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Cumulative Stress Restricts Niche Filling Potential of Habitat-Forming Kelps in a Future Climate

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Summary

1. Climate change is driving range contractions and local population extinctions across the globe. When this affects ecosystem engineers the vacant niches left behind are likely to alter the wider ecosystem unless a similar species can fulfil them.

2. Here, we explore the stress physiology of two coexisting kelps undergoing opposing range shifts in the Northeast Atlantic and discuss what differences in stress physiology may mean for future niche filling.

3. We used chlorophyll florescence (F_v/F_m) and differentiation of the Heat Shock Response (HSR) to determine the capacity of the expanding kelp*, Laminaria ochroleuca*, to move into the higher shore position of the retreating kelp, *Laminaria digitata*. We applied both single and consecutive exposures to immersed and emersed high and low temperature treatments, replicating low tide exposures experienced in summer and winter.

4. No interspecific differences in HSR were observed which was surprising given the species' different biogeographic distributions. However, chlorophyll florescence revealed clear differences between species with *L. ochroleuca* better equipped to tolerate high immersed temperatures but showed little capacity to tolerate frosts or high emersion temperatures.

5. Many patterns observed were only apparent after consecutive exposures. Such cumulative effects have largely been overlooked in tolerance experiments on

intertidal organisms despite being more representative of the stress experienced in natural habitats. We therefore suggest future experiments incorporate consecutive stress into their design.

6. Climate change is predicted to result in fewer ground frosts and increased summer temperatures. Therefore, *L. ochroleuca* may be released from its summer cold limit in winter but still be prevented from moving up the shore due to desiccation in the summer. *L. ochroleuca* will however likely be able to move into tidal pools. Therefore, only partial niche filling by *L. ochroleuca* will be possible in this system as climate change advances.

7.

Introduction

The world's oceans have warmed by 0.11 °C per decade for the last 40 years while short term rapid increases in ocean and aerial temperatures (heatwaves) have also increased in frequency and duration (Meehl & Tebaldi 2004; Christidis *et al.* 2005; Coumou & Rahmstorf 2012; Lima & Wethey 2012; Seneviratne *et al.* 2012; Hartmann, Tank & Rusticucci 2013). Together, these two types of warming are resulting in the global redistribution of marine species (Burrows *et al.* 2011; Poloczanska *et al.* 2013; Smale & Wernberg 2013; Hobday *et al.* 2016), altering the structure and functioning of entire ecosystems (Hawkins *et al.* 2009; Doney *et al.* 2012; Wernberg *et al.* 2016). In marine systems, organisms generally occupy the entirety of their 'thermal niche' meaning marine populations are particularly responsive to warming (Sunday et al. 2012). While local conditions can cause mosaic patterns of stress intensities in the intertidal (Helmuth *et al.* 2016; Lourenço *et al.* 2016), it is still range margins that are seen to be at the forefront of warming trends, resulting in poleward range expansions (leading edge) or contractions

(trailing edge) (Hampe & Petit 2005; Chen *et al.* 2011; Poloczanska *et al.* 2013). Understanding the physical determinants that set these boundaries is therefore critical for predicting the structure and functioning of ecosystems as climate change progresses (Gaston 2003).

Kelps dominate shallow temperate rocky reefs where they support high levels of primary productivity and modify environmental conditions such as light (Wernberg, Kendrick & Toohey 2005), water flow (Rosman *et al.* 2007), physical disturbance (Connell 2003) and sedimentation rates (Eckman, Duggins & Sewell 1989) allowing rich assemblages to persist (Steneck *et al.* 2002). The biogeographic ranges of kelp species are largely controlled by temperature (Lüning & tom Dieck 1990; Eggert 2012). As such, increases in mean temperatures and heatwaves can alter macroalgal distributions (Edwards & Estes 2006; Tuya *et al.* 2012; Smale & Wernberg 2013; Voerman, Llera & Rico 2013; Smale *et al.* 2015; Filbee-Dexter, Feehan & Scheibling 2016), which can have catastrophic consequences for the associated ecosystems they underpin (Wernberg *et al.* 2013; Wernberg *et al.* 2016).

The British Isles is an important Northeast Atlantic biogeographical transition zone (Forbes 1853). Along Britain's south coast, two structurally similar canopy-forming kelps occur in sympatry at opposing edges of their distributional ranges. *Laminaria digitata,* a boreal species, approaches its trailing edge while *Laminaria ochroleuca,* a Lusitanian species, reaches its leading edge (Bartsch *et al.* 2013; Smale *et al.* 2015). Both coexist on moderately-sheltered to moderately-exposed shores where their vertical distributions overlap. *L. digitata* dominates the low intertidal (< 1 m above *Chart datum*) before being replaced by *L. ochroleuca* further down the shore (Hargrave *et al.* 2016). These species are currently undergoing

opposing poleward range migrations, with *L. digitata* population declines observed along both sides of the English Channel, while *L. ochroleuca* has proliferated at its leading range edge (Raybaud *et al.* 2013; Smale *et al.* 2015). *L. digitata* is a key ecosystem engineer (Schultze *et al.* 1990) and its loss could significantly impact wider community structure, unless another functionally similar species, such as *L. ochroleuca,* is able to move into the vacated habitat left behind.

The environmental conditions a species can survive in (fundamental niche) are often wider than where it is found (realised niche). The reason for this disparity is often due to interspecific competitive exclusion (Hutchinson 1957; Hutchinson 1978). It is not known to what extent *L. ochroleuca's* vertical distribution on the shore is dictated by an inability to tolerate greater periods of low tide stress or through *L. digitata's* competitive dominance at higher tidal heights. Indeed, *L. ochroleuca* is found in much more varied habitats at lower latitudes that are currently occupied by *L. digitata* in Britain (e.g. tide pools & exposed coasts) (Pereira *et al.* 2015). The acquisition of such knowledge will allow for more accurate predictions of the structure and functioning of these communities in a future warmer world.

Optimal growth and maximal survival temperatures of macroalgae are well studied (Bolton & Lüning 1982; Lüning 1984; Bolton & Anderson 1987; Lüning & Freshwater 1988; Orfanidis 1991; Tom Dieck 1992; Simonson, Metaxas & Scheibling 2015; Hargrave *et al.* 2016) but the effects of consecutive low tide exposures remains relatively unknown (but see Pereira *et al.* 2015). This is surprising as low tide stress has long been known to be important in determining the vertical and latitudinal distributions of intertidal organisms (Evans 1948; Dring 1982) and increases in aerial temperatures can cause shifts in macroalgae distributions (Harley

& Paine 2009; Ugarte *et al.* 2009; Harley *et al.* 2012; Martínez *et al.* 2012). Here we examined the potential for *L. ochroleuca* to move into niches left behind by *L. digitata* as its biogeographic range contracts in response to warming. Firstly, we determined the thermal tolerance of both species by subjecting individuals to a single temperature shock using a traditional metric of stress, upregulation of *Hsp70* across a range of temperatures. Secondly, we used chlorophyll florescence (F_v/F_m) to investigate the resistance and resilience of each species to consecutive low tide scenarios. Understanding tolerances to consecutive low tide stress of these two species will provide important insight into how climate change can alter range edge dynamics whilst also providing foresight into the future species composition of kelp-dominated communities on Northeast Atlantic rocky shores.

Material and Methods

Mature *L. digitata* and *L. ochroleuca* sporophytes (n = 5) were collected on a low spring tide at St Mawes Bay, Cornwall, UK (50° 9'24.4764" N 5° 1' 4.1628" W) in April and September 2015. Heat shock experiments (qPCR) were conducted in April and consecutive stress assays were conducted in September. All individuals were taken from the overlapping zone where both species directly coexist (~ 0.4 m above chart datum). Individuals were transported to the laboratory in cool dark containers and held in aerated recirculating tanks for ~ 7 days under photosynthetic flux density of ~ 20 µmol m^{-2} s^{-1} (12:12 hr). Holding tanks were maintained at ambient sea temperatures, 9 °C (April) and 15 °C (September). Following well established methods in seaweed photophysiology and gene expression studies (García‐Mendoza & Colombo‐Pallotta 2007; Henkel & Hofmann 2008; Pearson, Lago‐

Leston & Mota 2009; Rothäusler *et al.* 2011; Jueterbock *et al.* 2014) we excised discs of tissue (diameter: 27 mm, area: 11.45 mm^2) from each kelp, using a cork borer with discs left for one hour before being used in stress assays. To establish that excised discs responded similarly to whole kelps we measured F_v/F_m values (a measure of stress see below), which were similar across tissue types.

Heat Shock Response

When environmental stresses cause proteins to denature and aggregate, organisms upregulate a suite of chaperone proteins known as Heat Shock Proteins (Hsps). These Hsps preserve normal cell function by ensuring appropriate protein folding during translation (Frydman 2001), membrane stability and transport (Hartl & Hayer-Hartl 2002) and protein refolding (Hendrick & Hartl 1993). The level of Hsp transcript is a proxy for cellular damage and quantifying it across a range of experimental treatments allows powerful inferences regarding stress tolerance to be made. The Heat Shock Response (HSR) is the induction of Hsps in response to temperature shocks (Parsell & Lindquist 1993). Induction follows a bell shaped curved and specific thermal set points along this curve (T_{peak} and T_{off}) indicate different important aspects of thermotolerance (Tomanek & Somero 1999; Tomanek & Somero 2002). T_{peak} represents an organism's maximum thermal tolerance as past this point protein synthesis can no longer keep up with the increasing thermal insult. T_{off} is an ultimate upper limit in the functioning of the translational machinery (Barua & Heckathorn 2004; Tomanek 2010). Both T_{peak} and T_{off} are fixed set points of the HSR and are not influenced by previous thermal history (Barua & Heckathorn 2004). Therefore, seasonal differences in sampling periods between HSR experiments

(spring) and chlorophyll florescence (summer) will not affect methodological comparisons.

To compare the HSR of *L. digitata* and *L. ochroleuca* n = 5 kelp discs per treatment were heat shocked at one of seven temperatures (8, 12, 16, 20, 24, 28 or 32 °C) for one hour. Discs were held in individual 100 ml sample pots in thermostatically controlled 35 L water baths with recirculating, aerated seawater. Discs were then removed, blotted dry and snap frozen in liquid N_2 and stored at -80 °C until RNA extraction.

RNA Extraction and qPCR

Total RNA was extracted following a protocol from (Pearson *et al.* 2006). All glassware and consumables were treated with RNAseAway (Ambion, UK) and rinsed with DEPC-treated water before use. All reagents were molecular grade and RNAase free. Half a disc (~300 mg) of heat shocked kelp tissue was ground to a fine powder under liquid N_2, using a pestle and mortar, placed in an extraction buffer (100 mM Tris, 50 mM EDTA, 2M NaCl and 2% CTAB pH 7.5), vortexed and left at room temperature (RT) for 10 – 15 min. To minimise endogenous RNase activity, DTT was added (from 1M stock) to the extraction buffer immediately prior to use to a final concentration of 50 mM. Total RNA was extracted from the resultant homogenate by chloroform extraction. One ml of chloroform:Isoamyl alcohol (24:1 v/v) was added, vortexed vigorously, centrifuged at 12,000g for 20 min at 20 °C and the upper aqueous phase transferred to a new tube. Absolute EtOH was added (0.3 volumes) and gently mixed by rocking. Another chloroform extraction was then performed on this upper aqueous phase/ EtOH mixture under the same conditions as the first.

RNA was precipitated with 0.25 vol 12M LiCl in the presence of 1 % mercaptoethanol at -80 °C for 30 min. Samples were centrifuged at 14,000g for 30 min at 4 °C and the pellet washed with 70 % EtoH and recollected by centrifugation at 14,000g for 10 min at RT. The RNA pellet was air-dried and then suspended in 50 µl DEPC-treated water. Contaminating gDNA was digested using 2U DNAse 1 (Turbo-DNAse free) (Ambion, UK) following the manufacturer's instructions and stored at -80 °C. Total RNA (100 ng) was reversed transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer's instructions and using random hexamer primers.

In order to determine the sequence of the *Hsp70* gene for *L. ochroleuca* and *L. digitata,* PCR primers were designed based on *Saccharina japonica* (accession no FJ375359.1). PCRs were run in 20 µL volumes using Bioline MyTaq Red PCR mix with 100 µM F and R primers and 1 µL cDNA template. Cycling parameters were 5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 60 s at 55 °C and 60 s at 72 °C and a final extension of 10 min at 72 °C. Amplicons were resolved on 1.5% agarose gels and products of the expected size were excised and extracted using Bioline Isolate II clean-up columns. All products were subjected to direct, Sanger sequencing in-house.

Primer 3 software (Life Technologies) was used to design all qPCR primers. *Hsp70* primers were based on the consensus sequences for *L. ochroleuca* and *L. digitata.* For *L. digitata* gene expression normalisation, two reference genes were used, 18s ribosomal RNA (accession no. JX442505) and Rubisco large subunit (accession no. AY157696). For *L. ochroleuca* expression normalisation only used 18s Ribosomal RNA (Table 1).

Quantitative PCR reactions were run in triplicate on 384 well plates on an Applied Biosystem Quant Studio 120-Flex platform, with each plate containing all targets and housekeeping genes for each species. Each well contained 1 µl of cDNA in 10 µl SYBR green reactions (Bioline Sensifast Lo-Rox kit). The PCR amplification protocol consisted of 95 °C for 2 min followed by 40 cycles of 95 °C for 5 s and 60 °C for 15 s (for all assays). Amplification specificity was verified by melt curve analysis (from 60 °C to 95 °C) and agarose gel electrophoresis. Four 10-fold serial dilutions of cDNA product were used to verify that all amplification reactions had efficiencies in the range of 90 – 105 %. No reverse transcriptase and no template controls were performed for each assay plate to ensure no contamination from gDNA was present. Relative mRNA levels were calculated as follows: Firstly, the difference in Ct values from *Hsp70* and the internal reference gene were calculated (ΔCT). The ΔCT value was then subtracted by the ΔCT of the control (8 °C) from each individual (ΔΔCT). Relative expression of *Hsp70* was then calculated by $e^{(-\Delta\Delta CT)}$.

Consecutive Stress Experiments

The experimental design for consecutive stress assays was based on field observations. Both species are found on the low shore and are exposed to low tide stress together only on spring tides. Aerial exposure usually last ~ one hour over each low tide period for four days before becoming immersed until the next spring tidal cycle.

Consecutive stress assays (n = 5) were conducted on two discs of tissue a mean value of which was used in all further analysis. Individual discs were placed under one of a number of different conditions for one hour, to simulate the low tide

and then placed back into the control tanks (15 °C) to simulate the returning tide. For immersed treatments discs were held in individual 100 ml sample pots in thermostatically controlled 35 L water baths with recirculating, aerated seawater. After 24 hrs recovery in control tanks discs were again exposed to their respective one hour treatments. In total, four simulated tidal cycles were performed on the same disc. Recovery was subsequently monitored daily for three days. Stress measurements (F_v/F_m see below) were taken directly before and after each 'low tide' treatment.

Treatments consisted of either emersed or immersed conditions and tested tolerance to either high or low temperatures. Conditions were: control at 15 °C, immersion at 24, 28, 32 °C (simulating summer tidal pools), 0 and 4 °C (simulating winter tidal pools), emersion at 24, 28, and 32 °C (simulating summer low tide aerial exposure) and -5 °C (simulating severe winter ground frost). These temperature ranges were chosen to represent realistic field exposures. Low shore tidal pools in the study area can reach 28 °C in summer (Martins *et al.* 2007) while blades of *L. ochroleuca* can be subjected to temperatures of > 30 °C in tidal pools in its southern range (Engelen *et al.* 2008). Similarly aerial temperatures can already reach 32 °C in summer and ground frosts are encountered during winter spring tides (Moore, unpub).

The experimental design allows clear interspecific comparisons to be made for different types of immersion and emersion stress, yet some caution should be taken when generalising to field conditions. For example, only temperature was elevated in warm-emersed treatments whereas in natural settings a number of environmental factors contribute to low tide stress including relative humidity, wind speed and solar radiation. Moreover, the abrupt temperature shocks used in this

experiment are realistic for emersion stresses but are more rapid than the gradual warming seen in tidal pools. However, such shocks still allow direct comparisons of interspecific thermal tolerance (Henkel & Hofmann 2008; Jueterbock *et al.* 2014; Smolina *et al.* 2016).

Stress was measured using pulse amplitude modulated (PAM) fluorometry (Walz Diving PAM, Germany). The maximum quantum yield of PSII (ratio of photochemical quenching (F_v) to total fluorescence from closed PSII reaction centres (F_m)) is proportional to its efficiency (Butler 1978) and F_v/F_m is an established method to quantify temperature stress in seaweeds (Pearson, Lago‐Leston & Mota 2009; Jueterbock *et al.* 2014; Saada *et al.* 2016). All F_v/F_m values were dark adapted for 15 min. The results for each species were normalised to one using the initial F_v/F_m values taken prior to the start of any treatments. This allowed small intrinsic interspecific differences in starting F_v/F_m to be accounted for.

For high temperature emersion treatments the level of desiccation experienced at each time point was expressed as relative water content (RWC %). Initial wet weights of fully hydrated discs were obtained at the start of the experiment prior to any experimental treatments (FW) and subtracted from their dry weights (DW) (estimated based on wet: dry weight of discs of both kelps, n = 12 for both *L. digitata* and *L. ochroleuca*, taken independently and dried at 60 °C for 48 hrs). Wet weight of kelp discs at each experimental time point (IW) (pre and post treatments) was subtracted from the dry weight and then divided by FW-DW. The resulting value was multiplied by 100.

$$RWC = [(IW - DW) / (FW - DW)] \times 100$$

IW = Wet weight of treated kelp.

FW = Initial wet weight of fully hydrated kelp (pre-treatments).

DW = Dry weight of kelp disc.

Statistical Analysis

Differences in upregulation of *Hsp70* to common garden temperature stress between species was measured using univariate Permutational Analysis of Variance using the PERMANOVA module (Anderson 2001) within Primer 6 software (Clarke & Gorley 2006). The model included the two fixed factors; species (2 levels: *L. digitata*, *L. ochroleuca*) and temperature (7 levels: 8, 12, 16, 20, 24, 28 & 32 °C). Permutations (9999 under a reduced model) were conducted on a similarity matrix constructed from Euclidean distances between untransformed data (relative *Hsp70* expressions). When conducting PERMANOVA analysis on univariate data using Euclidean distances, outputs (pseudo-F-statistics) are analogous to traditional least-squares ANOVA, without the same severity of assumptions regarding data distributions and homogeneity of variance (Anderson 2001; McArdle & Anderson 2001).

F_v/F_m values for consecutive stress assays were analysed using repeated measures Analysis of Variance (RM ANOVA) in SPSS v.22 (IBM). The models had three factors: species (2 levels: *L. digitata, L. ochroleuca*), temperature (heat emersion and immersion - 4 levels: 15, 24, 28 & 32 °C; cold emersion - 2 levels: 15 & - 5 °C and; cold immersion - 3 levels: 15, 4 & 0 °C) and time (11 time points). Relative water content values for heat emersion temperatures were also measured using RM ANOVA with three factors: species (2 levels: *L. digitata* & *L. ochroleuca*), temperature (4 levels: 15, 24, 28, 32 °C) and time (9 time points). Where the

assumption of sphericity was violated (Mauchly's test) degrees of freedom were corrected using Greenhouse-Geisser estimates of Sphericity. Where significant differences were found (at $p < 0.05$) *post hoc* analysis was employed to facilitate interpretation of the RM ANOVA results.

Results

Heat Shock Response

Upregulation of *Hsp70* to the temperature treatments followed a clear bell shaped response for both species (Fig. 1). There was no significant interaction between species and temperature (PERMANOVA $F_{(6,56)} = 0.10$ $p = 0.99$). In both species, thermal set points of the HSR were the same, T_{peak} 24 °C and T_{off} 28 °C (Fig. 1).

Consecutive Heat Shock

Immersed (Heat shock)

Normalised F_v/F_m values varied between time, species and temperature (RM ANOVA: Time × Species × Temperature $F_{(10.3, 109.6)} = 16.2$, $p < 0.0001$). A general pattern of a small reduction in F_v/F_m with each 24 °C heat shock followed by recovery was observed for both species (Fig. 2a & 2b). In initial heat shock treatments these were not significantly different from the control. However, by the 3rd heat shock for *L. digitata* and 4th for *L. ochroleuca* values were significantly different to control discs held at 15 °C. Whilst not returning to control values after 3 days of recovery both species showed patterns of resilience, indicating that heat stress was reversible.

At 28 °C species showed clear differences in their responses from the first heat shock treatment. Initial treatments (shocks 1 – 3) only caused minor reductions in F_v/F_m values for *L. ochroleuca* followed by a considerable reduction at shock 4 (Fig. 2d). However, there were signs of recovery after each 24 hour period and F_v/F_m levels almost reached those of controls after 3 days recovery. On the other hand, *L. digitata* was only able to recover from its first shock at 28 °C. By the fourth cycle values had reached < 40 % of the control and all discs non-viable 24 hrs after the final shock (Fig. 2c).

Neither species showed any ability to tolerate heat shocks of 32 °C with all discs non-viable by the second cycle (Fig. 2e & 2f).

Immersed (Cold Shock)

F_v/F_m values did not vary significantly between species, temperature and time (RM ANOVA: Time × Species × Temperature $F_{(6.3, 75)} = 1.9$ $p = 0.07$). At 4 °C, there was no pattern of reduced F_v/F_m values pre or post treatment (Fig. 3a). At 0 °C treatments began to affect F_v/F_m values but differences were not significantly different from control values, suggesting that these conditions were non-stressful (Fig. 3b).

Emersed (Heat shock)

F_v/F_m values varied significantly between species, temperature and time (RM ANOVA: Time x Species x Temperature $F_{(8.7, 92.3)} = 2.27$ $p = 0.03$). At 24 °C both species showed similar patterns of response with an almost full recovery from exposures 1 and 2. However, after the third exposure recovery was impeded. A

pattern of slow recovery was seen in both species yet neither reached close to control values after 72 hours (Fig. 4a & 4b).

At 28 °C a similar pattern of depression of F_v/F_m values followed by recovery was seen in both species for the first three cycles. However, recovery from the fourth cycle was only evident for *L. digitata* (Fig. 4c) with values for *L. ochroleuca* still declining after 72 hours (Fig. 4d).

At 32 °C a similar pattern to those seen above was observed for the first two cycles, with recovery occurring after 24 hours. However, after the third cycle recovery was considerably reduced. During the recovery period F_v/F_m values continued to decline in both species (Fig. 4e & 4f). This was most marked in *L. ochroleuca.*

Water loss

Water loss varied significantly between time, species and temperature (RM ANOVA: Time × Species × Temperature $F_{(9.6,102)} = 2.63$ $p = 0.007$). Kelp subjected to aerial temperatures followed a clear cycle of reduced relative water content (RWC) content after each exposure, followed by an increase after 24 hours (Fig. 5). The magnitude of reduction of RWC increased with increasing temperatures while the ability to recover to preheat shocked water content decreased. A cumulative reduction in RWC with each consecutive treatment was observed across all temperatures for both species. The rate of water loss was consistently greater in *L. ochroleuca,* which also failed to rehydrate fully after 24 hr recovery. In comparison *L. digitata* was generally almost fully hydrated after the recovery period (Fig. 5).

Emersed (Cold Shock)

L. digitata and *L. ochroleuca* exhibited very different tolerances to -5 °C emersion stress (RM ANOVA: Time x Species x Temperature $F_{(1.7, 27.1)} = 8.436$ $p = 0.002$). *L. digitata* showed reduced F_v/F_m values with each exposure recovering to near control levels after 24 hrs recovery for the first two cycles. After the third exposure values were still depressed after 24 hours and a subsequent exposure resulted in no further reduction. Following 72 hours recovery values had returned to almost that of control values indicating that this freezing stress was reversible (Fig. 6a). *L. ochroleuca* was much less tolerant to this treatment with large reductions in F_v/F_m values from the initial exposure which dropped further after 24 hours recovery. After the four exposures, values were down to 15 % (± 12.3% SE) of the control and there were no signs of recovery after 72 hours (Fig. 6b).

Discussion

Interspecific Difference in Consecutive Low Tide Tolerance

The two species exhibited varied responses to consecutive immersed heat shocks with the warmer-water species, *L. ochroleuca,* better equipped to deal with higher temperature treatments. At 24 °C a progressive erosion of resilience was seen in *L. digitata* that was not observed in *L. ochroleuca.* By the final treatment at 28 °C all *L. digitata* were non-viable whereas *L. ochroleuca* were able to return to control values after 3 days of recovery. Interestingly, a similar lethal response at 28 °C was not observed in *L. digitata* during emersed treatments which is likely a product of evaporative cooling reducing the tissue temperature or desiccation inducing an ametabolic state. Both species differed in their abilities to tolerate stress

related to emersion. Most strikingly *L. ochroleuca* showed no tolerance to even a single freezing exposure. Indeed, anecdotal evidence supports this with reports that over mild winters *L. ochroleuca* progressively advances up the shore becoming more abundant at higher shore heights, until a severe ground frost results in rapid die off of in the intertidal (G. Boalch, pers comm). *L. ochroleuca* was also less equipped to tolerate elevated aerial temperatures, which seemed related to the greater water loss experienced resulting in more severe desiccation. *L. digitata* has been shown to recover from 60 % water loss (Dring, 1982), which was also evident in this study. Whilst desiccation tolerance levels are unavailable for *L. ochroleuca*, it was where water loss fell below ~ 60 % that recovery was not possible.

Climate change in the UK is predicted to result in less summer cloud, increased air temperatures and sunny days, while winter ground frosts will become more infrequent (Jenkins *et al.*, 2010). This may result in *L. ochroleuca's* maximum tidal height switching from being cold limited in the winter to desiccation limited in the summer. Therefore, the habitat vacated by *L. digitata* may remain unoccupied by *L. ochroleuca* as conditions will still remain outside of its fundamental niche. However, it should be noted that conditions may change asymmetrically. Night temperatures are rising faster than day temperatures (Davy *et al.* 2017). As such, advancement up the shore may be possible at least for a period of time. One habitat where *L. ochroleuca* may be able to expand into is tidal pools. Tidal pools in SW England can already reach >28 °C in summer (Martins *et al.* 2007), a temperature that results in considerable stress to *L. digitata* but not *L. ochroleuca.* Currently, only *L. digitata* occupies these habitats suggesting *L. ochroleuca* is being competitively excluded. As temperatures rise and *L. digitata* becomes less competitively dominant or vacates

these tidal pools, *L. ochroleuca* will face few barriers to expanding its realised niche to encompass these habitats.

Comparing the Heat Shock Response and F_v/F_m (Single Exposures):

We observed a clear disparity between the HSR and F_v/F_m as metrics for stress. No interspecific differences in thermal set points of the HSR were found but differences were observed in F_v/F_m to similar single stress treatments. For example, *L. digitata* were able to recover from exposures that represent T_{peak} and T_{off} of the HSR, while *L. ochroleuca* showed no deviation away from control values. This is surprising given that T_{peak} and T_{off} are thought to represent the very upper limits of a species tolerance (Barua & Heckathorn 2004).

Mismatches between chlorophyll florescence and the HSR have been observed before (Jueterbock *et al.* 2014) and may be due to the photosynthetic apparatus having other protective mechanisms in place, allowing for greater thermotolerance compared to the rest of the cell (Downs *et al.* 2000). For example, the small HSP, cp-sHSP, is known to play an important role in protecting photosystem II during heat stress, and production levels are related to thermotolerance in higher plants (Neta-Sharir *et al.* 2005; Shakeel *et al.* 2012). Chlorophyll florescence may also differ from Hsp quantification as it measures different aspects of heat stress and not just symptoms of protein damage. For example, stress induced uncoupling of enzymes and metabolic pathways can cause the accumulation of reactive oxygen species that can lead to oxidative stress affecting F_v/F_m values (Liu & Pang 2010). Plants and seaweeds can upregulate genes with anti-oxidative functions during periods of heat stress to protect the photosystems (Collén *et al.* 2007) and as such maintain photosynthetic function.

Thus, different pressures and mechanisms may affect the two metrics differently and as such they should not be used interchangeably. Moreover, measuring the HSR alone is not sufficient to fully determine the levels of stress affecting macrophytes as they are likely to be more tolerant than levels of T_{peak}, and even T_{off} would suggest.

Single Vs. Consecutive Exposures

Intertidal stress studies have overwhelmingly focussed on absolute limits from single treatments with cumulative effects largely being ignored. We identified clear interspecific differences in tolerances to low tide scenarios that were only apparent after consecutive exposures. For example, if a single treatment had been conducted on *L. digitata* at 28 °C, we would conclude that such temperature shocks would be non-lethal as full recovery was attained within 24 hours. Moreover, when comparing this to *L. ochroleuca* we may conclude they exhibit similar tolerances. However, after the final treatment, all *L. digitata* were non-viable whereas *L. ochroleuca* had returned to near control values. Combined with data from the HSR indicating similar tolerances, measuring a single treatment would lead to potentially misleading interpretations.

Environmental Niche Models (ENMs also called distribution, envelope and bioclimatic models) are the most widely used tools to predict the impact of climate change in species distributions. The majority of ENMs determine where a species can exist in the future based on the conditions they exist in now. However, in recent years there has been an attempt to base ENMs on mechanistic cause and effect relationships with environmental variables (Jordà, Marbà & Duarte 2012; Sunday, Bates & Dulvy 2012; Martínez *et al.* 2015). Modellers seek to link critical temperature thresholds (e.g. CT_{max} and CT_{min}) with current and future warming scenarios. Here

we show an erosion of resilience leading to mortality at temperatures lower than CT_{max} with consecutive exposures. Therefore, such models may underestimate the effect of future warming on species distributions, with local extinctions potentially occurring at lower temperatures. This is especially true for intertidal and shallow subtidal species that experience discrete consecutive exposures on a daily basis or during a set of exposures that coincide with low spring tides. In such cases, smaller increases in temperatures or discrete 'heatwave' events (Hobday et al. 2016), could result in consecutive exposures lower than CT_{max} but great enough to result in considerable stress and subsequent mortality. Therefore, the effect of consecutive exposures on thermal tolerance should be factored into predictive models wherever possible.

Conclusion

Our study provides insight into whether a range expander can replace a range contractor at the interface where they coexist within a biogeographic boundary zone. We show that cumulative stress is a key factor in determining interspecific differences in stress physiology. Such knowledge serves to increase our understanding of the processes driving species redistributions, which is comparatively lacking for marine organisms. However, some caution should be taken when generalising results to the wider region. Although the magnitude of difference between species is quite striking and unlikely to arise from site specific local adaptations such factors cannot be discounted, as only a single site and time point were sampled. Moreover, we have only investigated physiological differences between species and species interactions (e.g. facilitation) have not been accounted for. Such interactions may be important in further elucidating species responses to warming.

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Author Contributions

NK, PM and DW conceived the ideas and designed methodology; NK and LH collected the data; NK and PM analysed the data; all authors contributed to data interpretation; NK led the writing of the manuscript, with equal input from all authors. All authors contributed critically to the drafts and gave final approval for publication.

Data Accessibility

Data available from Figshare online repository https://figshare.com/s/c2ad62334bdec04eb976.

Table 1: qPCR primer sequences used for assays on heat shocked *Laminaria digitata* and *Laminaria ochroleuca*.

	Forward	Reverse
L. digitata		
Hsp70	GCTGCGAGTCGTTGAAGTA	TGGTGCTCGTGAAGATGAAG
18s rRNA	CGGAAGGATCATTACCGAAA	CCCAACTTCGCATAACGAAT
Rubisco	GACATGGATTGGGCATCTCTT	GTAGAACCACATCGTCACCTA
L. ochroleuca		
Hsp70	GAACTTGCGCTTGAACTCTTG	GACGATCGAGGAGGGTATCT
18s r RNA	GATGAAGAACGAGCGAAATG	GTCAACAGACACTCCGACAA

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