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Multiple stressors drive convergent evolution of performance properties in marine macrophytes

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Summary

- Extreme environments have driven the evolution of some of the most inspiring adaptations in nature. In the intertidal zone of wave-swept shores, organisms face physical forces comparable to hurricanes and must further endure thermal and desiccation stress during low tides, compromising their physiological and biomechanical performance.
- We examine how these multiple stressors have influenced the evolution of tissue properties during desiccation using eight phylogenetically independent pairs of intertidal and subtidal macrophytes.
- Intertidal species generally lost water more slowly than their subtidal counterparts, presumably as an adaption to regular emersion. Under partial desiccation, breaking force, strength, and extensibility of intertidal species generally exceeded those of subtidal species, although important differences existed among phylogenetic pairs. This was often true even when subtidal relatives resisted greater forces or were more extensible under full hydration. The interacting effects of mechanical forces and desiccation during low tide are likely a major selective agent in determining macrophyte performance and fitness.
- Overall, we found that lineages that have independently evolved to occupy the wave-swept intertidal have converged on performance metrics that are likely to be adaptive to the interacting stressors associated with their extreme niches.

Introduction

The high-energy intertidal zone represents one of the most extreme environments on Earth in terms of mechanical stressors. Macrophytes (seaweeds and seagrasses) in the wave-swept intertidal zone must endure physical forces exceeding those that would be imposed by hurricane-force winds approximately every 5–10 sec (Denny & Gaylord, 2002; Mach *et al.*, 2011). The intense and chaotic nature of crashing waves imposes hydrodynamic forces (e.g. drag, lift and torsion) on macrophytes and can dislodge them from the substratum or break their tissues (e.g. Seymour *et al.*, 1989; Gaylord, 1999; Haring *et al.*, 2002; Denny & Gaylord, 2002; Demes *et al.*, 2013b). Although in some buoyant species, dislodgement or breakage may be an adaptive means of dispersal (e.g. Stewart, 2006), these processes usually have negative effects on macrophyte survival and reproductive fitness (Dudgeon & Johnson, 1992; Martone & Denny, 2008; Mach *et al.*, 2011; Bettignies *et al.*, 2012). While dislodgement or breakage of tissues near the substratum generally leads to mortality (Dudgeon & Johnson, 1992; Harder *et al.*, 2006; Denny, 2006; Demes *et al.*, 2013b), tissue breakage of any kind can lead to reduced growth and reproductive output since both photosynthesis and propagule production depend on a macrophyte's surface area (Denny, 2006; Wolcott, 2007). Thus, waves have presumably acted as a strong selective pressure on macrophyte traits, promoting the evolution of several biomechanical adaptations that are shared across macrophytes inhabiting these environments. For example, seaweeds and seagrasses have flexible materials that allow them to reconfigure in flow and thereby reduce the forces that they experience (Boller & Carrington, 2006; Martone *et al.*, 2012). Moreover, macrophytes must be structurally reinforced and variation in macrophyte traits – both within and across species – tends to correlate with exposure to crashing waves (e.g. Duggins *et al.*, 2003; Starko & Martone, 2016; Augyte *et al.*, 2018; Starko *et al.*, 2020).

Despite clear evidence that breakage and dislodgement occur in the field (Haring *et al.*, 2002; e.g. Mach *et al.*, 2011; Bettignies *et al.*, 2012), most numerical models predict that macrophytes should never be broken or dislodged under normal circumstances (Koehl & Alberte, 1988; Gaylord *et al.*, 1994; Johnson & Koehl, 1994; Friedland & Denny, 1995; Utter & Denny, 1996; Kitzes & Denny, 2005; Mach *et al.*, 2011) and may be 'overdesigned' to meet their mechanical challenges. This inconsistency between prediction and observation has resulted in a proliferation of

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hypotheses to explain the idiopathic dislodging of intertidal macrophytes, including the following: tissue fatigue from repeated wave loading (Mach, 2009; Mach *et al*., 2011), tissue compromise associated with aging and/or reproduction (Demes *et al*., 2013a; Krumhansl *et al*., 2015), crack initiation from herbivores (e.g. Black, 1976; Bettignies *et al*., 2012) and knotting and tangling of multiple individuals (e.g. Burnett & Koehl, 2018). Additional environmental stressors, notably desiccation, may also increase vulnerability to hydrodynamic forces by altering the structural integrity of macrophyte tissues and making breakage more likely (Haring *et al*., 2002). The biomechanical properties of *Fucus distichus* (formerly *Fucus gardneri*) are compromised by desiccation, and the cumulative effects of multiple stressors (i.e. desiccation and wave forces) may be critical in determining macrophyte survival (Haring *et al*., 2002). This is especially important because it is predicted that the strongest forces felt by an intertidal macrophyte are those shortly after low tide when waves crash directly onto exposed macrophytes (Gaylord, 1999) and when tissues are likely to be desiccated (Haring *et al*., 2002). If the forces from the wave impacts during a returning tide are enough to break the compromised tissues before they fully rehydrate, then we would predict that the evolution of an intertidal lifestyle is further associated with the evolution of improved mechanical performance when desiccated under conditions that resemble those experienced during low tide. However, not all macrophytes experience tissue compromise under normal low tide conditions (Kraemer, 1990), suggesting that some species may have adaptations to resist these effects, and it remains largely unclear how water content, which often makes up more than 80% of macroalgal tissues (Stagnol *et al*., 2016; Starko *et al*., 2020), influences the material properties of macrophyte tissues in general.

In this study, we use a phylogenetically informed experimental design to test whether intertidal macrophytes have evolved tissue traits that allow them to both avoid desiccation during aerial exposure and to tolerate wave forces following low tide. Marine macrophytes provide a rich landscape to test for associations between intertidal habitat use and tissue properties; across red (Rhodophyta), green (Chlorophyta), and brown algae (Ochrophyta), there are countless independent origins of intertidal habitat use from presumably subtidal ancestors. Despite these lineages exhibiting contrasting growth forms, they evolved to occupy an extreme environment with a common set of biomechanical challenges. We leverage this diversity to examine whether independently evolved intertidal lineages have converged on certain material or structural properties in response to the interacting effects of desiccation stress and wave forces following low tide. Moreover, given that intertidal habitats are associated with intense crashing waves, as opposed to the slower and more predictable flow of subtidal currents or rolling surge (Denny *et al*., 1985; Gaylord, 1999; Denny & Gaylord, 2002), we expect that intertidal species should have tissues that are generally better adapted to prevent breakage. We examined eight pairs of closely related intertidal and subtidal species spanning red algae, brown algae and marine angiosperms (seagrasses) to address the following questions: (1) Do intertidal lineages retain water for longer than their subtidal relatives as an adaptation to avoid desiccation stress? (2) Do tissues of intertidal species resist greater forces, or have material properties that better absorb wave forces (e.g. increased extensibility), than their subtidal relatives to better withstand greater wave forces? (3) Do intertidal species exhibit enhanced mechanical performance under desiccation relative to their subtidal relatives? Additionally, we assess the effect of tissue hydration on mechanical properties more generally by comparing mechanical properties between tissues that are fully hydrated and fully dehydrated.

Materials and Methods

Study species and sample collection

To assess whether responses to desiccation varied between intertidal and subtidal species, we selected phylogenetic clades where intertidal and subtidal species could be collected from otherwise similar habitats/sites and whose morphology allowed similar test-strips to be produced from their tissue. This resulted in eight phylogenetic blocks spanning brown algae (Division Heterokontophyta), red algae (Division Rhodophyta), and seagrasses (Division Liliopsida) (Fig. 1; Table 1). Collection sites (summarized in Table 1) included Botany Bay (BB), Port Renfrew, British Columbia (lat. 48°31′33″N, long. 124°26′50″W); Copper Cove (CC) near mainland Vancouver (lat. 49°22′43″N, long. 123°16′45″W); Sombrio Beach (SB), Port Renfrew, British Columbia, Canada (lat. 48°31′33.46″N, long. 124°26′50.22″W); and Roberts Bank (RB), Tsawwassen, British Columbia (lat. 49°01′49″N, long. 123°06′49″W). Upon arriving at the testing facility after collection, seaweeds were placed in running seawater until mechanical and desiccation tests, which were performed within 48 h of sample collection.

Determination of mechanical properties

All samples were taken from freshly collected, healthy individuals. A single dumbbell-shaped working section was cut from each individual in all but two species (*Sargassum muticum, Phyllospadix scouleri*) that were narrower than the working section of the dumbbell. The dumbbell working section provides grips that are necessary for tensile testing and more accurately controls strain localization, increasing the precision of material testing (e.g. Mach, 2009; Demes *et al*., 2013a,b). Moreover, the use of dumbbell shapes largely standardizes one dimension of the cross-sectional area (specifically width) across samples and species. Breaking force, as we present it here, is therefore an emergent property combining material (i.e. breaking stress) and morphological (i.e. thickness) traits that both resist breakage. To compare across samples, we standardized breaking force by the width of the sample (as in Read & Sanson, 2003; Onoda *et al*., 2011); thus breaking force in this study refers to the force-to-break per unit width (N mm^{-1}) rather than the force required to break off an entire blade (which varies depending on morphology, size and age, and is therefore not comparable across distantly related taxa with divergent growth forms). For *Phyllospadix* and *Sargassum*

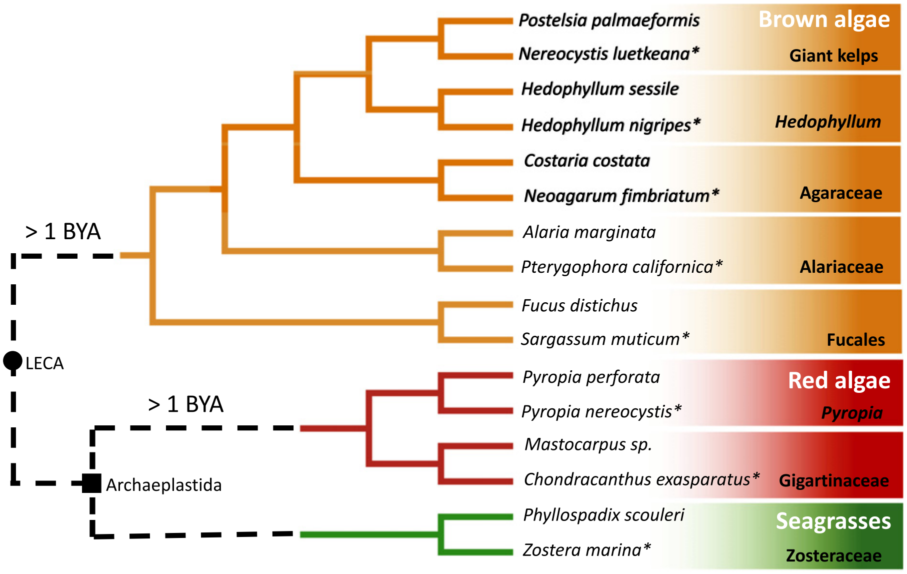

Fig. 1 Cladogram showing relationships between study taxa. Dotted lines indicate divergence near the root of all eukaryotic diversity. The last common ancestor of eukaryotes (LECA) is indicated with a circle, and the ancestor of all Archaeplastida is shown with a square. Divergence times between LECA and the brown algae as well as between red algae and seagrasses are greater than 1 billion years ago (BYA). For each independent species pair, the subtidal species is indicated with an asterisk (*) while the other is intertidal. Phylogeny adopted from Yang *et al*. (2016) and Starko *et al*. (2019).

Table 1 Species studied, their phylogenetic block, habitat (intertidal vs subtidal), site of collection, and mechanical traits of fresh tissues (mean ± SE, $n = 8$).

Phylogenetic clade	Species	Habitat	Site	Breaking force (N mm^{-1})	Breaking stress (MPa)	Elastic modulus (MPa)	Extensibility	Thickness (mm)
Alariaceae	*Alaria marginata*	Intertidal	SB	6.5 ± 0.5	2.9 ± 0.1	22.1 ± 1.2	0.48 ± 0.02	2.3 ± 0.15
	Pterygophora californica	Subtidal	SB	2.6 ± 0.2***	2.4 ± 0.2*	16 ± 1.1**	0.31 ± 0.03***	1.1 ± 0.05***
Agaraceae	*Costaria costata*	Intertidal	SB	1.1 ± 0.2	1 ± 0.1	8.4 ± 0.9	0.28 ± 0.02	1.1 ± 0.1
	Agarum fimbriatum	Subtidal	CC	2.2 ± 0.2***	2.7 ± 0.1***	20.6 ± 1.6***	0.34 ± 0.03$^{\mathrm{NS}}$	0.8 ± 0.05$^{\mathrm{NS}}$
Fucales	***Fucus distichus***	Intertidal	CC	0.8 ± 0.1	1.2 ± 0.2	7.5 ± 0.7	0.18 ± 0.02	0.8 ± 0.05
	Sargassum muticum	Subtidal	CC	7.5 ± 0.8**	5.4 ± 0.7***	41.7 ± 9.9**	0.21 ± 0.02$^{\mathrm{NS}}$	1.4 ± 0.05***
Giant Kelps	*Postelsia palmaeformis*	Intertidal	BB	1.1 ± 0.1	1.4 ± 0.2	3.9 ± 0.3	0.39 ± 0.04	0.8 ± 0.05
	Nereocystis luetkeana	Subtidal	BB	0.5 ± 0.1**	1.2 ± 0.1$^{\mathrm{NS}}$	11.7 ± 1.5***	0.29 ± 0.04$^{\mathrm{NS}}$	0.4 ± 0.05***
Gigartinaceae	***Mastocarpus papillatus***	Intertidal	CC	1.6 ± 0.1	5.2 ± 0.5	10.8 ± 1.3	0.48 ± 0.03	0.3 ± 0.04
	Chondracanthus exasperatus	Subtidal	CC	0.5 ± 0.02***	1.6 ± 0.1***	2.5 ± 0.2***	0.37 ± 0.02*	0.3 ± 0.02 $^{\mathrm{NS}}$
Hedophyllum	***H. sessile***	Intertidal	BB	0.5 ± 0.1	2.4 ± 0.2	3.9 ± 0.3	0.68 ± 0.03	1.1 ± 0.04
	H. nigripes	Subtidal	CC	0.4 ± 0.1$^{\mathrm{NS}}$	2.9 ± 0.2$^{\mathrm{NS}}$	9.4 ± 0.8***	0.48 ± 0.02***	0.8 ± 0.03***
Pyropia	*P. perforata*	Intertidal	SB	0.4 ± 0.1	5.2 ± 0.7	2.4 ± 0.4	0.80 ± 0.07	0.09 ± 0.01
	P. nereocystis	Subtidal	SB	0.2 ± 0.1***	4.9 ± 2.2 $^{\mathrm{NS}}$	4.1 ± 2.0$^{\mathrm{NS}}$	0.55 ± 0.06*	0.05 ± 0.002*
Zosteraceae	***Phyllospadix scouleri***	Intertidal	BB	1.5 ± 0.4	6.5 ± 1	383.4 ± 17.9	0.02 ± 0.002	0.2 ± 0.03
	Zosteria marina	Subtidal	RB	0.7 ± 0.1$^{\mathrm{NS}}$	2.4 ± 0.1**	66.8 ± 11.0***	0.05 ± 0.006***	0.3 ± 0.03*

Comparisons of mechanical or structural traits among intertidal and subtidal relatives are presented with the second species in each pair. NS, not significant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. BB, Botany Bay; CC, Copper Cove; PR, Port Renfrew; RB, Roberts Bank; SB, Sombrio Beach. Bold text denotes species for which completely desiccated samples were measured.

that have much narrower fronds, material properties were measured using entire blades (for *Phyllospadix*) or stipes (for *Sargassum*). The species sampled vary in their growth pattern, and because that results in differential tissue age and integrity across blades (Krumhansl *et al.*, 2015), we chose to target younger tissue in all species and therefore varied the location of the tissue sampled based on the species' growth forms. Working sections were cut: 5 cm from the apical meristematic region for apically growing species (i.e. Fucales, Gigartinaceae, Zosteraceae), 5 cm above the specimen's holdfast for species with pseudoparenchymatous growth (i.e. *Pyropia* spp.), and 5 cm above basal meristematic regions for species with predominately basal growth (i.e. *Alariaceae*, *Agaraceae*, Giant Kelps, *Hedophyllum* spp.). While the location of tissue sampled varied among clades, it was consistent between species within the same clade (as is growth form). Moreover, we note that young tissue (by virtue of being newly produced) is always found near the meristem, where breakage could have the largest effect on future growth.

Working sections were attached to a tensometer (5565; Instron, Norwood, MA, USA) via pneumatic clamps (90 psi). Strain was applied to tissues at a rate of 10 mm min^{-1} until failure occurred and the resisting force was measured at a frequency of 5 Hz. Tissues which failed at or near the clamps were discarded from analyses as they may have reflected mechanical failure from the clamps damaging tissues. For mechanical tests in all desiccation treatments, dimensionless tensile properties (i.e.

tensile modulus and breaking stress/strength) and strain (Δlength/length$_{\text{initial}}$) were calculated from the working section dimensions (length and cross-sectional area) immediately following the test and not as fresh tissues. Tissue thickness (diameter for *S. muticum*) and width of each sample were measured to the nearest 0.1 mm using digital calipers. From each tensile test, length of sample and the corresponding resisting force (N) data were extracted. Tissue width, thickness and length were used to calculate the following: breaking force (force per tissue width, N mm^{-1}), breaking strength (force per initial cross-sectional area, MPa), elastic modulus (initial linear slope of stress vs strain curve, MPa) and extensibility (strain required to break tissues) in analyses comparing tissue material properties.

Experimental desiccation methods

A number of environmental factors (e.g. temperature, light, association with canopy species) and macro-morphological traits (e.g. shape/size: Bell, 1995; frond density: Scrosati & DeWreede, 1998) could influence the rate at which tissues desiccate once out of water (Hay, 1981; Hunt & Denny, 2008). We isolated the effects of desiccation on tissue mechanical performance by minimizing extemporaneous environmental and macro-morphological factors. Thus, our experimental approach aimed to capture only the effects of tissue properties (thickness and composition) on desiccation rate. We note that some species may have additional morphological or ecological adaptations that may influence desiccation that were intentionally not considered to be within the scope of our study.

Working sections were cut from wet tissues (ensuring all specimens were the same shape and size) and all specimens were hung to dry at room temperature on clotheslines in a climate-controlled room. Two weeks of continuous temperature recordings (at 10 s intervals) showed that temperatures in the laboratory remained between 20.5°C and 26.5°C, with a mean (±SD) temperature of 23.3 ± 1.0°C. Samples were hung in a spatially random way relative to other samples (i.e. not spatially clustered by species) in order to reduce any possible effect of microclimatic differences within the room.

To test whether tissues of different species desiccated at different rates, we measured the relative water content (hereafter RWC) of tissues as a function of time out of water. We measured RWC for independent replicates (up to 10 but stopping once 0% RWC was achieved) of each species at a series of values of time out of water: 0, 15, 30, 45, 60, 90, 120, 150, 180 and 210 min. Relative water content was calculated after Slayter (1967):

$$\mathrm{RWC} = \frac{\text{Desiccated weight} - \text{Oven dry weight}}{\text{Initial fresh weight} - \text{Oven dry weight}}$$

Following each trial, we dried samples for 48 h in a 60°C drying oven to achieve a measure of ‘oven dry weight’ so as to back-calculate the RWC of a given sample. To test for differences in mechanical performance associated with desiccation stress among clades and habitat, we used the desiccation curve data to determine the time to 75% RWC for each species and measured mechanical performance of fresh tissues ($n = 8$) against tissues desiccated to 75% RWC ($n = 8$) for each species (total of 256 independent measurements). We chose 75% RWC because this represented an emersion time that is relevant to tidal cycles (~20–60 min depending on species). Given that intertidal macrophytes may be in air for multiple hours during a large tidal exchange, this is intended to represent a typical degree of desiccation experienced by intertidal species. To determine the amount of drying time required to achieve 75% RWC, we fitted exponential decay functions separately for each species: RWC ≈ exp ($-b \times$ time out of water) × 100 (where b is the decay factor). Because RWC data is determined as a percent of water relative to the samples' fully hydrated value, curve intercepts for all species are fixed at 100% and therefore we did not allow intercepts to vary. Individual curve parameters were used to estimate a time to 75% RWC for each species. To further explore possible drivers of variation in RWC decay curves, we also compared curves of total water content vs time out of water, and compared total wet weight and dry weight (in g) between intertidal and subtidal pairs visually. Results of analyses of total water content are presented in the Supporting Information (Figs S1, S2).

For a subset of species (those in bold in Table 1), we dried tissues to 0% RWC to evaluate tissue material properties under extreme desiccation. Although 0% RWC is likely to cause mortality in most species, the intention of this experiment was to determine whether the high flexibility and extensibility of macrophyte tissues more generally can be at least partially attributed to the high water content of macrophyte tissues relative to, for example, woody terrestrial plant tissues. Under severe desiccation, anisotropic tissue shrinkage may result in altered dimensional proportions and lateral and longitudinal curling. Because our working section is a thin beam, mechanical tests of the tissues after curling would be ruled by deformation from the curling process and not reflective of changes in tensile mechanics associated with desiccation. To test the tensile properties of tissues from each species at 0% relative water content (i.e. completely dry tissues), freshly cut working sections ($n = 8$ per species) were mounted in between two pieces of window screening and situated in a plant press before being placed in a herbarium dryer at 60°C for 48 h. The pressure of the plant press during drying maintained the shape and size of the original working section and eliminated curling during drying. The window screen kept the working sections from sticking to the herbarium press while drying. Fully desiccated seaweed tissues were then compared to a selection of terrestrial plant leaf and wood tensile properties taken from the literature (Lucas *et al.*, 1991; Al-Sulaiman, 2000; Köhler & Spatz, 2002; Balsamo *et al.*, 2003, 2006; Jeong *et al.*, 2009; Masselter *et al.*, 2016) to determine how the properties of hydrated and fully desiccated macrophytes compare to those of other plant-derived materials.

Statistical analyses

We used a model testing approach aimed first at testing our three hypotheses and second at determining the universality of trends across different clades. We first fit the model that best tested our hypotheses but then competed this model against more complex ones that allowed fixed effects and interactions to vary by clade. This approach allowed us to both test whether there is general support for our hypotheses and then also to identify lineages to which trends do not seem to apply. To test the hypothesis that desiccation rate depends on habitat (intertidal vs subtidal), we first fit a nonlinear mixed effects model using an exponential decay function (fixed effect = habitat; random effect = clade). Next, we competed this model (hypothesis 1) with a series of models: A null hypothesis where RWC was estimated only as a function of time; a hypothesis where desiccation rate could vary among clades, but not habitats; and a hypothesis where the effect of habitat on desiccation rate could vary among clades. For each model, the corrected Akaike information criterion (AICc) and ΔAICc were determined: the model with the lowest AICc value was considered to be the best model, and a difference of greater than 2 ΔAICc was used to confidently differentiate predictive power among models. Normality of each model was assessed using residual plots. Before the fitting of mixed-effects models, we log$_{10}$-transformed modulus and breaking stress data to ensure normality. For all other tissue traits, no transformation was required. We used a paired *t*-test on measures of time to 75% RWC to ask whether, on average, the tissues of intertidal species take longer to desiccate.

To understand how mechanical performance is associated with habitat (intertidal vs subtidal), desiccation stress (fully hydrated vs 75% RWC), and their interaction, we again used a model selection framework to evaluate how well the data supported a series of competing hypotheses. A null model including only breaking force and an intercept; a model where breaking force could vary between desiccation treatments (hypothesis 2); a model where breaking force varied between habitats (hypothesis 3); and two models where the relationship between desiccation treatment and breaking force was allowed to vary between habitats (one allowed the effects of habitat and desiccation to vary by clade; the other allowed the interaction between habitat and desiccation to vary with clade). For these analyses, we compared fully hydrated samples ($n = 8$) against samples desiccated to 75% RWC ($n = 8$) for each species. We performed this analysis for each material property of interest and then used an LSMEANS posthoc test, with a Tukey correction, to determine which means were significantly different after accounting for random effects.

All data analyses and visualizations were performed using R Statistical Software using the packages LSMEANS (Lenth & Lenth, 2018), NLME (Pinheiro *et al.*, 2017) and TIDYVERSE (Wickham, 2017).

Results

Table 2 Results of models aimed at testing the hypotheses in this study.

Response variable	*P*-value	*F*-statistic
Drying time (mins)		
Intercept	**0.0044***	8.386
Effect of habitat	**<0.0001***	96.660
Breaking force (N mm^{-1})		
Intercept	**0.0006***	12.078
Effect of desiccation	0.8136	0.056
Effect of habitat	**0.0225***	5.275
Habitat × desiccation interaction term	**0.0041***	8.416
Strength (MPa)		
Intercept	**<0.0001***	39.969
Effect of desiccation	0.8879	0.020
Effect of habitat	**0.0027***	9.163
Habitat × desiccation interaction term	**0.0012***	10.679
Modulus (MPa)		
Intercept	**<0.001***	22.406
Effect of desiccation	**<0.001***	40.789
Effect of habitat	0.1849	1.768
Habitat × desiccation interaction term	**0.0021***	9.709
Extensibility (dimensionless)		
Intercept	**<0.0001***	32.720
Effect of desiccation	0.2099	1.837
Effect of habitat	**<0.0001***	41.984
Habitat × desiccation interaction term	0.7872	0.073

'Habitat' refers to intertidal vs subtidal. In all cases, clade is included as a random effect. All models are fit to all clades ($n = 8$). Bolded values further demarcated by an asterisk (*) indicate statistically significant (i.e. <0.05) *P*-values.

Table 3 Model selection analyses for desiccation curve fitting.

Base model: RWC ~ 100 × exp(–*b* × time)	*P*-value	AICc	ΔAICc
Null hypothesis (effect of time)	<0.0001	1380.6	343.1
Varies by clade	0.0031	1151.7	114.2
Varies by habitat	<0.0001	1074.8	37.3
Effect of habitat varies by clade	<0.0001	1037.5	0

Overall, intertidal species tended to dry out at slower rates than subtidal species. We found that on average, intertidal species required significantly more time to dehydrate to 75% tissue RWC ($T_{1,7} = 2.515$, $P = 0.040$), with an average difference of 24.7 min between intertidal and subtidal relatives. Moreover, the model describing an effect of habitat was significant ($F_{1,141} = 96.6603$, $P < 0.0001$; Table 2). However, the best model describing tissue desiccation rate is one that allows the effect of habitat to vary among clades (Table 3). This result is graphically apparent in Fig. 2, where resistance to desiccation among intertidal species is clearly visible in five clades, no difference is discernable in two clades (Gigartinaceae and *Pyropia*), and one clade (seagrasses, Zosteraceae) shows a slightly opposing trend. Thus, although we found general support for the hypothesis that intertidal seaweeds are less prone to desiccation than subtidal relatives (Fig. 2a–g), this pattern is clade dependent and appears to be opposite for the two seagrasses considered (Fig. 2h). Analyses of this experiment using total water content (g) mirror these results in some clades, with differences in rate of bulk water loss varying between intertidal and subtidal species in clades such as Agaraceae and Alaraiceae (Fig. S1). However, in other clades, slower rates of change in RWC may be driven by higher levels of hydration in intertidal species than subtidal ones (Fig. S2).

A summary of mechanical and structural traits by species and statistical comparisons between intertidal and subtidal relatives is

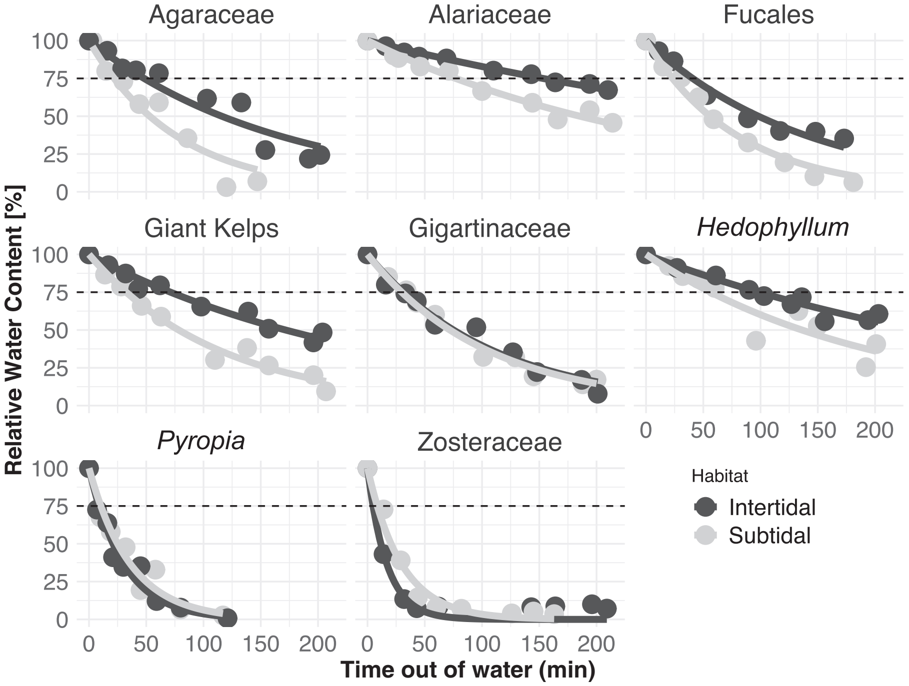

Fig. 2 Desiccation curves (RWC, or relative water content, of tissues as a function of time out of water) for intertidal (black points and lines) vs subtidal (grey points and lines) species in each phylogenetic block. Dashed horizontal line at 75% relative water content represents the tissue RWC at which mechanical tests were performed on desiccated tissues.

provided in Table 1. Only tissue extensibility displayed significantly greater values in intertidal species (compared to subtidal relatives) prior to any desiccation (LS Means Contrasts: Breaking force: $t = 0.427$, df = 245, $P = 0.9738$; Strength: $t = 0.745$, df = 245, $P = 0.8788$; Elastic modulus: $t = 0.614$, df = 245, $P = 0.5401$; Extensibility: $t = 4.391$, df = 245, $P = 0.0001$). While trends were evident for other tissue mechanical traits, there were clear exceptions to each of these patterns, and the exceptional clade(s) varied by trait. For example, while in most clades intertidal species displayed higher values of extensibility than their subtidal relatives, others showed no difference (e.g. Fucales) or the opposing trend (e.g. Zosteraceae). Similar patterns were found in all cases of significance. Although there were significant differences between the material properties of subtidal and intertidal species (Table 2), the best model tended to be the one that allowed the interaction among habitat and desiccation stress to vary among clades (Table 4). Statistical comparisons of the cross-sectional area of dumbbell working sections, the variation of which is explained by differences in tissue thickness, reveal that intertidal species are significantly thicker than subtidal relatives, with the exception of *Gigartinaceae* and *Hedophyllum* (Table 1). Overall, the general pattern that intertidal species are more resistant to external forces and more extensible than subtidal species is supported by our data, but this pattern is not canonical across all clades investigated. Moreover, increased breaking force in intertidal species is a consequence of the combined effects of stronger tissues in some species and thicker tissues in others, consistent with past work that has suggested that altering these properties may offer two different means of achieving the same emergent property (Martone, 2007).

Partial desiccation tended to impact the material properties of taxa investigated in this study, but this effect was rarely consistent across species and clades (Figs 3,4; Figs S3, S4). This led to few significant effects of desiccation or significant interactions between desiccation and habitat (Table 2). There were, however, significant desiccation × habitat interaction terms for models fit to breaking stress and elastic modulus; although not consistent across all taxa, partial desiccation tended to decrease strength and elastic modulus across subtidal species but had reduced or opposite effects across intertidal species (Figs S3, S4). Following partial desiccation, intertidal species tended to have higher values than subtidal species of every material property except elastic modulus (LS Mean contrasts: Breaking force: $t = 6.361$, df = 245 $P < 0.0017$; Strength: $t = 2.967$, df = 245, $P = 0.0173$; Elastic modulus: $t = 1.513$, df = 245, $P = 0.133$; Extensibility: $t = 4.773$, df = 245, $P < 0.0001$) and higher forces were generally required to break intertidal species than their subtidal relatives (except for *Fucaceae*), even for those species that were weaker under full hydration (e.g. Agaraceae, Fig. 3). Similarly, extensibility was consistently greater across intertidal algae than subtidal algae when they were partially desiccated, with Zosteraceae (seagrasses) as the only exception to this pattern (Fig. 4).

To assess the impact of water more generally on material properties, we fully dehydrated tissues from a subset of species. Under complete desiccation, the material properties (breaking stress and elastic modulus) of seaweed tissues were several orders of magnitude higher than when fully hydrated. Comparing these properties to some characteristic examples from land plants that tend not to have as high water contents, we found that the fully hydrated materials of marine macrophytes tended to be weaker and more flexible than the woody tissues of land plants, overlapping only with some leaf materials (e.g. *Prunus serrulatis* and *Heteromeles arbutifolia*) which are highly variable across embryophyte taxa (Fig. 5). However, the material properties of

Table 4 Model selection analyses comparing effects of habitat, hydration status, and their interaction among clades.

Model	AICc	ΔAICc	Model R^2
Breaking force (N mm^{-1})			
Null hypothesis (clade only; nonzero breaking force)	986.9	222.9	0.512
Varies by desiccation	990.4	226.4	0.511
Varies by habitat	985.3	221.3	0.521
Habitat × desiccation interaction term	982.7	218.7	0.535
Effect of habitat can vary by clade	768.8	4.8	0.844
Habitat × desiccation interaction term can vary by clade	**764.0**	**0**	**0.853**
Strength (MPa)			
Null hypothesis (clade only; nonzero breaking force)	38.5	116.7	0.321
Varies by desiccation	45.7	203.9	0.321
Varies by habitat	37.0	115.2	0.344
Habitat × desiccation interaction term	39.7	117.9	0.370
Effect of habitat can vary by clade	−64.3	13.9	0.658
Habitat × desiccation interaction term can vary by clade	**−78.2**	**0**	**0.701**
Modulus (MPa)			
Null hypothesis (clade only; nonzero breaking force)	184.6	187.6	0.702
Varies by desiccation	154.8	157.8	0.732
Varies by habitat	189.8	192.8	0.705
Habitat × desiccation interaction term	155.9	158.9	0.739
Effect of habitat can vary by clade	19.7	22.7	0.860
Habitat × desiccation interaction term can vary by clade	**−3.0**	**0**	**0.880**
Extensibility (dimensionless)			
Null hypothesis (clade only; nonzero breaking force)	−304.4	54.8	0.672
Varies by desiccation	−297.4	61.8	0.673
Varies by habitat	−334.6	24.6	0.719
Habitat × desiccation interaction term	−320.4	38.8	0.720
Effect of habitat can vary by clade	−361.7	2.5	0.787
Habitat × desiccation interaction term can vary by clade	**−359.2**	**0**	**0.797**

Bolded models were those considered to best fit the data based on ΔAICc. Clade is a random effect in each model and all model are fit to $n = 8$ clades. AICc, corrected Akaike information criterion; RWC, relative water content.

fully dehydrated macrophyte tissues overlapped with the range of material properties more commonly observed in wood and stiff leaves (Fig. 5).

Discussion

Although the impacts of environmental gradients on evolutionary processes have been well explored (e.g. Cavender-Bares *et al.*, 2009, 2018; Hipp *et al.*, 2018), the ways in which species adapt to multiple stressors remain poorly understood. The rocky intertidal zone imposes some of the most extreme abiotic conditions on the planet on its inhabitants; macrophytes occupying this environment not only face extreme hydrodynamic forces associated with crashing waves, but they also experience prolonged air exposure during low tide, which is likely to compromise aspects of their material properties (Haring *et al.*, 2002). In order to investigate how the interacting effects of desiccation and wave action influence trait evolution, we compared performance properties between intertidal and subtidal taxa, to test three hypotheses: (1) that intertidal species will lose water at a slower rate than their subtidal counterparts; (2) intertidal species will have tissue properties that are suited to high energy environments; (3) under mild desiccation stress, intertidal species will better maintain their material properties and remain stronger than their subtidal counterparts, as initial waves following low tide – those that crash against the shore before macrophytes have fully rehydrated – elicit the greatest forces and are thought to dislodge many intertidal species (Haring *et al.*, 2002). We evaluated all three of these hypotheses here using eight independent comparisons of intertidal and subtidal species across red algae, brown algae, and seagrasses and provided support for each of these hypotheses but with some clear outliers that likely represent biologically interesting exceptions.

Intertidal species are more desiccation resistant

Many have argued that repeated emersion at low tide should promote the evolution of desiccation resistance in intertidal macrophytes (e.g. Dromgoole, 1980; Abe *et al.*, 2005; Flores-Molina *et al.*, 2014). This prediction, by and large, is supported by our data. For five of the seven algal species pairs, intertidal species lost water at a slower rate than their subtidal counterparts. The two exceptions that did not differ between species were *Pyropia* and Gigartinaceae, both of which exhibit unique morphological properties. Both intertidal and subtidal *Pyropia*, for instance, are only one cell thick and thus there is little morphological potential to prevent swift desiccation, which has been negatively associated with thallus thickness (Bell, 1995). Gigartinaceae is likewise unique in that this lineage has evolved an anti-herbivory cuticle (Gaines, 1985) that incidentally prevents desiccation (Harvey & McLachlan, 1973). Along similar lines, we observed that seagrasses actually exhibit the opposite pattern to that predicted: the subtidal species (*Zostera*) loses water slower than its intertidal counterpart (*Phyllospadix*). Seagrasses are cuticle-possessing land plants that have secondarily returned to the seas, and this cuticle may influence rates of desiccation. Seagrasses desiccated more quickly than all seven macroalgal clades, however, demonstrating that a cuticle by itself is not an effective means of preventing desiccation. However, the faster desiccation of *Phyllospadix* relative to *Zostera* may nonetheless reflect differences in cuticle thickness or composition, perhaps with a reduced cuticle providing some other benefit, like enhanced nutrient absorption.

Differences in tissue thickness may also explain some of the observed variation in desiccation rate. Increases in thickness may limit desiccation through lower surface area to volume ratios. Indeed, many of the species pairs that differed significantly in desiccation rate also differed in tissue thickness (e.g. Alariaceae, *Hedophyllum*, Giant Kelps; Table 1). However, members of the Agaraceae differed significantly in desiccation rate, but not thickness, suggesting that multiple factors may be needed to explain patterns of desiccation resistance. Differences in tissue thickness could further explain why desiccation occurred faster in

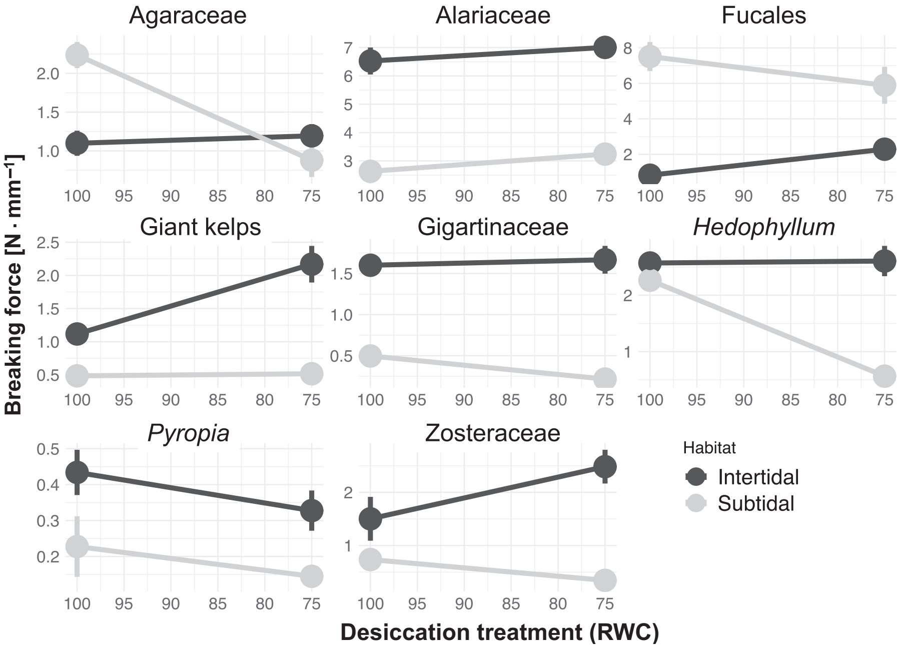

Fig. 3 Breaking force (N mm^{-1}) of intertidal (black dots) vs subtidal (grey dots) species in each of eight phylogenetic blocks when fully hydrated (relative water content, RWC = 100) vs moderately desiccated (RWC = 75). Data points show mean ± standard error of multiple independent samples (*n* = 8 per treatment).

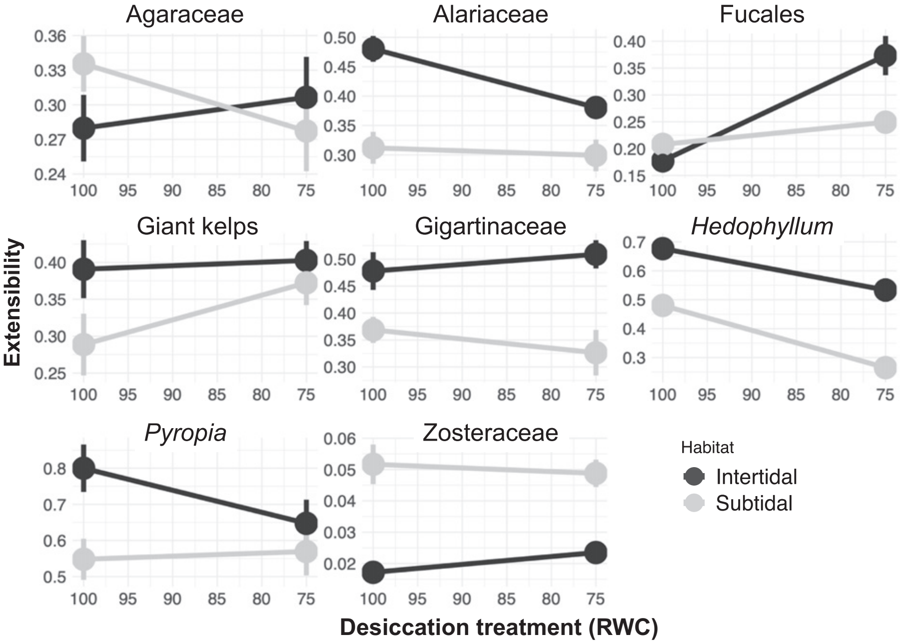

Fig. 4 Extensibility of intertidal (black dots) vs subtidal (grey dots) species in each of the eight phylogenetic blocks when fully hydrated (relative water content, RWC = 100) vs moderately desiccated (RWC = 75). Data points show mean ± standard error of multiple independent samples (*n* = 8 per treatment).

Phylospadix working sections than *Zostera* (Table 1). These two species are exposed to especially different habitats (i.e. sandy vs rocky habitats) and herbivore pressures (i.e. snails vs geese), and so further work is needed to understand the factors driving evolutionary differences in blade thickness. Anomalies aside, our comparative data are largely consistent with the prediction that intertidal living selects for desiccation resistance in macrophytes. This increased desiccation resistance seems to reflect a combination of morphological (tissue thickness) and potentially compositional adaptations to resist desiccation. Elucidating the extent to which different factors influence desiccation rate across macrophyte tissues would be an exciting avenue for future research.

Intertidal species have properties that prevent tissue breakage

Velocities associated with waves that crash in the rocky intertidal zone are far greater than those carried by the unbroken waves experienced in the subtidal zone (Denny *et al*., 1985; Gaylord, 1999; Denny & Gaylord, 2002). While high energy waves can carry velocities of approximately 2 ms^{-1} when unbroken, velocities of upwards of 25 ms^{-1} have been recorded in intertidal surf zones. Although these exact velocities vary considerably between localities, crashing waves are always expected to produce higher forces than rolling swell from the same waves

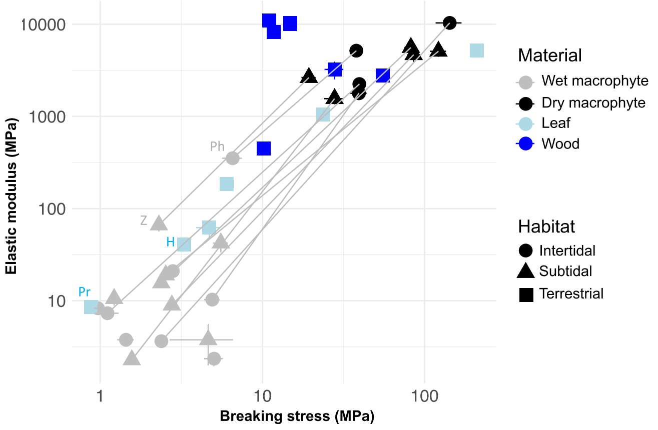

Fig. 5 Comparison of material properties of freshly collected (grey) and completely desiccated (0% relative water content (RWC); black) seaweed tissues to those of terrestrial plants (light and dark blue). Lines are drawn connecting 100% and 0% RWC samples of each species. Letters indicate seagrass species (Z = *Zostera*, Ph = *Phyllospadix*) and particularly flexible leaf tissue (Pr = *Prunus serrulatis*, H = *Heteromeles arbutifolia*).

(Denny & Gaylord, 2002). Moreover, while subtidal organisms generally experience more predictable drag forces, crashing waves elicit drag, lift, torsion, acceleration force and impingement on rocky intertidal organisms that amount to substantially greater forces than drag alone (Gaylord, 1999). Because of the intense and less predictable nature of the hydrodynamic forces in the intertidal zone compared to subtidal areas, we hypothesized that the tissues of intertidal species would have material and structural tissue adaptations to resist breakage. We tested four properties (breaking force, strength, elastic modulus and extensibility) of the tissue and found some support for this hypothesis on average but found that it was not universal. Of the properties that we measured, only the adaptive roles of breaking force and extensibility are understood. In particular, increased breaking force has clear ecological relevance to plant survivorship by directly preventing breakage (Demes *et al.*, 2013b), and increased extensibility is believed to be beneficial for reducing stress amplification in loaded tissues (Janot & Martone, 2016). Moreover, in a recent study of niche evolution in the kelps (Laminariales), extensibility was the only material property found to be significantly associated with a wave-exposed habitat, along with morphological characteristics such as holdfast mass fraction (Starko *et al.*, 2020). Evidence that strength and elastic modulus (i.e. material stiffness) are directionally adaptive in their own right is equivocal, since the emergent properties of tissues of which these properties are components (i.e. breaking force and flexural stiffness, respectively) depend also on tissue thickness; increasing material properties vs tissue thickness may be alternative strategies to achieve the same emergent outcome (Martone, 2007). Moreover, we note that, mathematically, tissue thickness contributes more to flexural stiffness (which predicts the ability of macrophytes to reconfigure to reduce drag; Demes *et al.*, 2011) than does elastic modulus; flexural stiffness is proportional to tissue thickness to the third power but only to elastic modulus to the first power.

In six out of eight clades, intertidal species had higher breaking forces than subtidal counterparts, but this effect was not significant on average. In Agaraceae, subtidal *Neoagarum fimbriatum* had a greater overall breaking force than intertidal *Costaria costata*, and in Fucaceae, subtidal *S. muticum* had a much higher breaking force than intertidal *F. distichus*. In Fucaceae, our subtidal samples were collected from stipe rather than blade tissues since *S. muticum* blades are too small to sample – this is likely the cause of the divergent result in this clade, and the overall effect is significant when this clade is not considered (LS Means: $t = 4.930$, $\mathrm{df} = 214$, $P < 0.0001$). While in some cases these differences in breaking force can be attributed to material breaking stress (e.g. *Pyropia*; Table 1), there was no significant effect of habitat on breaking stress across all clades. Thus, like desiccation rate, breaking force is explained by a combination of tissue thickness and compositional variation that, in this case, determines material strength. There were also significant effects of habitat on extensibility, consistent with past work suggesting that extensibility and overall breaking force (an emergent property of strength and thickness) are the tissue properties upon which selection acts to limit breakage (Demes *et al.*, 2013b; Janot & Martone, 2016). Overall, these results are consistent with the hypothesis that intertidal species have evolved tissue-level traits that allow for tolerance of immense wave forces. However, in cases where multiple tissue traits may achieve the same goal (e.g. thickness vs breaking stress), it may only be the emergent property (i.e. breaking force) that is consistently selected for, with ‘thickness’ and ‘strength’ strategies each utilized by different clades.

Adaptation to multiple stressors

Given that intertidal species must regularly endure desiccation during low tides followed by extreme wave forces with the returning tide (Kraemer, 1990; Haring *et al.*, 2002), we hypothesized that intertidal species would have evolved improved biomechanical performance following desiccation. Consistent with this prediction, we found that three of the tissue properties (breaking force, breaking stress, extensibility) investigated were greater in intertidal species than subtidal species following desiccation. In cases where subtidal species had higher breaking force when fully hydrated, intertidal species increased in breaking force following desiccation, while subtidal species decreased in breaking force. This included the Agaraceae, where desiccation reduced breaking force of *Neoagarum* by more than 50%, but did not affect breaking force of *Costaria* and Fucaceae, where desiccation increased *Fucus* breaking force and decreased *Sargassum* breaking force, although Sargassum tissue remained stronger overall (again, likely attributed to being sampled from stipe tissue). Enhanced breaking force, in particular, is important in wave-swept environments because it prevents the partial or complete loss of photosynthetic and reproductive tissues (Demes *et al.*, 2013b), which underlie the growth and fitness of an alga, respectively. We also found that extensibility was unilaterally higher across intertidal algae than subtidal algae following partial desiccation but showed the opposite trend in the seagrass clade. High extensibility is a general property of macroalgal tissues (Hale, 2002; Janot & Martone,

2016) and is often considered to be adaptive to high energy environments (Martone, 2007; Janot & Martone, 2016). Thus, the ability of intertidal species to maintain increased extensibility while desiccated is likely an adaptive response to the multiple stressors of crashing waves and aerial emersion. Extensibility in seagrasses is substantially lower than in algae overall (Fig. 4) and so selective pressures acting on this property may differ substantially between seagrasses and macroalgae. Finally, we found statistically significant interaction terms between desiccation and habitat for both elastic modulus and breaking stress, indicating that – although not universal – intertidal species tend to better maintain their material properties during desiccation.

Water content is central to macrophyte material properties

To test the role of hydration on tissue properties more generally, we compared the material properties of fully hydrated (100% RWC) and fully desiccated (0% RWC) macrophyte tissues. We found that, once fully desiccated, both strength and elastic modulus of macrophyte tissues increased by several orders of magnitude (Fig. 5). This dramatic effect of water content on material properties is substantially larger in macrophytes than has been found for land plant tissues in previous studies, where complete desiccation tends to result in less than an order of magnitude (e.g. between two- and seven-fold) increase in elastic modulus (Vincent, 1983; Gibson *et al.*, 1988; Al-Sulaiman, 2000; Hedderson *et al.*, 2009) and has mixed effects on strength (Vincent, 1983; Hedderson *et al.*, 2009).

We further compared the properties of fully hydrated and fully desiccated tissues to some available examples from land plants. Before any desiccation, macroalgal materials were largely distinct from those of land plants, with some overlap between the stiffest macrophytes and the weakest, most flexible leaves (e.g. *Prunus*). Once completely desiccated, the material properties of macroalgae fell within the range of tensile properties known from land plants, including both wood and leaves (Fig. 5). This might suggest that the high material flexibility of marine macroalgae is owed in large part to their high degree of hydration. Interestingly, for many macrophyte taxa, the effect of complete desiccation (stronger, stiffer tissues; Fig. 5) was the opposite of the effect of partial desiccation (weaker, more flexible tissues; Figs 2–3), suggesting that not only the strength but also the direction of desiccation effects varies depending on the degree of desiccation.

The fundamental load-bearing structures in red algae, green algae and streptophyte tissues are the same: cellulose microfibrils embedded in a matrix of polysaccharides (Kloareg & Quatrano, 1988). However, the matrix differs substantially between major lineages, with the inclusion of highly hydrophobic lignin composing large portions of streptophyte cell walls (Boerjan *et al.*, 2003) and sulfated polysaccharides that are highly polar and may interact with water making up substantial components of algal cell walls (Michel *et al.*, 2010; Deniaud-Bouët *et al.*, 2014; Starko *et al.*, 2018). These differences in chemical composition of terrestrial and marine tissues result in materials with substantially different properties, including their tendency to absorb or retain water (Balsamo *et al.*, 2006; Hedderson *et al.*, 2009). While complete desiccation is likely to have structural effects on the tissue (e.g. Lewicki & Pawlak, 2003; Oliver *et al.*, 2020), the magnitude of these observed effects strongly suggests that the ability of macrophyte tissues to retain water has a drastic effect on the properties of their materials. While fine-scale differences in materials are likely driven by variation in cell wall and extracellular matrix composition (Starko *et al.*, 2018; Martone *et al.*, 2019), the broad role of tissue hydration in determining material properties should not be overlooked.

Conclusions

In aggregate, the evolution of intertidal habitat use has resulted in a variety of correlated changes in the desiccation resistance and material properties of macrophytes in response to the combined effects of aerial emersion and wave action. We found that intertidal species generally exhibit greater desiccation resistance as well as higher values of all material properties investigated once tissues are partially desiccated. In particular, we showed that breaking force and extensibility were nearly universally higher under mild desiccation for intertidal macroalgae relative to subtidal relatives. Both breaking force and desiccation resistance were partially explained by variation in tissue thickness, which was generally greater among intertidal species. In particular, increased thickness was associated with slower desiccation and increased breaking forces and thus may be selectively advantageous in the face of both environmental stressors. These emergent tissue properties (i.e. desiccation rate, breaking force) of intertidal taxa are likely to be essential to their performance in these extreme habitats because desiccation compromises tissue properties, and the moments following low tide and the return of wave-swept conditions may be the most hydrodynamically stressful and thus most critical for macrophyte mechanical performance (Gaylord, 1999; Haring *et al.*, 2002). Our two seagrass species consistently showed different patterns from macroalgae, suggesting that niche specialization along the gradients of tidal height may cause selection for different emergent properties than in macroalgae, and/or differences in other factors essential for survival (e.g. anti-herbivory strategies targeted at gastropods vs geese) might overwhelm the benefits of adaptations that ameliorate mechanical stress under desiccation. Overall, intertidal macrophytes have risen to meet the challenges of living in highly stressful environments by evolving tissue traits that are uniquely resilient to multiple interacting stressors (i.e. desiccation and wave forces). These patterns hold despite large differences across focal lineages in growth form, physiology, and life history. Thus, life in the wave-swept rocky intertidal appears to have caused strong convergent evolution across distantly related lineages in response to multiple interacting stressors.

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Author contributions

KWD and SS led the writing of the manuscript. KWD and CDGH designed the study and guided data collection and analyses. KWD and SS collected the data. KWD and SS performed analyses and visualization of the data. CDGH provisioned resources to enable data collection. All authors contributed to editing of the manuscript. KWD and SS contributed equally to this work.

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Data availability

Data and R code used in this paper are available for download at (https://github.com/sstarko/Demes_et_al_2020Desiccation/).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Total water content as a function of time out of water for intertidal vs subtidal species in each phylogenetic block.

Fig. S2 Tisue mass of intertidal vs subtidal species in each of eight phylogenetic blocks when fully hydrated vs completely desiccated.

Fig. S3 Breaking stress (MPa) of intertidal vs subtidal species in each of eight phylogenetic blocks when fully hydrated vs moderately desiccated.

Fig. S4 Elastic modulus (MPa) of intertidal vs subtidal species in each of eight phylogenetic blocks when fully hydrated vs moderately desiccated.

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