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MORPHOLOGICAL PLASTICITY IN THE KELP *NEREOCYSTIS LUETKEANA* (PHAEOPHYCEAE) IS SENSITIVE TO THE MAGNITUDE, DIRECTION, AND LOCATION OF MECHANICAL LOADING[1]

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Nereocystis luetkeana **is a canopy-forming kelp that exhibits morphological plasticity across hydrodynamic gradients, producing broad, undulate blades in slow flow and narrow, flattened blades in fast flow, enabling thalli to reduce drag while optimizing photosynthesis. While the functional significance of this phenomenon has been well studied, the developmental and physiological mechanisms that facilitate the plasticity remain poorly understood. In this study, we conducted three experiments to characterize how the (1) magnitude, (2) direction, and (3) location of plasticity-inducing mechanical stimuli affect the morphology of *Nereocystis* blades. We found that applying a gradient of tensile force caused blades to grow progressively longer, narrower, less ruffled, and heavier in a linear fashion, suggesting that *Nereocystis* is equally well adapted for all conditions within its hydrodynamic niche. We also found that applying tension transversely across blades caused the growth response to rotate 90°, indicating that there is no substantial separation between the sites of stimulus perception and response and suggesting that a long-distance signaling mechanism, such as a hormone, is unlikely to mediate this phenomenon. Meristoderm cells showed morphological changes that paralleled those of their respective blades in this experiment, implying that tissue-level morphology is influenced by cell growth. Finally, we found that plasticity was only induced when tension was applied directly to the growing tissue, reinforcing that long-distance signaling is probably not involved and possibly indicating that the mechanism on display generally requires an intercalary meristem to facilitate mechanoperception.**

Key index words: **algae; biomechanics; growth; hydrodynamics; morphology; phenotypic plasticity; physiology; reaction norm; thigmomorphogenesis**

Abbreviations**: A_B, blade area; A_C, mean meristoderm cell area; A_S, kelp tissue sample area; BMSC, Bamfield Marine Sciences Centre; ΔA_B, change in blade area; ΔA_S, change in kelp tissue sample surface area; ΔL_B, change in blade length; ΔL_S, change in kelp tissue sample length; ΔM_B, change in blade wet mass; ΔM_S, change in kelp tissue sample wet mass; ΔR, change in blade ruffle; ΔT, change in blade thickness at 10 cm from the origin; ΔW_B, change in blade width at 10 cm from the origin; ΔW_S, change in kelp tissue sample width; L_B, blade length; L_C, mean meristoderm cell length; L_P, projected length of blade edge; L_S, kelp tissue sample length; L_T, total length of blade edge; M_B, blade wet mass; M_S, kelp tissue sample wet mass; R, blade ruffle; T, blade thickness at 10 cm from the origin; UBC, University of British Columbia; W_B, blade width at 10 cm from the origin; W_C, mean meristoderm cell width; W_S, kelp tissue sample width**

Moving water presents marine macroalgae with a complex evolutionary challenge. While high levels of water motion can be beneficial to these organisms by improving mass transfer rates across diffusion boundary layers and increasing primary productivity (Wheeler 1980, Gerard 1982, Hurd et al. 1996), excessively high levels can cause attachment or support tissues to fail, generally resulting in mortality (Koehl and Wainwright 1977, Blanchette 1997, Duggins et al. 2001, Demes et al. 2013). To reap the benefits of flow while mitigating its hazards, seaweeds have adopted a diverse range of biomechanical and evolutionary strategies (Koehl and Wainwright 1977, Denny and Gaylord 2002, Martone et al. 2012, Starko and Martone 2016a).

Nereocystis luetkeana (hereafter referred to as *Nereocystis*) is a canopy-forming annual kelp that grows in a broad range of hydrodynamic environments (Abbott and Hollenberg 1976, Johnson and Koehl 1994). This alga addresses the challenge of flow-induced mechanical forces, in part, by exhibiting morphological plasticity across hydrodynamic gradients (Koehl et al. 2008). When living in hydrodynamically forceful environments, thalli develop narrow, flat blades, and when living in sheltered environments, thalli develop broad, undulate blades (Koehl and Alberte 1988, Koehl et al. 2008). The narrow-bladed morphology causes blades to compress into

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a streamlined cluster in flow, which reduces drag, whereas the broad-bladed morphology causes blades to flap and oscillate in flow, preventing the formation of a cluster, which increases light interception by limiting self-shading (Koehl and Alberte 1988). Development of one morphology or the other is mediated by mechanical loading imposed by drag (Gerard 1987, Koehl et al. 2008) and, due to the ability of *Nereocystis* blades to elongate at rates as high as 10–14 cm $\cdot$ d^{-1} (Abbott and Hollenberg 1976, Kain 1987), the kelp can adjust its overall growth form extremely quickly (Koehl et al. 2008). Individual blades each have their own intercalary meristems located at their bases (Nicholson 1970, Kain 1987) and the morphology of each blade can be regulated independently (Koehl et al. 2008). Overall, morphological plasticity in *Nereocystis* blades allows the kelp to maximize photosynthetic output while minimizing the amount of drag it experiences in a given environment (Koehl and Alberte 1988).

While the functional consequences of this phenomenon have been well studied, there remains much that is not understood. For instance, as previous experiments have primarily utilized binary "weight" and "no weight" designs (Gerard 1987, Koehl et al. 2008), we have not yet characterized a full reaction norm (how trait phenotypes vary across an environmental gradient; Woltereck 1909, Stearns 1989) of blade morphology exhibited by a *Nereocystis* across a wide range of mechanical loading. A reaction norm would provide information on how selective pressures relating to hydrodynamic forces act on blade morphology (Gibert et al. 1998, David et al. 2004). If, for example, the kelp produced a linear reaction norm of blade shape across a wide range of flow conditions, we would infer that selection on blade morphology was the same across all flow conditions and that *Nereocystis* was equally well adapted for all tested environments (Gibert et al. 1998). Alternatively, if the kelp produced a logistic reaction norm, it would suggest that selection favored extreme phenotypes over intermediate ones and that *Nereocystis* was best suited for either very slow or very fast flow conditions (Gibert et al. 1998). Information like this may allow us to clarify the biogeographic range of *Nereocystis*, as well as enable us to predict shifts in those limits that might occur as global hydrodynamic environments continue to change (Young et al. 2011, Wang et al. 2014). Characterizing a reaction norm would also allow us to identify limits to morphological plasticity, which could help characterize hydrodynamic constraints on the kelp. If, for instance, we saw that blade morphology stopped responding to tensile force over a certain magnitude, it might suggest that the hydrodynamic benefit of altering blade shape is not constant across flow regimes. For instance, several studies on smaller wave-swept seaweeds have found that at higher flow velocities, morphology becomes a less important determinant of hydrodynamic performance than size (Milligan and DeWreede 2004, Bettignies et al. 2013).

While we know little about the range of morphologies that *Nereocystis* blades can achieve through phenotypic plasticity, we know even less about the physiological mechanisms that generate this plasticity. It has been shown in previous studies by Gerard (1987) and Koehl et al. (2008) that sustained tension induces kelp blades to grow narrower, longer, and less ruffled without any changes in thickness or biomass accumulation rate. This was hypothesized to be the result of mechanical forces inducing meristematic cells to preferentially divide in the longitudinal axis of the blade, thereby increasing elongation and reducing widening (Gerard 1987). However, this proposed mechanism has yet to be explicitly demonstrated and nothing more about the physiological processes that enable this control of cell division is known.

In contrast to kelps, land plants have a moderately well-understood set of physiological mechanisms for detecting and responding to mechanical stimuli (reviewed in Jaffe et al. 2002, Braam 2005, Telewski 2006, Chehab et al. 2008, Monshausen and Gilroy 2009, Monshausen and Haswell 2013, Sampathkumar et al. 2014, Moulia et al. 2015). It is widely believed that the initial step in plant mechanoperception is cell wall deformation (reviewed in Jaffe et al. 2002, Monshausen and Gilroy 2009). Such deformation applies tension to the plasma membrane, which initiates a signaling cascade (reviewed in Jaffe et al. 2002, Chehab et al. 2008, Monshausen and Gilroy 2009, Monshausen and Haswell 2013, Sampathkumar et al. 2014); this membrane tension and the responses it elicits can be localized and directionally specific (Gus-Mayer et al. 1998, Louveaux et al. 2016). When the signals generated in response to mechanical stimulation ultimately influence growth and development, the entire physiological process, from stimulus detection to ultimate response, is referred to as thigmomorphogenesis (Jaffe 1973). Phytohormones are thought to be among the various signaling molecules involved in mediating this process (e.g., Erner and Jaffe 1982, Biro and Jaffe 1984, Chehab et al. 2008, 2012, Malabarba et al. 2019). These substances are probably the reason that thigmomorphogenetic effects can be induced in tissue regions far from where a mechanical stimulus was actually applied (Erner et al. 1980, Coutand et al. 2000). The ultimate growth responses seen in cases of plant thigmomorphogenesis have been shown to result from changes in patterns of cell elongation and/or division (Biro et al. 1980, Louveaux et al. 2016).

Given that kelps and land plants show remarkable ecological and morphological similarity as a result of convergent evolution (Steneck et al. 2002, Keeling 2004, Starko and Martone 2016b, Graham et al. 2017), we propose that they might utilize common

cellular features in a similarly convergent manner to address comparable evolutionary problems. If *Nereocystis* were to perceive and respond to mechanical stimuli similarly to land plants at the cellular level, we hypothesize that thalli might (1) detect such stimuli via deformation of its cell walls, (2) utilize hormones in the signal transduction cascade that followed stimulus detection (previously suggested by Charrier et al. 2012), and (3) modify its blade morphology by altering meristematic cell elongation and/or division patterns. While we are not currently able to rigorously test these hypotheses due to the limited set of molecular and cell biological methods currently available for kelp systems, we can conduct experiments to better characterize the growth response of kelp blades to mechanical loading. This will help us assess which of our hypotheses, if any, are likely to be true and might merit further examination.

In this paper, we report on three experiments to better characterize the effect of tensile force on the growth and morphology of *Nereocystis* blades. Each involved the use of weights as a proxy for drag-induced tension (Gerard 1987, Kraemer and Chapman 1991, Koehl et al. 2008). In the first experiment, hereafter referred to as the "load magnitude experiment," we examined how blade morphology changed across a wide gradient of tensile forces. We hypothesized that, given the broad hydrodynamic niche of *Nereocystis* (Johnson and Koehl 1994), (1) the application of a linear gradient of tensile force would yield an equally linear gradient of morphologies and (2) there would be no limits to the kelp's attainable phenotypes within the range of mechanical loading applied. In the second experiment, hereafter referred to as the "load direction experiment," we examined how the morphologies of blades and their associated meristematic cells were affected by applying tensile force to the blade transversely instead of longitudinally. We expected that the growth response yielded under high transverse loading would be rotated 90° compared to that yielded under high longitudinal loading, which would be consistent with the behavior of cell wall-mediated thigmomorphogenetic responses observed in plants. We also predicted that effects of tension would be observed in the morphologies of both the blades and their respective cells, suggesting that tissue-level morphological changes were being driven by cell growth. In the third experiment, hereafter referred to as the "load location experiment," we investigated whether a growth response to tension could be induced in blade meristematic tissue by applying tensile force only to non-growing distal tissue. We hypothesized that applying mechanical loading to only distal tissue would not invoke plasticity in the meristematic tissue, suggesting that a long-distance signaling mechanism, such as a hormone, is unlikely to be involved.

MATERIALS AND METHODS

Load magnitude experiment. Two mature *Nereocystis* sporophytes were collected from a surge channel located north of Brady's Beach (48°49′57″ N, 125°8′59″ W) in Bamfield, British Columbia on July 17, 2017. These kelps were returned to the Bamfield Marine Sciences Centre (BMSC) and housed in flow-through sea tables for less than 24 h. For each of the kelps collected, five blades were haphazardly selected and cut off from the pneumatocyst such that a small piece of pneumatocyst tissue was left intact at each blade base (Fig. 1). All blades were cut to a standard initial length of approximately 70 cm and the morphology of each blade was characterized (Fig. 1). A tape measure was used to quantify midline blade length between the origin and a small hole initially punched 50 cm distal (L_B); this was measured to the nearest 1 mm. Blade width and thickness at 10 cm from the origin (W_B and T respectively) were measured to the nearest 0.1 mm using vernier calipers. Blade surface area (A_B) was estimated by photographing the blades and using the program ImageJ (Rasband 2019) to measure the area of the flattened projections of each blade. Ruffle (R) was quantified using the following equation:

$$R = \frac{\Sigma L_T}{\Sigma L_P} \quad (1)$$

where L_T is the "total length" of each of the two blade edges, incorporating all ruffles and irregularities, and L_P is the flattened "projected length" of the two edges (Fig. 1). This method is modified from one utilized by Koehl and Alberte (1988) to permit non-destructive measurement. L_T and L_P of each blade edge were measured by laying a string along the edge from the origin to the point 50 cm from the origin as measured from the midline, then measuring the length of string laid down with a measuring tape to the nearest cm. Blade wet mass (M_B) was measured to the nearest 0.1 g after wiping excess water from tissue surfaces. The mean initial morphological measurements across all blades (± SE) were mean $L_B = 491.9 \pm 1.4$ mm; mean $W_B = 42.1 \pm 1.5$ mm; mean $T = 0.6 \pm 0.01$ mm; mean $R = 1.07 \pm 0.01$; mean $M_B = 25.5 \pm 0.9$ g; mean $A_B = 425.9 \pm 9.6$ cm^2. Mean W_B for these blades is comparable to that of kelps found in a wave- and current-sheltered environment as described by Koehl and Alberte (1988).

The most distal 15 cm of tissue of each blade was looped around a short PVC tube and the loop was sewn closed, thereby securing the tube to the blade (Fig. 2). This method was modified from one described in Koehl et al. (2008) for attaching weights to kelp blades. All blades were then transferred to two outdoor growth tanks, with the blades originating from each of the two kelps being allocated to separate tanks. These tanks were positioned side by side and exposed to direct ambient sunlight. Fresh seawater was continuously pumped into the tanks from the bottom of the nearby Grappler Inlet. This water was consistently between 10 and 12°C and had a salinity of 35. Fast incoming flow was not pointed directly at the experimental blades in order to minimize additional mechanical loading being applied to growing tissues, but blades were still exposed to low levels of water motion.

To secure kelp blades into the growth tanks, the proximal ends of each blade were attached to PVC bars suspended below water by wrapping cable ties around the intact pieces of pneumatocyst. The tubes attached to the distal ends of each blade were then connected to free-hanging weights by monofilament lines that extended horizontally across the tanks, then, aided by a set of pulleys, up and over the tank edges (Fig. 2). Each of the six blades in the two tanks had a

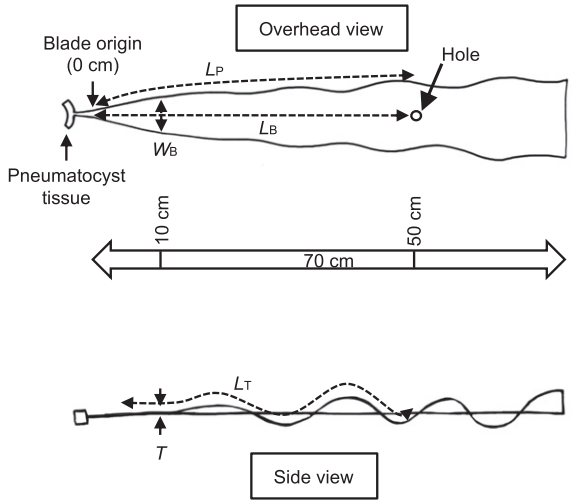

FIG. 1. Morphological measurements taken for experimental blades in load magnitude experiment. L_B = blade midline length; W_B = blade width at 10 cm from the origin; T = blade midline thickness at 10 cm from the origin; L_P = "projected" edge length; L_T = "total" edge length. All blades were cut to a standard initial length of 70 cm. L_B was measured between the blade origin and a small hole punched at an initial position of 50 cm distal. L_P and L_T were always measured only for the proximal 50 cm of the blade (as measured along the midline).

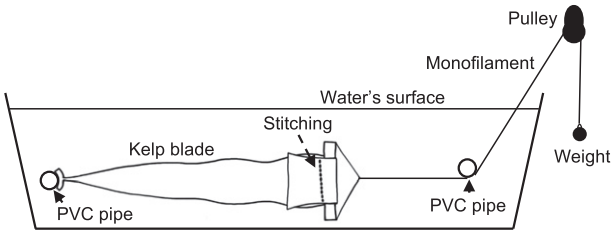

FIG. 2. Experimental setup used in load magnitude experiment.

different amount of weight attached (0, 0.5, 1.0, 1.5, 2.0, and 2.5 N). These weight levels were chosen to represent a range of loading that *Nereocystis* blades might naturally experience in flow due to drag as assessed by our own measurements of drag on single *Nereocystis* blades (L. Coleman, unpub. data) and observations that local populations can experience current velocities in excess of 3 $m \cdot s^{-1}$ (Canadian Hydrographic Service 2017). The position of each weight treatment within each tank was random. The blades were left in place to grow under constant longitudinal tension imposed by the weights for 4-5 d.

At the end of the growth period, the experimental blades were removed from the growth tanks and all morphological parameters were re-measured. All methods up to this point were then repeated for two more *Nereocystis*. Once the final measurements were collected for the additional kelps, ordinary least squares (OLS) regression was used to assess the effect of weight on all measured variables (expressed as percent change $\cdot$ d^{-1}; ΔL_B, ΔW_B, ΔT, ΔR, ΔM_B, and ΔA_B). Kelp of origin was incorporated into regression analyses as an interactive fixed effect due to the low number of individual kelps used. Additionally, the stress (in $MN \cdot m^{-2}$) experienced by each blade at the beginning and end of its growth period was calculated using the following equation:

$$\text{Stress} = \frac{\text{Weight}}{0.25\pi W_B T} \quad (2)$$

Blade cross-sectional area was modeled as an ellipse since the margins of kelp blades are known to be thinner than the midlines (Gerard 1987). To see if stress changed over the course of experimental growth periods as blade morphology changed, a linear model was used to test whether change in stress (in % $\cdot$ d^{-1}) across all weight treatments except the control group was significantly different from zero. OLS regression was also used to test whether the amount of weight applied (above 0 N) had a significant effect on change in stress. Finally, in order to check whether load was a useful predictor of kelp response, OLS regressions were used to assess the effect of stress (averaged between the beginning and end of growth periods) on ΔL_B, ΔW_B, ΔT, ΔR, ΔM_B, and ΔA_B; kelp of origin was incorporated into these regression models as a fixed interactive covariate.

All statistical analyses were performed in R (R Core Team 2020). All OLS regressions were performed using the lm() function from the R stats package (R Core Team 2020). ANOVA was performed on regression models using the anova () function from the R stats package (R Core Team 2020). Assumptions of linearity were verified with residuals vs. fitted plots generated using the plot() function from the R graphics package (R Core Team 2020). Assumptions of normality were verified with Shapiro–Wilk tests performed with the ols_test_normality() function from the olsrr package (Hebbali 2020). Assumptions of homoscedasticity were verified with Breusch–Pagan tests performed with the ols_test_breusch_pagan() function from the olsrr package (Hebbali 2020). Assumptions of independence of residuals were verified with Durbin–Watson tests performed with the durbinWatsonTest() function from the car package (Fox and Weisberg 2019). No data were found to deviate from any underlying assumptions of the analyses performed.

Load direction experiment. A single blade was haphazardly selected and removed at its base from each of 40 mature *Nereocystis* sporophytes growing at Stanley Park (49°18′10″ N, 123°07′35″ W) and returned to the University of British Columbia (UBC) on June 27, 2018. Collected blades were stored in a sea table for up to 48 h. An approximately 10 x 10 cm square of tissue was cut out of each blade at the most proximal position possible; a small notch was cut into the center of the distal edge of each of these tissue samples to denote the orientation of the original blade. Tissue samples were weighed to the nearest 0.1 g and photographed. The software ImageJ (Rasband 2019) was used to measure the midline length (L_S) and width (W_S) to the nearest 0.1 cm, as well as the surface area (A_S) to the nearest 0.1 cm^2, of each tissue sample (Fig. 3).

For each kelp tissue sample, two opposite edges were looped around short plastic rods and the loops were sewn closed. For 20 of the 40 samples, hereafter referred to as the "longitudinal" treatment group, the proximal and distal edges were folded over, while for the other 20, hereafter referred to as the "transverse" treatment group, the left and right edges were folded (Fig. 3). All tissue samples had weights attached to one of the two plastic rods; in the longitudinal group, the weight was always attached to the proximal rod. 10 of the 20 samples from each treatment group had a 1.8 N weight attached (the "high weight" group), while the remaining samples had 0.28 N weights attached (the "low weight" group). Each tissue sample's non-weight-bearing rod was tied to a PVC tube positioned over a growth tank such that the samples were suspended in mid water with their respective

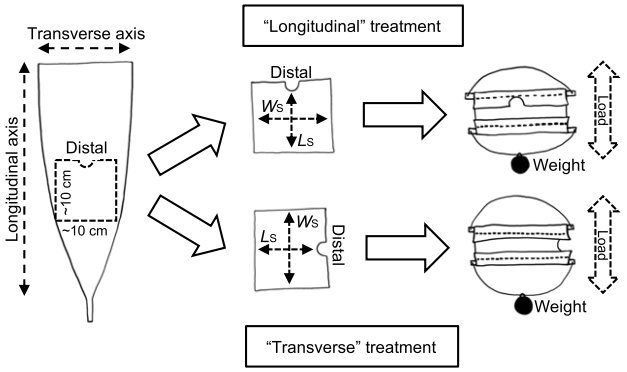

FIG. 3. Methods for preparation of kelp tissue samples in load direction experiment. L_S = tissue sample length. W_S = tissue sample width.

weights applying constant tension in either the longitudinal or transverse axis with respect to the original blades (Fig. 3). Tissues were left to grow for 6 d. The growth tank was maintained at a temperature of 10–11°C and a salinity of 31; kelps received 300–400 μmol photons · m^{-2} · s^{-1} of photons over an 18:6 h photoperiod and were cultured in half-strength f/2 growth medium. Water was continuously pumped through the tanks to maintain a low level of ambient motion; fast incoming flow was never pointed directly at experimental samples.

At the end of the growth period, the kelp tissue samples were re-weighed and re-photographed; final L_S, W_S, and A_S were measured in ImageJ (Rasband 2019). An approximately 1x1 cm^2 subsample was taken from the center of each tissue sample and fixed in 5% formalin seawater for histological examination. An Olympus BX51W1 microscope was used to image the top layer of meristoderm cells in each subsample. Photographs were taken of three random locations within each subsample using an Olympus DP21 camera. ImageJ was used to measure the area, length, and width of all meristoderm cells in each photograph. The means of these three variables were calculated for each photograph and these mean values were averaged across the three subsamples to yield measures of mean meristoderm cell length (L_C), width (W_C), and area (A_C) for each kelp square. Cell length and width were measured using the bounding rectangle method in order to maintain a fixed orientation with respect to that of the original blades (Fig. 3).

All data analysis was performed in R (R Core Team 2020). A two-factor ANOVA was used to analyze the effects of weight and orientation, as well as an interaction between the two, on kelp tissue and cell morphology data. Tissue morphology variables (wet mass [M_S], L_S, A_S, and W_S) were expressed as percent change · d^{-1} (ΔM_S, ΔL_S, ΔA_S, and ΔW_S respectively). PCA was used to identify axes of maximal variation in ΔM_S, ΔL_S, ΔA_S, and ΔW_S; a two-factor ANOVA was used to test the effect of weight and orientation on resulting principal components scores. All ANOVAs were performed using the lm() and anova() functions from the R stats package (R Core Team 2020). Assumptions of normality were verified with Shapiro–Wilk tests performed with the ols_test_normality() function from the olsrr package (Hebbali 2020). Assumptions of homoscedasticity were verified using F-tests performed with the ols_test_f() function from the olsrr package (Hebbali 2020). Assumptions of independence of residuals were verified with Durbin–Watson tests performed with the durbinWatsonTest() function from the car package (Fox and Weisberg 2019). Data were not found to deviate from any underlying assumptions of the analyses performed. Tukey–Kramer post hoc tests were performed using the lsmeans() function from the emmeans package (Lenth 2020) to identify significantly different treatment groups when any ANOVA identified significant interaction effects. PCA was performed with the prcomp() function from the R stats package (R Core Team 2020); data were centered and scaled.

Standard major axis (SMA) regression was used to assess whether L_C and W_C were significant predictors of final L_S and W_S respectively; this was performed using the lmodel2() function from the lmodel2 package (Legendre 2018). Assumptions of normality were verified with Shapiro–Wilk tests performed with the shapiro.test() function from the R stats package (R Core Team 2020). Assumptions of homoscedasticity were verified using Breusch–Pagan tests performed using the bptest() function from the lmtest package (Zeileis and Hothorn 2002). Assumptions of independence of residuals were verified with Durbin–Watson tests performed with the dwtest() function from the lmtest package (Zeileis and Hothorn 2002). Data were not found to deviate from any underlying assumptions of the analyses performed.

Load location experiment. One *Nereocystis* blade was haphazardly selected and removed at the base from each of 20 mature kelps growing near Brockton Point lighthouse in Stanley Park (49°17′56″ N, 123°17′56″ W) and returned to UBC on May 8, 2019. The collected blades were stored in a sea table for up to 72 h. Each blade was cut to a standard initial midline length of approximately 50 cm and W_B was measured to the nearest 0.1 mm using vernier calipers. Mean initial W_B across all blades (± SE) was 54.7 ± 2.1 mm, which is higher than that of kelps found in a wave- and current-sheltered environment as described by Koehl and Alberte (1988). Both the proximal and distal ends of each blade were looped around short plastic rods; these loops were sewn closed (Fig. 4). At the distal end, approximately 2 cm of tissue was folded over, while at the proximal end, 15 cm of tissue was.

Experimental blades were secured into two growth tanks. The proximal ends of the blades were tied to a PVC pipe via the plastic rod sewn into the tissue; this was done such that the most proximal 15 cm of blade tissue was always left slack. The distal end of each blade was tied, via the other plastic rod, to a line of monofilament that extended horizontally; these lines ultimately connected the experimental blades to weights hanging off the ends of the growth tanks, as in Figure 2. The weights applied differing degrees of loading to only the most distal ~35 cm of each blade; 10 of the 20 blades experienced 0.17 N of constant longitudinal tension

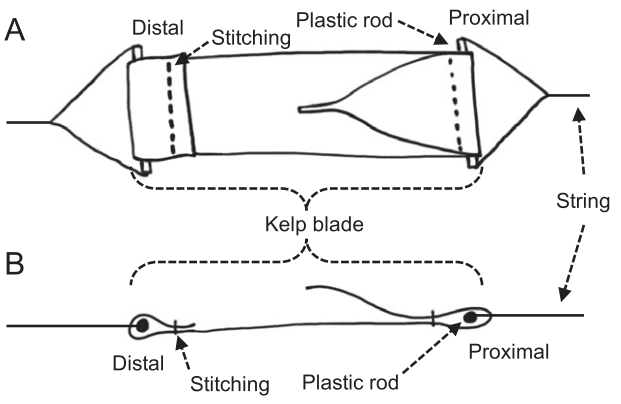

FIG. 4. Experimental methods for load location experiment. (A) Kelp blade configuration as viewed from above; (B) kelp blade configuration as viewed from the side.

(the "low weight" group), while the other 10 received 1.0 N (the "high weight" group). Five blades from each weight treatment group were assigned to each of the two growth tanks; blades in each tank were positioned randomly. Kelp tissue was left in place to grow for 5 d. Water was continuously pumped through the tanks to maintain a low level of ambient motion; fast incoming flow was never pointed directly at experimental samples.

At the end of the growth period, W_B was re-measured. The effect of weight on ΔW_B was assessed statistically using a two-way ANOVA; growth tank was incorporated into the analysis as an interactive fixed effect due to the low number of tanks. All data analysis was performed in R (R Core Team 2020). ANOVA was performed using the lm() and anova() functions from the R stats package (R Core Team 2020). Assumptions of normality were verified with Shapiro–Wilk tests performed with the ols_test_normality() function from the olsrr package (Hebbali 2020). Assumptions of homoscedasticity were verified using F-tests performed with the ols_test_f() function from the olsrr package (Hebbali 2020). Assumptions of independence of residuals were verified with Durbin–Watson tests performed with the durbinWatsonTest() function from the car package (Fox and Weisberg 2019). Data were not found to deviate from any underlying assumptions of the analyses performed.

RESULTS

Load magnitude experiment. Blades bearing higher amounts of weight showed significantly greater increases in L_B (ANOVA, $F_{1,13} = 16.1$, $P = 0.001$) and M_B (ANOVA, $F_{1,13} = 4.98$, $P = 0.044$), as well as significant decreases in W_B (ANOVA, $F_{1,13} = 23.3$, $P < 0.001$) and R (ANOVA, $F_{1,13} = 11.4$, $P = 0.005$), compared to those bearing lower amounts of weight (Table 1; Fig. 5). These changes in morphology appeared continuous and approximately linear across the loading gradient. There were no significant effects of weight on ΔT (ANOVA, $F_{1,13} = 0.66$, $P = 0.43$) or ΔA_B (ANOVA, $F_{1,13} = 4.27$, $P = 0.06$). There were no significant effects of kelp or weight:kelp interactions on any measured variables (Table 1). All but one sample of the 2.5 N treatment broke in the first 24 h of their growth periods; no sample loss occurred at other weight levels. In all instances where blade tissue failed, the failure occurred in the narrow region near the blade base, distal to the pneumatocyst; breaks never took place near the stitching securing the blades to the weights.

Mean change in stress was found to be significantly higher than zero (linear model, $P < 0.001$), but there were no significant effects of weight (ANOVA, $F_{1,9} = 2.81$, $P = 0.13$), kelp (ANOVA, $F_{3,9} = 1.91$, $P = 0.20$), or weight:kelp interactions (ANOVA, $F_{3,9} = 0.54$, $P = 0.66$) on change in stress (Table S1, Fig. S1 in the Supporting Information). Effects of mean stress on kelp morphological variables were found to be, for the most part, identical to the effects of weight (Table S2, Fig. S2 in the Supporting Information). The only exceptions were an instance where there was a significant effect of stress (ANOVA, $F_{1,13} = 5.55$, $P = 0.035$), but not of weight, on ΔA_B, and another instance where there was a significant effect of kelp on R (ANOVA, $F_{3,13} = 3.83$, $P = 0.036$) that was only detectable when stress was incorporated into the regression model instead of weight.

Load direction experiment. There was no significant effect of weight on ΔL_S (ANOVA, $F_{1,33} = 0.68$, $P = 0.41$), ΔW_S (ANOVA, $F_{1,33} = 1.18$, $P = 0.29$), ΔA_S (ANOVA, $F_{1,33} = 0.85$, $P = 0.36$), or ΔM_S (ANOVA, $F_{1,33} = 0.011$, $P = 0.92$; Table 2; Fig. S3 in

TABLE 1. ANOVA tables for load magnitude experiment

Response	Explanatory	df	Sum Sq	Mean Sq	F	P
Change in blade length (ΔL_B)	Weight	1	54.3	54.3	16.1	0.001
	Kelp	3	12.4	4.14	1.22	0.34
	Weight:Kelp	3	8.87	2.96	0.88	0.48
	Residuals	13	43.8	3.37		
Change in blade width (ΔW_B)	Weight	1	102.3	102.3	23.3	<0.001
	Kelp	3	30.5	10.2	2.32	0.13
	Weight:Kelp	3	26.3	8.75	2.00	0.16
	Residuals	13	57.0	4.38		
Change in blade thickness (ΔT)	Weight	1	3.20	3.20	0.66	0.43
	Kelp	3	6.23	2.08	0.43	0.73
	Weight:Kelp	3	6.87	2.29	0.48	0.70
	Residuals	13	62.6	4.82		
Change in ruffle (ΔR)	Weight	1	2.79	2.79	11.4	0.005
	Kelp	3	2.22	0.74	3.04	0.067
	Weight:Kelp	3	0.17	0.056	0.23	0.87
	Residuals	13	3.17	0.24		
Change in blade wet mass (ΔM_B)	Weight	1	12.0	12.0	4.98	0.044
	Kelp	3	15.9	5.31	2.20	0.14
	Weight:Kelp	3	4.47	1.49	0.62	0.62
	Residuals	13	31.4	2.42		
Change in blade area (ΔA_B)	Weight	1	16.8	16.8	4.27	0.059
	Kelp	3	24.1	8.02	2.04	0.16
	Weight:Kelp	3	27.2	9.05	2.31	0.12
	Residuals	13	51.0	3.92		

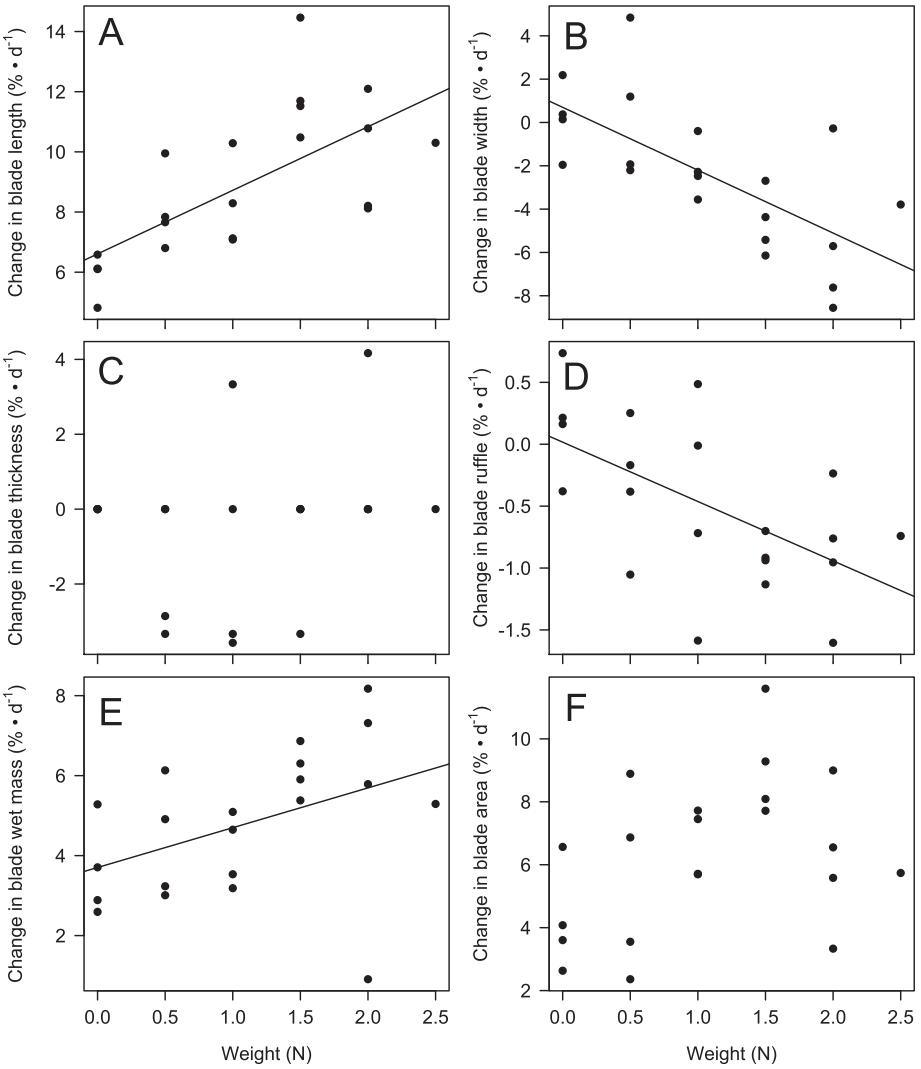

FIG. 5. Change in morphological characters of *Nereocystis* blades expressed as functions of weight applied during load magnitude experiment. OLS regression lines indicate a statistically significant relationship between weight and the specified response variable. Change in blade length = ΔL_B; change in blade width = ΔW_B; change in blade midline thickness = ΔT; change in blade ruffle = ΔR; change in blade wet mass = ΔM_B; change in blade area = ΔA_B.

the Supporting Information). There was no significant effect of orientation on ΔL_S (ANOVA, $F_{1,33} = 0.049$, $P = 0.83$), ΔW_S (ANOVA, $F_{1,33} = 1.34$, $P = 0.26$), ΔA_S (ANOVA, $F_{1,33} = 0.64$, $P = 0.43$), or ΔM_S (ANOVA, $F_{1,33} = 1.60$, $P = 0.21$). There was a significant effect of an interaction between weight and orientation on ΔL_S (ANOVA, $F_{1,33} = 7.85$, $P = 0.008$), but not on ΔW_S (ANOVA, $F_{1,33} = 0.077$, $P = 0.78$), ΔA_S (ANOVA, $F_{1,33} = 1.21$, $P = 0.28$), or ΔM_S (ANOVA, $F_{1,33} = 3.39$, $P = 0.074$). No pairwise combination of weight and orientation treatment groups were found to significantly differ in ΔL_S, but the high weight, transversely oriented kelp tissue samples had nearly significantly lower ΔL_S than the low weight, transversely oriented samples (Tukey, $P = 0.061$).

The first principal component (PC1) explained 91.4% of the variation in ΔL_S, ΔW_S, ΔA_S, and ΔM_S, while the second principal component (PC2) explained 5.6% (Table 3). PC1 was positively correlated with all four input variables and was interpreted as an index of overall growth. PC2 was negatively correlated with ΔL_S, but positively correlated with ΔW_S; it was interpreted as an index of shape change.

There was no significant effect of weight (ANOVA, $F_{1,33} = 0.57$, $P = 0.45$), orientation (ANOVA, $F_{1,33} = 0.80$, $P = 0.38$), or an interaction between the two (ANOVA, $F_{1,33} = 1.89$, $P = 0.18$) on PC1 scores (Table 4; Fig. 6). There was no significant effect of weight (ANOVA, $F_{1,33} = 1.79$, $P = 0.19$) or orientation (ANOVA, $F_{1,33} = 3.05$, $P = 0.090$) on PC2 scores, but there was a significant interaction effect (ANOVA, $F_{1,33} = 46.5$, $P < 0.001$). The low weight, longitudinally oriented tissue samples had significantly higher PC2 scores than the high weight, longitudinally oriented tissue

TABLE 2. ANOVA tables for tissue morphology data from load direction study

Response	Explanatory	df	Sum Sq	Mean Sq	F	P
Change in tissue sample length (ΔL_S)	Weight	1	1.21	1.21	0.68	0.41
	Orientation	1	0.086	0.086	0.049	0.83
	Weight:Orientation	1	13.9	13.9	7.85	0.008
	Residuals	33	58.5	1.77		
Change in tissue sample width (ΔW_S)	Weight	1	1.91	1.91	1.18	0.29
	Orientation	1	2.18	2.18	1.34	0.26
	Weight:Orientation	1	0.13	0.12	0.077	0.78
	Residuals	33	53.6	1.63		
Change in tissue sample area (ΔA_S)	Weight	1	7.01	7.01	0.85	0.36
	Orientation	1	5.26	5.26	0.64	0.43
	Weight:Orientation	1	10.0	10.0	1.21	0.28
	Residuals	33	273.0	8.27		
Change in tissue sample wet mass (ΔM_S)	Weight	1	0.081	0.081	0.011	0.92
	Orientation	1	12.0	12.0	1.60	0.21
	Weight:Orientation	1	25.3	25.3	3.39	0.074
	Residuals	33	246.4	7.47		

TABLE 3. Output of PCA performed for load direction experiment

Variable	Loadings			
	PC1	PC2	PC3	PC4
Change in square length (ΔL_S)	0.493	−0.619	−0.461	−0.402
Change in square width (ΔW_S)	0.489	0.741	−0.076	−0.454
Change in square area (ΔA_S)	0.518	0.115	−0.292	0.795
Change in square wet mass (ΔM_S)	0.499	−0.235	0.834	0.016
Proportion of variation	0.913	0.056	0.028	0.002

samples (Tukey, $P < 0.001$), whereas among the transversely oriented tissue samples, the low weight individuals had significantly lower PC2 scores than the high weight ones (Tukey, $P < 0.001$).

There was no significant effect of weight on L_C (ANOVA, $F_{1,33} = 2.46$, $P = 0.13$), W_C (ANOVA, $F_{1,33} = 0.11$, $P = 0.74$), or A_C (ANOVA, $F_{1,33} = 3.45$, $P = 0.072$; Table 5; Fig. S4 in the Supporting Information). There was no significant effect of orientation on L_C (ANOVA, $F_{1,33} = 2.43$, $P = 0.13$) or A_C (ANOVA, $F_{1,33} = 0.61$, $P = 0.44$), but transversely oriented tissue samples had significantly higher W_C than longitudinally oriented ones across both weight levels (ANOVA, $F_{1,33} = 4.84$, $P = 0.035$). There was a significant effect of an interaction between weight and orientation on L_C (ANOVA, $F_{1,33} = 8.67$, $P = 0.006$), but not on W_C (ANOVA, $F_{1,33} = 0.015$, $P = 0.90$) or A_C (ANOVA, $F_{1,33} = 1.53$, $P = 0.23$). The heavily weighted, longitudinally oriented tissue samples showed significantly increased L_C compared to both the lightly weighted, longitudinally oriented tissue samples (Tukey, $P = 0.023$) and the heavily weighted, transversely oriented tissue samples (Tukey, $P = 0.014$). See Figure S5 in the Supporting Information for distributions of cell morphologies in different treatment groups.

Final L_S was found to significantly increase with L_C (SMA regression, $P < 0.001$) and W_S was found to significantly increase with W_C (SMA regression, $P = 0.012$; Fig. 7).

Load location experiment. There was no significant effect of weight (ANOVA, $F_{1,13} = 0.41$, $P = 0.54$), growth tank (ANOVA, $F_{1,13} = 2.10$, $P = 0.17$), or weight:growth tank interactions (ANOVA, $F_{1,13} = 1.24$, $P = 0.29$) on ΔW_B (Table 6; Fig. 8).

DISCUSSION

Morphological plasticity across hydrodynamic gradients in the blades of *Nereocystis* has been

TABLE 4. ANOVA tables for principal components scores generated from tissue morphology data in load direction study

Response	Explanatory	df	Sum Sq	Mean Sq	F	P
Principal component 1 (PC1; growth)	Weight	1	2.08	2.08	0.57	0.45
	Orientation	1	2.89	2.89	0.80	0.38
	Weight:Orientation	1	6.85	6.85	1.89	0.18
	Residuals	33	119.7	3.63		
Principal component 2 (PC2; -elongation, widening)	Weight	1	0.17	0.17	1.79	0.19
	Orientation	1	0.29	0.29	3.05	0.09
	Weight:Orientation	1	4.46	4.46	46.5	<0.001
	Residuals	33	3.16	0.10		

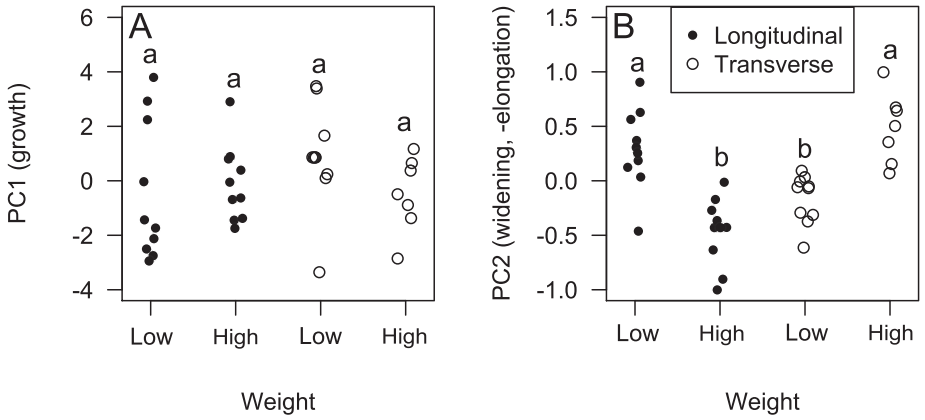

FIG. 6. Principal component 1 (PC1) and principal component 2 (PC2) scores generated from PCA performed for stress direction experiment expressed as functions of weight level applied and orientation of kelp squares; lowercase letters denote significantly different groups as indicated by a Tukey–Kramer post hoc test.

TABLE 5. ANOVA tables for cell morphology data from load direction study

Response	Explanatory	df	Sum Sq	Mean Sq	F	P
Mean cell length (ΔL_C)	Weight	1	0.31	0.31	2.46	0.13
	Orientation	1	0.31	0.31	2.43	0.13
	Weight:Orientation	1	1.11	1.11	8.67	0.006
	Residuals	33	4.22	0.13		
Mean cell width (ΔW_C)	Weight	1	0.012	0.012	0.11	0.74
	Orientation	1	0.54	0.54	4.84	0.035
	Weight:Orientation	1	0.002	0.002	0.015	0.90
	Residuals	33	3.65	0.11		
Mean cell area (ΔA_C)	Weight	1	25.5	25.5	3.45	0.072
	Orientation	1	4.49	4.49	0.61	0.44
	Weight:Orientation	1	11.3	11.3	1.53	0.23
	Residuals	33	243.9	7.39		

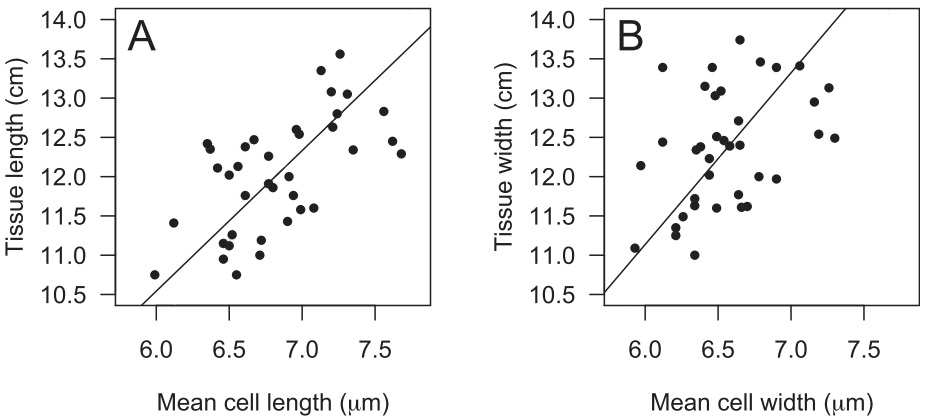

FIG. 7. Final tissue sample (A) length (L_S) and (B) width (W_S) expressed as functions of final mean meristoderm cell length and width respectively for kelp tissue samples in load direction experiment. Standard major axis (SMA) regression lines indicate that cell lengths and widths are statistically significant predictors of tissue lengths and widths respectively.

recognized for some time and its functional significance has been well studied (Koehl and Alberte 1988, Johnson and Koehl 1994). However, the nature of the growth response to tensile force that facilitates this phenomenon, including underlying physiological mechanisms, remains poorly understood. To better characterize these, we examined how morphological plasticity in *Nereocystis* blades was affected by the (1) magnitude, (2) direction, and (3) location of mechanical loading, using weights to

TABLE 6. ANOVA table for load location study

Response	Explanatory	df	Sum Sq	Mean Sq	F	P
Change in blade width (ΔW_B)	Weight	1	1.25	1.25	0.41	0.54
	Tank	1	6.47	6.47	2.11	0.17
	Weight:Tank	1	3.81	3.81	1.24	0.29
	Residuals	13	40.0	3.08		

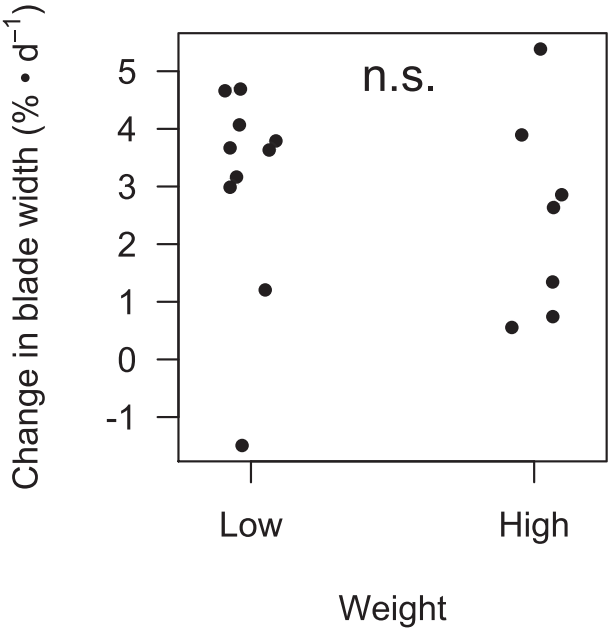

FIG. 8. Change in blade width (ΔL_B) expressed as a function of weight level applied during growth period in load location experiment; n.s. = not significantly different.

simulate drag imposed on the blades by moving water.

Nereocystis *is sensitive to the magnitude of mechanical loading.* As more tensile force was applied, experimental blades became longer, narrower, less ruffled, and heavier, but did not change in thickness and increased similarly in area across loading treatments. Furthermore, weight-induced changes in morphology scaled continuously and, especially in the case of blade width, approximately linearly with increasing loading. In other words, the reaction norms of measured blade characteristics across a loading gradient were linear, consistent with our hypothesis. Thus, *Nereocystis* thalli are able to perceive even minor changes in the ambient mechanical environment and respond proportionally, thereby achieving greater photosynthetic output for a given set of conditions while minimizing the risk of dislodgement (Koehl and Alberte 1988). Such phenotypic flexibility, coupled with exceptionally high growth rates (Abbott and Hollenberg 1976, Kain 1987), may be a key factor in the observed ability of *Nereocystis* to successfully colonize a wide range of hydrodynamic environments (Koehl and Alberte 1988) on an annual basis (Abbott and Hollenberg 1976). Our results are consistent with predictions from the land plant literature which suggest that plasticity is a favorable adaptive strategy in annual plants living in variable environments (Cook and Johnson 1968, Wilken 1977, Zangerl and Bazzaz 1983), especially when organismal response times to environmental changes are short (Padilla and Adolph 1996, Alpert and Simms 2002). The linear reaction norms observed suggest that selective pressure on *Nereocystis* blade morphology is equal across the loading gradient tested and that this kelp has no specific set of flow conditions within its hydrodynamic niche for which it is "best" adapted (Gibert et al. 1998, David et al. 2004).

Even though we found that stress was not constant throughout the growth periods due to progressive narrowing of blades, the facts that (1) individual weight treatments did not significantly differ in the magnitude of stress change observed and (2) the overall results of this experiment barely changed depending on whether weight or stress were used in our analyses suggest to us that mechanical load is indeed a useful predictor of kelp plasticity in this instance.

Because blades in the 2.5 N treatment group were close to their known mechanical limits (Johnson and Koehl 1994, Hale 2001) and almost all of these individuals broke within 24 h of their growth periods, we conclude that the range of weights used in this experiment covered the full breadth of tensile force that the blades could have withstood. The observation that blade morphology continued to respond to increasing levels of tension all the way up to the mechanical limits of the tissue suggests that there is no practical limit to the plasticity and that morphological adjustments are still functionally relevant even at high levels of water motion. Furthermore, given the highly linear nature of the reaction norms, we expect that subjecting blades to loading levels beyond those tested here would cause them to narrow even further if material properties had permitted. This interplay between hydrodynamic performance and material properties of blades is interesting and deserves further study, as recent work has demonstrated a clear trade-off between drag avoidance and drag tolerance in kelps (Starko and Martone 2016a). Drag avoidance via blade narrowing may compensate for the relative

weakness of kelp tissues (Hale 2001, Martone 2007), but ultimately may be constrained by these material properties. On the other hand, the benefits of blade narrowing may be limited at fast water velocities (Milligan and DeWreede 2004, Bettignies et al. 2013), so strengthening tissues to permit further narrowing would likewise not be beneficial.

As mechanical loading was applied constantly and unidirectionally in this experiment, our results are best considered to be reflective of how *Nereocystis* would behave when subjected primarily to strong currents. Wave-swept kelps, in comparison, would likely experience greater loading forces, but these forces would be applied transiently and repeatedly (Koehl 1984, Denny et al. 1998, Gaylord et al. 2008). It would be interesting to consider how our results might differ if we were to incorporate a punctuated loading regime such as this into our experimental design. Johnson and Koehl (1994) have shown that wave-exposed *Nereocystis* show a mean blade morphology that is intermediate to those of current-swept and sheltered individuals. This may indicate that wave-imposed mechanical loading does affect the kelp's blade morphology, but to a lesser degree than current-imposed loading, which suggests that blade morphology might be best predicted by the average mechanical loading experienced over a given time period. Based on this, an experiment incorporating transient, repeated mechanical loading treatments might be expected to produce results similar to those described here, but with shallower reaction norm slopes.

An unexpected finding of this experiment was that more highly weighted blades showed greater increases in wet mass than less weighted ones in spite of there being no significant effects of weight on blade area or thickness (and therefore volume). We see several possible explanations for this. One is that highly weighted kelps produced heavier tissue than the less weighted individuals. This could have been brought about by high loading inducing blades to incorporate more carbon into their cell walls, as described by Kraemer and Chapman (1991). Another possibility is that the morphological changes brought about by high tensile force facilitated an increase in productivity and growth (Gerard and Mann 1979, Koehl and Alberte 1988), resulting in an indirect positive effect of mechanical loading on wet mass accumulation rate. It is also possible that the additional wet mass found in highly weighted kelps could have been accounted for by thickened blade margins, which would have gone undetected in our measurements of blade midline thickness. However, we consider this unlikely, as Gerard (1987) found that mechanical loading had no effect on blade thickness as measured at either the midlines or the margins in *Saccharina latissima*. While we cannot explain our observations with certainty, we strongly feel that they merit further study. It would be of particular significance if mechanical loading directly increases kelp tissue mass via a mechanism similar to that proposed by Kraemer and Chapman (1991), as researchers have long been interested in relationships between water motion and primary productivity (reviewed in Hurd 2000).

Our biomass observations contrast with those of Gerard (1987), who found that *Saccharina latissima* blades became longer and narrower, but did not accumulate more biomass, when grown subject to high tensile force. This discrepancy may be due to methodological differences between the two studies. Our data include measurements of total wet mass accumulated after 4-5 d, whereas Gerard's data are estimates of biomass production calculated based on weight:length ratios measured during the sixth week of her experiment. Based on this, it is possible, for example, that we detected a proportional increase in biomass that only occurs for a brief period after the loading is first applied, or that Gerard's calculations systematically underestimated the biomass actually accumulated by highly weighted kelps.

Nereocystis *is sensitive to the direction of mechanical loading.* Our data indicate that the application of tension to *Nereocystis* blade tissue encourages growth in whichever axis is parallel to the tensile force while discouraging growth in the perpendicular axis. This is consistent with our hypothesis and has several important implications for our thinking on mechanisms facilitating morphological plasticity in *Nereocystis*. Firstly, it suggests that there is no substantial separation between the location of stimulus perception and that of the ultimate organismal response, which, coupled with the known ability of cell wall-mediated thigmomorphogenetic responses in plants to be directionally sensitive (Gus-Mayer et al. 1998, Sampathkumar et al. 2014, Louveaux et al. 2016), leads us to hypothesize that the entire set of physiological processes that facilitate morphological plasticity in *Nereocystis* blades takes place within individual cells and that cell wall deformation is the likely basis for the mechanoperception mechanism. Secondly, the lack of separation between the locations of stimuli and responses suggests that hormones (or some other long-distance signaling molecule) are unlikely to be involved in this process. We suspect that mechanical loading on the blade distends the walls of meristematic cells, initiating a signaling cascade that probably only serves to influence growth and/or division of those same cells.

While we cannot state definitively from the data at hand exactly how cell growth and division are affected by tension in this system, it does appear that growth of the tissue is somehow reallocated from the axis perpendicular to that of the tensile force into the axis parallel with it. This is consistent with, but does not explicitly confirm, the conclusions of Gerard (1987), who proposed that

longitudinal tension caused meristematic cells of *Saccharina latissima* to preferentially divide in the longitudinal axis over the transverse axis. Additionally, the final morphology of the experimental kelp tissues is reflective of the average morphology of those tissues' meristoderm cells, with longer tissues, for instance, also exhibiting longer cells. This suggests that changes in tissue morphology observed in this experiment represent the "sum" of changes in the morphologies of all growing cells. The tendency for meristoderm cells to be longer in the highly weighted longitudinal kelps and wider in the highly weighted transverse kelps most likely reflects increased, or at least more unified, cell elongation (possibly leading to division) in the principal direction of tensile force (Biro et al. 1980), as predicted by Gerard (1987). To verify this hypothesis more conclusively, we need to observe axes of meristoderm cell divisions directly and examine whether these were altered by the application of tension.

Nereocystis is sensitive to the location of mechanical loading. Our data indicate that only mechanical loading applied directly to actively growing meristematic tissue will evoke morphological plasticity in *Nereocystis* blades. This is consistent with our original hypothesis and our observations from the load direction experiment. The lack of response to a stimulus applied far from the tissue region where the response is generated reinforces our previously discussed conclusion that a long-distance signaling mechanism most likely does not mediate the effect of mechanical loading on kelp blade morphology.

If mechanical loads must be imposed directly on growing tissue by drag, then there must be tissue located distal to that growing tissue for drag to act upon. In other words, the mechanism being utilized by *Nereocystis* to facilitate its flow-induced plasticity may be reliant on intercalary meristems located at the proximal ends of blades. This may explain, in part, why morphological plasticity across hydrodynamic gradients in seaweeds has been best described and most consistently observed in kelps up to this point (e.g., Druehl and Kemp 1982, Gerard 1987, Buck and Buchholz 2005, Fowler-Walker et al. 2006, Koehl et al. 2008), as this whole group prominently exhibits intercalary growth (Fritsch 1923, Graham et al. 2017). It could also potentially explain why it has been historically difficult to convincingly demonstrate plasticity in seaweeds that rely on apical meristems, such as red algae (Floc'h 1969, Shaughnessy 2004) and the brown alga *Fucus* (Sideman and Mathieson 1983, 1985, Blanchette 1997). In a situation where growth was entirely apical, drag simply could not be imposed directly on the growing tissue. If plasticity were observed in spite of this, we would infer that some form of long-distance signaling mechanism would be needed to communicate the perception of the stimulus from more proximal tissue to the growing meristem.

CONCLUSIONS

In summary, we conducted three experiments to investigate how morphological plasticity in response to mechanical loading in *Nereocystis* blades was affected by the (1) magnitude, (2) direction, and (3) location of the applied force. We found that kelp blades subjected to a gradient of tensile force grew progressively narrower, longer, less ruffled, and, unexpectedly, heavier as the magnitude of loading increased; the linear reaction norms observed suggests that *Nereocystis* is equally well adapted for all flow environments tested. We found that when high tensile force was applied transversely across blade tissue, the response seen under high longitudinal tension was rotated 90°. This indicates that the entire set of physiological mechanisms that facilitate the plasticity most likely occurs within the same individual meristematic cells, suggesting against the involvement of a long-distance signaling mechanism. Furthermore, as the average morphology of a blade's meristoderm cells paralleled that of the tissue in this experiment, we infer that changes in blade morphology probably reflect altered cell elongation patterns. Finally, we found that the growth response to tension only occurred when mechanical loading was applied directly to the meristematic tissue, reinforcing that there is probably no long-distance signaling involved and indicating that response to hydrodynamic forces likely requires an intercalary meristem to facilitate mechanoperception. This study provides information on the evolutionary relationship between *Nereocystis* and water flow while lending insight into cellular mechanisms that might facilitate morphological plasticity in kelps.

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Abbott, I. A. & Hollenberg, G. J. 1976. *Marine Algae of California.* Stanford University Press, Stanford, CA, USA, 827 pp.

Alpert, P. & Simms, E. L. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? *Evol. Ecol.* 16:285–97.

Bettignies, T., Wernberg, T. & Lavery, P. 2013. Size, not morphology, determines hydrodynamic performance of a kelp during peak flow. *Mar. Biol.* 160:843–51.

Biro, R. L., Hunt, E. R., Erner, Y. & Jaffe, M. J. 1980. Thigmomorphogenesis: changes in cell division and elongation in the internodes of mechanically-perturbed or ethrel-treated bean plants. *Ann. Bot.* 45:655–64.

Biro, R. L. & Jaffe, M. J. 1984. Thigmomorphogenesis: ethylene evolution and its role in the changes observed in mechanically perturbed bean plants. *Physiol. Plant.* 62:289–96.

Blanchette, C. A. 1997. Size and survival of intertidal plants in response to wave action: a case study with *Fucus gardneri*. *Ecology* 78:1563.

Braam, J. 2005. In touch: plant responses to mechanical stimuli. *New Phytol.* 165:373–89.

Buck, B. H. & Buchholz, C. M. 2005. Response of offshore cultivated *Laminaria saccharina* to hydrodynamic forcing in the North Sea. *Aquaculture* 250:674–91.

Canadian Hydrographic Service 2017. *Juan de Fuca Strait and Strait of Georgia*. Fisheries and Oceans Canada, Ottawa, 141 pp.

Charrier, B., Le Bail, A. & de Reviers, B. 2012. Plant proteus: Brown algal morphological plasticity and underlying developmental mechanisms. *Trends Plant Sci.* 17:468–77.

Chehab, E. W., Eich, E. & Braam, J. 2008. Thigmomorphogenesis: a complex plant response to mechano-stimulation. *J. Exp. Bot.* 60:43–56.

Chehab, E. W., Yao, C., Henderson, Z., Kim, S. & Braam, J. 2012. *Arabidopsis* touch-induced morphogenesis is jasmonate mediated and protects against pests. *Curr. Biol.* 22:701–6.

Cook, S. A. & Johnson, M. P. 1968. Adaptation to heterogeneous environments. I. Variation in heterophylly in *Ranunculus flammula* L. *Evolution* 22:496.

Coutand, C., Julien, J. L., Moulia, B., Mauget, J. C. & Guitard, D. 2000. Biomechanical study of the effect of a controlled bending on tomato stem elongation: global mechanical analysis. *J. Exp. Bot.* 51:1813–24.

David, J. R., Gibert, P. & Moreteau, B. 2004. Evolution of reaction norms. *In* DeWitt, T. J. & Scheiner, S. M. [Eds.] *Phenotypic Plasticity: Functional and Conceptual Approaches*. Oxford University Press, New York, pp. 50–63.

Demes, K. W., Pruitt, J. N., Harley, C. D. G. & Carrington, E. 2013. Survival of the weakest: increased frond mechanical strength in a wave-swept kelp inhibits self-pruning and increases whole-plant mortality. *Funct. Ecol.* 27:439–45.

Denny, M. & Gaylord, B. 2002. The mechanics of wave-swept algae. *J. Exp. Biol.* 205:1355–62.

Denny, M., Gaylord, B., Helmuth, B. & Daniel, T. 1998. The menace of momentum: dynamic forces on flexible organisms. *Limnol. Oceanogr.* 43:955–68.

Druehl, L. D. & Kemp, L. 1982. Morphological and growth responses of geographically isolated *Macrocystis integrifolia* populations when grown in a common environment. *Can. J. Bot.* 60:1409–13.

Duggins, D., Eckman, J. E., Siddon, C. E. & Klinger, T. 2001. Interactive roles of mesograzers and current flow in survival of kelps. *Mar. Ecol. Prog. Ser.* 223:143–55.

Erner, Y., Biro, R. & Jaffe, M. J. 1980. Thigmomorphogenesis: evidence for a translocatable thigmomorphogenetic factor induced by mechanical perturbation of beans (*Phaseolus vulgaris*). *Physiol. Plant.* 50:21–5.

Erner, Y. & Jaffe, M. J. 1982. Thigmomorphogenesis: the involvement of auxin and abscisic acid in growth retardation due to mechanical perturbation. *Plant Cell Physiol.* 23:935–41.

Floc'h, J. Y. 1969. First results of a transplant experience in *Chondrus crispus* (L.) Lyngb. *Proc. Int. Seaweed Symp.* 6:171–7.

Fowler-Walker, M. J., Wernberg, T. & Connell, S. D. 2006. Differences in kelp morphology between wave sheltered and exposed localities: morphologically plastic or fixed traits? *Mar. Biol.* 148:755–67.

Fox, J. & Weisberg, S. 2019. *An R Companion to Applied Regression*, 3rd edn. Sage, Thousand Oaks, CA, USA, 607 pp.

Fritsch, F. E. 1923. *The structure and reproduction of the algae*. Cambridge University Press, Cambridge, 939 pp.

Gaylord, B., Denny, M. W. & Koehl, M. A. R. 2008. Flow forces on seaweeds: field evidence for roles of wave impingement and organism inertia. *Biol. Bull.* 215:295–308.

Gerard, V. A. 1982. In situ water motion and nutrient uptake by the giant kelp *Macrocystis pyrifera*. *Mar. Biol.* 69:51–4.

Gerard, V. A. 1987. Hydrodynamic streamlining of *Laminaria saccharina* Lamour. in response to mechanical stress. *J. Exp. Mar. Biol. Ecol.* 107:237–44.

Gerard, V. A. & Mann, K. H. 1979. Growth and production of *Laminaria longicruris* (Phaeophyta) populations exposed to different intensities of water movement. *J. Phycol.* 15:33–41.

Gibert, P., Moreteau, B., David, J. R. & Scheiner, S. M. 1998. Describing the evolution of reaction norm shape: body pigmentation in *Drosophila*. *Evolution* 52:1501.

Graham, L. E., Graham, J. M., Wilcox, L. W. & Cook, M. E. 2017. *Algae*, 3rd edn. LJLM Press, Madison, WI, 688 pp.

Gus-Mayer, S., Naton, B., Hahlbrock, K. & Schmelzer, E. 1998. Local mechanical stimulation induces components of the pathogen defense response in parsley. *Proc. Natl. Acad. Sci. USA* 95:8398–403.

Hale, B. 2001. *Macroalgal materials: foiling fracture and fatigue from fluid forces*. PhD, Stanford, 367 pp.

Hebbali, A. 2020. olsrr: tools for building OLS regression models. Version 0.5.3.

Hurd, C. L. 2000. Water motion, marine macroalgal physiology, and production. *J. Phycol.* 36:453–72.

Hurd, C. L., Harrison, P. J. & Druehl, L. D. 1996. Effect of seawater velocity on inorganic nitrogen uptake by morphologically distinct forms of *Macrocystis integrifolia* from wave-sheltered and exposed sites. *Mar. Biol.* 126:205–14.

Jaffe, M. J. 1973. Thigmomorphogenesis: The response of plant growth and development to mechanical stimulation: With special reference to *Bryonia dioica*. *Planta* 114:143–57.

Jaffe, M. J., Leopold, A. C. & Staples, R. C. 2002. Thigmo responses in plants and fungi. *Am. J. Bot.* 89:375–82.

Johnson, A. & Koehl, M. 1994. Maintenance of dynamic strain similarity and environmental stress factor in different flow habitats: thallus allometry and material properties of a giant kelp. *J. Exp. Biol.* 195:381–410.

Kain, J. M. 1987. Patterns of relative growth in *Nereocystis luetkeana* (Phaeophyta). *J. Phycol.* 23:181–7.

Keeling, P. J. 2004. Diversity and evolutionary history of plastids and their hosts. *Am. J. Bot.* 91:1481–93.

Koehl, M. A. R. 1984. How do benthic organisms withstand moving water? *Am. Zool.* 24:57–70.

Koehl, M. A. R. & Alberte, R. S. 1988. Flow, flapping, and photosynthesis of *Nereocystis leutkeana*: a functional comparison of undulate and flat blade morphologies. *Mar. Biol.* 99:435–44.

Koehl, M. A. R., Silk, W. K., Liang, H. & Mahadevan, L. 2008. How kelp produce blade shapes suited to different flow regimes: a new wrinkle. *Integr. Comp. Biol.* 48:834–51.

Koehl, M. A. R. & Wainwright, S. A. 1977. Mechanical adaptations of a giant kelp. *Limnol. Oceanogr.* 22:1067–71.

Kraemer, G. P. & Chapman, D. J. 1991. Effects of tensile force and nutrient availability on carbon uptake and cell wall synthesis in blades of juvenile *Egregia menziesii* (Turn.) Aresch. (Phaeophyta). *J. Exp. Mar. Biol. Ecol.* 149:267–77.

Legendre, P. 2018. lmodel2: model II regression. Version 1.7-3.

Lenth, R. 2020. Emmeans: estimated marginal means, aka least-squares means. Version 1.4.5.

Louveaux, M., Julien, J. D., Mirabet, V., Boudaoud, A. & Hamant, O. 2016. Cell division plane orientation based on tensile stress in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 113:E4294–303.

Malabarba, J., Reichelt, M., Pasquali, G. & Mithöfer, A. 2019. Tendril coiling in grapevine: jasmonates and a new role for GABA? *J. Plant Growth Regul.* 38:39–45.

Martone, P. T. 2007. Kelp versus coralline: cellular basis for mechanical strength in the wave-swept seaweed *Calliarthron* (Corallinaceae, Rhodophyta). *J. Phycol.* 43:882–91.

Martone, P. T., Kost, L. & Boller, M. 2012. Drag reduction in wave-swept macroalgae: alternative strategies and new predictions. *Am. J. Bot.* 99:806–15.

Milligan, K. L. D. & DeWreede, R. E. 2004. Morphological variations do not effectively reduce drag forces at high wave-exposure for the macroalgal species, *Hedophyllum sessile* (Laminariales, Phaeophyta). *Phycologia* 43:236–44.

Monshausen, G. B. & Gilroy, S. 2009. Feeling green: mechanosensing in plants. *Trends Cell Biol.* 19:228–35.
Monshausen, G. B. & Haswell, E. S. 2013. A force of nature: molecular mechanisms of mechanoperception in plants. *J. Exp. Bot.* 64:4663–80.
Moulia, B., Coutand, C. & Julien, J. L. 2015. Mechanosensitive control of plant growth: bearing the load, sensing, transducing, and responding. *Front. Plant Sci.* 6:52.
Nicholson, N. L. 1970. Field studies on the giant kelp *Nereocystis*. *J. Phycol.* 6:177–82.
Padilla, D. K. & Adolph, S. C. 1996. Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. *Evol. Ecol.* 10:105–17.
R Core Team 2020. *R: A Language and Environment for Statistical Computing. Version 3.6.3.* R Foundation for Statistical Computing, Vienna.
Rasband, W. 2019. *ImageJ. 1.52o.* U. S. National Institutes of Health, Bethesda.
Sampathkumar, A., Yan, A., Krupinski, P. & Meyerowitz, E. M. 2014. Physical forces regulate plant development and morphogenesis. *Curr. Biol.* 24:R475–83.
Shaughnessy, F. J. 2004. Contrasting patterns of allometry and realized plasticity in the sister species *Mazzaella splendens* and *Mazzaella linearis* (Rhodophyta). *J. Phycol.* 40:846–56.
Sideman, E. J. & Mathieson, A. C. 1983. Ecological and genecological distinctions of a high intertidal dwarf form of *Fucus distichus* (L.) Powell in New England. *J. Exp. Mar. Biol. Ecol.* 72:171–88.
Sideman, E. J. & Mathieson, A. C. 1985. Morphological variation within and between natural populations of non-tide pool *Fucus distichus* (Phaeophyta) in New England. *J. Phycol.* 21:250–7.
Starko, S. & Martone, P. T. 2016a. Evidence of an evolutionary-developmental trade-off between drag avoidance and tolerance strategies in wave-swept intertidal kelps (Laminariales, Phaeophyceae). *J. Phycol.* 52:54–63.
Starko, S. & Martone, P. T. 2016b. An empirical test of 'universal' biomass scaling relationships in kelps: evidence of convergence with seed plants. *New Phytol.* 212:719–29.
Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity. *BioSci.* 39:436–45.
Steneck, R. S., Graham, M. H., Bourque, B. J., Corbett, D., Erlandson, J. M., Estes, J. A. & Tegner, M. J. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environ. Conserv.* 29:436–59.
Telewski, F. W. 2006. A unified hypothesis of mechanoperception in plants. *Am. J. Bot.* 93:1466–76.
Wang, X. L., Feng, Y. & Swail, V. R. 2014. Changes in global ocean wave heights as projected using multimodel CMIP5 simulations. *Geophys. Res. Lett.* 41:1026–34.
Wheeler, W. N. 1980. Effect of boundary layer transport on the fixation of carbon by the giant kelp *Macrocystis pyrifera*. *Mar. Biol.* 56:103–10.
Wilken, D. H. 1977. Local differentiation for phenotypic plasticity in the annual *Collomia linearis* (Polemoniaceae). *Syst. Bot.* 2:99.
Woltereck, R. 1909. Weitere experimentelle untersuchungen über artveränderung, speziell über das wesen quantitativer artunterscheide by daphniden. *Verhandlungender Dtsch. Zool. Ges.* 19:110–92.
Young, I. R., Zieger, S. & Babanin, A. V. 2011. Global trends in wind speed and wave height. *Science* 332:451–5.
Zangerl, A. R. & Bazzaz, F. A. 1983. Plasticity and genotypic variation in photosynthetic behavior of an early and a late successional species of *Polygonum*. *Oecologia* 57:270–3.
Zeileis, A. & Hothorn, T. 2002. Diagnostic checking in regression relationships. *R. News* 2:7–10.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Change in stress experienced by *Nereocystis* blades at 10 cm from the origin over the course of experimental growth periods expressed as a function of weight applied.

Figure S2. Change in morphological characters of *Nereocystis* blades expressed as functions of mean stress applied during load magnitude experiment. OLS regression lines indicate a statistically significant relationship between mean stress and the specified response variable. Change in blade length = ΔL_B; change in blade width = ΔW_B; change in blade midline thickness = ΔT; change in blade ruffle = ΔR; change in blade wet mass = ΔM_B; change in blade area = ΔA_B.

Figure S3. Change in morphological characters of kelp tissue samples used in load direction experiment expressed as functions of weight and orientation. Change in tissue length = ΔL_S; change in tissue width = ΔW_S; change in tissue area = ΔA_S; change in tissue wet weight = ΔM_S. Lowercase letters denote significantly different groups as indicated by a Tukey–Kramer post hoc test.

Figure S4. Mean meristoderm cell morphological characters of kelp tissue samples used in load direction experiment expressed as functions of weight and orientation. Mean cell length = ΔL_C; mean cell width = ΔW_C; mean cell area = ΔA_C. Lowercase letters denote significantly different groups as indicated by a Tukey–Kramer post hoc test.

Figure S5. Density plots of (A) lengths and (B) widths of *Nereocystis* meristoderm cells as functions of weight and orientation applied during load direction experiment.

Table S1. ANOVA table for model of change in stress from stress magnitude experiment

Table S2. ANOVA tables for load magnitude experiment (with stress as predictor)