissolution rates, but that did not change the ranking of sites.

Projected area ( $A_{\text{projected}}$ ) of *Turbinaria ornata* differed between sites. Individuals collected from Fringing Reef had the largest  $A_{\text{projected}}$  (median =  $37 \text{ cm}^2$ ) and were significantly larger than the individuals from Back Reef B (median =  $32 \text{ cm}^2$ ; Kruskal–Wallis test,  $X^2_2 = 13.619$ ,  $P = 0.001$ ; Dunn's test,  $Z = 3.592$ ,  $P < 0.001$ ). Individuals from Back Reef A had intermediate  $A_{\text{projected}}$  (median =  $35 \text{ cm}^2$ ) but were still larger than individuals from Back Reef B (Dunn's test,  $Z = 2.440$ ,  $P = 0.044$ ; Fig. 2B). Breaking force ( $F_{\text{break}}$ ) of *T. ornata* also differed between sites.  $F_{\text{break}}$  was lower at Fringing Reef (median =  $25.0 \text{ N}$ ) than at both Back Reef A (median =  $28.5 \text{ N}$ ; Kruskal–Wallis test,  $X^2_2 = 20.687$ ,  $P < 0.001$ ; Dunn's test,  $Z = 2.573$ ,  $P = 0.030$ ) and Back Reef B (median =  $31.6 \text{ N}$ ; Dunn's test,  $Z = 4.548$ ,  $P < 0.001$ ; Fig. 2C).

Environmental safety factors (ESF) of *Turbinaria ornata* were not different between sites

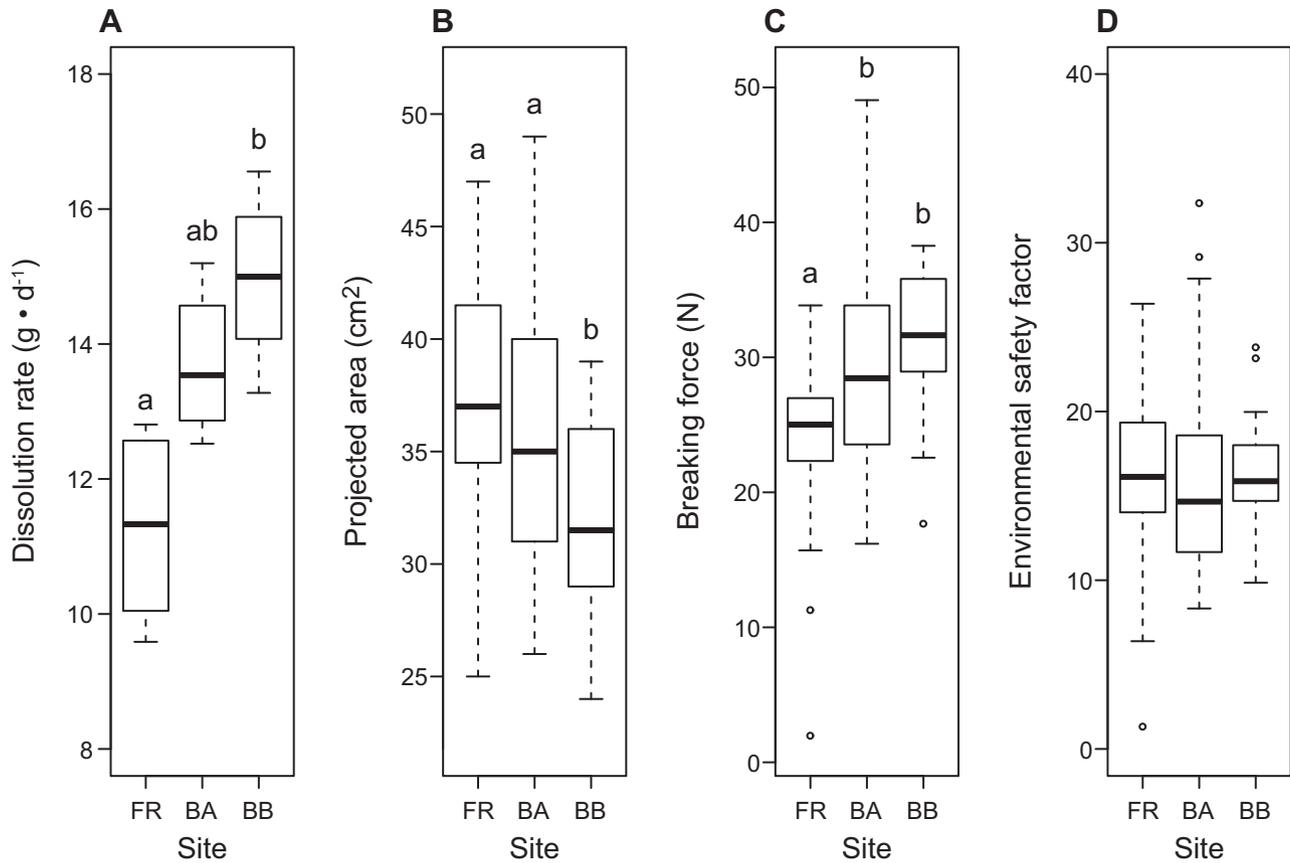


FIG. 2. Water motion and macroalgal morphology. (A) Clod cards' dissolution rates at each site. (B) Projected areas, (C) breaking forces, and (D) environmental safety factors of *Turbinaria ornata* at each site. Boxes show quartiles around the median, error bars show the most extreme data point  $\leq 1.5$  times the interquartile range from the box, and circles show values  $> 1.5$  times the interquartile range from the box. Lowercase letters in each panel show sites that are not statistically different (Kruskal–Wallis tests with post hoc Dunn's tests,  $P < 0.05$  for significance). In (A),  $n = 6$  at each site. In (B), (C), and (D),  $n = 24, 30,$  and  $30$  for FR, BA, and BB, respectively.

(Kruskal–Wallis test,  $X^2_2 = 0.930$ ,  $P = 0.628$ ; Fig. 2D). Median ESFs at the sites ranged from 14.7 (Back Reef A) to 16.1 (Fringing Reef).

Adult *Turbinaria ornata* varied their morphology ( $A_{\text{projected}}$ ) and strength ( $F_{\text{break}}$ ) in different flow regimes such that they were equally well-suited to withstand the forces of each flow regime. However, the adult life stage is when the stipes begin to weaken, which facilitates breakage and dispersal (Stewart 2006b), so these data can also be interpreted to show that *T. ornata* is equally likely to break in each flow regime. Our results agree with previous studies (Stewart 2004) that show that morphological plasticity of *T. ornata* allows the macroalga to inhabit a range of flow conditions. Decreasing  $A_{\text{projected}}$  can reduce the hydrodynamic forces that *T. ornata* experiences at any flow velocity (eq. 3) but increasing  $A_{\text{projected}}$  can benefit the macroalga's growth by increasing light and nutrient absorption (Hurd 2000). The change in  $A_{\text{projected}}$  between flow regimes could be due to a trade-off between the physiological benefit and physical penalty of having large  $A_{\text{projected}}$ .

In contrast to  $A_{\text{projected}}$ , we observed that  $F_{\text{break}}$  increased with flow velocities. Our values of  $F_{\text{break}}$  were larger than those reported in other studies (Stewart 2006b), which likely occurred because we applied loads stepwise at 15 s intervals rather than gradually increasing the load (i.e., adding loads rapidly rather than gradually can give less time for tissues to deform and break, resulting in larger breaking forces; Vincent 2012). Although we are not aware of other studies of *Turbinaria ornata* that measured  $F_{\text{break}}$  across flow habitats, *T. ornata* was similar to other macroalgae that increased  $F_{\text{break}}$  with increasing levels of applied forces (i.e., mimicking hydrodynamic forces; Kraemer and Chapman 1991). Thus, variation in  $F_{\text{break}}$  of *T. ornata* could be due to a strengthening response that is proportional to the hydrodynamic forces it experiences.

Median ESFs at each site ranged from 14.7 to 16.1 and were similar in magnitude to ESFs calculated for *Nereocystis luetkeana*, which also showed little variation in ESF between flow regimes because it varied its size and strength (Johnson and Koehl 1994). Similarities in ESF between *Turbinaria ornata*

and *N. luetkeana* are surprising because they are not closely related, they are different sizes (*N. luetkeana* can be > 10 m long while *T. ornata* is < 0.5 m long). Moreover, *T. ornata* decreases its strength and ESF with age while *N. luetkeana* does not. However, *T. ornata* and *N. luetkeana* do each have annual life spans and thalli with single stipes and buoyant air bladders (Johnson and Koehl 1994, Stewart 2004, 2006b).

We cannot attribute a single mechanism to the ESF of *Turbinaria ornata*. For instance, we cannot say whether an adult individual in one flow regime has a pre-determined  $F_{\text{break}}$  and grows its thallus until the hydrodynamic forces it experiences equal some critical level. Transplanting individuals between sites could reveal if  $A_{\text{projected}}$  and  $F_{\text{break}}$  are plastic and if they always co-vary to produce the same ESF, especially since *T. ornata* can decrease its strength over time (Stewart 2006b). Additionally, we cannot say how different flow habitats influence ESF for juvenile *T. ornata*, although within a single flow habitat, a juvenile can have a large ESF based on its small size and strong stipe tissue (Stewart 2006b). Although we cannot identify the exact mechanisms driving ESF, we did find that the mechanical design of *T. ornata* varied between sites, allowing the macroalga to be equally well-suited to withstand the hydrodynamic forces of its habitat – or alternatively, equally well-suited to break and disperse. Because these measurements and calculations have been made for too few species, we cannot say whether the ESF patterns reported here are widespread across macroalgae. Future studies on the ESFs of macroalgae could help us understand how macroalgae survive in different flow regimes and how they will respond to future changes in flow regimes.

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