Seasonal Impacts on the Breakage of *Nereocystis luetkeana*: A Break in Time

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Abstract

Nereocystis luetkeana is an ecologically important species in the marine environment and when it washes up as drift it becomes ecologically important to the terrestrial environment, providing a crucial link between the two ecosystems. The first appearance of *N. luetkeana* has been observed to be during early spring and then they reach their full height in early summer and soon after become reproductive. During this time, their chances of entanglement increase and so do their chances of detachment. Their blades also contain a large amount of mass, and when the current causes those blades to go in one direction, it causes tension in the stipe which in turn increases the risk of detachment. This study focuses on quantifying the location of bull kelp failure (stipe, holdfast, or substrate) throughout spring and seeing when the most breakage was observed in the San Juan Archipelago. Significant patterns in the data were variable; two locations found to have statistical significance in the average stipe length between early and late spring showed opposite trends compared to each other. The variation in data trends, and the observed variation in sizes and stages in the life cycle suggests that the life cycle of *N. luetkeana* does not follow a certain seasonal schedule.

Introduction

Kelp are Laminarialean brown algae that contain a holdfast, stipe, and blades (Graham, Graham, Wilcox, and Cook 301). They are ecologically important because they form kelp forests and shelter many species (Graham, Graham, Wilcox, and Cook 300, Rogers-Bennett et al. 2011, Duggins et al. 2016). Kelp provide shelter, refuge, and nutrients (Rogers-Bennett et al. 2011, Duggins et al.1989). The carbon that kelp fixes can be found throughout nearshore marine food webs through direct consumption of standing (living) kelp. Kelp biomass makes its way into the food web through particulate organic matter (POM) and dissolved organic matter (DOM) as the kelp decomposes (Duggins et al. 1989).

Bull kelp, *Nereocystis luetkeana*, have holdfasts that provide shelter for a variety species such as sea snails (Rogers-Bennett et al. 2011) while its stipe and blades provide a valuable canopy structure for species of shrimp, amphipods, urchins, and some coralline algae (Rogers-Bennett et al. 2011, Duggins et al. 2016). Bull kelp that remains attached to the substrate on the seafloor as well as drift kelp both provide habitats for other marine organisms (Duggins et al. 2016). However, once it washes up on shore, it becomes part of a different ecosystem. Bull kelp decomposes in the terrestrial environment and provides nutrition to organisms in this habitat (Orr et al. 2005). *N. luetkeana* that washes up on shores arrives in different conditions: stipe no longer attached to holdfast, stipe attached to holdfast, and stipe attached to holdfast that is still attached to substrate (pers. obs.).

Bull kelp thalli (the body of the kelp) have four main parts: the holdfast, the stipe, the pneumatocyst, and the blades (Graham, Graham, Wilcox, and Cook 290, Denny and Gaylord 2002). The gas-filled pneumatocyst, located between the stipe and the blades, acts as a float to give the photosynthetic blades access to the surface of the water (Denny and Gaylord 2002). *N. luetkeana* is an annual species (Maxell and Miller 1996, Denny et al. 1997). Bull kelp first appears during late winter or early spring and by early summer reaches its full height and reproduces (Maxell and Miller 1996, Denny et al. 1997). Stipe growth rate is at its highest as the kelp grows towards the surface, but once the blades reach the surface, stipe growth rates decline and blade growth takes over (Maxell and

Miller 1996). Young bull kelp have not yet reached the surface though, so wave motion has a smaller influence on them because they are living deeper in the water column (Maxell and Miller 1996, Denny and Gaylord 2002). However, once the adolescent bull kelp reach their full height at the surface, the influence of wave motion on their movement increases (Denny and Gaylord 2002).

The mass of the blades as well as hydrodynamic forces acting on the kelp contribute to the breakage of the stipe. The mass of the blades influences the momentum of the blades (Gaylord et al. 1994, Denny et al. 1997). For larger bull kelp the blades carry more mass, which means when the kelp is flowing in the direction of the wave's motion, it causes momentum (mass x velocity) in the blades. The blade's momentum creates tension at the point of attachment between the stipe and holdfast, which could potentially cause breakage (Gaylord et al. 1994, Denny et al. 1997). The different forces acting on *N. luetkeana* and its mass act as a single element (Denny et al. 1997). This element and the surrounding water cause drag and hydrodynamic forces that act on the thallus, depending on the motion of the waves. These forces acting on the thallus respond to resistance from the tension created in the stipe, and that imbalance of forces leads to acceleration of the mass of the kelp and could result in breakage (Johnson and Koehl 1994, Denny et al. 1997).

The purpose of this study is to quantify the location of bull kelp failure (stipe, holdfast, or substrate) throughout spring and see when the most breakage is observed (early or late spring). A key research question addressed is whether or not a difference in the breakage point of *Nereocystis luetkeana*, as evidenced by the presence or absence of a holdfast, can been seen during early spring (4/1/16-4/29/16) versus late spring (4/30/16-

5/27/16). As older bull kelp blades from senescing individuals begin to deteriorate, there will be less blade mass during early spring. Also during this time, young bull kelp begin to appear, but have not reached the surface yet. If there is less blade mass on the older kelp and younger bull kelp are still living deeper in the water column during early spring, then there should be less *Nereocystis luetkeana* breakage recorded in early spring compared to late spring.

I tracked and recorded the breakage of *Nereocystis luetkeana* at multiple sites in early spring, surveyed those same sites again at the end of spring, and then compared the type and amount of breakage seen as well as the size of the kelp between early and late spring. The blades mass and momentum will have a smaller influence on the detachment of the bull kelp and therefore drift kelp during early spring should have more with holdfasts and the stipes will be much longer as a consequence. Whereas when the blades are larger, they will have a greater impact on the detachment, so majority of bull kelp that washes up during late spring should not have holdfasts and the stipes will be shorter.

Methods

Drift *Nereocystis luetkeana* is defined as an individual that could be picked up with no work. Collection and measurements took place at 12 sites: Cattle Point, the Friday Harbor Labs Dock, Argyle Creek, Beaverton Cove, Collin's Cove, Eagle Cove, Picnic Cove, Jackson Beach, Botany Beach, Dead Man's Bay, Pacheedaht Campground, and Iceberg Point (Figure 1). Data was collected at these sites because they varied in exposure levels and substratum type. Measurements were taken during low tide to maximize the amount of data collected. Data collected included eleven parameters (Table

1). Early spring was from 4/1/16-4/29/16 and late spring was from 4/30/16-5/27/16. Bull kelp comprised of only a stipe or only a pneumatocyst were not measured.

Table 1. The different categories and options taken for each sample of *Nereocystis luetkeana* collected.

Date	Season	Location	Stipe Length (cm)	Bulb diameter (cm)	Holdfast State	Substrate	Freshness	Blade Mass (g)	Stipe Mass (g)	Entanglement
	Early				No		1-fresh			Tangled
	Late				Yes, unattached		2-degraded			Single
					Yes, attached		3-bleached			
							4-dessicated			

Drift bull kelp was collected during early and late spring at 6 sites: Friday Harbor Labs Dock, Collin's Cove, Argyle Creek, Eagle Cove, Beaverton Cove, and Cattle Point. To ensure that the same *N. luetkeana* was not measured repeatedly, we cut off the pneumatocyst after measurements of a specific kelp were complete. Collection during early spring (4/1/16-4/29/16) and in late spring (4/30/16-5/27/16) at the same sites was completed to determine if there was a difference in breakage throughout spring. Data collection during early spring and late spring was done so that about one month had passed between collections at the same sites.

Data analysis included only the six sites with data from early and late spring, and holdfast conditions of no holdfast and holdfast that was unattached to a substrate. A two by two contingency table (df=1) was made for each site and a chi squared value was calculated from it (Table 2). A two by two contingency table (df=1) was also done to calculate a chi squared value for combined early and combined late data from the six sites. If the chi squared value was greater than 3.84 and the p-value was less than 0.05 then it was significant. A two sample t-test was also done on the combined early and combined late site stipe data (Figure 3). A p-value was calculated for the different combinations of season and holdfast conditions (Table 3). If the p-value was less than 0.05, then the results were significant. A two-way ANOVA was done to compare the different means at the six different sites, and a follow-up Tukey test was done to compare which sites had significantly different means (Figure 4). If the site comparison had a p-value less then 0.05, then it was significant. Another two-way ANOVA was done to compare the different means at the six different sites during the early and late spring season. Then the twelve different means (six early and six late site means) were compared to each other to see which mean comparisons were significant (Figure 5). If the mean comparisons had a p-value less than 0.05, then it was significant.

a.

b.

Figure 1. a. Collection sites on the San Juan Archipelago and Vancouver Island . b. Close up of the collection sites on the San Juan Archipelago.

Results

Cattle Point showed a statistical difference in the proportion of bull kelp with holdfasts and without holdfasts between early and late spring (χ^2= 5.5742, df=1, and p-value= 0.018227) All other sites showed no statistical significance between early and late spring in the number of individuals with holdfasts compared to those without holdfasts (Table 2 and Figure 2).

Table 2. Chi squared and p-values for each of the six sites that were sampled in early and late spring.

Location	χ^2	p value
Argyle Creek	1.3177	0.251002
Beaverton Cove	1.92	0.165857
Cattle Point	5.5742	0.018227
Collin's Cove	0.0049	0.94422
Eagle Cove	0.6257	0.428945
FHL Dock	0.0384	0.844576

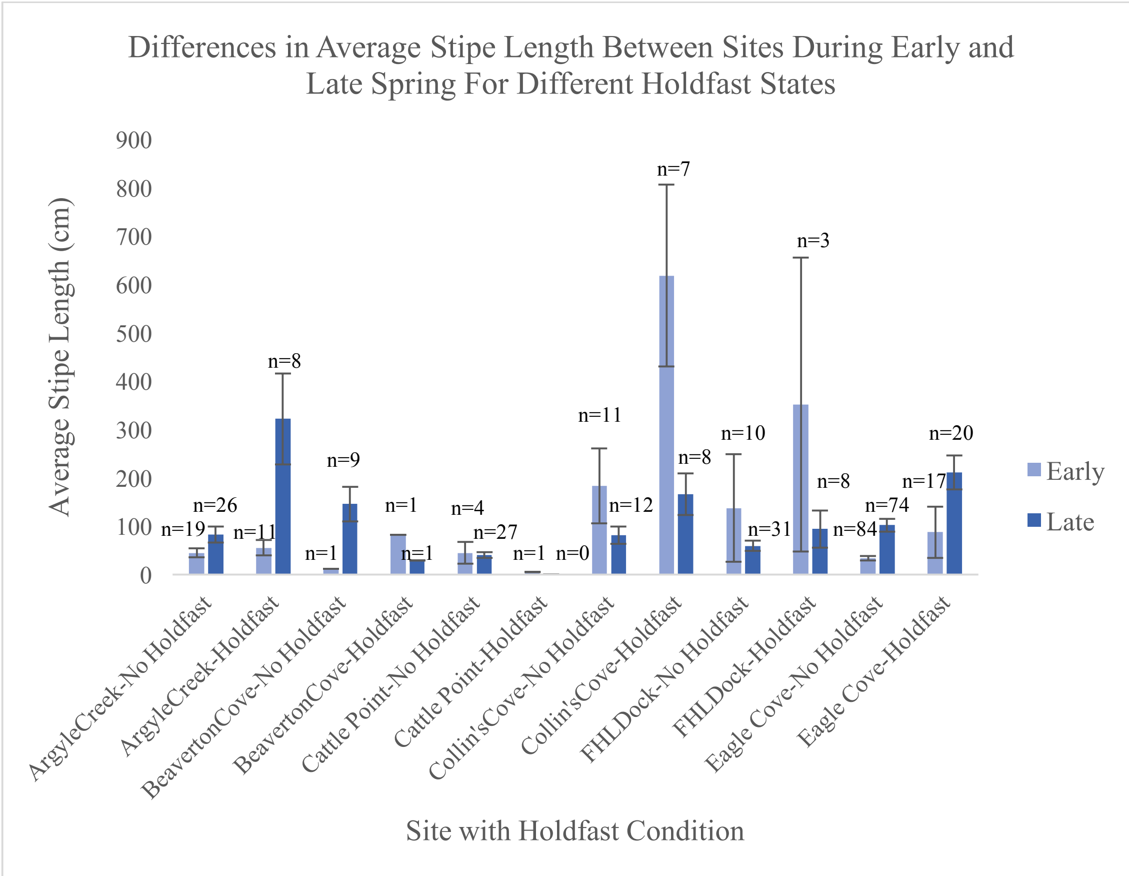

Figure 2. Average stipe length of bull kelp measured with and without holdfasts at each of the 6 collection sites during early and late spring. Error bars represent standard error.

The chi squared value for all 6 sites during early and late spring showed no statistical significance (χ^2= 0.7281, df=1, and p-value= 0.393513), so the proportion of bull kelp with and without holdfasts during early spring was about the same as during late spring.

The average stipe length of the bull kelp collected during late spring with and without holdfasts is statistically significant and so is the average stipe length of bull kelp collected during early spring with and without holdfasts (Table 3 and Figure 3). The overall stipe length of bull kelp collected with holdfasts during early and late spring was significantly larger than individuals collected during early and late spring without holdfasts (Figure 3). However, between seasons there is no statistical significance in average stipe length of bull kelp with and without holdfasts (Table 3 and Figure 3).

Table 3. P-values for Figure 3 showing what parings were statistically significant.

	Late	Early	
Late	5.75777E-09	0.887587007	Holdfast
Early	0.783738314	0.021557557	No Holdfast
	No Holdfast	Holdfast	

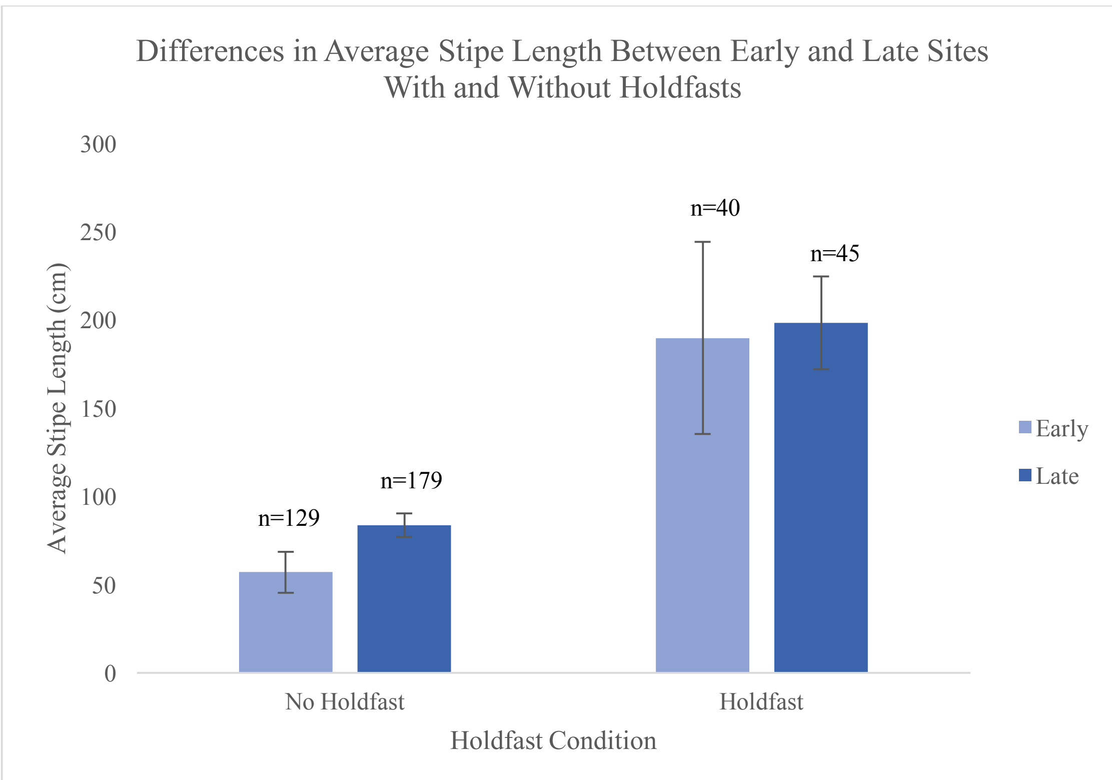

Figure 3. Average stipe length of collected bull kelp with and without holdfasts of all 6 sites during early and late spring. Error bars represent standard error.

When just looking at the overall pattern between sites throughout the whole season, the average stipe length (combining holdfast and no holdfast data) is significantly larger at Collin's Cove than at Argyle Creek (two-way ANOVA test=3.53e-6, df=5, p-value=0.0004686), Cattle Point (two-way ANOVA test=3.53e-6, df=5, p-value=0.0000062), Eagle Cove (two-way ANOVA test=3.53e-6, df=5, p-value=0.0000020), and the FHL Dock (two-way ANOVA test=3.53e-6, df=5, p-value=0.0009110). However, the overall average stipe length at Collin's Cove is not significantly larger than the overall average stipe length at Beaverton Cove (Figure 4); two-way ANOVA test= 3.53e-6, df=5, p-value=0.2661844.

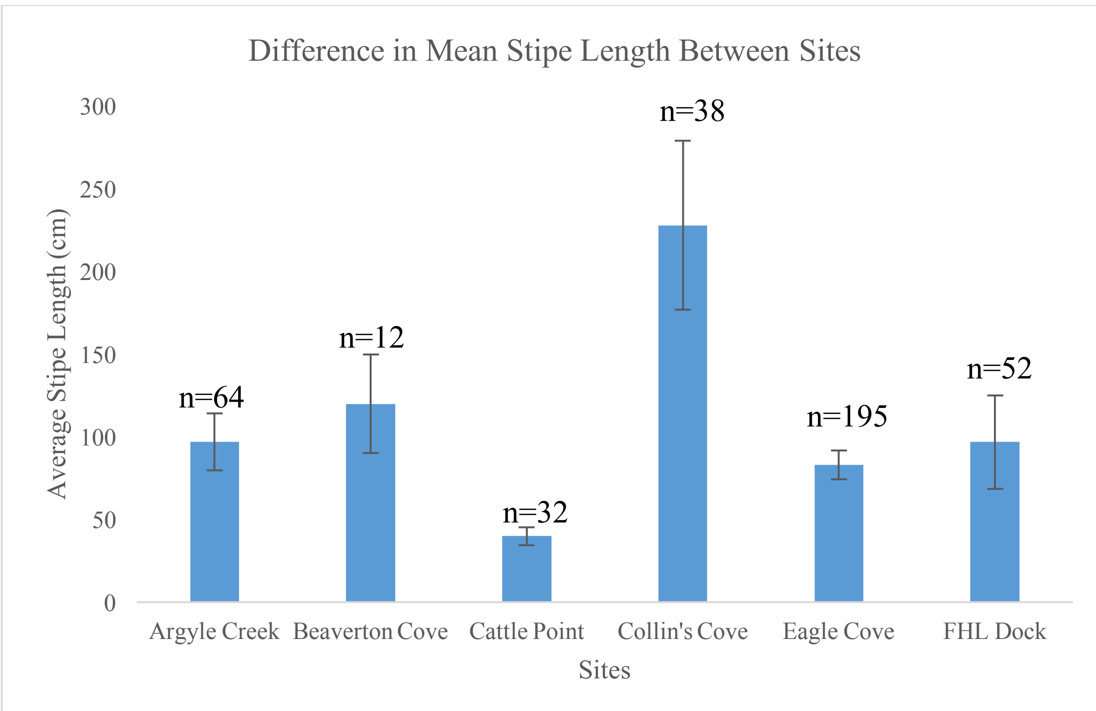

Figure 4. Overall average stipe length at each of the six sites that were visited multiple times throughout the season. Error bars represent standard error.

Comparing the sites during the early and late spring (combining holdfast and no holdfast data) yields statistically significant results (p-value < 0.05). The average stipe length at Collin's cove was significantly larger than at Argyle Creek during early spring (two-way ANOVA test=1.66e-8, df=5, p-value=0). Similar patterns were seen when average stipe lengths from Cattle Point (two-way ANOVA test=1.66e-8, df=5, p-value=0.0028986) and Eagle Cove (two-way ANOVA test=1.66e-8, df=5, p-value=0) were compared to the average stipe length at Collin's Cove during early spring. When comparing early and late spring average stipe lengths at Collin's Cove, the stipe lengths were overall larger during early spring than during late spring (two-way ANOVA test=1.66e-8, df=5, p-value=0.0001435). However, when comparing average stipe lengths during early and late spring at Eagle Cove, the overall stipe lengths were significantly larger during late spring (Figure 5); two-way ANOVA test=1.66e-8, df=5, p-value=0.0102602.

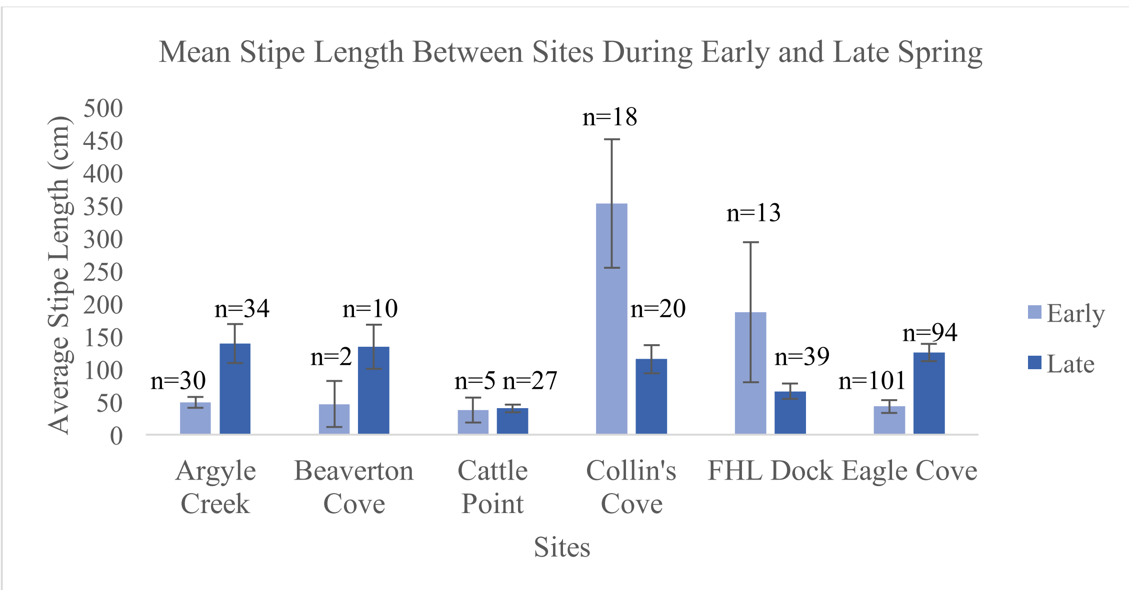

Figure 5. Combined holdfast data for each of the six sites during early and late spring. Error bars represent standard error.

Discussion

Reproductively mature *N. luetkeana* have been observed to get entangled because of their large and numerous blades, which may lead to holdfast failure (Maxell and Miller 1996, Koehl and Wainwright 1977). Bull kelp may also be more susceptible to detachment because of the substrates it attaches to, such as small rocks, cobble, or barnacles (Maxell and Miller 1996, Gaylord et al. 1994). If more mature bull kelp is washing up as they grow to the surface and begin reproduction, then fewer remain to spawn zoospores in a suitable location and give rise to the gametophytes of the next generation (Maxell and Miller 1996). This could negatively impact the amount of kelp available as habitat for other marine organisms. While entangled *N. luetkeana* was seen in beach wrack, it seemed to occur throughout the spring season, and a greater amount of large kelp was not seen later in spring than earlier. The sizes and stages of drift kelp varied, indicating that there is no overall significant pattern to the size and season in which bull kelp is washing up on beaches.

Even though *N. luetkeana* is an annual species, an individual bull kelp can release its zoospores at any time during the year once it reaches reproductive maturity. The data collected illustrates a different set of data than originally expected because of the variability in significant patterns seen. The two sites (Collin's Cove and Eagle Cove) that showed significant differences in average stipe length between early and late spring, illustrated opposite trends when compared to each other (Figure 5). Cattle Point was the only site with a statistically different proportion of bull kelp collected with holdfasts and without holdfasts between early and late spring (Table 2 and Figure 2). Young, drift bull kelp were seen at the start of spring, but continued to be seen throughout spring as well as older, drift individuals. Perhaps *N. luetkeana* does not have a strict life cycle as previously thought. This also may not have been enough time to achieve significant results about the seasonal impacts on the breakage of bull kelp. Further research could include this data and focus on another season to see if there are between season impacts on the breakage, or more seasonal data would also help to rule out season as a cue for the different stages in the *N. luetkeana* life cycle.

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