

THE ROLE OF MORPHOLOGICAL VARIATION IN BREAKING STRESS AND REPRODUCTIVE INVESTMENT IN THE DRIFTING MACROALGAE *TURBINARIA ORNATA* (PHAEOPHYTA) IN THE LAGOON SYSTEM OF MO'OREA

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Abstract. *Turbinaria ornata* is a brown macroalgae, distributed across subtropical and tropical waters, commonly found in the reef system of French Polynesia that uses detachment of propagules as a dispersal mechanism. This study explores the biomechanical survivorship of *T. ornata*, as a function of morphology and flow environment. Variations in the hydrodynamic regime at different geomorphic features in the lagoon pass system, was assessed by clod cards used as a proxy for an integrated index of hydrodynamic forces experienced by *T. ornata* fronds. Morphology, breaking strength, and reproductive investment were measured for each frond. Morphological measurements allowed for phenotypic comparisons of fronds from different flow environments. Breaking strength was measured by progressively adding weight to suspended fronds until breakage, allowing quantification of how easily fronds are dislodged from the substrate. Reproductive investment was quantified by determining relative number of reproductive receptacles found per frond. Differences in both morphology and breaking strength between fringing reef and back reef populations suggest that morphotypes of *T. ornata* exist as an adaptive measure in response to hydrodynamic regime found at each habitat. Additionally, examination into morphology and breaking strength reveals that stipe dimensions, specifically length is the strongest driver of breaking strength. Further investigation in reproductive investment revealed no significant correlation between breaking strength and reproductive output. The combination of differences in breaking strength and lack of correlation with reproductive investment suggests that the probability of dislodgement for each morphotype is uniform, ensuring the continual reproductive success of *T. ornata* in propagating fronds ecomorphologically adapted to a wide range of flow habitats.

Key words: *algae; Turbinaria; morphology; breaking strength; hydrodynamic regime; flow; reproductive propagation; Mo'orea, French Polynesia*

INTRODUCTION

Detachment is a common dispersal mechanism used by several sessile marine organisms for reproductive and dissemination purposes (Boller *et al.* 2002). For example, the calcareous sponge, *Clathrina aurea*, practices asexual fragmentation as a dissemination method to reach new areas for settlement (Padua *et al.* 2016). In sessile algae, detachment and drifting of propagules enable dispersal over larger distances (Norton 1992). Some macroalgae, resort to unicellular units such as zygotes, gametes, spores, etc. as a means for dissemination (Clayton 1992). In contrast, other macroalgae rely on detachment of entire or partial units from the parent individual for dispersal (Stiger and Payri 1999a). Regardless of unit size, propagation of drifting algal propagules is entirely contingent upon hydrodynamic forces found in their respective

habitats. The most mobile algal cells are capable of moving approximately 80- 100 μms^{-1} , an amount negligible in comparison to ocean driven flow rates (Norton 1992). Flow velocity and hydrodynamic regimes in wave swept environments vary with geomorphology; flow regimes range from unidirectional flow, to bidirectional or oscillating flow, and several other complex forms (Haring *et al.* 2002). More turbulent wave action, however, presents attached organisms with a tradeoff between greater potential for nutrient transport and biomechanical survivorship. (Carpenter *et al.* 1991, Falter *et al.* 2005).

The morphology of sessile marine organisms is a determinant of its biomechanical survivorship; therefore, apart from the influence of hydrodynamic force, the release threshold of propagules is also heavily influenced by morphology (Denny *et al.* 1998).

The morphotype of sessile organisms, especially erect organisms, governs drag, acceleration, and velocity forces experienced by the organism. Such hydrodynamic forces are principle factors that influence mechanical survivorship (Denny and Gaylord 2002). For instance, sea anemones maximize drag force (the resistance force acting in the opposite direction of flow velocity) to capture nutrients, and increase the probability of detachment (Koehl 1976). Similarly, structural and morphological properties of *Plexaura kuna*, a sessile cnidarian, also governs the breaking of *P. kuna* branches in the facilitation of asexual fragmentation (Boller *et al.* 2002).

Algae exist in morphologically diverse forms; however, all wave-swept algae follow a foundational structure consisting of a holdfast for attachment, stipe, and blades, and often in larger brown macroalgae, pneumatocysts (Denny and Gaylord 2002). The function of morphology in detachment can be quantified by breaking strength, a parameter that factors in strength of the organism's attachment to the substrate and strength of the impingement force acting on the organism (Stewart 2006a). Propagules of some algal species include an intact holdfast while propagules of other species break off at the stipe, without the holdfast still attached (Stewart 2006b). Because dislodgement is a method for reproductive dispersal in immobile algae, reproductive investment in algae is also associated with morphology (Denny and Gaylord 2002). For instance, studies on the intertidal macroalgae, *Mastocarpus papillatus*, have demonstrated an existing relation between breaking strength and reproductive investment. Across morphotypes of *M. papillatus*, individuals have equivalent stipe dimensions and breaking strength but different levels of reproductive investment (Carrington 1990).

Turbinaria ornata (Turner) J. Aghard is a drifting brown macroalgae species, distributed across subtropical and tropical regions in the Indian and Pacific Ocean (Wynne 2002). Specific to French Polynesia, *T. ornata* takes after the role of an invasive species (Stiger and Prayi 1999b) due to its dominance and high-density abundance over the reef system. Previously, in the 1980's, *T. ornata* was found only in small aggregates in the Society islands (Stiger and Prayi 1999a). *T. ornata* is characterized by its tubular upright stature, triangular shaped blades, thalli with both branched and unbranched fronds, and a holdfast attached to the substratum (Stewart 2008). Although, the thallus of *T. ornata* can

either be monoecious or dioecious, all mature fronds have reproductive receptacles that emerge at the base of the blades (Stiger and Prayi 1999a). Reproductive propagation of *T. ornata* can occur either by release of gametophytes from the parent frond or through detachment of fronds from the substratum (Stewart 2006a). Detachment of buoyant fronds allows for dispersal over much greater and longer distances than the direct release of germlings (Stewart 2006a). *T. ornata* thalli, detached from the substratum, remain reproductively viable up to 3 months (Stiger and Prayi 1999a). *T. ornata* can disseminate and drift in patches over very large distances, minimally ~200 km (Martinez *et al.* 2006), contributing to the species' reproductive success in interisland dispersal and propagation. Surveys of *T. ornata* in Paopao Bay Mo'orea, French Polynesia established presence of *T. ornata* in both the fringing reef and back reef (pers. obs.). Eulerian measurements in Paopao Bay shows unidirectional flow directed inwards of the lagoon in both the shallow fringing and back reef (Hench *et al.* 2008).

Morphology and hydrodynamic force both factor into the dislodgement of *T. ornata* from the substratum. Previous studies have demonstrated a correlation between different morphotypes at different ontogenetic stages of *T. ornata* with variations in breaking strength. Older more reproductively mature fronds have lower breaking strength, causing fronds to break more easily (Stewart 2006a); however little study has been done on morphological variation of *T. ornata* between heterogenous flow regimes. The first aim of this project is to expand upon studies on ecomorphology of *T. ornata* in its continually expanding geographic range across French Polynesia by (1) examining morphological plasticity of *T. ornata* across the lagoon system due to differences in hydrodynamic regimes. The second aim of this study is to address the life history strategy of *T. ornata* by (2) determining whether different *T. ornata* morphotypes affect breaking strength, and (3) whether there is an existing relationship between breaking strength and reproductive investment. Lastly, this project also aims to (4) examine which morphological characteristics in *T. ornata* morphotypes is the primary explanatory variable for breaking strength. Morphological plasticity, breaking strength, and reproductive investment of adult *T. ornata* fronds will be examined between two hydrodynamic settings: the fringing reef and the back reef in Paopao Bay Mo'orea.

METHODS

Study site

Collections and measurements of *T. ornata* were taken in Paopao Bay Mo'orea, French Polynesia. Paopao Bay, also known as Cook's Bay, encompasses a lagoon-pass system, in which a reef crest divides the bay from the ocean with an opening at Avaroa Pass (Hench *et al.* 2008). The study site is confined to the lagoon system found on the northeastern side of Paopao Bay, and includes two geomorphic features: the fringing reef and the back reef (Fig. 1). The average depth of the bay is approximately 20-25 m (Hench *et al.* 2008), but both the fringing and back reef are much shallower with depths ranging from 1-3 m. The fringing reef is approximately 103 m long and 1200 m wide, and the back reef is approximately 864 m long and 2154 m wide. The landscape of both sites is interspersed with coral rubble, and coral bommies lined with macroalgae, two of the most abundant being *Turbinaria ornata* and *Sargassum mangaravense*.

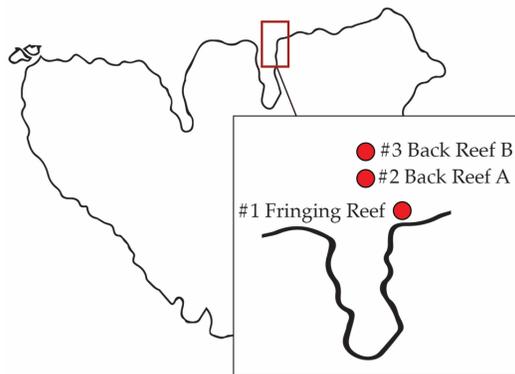


FIG. 1. Map of Mo'orea, French Polynesia, showing range of sampling site (red rectangle) and locality of each site (red dots) in the northeastern lagoon pass system of Paopao Bay.

Investigating flow habitats

To investigate hydrodynamic regimes in the vicinity of study sites, clod cards were constructed using blocks of plaster of Paris and the dissolution rate was used as a standard measure of water motion (Thompson and Glenn 1994). Clod cards were attached to plastic trays using epoxy; plastic trays were fastened with 2 kg weights to ensure clod cards remain submerged and stationary at the location of deployment. Because rate of clod

card dissolution is related to surface area, clod cards with uniform weights (within 2 g) ranging from 21- 23 g were used. At each sampling site, 6 clod cards were deployed on heads of coral bommies, at depths <1 m, adjacent to where *T. ornata* fronds are found attached and left for a total of 24 hours. Weights of clod cards were taken before deployment and again after collection using an electronic balance (JC1003PL), with a precision of 0.001 g. Reweighing of clod cards were taken after 24 hours of collection, to ensure that only dry weight was measured. Weight differences of deployed clod cards allows for a relative comparison of water velocity between sampling sites (Thompson and Glenn 1994). For a more descriptive statistic of flow regime dissolution rates of clod cards from the same batch as deployed clod cards was also measured under still water conditions (Leighter, pers. comm. 2018). Dissolution rates of clod cards under still water allows for water velocity to be expressed using the statistic, diffusion increase factor (DF), described by Doty (1971) as the quotient of the weight loss of deployed clod cards and weight loss of clod cards held under still water (Eq. 1). A calculated average flow velocity at each sampling site was also determined using a regression of clod card dissolution rate, obtained from a lab-controlled flow test (See Appendix A).

$$DF = \frac{\text{weight loss of deployed clod cards}}{\text{weight loss of clod cards in still water}} \quad (1)$$

Data collection

T. ornata fronds were collected between October 6-20, 2018 from random heads of coral bommies using transect sampling at three different sites: (1) Fringing Reef, (2) Back Reef A, (3) Back Reef B (Fig. 1). Transect sampling at site: Fringing Reef started at (S 17.48412 W 149.81737) and continued 30 m northeast along the fringing reef. Sampling in the back reef was divided into two localities: Back Reef A site extended from the end of the lagoon (S 17.48147 W 149.81833) to 30 meters northwest, and Back Reef B site continued from 30 meters to 60 meters northwest. A 50 x 50 cm quadrat was placed in intervals of 10 m along each of the 3 transects. A total of 3 quadrats were taken for sampling at each location. Within each quadrat, all mature adult fronds were collected. Adult fronds were chosen for the purpose of this study, since it has been demonstrated by Stewart (2006a) that fertile adult fronds are

most likely to become dislodged from the substratum. This was confirmed by personal observations of floating mats of *T. ornata*, which consisted mainly of adult fronds. Because of the high abundance of mature fronds found within each quadrat, at least 10 adult fronds were collected from each quadrat. Ontogenetic stage of *T. ornata* was determined using guidelines established by Stiger and Payri (2005): young fronds do not have reproductive receptacles, mature adult fronds have reproductive receptacles with <50% epiphytic coverage, and old fronds have reproductive receptacles with >50% epiphytic coverage. Adult fronds were manually pulled from the substrate with the holdfast still attached and deposited in buckets of seawater. After collection, samples were immediately transported back to lab, where they remained in seawater and were held in shaded conditions to minimize volume loss. From each quadrat sampling, 10 fronds were randomly chosen using a random number generator for measurements. All measurements were taken within 10 hours of collection.

Investigating morphological differences

To investigate morphological variability among adult *T. ornata*, a total of 30 fronds were measured from each of the 3 sample sites that varied in hydrodynamic regime. Morphological differences were explored using the following parameters: entire frond length, stipe length, stipe diameter, number of blades, and width of widest cross section. The chosen parameters follow a similar study on morphological plasticity adopted by Stewart (2006b). Relative figures on primary stipe measurements, relative stipe length, relative stipe width, and relative stipe area, were computed for further analysis (Eq. 2-4). Length, width and diameter measurements were taken using a caliper, with a precision of 0.01 mm. To standardize stipe diameter calculations, measurements were all taken at the base of each stipe, at the margin of the holdfast.

$$\text{Rel. stipe length} = \frac{\text{stipe length}}{\text{total frond length}} \quad (2)$$

$$\text{Rel. stipe width} = \frac{\text{stipe diameter}}{\text{total frond length}} \quad (3)$$

$$\text{Rel. stipe area} = \frac{\text{stipe diameter}}{\text{total length} \times \text{widest width}} \quad (4)$$

Examining breaking strength

To determine whether material properties of *T. ornata* fronds found in varying flow regimes, affect breaking strength, tensile strength tests were performed using a constructed tensometer. Constructed tensometer followed models of tensometers used by Koehl and Wainwright (1977) and Stewart (2006a). Because fronds of *T. ornata* break at the stipe, as observed in already dislodged floating fronds, tensile tests were performed by vertically suspending individual fronds onto the tensometer by clamping two ends of the stipe to the apparatus, using wingnuts. The stipe end, at the margin of the holdfast, was clamped to a horizontal, stationary, wooden beam. The opposite stipe end, at the margin of where the first blade begins, was clamped to a weight contraption. The weight contraption was positioned perpendicularly underneath the suspended frond. Weights in increments of 50 grams were consecutively added onto the weight contraption, in intervals of 15 seconds, until the stipe broke. Any tests, in which the stipe broke at point where clamp is attached were disregarded.

Preliminary trials were run to determine how securely wingnuts could be fastened to stipe ends without damaging the integrity of the stipe, which would cause the stipe to break at the point of fixation. Wingnuts were adjusted to match the diameter of the stipe at the two points of fixation.

Breaking strength (σ) was then calculated by taking the ratio of force required to break the stipe (F) and the cross-sectional area (A) of where the stipe breaks (Eq. 5) (Stewart 2006a). A caliper, with a precision of 0.01 mm, was used to measure the diameter of the stipe at the point of breakage. Stipe cross sectional area (A) was calculated using the equation for the area of a circle. Force required to break the stipe (F) was calculated as the product of the total amount of weight added, the summation of the weight contraption and amount of 50 gram weights added, multiplied by gravitational force (9.81 m/s^2) (Stewart 2006a). Measurements were performed on 30 randomly chosen adult fronds from each sampling site, within 10 hours of collection. Before tensile strength tests were performed, measurements of morphological characteristics of each frond were also taken for the purpose of investigating the relationship between morphology and breaking strength.

$$\text{Breaking strength } (\sigma) = \frac{F}{A} \quad (5)$$

Quantifying reproductive investment

T. ornata has reproductive tissues confined in structures called, receptacles, which are found on the base of the non-reproductive, vegetative blades (Stewart 2006a). To quantify reproductive investment, the number of receptacles per frond was counted. Relative number of receptacles was calculated by dividing total number of receptacles by total number of blades. To investigate whether tensile strength and reproductive investment in *T. ornata* are correlated, reproductive investment was examined on the same individuals that were also tested for breaking strength.

Statistical Analysis

All statistical analyses were performed using Past (V 3.21). (Hammer *et al.* 2001) For statistical tests alpha = 0.05 was used.

In exploring hydrodynamic regime, regression analysis was used to correlate water velocity with weight loss of clod cards in lab-controlled flow experiments. Although lab experiments measure for clod card weight loss as the dependent variable, flow velocity is

displayed as the dependent variable in the regression analysis figure. This is because extrapolation of the regression equation to the field deployment of clod cards assumes clod card weight loss as the independent variable.

An ANOVA analysis was used to analyze differences in flow between the three sampling sites. The ANOVA was performed on estimated water velocity. Water velocity was computed using regression analysis of clod card dissolution rates under lab fluid flow experiments, at each sample site. Normality and homoscedasticity were assumed based on data collected on flow regimes in Paopao bay by Hench (2008) and Washburn (2018).

To examine morphological variation of adult *T. ornata*, principle component analysis (PCA) was used. Morphological parameters that were examined under PCA include: widest width at cross section, number of blades, relative stipe length, relative stipe width, and relative stipe area.

A Shapiro- Wilk normality test (Shapiro and Wilk 1965) was performed on breaking strength data, revealing non-normal distribution, and the Levene's test revealed non-homoscedastic data resulting in the use of the non-parametric Kruskal Wallis test (Feir and Toothaker 1974) to test differences in breaking strength. Median breaking strength is compared between the three sampling sites that differ in hydrodynamic regime. A Mann Whitney pairwise post hoc test was used to

TABLE 1. Description of clod cards

Locality Name	Locality Coordinates (lat-long)	Deployment Date	Depth (m)	Average weight loss (g)	Average DF	Estimated water velocity (m/s)
Fringing Reef	S 17.48412, W 149.81737	Oct-29	<1	11.3 ± 1.40	2.52 ± 0.284	0.047 ± 0.009
Back Reef A	S 17.48147, W 149.81833	Nov-01	<1	13.71 ± 1.01	3.06 ± 0.206	0.062 ± 0.006
Back Reef B	S 17.48147, W 149.81833	Nov-03	<1	15.0 ± 1.23	3.34 ± 0.251	0.070 ± 0.008

Note: Values are expressed as mean ± standard deviation, correct to 3 significant figures. DF values are calculated using 4.4780 g as the average weight loss of clod cards held in still water. Estimated water velocity was computed using the regression equation $y=0.0062x-0.0232$

determine which groups differed significantly. Because breaking strength is a function of impingement force and cross sectional area at point of breakage (Eq. 5), an investigation of whether differences in breaking strength is a result of difference in force required for breakage to occur or difference in area at breakage point, was executed. This was carried out using a Kruskal Wallis test (Feir and Toothaker 1974). Cross sectional area of breakage point was compared between the three sample sites.

Breaking strength will also be examined in conjunction with morphology to investigate which morphological parameter is the strongest explanatory variable for breaking strength. Since breaking strength is a function of stipe dimension (Eq. 5), multiple regression was run only on force required for breakage (F) against multiple morphological parameters (independent variables). Experimental design used in this study to test tensile strength directly only addresses stipe integrity; therefore, only morphological measurements on stipe dimensions were used, namely, relative stipe length (Eq. 2), and relative stipe area (Eq. 4).

RESULTS

Hydrodynamic regime

Water velocity estimates were computed from regression analysis of dissolution rate of clod cards under controlled water flow experiments. The dissolution of clod cards under fluid velocity experiments yields a linear relationship (Fig. 2). Average weight loss of field deployed clod cards, DF values and estimated water velocity were calculated (Table 1.) ANOVA analysis of estimated water velocity, $F(2,15) = 14.09$, $p = 0.0003597$ reveals a statistically significant difference in water velocity at different sample sites. A Tukey's honestly significant difference (HSD) post hoc test reveals that flow velocity is significantly different between the Fringing Reef and Back Reef A, $p = 0.009634$ and between the Fringing Reef and Back Reef B, $p = 0.0002869$.

Morphological characteristics

Morphological variations among *T. ornata* found at different sampling sites was explored using principle component analysis (PCA) reduction analysis of 5 assessed morphological variables: number of blades, width of widest cross section, relative stipe length, relative stipe

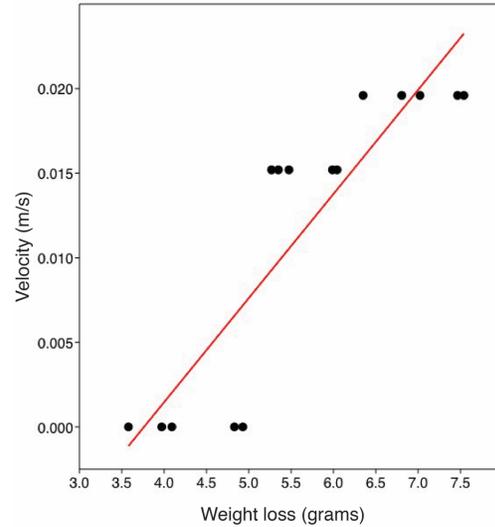


FIG. 2. Correlation between controlled laminar flow water velocity and clod card weight loss in 33.8 ppt seawater. Displayed regression line has a y-intercept of -0.0232 and a slope of 0.0062 with a R^2 value of 0.7826. Dissolution rates measured at unidirectional laminar flow velocities at 0.0152 m/s and 0.0196 m/s in a flow tank.

width, and relative stipe area. Correlations between 90 measured *T. ornata* individuals, 30 from each sampling site, are mapped onto the first two principle components capturing 85.0% total variance. (Fig 3.) Principle component 1 has an eigenvalue of 43.1, explaining 58.7% of total variance, and principle component 2 has an eigenvalue of 19.3, explaining an additional 26.3% of total variance. Separation of the fringing reef from the two back reef populations is best explained through principle component 2, and the separation between the two fringing reef populations (Back Reef A and B) is best explained through principle component 1. Further investigation into principle component 2, reveals widest width of cross section and relative stipe area as the greatest contributor with loading scores of 0.755 and -0.512, respectively.

Breaking strength

Breaking strength of *T. ornata* ranges from low, in the fringing reef with an average of 0.022 ± 0.554 , and to higher ranges in back reef A with an average of 0.023 ± 0.002 , and in back reef B with an average of 0.027 ± 0.001 (Fig. 4). Breaking strength in the fringing reef has the lowest average and the largest standard

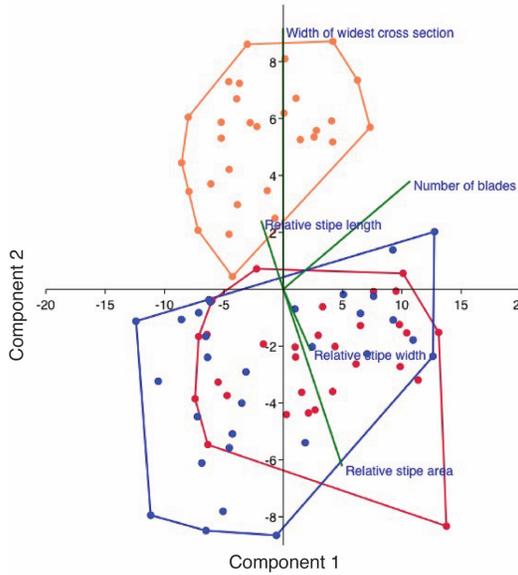


FIG. 3. Scatter plot of 90 collected *T. ornata* individuals. Orange points indicate individuals collected from the Fringing Reef, red points from Back Reef A and blue points from Back Reef B.

deviation, while breaking strength in the Back Reef A has the highest average and Back Reef B has the smallest standard deviation. A Kruskal Wallis analysis $\chi^2(2) = 8.042$, $p = 0.01793$ showed that there is a statistically significant difference in breaking strength between different locations in the lagoon system with different hydrodynamic regimes. A Mann-Whitney post hoc test reveals that statistically significant differences are found between the Fringing Reef and Back Reef B ($p=0.005825$) and Back Reef A and Back Reef B ($p=0.04593$). Further investigation on cross sectional area at breakage point reveals no statistically significant difference between sampling sites, $\chi^2(2) = 1.454$, $p = 0.477$.

Morphological drivers of breaking strength

Because statistically significant differences are found in breaking strength, an investigation into the morphological characteristics as explanatory variables for the difference in breaking strength was performed using multiple regression analysis. Since breaking strength is calculated as a function of stipe cross sectional area (Eq. 5), multiple regression was run between force required for breakage and relative stipe length, and relative stipe area. Multiple regression reveals

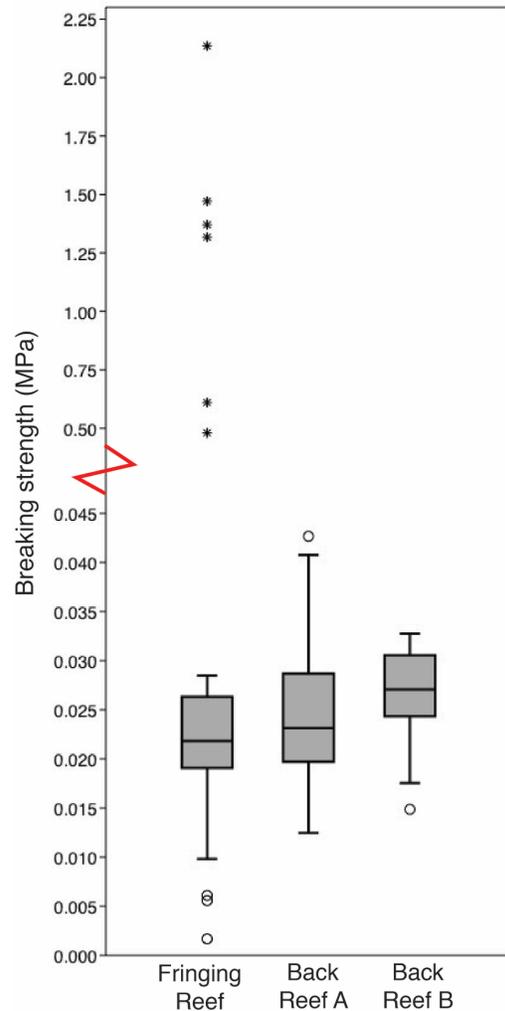


FIG. 4. Boxplot of breaking strength of adult *T. ornata* fronds at the three sample sites ($n=30$, $N=90$). Note: Because of strong outliers (marked as asterisks) found in the fringing reef, a discontinuity line (in red) is used to condense plotted information. Note that the scale on the vertical axis is different on each side of the discontinuity.

$R^2(\text{adj.}) = 0.047615$ with relative stipe length $R^2=0.038522$ ($p= 0.080936$), and relative stipe area $R^2=0.013482$ ($p = 0.36459$). (Fig. 5). A slight trend is observed between relative stipe area and force required for breakage ($p=0.36459$), and a stronger trend is observed between relative stipe length and force required for breakage ($p=0.080936$).

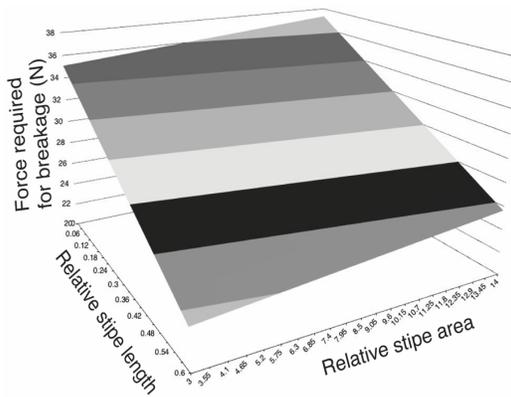


FIG. 5. 3-dimensional surface plot of predicted force required for breakage (z-axis) in response to relative stipe length (Rel. S.L.) and relative stipe area (Rel. S.A.) Predicted breakage force is equal to $0.18553(\text{Rel.S.A.}) - 19.53(\text{Rel. S.L}) + 34.493$. Shades on the surface plot are isometric. Within each individual shaded area, predicted force required for breakage is equal.

Breaking strength and reproductive investment

Regression between reproductive investment, quantified by relative number of reproductive receptacles per frond and breaking strength yields an insignificant correlation with $R^2 = 0.0031805$.

DISCUSSION

Correlations between breaking strength and morphological characteristics in different flow habitats, and the relationship between breaking strength and reproductive investment indicates the importance of ecomorphology on the life history of *Turbinaria ornata*. *T. ornata* has two distinct morphotypes: slender individuals from the fringing reef and stouter individuals from the back reef, that allows this species to successfully propagate across the Pacific.

Hydrodynamic regime

Differences in flow velocity between the fringing reef and back reef habitats in Paopao Bay affect the intensity of hydrodynamic force experienced by *T. ornata* fronds from fluid motion (see Table 1.). It is not uncommon for hydrodynamic regimes to differ with geomorphology (Ballantine 1961). Lower estimated water velocity (~ 0.047 m/s) in the

fringing reef indicates calmer hydrodynamic forces and higher estimated water velocity (~ 0.062 - 0.070 m/s) in the back reef indicates more intense conditions. Lack of statistically significant differences in flow velocity between the two back reef sample sites: Back Reef A and Back Reef B is supported by flow studies done by Hench *et al.* (2008), which confirmed that hydrodynamic regime found across the back reef is mostly uniform.

Morphotypes

Morphology of adult *T. ornata* fronds also varied with geomorphic feature, analogous to differences in flow velocity found across sample sites. Principle component analysis of the morphological data shows a clear separation in morphospace between *T. ornata* specimens of the fringing reef and back reef. Although there may be some differences in the morphospace occupation of specimens from the two back reef locations, overlap in morphological characters between both populations dominates. The morphological parameters widest width at cross section, and relative stipe area were the most significant morphological parameters in distinguishing *T. ornata* specimens sampled between the fringing reef and the back reef. *T. ornata* fronds found in the fringing reef have greater width at widest cross section, and lower relative stipe area, while fronds found in the back reef have narrower width at widest cross section and greater relative stipe area. Such intraspecific morphological variations, slender vs. stout, are commonly found in several plants, especially in marine algae. *Egregia menziesii*, a brown marine alga like *T. ornata*, have been found to morphologically vary in stipe length and number of branches with geographic location (Blanchette *et al.* 2002). The similarity in morphological dimensions that are distinguishing characteristics across brown marine alga morphotypes, indicates that stipe and frond properties are most influential to the mechanical survivorship of marine alga.

Pairing the results on hydrodynamic regimes and morphological data, this study has demonstrated that the two general *T. ornata* morphotypes that inhabit the Mo'orea reef system are adapted to different flow habitats, one adapted to calmer hydrodynamic regimes with a wider cross section and slim stipes (slender individuals), and one narrower in cross section with thicker stipes (stouter individuals). Such conclusions could,

therefore, be extrapolated to populations of *T. ornata* found in other similar flow habitats.

Correlations between hydrodynamic forces and biomechanical survivorship have been demonstrated to mechanically set limits to the morphology of marine macroalgae, in particular in terms of size (Denny 1999). This is corroborated by experiments on the Bladderwrack (*Fucus gardneri*), which showed an increase in size when individuals were relocated to calmer hydrodynamic conditions (Blanchette 1997). Equivalently, the results from this study also establishes that the longer, slender *T. ornata* morphotype was found in the less challenging hydrodynamic conditions of the fringing reef compared to the stouter morphotype found in the back reef. In contrast, studies performed on Sugar Kelp (*Laminaria saccharina*), has demonstrated that individuals found in stronger flow conditions often have narrower and longer blades for a more streamlined structure (Gerard 1987). This morphological adaptation diverges from what is observed in *T. ornata*, in which fronds found in stronger hydrodynamic conditions are stouter and more compact. The described disparity between the two species could be due to the differences in material properties of *L. saccharina* blades that allow for more flexibility than that of *T. ornata*. Greater length and flexibility have been shown to reduce the amount of fluid stress experienced by a submerged body (Gaylord *et al.* 2001). However, because *T. ornata* blades are not longitudinally shaped, and are calcified and corticated (Stewart 2008), elongation of blades would not accommodate streamlining. Instead the stouter *T. ornata* morphotype, adopted by back reef populations, reduces surface area exposed to hydrodynamic stress. To combat dislodgement, this study shows that marine macroalgae appear to employ two general strategies: altering shape of thalli for greater streamlining or reducing exposed surface area.

Breaking strength

T. ornata morphotypes occurring in the Mo'orea reef system motivated further examination into differences in breaking strength. Significant differences in breaking strength were found between the slender and stouter *T. ornata* morphotypes, but also, interestingly, found between the two populations of stouter individuals sampled. The difference in breaking strength between the slender and stout morphotype is not surprising given the differences in flow

velocity in the respective habitats of each morphotype (Table 1.) On the contrary, difference in breaking strength between the two populations of stout individuals contradicts numerical results of estimated flow velocity in the two back reef sample sites. The small difference (0.008 m/s) in flow velocity between the two back reef sites is negligible, and not statistically significant. A possible explanation for this observed result may be contingent on more subtle differences in flow regime. One of the back reef sites is located closer to the reef crest, while the other is further from the reef crest and closer to the opening of the Avaroa Pass; therefore, other confounding hydrodynamic forces that may only be present in one of the sites could account for the observed difference in breaking strength within the stout morphotype specimens.

Higher overall breaking strengths of stouter *T. ornata* specimens corresponds with higher flow velocities of the back reef, and lower breaking strengths of slender *T. ornata* specimens corresponds with lower flow velocities of the fringing reef (see Fig. 4). In effect, detachment of propagules is, on average, quantitatively uniform across flow habitat and morphotype. Variation in breaking strength was also noticeably much larger in the fringing reef than in the back reef (Fig. 4). A possible explanation for this observed difference is that the relatively calm hydrodynamic forces correspond to less environmental stress, thus lower selective pressure, allowing for more flexibility and variation in morphology to exist. On the other hand, in the back reef where higher flow velocity was measured, only more specialized and robustly adapted morphotypes of *T. ornata* can persist. The stouter morphotype, adapted to higher hydrodynamic stress are able to survive in calmer hydrodynamic conditions; conversely, the slender morphotype cannot persist in strong hydrodynamic conditions.

The differences in breaking strength (Eq. 5) can be due to two factors: impingement force or stipe area at breakage point. Lack of differences in cross sectional area of the stipe at breaking point indicates that differences in breaking strength is a result of differences in force required for dislodgement. Stipe diameter at breaking point does not account for the morphologically specific differences in breaking strength.

Breaking strength and morphology

Following conclusions that breaking strength differ across *T. ornata* morphotypes, this study also aimed to deduce morphological parameters that most strongly drives observed differences in breaking strength. Parallel differences in both morphology and breaking strength across habitats supports the claim that breaking strength is controlled by morphology. Because populations found in calmer flow habitats experience less hydrodynamic forces, less morphologically robust properties are required for fronds to remain attached to the substrate. Conversely, populations experiencing greater hydrodynamic forces require fronds to be more morphologically robust to remain attached to the substrate. Multiple regression results show that relative stipe length has the strongest effect on breaking strength (See Fig. 5). The negative correlation found between relative stipe length and breaking strength suggests that *T. ornata* individuals with longer stipes break off more easily than individuals with shorter stipes. Positive correlation between relative stipe area and breaking strength demonstrate that thicker stipes with greater diameter, are less likely to break. The results of this study present evidence that morphological characteristics, especially stipe length is a strong determinant of breaking strength. The conclusion drawn from this study is well supported by Denny (1999), that describes breaking strength in relation to plant size. If breaking strength is kept constant, dislodgement is found to be more likely to occur with larger individuals. More specifically, allometric growth of macroalgae, particularly, the ratio between stipe width or diameter and overall thallus size has been shown to have an impact on amount of stress encountered by the thallus (Carrington 1990). Macroalgae that fail to scale stipe diameter with increasing thallus size experience greater stress and are therefore more likely to experience breakage (Denny *et al.* 1997). This correlates with morphological results examined in this study. The slender *T. ornata* morphotype, that are, on average, wider and longer, experience much greater stress than the stouter morphotype, which are, on average, shorter and more compact. In regards to allometric growth of marine alga, conclusions can, therefore, be drawn on the close association that must exist between stipe morphology and other morphological dimensions, like that of the blade. Other hydrodynamic forces, such as drag and

acceleration forces, which was not included in the scope of this study, also increase in intensity with increased area or size of plant (Friedland and Denny 1995). The parallelism in intensity of other impingement forces and breaking strength opens more opportunities to study whether morphology has a direct influence on such forms of impingement force as well.

Other studies, on the contrary, concluded that morphological differences do not have a significant impact on breaking strength of macroalga (Milligan 2004 and Dewreede 2004, Bettignies *et al.* 2012). Milligan and Dewreede (2004) examined the macroalgae species, *Hedophyllum sessile*, and Bettignies *et al.* (2012) examined *Eckolonia radiata*, in both low and high flow habitats. Both studies revealed that morphology does not have a significant impact on mechanical survivorship of *H. sessile* and *E. radiata* found in habitats with high wave exposure and stronger fluid flow. This suggests that morphology may only significantly influence breaking strength in populations of macroalgae that occupy low flow velocity ranges, like *T. ornata* found in the Mo'orea reef system. It could, therefore, be interesting to explore whether morphology impacts *T. ornata* mechanical survivorship in higher flow settings. Apart from morphology, other factors, such as cellular or molecular properties, that were not explored in this study may also factor into breaking strength. For instance, studies on a green alga, *Cladophora glomerata*, have identified that the cylindrical cellular structure and arrangement affects tensile strength (Johnson *et al.* 1996).

Breaking strength and reproductive investment

For a sessile marine macroalgae, *T. ornata* relies on dislodgement for dispersal of reproductive propagules (Stewart 2006a). There is a lack of any substantial correlation between breaking strength and reproductive investment in mature adult *T. ornata* fronds across different flow habitats. The relative number of reproductive receptacles are equal across the two morphotypes presented in this study. Existing differences in breaking strength alone governs the quantity of propagules that are detached and dispersed. Therefore, it can be hypothesized that more energy expenditure is invested into morphology, to adapt to hydrodynamic condition, rather than in production of sexual receptacles. This ensures that the number of receptacles (reproductive investment) produced across morphotypes of

T. ornata remains consistent. Given this lack of correlation, number of reproductive receptacles dispersed from each morphotype would be approximately uniform, ensuring that morphological variations of *T. ornata*, each ecomorphologically adapted to certain flow habitats, are equally dispersed, allowing for continual settlement in a wide range of habitats.

The breadth of this study only covers influence of hydrodynamic flow on breaking strength; however, other dislodgement properties could also be expanded upon. For instance, to fully investigate breakage of *T. ornata* from the substrate further tests exploring drag forces and stipe extensions tests could be performed (Koehl 1976). Additionally, breaking strength tests carried out in this study does not consider other variables such as fatigue and frond flexibility. Moreover, this study only sampled *T. ornata* individuals from the same ontogenetic stage, namely, mature adult individuals. Extensions to this current study could investigate whether the same conclusions drawn from this study, could be applied to other ontogenetic stages in the life history of *T. ornata*. Numerical measurements used as a proxy for intensity of hydrodynamic flow obtained in this study only encompasses flow velocity, which may not be a complete representation of unidirectional flow. Given that deployment locations for both fringing and back reef sites are shallow (< 1m) flow reversals, thus accelerations, is expected (Leighter, pers. comm. 2018). Additional measurements on flow acceleration could help better characterization of hydrodynamic regime between the three sample sites to give more detailed figures such as maximum hydrodynamic forces. Nonetheless, the clod card technique that was used provides an integrated index of the hydrodynamic force experienced by *T. ornata*.

Future directions

Conclusions drawn from this study establish that *T. ornata* morphologically varies across different flow habitats; however, studies have not been performed to address whether *T. ornata* morphology differs seasonally. Studies have been performed exploring morphological changes in the brown algae *Hedophyllum sessile* in response to seasonal changes corresponding with different flow intensities (Milligan and Dewreede 2000). Future studies on *T. ornata* could aim to establish whether morphology responds to changes in seasonality or to

weather phenomena like the Southern Oscillation, known to carry a large impact on French Polynesia. Morphological variations can also be explored across other environmental gradients, such as depth, exposure to sunlight, or substrate type. In terms of breaking strength, this study conducted investigations into relationships between breaking strength and hydrodynamic regime. Preceding studies have examined breaking strength in relation to ontogeny (Stewart 2006a). However, no studies, have explored differences in breaking strength or influence of fluid forces in relation to position of *T. ornata* fronds within an aggregate or in relation to density of an aggregate. A study on a species of red alga, *Chondrus crispus*, has demonstrated that positioning of an individual within the canopy, and relative height of surrounding thalli influences hydrodynamic force experienced by the individual. *C. crispus* individuals that are solitary experience significantly greater hydrodynamic force than individuals positioned in the middle of the canopy (Boller and Carrington 2006). *T. ornata* on Mo'orea have been observed to exist in aggregates of different sizes; therefore, future studies could address questions on whether features of *T. ornata* aggregates such as, total number of individuals, distance between individuals, and height of neighboring individuals, causes significant differences in breaking strength. Furthermore, the widespread distribution of *T. ornata* in the lagoon system presents opportunities to study interactions of *T. ornata* within the community structure found in the reef system of Mo'orea. It is not uncommon for close symbiotic associations to develop within a community. Structurally, macroalgae, such as seaweed, provides more space for settlement of microalga and often mechanically deters herbivory, providing protective refuge for epiphytes (Al-Harbi 2017 and Bittick *et al.* 2010). Specific to *T. ornata* the most common epiphytes found includes: *Leptocylindrus minimus*, *Bacillaria paxillifer*, *Leptocylindrus danicus* etc. (Al-Harbi 2017). Other inhabitants on *T. ornata* includes macroinvertebrates, such as amphipods like *Metportella sandalensis* (Guerra-García and Koonjul 2004). The possible impact of epiphyte or macroinvertebrates species on stipe integrity or breaking strength could be an interesting area for further research.

Summary

Morphological plasticity, specifically correlations between stipe morphology and hydrodynamic performance of *T. ornata* accounts for the observed differences in breaking strength across flow habitats. The results of this study provide further support for the continuing reproductive success of *T. ornata*, best explained by the ability of this drifting perennial algae to morphologically adapt to a wide range of flow habitats. Therefore, it can be expected that dominance of macroalgae in the reef system across the Pacific will continue to proliferate.

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APPENDIX A

Clod cards are used as environmental proxies for flow strength. In the context of this study, clod cards were deployed at different geomorphic features in the lagoon pass system, namely the fringing reef and back reef to account for differences in flow environment. Dissolution of clod cards in water measured through weight loss of clod cards, allows for relative comparisons of hydrodynamic force at different sampling sites. In order to estimate water velocity from weight loss measured at each sample site, lab experiments in which water velocity was controlled for, was designed to obtain a regression equation between clod card weight loss and velocity. The implementation and design of clod card techniques practiced in field and in lab are described below.

Measuring clod card dissolution

Dissolution was quantified by measuring weight loss. For both field and in lab experiments, all clod cards were weighed before deployment with an electronic balance (JC1003PL), with a precision of 0.001 grams. Each clod card was placed in each flow setting for a period of 24 hours. After retrieval, clod cards were held in a shaded environment and reweighed after a period of 24 hours to ensure that only dry weight was taken. Dissolution rate of clod cards was computed by subtracting the difference of clod card weight before and after deployment.

Clod card in lab experimental controls

In lab clod card experiments were designed to best imitate hydrodynamic and climatic conditions of field deployment sites. Depth of experimental tanks were all standardized at <1m to prevent any significant effect of depth on hydrodynamic motions. Tanks were held outside allow for temperature to vary throughout the 24 hour period; temperature measurements of water in experimental tanks varied between 25 °C - 32°C, measured using a glass thermometer. Seawater was also used for lab experiments with a measured salinity of approximately 33.8 ppt, using a salinity probe (SPER Scientific Conductivity, TDS, Salinity Meter 850038).

Clod card construction technique

Clod cards for both field deployments and lab experiments were constructed using the same protocol. A mixture of one part water and two parts Plaster of Paris was poured into icecube trays (Fig. A1). A period of 24 hours was allowed for plaster to dry in ice cube trays before removal (Fig. A2). To ensure that surface area of all clod cards was uniform, only clod cards weighing between 21 and 23 grams were selected for field deployments and in lab experiments. Bottom surface of clod cards were sanded to adjust weight to 22 ± 1 grams (Fig A3).



FIG. A1. Mixture of Plaster of Paris in an icecube tray



FIG. A2. Selection of clod cards with approximately uniform surface area and weight removed from icecube trays

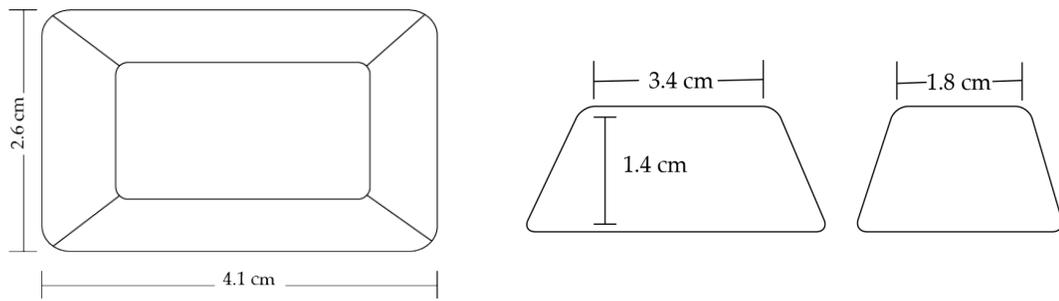


FIG. A3. Approximate dimensions of clod cards that were used in both field deployments and in lab experiments.

Field deployment of clod cards

Clod cards were fixed onto plastic trays using epoxy adhesive. Each plastic tray was attached to 2 kg weights to ensure that clod cards deployed in the field remained stationary and submerged (Fig A4). 6 clod cards were deployed at each sampling site, adjacent to where populations of *T. ornata* were found (Fig. A5). Plastic trays, in which clod cards were attached, were painted with bright nail polish to allow for easier identification of clod cards during retrieval.

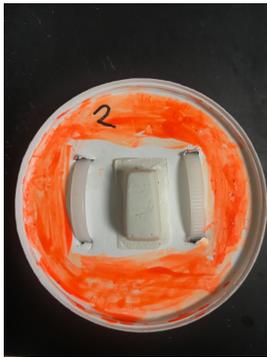


FIG. A4. Clod cards attached to plastic trays with 2 kg weights attached below



FIG. A5. Clod cards deployed at depths <1m on the same substratum *T. ornata* is found attached on.

In lab clod card experiments

Controlled fluid motion experiments were carried out to test dissolution rate of clod cards at different water velocities. Clod cards used in lab experiments were also attached to a plastic lid with weights, analogous to field deployments, to ensure consistency in dissolution rate with respect to the effect of exposed surface area to rate of dissolution. A total of 3 water velocities was explored in lab experimentation using 2 different experimental set ups described below:

1) Dissolution of clod cards under still water

Clod cards were placed in still seawater in a rectangular plastic tank, for a period of 24 hours. The rectangular tank is left outdoors to allow for daily temperature variations and was covered by a transparent plastic lid to prevent wind or rain induced water motion. Average weight loss of clod cards dissolution under standing water was used to calculate DF values.

2) Dissolution of clod cards under laminar flow

Fluid motion experiments were performed using a rectangular plastic tank, placed outdoors with a plastic lid, analogous to standing water experiments. A hose, discharging seawater was inserted into one end of the rectangular tank with an opening on the opposite end to ensure that water flows in one direction and depth is kept constant (Fig. A6). To create laminar flow, layers of mesh were placed in between the opening and the hose; flow following the mesh layer is perfectly laminar. Clod cards were positioned between the mesh layer and the opening for a period of 24 hours (Fig. A7). Controlled laminar flow was experimented using 2 flow velocities: 0.0196 m/s and 0.0152 m/s. A total of 5 clod cards were placed in each flow environment. Fluid velocity was computed by measuring average fluid discharge from the rectangular tank (Eq. A1-A2).

$$\text{Discharge (m}^3/\text{s)} = \frac{\text{fluid volume}}{\text{time}}$$

EQ. A1. Equation used to measure discharge from experimental tank. Fluid volume used was 12 L or 0.012 m³ and time was calculated from an average of 10 trials.

$$\text{Velocity (m/s)} = \frac{\text{discharge}}{\text{cross sectional area}}$$

EQ. A2. Equation used to compute velocity. Cross sectional area for the two different water velocities experimented were computed using the equation for area of a rectangle.



FIG. A6. Experimental set up of rectangular plastic tank, displaying hose discharging seawater from the opening on the right, and an opening on the left for water to exit the tank.



FIG. A7. Observable difference in flow between area before and after mesh layer. Clod cards placed in area behind mesh layer where flow was perfectly laminar.