KELP VERSUS CORALLINE: CELLULAR BASIS FOR MECHANICAL STRENGTH IN THE WAVE-SWEPT SEAWEED *CALLIARTHRON* (CORALLINACEAE, RHODOPHYTA)[1]

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Previous biomechanical studies of wave-swept macroalgae have revealed a trade-off in growth strategies to resist breakage in the intertidal zone: growing in girth versus growing strong tissues. Brown macroalgae, such as kelps, grow thick stipes but have weak tissues, while red macroalgae grow slender thalli but have much stronger tissues. For example, genicular tissue in the articulated coralline *Calliarthron cheilosporioides* Manza is more than an order of magnitude stronger than some kelp tissues, but genicula rarely exceed 1 mm in diameter. The great tissue strength of *Calliarthron* genicula results, at least in part, from a lifelong strengthening process. Here, a histological analysis is presented to explore the cellular basis for mechanical strengthening in *Calliarthron* genicula. Genicula are composed of thousands of fiber-like cells, whose cell walls thicken over time. Thickening of constitutive cell walls likely explains why older genicula have stronger tissues: a mature geniculum may be >50% cell wall. However, the material strength of genicular cell wall is similar to the strength of cell wall from a freshwater green alga, suggesting that it may be the quantity—not the quality—of cell wall material that gives genicular tissue its strength. Apparent differences in tissue strength across algal taxa may be a consequence of tissue construction rather than material composition.

Key index words: algae; biomechanics; breaking stress; cell wall; geniculum; histology; intertidal; material properties; tissue strength

Abbreviations: % cell wall$_{gen}$, proportion of geniculum containing cell wall; A_{cell}, cell cross-sectional area; A_{gen}, geniculum cross-sectional area; A_{lumen}, cross-sectional area of cell lumen; A_{wall}, cross-sectional area of cell wall; d, distance between cell centers; F_b, breaking force; N_{cells}, number of cells; r_{cell}, radius of cell; r_{lumen}, radius of cell lumen; w, cell wall thickness; r, breaking stress

To survive along wave-swept shores, intertidal macroalgae must have thalli whose breaking forces exceed the drag forces imposed on their fronds by breaking waves. Many studies of intertidal algal biomechanics have focused primarily on flow-induced forces (Koehl 1984, 1986, Denny 1994, 1999, Denny et al. 1997, 1998, Gaylord and Denny 1997, Bell 1999, Gaylord et al. 2001, Denny and Gaylord 2002) and how wave-swept thalli remain generally small (Denny et al. 1985, Gaylord et al. 1994, Blanchette 1997, Denny 1999) or reorient and reconfigure in flow (Koehl 1986, Bell 1999, Boller and Carrington 2006) to limit these forces. Many researchers have explored thallus breakage (Koehl and Wainwright 1977, Carrington 1990, Gaylord et al. 1994, Shaughnessy et al. 1996, Duggins et al. 2003, Kitzes and Denny 2005), but few studies have examined the dynamics of the supportive tissues and composite materials that allow macroalgae to resist drag forces as they grow.

Given an imposed force, algae can follow two basic growth strategies to avoid breaking: (i) increase cross-sectional area or (ii) increase tissue strength of thalli. Both strategies increase the ability of algal thalli to resist applied loads. Data from past biomechanical studies reveal that these two growth strategies are indeed traded against one another (Fig. 1). That is, algae with thick thalli tend to be composed of weaker tissues, while algae with slender thalli have the strongest tissues. This generalization even holds true for closely related sister species (Fig. 1, *Mazzaella splendens* [Setch. et N. L. Gardner] Fredericq and *Mazzaella linearis* [Setch. et N. L. Gardner] Fredericq).

In general, brown macroalgae (Ochrophyta, Phaeophyceae), including the kelps, follow the first strategy. While their tissues are quite weak (Koehl 1986, Hale 2001, see summary in Martone 2006), many brown macroalgae have secondary meristems, called ''meristoderms'' in kelps, which allow their stipes to increase greatly in girth (Graham and Wilcox 2000). Thus, brown algal stipes gain much of their strength from their large cross-sectional areas, rather than from inherently strong tissues, as a braided rope exceeds the strength of a single fiber. The giant intertidal alga *Durvillaea antarctica* (Cham.) Har. has taken this strategy to an extreme. Its tissues are among the weakest (0.7 MN · m^{-2}, Koehl 1986), but it can grow to >50 mm in diameter (Stevens et al. 2002) and can therefore resist >1000 N before breaking.

Red algae (Rhodophyta) generally follow the second strategy, constructing their thalli from stronger
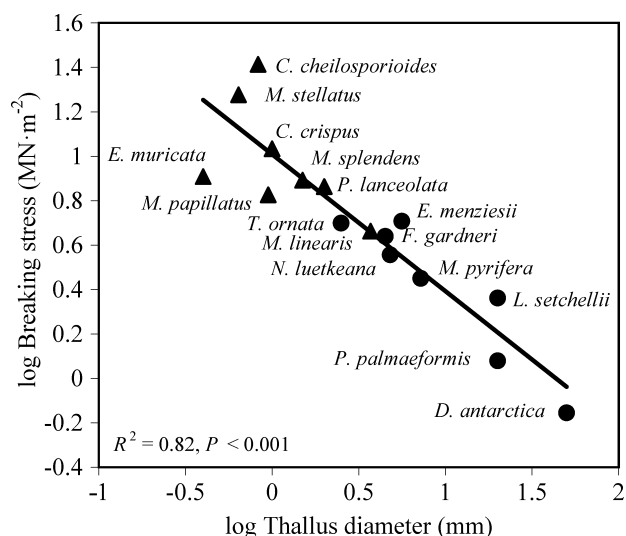
FIG. 1. Mean breaking stresses of various red macroalgae (triangles) and brown macroalgae (circles) as a function of mean thallus diameters, measured where thalli commonly broke (e.g., stipes). Data were extracted from the following references: *Calliarthron cheilosporioides* (Martone 2006), *Mastocarpus stellatus* (Dudgeon and Johnson 1992), *Chondrus crispus* (Carrington et al. 2001), *Endocladia muricata* (Hale 2001), *Mazzaella splendens* (Shaughnessy et al. 1996), *Prionitis lanceolata* (Hale 2001), *Mastocarpus papillatus* (Carrington 1990), *Egregia menziesii* (Friedland and Denny 1995), *Turbinaria ornata* (Stewart 2006a,b), *Mazzaella linearis* (Shaughnessy et al. 1996), *Nereocystis luetkeana* (Koehl and Wainwright 1977), *Macrocystis pyrifera* (Utter and Denny 1996), *Fucus gardneri* (Hale 2001), *Laminaria setchellii* (Klinger and DeWreede 1988, Hale 2001), *Postelsia palmaeformis* (Holbrook et al. 1991), and *Durvillaea antarctica* (Koehl 1986, Stevens et al. 2002). If diameters were not explicitly reported, mean thallus diameter was estimated by dividing mean breaking force (N) by mean breaking stress (N · m^{-2}) and assuming a circular cross-section. Diameter measurements of *F. gardneri* were taken by the author at Hopkins Marine Station (Pacific Grove, CA, USA). Error bars were excluded to increase the readability of the graph.

tissues but rarely growing large in cross-section (Fig. 1). Of particular interest are the flexible joints, or "genicula," in the wave-swept articulated coralline *Calliarthron cheilosporioides* (Fig. 2). Genicular tissue is extremely strong—more than 10 times

stronger than some kelp tissues—but genicula rarely exceed 1 mm in diameter (Martone 2006). In addition, *Calliarthron* genicula are formed secondarily via thallus decalcification and may be developmentally incapable of increasing in cross-section (Johansen 1981, but see Martone 2006). Despite this physical size limitation, genicular tissue strengthens as fronds age (Martone 2006). In a sense, *Calliarthron* genicula may grow stronger to compensate for their inability to grow larger.

Strengthening by growing in girth is easily understood, as meristematic growth in algae has been well studied (Klinger and DeWreede 1988, Kogame and Kawai 1996). But strengthening by altering material properties or tissue construction has been largely unexplored. We know relatively little about the material properties of algal tissues or about the effects of material properties or composition on tissue performance. Several studies have observed increases in algal tissue strength along gradients of wave exposure (Armstrong 1987, Johnson and Koehl 1994, Kitzes and Denny 2005), but whether these patterns resulted from selection or responses to environmental conditions is unknown. Few studies have observed changes in tissue properties associated with thallus ontogeny (Kraemer and Chapman 1991, Martone 2006, Stewart 2006b), but none has demonstrated a mechanism underlying such a shift in tissue performance. For example, hypothesized differences in cell wall polysaccharides (alginic acid) did not explain differences in tissue strength (Kraemer and Chapman 1991). Similarly, Carrington et al. (2001) were unable to link tissue properties to carrageenan content in tissue where thalli typically broke.

Genicula in the articulated coralline alga *Calliarthron* present an ideal system for studying mechanical strengthening at the cellular level. Genicula are composed of single tiers of elongated cells, which span the entire distance between calcified intergenicula (Fig. 2b; Johansen 1969, 1981). Thus, any change in genicular tissue is a direct result of changes to genicular cells. Unfortunately, the characteristics of these constitutive cells (e.g., dimen-

FIG. 2. (a) Basal segments of *Calliarthron* fronds, illustrating calcified intergenicula separated by uncalcified genicula (scale bar, 2 mm). (b) Long-section of *Calliarthron* geniculum (scale bar, 0.2 mm). (c) Cross-section of *Calliarthron* geniculum (scale bar, 0.1 mm), visualized by staining the surface of the resin block with methylene blue.

sions, quantities) are poorly described, severely limiting our understanding of genicular tissue and material dynamics. Yet, genicula comprise a wide range of sizes and breakage strengths and differ significantly in their mechanical abilities depending on their age (Martone 2006), all suggesting differences at the cellular level. Most notably, genicula enrich their cell walls with an unknown fibrillar substance as they develop, which makes them thicker than the calcified intergenicular cell walls from which they are derived (Johansen 1974, Borowitzka and Vesk 1978). Genicular cell walls may continue to thicken as genicula age (Yendo 1904), but data supporting this claim are scant. Such cell wall thickening would help explain differences in tissue strengths among young and old *Calliarthron* genicula (Martone 2006).

Here, results from a histological study are presented to characterize the cellular basis for the great tissue strength of *Calliarthron* genicula and to explore mechanisms underlying the tissue-strengthening process. Limitations to genicular growth are investigated by comparing equivalent genicula from young and old fronds. The characteristics of individual genicular cells are quantified, and the contribution of a single cell to overall geniculum strength is estimated. By measuring the proportion of genicular cross-section filled with cell wall, the material strength of the cell wall proper is estimated, and the effect of cell wall dimensions on observed tissue strengths is explored.

MATERIALS AND METHODS

Remarks on estimating growth. Ideally, to assess growth in algal thalli, one should monitor and repeatedly measure individual thalli over time. Unfortunately, this method is impractical to apply to genicula or to their constitutive cells. First, each geniculum is partially obscured from view by calcified flanges that grow down from adjacent intergenicula. Thus, accurate measurements of geniculum cross-sectional areas require destructive sampling (i.e., breaking the fronds). Furthermore, whole genicula are impervious to vital stains, such as Calcofluor White (personal observation), which previous studies used to prestain algal cells and to measure new growth after stain application (Waaland and Waaland 1975). This imperviousness is likely a consequence of the densely packed, thick-walled genicular cells. Thus, comparing genicula across different age classes may be the only practical option for estimating growth.

Sample collection and preparation. Twenty fronds of *C. cheilosporioides* were collected haphazardly from a single study site at Hopkins Marine Station in Pacific Grove, California (36°36′ N, 121°53′ W). The site was located in the low-intertidal zone at the landward end of a moderately wave-exposed surge channel. The collection site was also used and described by Martone (2006). Fronds consisted of two age classes, old ($n = 10$; 14.0 ± 2.5 cm, mean length ± SD) and young ($n = 10$; 4.0 ± 0.5 cm), corresponding to the large and small size classes used in Martone (2006).

Fronds were removed from their crustose bases by cutting the basal geniculum with a knife. Fronds were immersed in dilute fixative (1% glutaraldehyde, 1% formaldehyde, 98% filtered seawater) for 24 h and then decalcified in 1 N HCl for 24 h. The first and 10th genicula (counting up from the basal geniculum) were dissected out of young and old fronds by cutting through neighboring decalcified intergenicula. For additional comparison, genicula were also dissected near the tips of old fronds, ∼1 cm from apices. The distances from 10th genicula to the tips of young fronds were roughly equivalent to the distances from apical genicula to the tips of old fronds. Given that *Calliarthron* fronds exhibit apical growth (Johansen and Austin 1970), 10th genicula from young fronds and apical genicula from old fronds were assumed to be similar in age.

Samples ($n = 50$) were dehydrated with ethanol (25, 50, and 75%: 2 h each), infiltrated with Spurr's resin (Standard ''Firm'' recipe, 33, 50, 66, and 100%: 24 h each), and cured overnight in a 70°C oven. Thin cross-sections (4 μm) were cut through genicula using an MT2-B ultramicrotome (DuPont Instruments-Sorvall, Newton, CT, USA), stained with 2% methylene blue, mounted with Permount, and allowed to set for 24 h before imaging.

Histological calculations. Cross-sections of whole genicula were digitally photographed (Nikon Coolpix 4500 camera, Tokyo, Japan) under low magnification (×100; Olympus, CH compound microscope, Tokyo, Japan). The major and minor diameters of genicula were measured using an image-analysis program written in LabView (version 7.0; National Instruments, Austin, TX, USA), and the cross-sectional areas (A_{gen}) were calculated assuming elliptical cross-sections.

Genicular cross-sections were digitally photographed under high magnification (×400; Fig. 3, a and b). Methylene blue stained genicular cell walls but not cell lumens, allowing these components of individual cells to be distinguished and measured. Resin embedding had no measurable effect on cell dimensions, based on measurements of fresh genicula. In cross-section, cells resembled hexagons packed tightly together (Fig. 3c), an arrangement also noted by Yendo (1904). Preliminary measurements revealed that cells situated at the genicular periphery (within the outer one-sixth of any radius) were distinct from central cells (within the inner five-sixths of any radius) and were analyzed separately. An image-analysis program was written in LabView to measure genicular cells as follows. Representative regions, containing ∼100 cells, were selected from the center and periphery of each geniculum. Partial cells at the edge of the regions of interest were not measured, and data in excess of 100 cells per region were later discarded at random. Within each region, the program identified all cell lumens and measured their areas (A_{lumen}), based on the number of pixels (Fig. 3c). The program then measured the distance (d) between the center of each cell lumen and the center of its nearest neighbor (Fig. 3c). Thus the radius of each cell (r_{cell}) was estimated to be $d/2$, and the cross-sectional area of each cell (A_{cell}) was calculated according to the area of a hexagon:

$$A_{cell} = \frac{d^2\sqrt{3}}{2} \qquad (1)$$

Assuming circular cell lumens, the radius of each lumen (r_{lumen}) was calculated from its area:

$$r_{lumen} = \sqrt{\frac{A_{lumen}}{\pi}} \qquad (2)$$

The thickness of each cell wall (w) was calculated as the difference between radii

$$w = r_{cell} - r_{lumen}$$
$$= \frac{d}{2} - \sqrt{\frac{A_{lumen}}{\pi}} \qquad (3)$$

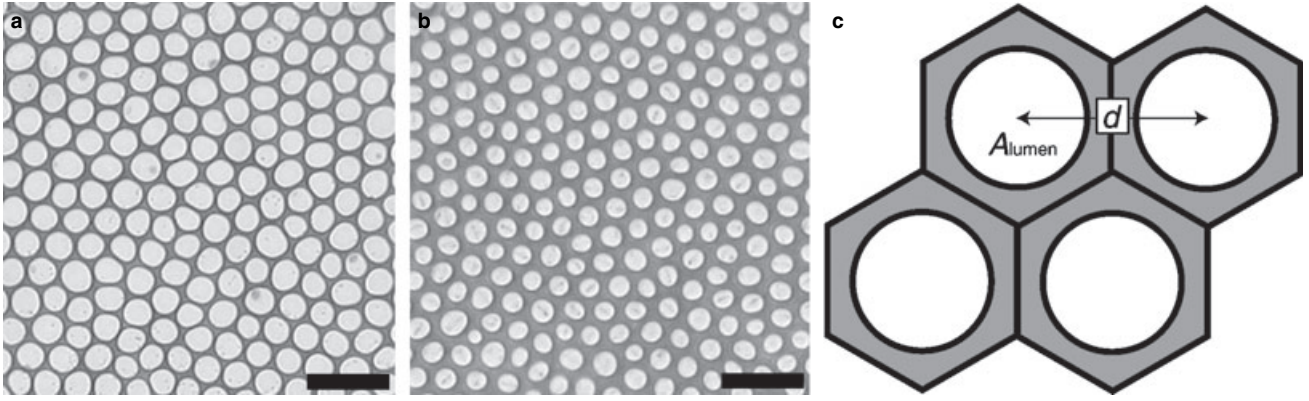and the area of each cell wall (A_{wall}) was estimated to be the difference between lumen and cell areas:

FIG. 3. Histological cross-sections of a young geniculum (a) and an old geniculum (b), both stained with methylene blue (scale bars, 20 μm). (c) Cells were assumed to be hexagonal in shape and were characterized by measuring areas of cell lumens (A_{lumen}) and distances to nearest neighbors (d).

$$A_{wall} = A_{cell} - A_{lumen}$$
$$= \frac{d^2\sqrt{3}}{2} - A_{lumen} \qquad (4)$$

The LabView program measured these cell characteristics in each geniculum ($n = 50$), thereby providing a complete characterization of 10,000 genicular cells.

The average cross-sectional area of central cells was used to estimate the number of cells (N_{cells}) in each geniculum:

$$N_{cells} = \frac{A_{gen}}{(mean\ A_{cell})_{center}} \qquad (5)$$

Since central and peripheral cells had similar cross-sectional areas, only central-cell measurements were used in this calculation.

The percent of genicular cross-sections occupied by cell wall was deduced from the percent of central and peripheral cell cross-sections occupied by cell wall:

$$\%\,cell\,wall_{gen} = \frac{5}{6}(\%\,cell\,wall_{center}) + \frac{1}{6}(\%\,cell\,wall_{periphery})$$
$$= \frac{5}{6}\left(\frac{mean\ A_{wall}}{mean\ A_{cell}}\right)_{center} + \frac{1}{6}\left(\frac{mean\ A_{wall}}{mean\ A_{cell}}\right)_{periphery} \qquad (6)$$

Since cell wall areas (A_{wall}) differed substantially among central and peripheral cells, measurements from both regions were used in this calculation.

Data analysis. The average girths of equivalent genicula (i.e., first genicula, 10th genicula) from young and old fronds were compared using Student's *t*-tests. The effects of cell position, age class, and frond identity on cell cross-sectional area (A_{cell}) were evaluated using three-way analysis of variance (ANOVA). To ensure independence, data were subsampled so that for each age class, central cells were analyzed from half the fronds (selected randomly), and peripheral cells from the other half. To retain statistical power, the first and 10th genicula were analyzed separately. Variances were significantly different (Cochran's test, $P < 0.05$), and transformations had negligible effects. Because ANOVA interpretation is generally robust given very large sample sizes (Underwood 1999), untransformed data were analyzed.

The effects of age class (fixed factor) and geniculum area (A_{gen}; covariate) on average cross-sectional area of central cells [mean (A_{cell})$_{center}$; $n = 50$] were determined using analysis of

covariance (ANCOVA). The effects of age class (fixed factor) and geniculum area (A_{gen}; covariate) on the numbers of genicular cells (N_{cells}; $n = 50$) were also determined by ANCOVA. In both analyses, variances were not significantly different (Cochran's test, $P > 0.05$), and slopes were not significantly different ($P = 0.40$ and $P = 0.29$, respectively). A single linear regression was used to predict the number of genicular cells comprising genicula of a given size.

Martone (2006) demonstrated that *Calliarthron* genicula resisted a breaking force (F_b) according to their cross-sectional area and age class, such that for old genicula, $F_b = 18.49\,A_{gen} + 2.81$ ($r^2 = 0.76$, $P < 0.001$), and for young genicula, $F_b = 14.42\,A_{gen} + 3.49$ ($r^2 = 0.72$, $P < 0.001$). These regressions were used to predict the breaking forces of all genicula measured in this study. Estimated breaking forces were plotted against the number of cells in each geniculum, and linear regressions were fitted to data from each age class. An ANCOVA (age class, fixed factor; N_{cells}, covariate) was used to compare regression slopes, which represented the breaking force per cell from a given age class.

The effects of age class, geniculum, and frond identity on cell wall thickness (w) were evaluated using three-way ANOVAs. In this case, central and peripheral cells were analyzed separately to retain statistical power. In addition, cell wall thicknesses of cells from apical genicula in old fronds and 10th genicula in young fronds were compared using a three-way ANOVA with age class, cell position, and frond as factors. All data were subsampled, as described above, to ensure independence. As with the first ANOVA, variances were significantly different (Cochran's test, $P < 0.05$), but given the very large sample sizes, untransformed data were analyzed.

Martone (2006) observed that old genicula were significantly stronger per cross-sectional area (mean $\sigma_{old} = 25.9$ MN · m^{-2}) than young genicula (mean $\sigma_{young} = 21.5$ MN · m^{-2}). Here, the average percent of genicular cross-sectional areas occupied by cell wall (mean % cell wall$_{gen}$) was compared across young ($n = 20$) and old ($n = 20$) genicula, averaging over the first and 10th genicula. To estimate the breaking stresses of genicular cell walls, mean breaking stresses of young and old genicula were divided by respective mean % cell wall$_{gen}$.

The effects of age class, cell position, and frond identity on cell wall thickness (w) were evaluated using three-way ANOVAs. Data were subsampled, as described above, and the first and 10th genicula were analyzed separately. Again, variances were significantly different (Cochran's test, $P < 0.05$), but the high degree of replication ensured that untransformed data could be interpreted reliably. ANOVAs were performed using GMAV

Table 1. Genicular cross-sectional areas and constitutive cell dimensions, means ± 95% CI, measured in three genicula (first, 10th, and apical), two age classes (young and old), and two cell positions (center and periphery).

	A_{gen} (mm^2)			A_{cell} (µm^2)			w (µm)		
	First	10th	Apical	First	10th	Apical	First	10th	Apical
Old									
Center	0.61 ± 0.10	0.64 ± 0.08	0.21 ± 0.03	38.8 ± 0.5	39.3 ± 0.4	38.9 ± 0.6	0.82 ± 0.02	1.01 ± 0.02	0.62 ± 0.02
Periphery				39.5 ± 0.5	35.5 ± 0.5	36.6 ± 0.5	1.28 ± 0.02	1.35 ± 0.02	1.28 ± 0.02
Young									
Center	0.69 ± 0.10	0.56 ± 0.06		40.1 ± 0.4	35.4 ± 0.5		0.46 ± 0.01	0.35 ± 0.01	
Periphery				37.4 ± 0.5	33.2 ± 0.5		1.04 ± 0.02	0.72 ± 0.02	

A_{gen}, geniculum cross-sectional area; A_{cell}, cell cross-sectional area; w, cell wall thickness.

software (Version 5, University of Sydney, Australia). All other statistical tests were performed using JMPIN software (Version 3.2.1; SAS Institute Inc., Cary, NC, USA).

RESULTS

Geniculum size. First genicula from young and old fronds were not significantly different in cross-sectional area (Table 1; Student's *t*-test, df = 18, P = 0.26). The same was true for 10th genicula from young and old fronds (Table 1; Student's *t*-test, df = 18, P = 0.13). Apical genicula in old fronds were smaller than all the first and 10th genicula (Table 1).

Cell area. On average, *Calliarthron* genicular cells measured 37.5 ± 0.2 µm^2 (mean ± 95% confidence interval [CI]; n = 10,000). Significant differences in genicular cell area (A_{cell}) were found among fronds, representing normal variability within the population, but this variability was not partitioned in any predictable way (Table 2). Cell area did not vary significantly among the centers and peripheries of genicula or among young and old age classes in either the first or 10th genicula (Tables 1 and 2). Moreover, large and small genicula were all composed of similarly sized cells (Fig. 4a; ANCOVA, $F_{1,46}$ = 0.64, P = 0.42). In sum, all genicula were made of cells of comparable cross-sectional area (Fig. 4a; Table 1).

Cell number. Differences in geniculum cross-sectional area can be explained by differences in

Table 2. Analysis of variance (ANOVA) results for the cross-sectional area of cells, A_{cell}, in the first genicula and 10th genicula.

Source of variation	df	F	P-value
First genicula			
Age	1	0.01	0.97
Position	1	0.02	0.88
Age × position	1	2.29	0.15
Frond (age × position)	16	123.84	**<0.001**
10th genicula			
Age	1	0.37	0.55
Position	1	0.63	0.43
Age × position	1	0.51	0.49
Frond (age × position)	16	159.76	**<0.001**

ANOVA factors were age class (two levels: young and old; fixed, orthogonal), position (two levels: center and periphery; fixed, orthogonal), and frond (five levels; random, nested) given 100 replicates. Boldface indicates significant effects.

numbers of genicular cells. That is, larger genicula were composed of significantly more cells (Fig. 4b; ANCOVA, $F_{1,46}$ = 133.86, P < 0.001). The smallest geniculum (A_{gen} = 0.16 mm^2), an apical geniculum from an older frond, had ~4242 cells, while the largest geniculum (A_{gen} = 0.93 mm^2), the first geniculum of an older frond, had ~23,806 cells. This correlation was independent of geniculum age; for a given geniculum size, young and old genicula had similar numbers of cells (Fig. 4b; ANCOVA, $F_{1,46}$ = 1.69, P = 0.20). In general, N_{cells} = 26,323 (A_{gen}); that is, a 1 mm^2 geniculum has ~26,323 cells (Fig. 4b).

Genicula with more constitutive cells resisted greater breaking forces (Fig. 5). This correlation was significant in both young genicula (F_b = 0.0004 N_{cells} + 5.42, r^2 = 0.63, P < 0.001) and old genicula
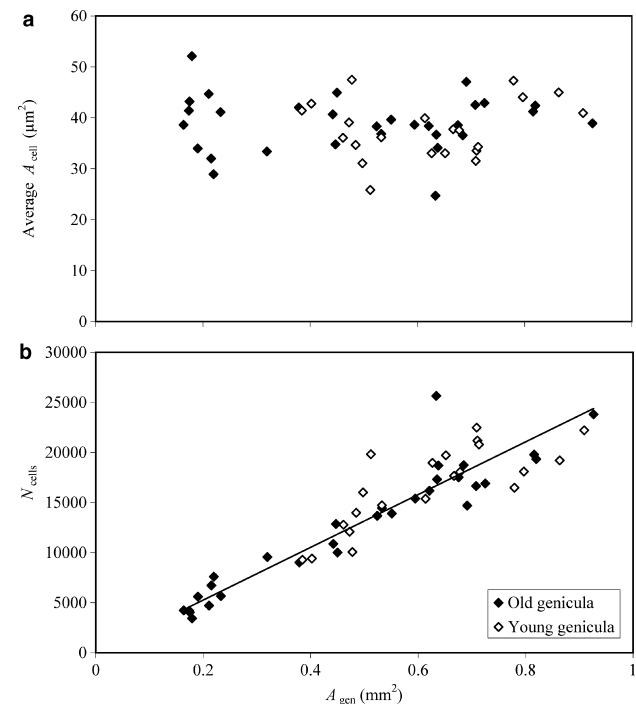

Fig. 4. (a) Average cross-sectional area of central cells and (b) number of constitutive cells as functions of geniculum cross-sectional area, comparing young genicula (white diamonds) and old genicula (black diamonds).
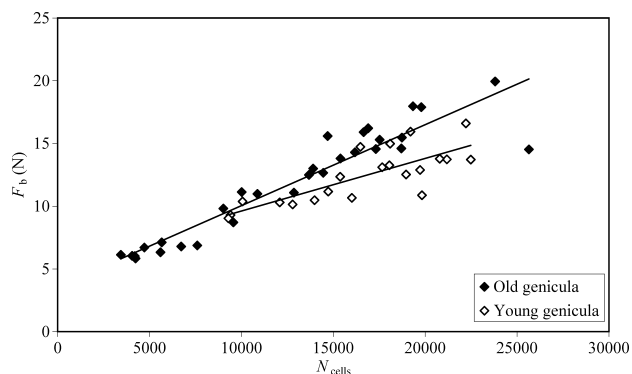
FIG. 5. Estimated breaking force (F_b) as a function of number of constitutive cells (N_{cells}), comparing young genicula (white diamonds) and old genicula (black diamonds). The slopes of these regressions are used to estimate the forces to break individual cells.

$$(F_b = 0.0006 \quad N_{cells} + 3.59, \quad r^2 = 0.89, \quad P < 0.001).$$
Regression slopes were significantly different (ANCOVA, $F_{1,46} = 6.26$, $P < 0.05$).

Cell wall thickness. Cell wall thickness varied significantly among cells in the various fronds (Table 3). Cells in old genicula had significantly thicker cell walls than cells in young genicula (Tables 1 and 3; Fig. 6). This pattern was evident in

TABLE 3. Analysis of variance (ANOVA) results for cell wall thickness, w, at the center and periphery of genicula.

Source of variation	df	F	P-value
Center			
Age	1	51.42	**<0.001**
Geniculum	1	0.29	0.60
Age × geniculum	1	3.15	0.10
Frond (age × geniculum)	16	78.75	**<0.001**
Periphery			
Age	1	15.65	**<0.001**
Geniculum	1	2.75	0.12
Age × geniculum	1	2.73	0.12
Frond (age × geniculum)	16	129.59	**<0.001**

ANOVA factors were age class (two levels: young and old; fixed, orthogonal), geniculum (two levels: first and 10th; fixed, orthogonal), and frond (five levels; random, nested) given 100 replicates. Boldface indicates significant effects.
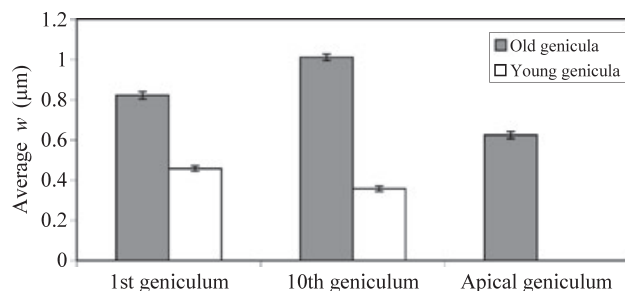

FIG. 6. Average thickness of cell walls (w) in central cells from the first, 10th, and apical genicula in old genicula (gray bars) and young genicula (white bars). Error bars represent 95% CI ($n = 1000$).

both the first and 10th genicula (Fig. 6), and parallel ANOVA results were obtained from both centers and peripheries of genicula (Table 3). Cells from the apical genicula in old fronds had significantly thicker cell walls than cells from the 10th genicula in young fronds (Tables 1 and 4; Fig. 6). This pattern was evident at both centers and peripheries of genicula (Table 4).

On average, young genicula were 33.6 ± 0.3% cell wall (mean ± 95% CI), and old mature genicula were 54.2 ± 0.3% cell wall (Fig. 7). Given the published tissue strengths of young and old genicula in *Calliarthron* (Martone 2006), young cell wall material was calculated to have a breaking strength of 64.0 MN · m^{-2}, and old cell wall material was calculated to have a breaking strength of 47.8 MN · m^{-2} (Fig. 7).

Cells near the periphery of genicula had thicker cell walls than central cells (Tables 1 and 5; Fig. 8). This pattern was consistent among both young and old genicula, although older genicula had significantly thicker cell walls (Tables 1 and 5; Fig. 8). Data from the first and 10th genicula gave parallel ANOVA results (Table 5).

TABLE 4. Analysis of variance (ANOVA) results for cell wall thickness, w, in genicula of approximately the same age: apical genicula from old fronds and 10th genicula in young fronds.

Source of variation	df	F	P-value
Geniculum	1	32.92	**<0.001**
Position	1	65.67	**<0.001**
Geniculum × position	1	7.18	**<0.05**
Frond (geniculum × position)	16	49.98	**<0.001**
SNK post hoc tests			
Apical > 10th (both cell positions)			**<0.05**
Periphery > center (both genicula)			**<0.01**

ANOVA factors were geniculum (two levels: 10th and apical; fixed, orthogonal), position (two levels: center and periphery; fixed, orthogonal), and frond (five levels: random, nested in geniculum × position) given 100 replicates. Boldface indicates significant effects.

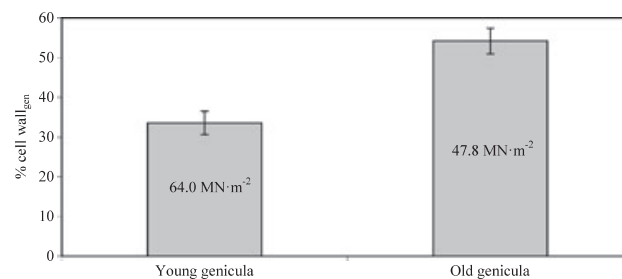SNK, Student–Newman–Keuls.

FIG. 7. Percent of old and young geniculum cross-sectional areas filled with cell wall (% cell wall$_{gen}$), averaged over the first and 10th genicula. Error bars represent 95% CI ($n = 20$). Calculated cell wall breaking strengths are also reported. Note that old cell wall is weaker, per cross-sectional area, than young cell wall.

TABLE 5. Analysis of variance (ANOVA) results for cell wall thickness, w, in the first genicula and 10th genicula.

Source of variation	df	F	P-value
First genicula			
Age	1	6.34	**<0.05**
Position	1	14.72	**<0.01**
Age × position	1	0.27	0.61
Frond (age × position)	16	189.32	**<0.001**
10th genicula			
Age	1	48.66	**<0.001**
Position	1	10.05	**< 0.01**
Age × position	1	0.12	0.73
Frond (age × position)	16	96.44	**<0.001**

ANOVA factors were age class (two levels: young and old; fixed, orthogonal), position (two levels: center and periphery; fixed, orthogonal), and frond (five levels: random, nested in age × position) given 100 replicates. Boldface indicates significant effects.
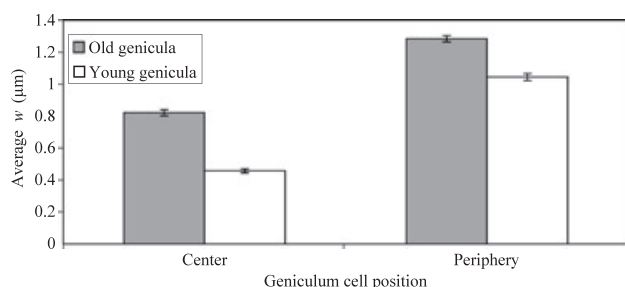

FIG. 8. Average thickness of cell walls (w) in central and peripheral cells from old first genicula (gray bars) and young first genicula (white bars). Error bars represent 95% CI ($n = 1000$).

DISCUSSION

Constraints on geniculum growth. Old genicula were not significantly larger than young genicula at either the first or the 10th geniculum position, suggesting that genicula do not grow in cross-sectional area once they have decalcified. This result highlights a major difference in growth—and thus strengthening—strategies between genicula and other algal tissues and is consistent with our understanding of genicular development: the cortical cells responsible for increasing thallus girth dissolve as the geniculum is revealed (Johansen 1969). Conversely, Martone (2006) reported significant, albeit slight, changes in cross-sectional area using similar methods to those reported here. Such conflicting results emphasize the difficulty in assessing growth via comparisons, where population variability may confound any effect of age. Thus, without direct measurements of individual genicula through time, whether genicula grow after maturation remains somewhat of an open question. However, such growth seems contrary to normal coralline development, and, even if it occurs, genicula remain quite small.

Building blocks of genicula. Calliarthron genicula are composed of thousands of long fiber-like cells. The large number of cells is routinely misrepresented, for the sake of simplicity, in drawings from previous publications (Johansen 1981, Martone 2006). In this study, an average geniculum (0.54 mm^2) was composed of ~14,300 cells. The average size of these constitutive cells is quite consistent across genicula, regardless of the size or age of the geniculum; genicular cells are ~37.5 μm^2 in cross-section or ~6.5 μm in diameter.

Geniculum cross-sectional area varies greatly (up to an order of magnitude), even within a single *Calliarthron* frond (Martone 2006). Given the uniformity of constitutive cell size, differences in cross-sectional area can be explained by differences in cell numbers. For instance, larger genicula, generally situated near the bases of fronds, may have five times as many cells as smaller genicula, situated near the frond apices. This trend suggests that each new geniculum has fewer cells than the one before it, possibly defining an inherent size limit to which *Calliarthron* fronds can grow. Whether any single geniculum can grow by increasing the number of constitutive cells is unknown but is unlikely; cell division is probably difficult, given that the ends of genicular cells are firmly calcified and embedded in adjacent intergenicula, and evidence of such a process would likely be seen in cells of varying size.

Larger genicula are capable of resisting more force than smaller genicula (Martone 2006), mainly because they are composed of more cells. It therefore seems pertinent to describe the breaking strength of a geniculum building block: a single cell. This can be estimated from the regression slopes in Figure 5, measured in force (N) per cell. In an old mature geniculum, an average cell can resist 0.0006 N, a seemingly modest strength, capable of supporting, for example, a few grains of rice. Yet when combined with 25,000 other cells in a mature geniculum, these cells can support a 2 kg hanging mass.

Cell wall thickening. Data clearly suggest that genicular cell walls thicken over time, a surprising result given that cell wall thickening has not been observed in other algal taxa and is generally considered a developmental process associated with terrestrial plant tissues, such as xylem (Niklas 1992, Raven et al. 2003). Nevertheless, older genicula had cell walls up to three times thicker than younger genicula. Because old and young genicular cells are not significantly different in size, new material must be added to the inside of each cell wall, as indicated by the smaller lumens of old genicular cells (Fig. 3). Cell walls provide structural support to algal cells, and differences in cell wall thickness help explain observed differences in genicular tissue strengths among young and old fronds (Martone 2006). For instance, judging from the differing regression

slopes in Figure 5, older thick-walled cells may be able to resist 50% more force than young thin-walled cells. Thus, by fortifying their cells with additional cell wall material, genicula increase their ability to resist breakage, helping fronds persist and reproduce for many years (Johansen and Austin 1970). Interestingly, cells from apical genicula in old fronds had significantly thicker cell walls than cells from 10th genicula in young fronds, even though these genicula are assumed to be approximately similar in age. This suggests that cell wall thickening may be more than a simple ontogenetic process and may also depend upon environmental parameters that may differ between basal and apical genicula, such as light interception, nutrient delivery, or drag force experienced by genicula in a given wave climate.

Within any geniculum, peripheral cells have thicker cell walls than central cells, a possible adaptation to resisting bending stresses. Intertidal *Calliarthron* fronds are constantly pulled back and forth by breaking and receding waves, and when genicula bend, the cells farthest from the center and nearest the periphery experience the most stress (see Denny 1988). Thus, by reinforcing these peripheral cells, fronds may resist bending. With few exceptions (Koehl and Wainwright 1977), most studies of algal biomechanics assume thalli are homogeneous in cross-section, and mechanical differences within algal tissues have largely been unexplored. Investigations into the distribution of materials within other macroalgal tissues may improve biomechanical models of algal breakage, especially those that consider complex loading regimes, such as bending.

Cell wall material strength. Because genicular tissue consists of cells packed tightly together, we can assume that applied forces are resisted directly by the cell walls of constitutive cells. This provides a unique opportunity to estimate the strength of geniculum cell wall—the true ''material'' strength of genicula—by adjusting the published tissue strengths of genicula by the percent cell wall, essentially factoring out the cell lumens, which presumably contribute little to tissue strength.

This novel approach to estimating mechanical strength at the subcellular level has provided insight into tissue biomechanics. First, young cell wall material is stronger than old cell wall material (Fig. 7). This difference implies that the thickening process is more complicated than simply accreting more of the same material in the same way into each cell wall. One explanation is that cell walls weaken over time as new material is added to wall interiors. Another hypothesis is that wall thickening is a 2-fold process, resulting in two distinct cell wall layers. For example, cell wall layers may be chemically similar but deposited differently, as microfibril orientation can differ among primary and secondary cell walls in terrestrial plants (Niklas 1992). Primary cell wall material is likely accreted as genicular cells elongate, suggesting that cell wall microfibrils might be oriented longitudinally and therefore ideal for resisting tensile stresses; whereas secondary cell wall material is added after genicular cells cease elongating, suggesting that secondary microfibrils might be oriented more radially, making them less effective in resisting tension. Alternatively, secondary cell wall material could be accreted in the same orientation but might be composed of a mechanically weaker substance—at least when stressed in tension. Although distinct layers have been documented within primary walls of other genicula (Borowitzka and Vesk 1979), the development of true secondary cell walls has never been described in marine algae, yet would be consistent with the findings of Yendo (1904). Additional experiments are currently underway to explore differences among these cell wall materials and to determine their molecular composition.

Martone (2006) reported that *Calliarthron* genicular tissue is stronger than other algal tissues. But is genicular tissue really made of uniquely strong materials? Cell walls in mature *Calliarthron* genicula have breaking strengths of 47.8 MN $\cdot$ m^{-2} (Fig. 7). This study is the first to report the strength of cell wall, independent of overall tissue strength, from a wave-swept macroalga. Interestingly, cell walls in the filamentous freshwater green alga *Chara corallina* have a breaking strength of 47.0 MN $\cdot$ m^{-2} (Toole et al. 2001), remarkably similar to *Calliarthron* cell wall strength, considering *Calliarthron* likely experiences significantly more hydrodynamic stress in the wave-swept intertidal zone. This comparison suggests that genicula probably gain their great strength from packing their cross-section full of cell wall (up to 50%) and not from using especially strong materials in their construction. This comparison also suggests that much of the variation in macroalgal tissue strength (e.g., Fig. 1) may be explained by tissue construction, rather than by material composition. Further exploration into other macroalgal tissues is needed to resolve these patterns.

Kelp versus coralline. That red macroalgae have more slender thalli than brown macroalgae (Fig. 1) may not be surprising, as brown macroalgae are often larger than red macroalgae in every dimension. However, size differences cannot explain the apparent pattern in tissue-breaking stress across this wide range of algal taxa. Such a pattern clearly suggests a trade-off between growing thicker thalli and developing stronger tissues (e.g., we have not identified macroalgae with both thick thalli and strong tissues). Kelps are generally composed of weak tissues but can grow large in cross-section to increase their mechanical ability, while *Calliarthron* genicula have relatively strong tissues but are probably incapable of growing in girth.

These two strengthening strategies are not entirely mutually exclusive. For example, the feather boa kelp, *Egregia menziesii* (Turner) Aresch. develops

stronger tissues when experimentally grown in high-flow conditions (Kraemer and Chapman 1991), although the ultimate breaking strength reported (2.5 MN · m^{-2}) was still not very strong: only half that reported for *Egregia* in Figure 1. Likewise, although red algae lack true secondary meristems, they are still generally capable of increasing their girth (Shaughnessy 2004), albeit less so than kelps. Future tests of the trade-off between girth and tissue strength will undoubtedly prove to be informative. Do small wave-swept brown algae, such as *Petalonia* or *Scytosiphon*, have strong tissues like small red algae? Can differences in tissue strengths be universally explained by differing tissue construction rather than by differing material composition? That is, are the strengths of all macroalgal cell walls comparable?

The growth strategies of kelps and corallines increase mechanical strength but differ widely in their scope. For instance, kelps grow outward in girth by adding new cells at the stipe surface, a process limited only by an ability to support underlying medullary tissue. Conversely, *Calliarthron* genicula grow inward by thickening their cell walls, a process limited by space, as the cells slowly compress their organelles and cytoplasm. Thus, in general, the brown algal strategy of growing in girth conveys a much greater potential for resisting drag forces. Perhaps as a consequence, kelps and other brown macroalgae can successfully produce the largest fronds in the wave-swept intertidal zone, while *Calliarthron* and most red algal fronds must remain relatively small.

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