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Evidence for different thermal ecotypes in range centre and trailing edge kelp populations.

Letter to JEMBE

Nathan G King[1,2*], Niall J McKeown[2], Dan A Smale[3], David C Wilcockson[2], Laura Hoelters[2], Emily A Groves[1], Thomas Stamp[4], Pippa J Moore[2,5].

[1]Centre of Applied Marine Science, Bangor University, Menai Bridge, LL59 5AB, UK.
[2]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, SY23 3DA, UK.
[3]Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK.
[4]School of Biological and Marine Sciences, Plymouth University, PL4 8AA, UK.
[5]Centre of Marine Ecosystems Research, School of Natural Sciences, Edith Cowan University, Joondalup 6027, Western Australia, Australia.

*Corresponding Author

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Abstract

Determining and predicting species' responses to climate change is a fundamental goal of contemporary ecology. When interpreting responses to warming species are often treated as a single physiological unit with a single species-wide thermal niche. This assumes that trailing edge populations are most vulnerable to warming, as it is here where a species' thermal niche will be exceeded first. Local adaptation can, however, result in narrower thermal tolerance limits for local populations, so that similar relative increases in temperature can exceed local niches throughout a species range. We used a combination of common garden temperature heat-shock experiments (8 - 32 °C) and population genetics (microsatellites) to identify thermal ecotypes of northeast Atlantic range centre and trailing edge populations of the habitat-forming kelp, *Laminaria digitata*. Using upregulation of *hsp70* as an indicator of thermal stress, we found that trailing edge populations were better equipped to tolerate acute temperature shocks. This pattern was consistent across seasons, indicating that between-population variability is fixed. High genetic structuring was also observed, with range centre and trailing edge populations representing highly distinct clusters with little gene flow between regions. Taken together, this suggests the presence of distinct thermal ecotypes for *L. digitata,* which may mean responses to future warming are more complex than linear range contractions.

1. Introduction

Temperature is one of the most important drivers of ecological patterns and processes (Hutchins 1947), dictating where a species can exist and how well it performs throughout its distribution (Brown 1984; Dunson and Travis 1991, Gaston 2003). Rising global temperatures, from anthropogenic greenhouse gas emissions, have already resulted in the altered performance and poleward range migrations of a range of biota and is set to continue as climate change advances (Walther et al. 2002; Parmesan and Yohe 2003; Burrows et al. 2012; Sunday et al. 2012; Poloczanska et al. 2013). As range migrations and altered performances can have serious implications for the structure and functioning of entire ecosystems (Walther et al. 2002, Parmesan 2006, Doney et al. 2012), understanding the effect of rising temperatures on species performance and distributions is a key goal in climate change ecology. However, if this is to be achieved then we must first understand the physiological traits and mechanisms that govern existing species distributions.

When forecasting future distributions, species are often treated as a single homogenous unit (Pearman et al. 2010; Reed et al. 2011), with populations assumed to exhibit similar thermal limits throughout the species' range. Therefore, thermal safety margins, the buffer between experienced temperatures and a species upper thermal limits (see Deutsch et al. 2008; Bennett et al. 2015), are assumed greatest at the range centre and lowest at trailing edges. As such, range centre populations are generally considered to be less vulnerable to predicted warming trends than trailing edge populations, where thermal safety margins will be exceeded first (Thomas et al. 2006; Thomas 2010). Along temperature gradients, however, thermal tolerances are not always consistent between populations, as local adaptation and/ or phenotypic plasticity can result in thermal limits being different across a species biogeographic range (Sanford and Kelly 2011). This may result in more complex responses to warming than simple linear range contractions from trailing edges (Sanford and Kelly 2011;

Valladeres et al. 2014; Bennett et al. 2015; Pontes-da-Silva et al. 2018), meaning central populations may also be vulnerable to ongoing warming.

Within the context of decadal scale warming, the significance of intraspecific variation in thermal niche largely depends on the mechanisms responsible (i.e. plasticity vs. adaptation). If the response is plastic then species are likely to be able to keep pace with climate warming, but if responses are a result of adaptation then the pace of warming is likely to be too fast for natural selection (Jump and Penuelas 2005; Quintero and Wiens 2013). Gaining an understanding of gene flow can provide valuable insight into whether adaptation or plasticity is favoured. Where gene flow is greater than the selection gradient, there is likely to be little selection for local thermal adaptation, resulting in a single plastic phenotype (Garcia-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997), whereas restricted gene flow facilitates the development of local ecotypes (Endler 1977).

Sessile organisms, that cannot modify their behaviour, rely on physiological mechanisms, underpinned by modulation of gene expression, to mediate periods of thermal stress. Whilst the mechanisms themselves are evolutionary conserved, patterns in gene expression are heritable and can vary considerably between populations (Lopez-Maury 2008) resulting in population level differences in thermal tolerance (e.g. Henkel and Hofmann 2008). Therefore, analysis of variation in gene expression offers a powerful tool to identify thermal set points of a population that are often apparent before higher level physiological differences are observed. The Heat Shock Response (HSR) is perhaps the most well studied mechanism for identifying differences in thermal physiology. When organisms are challenged by elevated temperatures that result in protein denaturation and aggregation they rapidly upregulate a suite of molecular chaperones 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 and Hayer-Hartl 2002) and protein refolding (Hendrick and Hartl 1993).

106 Kelps are large brown habitat-forming seaweeds that form extensive forests along
107 rocky coastlines in temperate and subpolar regions (Steneck et al. 2002; Smale et al. 2013;
108 Teagle et al. 2017). Kelp forests rank amongst the world's most productive and extensive
109 habitats, being distributed along one-quarter of the world's coastlines and rivalling the
110 productivity of tropical rainforests (Leith and Whittaker 1975; Mann 1973). Kelp exist over vast
111 temperature gradients and whilst long distance dispersal is possible (e.g. Fraser et al. 2018)
112 the majority of spores settle within a few meters of the parent alga (e.g. Norton 1992; Kendrick
113 and Walker 1995). Therefore, local adaptation may be a common feature throughout kelp
114 distributions. Given that climate mediated range shifts have already been observed in kelp
115 forests across the world (Marba and Duarte 2010; Wernberg et al. 2016; Krumhansl et al.
116 2017) and are predicted to continue as warming progresses (e.g. Martinez et al. 2012;
117 Jueterbock et al. 2013; Khan et al. 2018; Assis et al. 2018) understanding whether
118 intraspecific variation in thermal niche may make central populations vulnerable to future
119 warming trends has direct relevance for management and conservation of kelp forest
120 ecosystems.

121 In this study we investigated intraspecific variation in the Heat Shock Response (HSR)
122 of *Laminaria digitata* (Hudson) J.V. Lamouroux, a common transatlantic kelp. Specifically, we
123 compared populations from two thermally distinct regions in the United Kingdom (representing
124 East Atlantic range centre and trailing edge populations) and conducted experiments at the
125 coolest and warmest times of year, to characterise intraspecific and intra-annual variability in
126 HSRs. We also used neutral microsatellite markers to gain an understanding of gene flow
127 between populations. By adopting a multi-pronged experimental approach we aimed to
128 determine (i) whether populations show differentiation in thermal niche and (ii) whether such
129 differences are likely a product of plasticity or adaptation.

130 # 2. Methods

131 ## 2.1 Study Species

L. digitata is an important ecosystem engineer that forms highly productive stands supporting rich associated communities (Schultze et al. 1990). It has a transatlantic-boreal distribution stretching from the Arctic, where sea ice cover, light availability and low temperatures limit its leading edge, to the English Channel in the East Atlantic and Cape Cod along the US Eastern Seaboard, where it is limited by high summer temperatures. Currently, no climate related population declines have been observed in the West Atlantic (Merzouk and Johnson, 2011) but declines in abundance have been reported in the East Atlantic, although disentangling the roles of overexploitation and warming is difficult (Cosson, 1999; Simkanin et al. 2005; Gavaert et al. 2008). However, as climate change advances, population losses are predicted at both *L. digitata's* East and West Atlantic trailing edges (Raybauld et al. 2013; Khan et al. 2018). In the West Atlantic a lack of subtidal competition means *L. digitata* can extend well into the subtidal (> 20 m) but in the East Atlantic the presence of the subtidal congener *L. hyperborea* restricts *L. digitata* to the low intertidal/shallow subtidal. This means that East Atlantic populations will not be able to move to deeper locations as temperatures in the intertidal become inhospitable. However, the narrow linear distribution of *L. digitata*'s East Atlantic range make it ideally suited to understanding how temperature facilitates the development of thermal ecotypes as it is unconfounded by covarying factors associated with depth.

2.2 Heat Shock Experiment

2.2.1 Patterns of the Heat Shock Response

One of the most highly conserved and well-studied group of HSPs is the Hsp70 family. By measuring the thermal profile of *hsp70* upregulation (temperature at which HSPs are synthesised, T_{on}, maximally expressed, T_{peak}, and turned off, T_{off}) it is possible to investigate intra and inter specific differences in thermal tolerance (Tomanek and Somero 1999; Barua and Heckathorn 2004; Tomanek 2010). T_{on} represents the minimum temperature to cause stress, T_{peak} the upper thermal limit of tolerance beyond which protein synthesis can no longer

meet the cellular response demands and T_{off} the likely ultimate upper limit in the functioning of the translational machinery (Barua and Heckathorn 2004; Tomanek 2010). By comparing these profiles between populations at different latitudes within a species' biogeographic range, it is possible to quantify intraspecific variation in thermal tolerances and to begin to predict how populations, and species, may respond to continued warming.

2.2.2 Survey Periods and Sites

We sampled *Laminaria digitata* populations by employing a nested hierarchical design. Two regions were selected to represent range centre and trailing edge populations (Fig. 1); Within each region two sampling sites were randomly selected; Warbeth, northern Scotland (hereafter 'range centre 1'), Easdale western Scotland (hereafter 'range centre 2'), Trevone, southwest England (hereafter 'trailing edge 1') and St Mawes, southwest England (hereafter 'trailing edge 2'). These regions are thermally distinct with a mean temperature difference of ~ 2.5 °C in winter and ~ 4 °C in summer (Smale and Moore 2017; Pessarrodona et al. 2018). Although the absolute range edge of *L. digitata* extends to Brittany, (~ 48 °N), the temperature regimes either side of the English Channel are similar (mean September SST near St Mawes – 'trailing edge 2 = 17 °C, mean SST in southern Brittany, France = 17.4 °C), and as such, the southwest England sites sampled here were considered to support representatives of the wider marginal range-edge population (King et al. 2018). SST were obtained from the United States' National Oceanographic and Atmospheric Administration (NOAA) daily satellite readings (http://www.seatemperature.org).

2.2.3 Sample Collection and Thermal Assays

To investigate the plasticity of the HSR, the experiment was conducted across two sampling periods, spring (April) and summer (September), representing thermal minima and maxima in ambient seawater temperature (Smale and Moore, *unpublished*). The sampling periods also varied in the intensity of recent low tide heat shock events experienced, with

cooler aerial temperatures experienced over the winter and early spring compared to higher aerial temperatures and sunshine hours during the summer. Thermal assays were conducted for all sites in spring but in summer, it was only possible to conduct assays for range centre 1 and trailing edge 1.

At each site, five mature sporophytes were collected during low spring tides from within the *L. digitata* zone. Individuals were brought back from the field in cool dark containers and held in aerated recirculating tanks for 7 – 14 days under photosynthetic photon flux density of ~ 20 µmol m^{-2} s^{-1} (12:12 hr light: dark cycle). Acclimation tanks were maintained at 8 °C in spring and 12 °C in summer, representing the lowest monthly mean temperature experienced in the field by any population for that month (i.e. range centre 1) (Smale and Moore, *unpublished*). This acclimation period was necessary to ensure all individuals were not physiologically stressed prior to the thermal assays.

Thermal stress assays were conducted on discs of tissue (27 mm diameter, area of 11.45 mm^2) excised from each kelp using a cork borer. Such an approach is representative of the greater organismal response and is well established in seaweed gene expression studies (e.g. Henkel and Hofmann 2008; Pearson et al. 2009; Jueterbock et al. 2014; King et al. 2018). Each disc was heat shocked at one of seven temperatures (8, 12, 16, 20, 24, 28 or 32 °C) for one hour in thermostatically controlled water baths with recirculating, aerated seawater. After one hour's heat shock, discs were removed, blotted dry, snap frozen in liquid N_2 and stored at - 80 °C until RNA extraction.

2.2.4 RNA Extraction and qPCR

Total RNA was extracted following a protocol from Pearson et al. (2006). Primers and qPCR conditions for *hsp70* and two reference genes (18s ribosomal RNA and Rubisco large sub unit, Table S1) followed that of King et al. (2018). 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 $e^{(-\Delta\Delta CT)}$.

2.2.5 Defining Thermal Set Points

The thermal set points were defined as follows: $T_{(on)}$; the temperature at which upregulation first becomes evident, T_{peak}; the temperature of maximal expression and $T_{(off)}$; the temperature post T_{peak} where there is an obvious decline in expression, indicating the HSR has been turned off.

2.2.6 Statistical Analysis

Regional Comparison

Differences in upregulation of *hsp70* to heat stress between regions was assessed using univariate permutational ANOVA, using the PERMANOVA module (Anderson 2001) within Primer 6 software (Clarke and Gorley 2006). The experiment conducted in spring was first analysed in isolation to examine site and region-level variability. Here, the model included three factors; region (fixed factor; 2 levels – range centre and trailing edge), site (random factor; 2 sites nested within each region) and temperature (fixed factor; 7 levels - 8, 12, 16, 20, 24, 28 & 32 °C).

Seasonal Comparison

When comparing expression between regions and seasons only trailing edge 1 and range centre 1 were analysed. The model had three factors: region (fixed factor; 2 levels – range centre and trailing edge), season (fixed factor; 2 levels – spring and autumn) and temperature (fixed factor; 5 levels - 16, 20, 24, 28 & 32 °C). As expression values of *hsp70* were normalised to ambient sea temperatures for the time of year sampled (8 °C in spring and 12 °C in autumn) it was not possible to directly compare all temperatures between seasons.

Instead, temperatures 16, 20, 24, 28 & 32 °C were used for the formal analysis between seasons.

Permutations (9999 under a reduced model) were conducted on a similarity matrix constructed from Euclidean distances between untransformed data (relative hsp70 expressions) for each sampling period. When conducting PERMANOVA analysis on univariate data using Euclidean distances, outputs (F-statistics) are analogous to traditional least-square ANOVA, without the same severity of assumptions regarding data distributions and homogeneity of variance (Anderson 2001; McArdle and Anderson 2001).

2.3 *Population* Genetics

2.3.1 Sample Collection

Thirty individuals, at least 5 m apart from one another, were haphazardly sampled at each site by excising fresh tissue from directly above the meristem between May – June 2016. Samples were then dried in individual Eppendorf's with silica drying crystals until DNA extraction.

2.3.2 DNA Extraction and Microsatellite Amplification

Genomic DNA was isolated from 5 – 10 mg of dried tissue ground to a fine powder using a ball mill. 500 µl of extraction buffer (100 mM Tris, 25 mM EDTA, 1.4M NaCl, 1% PVP and 2% CTAB, pH8) was added and vortexed and left at room temperature (RT) for 10 minutes. RNA was digested by adding 2 µl RNAse A (10mg/ml) to the solution and incubating at 55 °C for one hour. Total DNA was extracted by chloroform extraction, 500 µl of chloroform:Isoamyl alcohol (24:1 v/v) was added, vortexed vigorously and centrifuged at 14000g for 10 minutes and the upper aqueous layer transferred to a new tube. DNA was precipitated out with 30 µl of ammonium acetate and 200 µl isopropanol at - 20 °C for 30 minutes. Samples were centrifuged at 14000g for 10 min at RT. Two EtoH washes were

255 performed at 70% and 95% and recollected at 14000g for 10 min at RT. Pellets were air dried
256 for one hour and then resuspended in 50 µl DEPC-treated water.

257 Twelve microsatellite markers previously developed for *Laminaria digitata* (Ld148,
258 Ld158, Ld167, Ld371, Ld531 and Ld704) and *Laminaria ochroleuca* (Lo4-24, Lo454-17,
259 Lo454-23, Lo454-24, Lo454-27, and Lo454-28) were used (Robuchon et al. 2014).
260 Microsatellites were amplified by individual PCR in 10 µl final volumes containing 1 X GoTaq
261 Flexi colourless reaction buffer, 2mM $MgCl_2$, 150 µm dNTPs, 0.35 U GoTaq DNA polymerase
262 (Promega) and 2 µl template (1:50 dilution) following the protocol of Robuchon et al. (2014).
263 For Ld-primers PCR cycling parameters consisted of an initial denaturation at 94 °C for 5 min,
264 five cycles of touchdown PCR (denaturation at 94 °C for 45s, annealing at 60 °C for 1 min and
265 extension at 72 °C for 45 s; 1 °C decrease of annealing temperature every cycle) followed 50
266 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s and extension at 72 °C
267 for 1 min and a final extension at 72 °C for 30 min. For Lo-primers PCR cycling parameters
268 consisted of an initial denaturation at 94 °C for 5 min, 5 cycles of denaturation at 94 °C for 30
269 s, annealing at 60 °C for 30 s and extension at 72 °C for 45 s followed by 30 cycles where all
270 conditions were similar apart from annealing temperature which was changed to 55 °C. A final
271 extension of 72 °C for 20 min was then performed. Forward primers were fluorescently labelled
272 with VIC (Ld167 and Lo454-17), PET (Ld148, Ld371, Lo454-23 and Lo454-28), NED (Ld158,
273 Ld371, Lo454-23 and Lo454-28) or FAM (Ld531 and Lo454-24) dye (Eurogentec). Amplicon
274 fragment size was analysed on an ABI PRISM 377 automated DNA sequencer (Applied
275 Biosystems) and alleles were scored manually using PEAKSCANNER software v 1.0.

276 **2.3.3 Estimating Genetic Structure - Statistical Analysis**

277 Genetic variation within samples was characterised using number of alleles (NA),
278 allelic richness (AR), observed heterozygosity (HO), and expected heterozygosity (HE) all
279 calculated using FSTAT 2.9.3 (Goudet 1995). Mean pairwise relatedness within samples was
280 calculated using the relatedness estimator, rqg, of Queller and Goodnight (1989) in GENALEX

6.2 (Peakall and Smouse 2006) with associated 95% confidence intervals determined by 1000 bootstraps. Permutation of genotypes among all samples (999 times) was used to calculate the upper and lower 95% confidence intervals for the expected range of rqg under a panmictic model. Genotype frequency conformance to Hardy-Weinberg equilibrium (HWE) expectations and genotypic linkage equilibrium between pairs of loci were tested using exact tests (10000 batches, 5000 iterations) in GENEPOP 3.3 (Raymond and Rousset 1995). The hierarchical Bayesian approach implemented in BAYESCAN (Foll and Gaggiotti 2008) was used to test for signals of selection at the loci. Genetic differentiation among samples was quantified by global and pairwise FST values, with associated significances evaluated by 10000 permutations using FSTAT. Pairwise FST values were also calculated incorporating the null allele correction method implemented in FreeNA (Chapuis and Estoup 2007). Isolation by distance effects were tested by assessing correlation between both sets of pairwise FST values (as well as their corresponding linearised [FST / (1- FST)] (Rousset 1997) values and the corresponding shortest sea distance between pairs of samples using Mantel tests with 10000 permutations in GENALEX. Genetic structuring was also investigated using the Bayesian clustering method implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000) to assess the most probable number of genetically distinct groups (K) represented by the data without *a priori* sample information. Each run had a burn-in of 100000 Markov Chain Monte Carlo samples followed by 1000000 MCMC repetitions. Simulations were run 3 times for each proposed value of K (1–5) to assess convergence with optimal models inferred using ΔK (Evanno et al. 2005). A PCoA of pairwise genotypic distances between individuals was performed using the methods implemented in GENELAEX 6.5 (Peakall & Smouse 2006).

3. Results

3.1 Heat Shock Experiment

The HSR of all experimental trials followed a clear pattern of upregulation; relative expression of *hsp70* increased with increasing temperature until a threshold was reached and

307 a marked decline was subsequently observed. As such, thermal set points of the HSR (T_{on},
308 T_{peak} and T_{off}) could readily be identified from the plotted data (Fig. 2).

309 **3.1.1 Regional Comparison (spring sampling only)**

310 When all four populations were compared in spring, there was a significant interaction
311 between region and temperature (Pseudo-$F_{6,12}$ = 3.03 p = 0.04; Table S2). Upregulation of
312 *hsp70* occurred at higher temperatures at the trailing edge compared to range centre
313 populations with this pattern consistent between sites within regions. Overall, magnitudes of
314 expression were similar between populations with maximal upregulation ranging from 8.8 (±
315 3.05 S.E) – 13.06 (± 4.48 S.E) compared to control values.

316 T_{on} ranged from 12 – 16 °C with no discernible link to population origin, however, T_{peak}
317 and T_{off} varied between regions occurring at lower temperatures in range centre populations
318 (range centre 1 T_{peak} 20 °C T_{off} 24 °C range centre 2 T_{peak} 16 °C T_{off} 20 °C) compared to
319 trailing edge populations (trailing edge 1 and 2 ; T_{peak} 24 °C T_{off} 28 °C) (Fig. 2).

320 **3.1.2 Seasonal Comparison (spring and autumn sampling)**

321 Thermal assays were conducted in both spring and autumn for range centre 1 and
322 trailing edge 1 to determine whether regional patterns were consistent across seasons. Here,
323 there was no significant interaction between temperature, region and season, indicating that
324 expression of *hsp70* was similar across seasons, in both regions (Pseudo-$F_{4,80}$ = 0.25, p =
325 0.92; Table S3) (Fig. 3). However, there was a significant interaction between temperature
326 and region (Pseudo-$F_{4,80}$ = 4.7, p = 0.001; Table S3), with *hsp70* upregulation of trailing edge
327 1 occurring at higher temperatures compared to range centre 1, reinforcing the pattern
328 observed when solely considering sampling undertaken in spring (Fig. 2).

329 T_{peak} and T_{off} remained fixed for both regions irrespective of sampling season (range
330 centre 1 -T_{peak} 20 & T_{off} 24 °C; trailing edge 1 - T_{peak} 24 & T_{off} 28 °C) but a difference in T_{on} was

observed. The HSR in summer was not evident until experimental temperatures were elevated to 16 °C at range centre 1 and 24 °C at trailing edge 1, representing an increase in $T_{(on)}$ of 4 and 12 °C for range centre 1 and trailing edge 1, respectively (Fig. 3).

3.2 Population Genetics

The total number of alleles per locus range from 5 to 28 (mean = 14.3). Basic summary statistics for each sample are reported in Table S4 and showed a general trend of higher variability (NA, AR, HE and HO) among the two range centre sites compared to trailing edge sites. 18 out of 48 locus/sample tests of HWE revealed significant results, in all cases due to heterozygote deficits. With the exception of locus LD158, no locus exhibited significant heterozygote deficiencies at all four sites. Global FST was highly significant (FST = 0.181, $p < 0.0001$). All pairwise FST (Table S5) values were significant and revealed a clear hierarchical pattern wherein (i) the range centre and trailing edge populations were highly differentiated and (ii) comparisons between the two trailing edge populations yielded much higher pairwise FST values than between the two range centre populations despite the greater geographic distance between the range centre sites. This hierarchical structure was also evident from the clustering analysis for which ΔK supported K = 2 (Fig. 4) wherein the range centre and trailing edge samples were partitioned into two distinct clusters, while at K=4 individuals robustly assigned to distinct clusters according to site.

4. Discussion

Here, we provide three lines of evidence that taken together, suggests the presence of locally adapted thermal ecotypes, in the kelp *Laminaria digitata*, at trailing edge and range centre sites of its distribution. Firstly, we show clear differentiation in *L. digitata*'s Heat Shock Response, with the thermal set points T_{peak} and T_{off} higher in trailing edge compared to range centre populations. Secondly, this pattern was mirrored across the warmest and coolest times of year, indicating that differences were fixed regardless of previous thermal history. Finally,

356 our microsatellite data revealed little gene flow between populations, meaning dispersal is far
357 exceeded by the selection gradient, and as such, local adaptation is likely favoured over
358 plasticity. The existence of these ecotypes may have serious implications for our
359 understanding of thermal safety margins and *L. digitata*'s potential vulnerability to warming.
360 Unlike many macrophyte species, *L. digitata* has not seen extensive population loss due to
361 ongoing climate change but poleward range contractions are predicted over the coming
362 century (Raybauld et al., 2013; Khan et al., 2018). As ecotypes possess a narrower thermal
363 tolerance range than that of the overall species, thermal limits may be exceeded throughout
364 *L. digitata's* range and not simply at its trailing edge. Moreover, *L. digitata*'s low dispersal
365 capacity may mean that the ability of ecotypes to track their niche in space is undermined
366 (Assis et al. 2017) which in turn could undermine recovery (Smale and Wernberg 2013).

367 The presence of local thermal ecotypes raises concerns for the effective use of current
368 tools to predict species responses to future warming. Environmental Niche Models (ENMs)
369 currently represent the most utilised tool to predict future species distributions under different
370 climate scenarios. However, they either base thermal niches on the temperatures experienced
371 by the species as a whole or source physiological data from a single climatic location (e.g.
372 Sunday et al. 2012). Thus, they fail to incorporate any intraspecific variation in thermal niche
373 that could increase the complexity and predictability of species responses (Harte et al. 2004;
374 Thomas 2004; Angert et al. 2011; Hällfors et al. 2016; Peterson et al. 2018). So far, ENMs for
375 macrophytes (e.g. Khan et al. 2018), including *L. digitata* (Raybauld et al. 2013; Assis et al.
376 2018), have taken a correlative approach, and as such, may perform poorly where local
377 ecotypes are present (King et al. 2018). In order to rectify this and effectively calibrate ENMs,
378 the relationship between ecotypes and temperature (genotype-by-environment interactions)
379 needs to be characterised. This has been successfully achieved in terrestrial forests (O'Neill
380 et al. 2008; Pearman et al. 2010; Doney et al. 2012) but has only been possible due to the
381 availability of comprehensive historic provenance datasets (Matyas 1996) that are distinctly
382 lacking in other systems.

383 Identification of thermal ecotypes may also have valuable applications for future
384 management of wild and farmed kelp populations. Transplantation of warm ecotypes to cooler
385 regions may boost local resistance to warming and allow populations to track their shifting
386 niche. Indeed, “assisted migration” is becoming an increasingly popular concept in terrestrial
387 forest systems with policy frameworks already being developed (McLachlan et al. 2007; Aitken
388 and Whitlock 2013; Williams and Dumroese 2013). Similar approaches are also being used in
389 commercial seaweed aquaculture in East Asia to combat recent crop failures in the kelp,
390 *Sacharina japonica* (Pang et al. 2007; Liu and Pang 2010). *L. digitata* is harvested from wild
391 populations as far north as Norway and introduction of warm tolerant ecotypes further north
392 could ensure successful harvests in the future.

393 Our data also provide insight into the adaptability of trailing edge populations
394 themselves. The greater differentiation between our trailing edge sites (despite closer
395 proximity) and lower genetic diversity could be seen as indicative of a classic trailing edge,
396 where populations have lower effective population sizes and are more fragmented (Eckert et
397 al. 2008). Pearson et al. (2009) found that such factors resulted in trailing edge populations of
398 the intertidal fucoid, *Fucus serratus*, being less tolerant to thermal shock than central
399 populations i.e. reduced genetic diversity resulted in maladaptation. While our trailing edge
400 populations did exhibit reduced genetic diversity, this did not prevent the formation of thermal
401 ecotypes. It is likely that regional biogeographic contexts can account for this. While both *F.*
402 *serratus* and *L. digitata* have similar range centre and trailing edge densities (King et al.
403 *unpublished*; Pearson. *pers comm*), they differ in their overall extent and isolation from central
404 populations. *F. serratus* occupies a small geographic area at its trailing edge, which is isolated
405 from the larger central population by a lack of suitable habitat in the Bay of Biscay. In contrast,
406 no such geographic barriers exist for *L. digitata* and it is contiguously distributed around the
407 rocky reefs of the western coastline of the UK. As such, differences in isolation time, population
408 connectivity and fragmentation may underpin variability in response patterns between these
409 two species.

410 One aspect of the HSR that exhibited plasticity was T_{on} that increased in the autumn
411 sampling period by up to 12 °C. Plasticity in T_{on} has been commonly documented in the
412 literature (see Barua and Hackerthorn 2004) and is thought to allow fine tuning of the HSR to
413 a highly variable environment, avoiding costly over production (Hotchachaka and Somero,
414 2002), which can decrease fitness (Feder et al. 1992; Krebs and Loeschcke 1994). The
415 cellular basis of this plasticity via the regulation of the transcription factor, heatshock factor-1
416 (*Hsf1*) which regulates expression of HSP genes (Voellmy 1996) in what is known as the
417 'cellular thermometer' model (Craig and Gross 1991). Under non-stressful conditions several
418 HSP's bind to *Hsf-1* supressing HSP transcription. When conditions become stressful, HSP's
419 dissociate from *Hsf-1* to perform their chaperoning roles (Parsell and Lindquist, 1993) and
420 thus *Hsf-1* is free to induce transcription of HSP's. Increased incubation temperature and heat
421 shocks increase the number of gene induction events which raises the standing level of HSP's
422 in the cell (Maloyan et al. 1999). This increased standing stock can maintain protein refolding
423 without dissociation of HSP's from the *Hsf-1* and so induction will occur at higher
424 temperatures. At the end of summer, when kelps may have been subjected to multiple heat
425 shock events, standing stocks will be at their greatest and thus responsible for an increase in
426 T_{on}

427 ## 5. Conclusion

428 Our study provides evidence for local adaptation in an important kelp, which has
429 implications for our understanding of thermal safety margins and how this species may
430 respond to warming. King et al. (2018) recently found intraspecific variation in thermal
431 tolerance and restrictive dispersal to be common traits in marine macrophytes (seaweeds and
432 seagrasses). Therefore, local adaptation may be a common trait for kelp and seaweeds in
433 general. Indeed, transplants that are the most direct method to detect local adaptation (Merilä
434 and Hendry 2014) have clearly demonstrated distinct physiological differences between native
435 vs transplanted populations (Gerard and Du Boise 1988; Saada et al. 2016). However, further

436 experimentation on the progeny of assumed ecotypes is still required to exclude any effects
437 from transgenerational plasticity that may mimic or obscure patterns of adaptation.

438 Quantifying what the presence of ecotypes means for the climate driven redistribution
439 of *L. digitata,* and other macrophytes, is currently constrained by a lack of fine scale empirical
440 data. The research effort required to attain the resolution needed to produce bespoke
441 calibrated ENMs is clearly unfeasible. Therefore, a first step should be to gather the relevant
442 data in a few ecologically representative species, such as *L. digitata*. In this way, it may be
443 possible to make generalisations across phylogenies and allow a more precise understanding
444 of thermal safety margins to be attained and areas of greatest vulnerability to be identified.

445 **Declaration of Interest**

446 The authors of the study declare that there is no conflict of interest.

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Supplementary Information

Table S1 qPCR primer sequences used for assays on heat shocked *Laminaria digitata.*

	Forward	Reverse
hsp70	GCTGCGAGTCGTTGAAGTA	TGGTGCTCGTGAAGATGAAG
18s RNA	CGGAAGGATCATTACCGAAA	CCCAACTTCGCATAACGAAT
Rubisco large sub-unit	GACATGGATTGGGCATCTCTT	GTAGAACCACATCGTCACCTA

ANOVA Output for Heat Shock Assays

Table S2. Univariate permutational ANOVA to test for differences in *hsp70* upregulation between region, site and temperature. Significant values ($p < 0.05$) are indicated in bold.

Source	df	SS	MS	F	p	Df
Temperature	6	678.9	113.2	3.1	**0.04**	12
Region	1	16.9	16.9	1.5	0.66	2
Site(Region)	2	22.1	11.0	0.4	0.72	112
Temperature x Region	6	660.5	110.1	3.0	**0.04**	12
Temperature x Site(Region)	12	436.0	36.3	1.2	0.23	112

Table S3. Univariate permutational ANOVA to test for differences in *hsp70* upregulation between region, temperature and season at range centre 1 and trailing edge 1

Source	df	SS	MS	F	p	Df
Temperature	4	451.6	112.9	4.5	**0.001**	80
Region	1	5.4	5.4	0.2	0.66	80
Season	1	33.7	33.66	1.3	0.26	80
Temperature x Region	4	475.6	118.9	4.7	**0.001**	80
Temperature x Season	4	42.1	10.5	0.4	0.81	80
Region x Season	1	8.58	8.6	0.3	0.57	80
Temperature x Region x Season	4	25.2	6.3	0.3	0.92	80

Summary Statistics for Population Structure

Table S4. Microsatellite data summary indices for each site. NA = number of alleles; Ar = allelic richness; H_E = expected heterozygosity and H_o = observed heterozygosity.

	Mean *NA*	Mean *Ar*	H_E	H_O
Range Centre 1	5.6	4.8	0.66	0.50
Range Centre 2	7.1	5.3	0.62	0.51
Trailing Edge 1	3.8	2.9	0.40	0.33
Trailing Edge 2	4.0	3.7	0.53	0.42

Table S5. Pairwise F_{ST} values between populations. All values calculated without null allele correction (below diagonal) and with null allele correction using the ENA method in FreeNA (above diagonal). All values were statistically significant ($p < 0.05$).

	Range Centre 1	Range Centre 2	Trailing Edge 1	Trailing Edge 2
Range Centre 1	-	0.050	0.358	0.132
Range Centre 2	0.051	-	0.29	0.130
Trailing Edge 1	0.263	0.272	-	0.127
Trailing Edge 2	0.138	0.127	0.133	-

Figure Legends

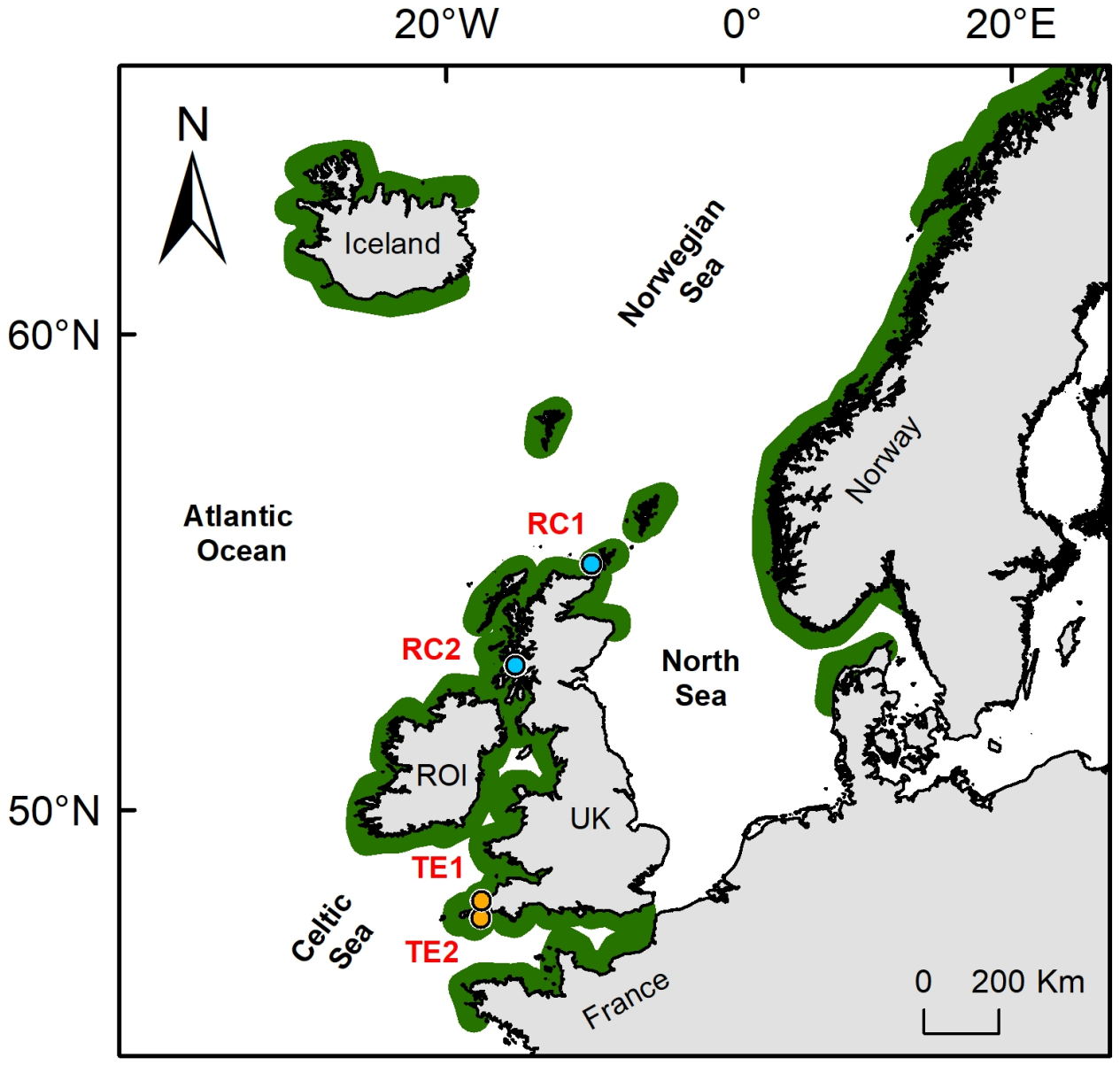
Fig. 1. Sampling sites for *Laminaria digitata.* Range centre 1 (RC1) = Warbeth Bay, Orkney. Range centre 2 (RC2) = Easdale, west-Scotland. Trailing edge 1 (TE1) = Trevone, Cornwall. Trailing edge 2 (TE2) = St Mawes, Cornwall. Green shaded area = approximate distribution of *L. digitata*

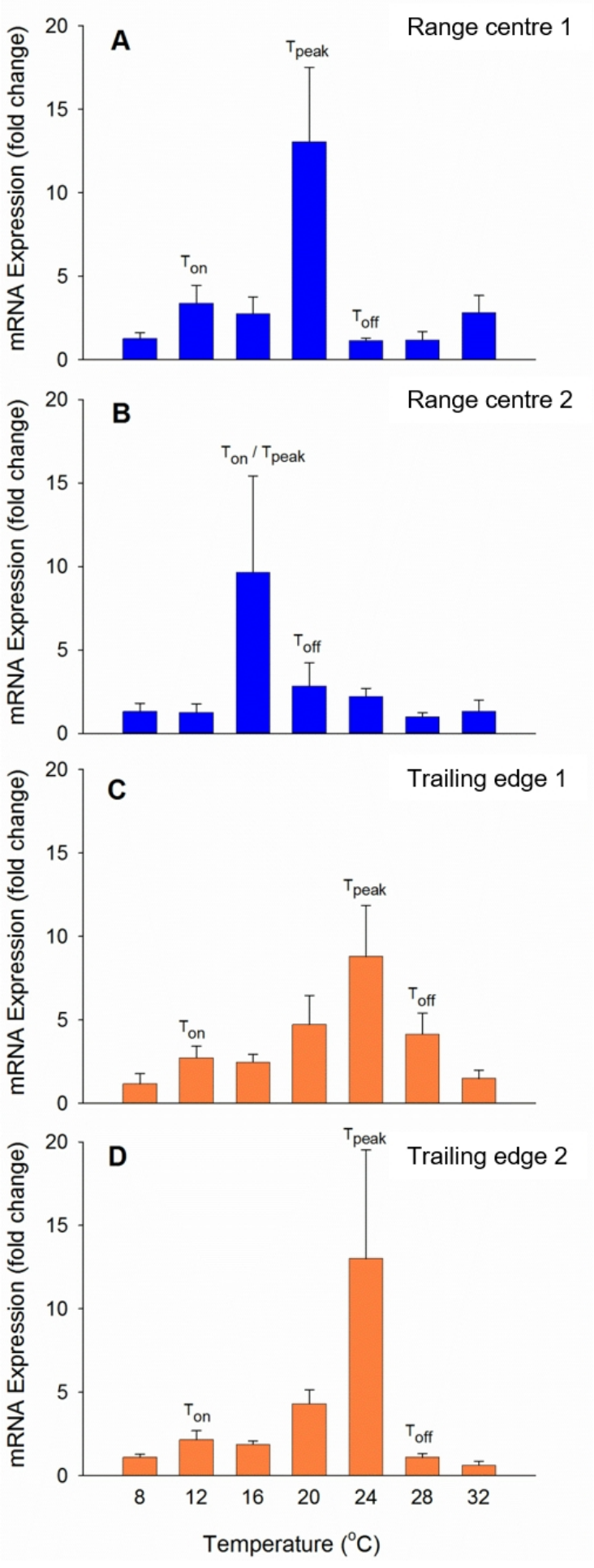
Fig. 2. Mean (± 1 S.E.) relative expression of *hsp70* in *Laminaria digitata* from range centre (blue) and trailing edge locations (orange) to acute 1 hr immersed temperature shocks in April 2015. Relative expression calculated against two housekeeping genes Rubisco and 18srRNA. n = 5 per temperature treatment.

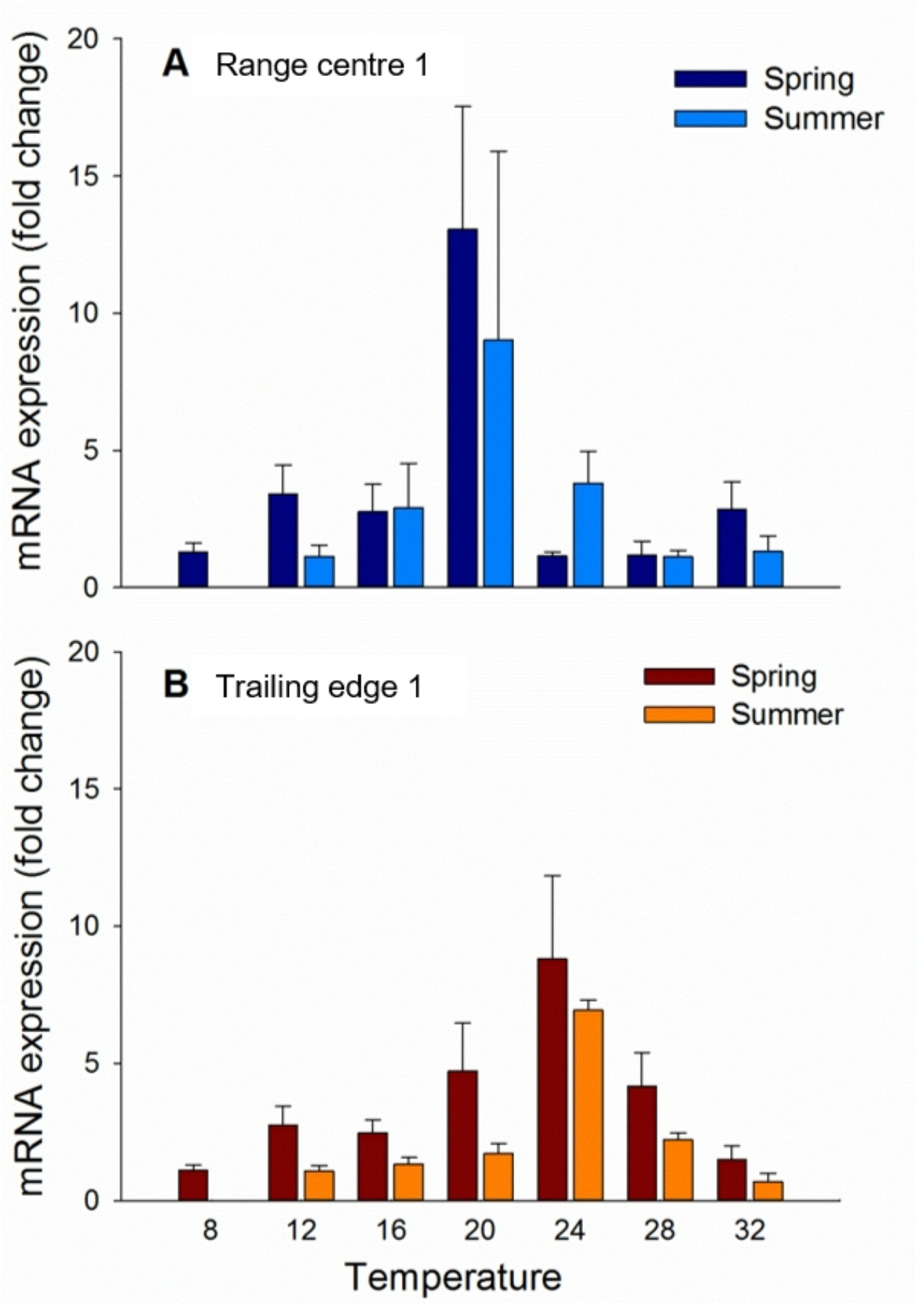
Fig. 3. Seasonal comparison of mean (± 1 S.E.) relative expression of *hsp70* in *Laminaria digitata* from range centre (blue) and trailing edge locations (orange) to acute 1 hr immersed temperature shocks in April and September 2015. Relative expression calculated against two housekeeping genes Rubisco and 18srRNA. n = 5 per temperature treatment.

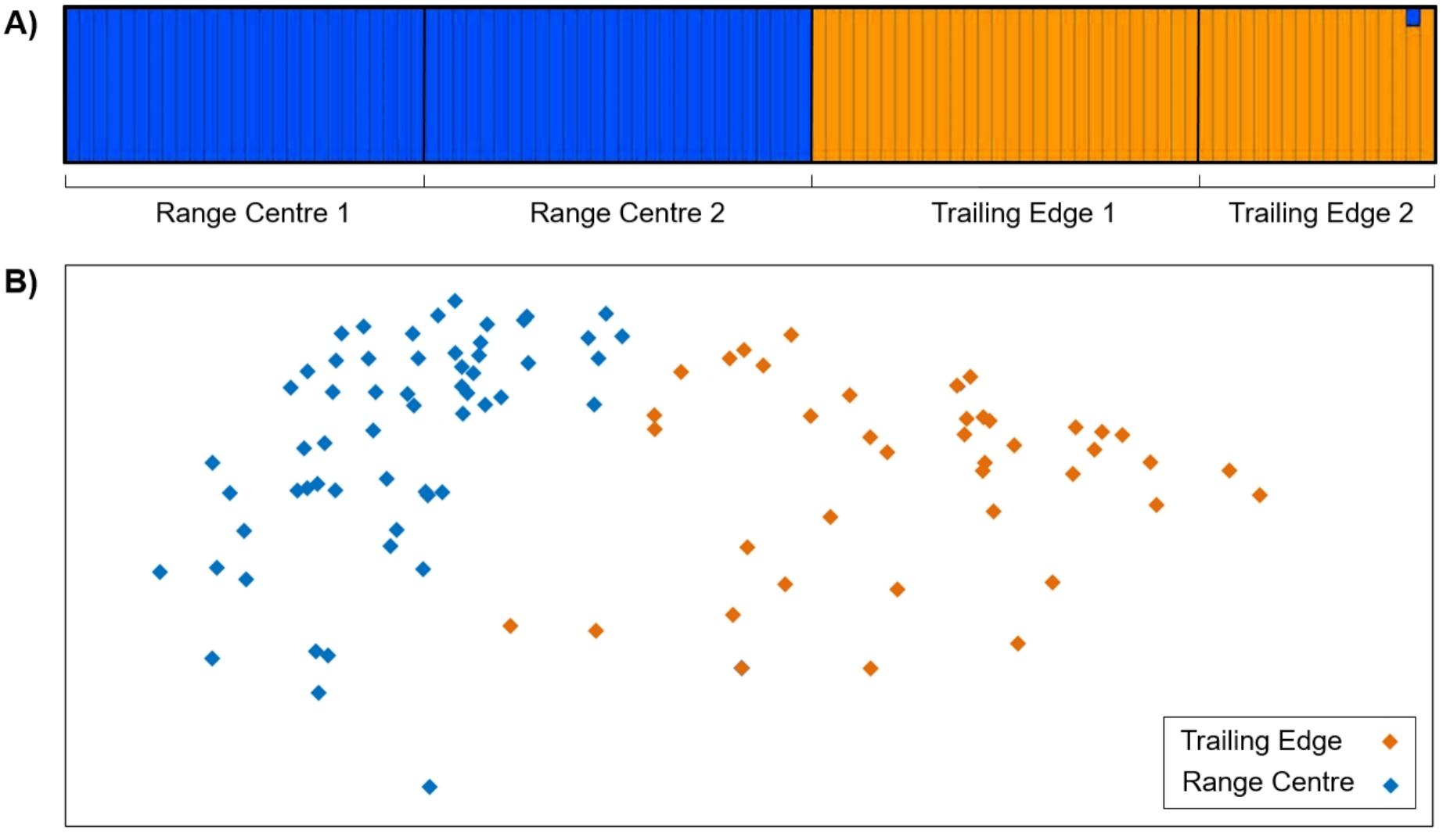
Fig. 4. A Genetic subdivision of *Laminaria digitata* based on STRUCTURE analysis. K1-4 were explored and K =2 represents the most likely number of clusters based in ΔK outlier identification. **B - Principal Coordinate analysis of pairwise genotypic distances between individuals.** Individuals are coloured according to their sampling location (i.e. trailing edge or range centre).

762
763


20°W
0°
20°E
N
60°N
50°N
Iceland
Norwegian Sea
Norway
Atlantic Ocean
RC1
RC2
North Sea
ROI
UK
TE1
TE2
Celtic Sea
France
0 200 Km


A
Range centre 1
B
Range centre 2
C
Trailing edge 1
D
Trailing edge 2
mRNA Expression (fold change)
Ton
Tpeak
Toff
Ton / Tpeak
0
5
10
15
20
8
12
16
20
24
28
32
Temperature (°C)


A
Range centre 1
Spring
Summer
mRNA expression (fold change)
20
15
10
5
0
B
Trailing edge 1
Spring
Summer
mRNA expression (fold change)
20
15
10
5
0
8
12
16
20
24
28
32
Temperature


A)
Range Centre 1
Range Centre 2
Trailing Edge 1
Trailing Edge 2
B)
Trailing Edge
Range Centre