

RESEARCH ARTICLE

Indefatigable: an erect coralline alga is highly resistant to fatigue

Mark Denny^{1,*}, Katharine Mach², Sarah Tepler^{2,3} and Patrick Martone⁴

¹Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950, USA, ²Carnegie Institution for Science, Stanford, CA 94305, USA, ³Department of Biology, University of California, Santa Cruz, CA 95064, USA and ⁴Botany Department, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

*Author for correspondence (mwdenny@stanford.edu)

SUMMARY

Intertidal organisms are subjected to intense hydrodynamic forces as waves break on the shore. These repeated insults can cause a plant or animal's structural materials to fatigue and fail, even though no single force would be sufficient to break the organism. Indeed, the survivorship and maximum size of at least one species of seaweed is set by the accumulated effects of small forces rather than the catastrophic imposition of a single lethal force. One might suppose that fatigue would be especially potent in articulated coralline algae, in which the strain of the entire structure is concentrated in localized joints, the genicula. However, previous studies of joint morphology suggest an alternative hypothesis. Each geniculum is composed of a single tier of cells, which are attached at their ends to the calcified segments of the plant (the intergenicula) but have minimal connection to each other along their lengths. This lack of neighborly attachment potentially allows the weak interfaces between cells to act as 'crack stoppers', inhibiting the growth of fatigue cracks. We tested this possibility by repeatedly loading fronds of *Calliarthron cheilosporioides*, a coralline alga common on wave-washed shores in California. When repeatedly loaded to 50–80% of its breaking strength, *C. cheilosporioides* commonly survives more than a million stress cycles, with a record of 51 million. We show how this extraordinary fatigue resistance interacts with the distribution of wave-induced water velocities to set the limits to size in this species.

Key words: fatigue failure, *Calliarthron cheilosporioides*, hydrodynamic forces, size limits, intertidal ecology.

Received 17 May 2013; Accepted 8 July 2013

INTRODUCTION

As ocean waves break on a rocky shore they are accompanied by water velocities that commonly exceed 10 ms^{-1} and at times can reach 35 ms^{-1} . These extreme flows can impose substantial hydrodynamic forces on intertidal organisms, with the potential to break or dislodge them (Denny, 1988; Denny, 1995; Vogel, 1994; Denny and Wethey, 2001). Unlike many animals, which use rigid shells and strong adhesives to resist drag and lift, most intertidal seaweeds cope by being flexible. Their blades, which at low velocities spread out to capture sunlight, reconfigure at high velocities. By bending with the flow they reduce the velocity of water relative to them and become more streamlined (e.g. Boller and Carrington, 2006; Carrington, 2007; Martone et al., 2012). Flexibility is so crucial for seaweeds that it has been deemed a 'necessary prerequisite' to survival in a wave-swept environment (Harder et al., 2004).

For most seaweeds, flexibility is an inherent consequence of the compliant materials from which they are constructed (Martone, 2007). Crustose coralline algae are an exception. By calcifying their cell walls, they make themselves rigid. The adaptive advantage of calcification is a matter for debate (e.g. Johansen, 1981; Padilla, 1993), but one of its disadvantages is clear. Lacking flexibility, crustose corallines cannot form structures that extend into flow, and they consequently run the risk of being shaded or overgrown. However, ~100 million years ago, crustose corallines developed flexible joints (genicula), which – when interspersed among the rigid intergenicula – allow the overall structure to bend in flow (Aguirre

et al., 2010). This strategy evolved separately in three lineages of coralline algae (the corallinoids, amphiroids and metagoniolithoids) (Johansen, 1981), and has been successful: at many wave-exposed sites around the globe, erect corallines are the dominant competitor for space in the low intertidal zone.

In order for the frond (technically, the thallus) of a coralline alga to have the same flexibility as that of non-calcified algae, the genicula (which comprise only ~15% of branch length) (Martone and Denny, 2008a) must be more flexible than the tissues of fleshy seaweeds. This increased flexibility is achieved through a combination of morphology and material properties (Martone, 2006; Martone and Denny, 2008a). In general, genicula have a smaller cross-section than the calcified parts of the thallus, which, by reducing the second moment of area, facilitates bending (Denny, 1988). To bend safely, however, the joint's material must be able to withstand the increased force per area (stress) and the resulting increase in proportional extension (strain) that accompany increased flexibility. Genicular material is indeed stronger and more extensible than material from other algae (Hale, 2001; Martone, 2007). For example, the genicular material of *Calliarthron cheilosporioides* Manza (a representative articulate coralline) is four times as strong and six times as extensible as the thallus material of its neighboring uncalcified red alga *Mastocarpus papillatus* Kützinger (Hale, 2001; Kitzes and Denny, 2005).

Flexibility of genicula is also augmented by the arrangement of cells in the joint. In corallinoids (of which *C. cheilosporioides* is the best-studied example), a geniculum is formed when the thallus

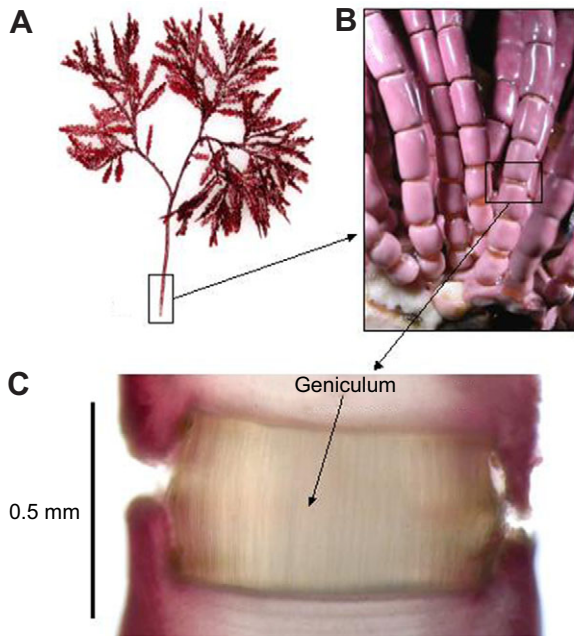


Fig. 1. *Calliarthron cheilosporioides*. (A) An entire frond. (B) A close-up of the basal portion of fronds showing flexible genicula separating the calcified intergenicula. (C) A cross-section through a representative geniculum showing the single tier of genicular cells.

locally decalcifies. The resulting joint comprises a single tier of cells, each anchored at its ends to a calcified intergeniculum (Fig. 1). Taking into account the size, shape and material properties of genicula and intergenicula, Martone and Denny (Martone and Denny, 2008a) formulated a numerical model of *C. cheilosporioides*'s thalli, which they tested by comparing its predictions with the flexibility of actual thalli. If the model assumed that genicular cells were firmly attached to each other as well as to the intergenicula, the predicted stiffness of the thalli was too high. Instead, flexibility was best predicted when the model assumed that each individual cell in the joints behaved as a separate cable with no substantial connection to its neighbors. As support for this assumption, Martone and Denny noted that the periphery of a geniculum is often frayed, with individual broken cells protruding (Martone and Denny, 2008a).

The apparent lack of connection between genicular cells has potential consequences beyond its contribution to joint flexibility. Several researchers (Denny et al., 1989; Hale, 2001; Mach et al., 2007a; Mach et al., 2007b; Mach, 2009) proposed that the breakage of intertidal seaweeds could be due not to the imposition of a single lethal force but rather to fatigue of the plant's material induced by the repetition of sublethal forces. As with other materials, algal tissues inevitably contain small flaws, traditionally referred to as 'cracks'. If a crack's tip is sharp, stress is amplified locally such that, even when the overall stress on a thallus is small, stress at the tip can exceed the material's strength, setting the stage for the crack to lengthen (Fig. 2A). Repeated application of small stresses can cause the crack to gradually grow – that is, to fatigue – and the material eventually breaks. Breakage can have serious consequences. Fatigue failure – rather than the imposition of extreme hydrodynamic forces – controls the rate of survival, and thereby the maximum size, of the common intertidal red alga *Mazzaella flaccida* (Mach et al., 2011).

A crack can grow, however, only if two criteria are met: (1) stress at the crack tip must exceed the material's breaking stress, and (2)

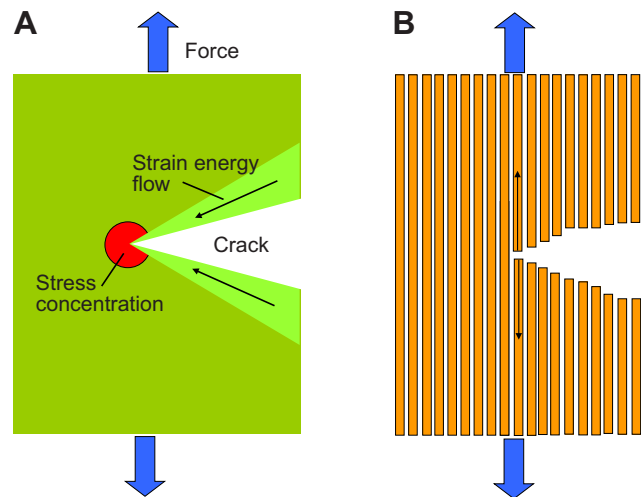


Fig. 2. Crack propagation in a homogeneous material (A) and a series of parallel cables (genicular cells) (B). In the homogeneous material, shear connection between cells allows strain energy to flow to the tip of the crack where, in conjunction with stress concentration, new crack area can be created. In the geniculum-like material (B), the crack tip is blunted by the interface between cells, and the lack of shear connection between cells ensures that when a cell breaks (as shown by the arrows), strain energy cannot be passed to the adjacent intact cell.

sufficient energy must be available to create new crack surface area. The morphology of *C. cheilosporioides*'s genicula appears to reduce the chance that these requirements can be met. First, if genicular cells act as separate cables, the tip of a crack will be blunted when it reaches the interface between cells (Fig. 2B), forming an extreme example of what is known as a Cook–Gordon crack stopper (Cook and Gordon, 1964; Gordon, 1976). Second, if genicular cells are disconnected, their independence likely inhibits or precludes the transfer of energy to the crack tip. Energy required for spontaneous crack growth is provided by the release of elastic potential energy (strain energy) as cells break at the crack tip (Fig. 2A). In *M. flaccida*, this is easily achieved because adjacent cells in the material are firmly attached in shear; as one cell breaks and attempts to slide by its neighbors, the resulting shear stress delivers strain energy to the intact cells. By contrast, if adjacent genicular cells act independently, energy released when one cell breaks cannot be transferred to a neighbor (Fig. 2B). If cells are weakly attached, some strain energy can flow to the crack tip, but flow will be curtailed if energy is dissipated by viscous processes as the loosely connected cells shear past each other. In short, even if local stress exceeds breaking stress in a geniculum, crack growth will likely be prevented by the lack of available strain energy at the crack tip. Together, these factors (crack blunting and inefficient energy transfer) suggest that articulated coralline algae should be resistant to fatigue failure. If so, their success on wave-swept shores may be due (at least in part) to their enhanced ability to cope with the repeated stresses imposed by waves.

Four questions thus arise. Are adjacent cells in the genicula of *C. cheilosporioides* disconnected? If connected, is substantial energy dissipated in shear? As a result of disconnection and/or dissipation, are genicula resistant to fatigue? And lastly, what role might fatigue resistance play in the survivorship of this seaweed? As we will see, genicular cells act as loosely connected cables that dissipate energy in shear, leading to an extraordinary resistance to fatigue failure. In

fact, *C. cheilosporioides* is essentially immune to fatigue, allowing it to grow to sizes that would otherwise be unattainable.

MATERIALS AND METHODS

Test species

Individual fronds of *C. cheilosporioides* were collected at Hopkins Marine Station (Pacific Grove, CA, USA), from the same site used in several previous experiments (Hale, 2001; Martone, 2006; Martone and Denny, 2008a; Martone and Denny, 2008b; Mach et al., 2011). Fronds were held in chilled seawater until tested, usually within 5 days.

Shear modulus and resilience

We explored the mechanical connection between genicular cells indirectly by comparing the material's tensile stiffness (its tensile modulus, E) with its shear stiffness (shear modulus, G). For an isotropic, elastic material, $E/G=3$ (Timoshenko and Gere, 1972; Wainwright et al., 1976; Vogel, 2009). If adjacent genicular cells are not connected – that is, if the cells behave as separate cables – the material's shear modulus should be substantially reduced and E/G should be significantly greater than 3.

To measure the energy dissipated as genicular cells are sheared, we measured the joints' resilience. Resilience is the fraction of the energy required to deform a material that is stored and released as the material returns to its initial state (Alexander, 1966; Wainwright et al., 1976). As noted above, high shear resilience facilitates effective crack growth, low resilience inhibits growth.

Both measurements were made by loading genicula in torsion, a regime that shears the joint material (Fig. 3A). A section of thallus was cut from near the base of a frond. One end of this specimen was glued (using cyanoacrylate adhesive) to the crossbar of the apparatus such that the specimen hung down like a rope. Its free

end was then glued to the top of a cylindrical polyvinylchloride bob. There were five to eight genicula between the glued portions of the 'rope', and both the suspended bob and test specimen were free to rotate around their common vertical axis. The rotational moment of inertia of the bob, J , was $1.61 \times 10^{-6} \text{ kg m}^2$. Two wire nubs on the bob allowed us to twist the test piece around its axis to an initial angular deflection. The bob was then released, whereupon it oscillated in rotation, and the instantaneous angle of rotation, θ , was recorded by a high-speed video camera ($250 \text{ frames s}^{-1}$, Fastcam 512 PCI, Photron USA, San Diego, CA, USA). The thallus was kept moist with seawater during the short duration of the test.

From the video record, we measured T , the period of oscillation, and Y_i , the amplitude of oscillation in each cycle (Fig. 3B). T was estimated as $2T_{1/2}$, the interval between successive zero crossings in the time record. After testing, the specimen was dissected to determine the dimensions of each of the n genicula: length, L_i , and the major and minor semi-axes of the cross-section, a_i and b_i , respectively. From a_i and b_i , we calculated the rotational second moment of area, I_i (m^4), for each geniculum (Roark and Young, 1975):

$$I_i = \frac{\pi a_i^3 b_i^3}{(a_i^2 + b_i^2)}. \quad (1)$$

These values allowed us to calculate the shear modulus and resilience of the material.

Shear modulus is (Timoshenko and Gere, 1972) (see Appendix):

$$G = \frac{4\pi^2 J \left(\frac{L_1}{I_1} + \frac{L_2}{I_2} + \dots + \frac{L_n}{I_n} \right)}{T^2}. \quad (2)$$

The modulus of a material often varies with strain (Wainwright et al., 1976), and to account for this possibility we recorded the strain for each of our measurements of G . In torsion, the strain of a material varies linearly with distance from the center of rotation. For the nearly circular cross-section of genicula, average strain γ is approximately:

$$\gamma = \frac{2\theta_{\max} a_{\text{avg}}}{3L_{\text{tot}}}, \quad (3)$$

where θ_{\max} is the maximum angular rotation of the free end of the specimen in an oscillation, and a_{avg} and L_{tot} are the average of the major and minor semi-axes and summed length of genicula in the specimen, respectively. In our calculation of E/G for *C. cheilosporioides* we used the value of E (27.6 MPa) measured by Hale (Hale, 2001). The ability of the system to measure G accurately was assessed by measuring the ratio of E to G for a cylindrical length of an isotropic elastic material (neoprene rubber); the mean value of E/G was 2.91 (95% confidence limits 1.79–3.85), not significantly different from the expected value of 3.00.

Resilience is (Alexander, 1966):

$$R = \frac{Y_2}{Y_1}, \quad (4)$$

where Y_1 and Y_2 are successive positive amplitudes (Fig. 3B). Some energy was lost to drag on the bob as it rotated through the air. To correct for this, we measured the resilience of the bob when it was suspended from a highly resilient fine tungsten wire. The hysteresis of the bob alone ($1-R=0.0715$) was added to the measured resilience of the bob–seaweed combination to correct for the loss to drag.

Thirty specimens were tested, each from different individuals, and three experiments were conducted on each specimen.

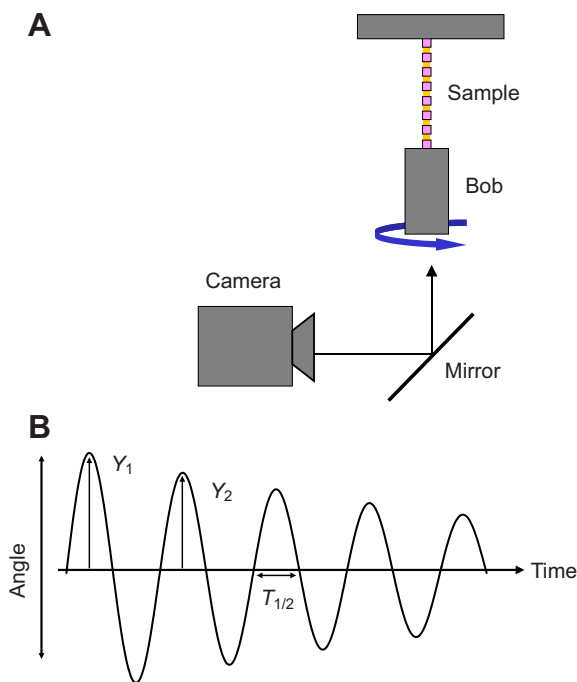


Fig. 3. Torsion tests on genicula. (A) A cylindrical bob is suspended by the test sample. After being twisted and released, the bob and sample oscillate in rotation, motion that is recorded by the high-speed camera. (B) The dimensions of a hypothetical damped oscillation. Y_1 and Y_2 , successive positive amplitudes of oscillation; T , period of oscillation.

Resistance to fatigue

In the field, genicula are loaded primarily in tension. The ability of genicula to resist fatigue from tensile loads was measured by subjecting sections of thallus to repeated tensile stresses and counting the number of repetitions required to break them. To perform these tests, an apparatus was constructed to apply precisely controlled stresses (Fig. 4). The test specimen – cut from the basal section of a thallus – was glued into small acrylic plastic sockets using underwater-setting epoxy putty (A-788 Splash Zone Compound, Kop-Coat, Rockaway, NJ, USA) with four to six genicula between sockets. One socket was then bolted to a strain-gauge force transducer (Fort100, World Precision Instruments, Sarasota, FL, USA) and the other was attached *via* a thin, stranded, stainless steel rope to the business end of an oscillating beam. Oscillation was effected by a cam, driven by a computer-controlled stepping motor rotating at 10.1 Hz. The amplitude of oscillation – and thereby the tensile strain imposed on the test specimen – was set by the translational position of the cam, which was controlled by a screw-driven slide attached to a second computer-controlled stepping motor. The position of the force transducer was similarly set by a computer-controlled slide. The force transducer was calibrated by hanging accurately known weights from it. Maximum strain rates in the fatigue-testing apparatus ranged from ~ 38 to 61 s^{-1} depending on peak strain, a function of peak stress.

Each test was performed as follows. Before the test specimen was glued into the sockets, the cross-sectional areas of its end genicula were measured and averaged as a prediction of the nominal area of genicula in the stressed portion of the specimen. Because much of each geniculum is hidden from view by the flanges of the intergenicula (Fig. 1C), it was not practical to measure genicular lengths directly. Instead, each geniculum was assumed to have a length equal to the average measured during previous experiments. The position of the cam was adjusted so that the minimum force of each oscillation was zero and the peak force was sufficient to impose a nominal stress in the test genicula equal to a set fraction of the known one-time average breaking stress of this species (25.9 MPa) (Martone, 2006). The apparatus was then allowed to apply repeated cycles of this stress, automatically adjusting the position of the cam and force transducer as necessary to account for any creep in the material. (Creep reduces the cross-sectional area of genicula, and therefore reduces the force required to achieve the desired stress.) The computer counted the number of cycles until the specimen broke. In the calculation of the change in cross-sectional area during creep, genicula were assumed to maintain a constant volume, and

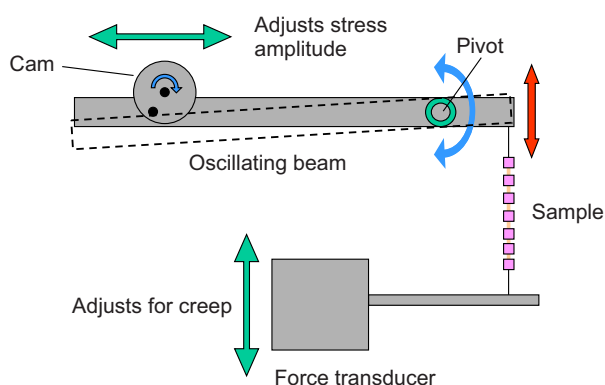


Fig. 4. An apparatus for measuring the fatigue characteristics of *C. cheilosporioides* through the application of repeated stresses. See Materials and methods for an explanation.

creep was assumed to be uniform across genicula. During a test, the specimen was continuously immersed in a seawater bath maintained at 12–13°C; water in the bath was changed daily. After breakage, the cross-section of the failed geniculum was measured and used to calculate the actual stress imposed during the experiment. Twenty-five specimens were tested.

Stress in the field

Water velocities on the shore where specimens were collected were estimated using a previously described device (Mach et al., 2011). In short, this apparatus measured (at 100 Hz) the force imposed on a small, roughened sphere of known drag characteristics held adjacent to the rock surface. Data were stored internally in the apparatus and subsequently read in the lab, where measured force was used to estimate water velocity. Measurements were made at 10 locations near that where *C. cheilosporioides* were collected (vertical height on the shore within ~ 0.5 m, horizontal location within ~ 20 m). Velocity records were then analyzed to give the maximum velocity, U , in each 10 s period. A total of $n=3,974,400$ 10 s periods were recorded. Velocities were sorted in ascending order: U_1 is the lowest, U_2 the next lowest, and so forth to U_n . From these data, we calculated the cumulative exceedance function, $P(U)$, the probability that – in a 10 s period chosen at random – velocity exceeds U (see Gaines and Denny, 1993; Denny and Gaines, 2000):

$$P(U_i) = 1 - \frac{i}{n+1}. \quad (5)$$

Each velocity was used to estimate the corresponding tensile stress, σ , on the genicula of a *C. cheilosporioides* frond. Stress imposed on a joint is governed by drag on the thallus, which is in turn a function of water velocity U (ms^{-1}) and density ρ (1025 kgm^{-3}), thallus planform area S (m^2), drag coefficient C_d , and basal genicular cross-sectional area A (assumed to be $0.87 \times 10^{-6} \text{ m}^2$) (Martone and Denny, 2008b):

$$\sigma = \frac{1}{2} \frac{\rho U^2 S C_d}{A}. \quad (6)$$

Drag coefficient depends on both S and U (Martone and Denny, 2008b):

$$\log_{10} C_d = -2.06 + 223.24 (\log_{10} Re_f)^{-3.58}, \quad (7)$$

where Re_f is frond Reynolds number:

$$Re_f = \frac{U \sqrt{S}}{\nu}. \quad (8)$$

The kinematic viscosity (ν) of seawater at 15°C (a typical sea-surface temperature at Hopkins Marine Station) is $1.17 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ (Denny, 1994).

RESULTS

Shear modulus and resilience

Shear modulus decreases with increasing strain (Fig. 5A). At low average strains, the ratio of E to G is close to 3, indicating that there is some connection between adjacent cells, but E/G increases rapidly with increasing strain (Fig. 5B). For $\gamma > 0.15$, E/G is ~ 10 , suggesting that shear connection between genicular cells is disrupted at these strains.

The average resilience of genicular material in shear is 0.399 (95% confidence limits ± 0.018), indicating that only $\sim 40\%$ of the energy that goes into deforming the material in shear is stored and available for crack propagation. There is no significant correlation between resilience and initial average strain ($r=0.0298$, 81 d.f., $P>0.5$).

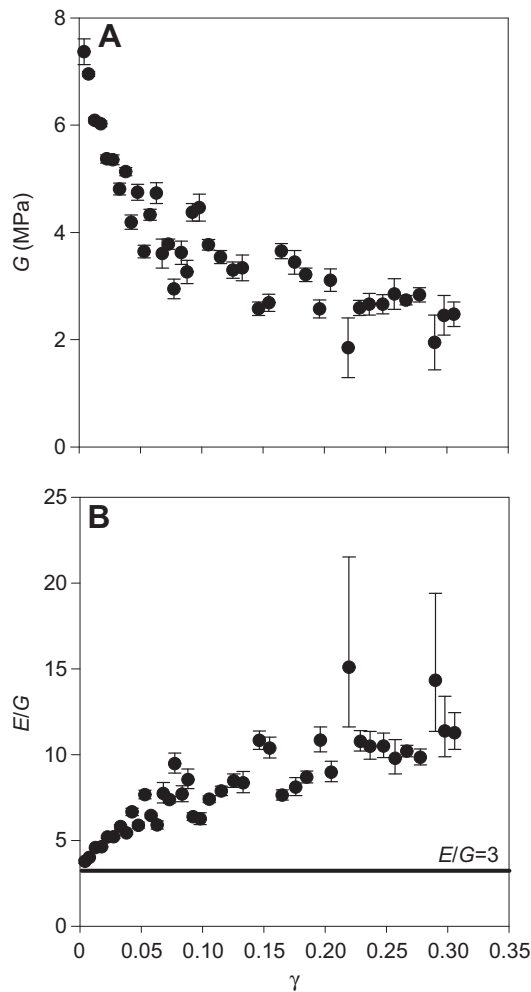


Fig. 5. (A) The shear modulus (G) of *C. cheilosporioides* genicular material decreases with increasing average strain (γ). The points shown here are binned averages across all tests; the error bars are standard errors. The exceptionally large standard errors at strains of ~ 0.21 and 0.29 are due to the chance occurrence of a small number of measurements in these bins. (B) The ratio of tensile (E) to shear modulus increases with increasing average strain, and except at very low strains is well above the ratio of 3 expected for an isotropic material (shown by the solid line). Again, the error bars are standard errors.

Resistance to fatigue

The genicula of *C. cheilosporioides* are highly resistant to fatigue (Fig. 6). Even when loaded to 80% of their one-time breaking stress (20.1 MPa, $\log_{10} N=7.30$), the joint material can, on average, withstand more than a thousand loading cycles, and at 60% of one-time breaking stress (15.1 MPa, $\log_{10} N=7.18$) it can survive more than 10 million cycles. The record longevity (51 million cycles at 50% of one-time breaking stress) could have been even more impressive; in order to proceed with the experiments, this test was terminated after 58 days with the specimen still intact. Specimens always broke in the genicula rather than in the intergenicula.

Stresses in the field

For an individual wave chosen at random, the probability of encountering a stress high enough to threaten *C. cheilosporioides* is low (Fig. 7). The distribution shown here is for a thallus with a planform area of 20 cm^2 , a typical size found at this site.

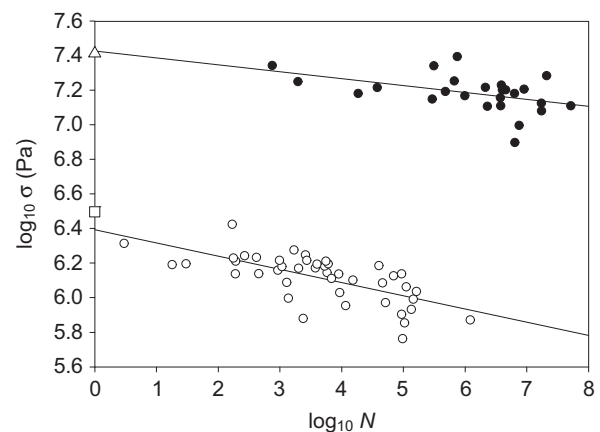


Fig. 6. *Calliarthron cheilosporioides* (filled circles) is highly resistant to fatigue. The equation for the regression line is $\log_{10} \sigma = -0.0399 \log_{10} N + 7.428$ ($r^2=0.210$), where σ is the peak stress applied in each cycle and N is the number of cycles required to break the specimen. The open triangle is the average one-time breaking stress for *C. cheilosporioides*. For comparison, analogous data are shown for male *Mazzaella flaccida* (from Mach, 2009) (open circles). The equation for this regression line is $\log_{10} \sigma = -0.0766 \log_{10} N + 6.394$ ($r^2=0.455$). The open square is the one-time breaking stress for *M. flaccida*.

In 220 of the recorded intervals (0.0055%), exceptionally high forces were registered, corresponding to apparent velocities $>30 \text{ m s}^{-1}$ and as high as 53 m s^{-1} . These extreme forces were likely due either to impingement [the brief impulse described by Gaylord (Gaylord, 1999)] or to impact with a solid missile (Shanks and Wright, 1986). The stiff force transducer was capable of recording this sort of near-instantaneous force, whereas the reorientation of flexible algae makes it highly unlikely that they can experience such brief loads (Gaylord et al., 2001). Evidence to this effect can be drawn from the measurements made by Martone and Denny at the same site (Martone and Denny, 2008b). Using spring dynamometers – which more closely match the response time of algae than does the stiff force transducer – they measured forces corresponding to a maximum velocity of 22.1 m s^{-1} . In short, the stresses calculated from these 220 extreme measured forces (denoted by the red portion of the curve in Fig. 7B) are in all likelihood spuriously high.

DISCUSSION

Predicting fatigue in the field

As hypothesized, *C. cheilosporioides* is highly resistant to fatigue failure. At a comparable fraction of one-time breaking stress (e.g. 60%), the joints of *C. cheilosporioides* can withstand 100,000 times the number of stress cycles of the blades of male *M. flaccida*, the most fatigue-resistant alga previously tested (Mach, 2009).

To estimate the fatigue a frond would suffer in the field, we use Miner's law (Mach et al., 2011). If, for a given stress applied to a geniculum, N cycles are required (on average) for the joint to fail, each cycle contributes a fraction $\Delta F=1/N$ to the failure of the joint. If M stresses are applied to the joint – each with its own particular stress – the overall fraction of the joint's fatigue resistance is the sum of the individual fractions:

$$F_{\text{tot}} = \sum_{i=1}^M \Delta F_i \quad (9)$$

Fatigue accumulates until $F_{\text{tot}}=1$, at which point the frond breaks.

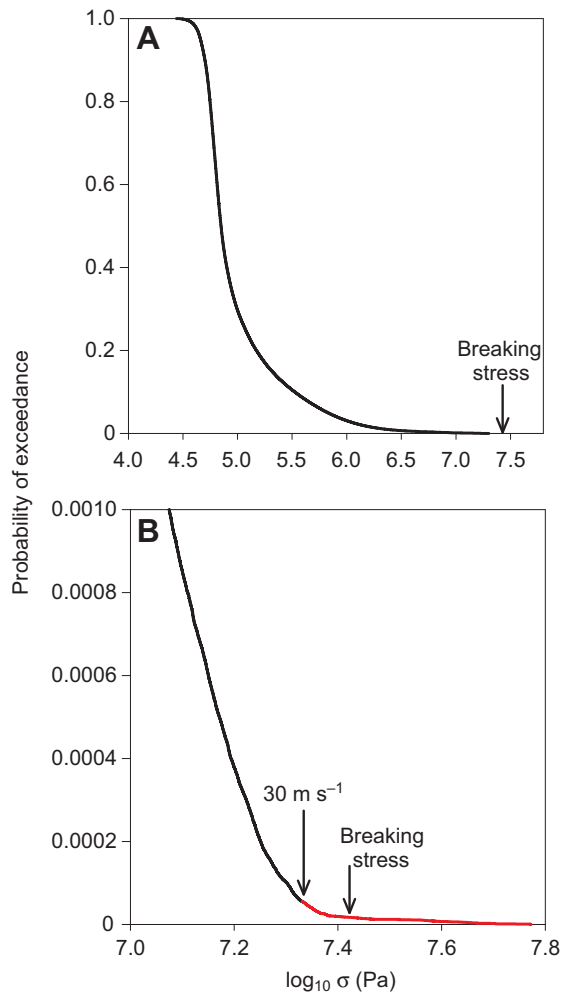


Fig. 7. The distribution of stresses imposed by waves at the field site, calculated for a frond with planform area $S=20\text{ cm}^2$. (A) The complete distribution. (B) Distribution of the right-hand tail of highest stresses. The arrow labeled '30 m s⁻¹' denotes the stress imposed by a velocity of this magnitude. Larger stresses (the red portion of the curve) probably are not imposed on *C. cheilosporioides* in this system (see Results).

From Fig. 6, we know that for *C. cheilosporioides*:

$$N = 10^{\left(\frac{-\log_{10}\sigma - 7.428}{0.0399} \right)}. \quad (10)$$

Thus,

$$F_{\text{tot}} = \sum_{i=1}^M \left[\frac{1}{10^{\left(\frac{-\log_{10}\sigma_i - 7.428}{0.0399} \right)}} \right]. \quad (11)$$

Given this information, we picked a velocity at random from our measured data and calculated the corresponding stress for a plant of given frond area. Repeating this process 3,153,600 times (the number of 10s intervals in a year), we calculated F_{tot} for a year. Because we are interested in the effects of fatigue separate from those of one-time breakage, any stress that exceeded breaking stress was not included in the calculations. The calculation of F_{tot} was repeated 10 times for each planform area, and the results were averaged (Fig. 8, red line). The likelihood of fatigue failure is very low ($F_{\text{tot}} < 1$) until frond area reaches $\sim 20\text{ cm}^2$.

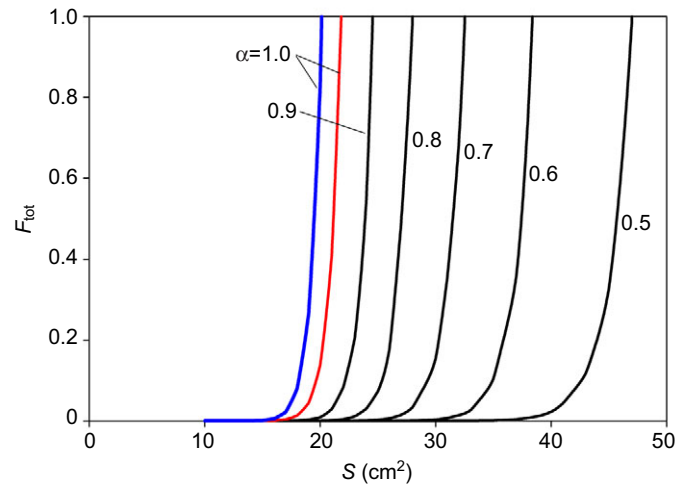


Fig. 8. Annual summed contributions of fatigue (F_{tot}) as a function of frond planform area (S). The lines are calculated for fronds with different fractions (α) of their area exposed to flow. The blue line depicts F_{tot} for $\alpha=1.0$ recalculated for 6 years, rather than 1 year, of exposure.

These data are for a 1 year exposure to waves, and the results of Martone (Martone, 2010) suggest that *C. cheilosporioides* can live for approximately 6 years. One might suppose that longer exposure would lead to stricter limits on size. However, calculated size limits change only slightly for multiple years of exposure. For example, the 6 year estimate of F_{tot} is shown by the blue line in Fig. 8.

These calculations assume that the entire thallus is exposed to mainstream flow. *Calliarthron cheilosporioides* grows in tightly packed clumps, so it is likely that only a fraction α of frond area is exposed to flow (Johnson, 2001). If so, larger sizes can be reached. For reasonable values of α (0.5–1.0), the size at which plants are predicted to fail due to fatigue in a year (20–40 cm², Fig. 8, black lines) encompasses the range of maximum sizes found at this site (Martone and Denny, 2008b). Note that these calculations do not take growth into account, and therefore overestimate F_{tot} . For example, a plant that has a frond area of 20 cm² at the end of a year would have had a smaller area earlier in the year, and thus smaller ΔF values.

Fatigue failure (as calculated here) is due primarily to contributions from a small number of exceptionally high velocities that load the plant nearly to its breaking stress. Smaller loads have negligible effect. To see this, consider ΔF as a function of load, where load is expressed as a fraction of one-time breaking stress, σ_{break} (Fig. 9). For loads as high as 60% of σ_{break} , a single cycle contributes a vanishingly small ΔF of 2.85×10^{-7} to the fatigue sum for *C. cheilosporioides* (Eqn 9). A load cycle in excess of 86% of σ_{break} is needed to contribute 1% of the total needed for breakage. If we exclude from consideration velocities greater than 30 m s⁻¹ (which are unlikely to apply correspondingly large hydrodynamic forces, see above), effective velocities high enough to load thalli to 86% of σ_{break} (22.3 MPa, $\log_{10}=7.35$) do not occur at this site unless frond area is greater than $\sim 20\text{ cm}^2$ (see Fig. 7). In short, *C. cheilosporioides* can be broken only by the imposition of a single fatal stress or a few near-fatal stresses; and these high stresses occur only in large plants.

This conclusion is compatible with that of Martone and Denny (Martone and Denny, 2008b), who used maximum velocity (and the resulting one-time maximum stress) to predict maximum frond area. Their narrow theoretical prediction closely matched the

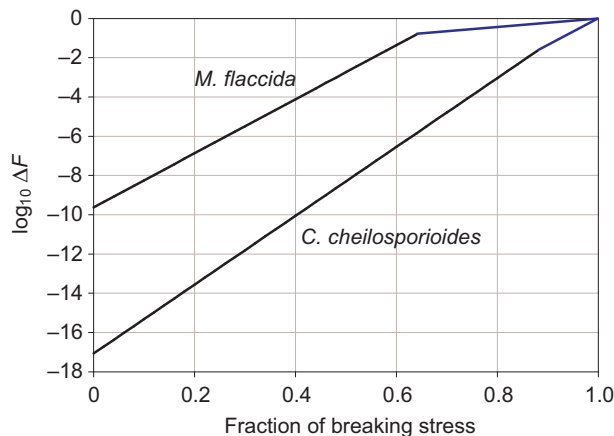


Fig. 9. The regressions of Fig. 6 are replotted to show ΔF , the contribution of individual stresses to fatigue, as a function of the magnitude of stress, where stress magnitude is expressed as a fraction of one-time breaking stress. Each individual stress in *M. flaccida* contributes much more to fatigue than does a comparable relative stress imposed on *C. cheilosporioides*. The blue lines are extrapolations for applied stresses larger than those used in the experiments. Because, by definition, a single cycle is needed to break a sample at its one-time breaking stress, these extrapolations extend from the end of the measured data to the point $\Delta F=1$, fraction of breaking stress=1.

average maximum size found in the field, a match possible only if maximum velocity or a few near-maximum velocities – rather than the accumulated fatigue from multiple low velocities – is the cause of frond breakage.

By contrast, fatigue controls survival in *M. flaccida*. For instance, each time *M. flaccida* is loaded to 60% of σ_{break} , it notches another 4% toward breakage (Fig. 9). As a result, a few tens of relatively small stresses can cause failure, explaining why Mach and colleagues (Mach et al., 2011) found that the maximum size of *M. flaccida* observed in the field was substantially smaller than that predicted on the basis of maximum velocity alone.

Resilience

When loaded in tension rather than shear, *C. cheilosporioides* has a resilience of ~ 0.89 [calculated from the data reported in Gaylord et al. (Gaylord et al., 2001)], similar to values measured for 25 other species of intertidal macroalgae (mean \pm s.d.= 0.749 ± 0.118 , range=0.529 to 0.971) (Hale, 2001). The disparity between resilience in tension (0.89) and shear (0.40) for *C. cheilosporioides* suggests that the material from which genicular cells are constructed is capable of effectively storing elastic potential energy, but the sliding of cells past each other in shear introduces substantial additional viscosity into the system. Shear resilience data in other algal species are not available for comparison.

Evolution of fatigue resistance

The indefatigable nature of *C. cheilosporioides* joints raises an obvious question: if fatigue failure limits the survival of other algae, why have they not evolved the sort of resistance found in *C. cheilosporioides*? There is an obvious answer: something has to hold the organism together. Genicular cells can function effectively as separate cables only because their ends are firmly attached to intergenicula. Uncalcified blades have no supporting structures analogous to intergenicula. Thus, blades can maintain their integrity only if cells are well connected in both tension and shear, concomitantly increasing the likelihood of fatigue failure.

It remains to be seen whether the joints of other articulated corallines have fatigue resistance on a par with that of *C. cheilosporioides*. Because other corallinoids have joint ontogeny and morphology similar to that of *C. cheilosporioides*, we suspect that they are similarly fatigue resistant. Metagoniolithoids and amphiroids develop their joints differently, however (Johansen, 1981). In particular, because they have multiple tiers of cells they may not have the independence in shear exhibited by *C. cheilosporioides*, and therefore may be less resistant to fatigue.

Caveats

Several aspects of the fatigue tests reported here differ from conditions found in the field. In our experiments, peak stresses were applied 10.1 times per second. By contrast, waves arrive only every 4–20 s when the tide is in and (at the low level where *C. cheilosporioides* occurs on the shore) sporadically when the tide is out. It is possible that, in the intervals between applied loads (especially at low tide), plants in the field could repair fatigue damage as it occurs; in our experiments, repair seems unlikely. It should be noted, however, that genicular cells lack much of the intracellular apparatus found in the rest of the plant, so it is uncertain whether joints can repair themselves even under the most favorable circumstances. In the field, *C. cheilosporioides* thrives only where there is substantial water motion. By contrast, water surrounding the test specimens in the laboratory was still and was replaced only once per day. Test specimens often began to bleach (a sign of stress) after a week in the apparatus. Lastly, all our test specimens had been subjected to an unknown number of stresses in the field prior to collection. Together, these factors suggest that *C. cheilosporioides* can survive even more cycles of stress in the field than we observed in the laboratory.

Because of our inability to measure geniculum length prior to a test, and the assumption that creep is uniform across all genicula, there is some uncertainty in the precise stress applied during the fatigue tests. As the specimen lengthens, the force required to maintain a constant stress, σ , is:

$$F = \sigma A_0 \frac{L_{\text{tot}}}{L}, \quad (12)$$

where A_0 is initial cross-sectional area, L_{tot} is the estimated initial summed length of genicula in the test thallus and L is instantaneous length. Any mismeasurement of L_{tot} affects those calculations. For instance, if genicula are longer than the assumed average length, the calculated effect of an increasing L is smaller than it should be, and the applied force is smaller than necessary to maintain constant stress. Similarly, if some genicula creep more than others, the stress applied will vary among genicula, with those genicula that creep the most receiving more stress than those that creep less. Future experiments might consider taking X-ray images of the thallus during testing to measure genicular length and width directly.

Conclusions

Genicular cells act as loosely connected cables that dissipate energy when loaded in shear. As a result, it is difficult for strain energy to be delivered to the tip of a fatigue crack. We propose that this inhibition of energy flow – along with the blunting of a growing crack tip at the interface between cells – accounts for the extraordinary resistance of *C. cheilosporioides* to fatigue failure. The apparent immunity of *C. cheilosporioides* to fatigue allows it to grow to sizes that would otherwise be unattainable, enhancing its ability to compete for space and light in its intertidal environment.

APPENDIX

Calculating shear modulus, *G*

Shear modulus is calculated by combining information about the natural period of oscillation of a thallus in torsion and the rotational stiffness of its genicula. The natural period, *T*, of a rotational oscillator such as the bob–thallus system of Fig. 3 is (Thomson, 1981):

$$T = 2\pi\sqrt{\frac{J}{k}}, \tag{A1}$$

where *J* is the system’s rotational moment of inertia (in this experiment, the bob’s *J*) and *k* is its rotational stiffness. Rotational stiffness is the ratio of applied torque (Γ) to the resulting angular deflection (θ_{tot}), a ratio that (in this system) is determined by two factors: (1) the intrinsic torsional stiffness of genicula (the product of the shear modulus of genicular material, *G*, and the genicula’s rotational second moment of area, *I*) and (2) the length, *L*, of the structure being twisted. If all genicula had the same *I*, thallus stiffness would be:

$$k = \frac{\Gamma}{\theta_{\text{tot}}} = \frac{GI}{L_{\text{tot}}}. \tag{A2}$$

However, because *I* can vary among genicula, we need to account for each individual geniculum’s contribution to θ_{tot} . For a geniculum of length *L*₁ and rotational moment of area *I*₁, the angular deflection resulting from the application of torque Γ is:

$$\theta_1 = \frac{\Gamma L_1}{GI_1}. \tag{A3}$$

For *n* such segments connected in series, the total angular deflection is:

$$\theta_{\text{tot}} = \frac{\Gamma L_1}{GI_1} + \frac{\Gamma L_2}{GI_2} + \frac{\Gamma L_3}{GI_3} + \dots + \frac{\Gamma L_n}{GI_n} \tag{A4}$$

$$= \frac{\Gamma}{G} \left(\frac{L_1}{I_1} + \frac{L_2}{I_2} + \frac{L_3}{I_3} + \dots + \frac{L_n}{I_n} \right). \tag{A5}$$

Substituting this expression for θ_{tot} in Eqn A2, we find that:

$$k = \frac{G}{\left(\frac{L_1}{I_1} + \frac{L_2}{I_2} + \frac{L_3}{I_3} + \dots + \frac{L_n}{I_n} \right)}, \tag{A6}$$

and inserting this expression into Eqn A1 we conclude that:

$$T = 2\pi\sqrt{\frac{J \left(\frac{L_1}{I_1} + \frac{L_2}{I_2} + \frac{L_3}{I_3} + \dots + \frac{L_n}{I_n} \right)}{G}}. \tag{A7}$$

Solving for *G* brings us to our destination:

$$G = \frac{4\pi^2 J \left(\frac{L_1}{I_1} + \frac{L_2}{I_2} + \dots + \frac{L_n}{I_n} \right)}{T^2}. \tag{A8}$$

Thus, by measuring the dimensions of genicula and the rotational moment of inertia and natural period of the system, one can calculate the shear modulus.

LIST OF SYMBOLS AND ABBREVIATIONS

<i>A</i>	cross-sectional area of a basal geniculum
<i>A</i> ₀	initial cross-sectional area

<i>C</i> _d	coefficient of drag
<i>E</i>	tensile modulus
<i>G</i>	shear modulus
<i>I</i>	rotational second moment of inertia
<i>J</i>	rotational moment of inertia
<i>k</i>	rotational stiffness
<i>L</i>	length
<i>N</i>	number of cycles
<i>R</i>	resilience
<i>Re</i> _f	frond Reynolds number
<i>S</i>	planform area
<i>T</i>	period of oscillation
<i>U</i>	velocity
<i>Y</i>	amplitude of oscillation
α	fraction of frond area
γ	average strain
θ	instantaneous angle of rotation
ν	kinematic viscosity
ρ	density
σ	tensile stress

ACKNOWLEDGEMENTS

We thank Tad Finkler for writing the program to control the fatigue-testing apparatus and Anton Staaf for design and construction of the recording wave-force dynamometers.

AUTHOR CONTRIBUTIONS

This study grew out of P.M.’s longstanding interest in, and research of, the mechanics of articulated coralline algae; he contributed ideas and guidance throughout. K.M. conducted and analyzed the wave force measurements. S.T. conducted and analyzed the torsional experiments. M.D. conceived the fatigue hypothesis, designed and built the fatigue testing apparatus, conducted and analyzed the fatigue experiments, and wrote the manuscript.

COMPETING INTERESTS

No competing interests declared.

FUNDING

This research was supported by a National Science Foundation grant [IOS-1052161 to M.D.].

REFERENCES

Aguirre, J., Perfectti, F. and Braga, J.C. (2010). Integrating phylogeny, molecular clocks, and the fossil record in the evolution of coralline algae (Corallinales and Sporolithales, Rhodophyta). *Paleobiology* **36**, 519–533.

Alexander, R. M. N. (1966). Rubber-like properties of the inner hinge-ligament of Pectinidae. *J. Exp. Biol.* **44**, 119–130.

Boller, M. L. and Carrington, E. (2006). The hydrodynamic effects of shape and size change during reconfiguration of a flexible macroalga. *J. Exp. Biol.* **209**, 1894–1903.

Boller, M. L. and Carrington, E. (2007). Interspecific comparison of hydrodynamic performance and structural properties among intertidal macroalgae. *J. Exp. Biol.* **210**, 1874–1884.

Cook, J. and Gordon, J. E. (1964). A mechanism for the control of cracks in a brittle system. *Proc. R. Soc. A* **282**, 508–520.

Denny, M. W. (1988). *Biology and the Mechanics of the Wave-Swept Environment*. Princeton, NJ: Princeton University Press.

Denny, M. W. (1994). *Air and Water: The Physics of Life’s Media*. Princeton, NJ: Princeton University Press.

Denny, M. W. (1995). Predicting physical disturbance: mechanistic approaches to the study of survivorship on wave-swept shores. *Ecol. Monogr.* **65**, 371–418.

Denny, M. W. and Gaines, S. D. (2000). *Chance in Biology*. Princeton, NJ: Princeton University Press.

Denny, M. W. and Wethey, D. (2001). Physical processes that generate patterns in marine communities. In *Marine Community Ecology* (ed. M. Bertness, S. Gaines and M. E. Hay). Sunderland, MA: Sinauer.

Denny, M. W., Brown, V., Carrington, E., Kraemer, G. and Miller, A. (1989). Fracture mechanics and the survival of wave-swept macroalgae. *J. Exp. Mar. Biol. Ecol.* **127**, 211–228.

Gaines, S. D. and Denny, M. W. (1993). The largest, smallest, highest, lowest, longest, and shortest: extremes in ecology. *Ecology* **74**, 1677–1692.

Gaylord, B. (1999). Detailing agents of physical disturbance: wave-induced velocities and accelerations on a rocky shore. *J. Exp. Mar. Biol. Ecol.* **239**, 85–124.

Gaylord, B., Hale, B. B. and Denny, M. W. (2001). Consequences of transient fluid forces for compliant benthic organisms. *J. Exp. Biol.* **204**, 1347–1360.

Gordon, J. E. (1976). *Structures: Why Things Don’t Fall Down*. London: Penguin Books.

Hale, B. B. (2001). Material properties and fatigue fracture mechanics in intertidal macroalgae. PhD dissertation, Stanford University, Stanford, CA, USA.

- Harder, D., Speck, O., Hurd, C. and Speck, T.** (2004). Reconfiguration as a prerequisite for survival in highly unstable flow-dominated environments. *J. Plant Growth Regul.* **23**, 98-107.
- Johansen, H. W.** (1981). *Coralline Algae: a First Synthesis*. Boca Raton, FL: CRC Press.
- Johnson, A. S.** (2001). Drag, drafting, and mechanical interactions in canopies of the red alga *Chondrus crispus*. *Biol. Bull.* **201**, 126-135.
- Kitzes, J. and Denny, M. W.** (2005). Red algae respond to waves: morphological and mechanical variation in *Mastocarpus papillatus* along an exposure gradient. *Biol. Bull.* **208**, 114-119.
- Mach, K. J.** (2009). Mechanical and biological consequences of repetitive loading: crack initiation and fatigue failure in the red macroalga *Mazzaella*. *J. Exp. Biol.* **212**, 961-976.
- Mach, K. J., Nelson, D. V. and Denny, M. W.** (2007a). Techniques for predicting the lifetimes of wave-swept macroalgae: a primer on fracture mechanics and crack growth. *J. Exp. Biol.* **210**, 2213-2230.
- Mach, K. J., Hale, B. B., Denny, M. W. and Nelson, D. V.** (2007b). Death by small forces: a fracture and fatigue analysis of wave-swept macroalgae. *J. Exp. Biol.* **210**, 2231-2243.
- Mach, K. J., Tepler, S. K., Staaf, A. V., Bohnhoff, J. C. and Denny, M. W.** (2011). Failure by fatigue in the field: a model of fatigue breakage for the macroalga *Mazzaella*, with validation. *J. Exp. Biol.* **214**, 1571-1585.
- Martone, P. T.** (2006). Size, strength and allometry of joints in the articulated coralline *Calliarthron*. *J. Exp. Biol.* **209**, 1678-1689.
- Martone, P. T.** (2007). Kelp versus coralline: cellular basis for mechanical strength in the wave-swept seaweed *Calliarthron* (Corallinales, Rhodophyta). *J. Phycol.* **43**, 882-891.
- Martone, P. T.** (2010). Quantifying growth and calcium carbonate deposition of *Calliarthron cheilosporioides* (Corallinales, Rhodophyta) in the field using a persistent vital stain. *J. Phycol.* **46**, 13-17.
- Martone, P. T. and Denny, M. W.** (2008a). To bend a coralline: effect of joint morphology on flexibility and stress amplification in an articulated calcified seaweed. *J. Exp. Biol.* **211**, 3421-3432.
- Martone, P. T. and Denny, M. W.** (2008b). To break a coralline: mechanical constraints on the size and survival of a wave-swept seaweed. *J. Exp. Biol.* **211**, 3433-3441.
- Martone, P. T., Kost, L. and Boller, M.** (2012). Drag reduction in wave-swept macroalgae: alternative strategies and new predictions. *Am. J. Bot.* **99**, 806-815.
- Padilla, D. K.** (1993). Rip stop in marine algae: minimizing the consequences of herbivore damage. *Evol. Ecol.* **7**, 634-644.
- Roark, R. J. and Young, W. C.** (1975). *Formulas for Stress and Strain*, 5th edn. New York, NY: McGraw-Hill.
- Shanks, A. L. and Wright, W. G.** (1986). Adding teeth to wave action: the destructive effects of wave-borne rocks on intertidal organisms. *Oecologia* **69**, 420-428.
- Thomson, W. T.** (1981). *Theory of Vibration with Applications*, 2nd edn. Englewood Cliffs, NJ: Prentice-Hall.
- Timoshenko, S. P. and Gere, J. M.** (1972). *Mechanics of Materials*. New York, NY: Van Nostrand.
- Vogel, S.** (1994). *Life in Moving Fluids*. Princeton, NJ: Princeton University Press.
- Vogel, S.** (2009). *Glimpses of Creatures in Their Physical Worlds*. Princeton, NJ: Princeton University Press.
- Wainwright, S. A., Biggs, W. D., Currey, J. D. and Gosline, J. M.** (1974). *Mechanical Design in Organisms*. Princeton, NJ: Princeton University Press.