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RESEARCH ARTICLE

Mechanical properties of the wave-swept kelp *Egregia menziesii* change with season, growth rate and herbivore wounds

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ABSTRACT

The resistance of macroalgae to damage by hydrodynamic forces depends on the mechanical properties of their tissues. Although factors such as water-flow environment, algal growth rate and damage by herbivores have been shown to influence various material properties of macroalgal tissues, the interplay of these factors as they change seasonally and affect algal mechanical performance has not been worked out. We used the perennial kelp *Egregia menziesii* to study how the material properties of the rachis supporting a frond changed seasonally over a 2 year period, and how those changes correlated with seasonal patterns of the environment, growth rate and herbivore load. Rachis tissue became stiffer, stronger and less extensible with age (distance from the meristem). Thus, slowly growing rachises were stiffer, stronger and tougher than rapidly growing ones. Growth rates were highest in spring and summer when upwelling and long periods of daylight occurred. Therefore, rachis tissue was most resistant to damage in the winter, when waves were large as a result of seasonal storms. Herbivory was greatest during summer, when rachis growth rates were high. Unlike other macroalgae, *E. menziesii* did not respond to herbivore damage by increasing rachis tissue strength, but rather by growing in width so that the cross-sectional area of the wounded rachis was increased. The relative timing of environmental factors that affect growth rates (e.g. upwelling supply of nutrients, daylight duration) and of those that can damage macroalgae (e.g. winter storms, summer herbivore outbreaks) can influence the material properties and thus the mechanical performance of macroalgae.

KEY WORDS: Macroalgae, Material properties, Wave exposure, Damage, Hydrodynamic forces

INTRODUCTION

On many shorelines around the world, water motion from waves and currents exerts hydrodynamic forces on the organisms living on those shores (reviewed in Denny, 1988). Macroalgae, which are important sources of food and habitat for many other marine organisms, must withstand those forces, otherwise they can be damaged or completely dislodged from the substratum (e.g. Koehl and Wainwright, 1977; Carrington, 1990). The magnitude of the hydrodynamic forces that a body experiences depends on its size and shape, and the velocity and acceleration of the water relative to the body (e.g. Vogel, 1994; Denny et al., 1985). However, hydrodynamic forces on flexible macroalgae can be influenced by whether the macroalgae move with the ambient water or reconfigure into streamlined shapes when subjected to rapid water motion (Koehl, 1984, 1986; Carrington, 1990; Martone et al., 2012; de Bettignies et al., 2013). The water motion around macroalgae can be reduced when those macroalgae live in dense aggregations, such that macroalgae living in groups experience smaller hydrodynamic forces than do isolated individuals (e.g. Jackson and Winant, 1983; Koehl and Alberte, 1988; Johnson, 2001; Gaylord et al., 2007). Additionally, if macroalgae live in a subtidal area exposed to alongshore currents, those currents can push the macroalgae in a different direction from that of the dominant ocean waves and reduce the ambient water motion and hydrodynamic forces due to waves that the macroalgae experience (Gaylord et al., 2003). The size and shape of macroalgae change as they grow and reproduce, and many species of macroalgae undergo growth and reproduction during seasons when waves are small and water motion is slow (e.g. summer) (Johnson and Koehl, 1994; Koehl, 1999; Wolcott, 2007; Demes et al., 2013a).

Whether macroalgae deform (e.g. bend, stretch) or break when exposed to hydrodynamic forces depends on the material properties of their tissues (Vincent, 2012), as well as on the duration and magnitude of the hydrodynamic forces they experience (Koehl and Wainwright, 1977). The mechanical stress (σ) in a tissue is the force per cross-sectional area of tissue bearing a load. The resistance of an alga to being deformed is affected by the stiffness of its tissues. For example, the stiffness of a tissue when stretched is its tensile elastic modulus (E), which is the slope of the plot of stress in the tissue as a function of how far it has been stretched, where the degree of stretching is given by the extension ratio λ (=length of tissue at a given stress/length of tissue before stress was applied). A number of tissue properties affect whether macroalgae can resist breakage by hydrodynamic forces. For example, the breaking force (F_{brk}, force to break) of an algal stipe or rachis depends both on the strength of the algal tissue (breaking stress σ_{brk}, the breaking force per cross-sectional area of tissue) and the cross-sectional area of the structure bearing the hydrodynamic load. However, if the peak magnitude of a hydrodynamic force is brief, such as in a wave, macroalgae can resist breaking by being extensible (Koehl and Wainwright, 1977). The extensibility of a tissue (i.e. how far the tissue stretches before breaking) is given by its breaking extension ratio λ_{brk}. The toughness of an algal tissue is the mechanical work that moving water must do to break the tissue (Wainwright et al., 1976; Vincent, 2012). One measure of the toughness of a tissue is work per volume to fracture the tissue (W/V, the area underneath the curve in a plot of σ as a function of extension for a piece of tissue pulled until it breaks). Thus, both strength and extensibility contribute to tissue toughness. Many studies of the material properties of macroalgae have focused only on tissue strength, giving only a partial explanation for how macroalgae survive harsh hydrodynamic conditions.

The material properties of macroalgal tissues can be influenced by the alga's habitat, the age of the tissue and the alga's response to

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wounds (e.g. due to herbivory or abrasion). A number of species of macroalgae have different tissue material properties in habitats exposed to rapid water flow than in more protected habitats (e.g. Armstrong, 1987; Johnson and Koehl, 1994; Koehl et al., 2008). For example, the kelp *Nereocystis luetkeana*, growing in different hydrodynamic habitats, adjusts both its blade morphology (which affects the drag force imposed on the kelp by ambient water flow) and the breaking strength of its stipe tissue such that the ratio of the breaking stress of the stipe relative to the maximum stress imposed on the stipe due to drag is the same at all sites (Johnson and Koehl, 1994). As macroalgae grow, young tissue (tissue near the meristematic region) tends to be less stiff and less strong than old tissue, and the particular growth pattern (i.e. the location of the meristematic region on a macroalga) can therefore influence how the macroalgae deform in moving water and the positions in the alga where breakage generally occurs (Armstrong, 1987; Stewart, 2006; Krumhansl et al., 2015). When macroalgae are wounded by herbivores or abrasion against the substratum (Black, 1976; Kennelly, 1989; Hughes, 2010), the wounds initiate cracks in the tissues, leading to an increased concentration of mechanical stress at the crack tips that can then exceed the tissue's strength. As a result, wounded tissues break more easily than do unwounded tissues (Black, 1976; Koehl and Wainwright, 1977). Over time, macroalgae can accumulate small wounds that effectively reduce the strength, stiffness or extensibility of the tissues, such that old, and usually large, macroalgae with many wounds may break more easily than young macroalgae with few wounds (Johnson and Koehl, 1994; de Bettignies et al., 2012). While some macroalgae have arrangements of different types of tissues that prevent cracks from propagating (Denny et al., 2013), other macroalgae can heal from wounds by increasing the strength of the tissue around the wound (Lowell et al., 1991). Knowing how macroalgae heal from wounds can help us understand the long-term effects of wounds on macroalgae. Individual studies of macroalgae have examined how each of these factors (environment, growth, wounds) in isolation affect the material properties of macroalgae, but comprehensive analyses of all of these factors for single species are lacking.

The factors described above that can affect algal material properties can change with season. For example, wave action, one of the most relevant environmental factors for macroalgal breakage, tends to be greatest during winter storms and lowest in summer and autumn (Wolcott, 2007). In contrast, macroalgal growth tends to be highest in summer and autumn (seasons with long periods of daylight and higher temperatures) and lowest in winter (i.e. short periods of daylight and cooler temperatures) (Black, 1974). Wounds from herbivory follow seasonal patterns of herbivore populations, and occur frequently in summer when herbivore populations are large (Gunnill, 1983; Burnett, 2017). Because many studies of the material properties of macroalgae are short term, we know little of how the material properties of macroalgae change with season, growth and wounds. The goal of the present study was to fill in this gap in our knowledge using an ecologically important kelp, *Egregia menziesii*, and focusing on material properties that contribute to the tissue's strength, extensibility and toughness.

The kelp *Egregia menziesii*

We use the intertidal kelp *E. menziesii* in the present study because it is a dominant kelp on wave-swept rocky shores where few other organisms can grow to similar size, and its large size makes it an ecologically important habitat and food source for many other organisms (Abbott and Hollenberg, 1976). The range of *E. menziesii* runs along the west coast of North America from Baja California to southeastern Alaska (Abbott and Hollenberg, 1976). Each *E. menziesii* is perennial, with numerous strap-like fronds growing from a single holdfast (Burnett, 2017). A frond, which consists of a strap-like rachis bearing lateral blades and pneumatocysts along each edge, can grow to several meters in length. The intercalary meristem (IM) of each frond is located distally and thus rachis tissue just proximal to the IM is younger than rachis tissue closer to the holdfast of the kelp (Black, 1974; Abbott and Hollenberg, 1976) (Fig. 1).

Egregia menziesii is a good model organism for measuring and understanding material properties because growth of the fronds occurs mostly as elongation of the rachis, allowing us to easily quantify growth and approximate the relative age of different tissues, and the rachis has a nearly constant cross-sectional area and shape along its length, permitting us to measure a wide range of material properties with minimal modifications to the rachis. From previous work, we know that the rachises of *E. menziesii* can be exceptionally strong compared with those of other macroalgae (Friedland and Denny, 1995), but they are also flexible, which allows the fronds to move back and forth with the waves (Burnett and Koehl, 2017). However, we do not know how material properties of the rachis vary with seasonal changes in the environment and growth. Wounds caused by grazers, such as the specialist limpet *Lottia insessa* (Black, 1976; Kuo and Sanford, 2013) and at least seven genera of gammarid amphipods known to graze on *E. menziesii* (Chapman, 2007), can weaken the rachis (Black, 1976; Burnett and Koehl, 2018), but we do not know how the material properties of the rachises change as the tissues respond to, or heal from, those wounds.

Objectives of this study

The goal of this study was to determine how seasonal variation in the environment, growth and wounds affects the mechanical properties of the support tissues of a perennial macroalga. We focused on the rachis tissue of fronds of *E. menziesii* to test the following hypotheses: (1) material properties do not change with season; (2) material properties do not depend on tissue growth rate; and (3) material properties do not change in response to wounding. Based on previous studies of other macroalgae (e.g. Lowell et al., 1991; Krumhansl et al., 2015; Starko et al., 2018), we expected that: (1) rachis tissue would be stronger and tougher, but less extensible, in winter than in summer; (2) young, rapidly growing tissue would be weaker and less tough, but more extensible, than old, slowly growing tissue; and (3) *E. menziesii* would respond to wounds by increasing the strength of the tissues around the wound.

MATERIALS AND METHODS

Field sites

Site descriptions

Fronds of *Egregia menziesii* (Turner) Areschoug 1876 were studied at two sites in northern California: Miwok Beach (38°21′25″ N, 123°4′2″ W) near Bodega, CA, USA, and McClures Beach (38°11′3″N, 122°58′2″W) in the Point Reyes National Seashore (Fig. S1). Both sites were westward facing shorelines that consisted of boulder fields directly exposed to the open ocean. Miwok Beach was bounded by a sandy beach to the south and rocky outcroppings to the north, while McClures Beach was bounded by rocky headlands to the north and south. The intertidal zones at the sites showed similar biological communities, which included dense aggregations of the mussel *Mytilus californianus* that dominated most available space above the *E. menziesii* zone (Burnett, 2017). During the study period (June 2015–May 2017), data from the Coastal Data

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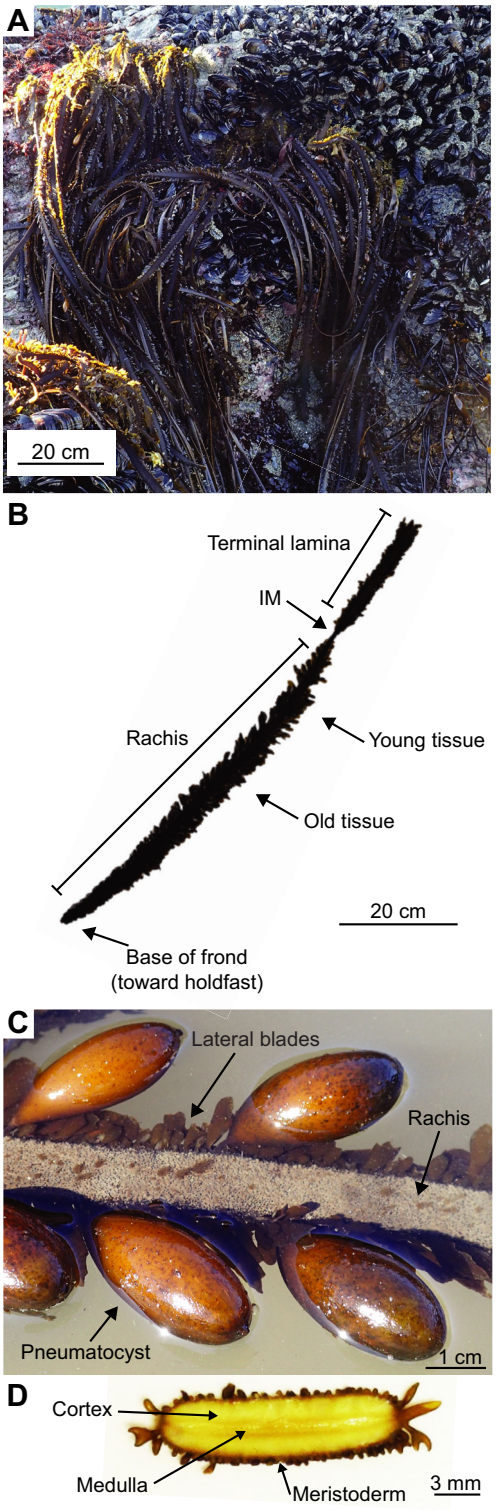

Fig. 1. Anatomy of ***Egregia menziesii.*** (A) *Egregia menziesii* in the rocky intertidal zone. (B) The majority of the growth of each strap-like frond occurs at the frond's intercalary meristem (IM) such that tissue near the meristem is younger than tissue further away from the meristem. (C) The anatomy of a frond, showing the rachis, the lateral blades and the pneumatocysts. (D) A cross-section of the frond's rachis, showing the major tissue types of the frond.

Information Program (Scripps Institution of Oceanography, http://cdip.ucsd.edu) showed that estimates of significant wave heights (the mean of the largest 33% of waves measured in a given time period) near shore to McClures Beach were only 22 cm larger on average (s.d.=20 cm) than the significant wave heights near shore to Miwok Beach (paired *t*-test, $P<0.05$).

Seasonal variation in the environment

The study period encompassed an El Niño Southern Oscillation (ENSO) from autumn 2015 to spring 2016 (Barnard et al., 2017). To characterize the oceanographic environment in the area around the study sites during the study period, we compared local measurements of significant wave height, water temperature, upwelling and photosynthetically active radiation (PAR) between seasons and years. Hourly measurements of offshore significant wave height and water temperature were downloaded from the National Buoy Data Center (Buoy 46013; www.ndbc.noaa.gov). Daily measurements of upwelling, which describe the delivery of cold, nutrient-rich water to the shore, for a nearby offshore region (39°N, 125°W) were downloaded from the Pacific Fisheries Environmental Laboratory (www.pfeg.noaa.gov). Daily averages of PAR were downloaded from the Bodega Ocean Observing Node (boon.ucdavis.edu; University of California, Davis, Bodega Marine Laboratory). The environmental data did not satisfy assumptions of normality and homogeneity of variances for an analysis of variance (ANOVA), so we compared data between seasons and years using Kruskal–Wallis tests with *post hoc* Dunn's tests.

Seasonal changes in material properties

Frond collection

To test how material properties of the fronds changed seasonally, we collected fronds each month from June 2015 to May 2017. At each site, individual *E. menziesii* were selected by walking along a transect that ran parallel to the shoreline and choosing approximately every third holdfast encountered. Transects remained within a single boulder field at each site and were between 20 and 50 m in length. At both sites, the distance between the selected holdfasts ranged between approximately 0.5 and 3.0 m. A single frond was haphazardly chosen on the selected kelp, and that frond was collected if its length between the IM and the base of the frond (i.e. where the frond branched from another frond or from the holdfast) (Fig. 1) was at least 40 cm, and if it was free of any visible wounds. Fronds were removed from the kelp by cutting them as close to the base of the frond as possible. Only one frond was taken from an individual kelp. Between 4 and 11 fronds were collected during each visit, for a total of 242 fronds over 40 visits to the field sites. They were stored at 11–15°C in an air-filled cooler with just the water that was trapped on the fronds until their material properties were measured. If overnight storage was required, the fronds were placed in the cooler at 4°C and then returned to 11–15°C the next day. Preliminary comparisons showed that overnight storage did not affect the material properties of the rachises (Mann–Whitney *U*-test, $P>0.05$, $n=5$ for same-day measurement and $n=5$ for overnight storage). Material properties were always measured within 24 h of collection. Before measuring material properties, the lengths of the rachises were measured to the nearest 1 cm.

Material properties

Material properties of *E. menziesii* were measured on regions of the rachis, distinguished by the distance of the tissue from the IM (Armstrong, 1987; Demes et al., 2013b; Krumhansl et al., 2015), such as 0–10 cm from the IM. The specific region used as a sample depended on the experiment (see below). The rachis is composed of several types of tissue distributed across the cross-section (Fig. 1D); therefore, we did not cut the samples into the traditional 'dumbbell'

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shape for strength measurements but instead used the whole rachis cross-section (Niklas, 1992). The lateral blades and pneumatocysts were trimmed away from each rachis sample. Material properties were measured using an Instron materials-testing machine (Model 5544, Norwood, MA, USA). To prevent slipping and damage when a specimen was held by the grips of the Instron, the ends of each sample were wrapped with a strip of paper towel attached to the tissue by cyanoacrylate glue. Each sample was secured in the grips of the Instron and strain (increase in length pulled divided by the original length of the sample between the grips) was applied at a fixed rate of 3.3×10^{-3} s^{-1} until the sample failed, following Burnett and Koehl (2018). This rate is representative of the strain rate that fronds may experience in the surge portion of a wave, and is also similar to strain rates used in previous studies of macroalgal material properties (Demes et al., 2013a; Krumhansl et al., 2015). The force with which the sample resisted the strain was measured at a rate of 10 Hz to the nearest 0.1 N. After failure, we cut a cross-section of the sample next to the break, photographed the cross-section, and used ImageJ (US National Institutes of Health, Bethesda, MD, USA) to measure its area. The Instron measurements of force (F) and length (L), and measurements of sample length between the grips before the experiment started (L_o) and of the cross-sectional area of the sample (A) were used to calculate the instantaneous extension ratio ($\lambda=L/L_o$), the instantaneous stress in the tissue ($\sigma=F/A$), and the following material properties (Fig. 2). (1) Breaking force, F_{brk} – the force with which the sample resisted extension at the moment when the sample broke. (2) Breaking stress, σ_{brk} – the stress (F_{brk}/A) at which the sample broke. (3) Yield stress, σ_{yield} – the stress at which the sample exhibited plastic deformation after it was extended beyond the range of elastic behavior (i.e. the initial linear portion of the stress–extension ratio curve). We identified σ_{yield} using Considère's construction: the stress reached by a line that is tangent to the stress–extension ratio curve and that intersects $\lambda=0$ (e.g. Vincent, 2012). (4) Elastic modulus, E – a measure of the stiffness of the sample, given by the slope of the plot of stress versus extension ratio. We called the modulus of the sample at low extensions (extension ratios 1.0 to 1.1) 'modulus 1' (E_1) and the modulus of the sample at high extensions (extension ratios 1.2 to 1.3) 'modulus 2' (E_2) (Johnson and Koehl, 1994). (5) Breaking extension ratio, λ_{brk} – the extension ratio at which the sample broke. (6) Work per volume to fracture, W/V – the area underneath the curve in a plot of stress versus extension. This value describes the mechanical work per volume (AL_o) of the sample and is commonly referred to as toughness.

Each of these properties provides valuable information about the behavior of the rachis when loaded by hydrodynamic forces. F_{brk}, σ_{brk} and σ_{yield} provide information on the mechanical loads that the rachis can tolerate, and E and λ_{brk} provide information on the type and amount of extension that the rachis can tolerate. W/V is an overall measure of how tough the rachis is, incorporating the magnitude of forces it can withstand and how far it can extend before breaking.

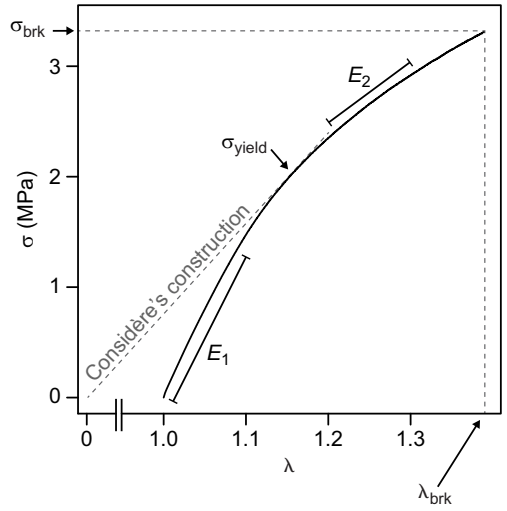

Fig. 2. A representative stress–extension curve for *E. menziesii*. Each of the material properties can be calculated from this curve, including work per volume to fracture (W/V), which is the area underneath the stress–extension curve. The yield stress (σ_{yield}) is calculated by finding the tangent to the curve that also runs through the graph's origin, using Considère's construction. σ, stress; λ, extension ratio; σ_{brk}, breaking stress; λ_{brk}, breaking extension ratio; E_1 and E_2, modulus of the sample at low and high extension, respectively.

Differences in material properties along the length of a frond

We tested whether material properties of the rachis of a frond changed with distance from the IM along the length of the rachis. In summer and winter of the first year of the study, we used a random subset of the collected fronds to measure the material properties at five regions on each rachis: 0–10, 20–30, 40–50, 60–70 and 80–90 cm from the IM. We used a mixed-effects ANOVA to test for an effect of tissue region on material properties, with the individual rachis as a random effect to control for the lack of independence of measurements on the same rachis. A separate mixed-effects ANOVA was done for each material property during each season. Assumptions of homogeneity of variances were checked with Levene's tests, and assumptions of normality were checked with Shapiro–Wilks tests and quantile–quantile plots. Data transformations were made when necessary. In summer and winter, all material properties except λ_{brk} showed a strong effect of tissue distance from the IM ($P<0.005$; Fig. 3; Table S1). Therefore, in all subsequent comparisons of rachis material properties, we did two separate analyses, one of rachis tissue that was close to the meristem (0–10 cm proximal of the IM; hereafter, tissue region $TR_{0–10}$), and the other of rachis tissue that was 40–50 cm from the IM (hereafter, $TR_{40–50}$). In each of these separate analyses, only one tissue sample per kelp was used (see below).

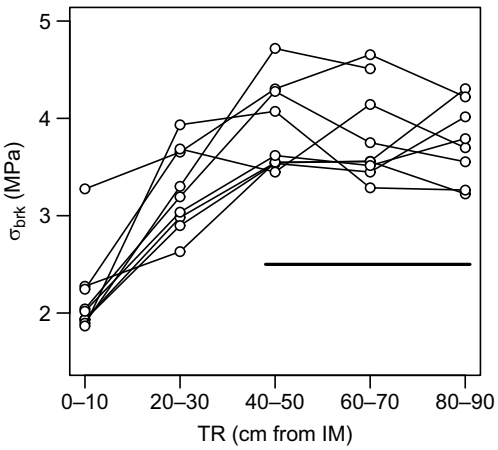

Fig. 3. Breaking stress of rachis tissue plotted as a function of the tissue's distance from the IM. Points connected by lines indicate data from a single rachis. The solid line below the data shows statistically similar tissue regions (TR; mixed-effects ANOVA with Bonferroni P-value adjustments for multiple comparisons, $P=0.05$ for significance).

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Seasonal changes in material properties

Over 2 years, we collected data from TR0–10 and TR40–50 during eight seasons (spring: March–May, summer: June–August, autumn: September–November, winter: December–February). Within each of the two tissue regions and each of the eight seasons, we compared each of the seven material properties between sites using Mann–Whitney *U*-tests (112 comparisons; no data were used in multiple comparisons). For 99 of the 112 comparisons, there were no differences between sites ($P>0.05$). For the remaining comparisons, there were statistical differences between sites ($P<0.05$), but for those material properties, the variation (i.e. interquartile ranges) at each site was greater than the difference in medians between the sites, suggesting that the statistically significant differences were not biologically significant. Based on those comparisons, the proximity of the sites to one another and the similarity in wave exposure between the sites, we pooled data between sites for all subsequent comparisons.

Next, we tested whether material properties of *E. menziesii* changed with season and year, separately analyzing the two tissue regions ($TR_{0–10}$ and $TR_{40–50}$). We used a separate ANOVA to test for effects of season and year on each material property. Years were defined as year 1 (June 2015 to May 2016) and year 2 (June 2016 to May 2017). Assumptions of ANOVA were checked as described above, and data transformations were made when necessary.

Last, we tested for correlations between the material properties of each tissue region and the environmental data, using the seasonal means of each feature (i.e. $n=8$ observations per feature). We calculated a Pearson's correlation coefficient for each pair of features.

Effects of growth on material properties

Seasonal changes in growth rate

We measured growth rates of fronds at the field sites for 2 years, from the spring of 2015 to the winter of 2016, following Black (1974). During visits to each site, kelp were selected following the sampling protocol described above, and individual kelp were tagged with a nylon cord and an acrylic identification tag. On each tagged kelp, one frond with an IM was selected haphazardly and tagged with nylon string. The distance between the base of the frond and the IM was measured to the nearest 1 cm. The same frond was measured again on subsequent visits to the site and the growth rate was calculated as the change in frond length divided by the number of days. Data were discarded if the frond had broken between measurements (i.e. lost its IM).

We used an ANOVA to test for effects of season and year on growth rate. Growth rate is proportional to the initial length of the frond (Black, 1974), so those data were first converted to relative growth rate (% length day^{-1}) by dividing the growth rate by the initial frond length and multiplying that value by 100. Data were transformed to satisfy assumptions of ANOVA, which were checked as described above.

We tested for correlations between growth rate and environmental data, and between growth rate and material properties of each tissue region, using seasonal averages of each feature. There were only seven seasons to compare seasonal averages of growth rate and material properties because growth rate was not measured in the spring of year 2. We calculated a Pearson's correlation coefficient for each pair of features.

Correlations between growth rate and material properties of individual rachises

Growth rate averaged within seasons and between sites was strongly correlated with seasonal averages of material properties (see Results), so we tested whether growth rate and material properties were correlated for individual rachises. We used growth rate data of fronds at each field site to estimate the growth rate of fronds for which material properties were measured. Following Black (1974), we calculated the linear regression of the growth rate of tagged fronds at each site as a function of their initial length. We calculated regressions separately for each combination of season, year and site that occurred in the seasons when we measured material properties. When there was a significant linear correlation between growth rate and initial frond length ($P<0.05$), we used the regression equation to estimate the growth rate of the fronds collected for material properties in that same season and from that same site. When there was no correlation between growth rate and initial frond length, we calculated the mean growth rate for all tagged fronds within that time and site and used that mean value as the estimated growth rate for collected fronds.

We tested for linear correlations between frond growth rate and material properties, using separate analyses for each tissue region and material property. Growth rate data were log-transformed to be normally distributed.

Effects of wounds on material properties

Instantaneous effects of wounds on material properties

We compared the instantaneous effects on material properties of differently sized wounds that mimicked amphipod grazing. Grazing by amphipods is the most prevalent form of herbivory at the field sites (Burnett, 2017; Burnett and Koehl, 2018), and when these herbivores feed on and burrow into the rachis, they create large, ellipsoidal holes of length 8.9±8.3 mm and width 2.7±0.7 mm (mean±1 s.d., $N=8$). The large size of these holes can be caused by numerous individuals feeding and burrowing in one location on the rachis (Burnett and Koehl, 2018). The holes can frequently go all the way through the rachis, leaving tissue at the edges of the rachis (Fig. 4). Fronds were randomly collected from separate kelp at each field site, as described above. A metal hole punch was used to remove different amounts of tissue from the rachis, leaving a large hole in the middle of the rachis with smooth, rounded edges. We measured the material properties of the remaining tissue on the edge of the rachis (i.e. the 'edge' tissue) and, for simplicity, only examined one of the two edges on each side of the wound. To control for variation in the baseline material properties of the rachis, we normalized our measurements by dividing the material properties of the edge tissue by the material properties of the adjacent, unwounded rachis (averaged between the tissue regions on either side of the wounded tissue region). We tested for a linear correlation between the relative material properties of the edge tissue and the relative size of the edge tissue (cross-sectional area of the edge tissue divided by the cross-sectional area of the adjacent, unwounded rachis).

Healing response to wounds

We tested how the material properties of the rachises of *E. menziesii* responded to wounds. Briefly, we inflicted an ecologically relevant wound on the rachis and measured how the material properties of the tissue around the wound changed over 4 weeks. Kelp were randomly selected at the sites and marked with a nylon cord and acrylic tag, as described above. On each kelp, two fronds that each had their IM and were at least 30 cm in length were selected and tagged with a plastic band at the base of the frond. Kelp that did not have two fronds meeting these requirements were removed from the study. Following Burnett and Koehl (2018), we used a metal hole punch to inflict a wound 15 cm from the IM on each frond. The wound was ellipsoidal, measured approximately 1 cm×4 mm, and was punched all the way through the rachis (Fig. 4C). Of the two

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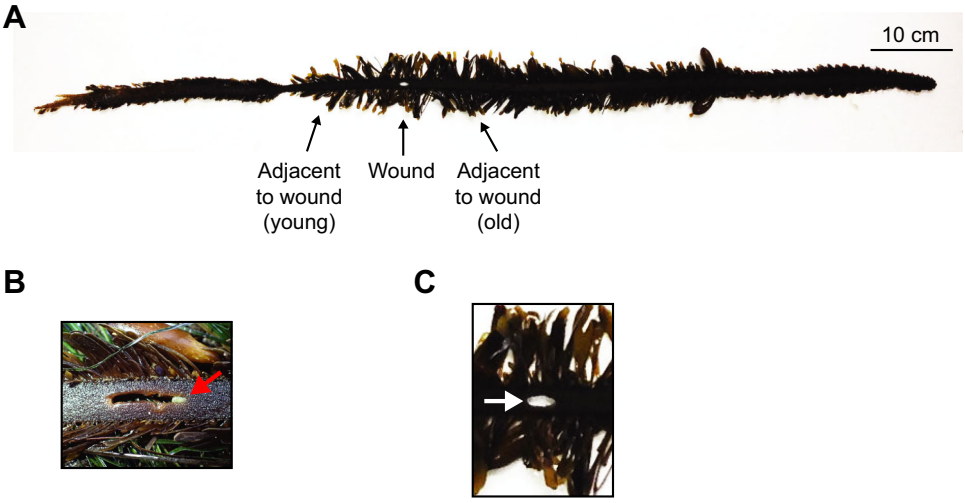

Fig. 4. Mimicking wounds on fronds. (A) When measuring the material properties of a wounded section of frond, we controlled for age by also measuring the properties of the older and younger tissue that were adjacent to the wounded tissue. (B) Amphipods create ellipsoidal wounds when they feed on or burrow into the fronds and these wounds can go all the way through the rachis. The red arrow shows an amphipod at the edge of the wound. (C) These wounds were mimicked for the wound-healing experiment using an ellipsoidal hole punch (white arrow).

wounded fronds on each kelp, we randomly selected one frond to collect immediately after the wound was inflicted, and then we collected the second frond after 4 weeks. After each frond was collected, we measured the frond's length and the distance of the wound from the IM to the nearest 1 cm, and then we measured the material properties of the tissue around the wound. Because of the shape of the wounded rachis, we only measured the breaking force and breaking stress of the tissues. We conducted this experiment twice (April 2015 and April 2016), during which we measured material properties at a strain rate of 3.3×10^{-3} s^{-1} (the same strain rate used in the earlier parts of the study). We also repeated this experiment a third time (April 2017), during which we measured material properties at a strain rate that was 100 times faster (i.e. 3.3×10^{-1} s^{-1}) to test how the wounded and healed tissue responded to fast loading regimes (e.g. crashing waves) as the properties of some biomaterials are known to change with strain rate (Vincent, 2012).

To control for the effect of tissue region on the material properties around the wound, we normalized the material properties of the wounded tissue by the material properties of the unwounded tissue adjacent to the wound (Fig. 4) by calculating the percentage difference between the tissue around the wound and the adjacent, unwounded tissue:

$$\text{Normalized MP}_\text{w} = \frac{\text{MP}_\text{w} - \text{MP}_\text{u}}{\text{MP}_\text{u}} \times 100, \quad (1)$$

where the subscripts u and w refer to the material properties (MP) of unwounded and wounded rachis tissue from the same frond, respectively. We then used paired *t*-tests to compare the normalized material property between fronds that were collected immediately after the wound was inflicted and fronds that were collected 4 weeks afterward, pairing measurements by the individual kelp from which each frond originated.

Statistical analyses

R statistical software was used for all analyses (http://www.R-project.org/). Dunn's tests were performed after Kruskal–Wallis tests using the dunnTest function in the FSA package (https://CRAN.R-project.org/package=FSA). Levene's tests were run using the leveneTest function in the car package (Fox and Weisberg, 2011). Mixed-effects ANOVA were run using the lmer function in the lme4 package (Bates et al., 2015). Multiple comparisons of ANOVA models were made using the lsmeans function in the lsmeans package (Lenth, 2016), and multiple comparisons of mixed-effects ANOVA models were made using the glht function in the multcomp package (Hothorn et al., 2008). Bonferroni *P*-value adjustments were made for all multiple comparisons, and a critical *P*-value of 0.05 was used for significance in all tests.

RESULTS

Seasonal variation in the environment

The four environmental factors that we examined each showed significant seasonal variation, but only two factors showed inter-annual differences (Kruskal–Wallis tests with *post hoc* Dunn's tests). In each year of the study, significant wave heights were smallest in summer and autumn, and largest in winter ($P<0.005$). Compared with those in year 2, the wave heights in year 1 were smaller in the summer and autumn but bigger in the winter and spring ($P<0.005$). Water temperature was warmest in the autumn and coolest in the spring of each year, and every season in year 1 was warmer than the same season in year 2 ($P<0.005$). Upwelling was strongest in the summer and weakest in the winter of each year ($P<0.05$), and there were no differences in upwelling between study years ($P>0.05$). PAR was highest in the summer and spring, and lowest in the winter of each year ($P<0.005$), and there were no differences in PAR between study years ($P>0.05$). The study period encompassed an ENSO event in the late autumn, winter and early spring of year 1 (Barnard et al., 2017). ENSO events are characterized by warm waters and increased wave action, which explains the inter-annual differences we observed in water temperature and significant wave height.

Seasonal changes in material properties

We examined seasonal variation in the material properties of two regions of the rachis of *E. menziesii*: $TR_{0–10}$ and $TR_{40–50}$. In general, material properties of $TR_{0–10}$ and $TR_{40–50}$ (Fig. 5) showed similar seasonal variation (ANOVA results in Table 1). In each tissue region, F_{brk}, σ_{brk}, σ_{yield}, E_1 and E_2 were largest in autumn and winter, and smallest in spring and summer, while λ_{brk} was largest in spring and summer, and smallest in autumn and winter. λ_{brk} and σ_{yield} were the only properties that showed an effect of year: at each tissue region, λ_{brk} was smaller in year 1 than in year 2, and σ_{yield} at $TR_{0–10}$ was larger in year 1 than in year 2. W/V showed different seasonal patterns between the two tissue regions. W/V of $TR_{0–10}$ was largest in spring and summer, and smallest in autumn and winter, while the opposite was true for $TR_{40–50}$.

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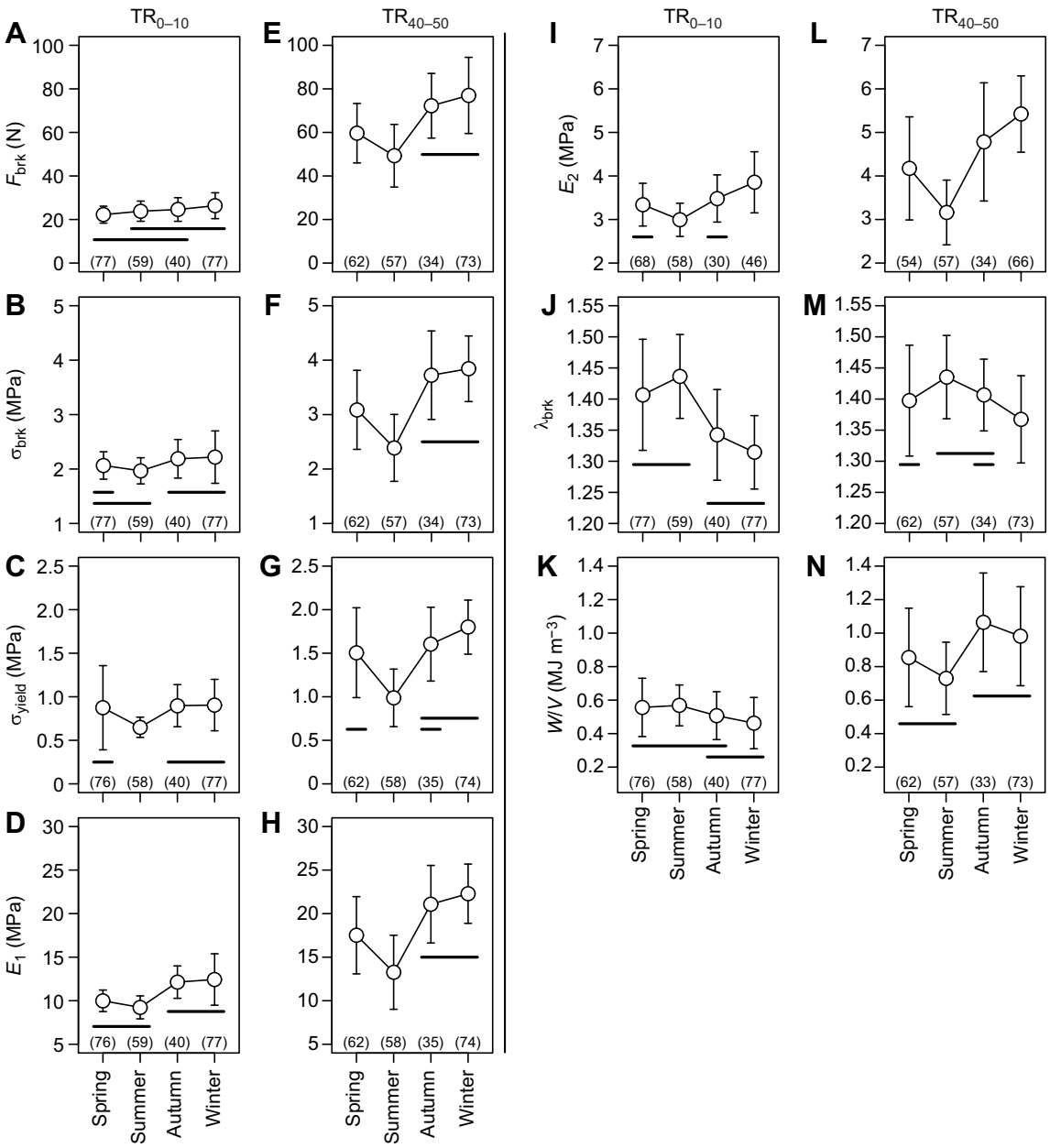

Fig. 5. Seasonal changes in material properties. Material properties plotted as a function of season for rachis tissue 0–10 cm from the IM ($TR_{0–10}$; A–D, I–K) and 40–50 cm from the IM ($TR_{40–50}$; E–H, L–N). F_{brk}, breaking force. Data points are means and error bars show 1 s.d. Solid lines at the same vertical position below the data indicate statistically similar seasons (ANOVA with Bonferroni *P*-value adjustments for multiple comparisons, P=0.05 for significance). Numbers in parentheses below data points are sample sizes.

Most of the material properties of each tissue region, averaged by season, were strongly correlated with significant wave height, upwelling and PAR, with the direction of the correlation depending on the property (see Pearson's correlation coefficients reported in Table 2). No material properties showed strong correlations with water temperature.

Effects of growth on material properties

Seasonal changes in growth rate

Growth rate of fronds showed an effect of season and year (ANOVA results in Table 1). Growth rate was faster in spring and summer than in autumn and winter (P<0.005; Fig. 6), but overall, growth rate was faster in 2015 than in 2016 (P=0.019).

Seasonal averages of growth rate were strongly correlated to upwelling (Pearson's r=0.79, P<0.05) and PAR (r=0.85, P<0.05), but not correlated to significant wave height (r=−0.54, P>0.05) or water temperature (r=−0.52, P>0.05). Material properties that showed a strong correlation with upwelling and PAR also showed a strong correlation to growth rate (Table 2).

Correlations between growth rate and material properties of individual rachises

Material properties of $TR_{0–10}$ and $TR_{40–50}$ were correlated to frond growth rate (Fig. 7). At both tissue regions, F_{brk}, σ_{brk}, σ_{yield}, E_1 and E_2 decreased with growth rate, and λ_{brk} increased with growth rate. However, W/V increased with growth rate at $TR_{0–10}$ but decreased with growth rate at $TR_{40–50}$.

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Table 1. ANOVA results for effects of season and year on material properties of $TR_{0–10}$ and $TR_{40–50}$, and on growth rates with Type III sum of squares

	$TR_{0–10}$					$TR_{40–50}$				
	d.f.	SS	MS	*F*	*P*	d.f.	SS	MS	*F*	*P*
F_{brk}										
Season	3	5.78	1.93	8.116	<0.005	3	110.84	36.95	42.421	<0.005
Year	1	0.34	0.34	1.433	0.23	1	0.13	0.13	0.148	0.70
Error	248	58.91	0.24			221	192.48	0.871		
σ_{brk}										
Season	3	0.260	0.087	6.60	<0.005	3	6.237	2.079	59.604	<0.005
Year	1	1.600×10^{-5}	1.600×10^{-5}	1.20×10^{-3}	0.97	1	0.013	0.013	0.380	0.54
Error	248	3.26	0.013			221	7.709	0.035		
σ_{yield}										
Season	3	0.419	0.140	18.025	<0.005	3	3.981	1.327	50.913	<0.005
Year	1	0.126	0.126	16.272	<0.005	1	0.033	0.033	1.279	0.26
Error	246	1.908	0.008			224	5.838	0.026		
E_1										
Season	3	10.31	3.44	44.164	<0.005	3	405.74	135.25	62.914	<0.005
Year	1	0.09	0.09	1.161	0.28	1	5.39	5.39	2.505	0.11
Error	247	19.21	0.08			224	481.53	2.15		
E_2										
Season	3	1.372	0.457	23.111	<0.005	3	9.863	3.288	59.608	<0.005
Year	1	0.008	0.008	0.395	0.53	1	0.111	0.111	2.016	0.16
Error	197	3.898	0.020			206	11.362	0.055		
λ_{brk}										
Season	3	0.655	0.218	41.248	<0.005	3	0.032	0.011	11.577	<0.005
Year	1	0.030	0.030	5.583	0.019	1	0.008	0.008	9.048	0.003
Error	248	1.312	0.005			221	0.202	0.001		
W/*V*										
Season	3	0.277	0.092	8.680	<0.005	3	0.767	0.256	12.727	<0.005
Year	1	0.031	0.031	2.914	0.089	1	0.060	0.060	2.997	0.085
Error	246	2.617	0.011			220	4.419	0.020		
Growth rate										
Season	3	14.775	4.925	83.619	<0.005					
Year	1	0.328	0.33	5.560	<0.005					
Error	326	19.201	0.059							

$TR_{0–10}$, tissue region 0–10 cm from the intercalary meristem (IM); $TR_{40–50}$, tissue region 40–50 cm from the IM; F_{brk}, breaking force; σ_{brk}, breaking stress; σ_{yield}, yield stress; E_1, E_2, sample modulus at low and high extension, respectively; λ_{brk}, breaking extension ratio; *W*/*V*, work per volume to fracture.

Effects of wounds on material properties

Instantaneous effects of wounds on material properties

As the size of a wound in the middle of the rachis increased and the size of the tissue remaining at the edge of the rachis decreased, the relative strength of the remaining edge tissue increased (Fig. 8A). That is, edge tissue has a higher breaking stress than tissue in the middle of the rachis, such that when a rachis is wounded (e.g. feeding or burrowing by amphipods) the amount that the rachis is weakened (i.e. reduction in σ_{brk}) is not directly proportional to the size of the wound.

Healing response to wounds

Rachises responded to wounds by increasing the cross-sectional area of the tissue around the wound (from 58% to only 28% smaller than the adjacent tissue, paired *t*-test, $P<0.005$, d.f.=14), but not by changing the σ_{brk} ($P=0.72$). Thus, wounded rachises increased the force required to break them, F_{brk} (from 43% to only 8% smaller than the adjacent tissue, $P<0.005$) by making more tissue around the wound rather than increasing tissue strength (Fig. 8B–D). Rachises tested at the faster loading regime (strain rate=3.3×10^{-1} s^{-1}) showed similar results: no change in σ_{brk} ($P=0.07$, d.f.=9) but an increase in F_{brk} ($P<0.005$) due to an increase in cross-sectional area ($P=0.02$).

DISCUSSION

We tested whether the material properties of the rachis of *E. menziesii* changed with season, with growth of the fronds and after wounding. Overall, we found that material properties changed seasonally and were correlated with growth rate, and fronds responded to wounds by increasing the cross-sectional area of the tissue around wounds rather than by changing the material properties of the tissue.

Effects of season and growth on material properties

The material properties of the rachis showed strong seasonal patterns but little variation between years. Properties relating to strength (F_{brk}, $\sigma_{brk,}$), stiffness (E_1, E_2) and toughness (*W*/*V*) showed no inter-annual differences at either of the tissue regions that we studied, while extensibility (λ_{brk}) and yield stress (σ_{yield}) changed with season and year. The low inter-annual variation in material properties is surprising because the study period encompassed an ENSO event that led to increased water temperatures and increased wave height near the study sites. In contrast, upwelling and PAR near the study sites did not differ between years, but – like water temperature and wave height –did show strong seasonal patterns. Upwelling and PAR are known to influence kelp growth: upwelling promotes kelp growth by delivering nutrient-rich water to the shore, and PAR provides the correct wavelength of light for photosynthesis (Kain, 1989; Hurd et al., 1996; Dayton et al., 1999; Dean and Hurd, 2007). Wave-driven water motion can promote kelp growth by mixing the water column and increasing the delivery and uptake of nutrients by the kelp, but this is most effective in places where nutrients are limited (Hurd et al., 1996). Additionally, water temperature has been found to have a minimal effect on kelp growth when compared with other environmental factors such as nutrients and light availability (Kain, 1989; Brown et al., 1997).

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Table 2. Pearson correlation coefficients between the seasonal averages of material properties, growth rate and environmental factors

Tissue region	Property	Significant wave height	Temperature	Upwelling	PAR	Growth rate
Whole rachis	Growth rate	−0.54	−0.52	0.79*	0.85*	–
$TR_{0–10}$	F_{brk}	0.37	0.18	−0.69	−0.74*	−0.70
	σ_{brk}	0.78*	0.17	−0.82*	−0.93**	−0.89**
	σ_{yield}	0.55	−0.51	−0.55	−0.40	−0.87*
	E_1	0.75*	0.31	−0.86*	−0.95***	−0.94**
	E_2	0.88**	−0.07	−0.85*	−0.89**	−0.74
	λ_{brk}	−0.79*	−0.26	0.87**	0.94**	0.89*
	W/*V*	−0.75*	−0.30	0.83*	0.88**	0.82*
$TR_{40–50}$	F_{brk}	0.74*	0.15	−0.93**	−0.89**	−0.97***
	σ_{brk}	0.73*	0.17	−0.88**	−0.86*	−0.96**
	σ_{yield}	0.82*	−0.14	−0.90**	−0.80*	−0.92**
	E_1	0.79*	0.16	−0.91**	−0.88**	−0.93**
	E_2	0.81*	0.09	−0.91**	−0.88**	−0.89*
	λ_{brk}	−0.83*	0.00	0.69	0.65	0.41
	W/*V*	0.41	0.20	−0.66	−0.66	−0.96***

n=8 seasons for each correlation, except between growth rate and material properties, where only seven seasons were used for each correlation. Asterisks indicate significant correlations: **P*<0.05, ***P*<0.005, ****P*<0.0005.

Correspondingly, we found that kelp growth was most strongly correlated to upwelling and PAR, but not temperature or wave action (Table 2). Our results also showed that, for populations averaged within seasons, material properties were correlated to both the environment and frond growth, suggesting that the environment indirectly affects material properties by influencing growth rate. The specific mechanism by which growth may influence the material properties of the rachis is likely related to changes in the thickness or composition of the tissue cell walls that occur with growth (e.g. Koehl and Wainwright, 1977; Vincent, 1982; Kokubo et al., 1989; Martone, 2006; Martone et al., 2009; Starko et al., 2018).

Seasonal variation in the growth rate of fronds appeared to be a strong driver of the seasonal variation in material properties, as well as the distribution of material properties along the length of rachises. As in many species of macroalgae, material properties of *E. menziesii*, such as strength and stiffness, tend to increase in magnitude with the tissue age (Krumhansl et al., 2015; Starko et al., 2018). Frond growth on *E. menziesii* occurs mostly as elongation of the rachis near the IM. Therefore, tissue adjacent to the IM ($TR_{0–10}$) is the youngest tissue on the rachis, which supports our findings that show it is also the weakest, least stiff and least tough tissue on the rachis. When growth rates are high (i.e. in spring and summer), there is an increased amount of 'new' or 'young' tissue on the rachis, such that an increased amount of the rachis' length will have material properties that are similar to those of young tissue. Thus, the seasonal patterns and the spatial distribution of material properties along the length of the rachis can be attributed to the true age of the tissue (Fig. 3).

The mechanical strategy (being strong or being extensible) that allowed fronds to withstand hydrodynamic forces differed between regions of the rachis. Seasonal patterns of toughness for $TR_{0–10}$ closely mirrored the tissue's extensibility, whereas seasonal patterns of toughness for $TR_{40–50}$ closely mirrored the tissue's strength. That is, it appears that extensibility contributed more than strength to the toughness of tissue near the IM, and strength contributed more than extensibility to the toughness of tissue near the holdfast. This pattern is not surprising because tissue regions near the holdfast (i.e. far from the IM) are where stresses in the rachis are highest. As the flexible fronds move back and forth in wave-driven flow, the hydrodynamic forces on the fronds pull on them for a large portion of each wave cycle and the tensile stresses are largest in the rachis tissue adjacent to the holdfast (Friedland and Denny, 1995). Correspondingly, we found that the strength, stiffness and toughness of rachis tissue increased with distance from the IM, such that the parts of the frond being loaded most by tension while the frond is pulled by waves are also the parts of the frond most capable of resisting that tension. The difference in mechanical behavior between $TR_{0–10}$ and $TR_{40–50}$ could therefore be due to the unique loading regimes that each tissue region

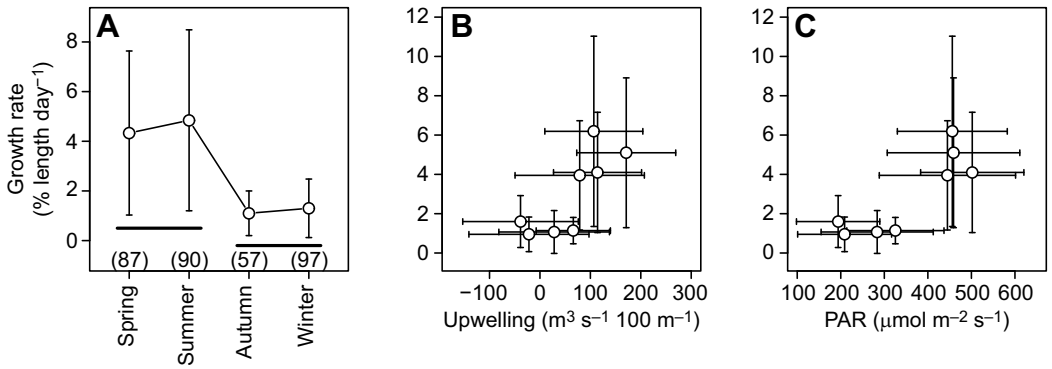

Fig. 6. Frond growth rate. (A) Growth rate of fronds plotted as a function of season. Solid lines below data indicate statistically similar groups (ANOVA with Bonferroni *P*-value adjustments for multiple comparisons, *P*=0.05 for significance). Numbers in parentheses below data points show sample sizes. (B) Seasonal growth rate plotted as a function of seasonal upwelling (Pearson's *r*=0.79, *P*<0.05). (C) Seasonal growth rate plotted as a function of seasonal photosynthetically active radiation (PAR) (*r*=0.85, *P*<0.05). In each panel, data are means and error bars show 1 s.d.

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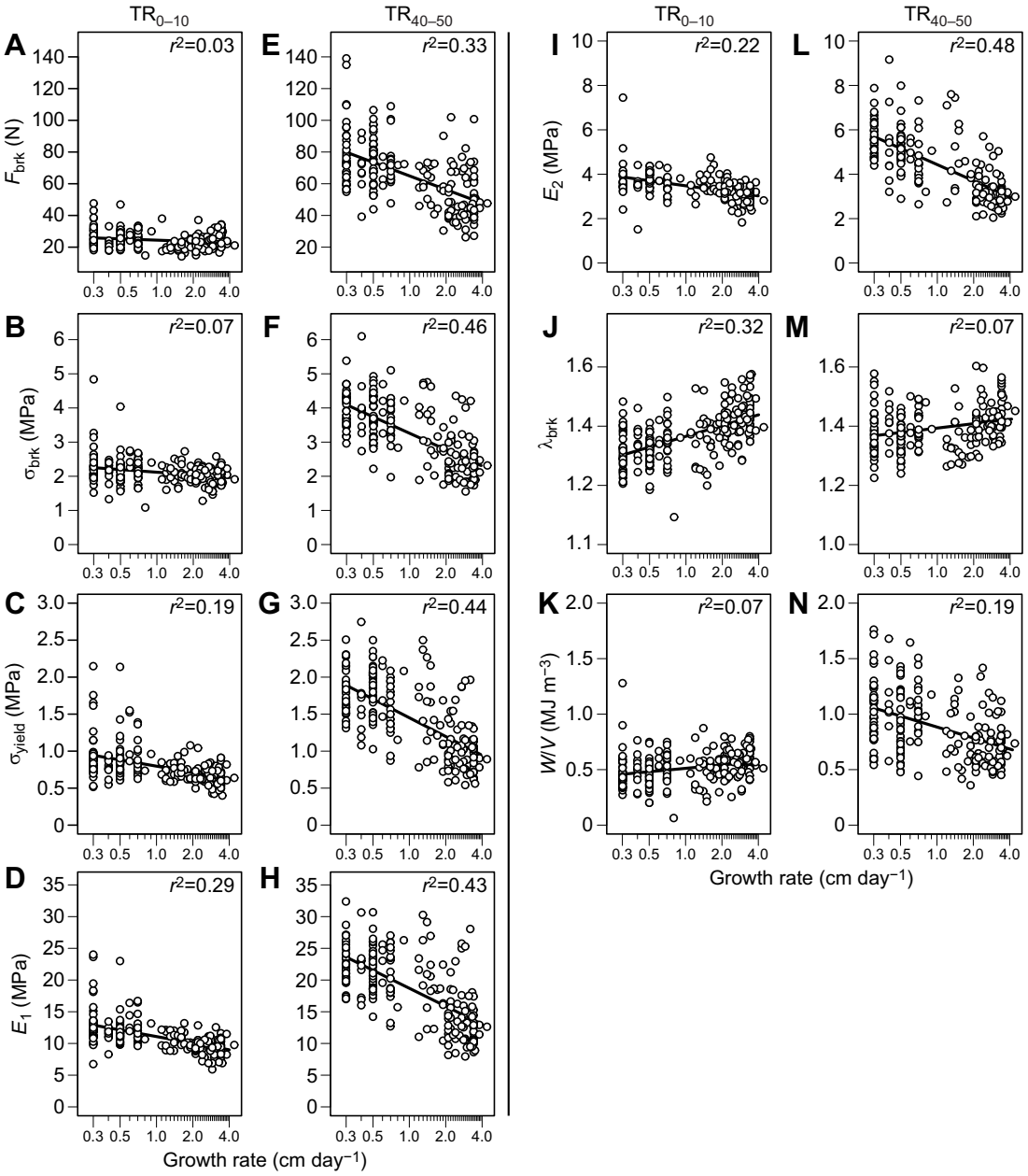

Fig. 7. Correlation between growth rate and material properties. Material properties plotted as a function of growth rate for $TR_{0–10}$ (A–D, I–K) and $TR_{40–50}$ (E–H, L–N) of individual fronds. Within each tissue region, each data point is from a separate kelp. Lines show statistically significant linear regressions (P<0.005 for each). Sample sizes in each panel range from 170 to 221 kelp.

experiences. $TR_{0–10}$ is not heavily loaded by hydrodynamic forces and is also not very strong, suggesting that the kelp can modulate the properties of its tissues along the length of the frond in response to applied loads (Johnson and Koehl, 1994), possibly by modifying the arrangement or thickness of its cell walls. For instance, when grown with an applied load, fronds of *E. menziesii* assimilated more carbon into their cell walls and were also stronger than when the kelp were grown without an applied load (Kraemer and Chapman, 1991). Similarly, cellular architecture, such as the arrangement of cells, cell wall thickness and the presence of cell wall polysaccharides (e.g. alginic acid, carrageenans), has been shown to alter mechanical properties of other macroalgae (Koehl and Wainwright, 1977; Carrington et al., 2001; Martone, 2006; Martone et al., 2009; Starko et al., 2018).

Effects of wounds on material properties

Values of material properties of tissue near the edge of a rachis were larger than those near the middle of the rachis, and this can help the frond resist breakage when the middle of the frond is wounded. That is, a wound in the middle of the rachis (e.g. from grazing or burrowing by amphipods; Fig. 4) will have a disproportionately small effect on the strength of the rachis relative to the size of the wound. Similarly, the limpet *Lottia insessa* also frequently wounds the rachises of *E. menziesii*, creating a homescar in the middle of the rachis while leaving tissue on the edge of the rachis intact (Black, 1974, 1976). The mechanical differences between the middle and edge of the rachis are likely due to the multiple tissue types within the rachis that each have different material properties (Harder et al., 2006). The stipes and fronds of many species of kelp can be divided

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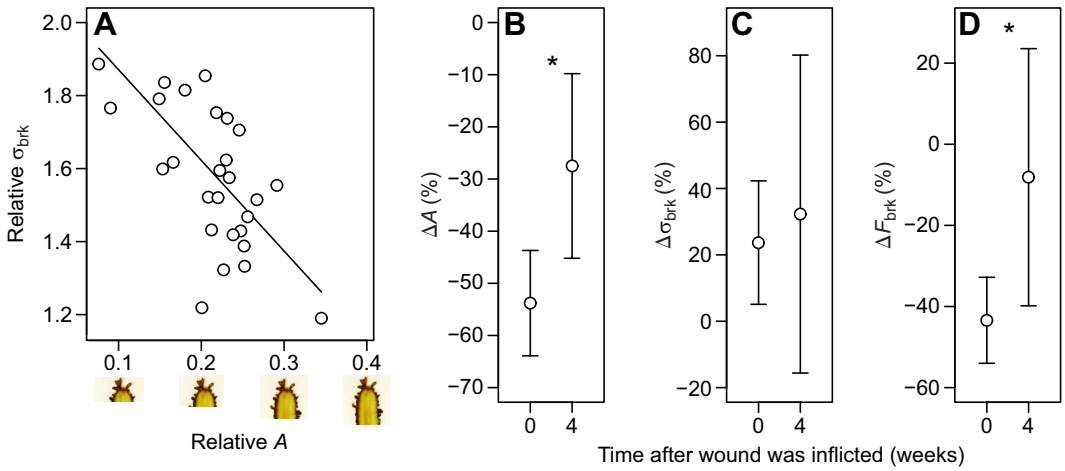

Fig. 8. Effects of wounds on material properties. (A) The relative σ_{brk} of the tissue (sample σ_{brk}/rachis σ_{brk}) remaining at the edge (e.g. after a wound) plotted as a function of the relative cross-sectional area (*A*) of the tissue (sample *A*/rachis *A*). The line shows a statistically significant correlation (P<0.005, r^2=0.39, n=28). (B,C) Change in the percentage difference (unwounded tissue versus wounded tissue, Eqn 1) of (B) CSA, (C) σ_{brk} and (D) F_{brk} for fronds 0 and 4 weeks after being wounded. Data points show mean values and error bars show 1 s.d. Asterisks indicate statistically significant changes over time (paired *t*-test, P<0.005, d.f.=14).

into three tissue types with different cell structure: the meristoderm, cortex and medulla (e.g. Schiel and Foster, 2015) (Fig. 1D). The meristoderm is the outermost layer and is composed of a few layers of epidermal cells. The cortex, which is just inside the meristoderm, is composed of cells that are aligned with their long axes perpendicular to the length of the structure. The medulla, which is just inside the cortex, is composed of elongated cells that run parallel to the length of the structure. Although these tissue types are difficult to separate in many kelp species, previous work has shown that the cortex can be stiffer (higher E_1 and E_2) and stronger (higher σ_{brk} and W/V) than the medulla (Koehl and Wainwright, 1977; Harder et al., 2006), which may be due in part to the alignment and directionality of cells in each tissue type. In total, the tissue-specific trends in material properties reported by these earlier works support the data collected in the present study (Fig. 8). The arrangement of the tissues within *E. menziesii* fronds allows the frond to remain strong even after being wounded, and this is an important trait for a kelp that is frequently wounded by amphipods and other herbivores (Black, 1976; Sotka, 2007; Burnett and Koehl, 2018).

After being wounded, the rachis of *E. menziesii* responded to the wound by increasing the cross-sectional area of the tissue around the wound, but not by increasing the σ_{brk} of the tissue. As a result, the tissue around the wound increased its F_{brk} over time, and this response differed from that of other seaweeds that increased the σ_{brk} of wounded tissues over time (Lowell et al., 1991). The wound response of *E. menziesii* appears to be coupled to the growth of the frond because the tissue around the wound, as well as the unwounded tissue on other parts of the rachis, all increased cross-sectional area and F_{brk} over 4 weeks. Therefore, the response of the kelp to wounds may not be a specialized 'healing' response (e.g. Lowell et al., 1991) but rather the result of a general growth pattern that can compensate for structural damage. Wounds can alter the translocation of materials (sugars, nutrients) within the frond or, if significant enough, cause the kelp to devote energy toward growing new fronds (i.e. a branching response) rather than continue growing the wounded frond (Sargent and Lantrip, 1952; Black, 1974; Fox, 2013; Knoblauch et al., 2016), but there was no evidence of altered growth in the individuals used in our study. Overall, wounded and healed tissues were able to resist both slow and fast strain rates, suggesting that the kelp's healing mechanism is sufficient to reduce the risk of the frond breaking from different loading regimes, such as the wave surge (slow rate of loading) and wave impingement (fast rate of loading) portions of a wave cycle (Gaylord, 1999; Gaylord et al., 2008; Jensen and Denny, 2015).

Material properties and the kelp's perennial lifespan

Over its lifetime, *E. menziesii* experiences seasonal increases in wave action and seasonal increases in herbivory (Wolcott, 2007; Burnett, 2017). Waves, and the consequent hydrodynamic forces, are largest in winter, while herbivory is greatest in summer and autumn. However, wounds from herbivory, in combination with the seasonal patterns of the rachis tissue's material properties and growth rate, may actually help *E. menziesii* survive the large waves of winter storms. First, as the kelp grows through summer and autumn, the fronds accrue wounds that eventually make them break in the waves, resulting in a decrease in the kelp's total size (de Bettignies et al., 2012; Burnett, 2017). Second, the slowed growth of the kelp in winter means that the fronds, and the whole kelp, stay small throughout the season. Being small when waves are large minimizes the hydrodynamic load acting on the entire kelp and consequently reduces the risk that the entire kelp will be dislodged from the substratum (Black, 1976; Demes et al., 2013b). Lastly, the slowed growth and strong material properties of the fronds means that the remaining fronds on the kelp are able to resist further breakage. Surviving through winter with strong fronds allows the kelp to maintain its dominance as a canopy-forming kelp in the intertidal zone and to begin growing to a large size immediately after winter ends (Burnett, 2017).

Considerations for studying kelp material properties

It is thought that *E. menziesii* can grow to large sizes and survive through multiple years in hydrodynamically challenging habitat because of the exceptional strength of its rachis tissue (Friedland and Denny, 1995). It is difficult to make an accurate comparison of *E. menziesii*'s material properties with those of other macroalgae because most studies of macroalgal material properties use different techniques and often do not report the position, season or growth rate of the tissue being tested; thus, comparing data across species from multiple publications can be misleading. If we overlook methodological differences between studies, it appears that rachis tissue of *E. menziesii* is not exceptionally strong compared with that

of other macroalgae. The values that we measured for λ_{brk}, σ_{brk} and E_1 were within the range of those properties for other macroalgae (Johnson and Koehl, 1994; Koehl, 2000; Harder et al., 2006; Krumhansl et al., 2015). Our results showed that *E. menziesii* had a smaller E_2 than *N. luetkeana* and some observations of *Lamarina* spp. (Johnson and Koehl, 1994; Harder et al., 2006), but it had a similar E_2 to the macroalgae *Macrocystis pyrifera*, *Pterygophora californica*, *Durvillaea antarctica* and *Alaria marginata* (Koehl, 2000; Harder et al., 2006; Krumhansl et al., 2015). Lastly, *E. menziesii* appears to be tougher (W/V) than *Laminaria* spp. but equal in toughness to *D. antarctica*, *Lessonia nigrescens*, *Postelsia palmaeformis* and *N. luetkeana* (Johnson and Koehl, 1994; Koehl, 2000; Harder et al., 2006). However, data on the toughness of macroalgae are not as widely reported in the literature as stiffness and strength. Without similar examinations of other macroalgae, it is difficult to pinpoint whether *E. menziesii* has unique material properties, or unique combinations of material properties, that enable its survival in such a physically and biologically challenging habitat.

Our data indicate that the material properties of *E. menziesii* were sensitive to numerous factors that have often been overlooked by other studies investigating the mechanics of this large kelp and how it survives on wave-swept rocky shores. We found that the material properties of *E. menziesii* changed with season, growth and wounds. The values for λ_{brk}, σ_{brk} and E_1 of *E. menziesii* rachises reported here are similar in magnitude to those measured by Demes et al. (2013b) on a similar materials-testing machine, whereas our measurements of F_{brk} and σ_{brk} are lower than those reported by others who broke the kelp in the field using spring scales (Black, 1976; Friedland and Denny, 1995; Blanchette et al., 2002). Differences between material properties of the rachis reported in the present study and those reported in previous *in situ* studies could be due to the difficulty of controlling strain rate during field measurements with spring scales and to differences in season, growth, tissue region and wounding of the tissue used. Because the material properties of *E. menziesii* were strongly linked to frond growth rate, we can conclude that future changes in oceanographic conditions that alter kelp growth will have consequences for the material properties of the rachis tissue and its ability to withstand hydrodynamic forces and biological stressors such as herbivory (Hurd et al., 1996; Dayton et al., 1999; Simonson et al., 2015). For instance, the frequency and severity of storms (Cai et al., 2014), as well as the intensity of herbivory (O'Connor, 2009; Poore et al., 2013, 2014), are expected to increase with climate change. If storms occur in a period when *E. menziesii* is growing rapidly, such as during periods of increased upwelling or PAR (Fig. 6), the fronds could be more susceptible than normal to breakage. If herbivory wounds occur in a period when *E. menziesii* is growing slowly, such as during periods of decreased upwelling or PAR, the rachis may not be able to heal as rapidly and could be more susceptible than normal to breakage. Overall, material properties can be affected by a variety of endogenous and exogenous factors, and understanding those relationships, through careful baseline monitoring and mechanistic studies, can help us predict how macroalgae and their associated ecosystems will be affected by future changes in our climate (e.g. Harley et al., 2012).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: N.P.B.; Methodology: N.P.B., M.A.R.K.; Software: N.P.B.; Validation: N.P.B.; Formal analysis: N.P.B.; Investigation: N.P.B.; Resources: N.P.B.; Data curation: N.P.B.; Writing - original draft: N.P.B., M.A.R.K.; Writing - review & editing: N.P.B., M.A.R.K.; Visualization: N.P.B.; Supervision: M.A.R.K.; Project administration: N.P.B.; Funding acquisition: N.P.B., M.A.R.K.

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Data availability
Data are available from N.P.B. upon request.

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